Proceedings of The First International Conference on Pesticidal Plants

Volume 1 (August 2013)

Joshua O. Ogendo; Catherine W. Lukhoba; Philip K. Bett; Alex K. Machocho (Editors)

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The 1st ICPP is the brainchild of the ADAPPT project, supported by a European Union project that focusses on building scientific capacity in Africa. See www.nri.org/adappt for more information

NRI is the lead partner of ADAPPT and leader of the ICPP's International Scientific Committee. NRI has been at the forefront of research on pesticidal plants in Africa for more than 50 years.

The International Centre for Insect Physiology and Ecology is our host for the ICPP. *Icipe*- African Insect Science for food and health is the editorial home of the *International Journal of Tropical Insect Science*.

Egerton University is a partner and WP 4 leader of ADAPPT Network, leader of the ICPP's national scientific organizing committee and the ICPP's Primary Host Institution in Kenya. Egerton University is a premiere institution in agricultural research and training in east and central Africa.

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The Koul Research Foundation is the editorial home of *Biopesticides International*, one of the journals accepting invited papers in a special a conference proceedings issue.

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Opening address

Prof. J. O. Ogendo. Chair, ADAPPT Network, Kenya Chapter

The Chair welcomed the Guest of honour, invited guests and all participants and exhibitors. He especially recognized the contribution by conference sponsors and the national and international organizing committees towards the ICPP success. He introduced the African Dry-land Alliance for Pesticidal Plant Technologies (ADAPPT) as a network for optimizing and promoting the use of pesticidal plants for food security and poverty alleviation in Africa. The network, he noted was funded by the European Commission Europe Aid ACP S&T Programme. The ADAPPT partners included the Natural Resources Institute (UK) as the lead partner institution and seven African countries namely, Ghana, Kenya, Malawi, South Africa, Tanzania, Zambia and Zimbabwe.

The overall objective of the ADAPPT Network was to strengthen scientific and technological capacity of African nations to exploit and promote pesticidal plants for agricultural development and poverty alleviation. The specific objectives include: training and capacity building, dissemination and promotion of pesticidal plant technologies, investigating sustainable production and use, and developing commercialization and marketing opportunities.

The idea of the 1st International Conference on Pesticidal Plants was proposed as one of the outputs of ADAPPT network to help improve networking and build scientific expertise in the region. The ADAPPT partners unanimously voted to hold this scientific conference in Kenya since Egerton University was the lead institution for dissemination activities in the ADAPPT network. Egerton University was therefore selected to be the host institution for the ICPP. The NOC identified ICIPE as the perfect place to hold the conference because of its location, excellent facilities and long history of research on pesticidal plants and chemical ecology.

The Chair thanked the members of the NOC, the international ADAPPT team and *icipe* for all the hard work they had put in making this a successful conference. He noted with gratitude the generosity and involvement of many other sponsors who made it possible to hold the conference. He particularly thanked AAS-TWAS for sponsoring over 30 young scientists to present their findings. The 1st ICPP had attracted nearly 100 talks, 30 posters and many displays, with delegates arriving from more than 25 countries representing researchers, scholars, traditional herbalists, farmer organizations, NGO/CBOs, industry and suppliers (scientific general).

He again welcomed all members to the conference and encouraged all to interact and network widely in order make this time both productive and enlightening.

Prof. Phil Stevenson, Chair, ADAPPT Network

The network chair welcomed all participants gathered and thanked them for their attendance. He thanked *icipe* and Egerton University for hosting conference. He informed the audience that ADAPPT was an EU funded project awarded to NRI- University of Greenwich. He noted this was and inter -disciplinary project in applied research and management. Some of the outputs included providing seed money to farmers, bringing scientists together and publication of high quality papers in peer reviewed journals. He revealed that there were plans to hold ICCP2 and hence the other member countries may bid to host. He gave credit to the NOC for organizing the conference, to AAS for funding a large number of young scientists, to EU for funding several participants to the conference and to the many willing sponsors who contributed generously to make this conference a success. He once again thanked all members for participating and wished all a good conference.

Prof. Berhanu Abegaz, Executive Director, AAS-TWAS ROSSA

This first International Conference on Pesticide plants which seeks to strengthen scientific capacity and increase knowledge on the use of plants for pest management. The conference comes at an important time in the History of Africa given as you may already be aware that food security has become a major concern Across Africa. The challenge Africa faces now is how to sustainably increase food production to meet the demands of the growing population.

The African Academy of Sciences (AAS) and particularly the World Academy of Sciences (TWAS) Regional Office for sub-Saharan Africa (TWAS-ROSSA) (whose activities in Africa are coordinated by AAS) and this conference is one of its activities. AAS-TWAS/ROSSA is pleased to be part of the organizing team led by Dr. J. Ogendo to put together this exciting event.

The Academy's leadership is deeply aware not only of the need to increase in the amount of research coming from Africa but also the importance of sharing information generated from such research so that in can inform policy and decision making. Currently the level of information exchange among African Researchers is low something that compromises development.

The conference was going to be an opportunity for scientists and other stakeholders to share available knowledge around sustainable use of plants in pesticide control. Coming at a time of heightened debate over the long time safety of chemical pest control, this conference would be wise to assess the viability of this method and provide some valuable advice that can translate into policy action. He said he was aware that some of chemicals that have been in use in Africa for years now to control pests have been known to have a lasting and undesirable effect on the ecosystem. He therefore, urged all of those in this conference to take these deliberations as a way of responding to the ongoing conversation on the safe control of pesticides, and be able to advice the policy makers on the use of this approach.

He briefly shared the deep and inter-linked history of the three organizations present at this conference-the Academy, TWAS and *icipe*. The latter is of course the oldest of the three organizations, founded by the late Prof. Thomas Odhiambo. He also happened to be the founding President of the Academy, an ideas that was initiated in 1985,at a TWAS Congress in Triesete, Italy, when TO persuaded other 33 Africans to establish a pan-Africa-wide, merit-based academy. TRO as he was fondly known was passionate about the subject of sustainable pest control in Africa to boost Agricultural Production and spend so much time on research in this area. It is therefore befitting for *icipe* to host the conference.

He was pleased at the continued collaboration between the three organizations, TWAS, AAS and *icipe* over the years. The three organizations share a lot of history from their formative years. The engagement in this Conference is one of our regular annual activities under the theme – young scientist's conference, sponsored by TWAS ROSSA. The Academy attempted to bring young scientists from various Africa countries and also invite senior ones like TWAS Fellows and AAS Fellows to meet with them, mentor them and hopefully establish useful collaborative arrangements.

The conference provided opportunities to (1) award two prizes (a) for an outstanding young scientist, Dr. Emmanuel I. Unuabonah through an Africa-wide competition, and (b) also for a senior scientist, Prof. Charles O. N. Wambebe who is receiving a prize for his contributions in the building of scientific institutions. He also reported that there was going to be a public lecture on *"Innovative Pathway to Drug Development: From Plants to Medicines"*

He further added that AAS/TWAS-ROSSA are committed to bringing more young scientists into scientific discourse through a mentorship programme that will link young scientists in both at various research Centre and institutions of higher learning with well-established scientists. He concluded by urging young scientists all over the continent to take up the challenge of using their skills to help find solutions to Africa's problems.

Prof. J. Gowland Mwangi, PhD, Deputy Vice Chancellor, Research & Extension- Egerton University

In his speech on behalf of the vice Chancellor, Egerton University, the DVC welcomed all the ICPP participants, exhibitors, partners and sponsors to the first international conference on pesticidal plants. Egerton University was pleased and honoured to host the ADAPPT network in Kenya and to host the 1st ICPP. Egerton University puts great emphasis on research especially in the areas of agriculture, food and water security and climate change. Currently, global food requirements outstripped the supply of food. It is documented that over 50% of Kenyans are unable to get three meals a day. At the same time there is 50% reduction of water resources. The leadership of Egerton University was happy that the conference was addressing important issues related to food production through utilization of plant pesticides. This natural resource, he noted would play an important role in controlling the emergence of insect pests as a result of climate change. He encouraged scientists in Kenya and Africa in general to focus their research on increasing food production through viable and safe agricultural processes.

Mr Bernard Odanje, Deputy Director of Agriculture- The Guest of Honour

This First International Conference on Pesticidal plants will enable researchers, scholars, extension agents, farmers organizations, industry personnel and suppliers of scientific equipment and consumables to interact and share knowledge and experiences as well as research outputs from diverse fields and geographic locations across the globe. This will ultimately lead to communities' improved socio-economic status and quality of life.

Research and Development (R&D) are often driven by three key objectives. These are creation of knowledge; expansion of existing knowledge and verification of already established empirical facts. Africa in general and Kenya in particular is endowed with a highly trained human resource capable of contributing significantly to the development and dissemination of high quality innovations and products. Despite this endowment, the country still faces surmountable odds in its efforts to translate high-level training and technical skills into tangible and sustainable improvement in the quality of life of its resource-poor communities. It is the duty and responsibility of the scientific community, most of which is represented here today, to find the reasons why researchers' expertise does not necessarily translate into measurable positive outputs/ impact in society. It is hoped that in this Forum, will find better ways of overcoming this disconnect between theory and practice and between what is recommended and what is actually achieved.

Egerton University is commended for playing a key role in R&D in this region for development of patented, high-yielding, two-bean and two-chickpea varieties that are well adapted to arid and semi-arid areas (ASAL). This augurs very well with my Ministry's efforts to align its activities with the aspirations of Vision 2030. The University has also developed a biolarvicide for controlling malaria-causing mosquitoes. Furthermore, the University engages in demand-driven research and outreach activities that address issues of water and sanitation, supply of affordable energy, post-harvest handling and storage, as well as promotion and use of good agricultural practices for optimized and sustained farm productivity. In order to mitigate against the effects of climate change and to protect our environment, the University has embarked on a successful campaign of rehabilitation of the Mau catchment by planting indigenous trees along the Njoro River and its neighbourhood. So far over 400,000 trees have been planted and these have increased the River's water level.

Keynote Presentations

Harnessing pesticidal plant technologies for improved livelihoods

Professor Murray Isman
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Stringent regulatory requirements governing the use of pesticides in G20 countries have created renewed interest in insecticides based on natural products, including botanical insecticides. Commercialization of neem (azadirachtin)-based insecticides in the 1990s and pesticides based on plant essential oils in the past decade have raised the profile of botanicals and demonstrated their ut ility in certain pest management contexts. However the numbers of successful botanical insecticides in use are dwarfed by the volume of research publications on this subject, based on recent bibliometric analyses, suggesting a big "disconnect" between theory and practice. Botanical insecticides may lack the absolute efficacy and residual action of many conventional insecticides, but they are finding increasing acceptance control of domestic and public health pests, and greenhouses. Other uses include ectoparasite management on companion animals and domesticated livestock, mosquito abatement, and as personal repellents against blood -feeding arthropods. In developing countries botanicals enjoy more widespread use in agriculture. Many tropical plants have insecticidal properties, are readily available locally, affordable, and can be used with minimal preparation. Due to pesticide-related poisonings and deaths in developing countries, the benefits of botanical insecticides are best realized. I argue that we already have enough candidate plants available to improve the livelihoods of smallholder farmers in sub-Saharan African. Therefore, emphasis should be placed on demonstrating the practical utility of these plants and simple plant preparations useful for pes t management.

Pesticidal plant bio-prospecting: ethno-veterinary and vector control

Professor Ahmed Hassanali
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The presentation was on on-going studies on two disease vectors: *Anopheles gambiae*, which transmits malaria, and *Rhipicephalus appendiculatus*, which transmits East Coast Fever (ECF). Both sets of studies included research on the efficacy of phytochemicals-based ethno-practices for the control of the vectors and explored methods of improving upon these. However, R&D in the development of effective vector control strategies constitutes a multi-disciplinary subject. The importance of 'bio-rational' R&D approaches that integrate bioprospecting for active phytochemicals with behavioural/physiological manipulation of the target vectors and chemo-ecological insights and tools was highlighted. Fruitful bio-prospecting needs to take into account consequences of co-evolutionary interactions mediated by secondary metabolites between plants and their predators. Important consequences of such interactions include (i) structural and analogue diversity of compounds often acting as blends to provide effective protection against specific predators and to mitigate against speedy resistance development; and (ii) subtlety in the actions of secondary metabolites, often relying on repelling/deterring specific functions of predators or inhibiting their normal physiology and development, and rarely on their acute toxicity. These have important implications on experimental approaches used to discovering potentially useful phytochemicals and to their effective exploitation.

Plants-derived compounds in pest control: challenges and opportunities

Professor Monique Simmonds
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Despite the interest in plant-derived compounds for the control of pests very few have been developed as commercial products. Plants have a significant influence on the selection behaviour of insects and as antifungal agents yet few are used in pest control. Therefore, there is need to address legal and scientific challenges in the development of a product for pest control. Challenges will vary depending if the products are for local use or for the commercial market. Traditionally the companies have been interested in the development of compounds that will show selective toxicity against target organisms. More recently new products that modulate the behaviour of pests such as plant extracts, not just single compounds. Our increased knowledge about the ecological role of plant-derived extracts/compounds in plant-pest interactions could open the door to an increased use of natural products in pest control strategies. However, in the development of products the scientist has to cover issues that relate to the access of biological resources for testing and to the use of any intellectual property about the traditional uses of these resources. The main international agreements that relate to intellectual property about the uses of plants are "Trade-Related aspects of Intellectual Property rights (TRIPS) and the Convention on Biological Diversity (CBD). These agreements differ fundamentally in their approaches to intellectual property. Greater knowledge about how to deal with these treaties in the early stages of a project should assist scientists focus on finding robust new leads and mitigate the potential of being called a bio-pirate should they discover the next international pesticide or a local control agent that they want to share with their colleagues in other countries.

Symposium 1 Pre-harvest Plenary

Evaluation of the Efficacy of *Alium sativum, Albizia versicolor* and *Lantana camara* for the Control of Coffee Leaf Rust Pathogen

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Abstract

Coffee leaf rust, caused by *Hemileia vastatrix* Berkeley & Broome., remains one of the most economic important diseases of coffee causing losses of up to 80% in plantations if uncontrolled. Three experiments were carried out to evaluate the efficacy of three botanicals: garlic (*Allium sativum*), lantana (*Lantana camara*) and Albizia (*Albizia versicolor*) against Coffee Leaf rust at Coffee Research Institut e laboratory, Chipinge, Zimbabwe. Aqueous leaf extracts for each botanical were prepared into concentrations of 100, 50, 25 and 12.5%. Copper Oxychloride 85% WP at 10g/litre water and distilled water were used as the standard and negative control respectiv ely. Spore suspensions of *H. vastatrix* were germinated in petri dishes containing sterile deionized water agar and each botanical in three separate experiments. Germinated spore counting was done by microscopic examination of 100 spores per slide after inc ubating for 16 hours in the incubator. There were highly significant differences ($p < 0.001$) in leaf rust spore germination inhibition due to *A. sativum, L.camara* and *A. versicolor* concentrations. Copper oxychloride and the neat concentration (100% aqueous extract) were not significantly different for all the botanicals tested. Distilled water was not effective in inhibiting spore germination and was significantly different from the rest of the treatments. The results showed that efficacy increases with inc rease in concentration for all the three botanicals. Garlic, lantana and albizia showed great potential in controlling coffee leaf rust under laboratory conditions. More work is required to design strategies for integrating the botanicals in disease manage ment programs.

Introduction

Production of coffee has become a very important unique source of income for smallholder farmers in developing countries. However, the coffee sector increasingly faces challenges. Among the major challenges is the need to control coffee diseases such as coffee leaf rust (Zambolin *et al.*, 2005). Coffee leaf rust caused by *Hemileia vastatrix* Berk. and Br., is one of the main diseases of Arabica coffee in Africa. *H. vastatrix* Berk. and Br., is an obligate biotrophic fungus which occurs worldwide in coffee growing countries including Zimbabwe and its effects were found to be potentially devastating (Manuela *et al.*, 2005). The pathogen penetrates through the stomata and later develops powdery orange pustules below the leaf surface. The disease is most severe on *Coffea arabica L.*, which accounts for about 70% of the world coffee production and supply (Dinesh *et al.*, 2011). Coffee leaf Rust is effectively managed using fungicides and host plant resistance (Coste, 1992; Zambolin *et al.*, 2005). The use of fungicides has become the major practice in coffee leaf rust control in the corporate and smallholder farms. Despite the effectiveness of fungicides, they are environmentally unfriendly and expensive for resource poor farmers. This has prompted the need to evaluate new products such as plant extracts for the control of coffee leaf rust.Plant extracts are locally available, cheap and environmentally friendly. In addition, the use of botanicals could be important in sustainable coffee production where the use of inorganic fungicides is no longer encouraged. These trials were set up to evaluate the effectiveness of three botanicals, namely garlic (*Allium sativum*), lantana (*Lantana camara*) and albizia (*Albizia versicolor*) in the control of coffee leaf rust under laboratory conditions.

Literature Summary

Due to high demand for ecologically and environmentally friendly pesticides, a lot of research has been done to evaluate plant extracts for their effi cacy against various pests and diseases. Natural products such as *Carica papaya* leaf extracts (Kutywayo *et al.*, 2008), *Lantana camara* (Triphyani *et al.*, 2009) and *Allium sativum* (Masum *et al.*, 2009) have been used in controlling different plant pathogens at different concentrations and were found to be effective. Garlic tablets were found to be very effective in the control of various seed borne pathogenic fungi in sorghum, with about 90% inhibition of the fungi present (Masum *et al.*, 2009). In a study done in India, *L. camara* was used in the control of coffee leaf rust under greenhouse and field conditions. A spore germination inhibition of 69.9% was obtained with *L. camara*, and was not significantly different from the most effective botanical *A. vasica* evaluated along with *L. camara* (Barros *et al.*, 1999). *Albizia versicolor* has not been used in the control of coffee leaf rust. *Albizia spp* was found to possess important antiarthritic properties in some rats (Pathak, *et al*., 2010).The leaves, roots and root-bark are also reported as being useful in curing of stomach pains in Zimbabwe.

Description of Research

The trial was carried out at Coffee Research Institute in the Laboratory. Three botanicals were evaluated; namely *A. sativum* (Garlic), *L. camara* (Lantana) and *A. versicolor* (Albizia). Each botanical was evaluated in a separate experiment/ Half kilogram of each botanicals' leaves were collected and prepared by crushing using a pestle and mortar. Aqueous leaf extracts were obtain ed by soaking crushed 100g leaves of each botanical in 100 ml distilled water (aqueous) for 16 hours. Crude extracts were filtered through a fine cloth and diluted into different concentrations for each botanical as 100% (undiluted), 50%, 25% and 12.5%. Co pper Oxychloride 85% WP was used as the standard control and distilled water was the negative control. *H. vastatrix* spores were collected from naturally infected coffee plants in coffee fields. A spore suspension of *H. vastatrix* (8×10^6 spores/ ml) was prepared in sterile deionized water and spores were counted using a haemocytometer. The experiments were set up in a completely randomized design with three replicates for each botanical. Garlic contains three replicates, each replication with three petri di shes to make nine petri dishes per treatment. Lantana and Albizia had three replications each, where one replication had one petri dish to end up with three petri dishes per treatment. 1ml of each plant extract concentration was added to 9ml of sterile wat er agar in a test tube and shaken well to mix. The test solution was poured into Petri dishes followed by 0.5ml *H. vastatrix* spore suspension in each petri dish. The inoculated Petri dishes were incubated for 16 hours at 24 ^{o}C. After 16 hours, germination was interrupted by adding one drop of 0.1% Mercuric chloride into each Petri dish. Germinated spore counting was done by microscopic examination of 100 spores per slide. Uredospores with germ tubes of at least one half the lengths of their diameter were con sidered germinated. The results were expressed as percentage spore germination in relation to germination recorded in the control. Analysis of variance was done on transformed percentage data (ArcSine) for each botanical in Genstat 14 th Edition (VSNI, 2011). Least significant difference was used to separate the means. The effective dose that will inhibit spore germination by 50% (ED_{50}) and 95% (ED_{95}) were determined using probit analysis in Genstat 14.

Research Results and Application

There were highly significant differences in coffee leaf rust germination inhibition due to garlic concentrations ($p<0.001$). Copper Oxychloride 85%WP, undiluted garlic extract (100%) and the 50% concentration had the highest number of ungerminated spores and were not signif icantly different from each other. The performance of garlic was found to be in line with other studies done on effectiveness of garlic against *Pythium* spp., *Phytopthora* spp. and *Fusarium* spp. where spores failed to grow in nutrient solutions containing 10% or higher levels of garlic extract (Sealy *et al.*, 2007). The higher inhibitory effects exhibited by garlic at higher concentrations suggests the botanical has great potential in coffee leaf rust control equally the same as the standard treatment of Copper Oxychloride. Garlic extracts were found to contain many biologically active compounds responsible which possess antimicrobial properties (Avato *et al.*, 2000) and the diversity of compounds in garlic may explain the high spore germination inhibitory effects on coffee leaf rust. Inhibition of spore germination increased with increasing extract concentration for garlic. Distilled water (control) was not effective in inhibiting spore germination and was significantly different from the 12.5 and 25% conce ntrations (Table 1). The non-inhibitory effect of distilled water alone suggests that the spores were viable.

Table 1: Efficacy of *A. sativum, Lantana camara* and *Albizia versicolor* aqueous leaf extracts against Coffee Leaf Rust under laboratory conditions

Treatment	Mean spore germination inhibition		
	A. sativum	*L. camara*	*A. versicolor*
Control	0d *	0d	0e
12.5% Garlic	45.5c	65.5c	70.6d
25% Garlic	68.6b	78.1b	80.1c
50% Garlic	92.8a	98.2a	87.2b
100% Garlic	97.9a	100a	95.9a
Copper oxychloride 85% WP	90.8a	100a	97.1a
P	<0.001	<0.001	<0.001

*** Means followed by the same letter(s) are not significantly different using LSD.**

There were significant differences in percentage spore inhibition due to the effect of different concentrations of *L. camara*. Copper Oxychloride 85%WP, 100% (neat extract) and 50% extract had the highest mean number of spores inhibited of 100, 100 and 98.24%, respectively, which were not significantly different from each other. *L. camara* was found to be very effective in inhibiting colony growth and spore germination of leaf blight of wheat (Patil and Kulkarni, 2002) and *Fusarium spp*. (Saraf *et al.*, 2011). The results on the effectiveness of *L. camara* agree with studies done by Santos *et al* .(2007) on coffee rusts control where coffee leaf rust spores inhibition was 69.91% using 15% concentration of lantana. The results were confirmed by the fact that even lowest concentration of *L. camara* (12,5%) managed to inhibit 65.5% of spores from germinating. The results in this study show that 25% of *L. camara* inhibited more than 50% of the spores. The effectiveness of *L. camara* in low concentrations from this study means the botanical is very effective. The control did not in hibit any spores and was significantly different from the rest of the treatments (Table 1).

There were significant differences ($p< 0.05$) in percentage spore germination inhibition due to the effects of *A. versicolor*. Copper Oxychloride 85%WP and the neat extract (100%) had the highest mean number of spores inhibited of 97.1and 95.9%, respectively, which were not significantly different from each other. All the other treatments except the control managed to inhibit spore germination. There was evidence of a linear relationship between concentration and inhibition of spore germination (Table 2). *Albizia versicolor* has not been used in the control of coffee leaf rust before. However, the effectiveness of this plant extract in other diseases has prompted its evaluation against coffee leaf rust. Twenty plant extracts including *Albizia* spp. were used to control disease causing pathogens in humans. *Albizia* spp. had more than11 mm inhibition zone of microbial activity in cultures suggesting that the botanical is very effective for treatment of infections (Maji *et al*., 2010) and can therefore be a new source of treatment. *Albizia* species were also found to possess significant antiarthritic activity in rats (Pathak *et al.*, 2010) and listed among the poisonous plants in Southern Africa (Bothaa and Penrith, 2008). The effectiveness of *Albizia* species in inhibiting pathogen growth shows potential for using it in integrated disease management in plants.

L. camara had the lowest ED_{50} of a concentration of 14.22% followed by Garlic with 14.43% (Table 5). The lowest ED_{95} was also for *L. camara*, which showed that 33.89% concentration of *L. camara* leaf extracts are able to destroy up to 95% of coffee leaf rust spores. Although *A. sativum* was the second best after *L. camara* in terms of ED_{50}, it required the highest concentration to kill 95% of coffee leaf rust spores (66.02), with *Albizia versicolor* requiring a concentration of 56.08%.

Table 2: ED_{50} and ED_{95} for *L. camara, A.sativum* and *A. versicolor* for controlling coffee leaf rust

Botanical	ED_{50}	ED_{95}
L. camara	14.22	33.89
A. sativum	14.43	66.02
A. versicolor	20.77	56.08

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Differential Responses of the Cowpea Aphid, *Aphis craccivora* to Host Plant and Pesticidal Plant Odours

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Abstract

Olfactometer experiments were conducted in the laboratory to study the effects of host plant odours on apterae and alataeforms of *Aphis craccivora*. The host plants were cowpea, *Vigna. unguiculata* and groundnut, *Arachis hypogaea* fresh leaves, while the non-host plants were neem, *Azadirachta indica* and Pepper fruit, *Dennettia tripetala* seed oils. Both alatae and apterae significantly (P<0.001) spent more time and made more number of visits to the olfactometer arm emitting host pla nt odours compared to control in single choice bioassay.In response to non -host odour perception, the aphids significantly exhibited avoidance behaviour in time spent and number of visits compared to the control. The aphids were also significantly (P< 0.001) repelled by *A.indica* and *D. tripetala* volatiles compared to control arms in choice tests. This study showed that *A. craccivora* responded with positive anemotaxis to kairomonal cues emanating from its host plants, *V.unguiculata* and *A. hypogaea*, but was repelled by non-host volatiles.

Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers, is an important indigenous leguminous food crop that provides a relatively inexpensive source of high quality protein for humans and animals in many African countries. I n Nigeria for instance, cowpea is cultivated by resource -poor farmers on small farm holdings but who produces almost all the cowpeas consumed in the country. But aphids are a perennial limitation to sustainable cowpea production especially in Africa. The black cowpea aphid, *Aphis craccivora* Koch, (Homoptera: Aphididae) is a key pest of *V. uniguiculata* , groundnut or peanut, *Arachis hypogaea* (L.) and other related leguminous crops in the field. Groundnut is the fourth most important oilseed crop in the world, grown mainly in tropical, subtropical and warm temperate climates. It is an important leguminous cash and crop for the tropical farmers and its seeds contain high amounts of edible oil (43–55%) and protein (25–28%) (Reddy *et al.,* 2003).*Aphis craccivora* nymphs and adults infest *V. unguiculata* seedlings feeding on the leaves, shoots, inflorescence and green pods causing malformation of the affected parts, stunted growth, curling up of leaves, flower and pod abortion, and poor yield (Ofuya, 1995; Pettersson*et al*., 1998).This laboratory study was designed to investigate the behavioural responses of *A. craccivora* to two leguminous crops, (*V. unguiculata* and *A. hypogaea*) and non-host plants (*A. indica* and *D. tripetala*) volatiles in an air flow olfactometer.

Literature Summary

Pest control in smallholder farms has become imperative if sustainable food production must be accomplished for the ever-increasing populations in the sub-Saharan Africa. *Aphis craccivora* has been reported to transmit the cowpea mosaic virus (CMV), groundnut rosette virus (GRV), !frica's most devastating groundnut disease, peanut mottle virus (PMV), peanut stripe virus (PSV) and peanut green mottle virus (PGMV) (Schilling and Gibbons, 2002). In Nigeria, *V. unguiculata* is protected against insect pests such as *A. craccivora* by the application of synthetic insecticides such as lambdacyhalothrin and monocrotophos. But environmental concerns regarding the continuous application of these insecticidesin crop protection,such as the development of resistance by pest organisms, persistence in the environment, detrimental effects on beneficial organisms, mammalian toxicity, issues with residues on food (Daglish, 2004; Sousa *et al*., 2009), higher cost of crop production and scarcity at times of need (Umoetok *et al*., 2009),have prompted the need to evaluatebotanical products to manage pests of crops in Africa. Different Pesticidal plant products in the form of essential oils (EO), powders, pellets, extracts or distillates could be harnessed as potential toxicants, deterrents, antifeedants and repellents to prevent insect feeding and oviposition (Isman , 2006; Rajendran and Sririnjini, 2008).Neem (*Azadirachta indica*A. Juss) (Meliaceae) is perhaps the most useful traditional medicinal plant in Africa and Asia. Neem extracts generally possesses anti-fungal, anti-bacterial and insecticidal properties (Mordue *et al*., 1998; Isman, 2006; Umoetok *et al*., 2009).Pepper fruit, *Dennettia tripetala* (G.) Baker (Annonaceae) is a woody forest and spicy plant, cultivated in Southern states of Nigeria, where the leaves and fruits are used in combination with other herbs for the treatment of cough and stomach ache (Ejechi and Akpomedaye, 2005). *D. tripetala* extracts have also been reported to exhibit insecticidal (Ukeh *et al*., 2011) and antifungal properties (Nwachukwu and Osuji, 2008).

Description of Research

Bioassays were carried out using a Perspex four-arm olfactometermodified after Pettersson (1970) to determine the responses of alatae and apterae forms of *A. craccivora* to volatiles from *V. unguiculata* and *A. hypogaea* fresh leaves, and *A. indica* and *D. tripetala*essential oil solutions. Detailed construction of the olfactometer and procedures of bioassays have been described (Ukeh *et al*., 2010). In the single choice tests, one arm of the olfactometer contained the treatment (odour source) while the remaining 3 arms served as controls. The stimuli tested included 3 grams whole fresh leaves of *V. unguiculata* and *A. hypogaea*, 10 µl essential oil solutions of *A. indica* and *D. tripetala* loaded on filter paper disc in the test arm against 10 µl solvent (hexane) impregnated on filter discs as controls. Actively moving *A. craccivora* alatae and apterous individuals were selected for the behaviour studies. Aphids were kept singly in Petri dishes and starved for 24 h prior to the bioassays, and a single aphid was introduced through a hole in the top of the olfactometer with a fine hairbrush. Air was drawn through the central hole at a rate of 400 ml min $^{-1}$ and subsequently expelled from the room. Each aphid was observed for 16 min, and the olfactometer was rotated 90º every 2 min in a clockwise direction to control for any directional bias in the behaviour chamber. The test aphids, olfactometers and odour samples or stimuli were changed after every replication. All experiments were replicated 10 times, while data on time spent and number of entries made by *A. craccivora* to test and control arms were recorded with Olfa computer software (F. Nazzi, Udine, Italy). Data on the mean time spent in and number of entries into treated and control arms were compared to a test mean of zero using a paired t-test after checking that data were normally distributed Genstat 13).

Research Results and Application

The present study has demonstrated behavioural responses of *Aphis craccivora* towards host and non-host plant materials in olfactometer bioassays.

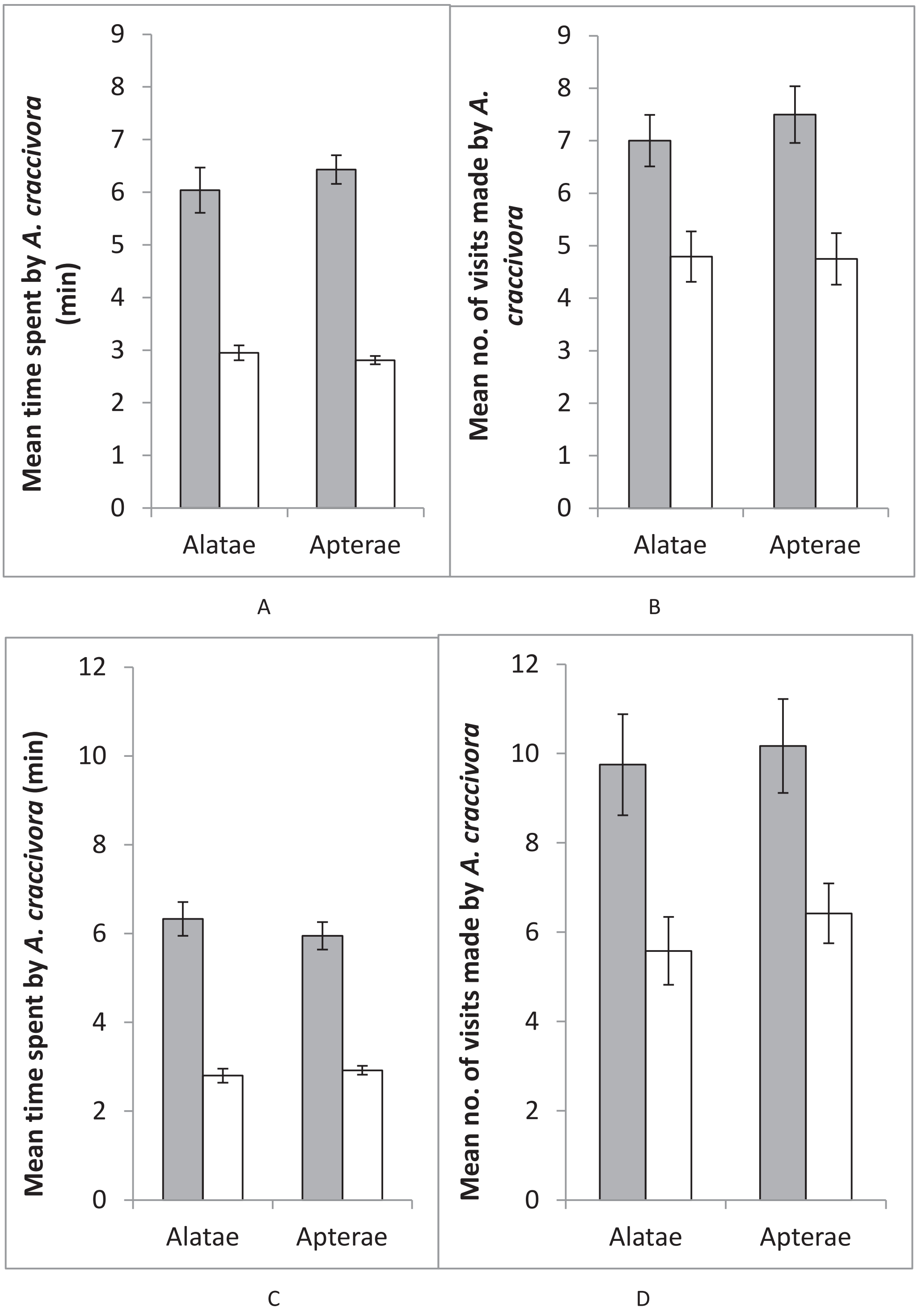

Figure 1: Behavioural responses of alatae and apterous *Aphis craccivora* measured as (A) mean time spent (min ± SE) and number of visits (B) to *V. unguiculata* fresh leaf volatiles, and (C) time spent (min ± SE) and number of visits (D) to *H. hypogaea* leaf volatiles in a 4-way olfactometer

■ = Treated □ = Control.

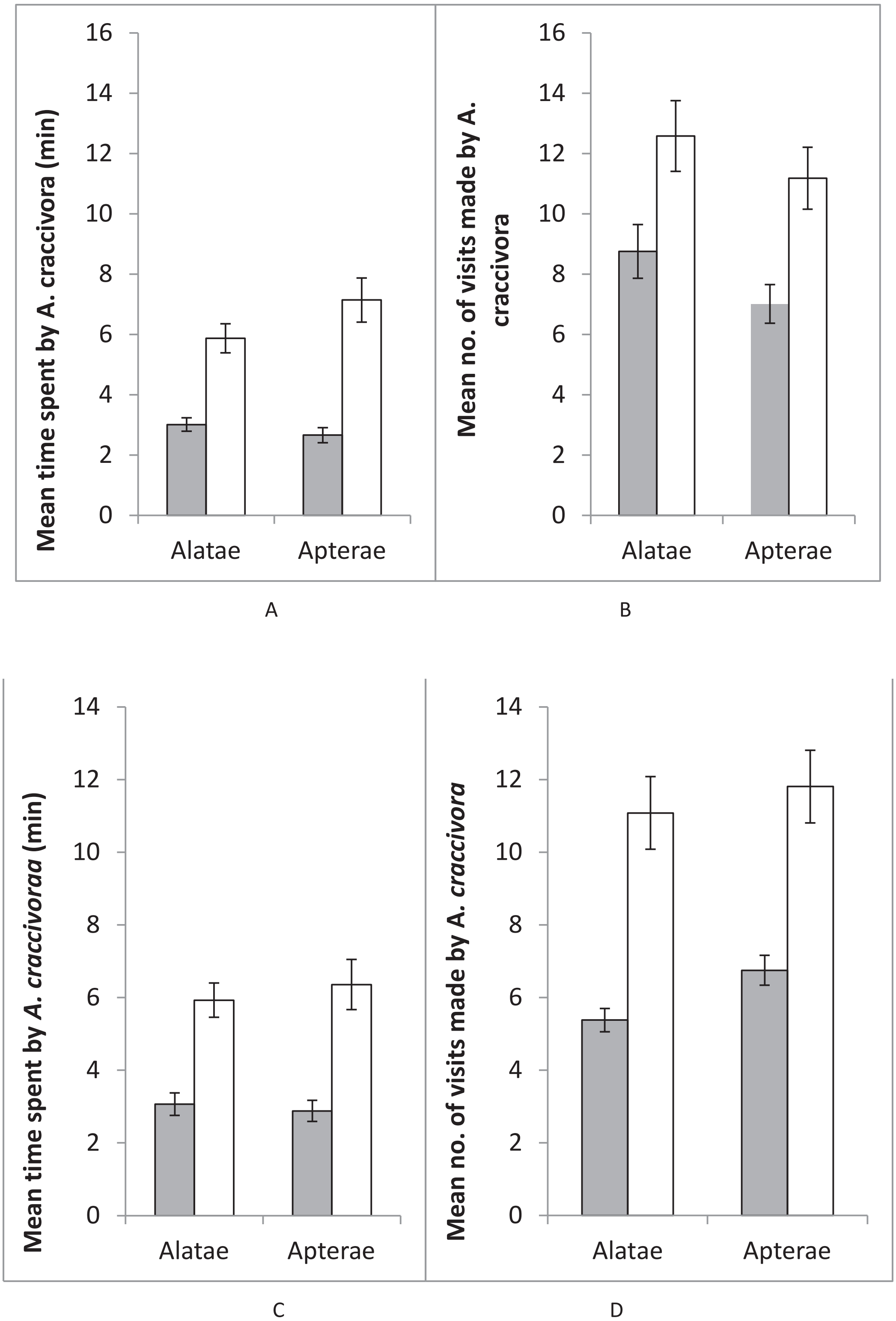

Figure 2: Behavioural responses of *A. craccivora* measured as (A) time spent (min ± SE) and number of visits (B) to *A. indica*, and (C) time spent (min ± SE) and number of visits (D) to *D. tripetala* essential oil solutions in a 4-way olfactometer

▆ = Treated ☐ = Control

The results showed that both the alatae and apterous forms of *A. craccivora* significantly (P<001) spent more time and made more number of visits to the olfactometer arm treated with *V. unguiculata* fresh leaves than the control (Fig. 1 A, B). Similarly the aphids significantly (P<0.001) preferred the arm containing *A. hypogaea* leaf than the controls (Fig. 1C, D) in tine spent and number of entries (visits). Data from this study showed that adult *A. craccivora* has the ability to recognize its host plants through olfaction. Behavioural and electrophysiological studies have shown that aphids, like other phytophagous ins ects detect olfactory cues which they exploit for host location, escape responses and materecognition (Pickett *et al*., 1992; Park *et al*., 2000). Using non-host plants, the aphids significantly (P<0.001) spent less time and made fewer number of entries or visits to the treated arm containing *A. indica* essential oil solution (Fig. 2 A, B). A similar behavioural response was recorded when *D. tripetala* essential oil solution was assayed against *A. craccivora* (Fig. 2C, D). The result indicated that both plants were repellent to *A. craccivora* in olfactometer bioassays. Neem derivatives have also been used for the management of five key pests of okra in India (Dhingra *et al*., 2008) and *D. tripetala* has also been reported repellent and insecticidal against stored products insects (Ukeh *et al*., 2012)/ This implies that aphid's antennae possess olfactory receptors which respond to and differentiate between plant volatiles as attractants and repellents (non -hosts). Findings from this study also showed that both alatae and apterae exhibited similar behavioural responses to host and non -host plants' volatiles/ Adequate knowledge of *A. craccivora* olfaction could be useful in the deployment of environmentally friendly approaches as an aspect of integrated pest management of the insect in tropical fields. On -going studies are underway to identify the bioactive kairomones from host plants such as *V. unguiculata* and *H. hypogaea*. The identified attractive kairomones could be incorporated as baits in traps for monitoring aphids, and known repellents used to reduce pest populations in small scale cultivated crops.

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Investigations on the performance of potential botanicals against cashew powdery mildew disease in Tanzania

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Abstract

Studies on five potential botanicals namely Kimbinga (*Morinda morindoides*), Mnyaa (*Senna occidentalis)*, Mwarobaini (*Azidarachta indica)*, Lipangati *(Opuntia cactus*), Kibamba (*Opuntia vulgaris*) were conducted against cashew powdery mildew disease caused by the fungus *Oidium anacardii* in the field and *in vitro* during 2012/2013 cashew season. Progressive disease assessment showed that panicles sprayed with Mwarobaini extracts had the least mildew infection level (31.5%), followed by Kibamba (35.5%), Mnyaa (36.5%) and Lipingati (41.8). Kimbinga plant extracts attained the highest mildew infection (80.8%) among the treatments close to 100% level recorded on the control. *In vitro* studies revealed that all tested plant extracts had significantly reduced the growth and development of the mildew fungus. These preliminary results clearly indicated that the four botanicals Mwarobaini, Kibamba, Mnyaa and Lipangati appear to have some potential in controlling powdery mildew disease on cashew. Thus, further field and laboratory studies on these botanicals will be conducted in the coming seasons to identify and possibly recommend some for commercial use.

Introduction

Powdery mildew caused by the fungus *Oidium anacardii* Noak is among the most devastating cashew diseases in Tanzania. This disease was first identified in Tanzania in the late 70s and since then has been considered as the most important cashew disease in the country. The disease infects young growing tissues on all aerial parts of the tree, including shoots, leaves and flowers (Waller *et al*. 1992). All affected tissues are easily observed to be covered by the whitish powder of the fungal material. The main damage to cashew productivity is caused by infection on flowers, which may be attacked even before opening. Infected flowers often become necrotic, fail to open for fertilization and frequently abscise. When flowers succeed to open, the emerging nuts are usually scarified leading to low quality. In situations where effective control measures are not taken the disease may cause a crop loss ranging between 70 – 100% (Sijaona and Shomari, 1987). Presently in Tanzania the disease is controlled mainly by sulphur dust and water based fungicides. Generally all these chemicals are very expensive to the extent that most farmers are not able to purchase them and they also pollute the environment. Due to these environmental and economic considerations, there is a need to search for other control measures which are affordable to farmers and more environmentally friendly.

Literature Summary

Currently all over the world, scientists are involved in finding the cheaper and more environmentally friendly bio-compounds for the control of plant diseases using different types of botanicals ((Mothana *et al.* 2005). In the past, several higher plants have proved their usefulness against a number of fungi (Dixit *et al*. 1983; Sigh *et al*. 1983).

The systematic search of higher plants for antifungal activity has shown that plant ex tracts have the ability to inhibit spore germination and mycelia growth in many fungal species (Guerin and Reveille, 1984; Natarajan and Lalithakumari, 1987; Sigh an Dwivedi, 1987). During recent years, use of plant extracts particularly neem derivatives is gaining importance for their antifungal and antibacterial properties (Yin and Cheng, 1998; Mishra *et al*., 2003; Janagiri and Naiik, 2005). Many plant extracts are reported to specifically inhibit the germination of fungal spores (Babu *et al*., 2001). Hence, in the present studies, extracts from locally available plants were evaluated both in the field and *in vitro* against cashew powdery mildew disease.

Description of Research

The five plant materials tested were collected from a botanical garden establi shed from 2010 at Naliendele Agricultural Research Institute, Mtwara, Tanzania. For field studies six trees were selected from one of the vegetatively propagated cashew blocks at Naliendele Agricultural Institute, Mtwara, Tanzania. These were tagged showing treatment numbers together with the name of the treatment. About 250g of leaf material of each of the tested botanical plants (Table 1) were collected one day prior to the preparation of the extract solutions. These were later grinded and sieved separate ly. The fine powder obtained from each lot was mixed with 1 litre of water and left on bench for about 24 hours to allow the pesticide extraction to take place.

Table 1: Plant species tested against cashew powdery mildew disease

No	Vernacular name	Plant species	Family	Parts used
1	Kimbinga	*Morinda morindoides*	Rubianceae	Leaf
2	Mnyaa	*Senna occidentalis* (L.)	Leguminosae	Leaf
3	Mwarobaini	*Azidarachta indica* (A.) Juss	Meliaceae	Leaf
4	Lipangati	*Opuntia cactus*	Cactaceae	Leaf
5	Kibamba	*Opuntia vulgaris*	Cactaceae	Stem

After 24 hours, about 500mls were collected from each of the extract solutions and sprayed on the ten panicles of the respective cashew trees using a one litre hand sprayer. One tree was not sprayed and this was used as a control. This exercise was repeate d for seven consecutive weeks. Each of the ten panicles was assessed for mildew infection at weekly intervals. During disease assessment the percent of flowers and flower buds affected by mildew was estimated using the 0 – 6 disease severity key (Nathaniels, 1996). Scoring was done on the four oldest laterals of a panicle.

In vitro studies were also conducted at Naliendele Agricultural Research Institute, Mtwara, Tanzania. Collected plant materials were surface sterilized with 0.1% sodium hypochlorite, washed and shade-dried for 5-6 days. These were finally powdered using wooden pestle and mortar. The extracts of the botanicals were prepared following the method of Rezaul Karim *et al.* (1992). Each of the five plants extracts was prepared at a concentration of 10% w/v. The resulting solution was stirred continuously for 10 minutes and left to stand for 12 h before filtration. Cashew leaves with natural mildew infection were selected 24 h before inoculation date and shaken to dislodge old spores and encoura ge production of fresh spore overnight. To determine the effect of the plant extracts on conidial germination, conidia of powdery mildew harvested were mixed with sterile water amended with the various plant extracts. Inhibition of spore germination technique was adopted. Single drop of conidial suspension of *O. anacardii* was added to sterilized slides, to which a single drop of double the concentration of different plant extracts was added to get the required concentrations. Later cover slip was placed on the cavity slide. Each control treatment was maintained with distilled water. These sterilized slides were kept in the Petri dishes lined with moist blotting paper and were incubated at room temperature (27±1^{o}C). After 24 h, observations were taken in ten microscopic fields for each slide and the total numbers of spores germinated in each microscopic field were recorded and per cent germination was calculated. Further, the per cent inhibition of spores was calculated by using formula given by Vincent (1947). Data were subjected to one-way analysis of variance (ANOVA) while differences in treatment means were separated by Student

Newman Keul's (SNK) test at 5% level of significance/ !ll statistical analyses were done by SPSS version 17/0 for windows.

Research Results and Application

Table 2 shows field mildew development expressed as percentage infection levels. Generally, it appears that four plant extracts had suppressed the development of the disease. It was observed that panicles sprayed with Mwarobaini extract solution had the least mildew infection level (31.5%), followed by Kibamba (35.5%), Mnyaa (36.5%) and Lipingati (41.8). Kimbinga attained the highest mildew infection (80.8%) close to 100% level reached in the control.

Table 2: Powdery mildew disease development expressed as percentage infection levels

Plant species	Scoring Dates for Mildew development						
	2 Aug	9 Aug	17 Aug	25 Aug	2 Sep	10 Sep	17 Sep
Kimbinga (Morinda morindoides)	2.9	10.0	21.9	30.3	36.1	67.3	80.8
Mnyaa (Senna occidentalis)	0.6	3.0	6.5	32.5	17.7	29.5	36.5
Mwarobaini (Azidarachta indica)	0.0	1.5	2.0	4.2	8.5	25.1	31.5
Lipingati (Opuntia cactus)	0.3	1.2	2.7	6.3	18.8	29.8	41.8
Kibamba (Opuntia vulgaris)	0.0	1.3	8.3	13.6	16.2	38.4	35.5
Control	0.7	7.5	34.5	49.9	70.4	77.3	100

Results from i*n vitro* studies indicated that all plant extracts had significantly inhibited spore germination of *Oidium anacardii* (Table 3).

Table 3: Percentage spore germination inhibited by different plant extracts

Plant Species	**Mean ± S.E**
Kimbinga (Morinda morindoides)	15.24 ± 5.8
Mnyaa (Senna occidentalis)	46.48 ± 3.5
Mwarobaini (Azidarachta indica)	64.91 ± 2.3
Lipingati (Opuntia cactus)	50.81 ± 3.6
Kibamba (Opuntia vulgaris)	56.82 ± 1.8
Control	0

In vitro results (Table 3) further indicated that the five plant extracts significantly ($P < 0.05$) inhibited spore germination of the tested fungus. The five botanical extracts tested showed varied degree of inhibition over control in spore germination of the pathogen *O. anacardii*. The maximum inhibition of spore germination was recorded in Mwarobaini with 64%. This was followed by Kibamba with 56%, Lipangati (50%), Mnyaa (46%) and Kimbinga (15%).

Microscopic examination was also undertaken to compare mildew growth on treated and untreated mildew spore slides. It was observed that most of the *O. anacardii* spores germinated and formed a number of primary and secondary hyphae on slides where no extracts were applied. On slides where plant extracts had been applied the growth and development of *O.anacardii* were highly reduced.

These studies showed that extracts from different plants vary in their effect on the germination of *O. anarcadii*. The most promising plant extracts against *O. anarcadii* spore germination were from Mwarobaini and Kibamba plant species. Mwarobaini inhibiting growth of *A. alternate, Bipolaries sorokiniana* and several other fungi have been reported (Singh and Dwivedi, 1990; Alam *et al*., 2002a).

The present *in vitro* studies conducted on five plants show inhibition of spore germination of *O. Anacardii.* Possibly the inhibitory effect of the plant extracts on spore germination of *O. anacardii* might be attributed to the presence of some partially effective antifungal ingredients. The inhibitory effect of the plant extracts has been clearly illustrated by Mwarobaini and Kimbinga compared to the control. With the control slides most spores had germinated producing an aggregate of hyphal strands. Usually the fungal mycelia produce appressoria which attach to the host surface. From the appresorium, haustorium (a hyphal branch) is produced which penetrates the host cells of the plant to obtain nutrients. Most spores on slides that contained Mwarobaini extract had been inhibited from germinating although very few had germ tubes. The slides applied with Kibamba extract had good spore germination but there were far less secondary hyphal formation as compared to the control slides.

The results of the present investigation show that spores germination of *Oidium anacardii* was restricted *in vitro* by extracts of Mwarobaini, suggesting the presence of antifungal substances in the plant tissue, which agreed with the results reported by other workers on different pathogens and plants (Qasem *et al*, 1996; Amadioha 2003). The results obtained are in conformity with the Rettinassababady (2002) who have reported that the leaf extract of Mwarobaini (neem) was effective against the control of powdery mildew of blackgram.

From these preliminary studies we can conclude that the tested plant extracts have the potential to inhibit spore germination of *Oidium anacardii*. These plant extracts prove to be better substitutes under field conditions to the management of cashew powdery mildew fungus. Therefore, there is a need to conduct further evaluation on these botanicals in the field and *in vitro* conditions so as to identify and possibly recommend some for commercial use.

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Comparative effect of neem seed kernel extract and two synthetic insecticides against the new major pest of sorghum, *Poophilus costalis* in Togo

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Abstract

Since 2001 in Northern Togo, most varieties of sorghum are damaged by severe attacks of the new major pest *Poophilus costalis* (Walker) (Hemiptera: Aphrophoridae). This pest was not important for sorghum before 2001. Both adults and larvae feed on leaves of Sorghum by sucking the sap. The symptoms are chlorosis and necrosis of sorghum leaves. Severe attack could induce in farm 100% crop losses. Bioassays with a field population of *P. costalis* were conducted in laboratory to compare the efficacy of crude aqueous Neem (*Azadirachta indica*) Seed Kernel Extract (local material) to chemicals, cypermethrin and carbofuran. Field experiments were carried out at the same time in research station. In laboratory, the three insecticides were tested against the third instars (L_3) of *P. costalis* but, in field, all the stages were observed. The result indicated a lower efficacy of Neem Kernel Extract, compared to cypermethrin and carbofuran to control *P. costalis*. The LC_{50} for Neem Kernel was 16.34 g seed kernel/l and those of cypermethrin and carbofuran were 2.26 mg/l and 6/80 mg/l respectively/ On field experiment, the concentration of 50 g/l of Neem kernel (≈0/160.31 g azadirachtin), 0.31 g/l of Cypermethrin and 0.004 g/m^2 of Carbofuran contributed to reduce the population of *P. costalis* by 73%, 87% and 94% respectively. Compared to the synthetic insecticides, Neem Seed Kernel extract could be a natural alternative for sorghum protection against *P. costalis*. Further field experiments are necessary to study the effect of neem seed kernel extract on secondary pests and useful insects.

Keywords: Sorghum, *Poophilus costalis*, Neem Seed Kernel Extract, Cypermethrin, Carbofuran

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the principal cereals growing in northern Togo. Sorghum is damaged by a wide range of insect species. The major pests are, *Contarinia sorghicola* (Coquillett) (Cecidomyidae: Diptera), *Poophilus costalis* (Walker) (Hemiptera: Aphrophoridae) and *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae). The spittlebug *P. costalis* is an endogenous insect pest in Togo. Since 2001 *P. costalis* became an important destructive pest of sorghum plant in Northern Togo. Today, most varieties of sorghum are damaged by severe attacks of *P. costalis*. Both adults and larvae feed on leaves of sorghum by sucking the sap. The species was reported to be also a sorghum pest in Benin, Burkina Faso, Ghana, Niger and Nigeria (Tanzubil and Yakubu, 1997; Ajayi and Oboite, 1999; Hautier *et al*., 2002). Infestation rate of 22 to 100% was reported in Nigeria in 1991 (Anon, 1991). Other crops like maize, millet, rice and sugar cane could also be seriously attacked by *P. costalis* (Tanzubil and Yakubu, 1997; Thompson, 2004; Robinson, 2005).

The challenge is to develop a suitable control strategy against *P. costalis*. The research is then focused on the valorization of a local pesticidal plant called neem (*Azadirachta indica* A. Juss). Several studies demonstrated that neem seed kernel extract is safe for some natural enemies by it selective properties. It is also biodegradable, suggesting that its use for *P. costalis* control would be environmentally acceptable and compatible with IPM programs (Aggarwal and Brar, 2006). Moreover, it is difficult for pest to develop resistance against neem seed kernel extract because it contain in addition to the tetranortriterpenoid azadirachtin, a high number of active ingredients (Völlinger and Schmutterer 2002).

This study presents the first report on the evaluation of the potential of neem seed kernel extract, cypermethrin and carbofuran to control *P. costalis* in Togo.

Description of Research

Insect
During its life cycle, *P. costalis* has five instars which live in their spittle until adult emerged. The spittle is very often located in the plant whorl. In laboratory experiments, the third instar larvae (L_3) of *P. costalis* were collected on untreated sorghum plants (var. SORVATO 1) on farm. The collection was carried out in Dapaong in the savanna Region of Togo on 28 August 2012. The larvae were put in jars containing a piece of young leaf of sorghum. The jars were put in cool box and carried immediately in laboratory for toxicity tests. For field efficacy tests, both various instars of larvae and adult were considered.

Biopesticide: Neem Seed Kernel Extract
Neem is one of the most abundant trees in the savanna region of Togo. It is an important natural resource that could help for pest control (Liang *et al*., 2003). Studies of Schmutterer (1992) revealed that neem seed kernel from various regions of Togo contained about 3.1 -6/2 µg azadirachtin/g of seed kernel/ In this study, neem seeds flour was mixed with water to obtain various concentrations of aqueous Neem Seed Kernel extract. The mixture stayed in room under ambient temperature 24 H before filtered by filter paper and used for toxicity tests in laboratory and field.

Bioassay for susceptibility tests
A leaf dip bioassay method was used to evaluate the toxicity of insecticides against *P. costalis* (Cahill *et al*., 1995). The insecticides tested were cypermethrin (CYPERCAL 50 g/l EC ; Arysta LifeScience, France), carbofuran (DIAFURAN 5% GR ; Arysta LifeScience, France) and Neem Seed Kernel from Togo (major a.i.: azadirachtin). Chemicals are rarely sprayed by farmers against sorghum pests. Cypermethrin and carbofuran are used by some of them. The insecticides were prepared with distilled water to obtain various concentrations. Six concentrations of cypermethrin (1-500 mg/l), carbofuran (0.05-3000 mg/l) and Neem Kernel (10000-150000 mg/l) were tested in 3 replications. Piece of fresh young leaf (30 cm^2) of sorghum (SORVATO 1) collected in greenhouse were washed with distilled water and dried before immersed in the test solution for 20 s. They were dried again at ambient temperature before transferred to plastic petri dishes (90 mm) containing a single moistened Whatman No1 filter paper. Control leaves were treated with distilled water. Ten larvae L_3 were released in each petri dish and mortality counts were made after 36 h exposure to insecticides.

Field experiments
The field experiment was conducted on station during the rainy season, in Dapaong in the northern Togo (N 10° 52'56/6''- E 000° 09' 53/2'')/ The area receives usually about 1000 mm rainfall per annum/ During the rainy season, we often noticed some gaps of drought, favorable for pest development, especially *P. costalis*.
The sorghum variety SORVATO 1 was used for the test of insecticides efficacy on station because of its great sensibility for *P. costalis*.
A randomized complete block design with four treatments (cypermethrin, carbofuran, neem kernel and control) replicated four times was used. The plot size was ten rows of 4 m length each with a spacing of 40 cm between plants and 80 cm between rows. The cultural practices (fertilization, weeding, etc.) were carried out according to the recommendation of the national agriculture research center. The multinutrient fertilizer NPK (15% N, 15% P_2O_5, 15% K_2O) was applied 15 days after sowing at the rate of 200 kg/ha and on 45 days after sowing, 100 kg/ha of the amide fertilizer urea was applied. The spacing between plot and replication were 3 m. The efficacy of the botanical neem kernel was tested in comparison with two synthetic insecticides, cypermethrin and carbofuran. The botanical extract was applied weekly at the concentration of 50 g/l of neem kernel. The synthetic insecticides were applied every two weeks, according to the recommendation on their tags (0.31 g/l and 0.004 g/m^2 for cypermethrin and carbofuran respectively). Evaluations of *P. costalis* infestations were done on 16 plants sampled randomly on each plot and number of individuals was recorded. The evaluation was done weekly and preceded each application of insecticide in the same date. The percentage of the pest population reduction was calculated using the formula:

$$R=[(No-Nx)/No]\text{x}100$$

Where *R* is the reduction rate of *P. Costalis* population, N_0 the total number of *P. Costalis* individuals in the control plot and *Nx* the total number of *P. Costalis* in the treated plot.

Statistical Analysis

Mortality data were analyzed with the software WINDL32 (DL50 Version 4.6) to estimate the lethal concentration (LC) values at 50%. Field data analyses were performed using the STATISTICA (StatSoft, 1995). Insect number was expressed in density (individuals per 16 plants) in each replication of treatment and then transformed into log(x +1). Means were separated using least significant difference (LSD) (p = 0.05).

Research Results and Application

Insecticides toxicity to *P. costalis*

The toxicity of the insecticides against *P. costalis* was indicated by the LC_{50} value (table 1). The pest was more susceptible to chemicals than to the botanical extract. Neem Seed Kernel extracts had the highest LC_{50}, compared to the synthetic insecticides, cypermethrin and carbofuran ($P < 0.05$). That could be explained by the crude extraction that was not concentrated in active ingredients before used. Consider the 95% confidence intervals, the insecticides cypermethrin and carbofuran showed a similar toxicity to *P. costalis* (table 1). The LC_{50} of cypermethrin and carbofuran was 2.26 µg/ml and 6.80 µg/ml respectively, indicating that the species was very sensible to the two chemicals.

Table 1: Toxicity of insecticides (after 36 H) against *P. costalis* third instar larvae

Group of insecticides	Insecticides	N*	LC_{50} (95% FL) (mg/l)	Chi-square (χ^2)
Botanical	Neem seed kernel**	180	16,337 (1,837-37,163)	6.78
Pyrethroid	Cypermethrin	180	2.26 (0.25-5.95)	4.64
Carbamate	Carbofuran	180	6.80 (1.25-20.23)	4.99

* Number of larvae tested per insecticide
** Crude aqueous neem seed kernel extract was used; the amount of azadirachtin, the most important active ingredient of neem seed kernel from Togo is about 3.1-6/2 µg /g of seed kernel (Schmutterer, 1992)/

Efficacy of insecticides on field

The number of *P. costalis* for all the treatments including the control was comparable before insecticide application (*F = 0.58; P = 0.642*) (fig. 1). One week after the first application of neem kernel extract, cypermethrin and carbofuran, the poplulation of *P. costalis* decreased significantly, compared to the control. The population decreased with neem kernel extract as with synthetic insecticdes, cypermethrin and carbofuran. From the second to the last application of insectides, the density of *P. costalis* was maintained at a low level (< 2 larvae/16 plants) in the treated plots (*F = 3.89; P = 0.037*). Overall, the three insecticides contributed to reduce the population of *P. costalis* in the field test. Neem kernel extract, cypermethrin and carbofuran reduced the population by 73%, 87% and 94% respectively (fig. 2).

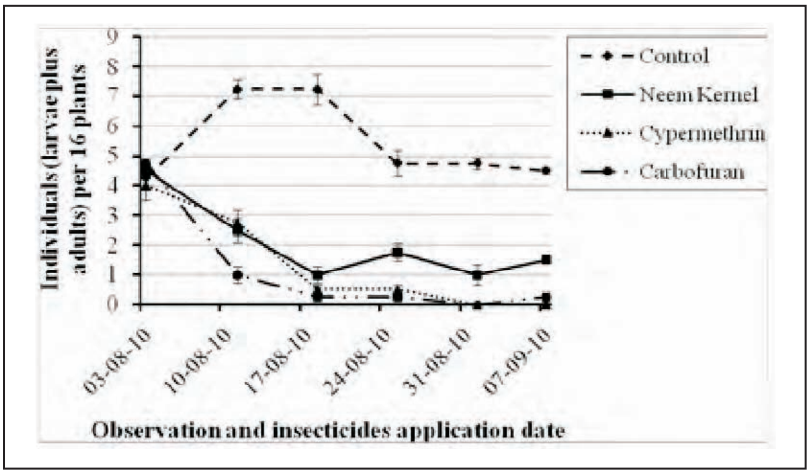

Figure 1: Efficacy of treatments against *P. costalis* (density for 16 plants ± SD)

N = 4 replications

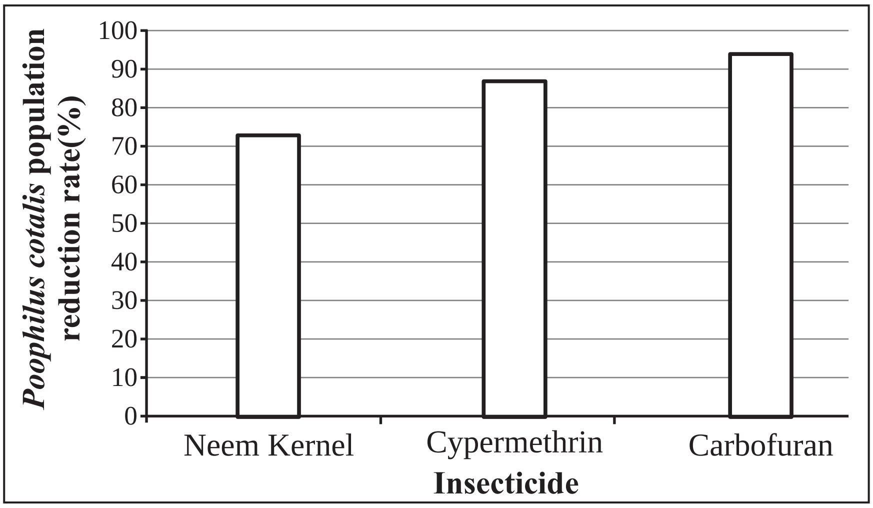

Figure 2: Capacity of insecticides to reduce the infestation proportion of *P. costalis* on field

Our results from laboratory and field experiments showed that the new major pest of sorghum, *P. costalis* may be controlled by the botanical neem seed kernel extract and the synthetic insecticides, cypermethrin and carbofuran. The efficacy of neem seed kernel extract was possible because of the capacity of its actives ingredients, especially azadirachtin to inhibit appetite (Singha *et al*., 2007) and to prevent the pest from producing spittle during the tests. Therefore the larvae died of starvation and absence of spittle. Schmutterer (1992) found that the amount of azadirachtin in neem seed kernel from various regions of Togo is about 3.1-6/2 µg /g of seed kernel, indication that the crude neem extract must contain a small amount of active ingredients. For Liang *et al*. (2003) insecticides derived from neem could inhibit the growth of insects larvae, explaining also the low density of *P. costali* observed in sorghum plots treated by neem kernel extract in our study. The small use of chemicals against sorghum pests by farmers in savanna region of Togo could explain the great sensibility of *P. costalis* to chemicals, cypermethrin and carbofuran. The research is going on to study the effect of neem seed kernel extract on secondary pests and useful insects.

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The efficacy of *Gmelina* and Neem seeds extract for the management of American cockroach (*Periplanata amaricana)* (Dictyoptera: Blattidae)

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Abstract

Two laboratory trials were conducted to evaluate the efficacy of Neem (*Azadirachta indica*) and Gmelina (*Gmelina arborea*) seeds for the control of American cockroach (*Periplanata americana*). The first experiment consisted of four levels (0, 5, 10 and 15g) of Gmelina (G) and Neem (N) seeds paste mixed with 20g of bread and fed as food. The second experiment consisted of the extract solutions of the same levels (0, 5, 10 and 15g) of Gmelina and Neem seeds in one litre of water applied as spray biweekly for 42 days using hand sprayer. In both experiments 20 (synchronized 180 days) old adults were used. The experiments were laid out in a 4x4 factorial arrangement in a Complete Randomized Design (CRD) and replicated four times. Significantly ($P < 0.05$) higher mean percentage mortality of 96.70 and 80.00 were recorded in the $G_{15}N_{15}$ and $G_{10}N_{15}$ treatments combination respectively. No mortality was obtained in the control G_0N_0. In the spray experiment, significantly ($P < 0.05$) higher adult percent mortality was recorded in the $G_{15}N_{15}$ (96.67) combination. No significant ($P > 0.05$) difference in percentage weight loss was observed between $G_{15}N_{15}$, $G_{15}N_{10}$ and $G_{10}N_{15}$ (i.e. 60.15, 59.46 and 58.99). This study showed that neem and gmelina products can be used for the control of *P. americana*.

Keywords: Gmelina, Neem, *Periplanata americana*, mortality, weight loss

Introduction

Cockroaches are the most abundant insect pests of public importance. They infest hospitals, food manufacturing industries, kitchens and residential apartments (Vahabi *et al*., 2007). Their infestation has always raised safety concerns, especially as carriers of food borne pathogens and food spoilage organisms (Mpuchane *et al*., 2008). This study was carried out to evaluate the efficacy of gmelina and neem seed extracts in the control of *P. americana*.

Literature Summary

The American cockroach *Periplaneta americana L*. is the largest species of common cockroach and often considered a pest. It is an omnivorous and opportunistic feeder and also consumes decaying organic matter (Bell and Adiyodi, 1981) and being a scavenger, it will eat almost anything. It can become a public health problem due to their association with human waste and disease, and ability to move from sewers into homes and commercial establishments, thus regarded as one of the commonest household insect pest (Brenner *et al*., 1987).

Presently the use of synthetic insecticide is the most popular and effective method of controlling the menace of this pest. However, chemical control has become increasingly less popular. This is primarily due to the development of multi-chemical resistance among the cockroach populations (Hamman and Gold, 2002; Miller and Koeler, 2004) and increased public concern about pesticide exposure to the living environment (Miller and Koeler, 2004). Biopesticides have been widely used around the world in the control of insect pests. The seed of neem has been used for the control of insect pests due to its pesticidal active ingredient "azadirachtin and salanin (Emosairue and Ukeh, 1996; Oparaeke, 2006) where it is reported to have ovicidal and larvicidal toxicity. Oparake (2006) reported that gmelina tree suffers less attack by insect pests due to its high alkaloid and tannin contents. Thavara *et al*. (2007) studied seven commercial essential oils for repellency against cockroaches and found *Citrus hystix* exhibited complete repellency against *P. americana* and *B. germanica.*

Description of Research

Forty eight square-shaped cages each measuring 17.8 by 17.8 cm were constructed using a ply wood and a fine wire net and a door measuring 7 by 7 cm. One hundred adult American cockroaches were captured into perforated containers and fed with domestic waste (crayfish, tomatoes fruits, yam pills, Irish potato, bread, cooked rice, and biscuit). The adult cockroaches were allowed in the perforated containers for six weeks to lay eggs after which they were removed. About 35-40 days later the eggs hatched into nymphal instars and were transferred into empty cages. The instars matured in five to six (5 -6) months (150 -180 days) into adults, which were used for the experiments.

Ripe fruits of neem (*A. indica*) and gmelina (*G. arborea*) were collected from neem and gmelina trees around the University of Calabar compound. The fruits were washed to remove sand and other contaminants before drying in the shade. The first experiment consisted of four levels (0, 5, 10 and 15g) of gmelina and neem seed paste mixed with 20g of bread and applied as food. The second consisted of the extract solutions (1litre of sterile water) of the same levels (0, 5, 10 and 15g) applied as spray biweekly for 42 days using hand sprayer. In both experiments, 20 (synchronized 150 days) old adults placed in cages were used. The experiments were laid out in a 4 x 4 factorial in a Complete Randomized Design (CRD) arrangement and replicated four times.

Data Collection

Data on mortality and weight loss or gain were collected weekly for 6 weeks. The weight of all the cockroaches in each cage was recorded in grams after subtracting with the initial weight. Data obtained for adult mortality were transformed using square root transformation. The data collected were subjected to the Analysis of variance (ANOVA) and Fisher,s Least Significant Difference used to determine the difference between significant treatment effects using the statistical software MINITAB version 15.

Research Results and Application

Significant ($P < 0.05$) weight loss was recorded in all the treatment combinations with the highest observed on the $G_{15}N_{15}$ and $G_{15}N_{10}$ while the least was observed in the control (Table 1). The highest adult mortality was observed when 15g each of neem and gmelina treated food was applied respectively (Table 2). The highest adult mortality was observed in $G_{15}N_{15}$ and $G_{10}N_{15}$ while the lowest was in the control (Table 2).Mortality was significantly ($P < 0.05$) higher when neem was sprayed at 15g and lowest in the control. The interaction was significant ($P < 0.05$) with highest percent mortality observed when $G_{15}N_{15}$ was applied. No mortality was observed in the control.

Neem and gmelina mixed with food or sprayed directly on the cockroaches successfully controlled the pest . So far neem and gmelina products are only utilised for research purposes in Nigeria (Ukeh *et al*., 2007; Umoetok *et al*., 2009) with little usage among the resource -poor-farmers as protectants. This renders the fruits underutilized during their fruiting seasons. The observation made on the treated food was that all the cockroaches stopped feeding and moved to the sides of the cages, away from the treated food and this could be attributed to the antifeeding or repellent actions of the treatments (Oparaeke, 2006). Several studies have employed the use of plant powder and oil extracts such as neem (Ukeh *et al*., 2007, Umoetok *et al*., 2009) for the control of insect pests. According to Mordue and Nisbet, (2000) and Isman (2006) bio- pesticides stimulate specific deterrent cells in chemoreceptors and block the firing of sugar receptors cells which normally stimulate feeding, these results to starvation and death of these pest species by feeding deterrency. This study showed that botanical products such as neem and gmelina can be used for the control of *P. americana*.

Table 1: Effects of Neem and *Gmelina* seed extracts on the percentage weight loss of *Periplanata americana*

Neem (N)/kg *Gmelina* (G) /kg	0	5	10	15	Mean (G)
0	8.97	35.68	42.21	48.04	33.73
5	27.93	40.09	50.24	52.16	42.61
10	28.01	45.39	52.10	58.99	46.12
15	33.56	51.39	59.46	60.15	51.14
Mean (N)	24.62	43.14	51.00	54.84	
F-LSD 0.05 (GxN) = 5.47					
F-LSD 0.05 (N) = 15.00					
F-LSD 0.05 (G) = 26.90					

Table 2: Mean percentage mortality of adult *Periplanata Americana* fed with treated food of *Gmelina* and Neem seed formulations

Neem (N)/kg *Gmelina* (G) /kg	0	5	10	15	Mean (G)
0	0.00	20.56	40.67	60.67	30.48
5	26.70	40.00	56.70	70.00	48.35
10	33.30	58.00	70.00	80.00	60.33
15	46.70	62.00	76.70	96.70	70.53
Mean (N)	26.68	45.14	61.02	76.84	
F-LSD 0.05 (GxN) = 11.77					
F-LSD 0.05 (N) = 14.76					
F-LSD 0.05 (G) = 3.49					

Table 3: Mean percentage mortality of adult Periplanata Americana sprayed with Neem and *Gmelina* extracts

Neem (N)/kg *Gmelina* (G) /kg	0	5	10	15	Mean (G)
0	0.00	40.00	43.33	70.00	38.33
5	30.00	40.00	50.00	76.39	49.10
10	33.33	46.67	60.00	80.00	55.00
15	66.67	73.33	83.33	96.67	80.00
Mean (N)	32.50	50.00	59.17	80.77	
F-LSD 0.05 (GxN) = 2.88					
F-LSD 0.05 (N) = 8.93					
F-LSD 0.05 (G) = 8.18					

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Evaluating efficacy of Fresh leaf extract *Tephrosia vogelii* with and without soap in the control of Aphids, Forage Thrips and Jassids In Cowpeas.

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Abstract

One of the challenges in agricultural production is costly management of insect pests, using synthetic insecticides which are not readily available for the local farmers. This study presents an attempt to assess the effectiveness of different concentration s of *Tephrosia vogelii* crude leaf extracts with and without soap in control of aphids (*Aphids craccivora)*, foliage thrips(*Soricothrips acipitaris)* and Jassids in cowpeas. The major findings that emerged were as follows: **a)** no significant differences in insect pest populations before treatment, **b)** with respect to the concentration of the extract (with soap and without soap) there was varying effectiveness of the extract against insect populations at $P<0.05$ **c)** high insect pest reduction in tephrosia extract mixed with soap at $P<0.05$ **d)** varying efficacy of the extract (with and without soap) on different insect pests **e)** observed burnt leaves in plants treated with high concentration of soap. The study has shown that tephrosia leaf extract mixed with soap is more efficient that the one without soap and the efficacy varies with insect pest species. Further investigations on the selective toxicity of the extract are recommended. Moreover, investigation of soap and the leaf extracts residues on the plants ca nnot be over emphasised.

Key words: *Tephrosia, leaf extract, soap, Jassids, aphids, foliage thrips*

Introduction

Cowpea (*Vigna unguilata* (L) Walp) production in Malawi is constrained by insect pest attack. Studies conducted elsewhere by Edipala and coathors, 2005, attributed 70% yield loss in cowpeas to insect pest infestation. Among the insect pests that constrain cowpea production are; *Aphids craccivora,* and Jassids Control of these insect pests in Malawi, typically consists of treatments by synthetic insecticides which fall in the classes of pyrethroids, organophosphates and carbamates. Though, currently very reliable, purchasing power, safety and access to these synthetic insecticides by small holder rural farmers may be not be guaranteed. In addition, their use is becoming unpopular because of associated environmental and health hazards. Ranger *et al*., 2009, emphasize on efforts to identify environmentally sound and sustainable alternatives. One such alternative is the use of plants with insecticidal properties such as *Tephrosia Vogelii*. Due to their high degree of biodegradation, plant extracts are attracti ve as replacements for synthetic insecticides because of their potential to minimize health and environmental hazards (Sutthanont *et al*., 2010). There is need however there is need further Developing the plant extracts as alternatives, to reduce insect attack that cause low productivity in important crops like cowpeas. (Sjostedti *et al*., 2011). This research aimed at comparing efficacy of fresh tephrosia leaf extracts with and without soap in control of Jassids, aphids and Foliage thrips in the field.

Research Description

Tephrosia Vogelii Formulations and extraction

Leaves were harvested from tephrosia trees and pound in a mortar with a Pistil and then soaked overnight. Required amounts of the pounded material were weighed in duplicate (30g/l, 60g/l and 90g/l) and soaked in tap water in respective beakers overnight. Sunlight dish washing soap (125 ml) was measured and added in the other half of the containers, to make, an extract of tephrosia leaves only and of soap plus tephrosia that is thoroughly mixed. The following day, the mixtur es were sieved using a clean, white cloth to remove the residues.

Insecticide Application

All botanical insecticides and their rates in the trial are indicated in Table 1. Application rates were decided arbitrary because the specific dosages for specific insect species are not yet known. The test was field based and was arranged in a randomized complete block design with three replicates. To evaluate the efficacy of the two tephrosia extract formula tions, insect pest counts for (Jassids, Aphids and Foliage Thrips) were collected twice a week from randomly sampled 5 plant stations in each of the treatments. Knapsack sprayer was used and spraying was restricted to very early morning hours. Buffer rows were used. In addition, a plastic sheath was used to demarcate the boundaries during the spraying, to minimize cross boundary effects.

Plant Damage

Damage of plants caused by insect pests was evaluated on weekly basis on each of the treatments and replication. Actual numbers on damaged plants were collected.

Insect sampling

Scouting for insect population done on weekly basis, before and after spraying. Five planting stations were selected randomly in each of the treatment from which insect population data was collected. Insect population data was collected at each of the growth stages of the cowpea, this paper presents insect pests that were studied at a vegetative stage. Number of attacked plants were also collected and recorded. Analysis of Variance (ANOVA) and comparison of means was performed in Genstat. Mean insect populations that were collected on weekly basis per five stations were corrected and separated using the least significant difference (LCD) at 5% significant level using Genstat statistical package.

Research Results and Application

There was no significant difference in insect pest populations between treatments before spraying started. In contrast, application of tephrosia extract gave significant results even at lowest concentrations regardless of formulation after successive sprays. All th e extracts irrespective of formulation, exhibited increased efficacy with increase in concentration. The effectiveness of the plant extract was mainly observed at the highest concentration (90g/l) (figure 1).

However, results displayed a significant redu ction of insect pest numbers in plots sprayed with tephrosia mixed with soap compared to the ones sprayed with tephrosia only. At similar dosages, it was clear that there were significant increases in insect populations, in plots that were treated with tep hrosia leaf extract without soap

Insect pests, responded differently to the treatments. Comparatively, Aphids and Jassids were more responsive to the insecticide than Foliage thrips. At higher concentrations, 60g/l and 90g/l the population of foliage thrips persisted when treated with a mixture of soap and tephrosia. It was surprising though, that when treated with tephrosia leaf extract only without soap, there were no significant differences in insect pest populations at same concentration.

Observations of insecticide efficacy were extended to treatment plots that were subjected to soap only and no spray. In contrast with plots that were not treated, it was evident that soap had a significant impact on the insect pest populations. It was clear that, besides being used as an adhesive to increase the residual effect of the tephrosia extract, soap, on its own worked as an insecticide. However, it was noted that plots that were treated with soap only and soap-tephrosia mixture had some leaves burnt.

Varying numbers of infested plants was evident in plots subjected to different treatments, the number of attacked plants reduced with increase in treatment concentration of the extracts regardless of formulation. However results revealed a decreased number of infested plants in plots that were treated with a mixture of tephrosia and soap, than those that were treated with fresh tephrosia extract without soap (see figure 3). Beside tephrosia treatments, comparing the two control experiments, of soap and no treatment, there was a decrease in the number of infested plants in plots that were treated with soap.

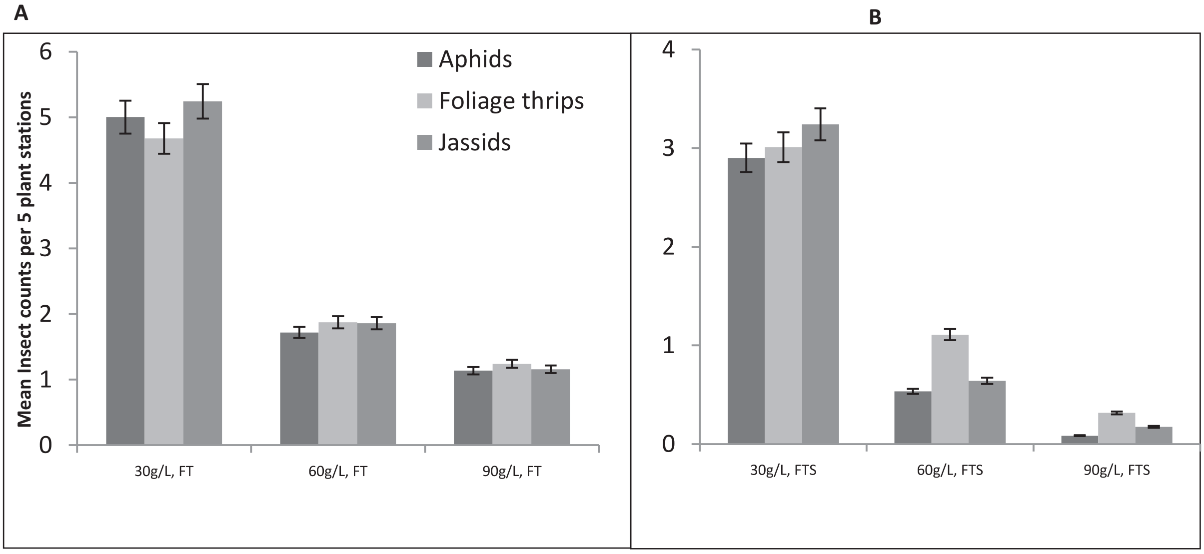

Figure 1: Variations in insect pests populations, subjected to different treatments of *Tephrosia* extracts at vegetative stage,(A)Changes in population numbers of Foliage Thrips, Jassids subjected to Fresh *Tephrosia* without soap(FT), (B) Fresh *Tephosia vogelii* with soap (FTS)

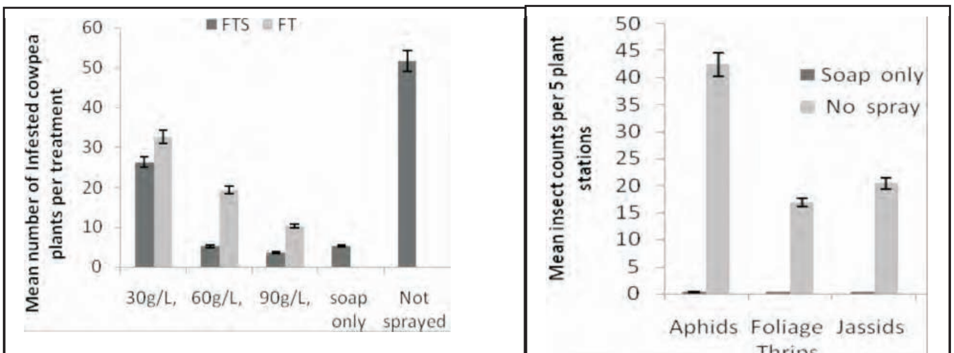

Figure 2: Variations in infested plant populations, subjected to different treatments of *Tephrosia* extracts at vegetative stage, (A)

FTS (fresh tephrosia with soap), FT (Fresh Tephrosia without soap)
Changes in population numbers of Foliage Thrips, Jassids subjected to no spray and soap spray only.

Our study has demonstrated a unique feature of tephrosia action on jassids, aphids and foliage thrips. The study has revealed varying effectiveness of tephrosia extract with concentration and formulation. It was clear that the efficacy of the extracts was dependent on the dosages applied. Insect pest population decreased with increase in the dosage of the extracts. The application of tephrosia mixed with soap proved more effective than when not mixed with soap, against the three target insect pests. This could be attributed to action of soap in extract as a spreading agent of extract during spraying on plant surfaces that are hydrophobic; this makes even distribution of the extract over plant canopy (Nyirenda, 2009). Besides, increased efficacy of the extract mixed with soap could be attributed to activity of soap in itself as an insecticide. Worth noting is the significant reduction of insect pest population in plots treated with soap only, compared to those that were not treated at all. In addition, the difference in response of the three target insects when treated with tephrosia and soap mixture could be associated with the ability of each of the insect pests to with stand activity of the formulation with increased concentration. Persistence of foliage thrips populations, even when treated with soap and tephrosia mixture revealed an essential result. Although, Olaitan and Abiodun, (2011), emphasize that insecticides that are of plant origin are generally pest specific and are relatively not harmful to non-target organisms, these study results confirm the need to further study the insects' defensive systems towards the botanical extracts, to characterize their mode of action, specific and comparative toxicity. Intensive work on effective formulations and toxicology of extracts from plants such as *Tephrosia* need to be done to maximize their potential without compromising their potency over time. Significant reduction of damaged plants at vegetative stage in plots that were treated with fresh tephrosia with soap, and soap only revealed an important result. The results appreciate the potential that botanical extracts, especially tephrosia, have to reduce pest infestation in crops if they are improved. What this study could not do was to further tease out the effective concentration of soap to be mixed with *Tephrosia* to reduce the burning effect on the leaves.

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Use of Pymarc in management of bean fly (*Ophiomyia species*) on common beans (*Phaseolus vulgaris* L.)

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Abstract

Bean fly (*Ophiomyia species*) is a major pest of common beans (*Phaseolus vulgaris* L.) causing up to 100% yield losses, during dry seasons. Use of chemical sprays to control bean fly on beans has been used by farmers with limited success. An alternative management procedure exploiting the principle of cultural control, a seed dress insecticide and use of Pymarc (a by-product of pyrethrum) were investigated. Results showed that Pymarc and gaucho 350FS significantly ($P < 0.05$) reduced bean fly population and damage. Gaucho 350FS reduced plant mortality significantly ($P < 0.05$), while Pymarc enhanced yields during the long than short rains seasons. Thus, application of Pymarc has a potential to reduce bean fly damage and improve bean yields.

Introduction

Common beans (*Phaseolus vulgaris* L.) is an important legume crop in East and Central Africa, providing protein, calorie and cash income for rural households and that its consumption exceeds 50 kilograms per person per year (Mauyo *et al.* 2007). Kenya consumes approximately 450,000 tons of beans against a local production level of between 150,000 to 200,000 tons harvested from about 800,000 ha. The country imports the deficit mainly from Uganda, Tanzania and Central Africa (African Agriculture, 2008) due to many production constraints including insect pest damages (Abate *et al.* 2000). Bean fly has been identified as the most important pest of beans in Africa and particularly Kenya (Kibata, 1990; Abate and Ampofo, 1996). Recent research indicates that late-sown beans have significantly higher infestation than early planted levels (Sariah and Makundi, 2007). This is critical, as farmers do not have specific planting time due to resource constraints. This study was therefore carried out to evaluate the use of phosphorus, at different levels and plant insecticide, Pymarc, singly and in combination to manage bean fly in two common bean varieties with an aim of improving bean yields.

Literature Summary

Bean fly is particularly serious in infertile soils, during short rains and late planted beans leading to losses of up to 100% if not controlled (Letourneau, 1994 and Byabagambi *et al.,* 1999). Recent research indicates that late-sown beans have significantly higher infestation than early planted levels (Sariah and Makundi, 2007). This therefore calls for a research into control measures which are cheaper and available to resource poor farmers.

Crop fertilization can affect susceptibility of plants to insect pests via altering plant nutrient and that excess use of inorganic fertilizers can cause nutrient imbalances and lower pest resistance compared to organic fertilizers (Altieri and Nicholls, 2003). Further, Yu-Tzu *et al.* (2009) suggested that proper organic treatment can increase a plant's biomass production and may have a lower pest occurrence. Research done by Byabagambi *et al.,* (1999) indicated that uses of inorganic fertilizers, especially those with high nitrogen increase bean fly population density and percentage plant mortality. Phosphorus (P) is an essential element for plant growth and that common bean is often limited by low P in the soil (Hernández *et al.,* 2007). It was estimated that over 50% of common bean production in the tropical soil is limited by phosphorus deficiency (CIAT, 1992). Furthermore, unlike nitrate, which readily moves in soil towards the roots via both mass flow and diffusion, phosphate (P_i) is highly immobile (Lambers *et al*., 2006) where diffusion is also a very slow process. This is worsened when the soils are dry. According to KARI, (1999), the application of phosphorus in combination with FYM and seed dressing reduced bean fly. In addition, phosphorus application was shown to be negatively correlated with bean fly pupae density (Letourneau, 1994).

Application of seed dressing and soil-applied pesticides are easy to apply. Despite this they are expensive to poor small scale farmers and environmentally unsafe. In addition, continuous use of particularly one pesticide leads to pests developing resistance (Fraser, 2005). Botanicals have been found to have broad spectrum insecticidal properties with reduced persistence and toxicity in relation to organochlorines and organophosphorus compounds (Reuben *et al.,* 2006). According to Katsvanga *et al*., (2006) use of these pesticides provides an environmentally user friendly alternative to conventional pesticides and that these can be exploited in three ways namely; use of whole parts of a plant, in powder (dust) or as crude extracts in water or other solvents, and as purified extracts like rotenone. The use of pyrethrum (*Chrysanthemum cinerariaefolium* Trev.) by-product (pymarc dust) is still at low levels. It has been mainly used in controlling maize stalk borer and in livestock, as a feed and controlling helminths and applied to crops as manure. Report by The organic farmer (2007), indicated that the pymarc dust has been used at planting and later applied around the bean stem to control bean fly.

Other botanicals such as neem seed extract in alcohol (NSE-AL) and Tephrosia leaf juice solution masked to 10% and sprayed on bean plants until dripping at three day intervals from emergence to flower bud initiation reduced bean fly oviposition/feeding puncture counts, indicating a deterrent effect from these substances (Abate, 1990). Obeng-Adu (2005) reported that neem seed extract was found to be cost effective as alternate insecticide to control French bean insect pests other than controlling bean fly/ Further, it's reported that neem and pyrethrum biopesticides have been used to control bean flies (The organic farmer, 2007). The use of such plant extracts to control pests is not a new innovation; it has been used by small-scale subsistence farmers (Moyo *et al*., 2006). Farmers have also used intercropping, where the intercrop needs to be chosen well, as research had shown that intercropping beans with maize and sunflower led to prolonged bean fly oviposition due to leaves remaining green for long period (Tuey and Lelgut, 2002)., However, mixed cropping of beans with leek significantly reduced adult bean fly settling, emergence, and death of bean plants compared with a mono crop (Bandara *et al.* 2009). Other control measures include mulching, ridging, among others. Hence need to develop integrated practices, which are sustainable and affordable to resource poor small-scale farmers.

Description of Research

The field experiments were carried out at Kenya Agricultural Research Institute (KARI), Njoro, Kenya during the short and long rains. The centre is located on grid 35^o 31'E and 0^o 21' S at an altitude of 2164 m above sea level (ASL). The region is classified in Agro – ecozone lower highlands 3 (LH3 (Jaetzold and Schmidt, 1983). The centre received an average of 193.4mm and 369.4mm rainfall in short and long rains seasons respectively giving a total of 552.8mm. Treatments investigated were: Insecticide: no insecticide (control), Gaucho 350FS (imidacloprid) at rate of 8 ml/kg of beans as described in Bayer manual (2000) and pymarc dust at 200 kg/ha applied 7 days after emergence (DAE); Phosphorus (TSP) at three levels: 0, 40 and 80 P_2O_5 kg/ha applied in the holes at planting. Two bean varieties; Rose coco and Red haricot were used. These were laid out in a Randomised Complete Block (RCBD) in a split-split plot arrangement replicated three times. The main plot measured 36m x 3m with three sub plots each measuring 12m x 3m and six sub-subplots of 2m x 3m each. The inter-row and intra-row spacing was 0.5m and 0.2m respectively with two seeds per hill and a one-meter path between the replicates. Insecticides were allocated randomly to the main plots, phosphorus levels and varieties were randomly allocated to subplots and sub sub-plots respectively. Data was collected from five middle rows on plant stand count both at 7 DAE and at harvesting, plant height, number of plants showing bean fly symptoms and dead plants at 21 and 35 DAE, number of larvae and pupae done at 21 and 35 DAE, number of pods/plant and seeds/per pod from ten plants and 100 seed weight and total grain yields were measured using electric weighing balance. All counted parameters were transformed using square root transformation, $\sqrt{x+1}$ before subjecting to ANOVA to homogenise their variances. Statistical package SAS (1999-2000) was used during analysis.

Results and Application

Varieties responded significantly ($P < 0.05$) at 7 DAE during the long rains with lower counts in red haricot (87.3b) than in Rose coco (88.6a) (Table 1). Application of pymarc and gaucho 350FS significantly ($P < 0.05$) reduced bean fly density at 21 and 35 DAE during both seasons with the highest bean fly densities obtained in controlled plots. Gaucho 350FS treated plots had the lowest bean fly population but did not differ significantly from pymarc at 35 DAE during the short rains season compared to long rains season. Phosphorus application did not reduce bean fly population density significantly during the two sampling dates in both seasons although higher densities were recorded in controlled plots. Infestation of the varieties did not show significant difference in the short rains except the long rains where Rose coco had significantly ($P < 0.05$) higher bean fly densities (Table 2).

Table1: Effect of insecticide and phosphorus application on bean fly population density during the short rains 2004 and long rains 2005 at KARI, Njoro

	Short rains season		Long rains season	
Insecticide	**21 DAE**	**35 DAE**	**21 DAE**	**35 DAE**
Control	2.1 (1.7^a)	3.6 (2.1^a)	1.2 (1.4^a)	3.9 (2.2^a)
Pymarc	1.3 (1.5^{ab})	2.5 (1.9^b)	0.4 (1.2^b)	3.8 (2.2^a)
Gaucho 350FS	1.1 (1.4^b)	2.5 (1.9^b)	0.0 (1.0^c)	1.7 (1.7^b)

LSD (5%)	**0.27**	**0.21**	**0.03**	**0.13**
P(P_2O_5) 0	1.4 (1.5)	1.9 (2.0)	0.5 (1.2)	3.2 (2.0)
P(P_2O_5)40	1.6 (1.6)	2.2 (1.9)	0.6 (1.2)	3.3 (2.0)
P(P_2O_5)80	1.5 (1.5)	1.8 (1.9)	0.4 (1.7)	2.9 (1.9)
LSD (5%)	**ns**	**ns**	**ns**	**Ns**
Red haricot	1.5 (1.5)	2.7(1.9)	0.5 (1.2)	2.4 (1.8^{b})
Rose coco	1.5 (1.5)	3.1 (2.0)	0.6 (1.2)	3.8 (2.9^{a})
LSD (5%)	ns	**ns**	**ns**	**0.17**
C.V%	**24.84**	**15.14**	**15.90**	**9.37**

Means followed by the same letter(s) within the column are not significantly different (P < 0.05) using Least Significant Difference (LSD). Values in parentheses are means after transformation.

Table 2: Effects of insecticide and phosphorus on percentage *bean fly* infestation during the short rains (2004) and long rains (2005) seasons at KARI, Njoro

	Short rains season	Long rains season	
Insecticide	**35 DAE**	**21 DAE**	**35 DAE**
Control	16.0 (4.0^{a})	9.7 (3.2^{a})	17.8 (4.2^{a})
Pymarc	11.7 (3.5^{ab})	8.6 (2.5^{b})	6.2 (2.6^{c})
Gaucho 350FS	10.4 (3.3^{b})	5.7 (3.0^{a})	10.0 (3.2^{b})
LSD (5%)	**0.56**	**0.29**	**0.46**
P (P_2O_5) 0	15.5 (3.9)	9.6 (3.2^{a})	12.9 (3.5)
P (P_2O_5) 40	11.6 (3.4)	7.8 (2.9^{b})	11.2 (3.3)
P (P_2O_5) 80	10.9 (3.4)	6.5 (2.7^{b})	9.7 (3.1)
LSD (5%)	**Ns**	**0.29**	**Ns**
Red haricot	12.9 (3.6)	7.3 (2.8^{b})	7.2 (2.7^{b})
Rose coco	12.6 (3.6)	8.6 (3.0^{a})	15.5 (3.9^{a})
LSD (5%)	**Ns**	**0.24**	**0.38**
C.V%	**22.19**	**14.11**	**19.91**

Means followed by the same letter(s) within the column are not significantly different (P < 0.05) using Least Significant Difference (LSD).

During the short rains season results obtained at 35 DAE showed a significant ($P < 0.05$) reduction in Gaucho 350FS and pymarc treated plots but negligible symptoms were observed at 21 DAE. However, during the long rains season pymarc treated plots reduced bean fly infestation significantly ($P < 0.05$) than gaucho 350FS treated plots. Plots treated with40 P_2O_5 kg/ha and 80 P_2O_5 kg/ha phosphorus significantly ($P < 0.05$) reduced bean fly infestation during the long rains season than controlled plots. The two varieties significantly ($P < 0.05$) indicated more damaged plants in Rose coco than Red haricot during the long rains seasons. The positive performance of pymarc on plant stand count indicated that pymarc has a potential of improving crop growth probably by controlling bean fly. This could be attributed to pymarc odour, which could have repelled bean fly adults. In addition, pymarc dust held on leaf trichomes could have disoriented bean fly adults hence less oviposition.

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Incorporating a botanical based bait in an integrated pest management program of fruit flies (Diptera: Tephritidae) attacking mangoes in Tanzania

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Abstract

Trials were conducted in eight selected orchards located in the mango growing areas of Morogoro Region, Tanzania, to evaluate the effectiveness of three IPM programs against fruit flies. Split plot design was used, with season being the main plot factor and treatment being the sub-plot factor. A total of six traps (replicates) were placed in a 1 ha area for monitoring adult flies. The treatments included (i) orchard sanitation (ii) orchard sanitation + spray of dimethoate 480EC + early harvesting (iii) orchard sanitation + spray of GF 120 containing 0.02% spinosad + male annihilation using methyl eugenol + early harvesting (iv) orchard sanitation + spray of a locally formulated bait (containing crude extracts of *Derris elliptica* as a toxicant) + early harvesting. Each treatment was applied in an individual orchard and replicated twice. Populations of fruit flies in each orchard were monitored using torula yeast placed in McPhail traps. The population of fruit flies was determined as the number of adult fruit flies per trap per week. Further, fruits were sampled at ripening and individually placed in containers to determine incidences and infestation rates of emerged fruit fly species. The IPM program containing Derris bait has shown potential for reducing infestation rates of fruit flies in mango.
Key words: Botanical, IPM, fruit flies

Introduction

Tanzania's agro-based economy can be boosted by finding reliable markets for its non-traditional export crops like fruits. However, quick movement and opening of new markets for agricultural products also offer greater opportunities for the movement of pests that have deleterious consequences to crop production (Griffin, 2000). Reliable markets for fruits can be secured only when a country is able to produce high quality fruits, free from pests and diseases. Production of high quality fruits in Tanzania is hampered by, among other things, insect pests, especially fruit flies (Diptera: Tephritidae). Infestation by fruit flies is a major constraint to fruit production, for example in East Africa, fruit flies is a cause to losses of up to 40% in mangoes (Lux, 1999). Monetary losses due to fruit flies are not readily available from many countries. Existing data show that losses are very large, for example exports of banana from Mozambique to neighboring South Africa, are estimated to be worth around USD 20 million annually (Cugala *et al*., 2011) and this market is jeopardized by presence of the invasive fruit fly *Bactrocera invadens* Drew Tsuruta & White. The damage to fleshy fruits is mainly caused by a limited number of highly polyphagous species. Many fruit flies are of quarantine importance globally and control programs which aim more at increasing access to market through compliance with the importing country's phytosanitary measures are necessary, rather than aiming at just the reduction of the damage from fruit fly pests (Quinlan, 2004). Exporting pest-infested fruits could result into loss of international markets, because some of these pests are of quarantine importance. In Tanzania, fruit flies (Diptera: Tephritidae) are among the important pests in the major fruit growing areas. The initial surveys in Morogoro Region indicated the presence of many fruit fly species, both native and exotic, and follow up studies showed dominance of an invasive species *B. invadens*. Ecologically based Integrated Pest Management (IPM) programs were was formulated and their effectiveness was evaluated tested in farmers, fields in Morogoro. However, since most the materials used in fruit flies management are imported and mostly inaccessible to smallholder farmers, locally formulated bait containing *Derris elliptica* as a toxicant, was included in the IPM program in one of the evaluated IPM programs.

Literature Summary

Managament of fruit flies, in particular *B. invadens* is ongoing in many African countries, although data on effectiveness of the tested IPM programs are not readily available. Vayssières *et al.,* (2009) reported the effectiveness of siponsad bait in controlling mango infesting fruit flies in Benin. Furthermore, Ekesi *et al.,* (2010) reviewed the status of *Bactrocera invadens* management in Africa. According to Aluja (1996) any designed IPM program for managing fruit flies should be viewed as a transition from a chemical dependent control to an ecological model pest management. Natural products like neem should be researched on their ability to reduce populations of fruit flies. In this regard in the study by Verghese *et al.,* (2006) included a neem-based product, Azadirachtin, in their IPM program for *B. dorsalis*. Botanical insecticides like neem are safer to use and are compatible with organic farming. Many new tools and approaches recently developed may be very effective at controlling the fruit fly problem, but whether these tools are compatible with the entire crop production scheme in respect to cost effectiveness is rarely considered (Aluja, 1996). Botanical pesticides and less toxic synthetic pesticides and those with promising results should be recommended to farmers. What should be done at this stage is to adopt a control program from other areas for testing and refining to suit our farming systems. Programs like those by Verghese *et al.,* (2004; 2006) can be adopted and proposed to farmers while ecologically based pest management systems, compatible with Tanzanian farmer situation are being designed (Mwatawala *et al.,*, 2009a).

Description of Research

Trials were conducted in Morogoro Region, Ea stern Central Tanzania, for two mango seaqson, 2008/9 and 2009/10. A total of eight mango orchards were selected and used for the study. Split plot design was used, with season being the main plot factor and treatment being the sub-plot factor. A total of six traps (replicates) were placed in a 1 ha area for monitoring adult flies. The treatments included (i) orchard sanitation (ii) orchard sanitation + spray of dimethoate 480EC + early harvesting (iii) orchard sanitation + spray of GF 120 containing 0.02% spinosad + male annihilation using methyl eugenol and early harvesting (iv) orchard sanitation + spray of a locally formulated bait (containing crude extracts of *Derris elliptica* as a toxicant) + early harvesting. *Derris elliptica* roots were obtained from Muheza, Tanga, Tanzania. The roots were washed, and cut into pieces and dried under shade for five days/ They were later ground and mixed with molasses, brewer's yeast and water (360gm. 1L molasses. 360gm of brewer's yeast. 20L of water)/ The mixture was left for 12 hours. Each treatment was applied in an individual orchard and replicated twice. Populations of fruit flies in each orchard were monitored using torula yeast placed in McPhail traps. The population of fruit flies was determined as the number of adult fruit flies flies per trap per week. Data collected weekly from orchards receiving similar treatments were averaged. Additionally, mango fruits were harvested and taken to the laboratory at SUA, were they were washed, weighed and each fruit was place d in an individual rearing container to monitor the number and type of emerging flies. Procedures used by Mwatawala *et al*., (2009b) were largely followed. Split plot design was used; main plot being fruit fly species and sub-plots being the treatment. Infestation rate was determined as the number of adult fruit flies per unit weight of fruits. Adult flies were identified using standard keys.

Research Results and Application

Infestation rates of fruit flies in mango

Results show that there was a significant difference on infestation rates among species (df=2, p< 0.001). However, the infestation rates among treatments were not significantly different (df 3, p= 0.633). The lowest infestation rates were recorded in fruits harvested from plots treated with an insecticide based IPM, followed by GF 120 and Derris bait. Furthermore, significant differences on number of trapped *B. invadens* between season were observed (df=1, p<0.001). An IPM program incorporating a Derris bait performed better than orchard sanitation alone, but the performance was lower compared to insecticide and GF 120 based IPM programs.

Number of trapped fruit flies

Number of trapped *B. invadens*

Significant differences on number of trapped *B. invadens* treatments (df-3, p=0.02). Least numbers of *B. invadens* were trapped from plots treated with an insecticide based IPM, followed by GF 120 and Derris bait.

Number of trapped *C. rosa*

Furthermore, the number of trapped *C. rosa* were not significantly between seasons (df=1, p=0.162). However the number of trapped *C. rosa* were significantly different among treatments (df=3, p<0.001). Significantly fewer flies were recorded in plots treated with an insecticide based IPM compared to GF 120 and Derris Bait. The infestation rates of *C. cosyra* significantly differed between seasons (df=1, p<0.001) and among treatments (df=3, <0.001).

Number of trapped *C. cosyra*

Results also show that more *C. cosyra* adults were trapped in 2008/9 season compared to the 2009/10 season. The effects of interaction between season and treatment was also significant (df=3, p<0.001). More *C. cosyra* adults were trapped in orchards were sanitation alone was practiced.

These results suggest, that the locally formulated bait has a potential of being used in IPM programs of fruit flies, as it performed better that orchard sanitation in most cases. However, the performance has been less compared to insecticide and GF 120 based IPM programs. Further, studies on the stability and effectiveness of the toxicant over time in the field should be investigated. Other botanically based toxicants, e.g. neem *Azadirachat indica* and *Tephrosia vogelii* should be researched for their suitability as toxicants in the bait.

Smallholder farmers can easily adopt the use of the bait because all the materials used are cheap and locally available.

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Efficacy of some Botanicals on Stemborers (*Busseola Fusca* (Fuller) and *Chilo Partellus* (Swinhoe) on Sorghum in Ethiopia under field condition

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Abstract

Two Lepidopteran stem borers, the maize stem borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and the spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) are the primary pests of sorghum in Ethiopia. Studies on management of these stem borers using botanical pesticides on sorghum were conducted at Sirinka and Chefa, north-eastern Ethiopia in 2009 and 2010. The experiments were designed in randomized complete block with three replications. The treatments were *Tanacetum cinerariaefolium*, *Nicotiana tobacum*, *Azadirachta indica*, *Jatropha curcas, Cissus quadrangularis*, *Chenopodium ambrosioids* and *Euphorbia schimperiana*. *T. cinerariaefolium*, *N. tobacum*, *J. curcas* and *E. schimperiana* were better than the standard and untreated controls in reducing damage and increasing yield. Percent damage of sorghum was significantly (P<0.0001) reduced from 66–95% in control to 3–19%, 3-15%, 2-10% and 7–15%% in *J. curcas* seed powder, *T. cinerariaefolium* flower powder, *N. tobacum* leaf powder and *E. schimperiana* leaf powder treatments, respectively, in all locations and years. Significantly higher yield was recorded on sorghum treated with these botanicals than the untreated control and the rest of the botanicals. Sorghum yield advantages of 46 -69%, 48-63%, 49-57% and 39-63% over the untreated control were recorded in *J. curcas* seed powder, *T. cinerariaefolium* flower powder, *N. tobacum* leaf powder and *E. schimpriana* leaf powder treatments, respectively. The results suggest that powder form of different parts of th ese plant species can be used for the management stem borers on sorghum.

Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, produced in sub-Saharan Africa is severely constrained by Lepidopteran stem borers which cause yield losses of 28-100% (Kassahun, 1993; Adane and Abraham, 1998; Emana and Tsedeke, 1999; Kfir *et al.,* 2002; Charles *et al*., 2006; Asmare *et al*., 2007; Asmare, 2008). The most important species in Ethiopia are *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in the low land and dry prone areas and *Busseola fusca* Fuller (Lepidoptera: Noctuidae) in highland and moist areas (Assefa *et al*., 1989; Emana, 2002, Asmare, 2007). Recently Assefa *et al.,* (2006) reported that these stem borers and others like *Eldana saccharina* and *Sesamia calamistis* were important on sugarcane estate at Metehara, Wonji and Fincha, Ethiopia.

Literature Summary

Successful control of stem borers of sorghum has been achieved through the use of conventional insecticides in Ethiopia (Emana and Tsedeke, 1999; Asmare *et al*., 2007**;** Asmare, 2008, Emana *et al*, 2008). Although, studies are rare in Ethiopia, the indiscriminate use of insecticides believed to cause a number of undesirable side effects such as the emergence of resistant species of the insects, environmental pollut ion and hazard to farmers. Moreover, the cost of agro chemicals can limit their use by resource poor farmers (Isman , 2006; Aroga and Ajala, 2007). Therefore, more fundamental strategies to pest management such as cultural, biological control and the use of botanical pesticides are emphasized in the control of stem borer complex in sorghum (Assefa and Ferdu, 1999; Emana, 2002; Aboubakary *et al*., 2008, Adane and Asmare, 2007; Asmare, 2008, 2010; Shelley, 2010).

Indigenous use of botanical pesticides could be one way of mitigating the problems associated with the inappropriate use of synthetic pesticides (Koul, 2008; Koul and Walia, 2009). Interest in the use of bio-pesticides with selectivity towards phytophagous insects has increased in recent years, particularly in cropping systems that rely on natural enemies as a major component of integrated pest management (Rau sell *et al.,* 2000). Further more, the demand for pesticide-free food, the biodegradability of natural products and the greater selectivity of natural products favoring non-target organisms have encouraged researches to use crude, bioactive plant extracts in pest management (Alphonsus, 2007).
Use of these natural compounds in place of conventional insecticides can reduce environmental pollution, preserve non-target organisms, and avert insecticide-induced pest resurgence. In this regards, the use of different parts of *Azandracta indica*, *Mexican marigold, Ricinus communis*, *Monodora myristica*, pyrethrum, *Melia azedarach,* pepper tree and *Jatropha curcas* were reported for the management of Lepidopteran insect species (Assefa and Ferdu, 1999; Sohail *et al.*, 2002; Amaugo and Emosairue, 2003; Aboubakary *et al*., 2008; Asmare, 2008; Brigitte, 2009**)**. As a result attention has to be given to environmentally friendly, economically sound, easy to use and socially acceptable control practices. Therefore, the exploitation o f bio-pesticide strategy could be vital and important in integrated management of stem borers. Thus the main objective of these studies was to look for ecologically sound, cheap and easily available botanical pesticides contributing vital role in an integrated management of stem borers.

Description of Research

The experimental fields were mechanically cleared, disc ploughed and disc-harrowed. Susceptible improved sorghum variety (Gambela 1107) and local cultivar (Jigurty) were sown on a plot size of 3.75 m × 5 m with 0.2 m between plants and 0.75 m between plots at Chefa and Sirinka, respectively in 2009 and 2010 main cropping seasons. Sorghum was sown in the first week of July in each location.

Table 1: Various plants and their parts used for treatments

	Treatments	Common/local names	P art used	Coded as
1	*Jatropha curcas*	Physics nut/Ayderke	Seed (SP)	JCSP
2	*Tanacetum cinerariaefolium*	Pyrethrum	Flower (FP)	TCFP
3	*Nicotiana tobacum*	Tobacco/Tinbaho	Leaf (LP)	NTLP
4	*Azadirachta indica* (standard control)	Neem/kinin	Seed (SP)	AISP
5	*Jatropha curcas*	Physics nut/Ayderke	Leaf (LP)	JCLP
6	*Cissus quadrangularis*	Veldt-grape /cheobe	Stem and leaf (S and L)	CQSLP
7	*Chenopodium ambrosioids*	Wormseed/Gime	Leaf (LP)	CALP
9	*Chenopodium ambrosioids*	Wormseed/Gime	Seed (SP)	CASP
9	*Euphorbia schimperiana*	----/Antarfa	Leaf (LP)	ESLP
10	endosulfan 5% dust (positive control)	---------	--------	end
11	Untreated control	---------	--------	UC

Dose and Treatment Application

Treatment application was started at two weeks after the crop emergence or when tiny white to dark brown larvae feed on the leaves causing damage symptom of 'leaf windowing'/ Hand application of one pinch or a teaspoon (0.65-1.0 gm) (Asmare, 2008) of each botanical powder was inserted in the funnel (central shoot) of sorghum seedling, where the neonate larvae feeding on. The application was done 3-4 times with seven days interval during the season (Adane, 2007; Asmare, 2008). Treatments were applied in the afternoon to avoid wind disturbance, evaporation and to moist the powder for better efficacy.

Research Results and Application

Sirinka: Damage parameters, percent chaffy head, peduncle damage, dead heart, mean exit hole and leaf damage as well as larval density per 10 plant, were significantly (P<0.0001) low in plots treated with *J. curcas* SP (1-8, 8-9, 5-8, 1-9-2, 7-8 and 1-10%), *T. cinerariaefolium* FP (1-5, 4-5, 5-6, 2-4, 3-7% and 7-10) and *E. schimperiana* LP (1-4, 8-12, 9-10, 6-7, 7-15%, and 10-21) and *N. tobacum* LP (3-17, 4.5-5.4, 3-6, 2-3,2-7% and 1-10) as compared to the untreated control (3-17, 69-78, 59-65, 17-36, 68-95%, and 20-40) and other tested botanicals at Sirinka in 2009 and 2010 (Tables 2-3 & Fig. 1-2). Leaf damage was significantly (P<0.0001) higher in plots receiving *C. ambrosioids* SP and LP (38-83% and 34-88%), *C. quadrangularis* (34-69%), *J. curcas* LP (31-35%) and *A. indica* SP (16-25%) as compared to the above promising botanicals in both years. Among botanicals, the highest percent damage and chaffy head were recorded in plots treated with *C. ambrosioids* (38-88 and 2-14%) and *C. quadrangularis* (34-69 and 17%), respectively in both years. There was no significant (P>0.05) difference between botanicals in percent chaffy head in 2010. Endosulfan 5% dust was as effective as the promising botanicals in reducing leaf damage (1-5%), chaffy head (0-6%) and larval density (0-5) (Table 2 & Fig. 1-2).). Number of larvae had significantly high positive correlation with percent chaffy head (r= 0.80, P<0.001) and percent damage (r= 0.89, P<0.001). Percent chaffy head of sorghum was significantly (P<0.001) reduced from 3-17% in control to 1-8, 1-5, 1-4 and 1-4% in *J. curcas* SP, *T. cinerariaefolium* FP, *N. tobacum* LP and *E. schimperiana* LP respectively.

Compared to the untreated control, all the tested botanicals contributed significantly to sorghum yield enhancement. However, significantly higher yield (mean ±SE, P< 0.05) was obtained by applying physic nut SP (40-42 qt/ha), pyrethrum FP (37-43 qt/ha), tobacco LP (38-44 qt/ha), *E. schimperiana* LP (37-40 qt/ha) and neem SP (36-38 qt/ha) than the untreated control (24-29 qt/ha) and the other tested botanicals. These botanicals were as effective as endosulfan 5% dust in boosting sorghum grain yield. In addition, sorghum yield was significantly increased by applying the other botanicals and their different parts as compared to the untreated control. Sorghum yield increment of 46-69 and 48-55, 51-56, 31-53, 39-57 and 66-71% over the untreated control were recorded in physic nut SP, pyrethrum FP, tobacco LP, neem SP, *E. schimperiana* LP and endosulfan 5% dust, respectively in 2009 and 2010 (Table 2). Sorghum yield increment of 13-27, 2-30, 4-19 and 7-18% were also obtained using physic nut LP, wormseed SP, wormseed LP and *C. quadrangularis* over untreated control **(**Table 2). Percent damage, chaffy head and number of larvae had significantly high inverse correlation with yield (r = -0.78, P<0.001, r = -0.71, P<0.001 and r = -0.80, P<0.001, respectively).

Chefa: Damage parameters, percent chaffy head, leaf damage, peduncle damage, dead heart, tunnel length and mean exit holes as well as larval density per 10 plant, were significantly (P<0.001) reduced in plots treated with physics nut SP (3-6%, 6-19%, 8-9%, 5-8%, 5-6, 1.9-2 and 0-25), pyrethrum FP (3-4%, 4-15%, 5-7%, 5-6%, 6-8, 2-3 and 3-53), tobacco LP (2-3%, 4-10%, 4-5%, 5-6%, 6.6-7, 4-4.2 and 1-70) and*, E. schimperiana* (5-6%, 7-11%, 11-12%, 11-13%, 6-7, 4-5 and 4-52) over untreated control (13-32%, 66-77%, 13-32% 79-89%, 59-67%, 13-15, 21-44 and 26-141) and other tested botanicals at Chefa in 2009 and 2010 (Tables 4 and 5). The highest and the lowest larval density of *C. partellus* were recorded at seedling and harvesting stage of sorghum, respectively (Fig. 3&4). Percents leaf damage were significantly ($P<0.005$) high in plots treated with *C. ambrosioids* SP and LP (27-71 and 38-73%), *C. quadrangularis* (38-68%), *J. curcas* LP (38-50%) and *A. indica* SP (41-44%) as compared to the above promising botanicals in both years (table 3). Endosulfan 5% dust was as effective as the promising botanicals in reducing leaf damage (0-10%), chaffy head (3-4%) and larval density (0-43). Number of larvae had significantly high positive correlation with percent damage ($r = 0\ 0.81$, $P<0.001$). Percent chaffy head of sorghum was significantly ($P<0.001$) reduced from 13-32% in control to 3-4%, 3-4%, 2-3% and 5-6% in *J. curcas* SP, *T. cinerariaefolium* FP, *N. tobacum* LP and *E. schimperiana* LP.

Compared to the untreated control, all the tested botanicals significantly increased sorghum yields. However, significantly higher yield was obtained using physic nut SP (40.5-41.3 qt/ha), pyrethrum FP (42-43 qt/ha), tobacco LP (40-42 qt/ha), *E. schimperiana* LP (41qt/ha) and neem SP (39-41 qt/ha) than the untreated control (25-28 qt/ha) and the other tested botanicals. These botanicals were as effective as endosulfan 5% dust in boosting sorghum grain yield. Sorghum yields were significantly increased using the other botanicals and their different parts as compared to the untreated control. Sorghum yield increment of 46-59 and 50-63%, 49-57, 45-55, 45-63 and 71-86% over the untreated control were recorded in physic nut SP, pyrethrum FP, tobacco LP, neem SP, *E. schimperiana* LP and endosulfan, respectively in 2009 and 2010 (Table 4). Sorghum yield increment of 12-41, 8-23 and 0-21% were obtained using physic nut LP, wormseed LP and *C. quadrangularis* over untreated control **(**Table 4). Percent damage and number of larvae had significantly high inverse correlation with yield ($r = -0.82$, $P<0.001$ and $r = -0.72$, $P<0.001$, respectively). In this study, we showed that the topical application of powder form of botanicals in the central shoot of sorghum at 2WAE (weeks after the crop emergence) for 3-4 times with a week interval were promising and substantially reduced larvae density, damage parameters and increased sorghum yields as compared to the standard botanical (neem) and untreated control. In the previous study, Asmare (2008) also recommended 3-4 applications of neem and chinaberry seed powder at dose of about a teaspoon or 0.65-1 gm for stem borers management.

The effect of various botanicals and their parts evaluated for the management of stemborers (*B. fusca* and *C. partellus*) on sorghum in Ethiopia showed that *T. cinerariaefolium*, *N. tobacum*, *J. curcas* and *E. schimperiana* were the most promising botanicals as compared to the standard and untreated controls and as effective as the synthetic chemical in reducing damage and increasing yield. Jatropha oil or a methanol extract of Jatropha oil containing phorbol esters has been shown to have strong insecticidal effects against *Busseola fusca* and *Sesamia calamistis* larvae (Mengual, 1997) and *Lipaphis erysimi*. The pesticidal effects against *Sitophilus zeamays* and *Callosobruchus chinesis*, deterring their oviposition on sprayed corn and mungbean seeds are also known.The leaf and seed extracts various botanicals can be highly toxic to neonate larvae of *B. fusca and C. partellus* (Harinder and Klaus, 2009**;** Brittaine and Lutaladio, 2010). Higher yields were recorded on sorghum treated with these botanicals than the untreated control and with the botanicals tested. Sorghum yield advantages over the untreated control were recorded with *J. curcas* seed powder, *T. cinerariaefolium* flower powder, *N. tobacum* leaf powder and *E. schimpriana* leaf powder treatments both at Sirinka and Chefa in both years. These results are in agreement with previous reports by Arnubio *et al.,* (2006), Adane and Asmare (2007), Asmare (2008), Wiratno (2008), Mahmoud *et al*. (2009), Orwa *et al*. (2009*),* Brittaine and Lutaladio (2010), Dubey *et al.* (2010) and Gupta *et al*. (2010), which showed that various extracts of neem, physic nut, tobacco and pyrethrum flower can be as effective as commercial insecticides when used on maize and sorghum to control Lepidopteran stemborer species.

Fortunately, some of these botanicals are readily available in the tested localities, cheap, safe to handle, biodegradable and selective and the technology for its preparation is simple. Thus, these materials can provide a suitable cost effective alternative to the synthetic insecticide for the management of stem borer larvae in low input farms managed by limited resource farmers in tropical and subtropical countries like Ethiopia. Therefore, these biopesticides could be considered as useful option in integrated management of stem borers on sorghum based farming system of resource poor farmers in Ethiopia.

Table 2: The effect of botanical pesticides on damage parameters, sorghum grain yield (qt/ha) and yield advantages (%) due to *B. fusca* at Sirinka in 2009 and 2010

Treatments	Leaf Damage %		Chaffy head (%)		Yield (qt/ha)		Yield adv. (%)	
	2009	2010	2009	2010	2009	2010	2009	2010
J. curcas, SP	7.5±1.9[c]	6.7±1.5[d]	7.5±1.6[b]	0.9±0.1[cde]	42.1±1.9[ab]	40.1±4.5[a]	45.7	68.5
T. cinerariaefolium, FP	6.6±2.2[c]	3.3±0.4[d]	4.9±1.3[b]	1.4±0.2[bcd]	42.7±3.1[ab]	36.9±6.2[ab]	47.8	55.0
N. tobacum, LP	7.2±0.8[c]	1.9±0.2[d]	4.1±1.5[b]	0.8±0.3[de]	43.7±2.7[ab]	37.6±5.1[ab]	51.2	56.0
A. indica, SP	24.6±2.6[b]	16.2±1.7[cd]	9.2[a]±0.6[b]	1.9±0.4[bc]	37.9±4.5[bc]	36.3±5.1[ab]	31.1	52.5
J. curcas, LP.	31.1±3.4[b]	35.1±4.2[c]	11.2±3.9[a]	1.5±0.3[bcd]	32.7±1.7[cd]	30.3±6.1[c]	13.1	27.2
C. quadrangularis S &L	34.2±12.8[b]	68.5±8.2[b]	17.4±4.2[a]	1.1±0.1[cde]	30.9±2.4[cd]	28.0±5.2[c]	6.9	17.7
C. ambrosioids, LP	37.6±7.8[b]	87.8±11.0[ab]	12.3±0.7[a]	2.1±0.3[ab]	29.4±1.0[d]	30.2±3.1[c]	1.7	26.9
C. ambrosioids, SP	37.9±4.0[b]	83.3±10.1[ab]	14.3±2.0[a]	1.2±0.1[bcde]	30.3±1.0[d]	28.2±6.6[c]	4.2	18.5
E. schimperiana, LP	7.1±0.8[c]	15.2±1.1[cd]	3.6±2.0[b]	1.2±0.1[bcde]	40.2±2.0[ab]	37.4±4.3[ab]	39.1	57.1
endosulfan 5% dust	4.8±0.2[c]	0.6±0.1[d]	5.7±0.4[b]	0.3±0[e]	47.9±1.4[a]	40.7±8.7[a]	65.7	71.0
Untreated control	68.4±6.9[a]	94.6±9.4[a]	17.2±1.2[a]	2.5±0.4[a]	28.9±1.4[e]	23.8±5.4[c]	---	---

S and L=stem and leaf, FP=flower powder. SP=seed powder, LP=leaf powder, Means followed by different letters are significantly different (P < 0.0001), one -way ANOVA, Tukey's HSD

Table 3: The effect of botanical pesticides on damage parameters, sorghum grain yield (qt/ha) and yield advantages (%) due to *C. partellus* at Chefa in 2009 and 2010

Treatments	Leaf damage %		Chaffy head (%)		Yield (qt/ha)		Yield adv.(%)	
	2009	2010	2009	2010	2009	2010	2009	2010
J. curcas, SP	19.0±2.1[c]	5.6±1.3[c]	6.4±1.8[b]	3.2±1.2[c]	41.3±7.4[a]	40.5±1.9a	45.9	59.4
T. cinerariaefolium, FP	15.2±4.5[c]	4.0±1.2[c]	4.0±1.0[b]	2.8±1.0[c]	42.5±1.1[a]	41.5±1.0a	50.2	63.4
N. tobacum, LP	9.7±3.9[c]	3.9±1.7[c]	2.0±0,4[b]	3.0±1.3[c]	42.1±3.1[a]	39.8±1.9a	48.8	56.7
A. indica, SP	44.0±3.2[b]	41.1±3.2[b]	5.0± 1.1[b]	5.0±2.0[c]	40.9±4.6[a]	39.3±1.7a	44.5	54.7
J. curcas, LP.	37.6±3.8[b]	50.0±2.1[ab]	14.2± 4.0[a]	21.1±4.4[a]	31.6±0.8[b]	35.9±1.4b	11.7	41.3
C. quadrangularis S &L	37.5±7.8[b]	68.1±6.9[ab]	11.9±5.7[a]	17.2±6.1[b]	26.4±3.2[bc]	30.8±5.3bc	--	21.3
C. ambrosioids, LP	37.6±6.3[b]	72.9±3.0[ab]	10.0±1.8[a]	23.4±3.4[a]	34.9±9.9[b]	27.4±1.5c	23.3	7.9
C. ambrosioids, SP	27.4E7.8[b]	70.6±9.2[ab]	5.6±2.9[b]	26.7±2.1[a]	28.1±3.5[bc]	23.4±1.1c	--	--
E. schimperiana, LP	11.2±3.2[c]	6.7±1.1[c]	6.0±1.2[b]	4.5±3.1[c]	41.1±9.1[a]	41.3±2.1a	45.2	62.6
endosulfan 5% dust	9.8±3.9[c]	0.0±0.0[c]	4.0±1.6[b]	3.2[c]±1.3	52.6±4.8[a]	43.5±1.1a	85.9	71.3
Untreated control	66.4±14.3[a]	76.9±9.6[a]	13.0±2.6[a]	32.1±5.3[a]	28.3±3.5[c]	25.4±1.2c	0.0	0.0

S and L=stem and leaf, FP=flower powder. SP=seed powder, LP=leaf powder, Means followed by different letters are significantly different (*P* < 0.0001), one-way !NOV!, Tukey's HSD

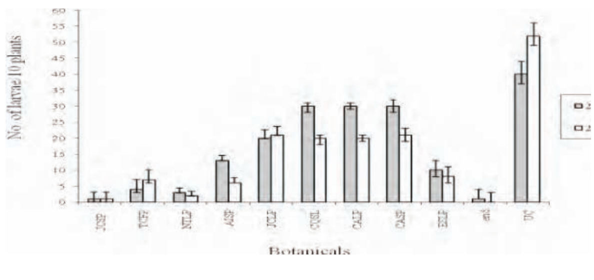

Figure 1: The effect of various botanical pesticides on larval density of *B. fusca* on sorghum at Seedling stage at Sirinka in 2009 and 2010 (See Table 1 for botanicals captions).

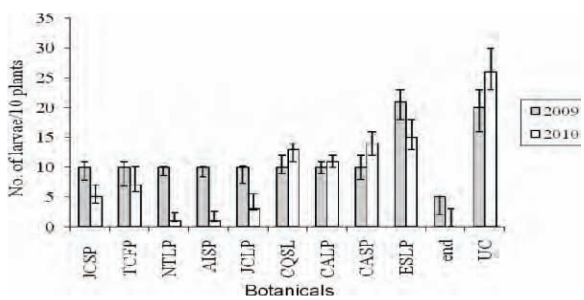

Figure 2: The effect of various botanical pesticides on larval density of *B. fusca* on sorghum at harvesting stage at Sirinka in 2009 and 2010 (See Table 1 for botanicals captions).

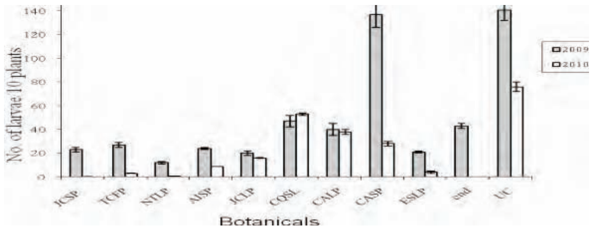

Figure 3: The effect of various botanical pesticides on larval density of *C. partellus* on sorghum at seedling stage at Chefa in 2009 and 2010 (See Table 1 for botanicals captions)

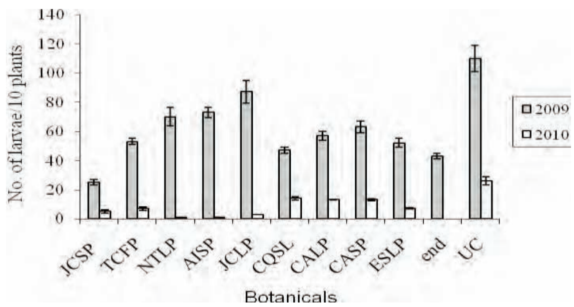

Figure 4: The effect of various botanical pesticides on larval density of *C. partellus* on sorghum at harvesting stage at Chefa in 2009 and 2010 (See Table 1 for botanicals captions)

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Evaluation of selected crude aqueous botanical extracts against sap-sucking pests in vegetable production

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Abstract

Cabbage aphid (*Brevicoryne brassicae* L) is the most problematic pest in smallholder vegetable production, causing 9-77% yield loss in heavy infestations. Current control strategy of using synthetics is widely used but consequently leads to decimation of natural enemies and development of inse ct's resistance/ Botanicals have been widely used in smallholder farming but not much literature exists on efficacy of the botanical products. Application of these natural insecticides has had a long and difficult road because of the earliest data gathered from researches by farmers and natives which revealed a lot of practices based on superstition. This paper discuss efficacy of selected crude aqueous botanical extracts in control of *Brevicoryne brassicae* L. in vegetable production by small scale farmers in Zimbabwe.

Introduction

Rape, *Brassicae napus L,* is one of the most important and widely grown vegetable crop by resource poor and small scale farmers of Zimbabwe for subsistence as well as for income generation, mainly due to its early maturity capacity. However pests and diseases are major constraints in *brassica* production in general, causing loss in quality, marketability and up to 80% on yield (Dobson *et al*., 2002). Due to the operation in resource poor environments in terms of inputs such as pesticides, the management and control of pests and diseas es is poor in small scale. Cabbage aphid (*Brevicoryne brassicae* L.) is the most destructive aphid species of the three and generally causing a loss of 9-77% in heavy infestations (Kelm *et al*., 1995). In Zimbabwe, aphid control in horticulture is heavily centered on the use of synthetic insecticides such as malathion, diazon, dimethoate which are efficient in eradicating pests but effectiveness decreases with time (Dube *et al*., 1998). Decrease in their efficiency can be attributed to decimation of natural en emies that help to reduce aphid numbers and development of insect resistance (Maas and Elwell, 1995). The sky rocketing of prices of inputs in Zimbabwe and the erratic market supply has seen the prices of synthetic pesticides inflated far beyond the reach of majority of small-scale farmers, thus the need to seek for affordable alternatives to synthetics. Thus natural methods of plant protection, which use local available resources, have assumed a new importance in an age when a host of commercial products are available. This paper responds to the continuing need to combat insect pests which have not yielded to effective control, thus the need to find alternative for existing chemicals and to provide a ready supply in future to meet insecticide shortages.

Literature Summary

Rape is an important leafy vegetable which is mostly grown by small scale farmers and gardeners having peak production during cool dry period from April to August and volumes decline in the hot dry periods from August to October (Jackson, *et al*., 1997) due to increased pest and diseases infestations resulting from warm temperatures. Pests and diseases are major constraints in *brassica* production in general, causing loss in quality, marketability and up to 80% on yield (Dobson, *et al*., 2002). It is because of the poor control that vegetables of small scale farmers become highly infested with pests such as aphids (Berger, 1994). According to Mariatou and Kwaramba (1999), aphids represent pests that are not effectively controlled by the curren t pest management measures in small holder farming. In Zimbabwe aphid control in horticulture is heavily centered on the use of synthetic insecticides. Reliance on synthetic chemicals to control pests has given rise to a number of predicaments such as destruction of beneficial non-target organisms (parasitoids and predators) thereby affecting the food chain and impacting on biological diversity. In addition, due to problems such as health hazards, undesirable side effects and environmental pollution caused by the continuous use of synthetic chemical pesticides (Nas, 2004), there is renewed interest in the application of botanical pesticides for crop protection. These Botanical extracts are naturally occurring insecticide compounds derived from plants which contain groups of active ingredients of diverse chemical nature and have an average residual life of 2-5 days. The use of botanicals in pest control has been practiced for a long time and people have been experimenting on different plants to reduce infestation of pests on their crops especially in small scale subsistence farming (Pedigo, 1999). As plants evolved, the selection pressures exerted by pests and pathogens resulted in the evolution of plant chemical defenses which inhibit pest attack and these chemical defenses derived from plants were some of the first known pesticides (Norris, 2003). Application of these natural insecticides has had a long and difficult road because the earliest data gathering done by researchers from farmers and natives revealed a lot of practices based on superstition, which when tested by scientific methods were shown not to be effective (Aguayo, 2000). Botanical pesticides are biodegradable (Devlin *et al*., 1999) and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators (Grange *et al*., 1988), and reduces environmental contamination and human health hazards. Research on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides are a prerequisite (Buss and Park - Brown 2002) for sustainable agriculture.

Description of Research

The research was done at Fambidzanai Permaculture Centre, located at 17° 51' 50 S and 31° 1' 47 E, west of Harare being necessitated by the results of evaluation of the organic farmers in Mashonaland East Province of Zimbabwe in their organic farming experience. A field trial, laid out in a randomized complete block design, was done to evaluate the efficacy of crude aqueous extracts of *Tephrosia vogelii, Allium sativum* and *Solanum incanum* in controlling cabbage aphids. An isolated garden was used to avoid the spread of aphids and twenty beds were prepared each measuring 2 m^2. Ten wingless female aphids were inoculated to each plot 3 weeks after transplanting of seedling and left for 14 days to allow them to acclimatize and reproduce. Spraying began 5 weeks after transplanting at weekly interval for 4 weeks. Bulbs of *A. sativum* were peeled first and all other plant material were washed, and sterilized using sodium hypo chloride before the preparation of the remedy. The *T .voguelli* fresh leaves, *A. sativum* cloves and fresh fruit and leaves of *S. incanum* were pounded using a mortar, measured into 200g, 300g and 500g respectively then soaked in 1 liter of water (25 ^{o} C) to give mass per unit volume concentrations. The mixtures were thoroughly stirred with a spatula and left to settle for 24 hours. A sieve was used to separate residue from crude extracts before spraying using a hand sprayer. Two sample plants were randomly selected from each seedbed and aphid count was done on the third leaf from the aerial plant part. Enumeration of aphids in situ was done using hand lenses and a pair of clippers, 24 hours after application of treatment to collect data on nymph and adult aphid population. As a positive control, 10 ml of 40% EC was dissolved in 1 litre of water and applied at an area of 4m^2, which are 2 seedbeds. 1 litre of borehole water, same as used for remedy preparation and dilution of synthetic, was sprayed in each seedbed as a negative control. Data on fresh weight was obtained through rouging sample plants and measuring them using a digital scale.

Results

Efficacy of botanical extracts on controlling nymph aphid population

The effects of different treatments of crude botanical extracts on rape nymph aphids are presented in Figure 1

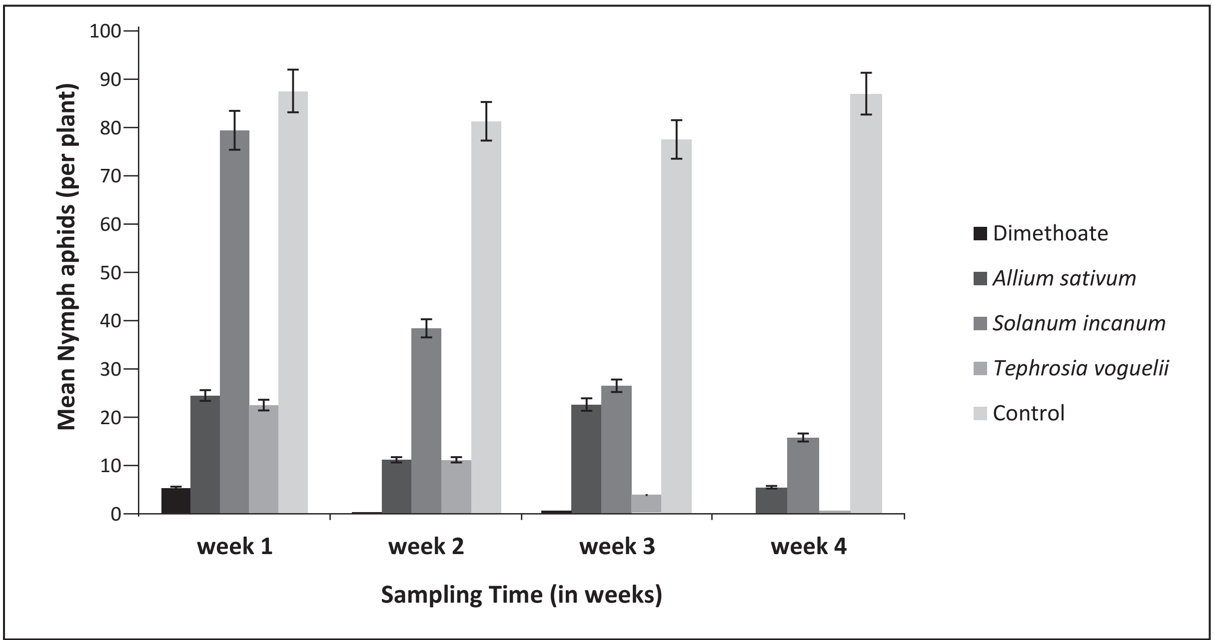

Figure 1: Effect of different treatments on mean nymph aphid population

The treatments applied had a significant effect on nymph aphid population (p<0.05.) Control was significantly different (p<0.05) from other treatments except in week one where it had no significant difference (p>0.05) on the effect as *S. incanum. T .voguelii* treatments were significantly different (p<0.05) from other botanicals. Dimethoate significantly differed from all botanical treatments throughout the sampling period except in week four were it had no significant difference (p>0.05) with *T. voguelii.*

Efficacy of botanical extracts on controlling adult aphid population
The effects of different treatments of crude botanical extracts on rape adult aphids are presented in Figure 2

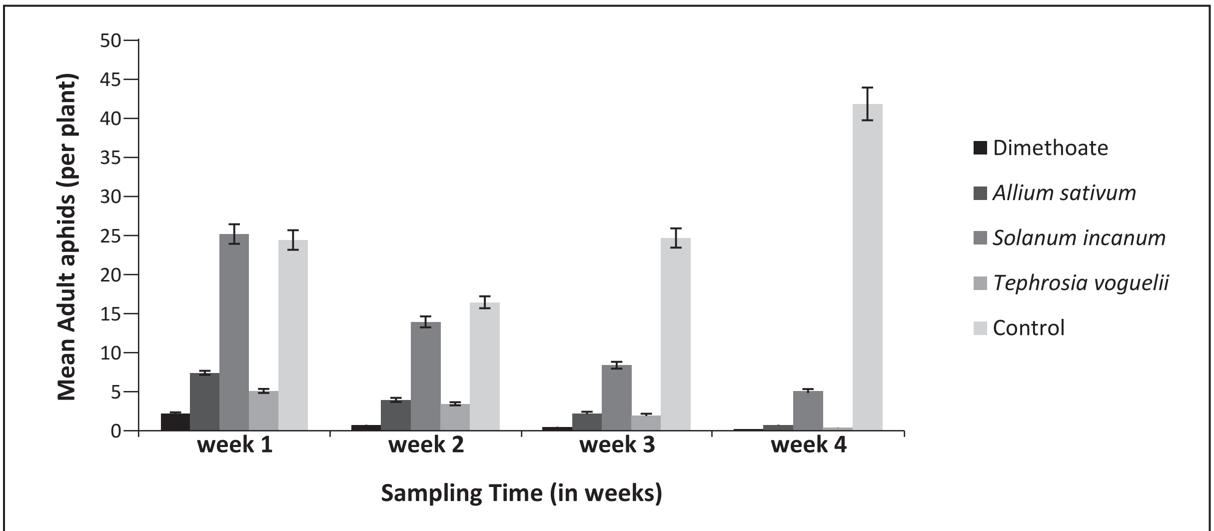

Figure 2: Effect of different treatments on mean adult aphids

The treatments applied had a significant effect on adult aphid population (p<0.05). The negative control, borehole water, treatment showed no significant difference (p>0.05) with *S. incanum* treatment in the first week. Dimethoate significantly differ (p<0.05) from all treatments in the first three weeks of the sampling period but has no significant different effect (p>0.05) with *T. voguelii* and *A. sativum* in the forth week.

Effect of aphid infestation on yield
The effects of different treatments of crude botanical extracts on yield of rape adult presented in Figure 3

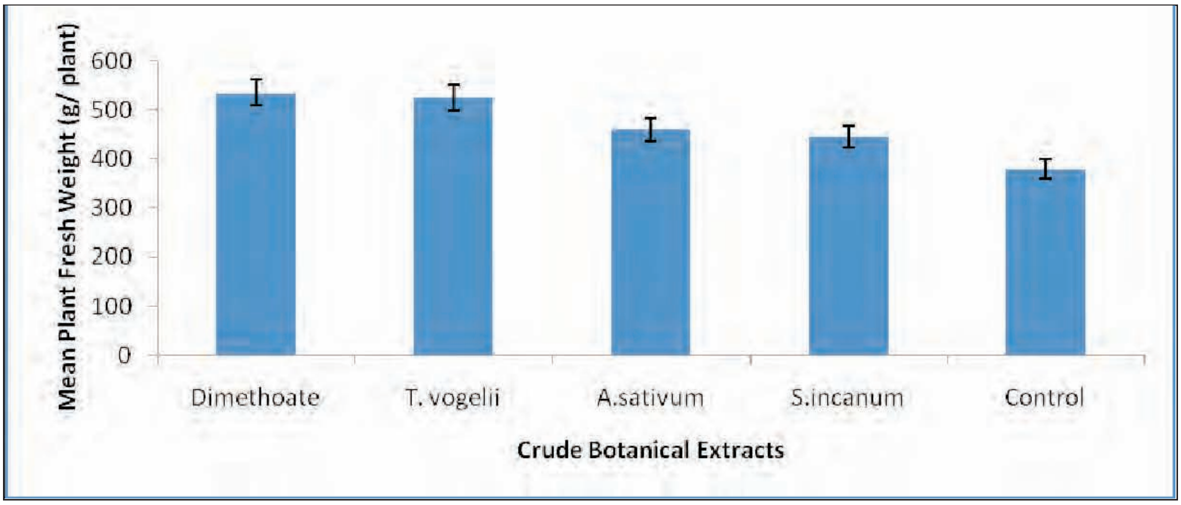

Figure 3: Effect of different treatments on yield of rape

The treatments applied had a significant effect on the yield of rape ($p<0.05$) Figure 3. The highest mean yield was recorded on Dimethoate treatments followed by *T. vogelii, A. sativum* and *S. incanum.* There was no significant difference ($p>0.05$) in yield on *T. vogelii* and Dimethoate treatments and between *S.incanum* and *A. sativum* treatments.

Research Application

The research has shown that the crude botanical extracts of *T. vogelii, S.incanum* and *A. sativum* can be effectively used for aphid control in *B napus* production without causing significant reduction in fresh weight, which is yield, due to aphid infestation. *S.incanum* is effective but it may be recommended to increases the frequency of its application and concentration of the extract for improved efficacy. These results indicate the potential for on-farm cultivation, extraction, formulation and use of botanical extracts in crop protection within the framework of sustainable pest management. Based on the findings of this research, farmers can be recommended to use *T. vogelii* at a rate of 200g of crushed fresh leaves per 1 litre of water and *A. sativum at* 300g of crushed cloves per litre of water which were effective in controlling aphid populations. These are locally available plants that can provide an easy alternative to the use of synthetics to minimize the detrimental effects of synthetics to human health and environment. Farmers who opt for the use of these botanical pesticides may be warned that despite being natural, some of the active ingredients in some plants extracts can have negative impacts on human health and can cause loss of life if not properly handled.

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LC/MS-ESI characterization of flavonoids from two food legumes: chickpea (*Cicer arietinum* L.) and mung beans (*Vigna radiata* L.), possible candidates for controlling *Striga* weed

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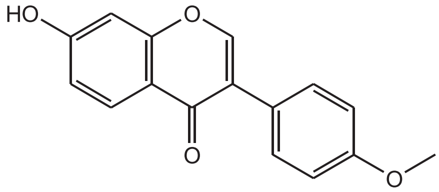
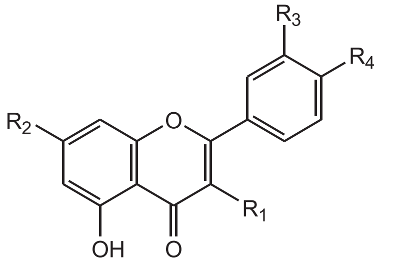
Abstract

A standardized profiling method based on Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) and Liquid Chromatography coupled with Mass Spectrometry (LC/MS) was used to identify flavonoids in extracts of chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*), potential trap crops for *Striga* weed. Nine flavonoids including mono- and diglycosyl flavanone and anthocyanidin derivatives of luteolin, apigenin, isorhamnetin, formonometin, quercetin, isosakuranetin, and sakuranetin were tentatively identified. The detected phenolics were present at concentrations greater than 0.001% of the dry materials. Many of these phenolics have been reported to have human health benefits. Some of the compounds, including glycosylated flavones are reported for the first time in these two species. The comprehensive analysis of the aqueous secondary metabolites in these leguminous plants is helpful for understanding their traits. Similar traits to *Desmodium uncinatum*, a fodder legume interfering with *Striga* development, could either be exploited or modified genetically to enable the food legumes *C. arietinum* and *V. radiata* attain *Desmodium's* allelopathic ability.

Formononetin





Apigenin: R_1=H R_2=OH R_3=H R_4=OH
Luteolin: R_1=H R_2 =OH R_3=OH R_4=OH
Isorhamnetin: R_1=OH R_2 =OH R_3=OCH3 R_4=OH
Quercetin: R_1=OH R_2 =OH R_3=OH R_4=OH
Isosakuranetin:R_1= H R_2=OCH_3 R_3= H R_4=OH
Sakuranetin: R_1= H R_2=OH R_3= H R_4=OCH_3

Key words: LC/MS-ESI, *Cicer arietinum, Vigna radiata, Striga,* phenolics, allelopathy.

Introduction

Some legume varieties, for example chickpea, mung beans, cowpea, groundnut and soybeans have potential to cause suicidal germination of *Striga. hermonthica,* reduce attachment to host and improve fertility of soil (Kureh *et. al.,* 2003) by enriching the soil with nitrogen and organic matter. These pesticidal-promoting effects may be attributed to the presence of bioactive compounds such as flavonoids and phenolic acids in the roots and leaves of *Cicer arietinum* and *Vigna radiata*. However, variety in the two plants remains uncertain and need to be investigated. Some understanding has been gained of the secondary metabolism involved in the mechanism by which *Desmodium* suppresses *Striga* (Tsanuo *et. al.,* 2003). Phytochemical studies of various plants of *desmodium* species have progressively isolated phenolic components (Tsai *et. al.,* 2011). From *Desmodium uncinatum* root, 2'-O-glucosylvitexin, vitexin, isovitexin and apigenin have been isolated (Tsanuo *et. al.,* 2003 and Guchu 2007). Tsanuo *et. al.,* (2003) and Hooper *et. al.,* (2010) isolated three isoflavanones, 5, 7, 2',4'- tetrahydroxy-6-(3-methylbut-2-enyl)-isoflavanone, 4",5"-dihydro-5,2',4'-trihydroxy-5"-isopropenylfurano-(2",3",7,6)- isoflavanone and 4", 5"-dihydro-2'-methoxy-5,4'-dihydroxy-5"-isopropenylferano-(2",3",7,6)-isoflavone from the root exudates of *D. uncinatum*. Isolated fractions containing compound B induced germination of *Striga* seeds while fractions containing compound C inhibited radical growth. Pickett *et al.,* (2007) and Hooper *et al.,* (2009) recently characterized another key haustorium growth inhibitor, di-C-glycosylflavone-6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranosylapigenin, also known as isoschaftoside from polar fraction of *D. uncinatum* root exudates (Khan *et al.,* 2010). The biosynthetic pathway of this class of compounds is already present in edible legumes and in cereals, so characterization of the enzyme and genes that control *C*-glycosylflavone biosynthesis has the potential to create this protection mechanism in other agriculturally important plants (Hooper *et. al*., 2009). The active ingredients in *Desmodium* roots exudates that have been characterized are suspected to be present in traditional food legumes. If similar traits are found in the food legumes, then conventional breeding could then be used to select and enhance the traits. Alternatively, if these traits are lacking, a gene could be transferred directly from *Desmodium* into food legume via genetic modification to enable legumes suppress *Striga* ssp.. In the longer term, it may be possible to transfer the same trait to cereal crops through heterologous gene expression, principally to the open pollinated varieties, rather than hybrids for easier accessibility to small scale farmers (Pickett *et. al.,* 2010).

This study sought to isolate using column chromatography and RP-HPLC-PDA, purify and characterize using column chromatography, RP-HPLC-PDA and LC/MS substantial concentrations of secondary metabolites in *C. arietinum* and *V. radiata* leaves and roots exudates. This will enable assessment the aqueous phase of the two plants. This information could also be useful when considering the possibility of transferring relevant biochemical traits involved to edible legumes, such as chickpea and mung beans.

Experiment Description

Data in this study were acquired using three analytical instruments; A high performance liquid chromatograph (HPLC) consisting of quaternary pump, column compartment, auto-sampler and variable wavelength PDA detectors (Data system, version 1.24 ACE, Shimadzu Corporation England), A Liquid Chromatograph equipped with a quaternary LC pump (Model 1200) coupled to an Agilent MSD 6120-Single quadruple mass spectrometer with electrospray source and another LC-MS Hewlett-Packard 1090 photodiode array detector (Agilent Technologies) and a Perkin-Elmer SCIEX API III triple-quadrupole mass spectrometer (Perkin-Elmer, Toronto, Ontario, Canada) equipped with an ion spray source (ISV = 5500, orifice voltage = 50) in positive ion mode.

After air drying then oven drying, leaves and roots of *C. arietinum* and *V. radiata* were ground to powder weighed and extracted with 50:50 MeOH/CH_2Cl_2 at room temperature for 3 days in the ratio of one gram herbage: 7.5 ml solvent. The mixture was filtered to separate the filtrate from resid ue then using the residue the extraction was repeated twice using the same solvent system. The filtrates were evaporated *in vacuo*, the residue weighed. The constituents purified and isolated using column chromatography. Each extract was analyzed using TLC and HPLC then further purified using HPLC.

The initial spectral scan of phenolics from nine samples of the leaf and root extracts of Chickpea (*C. arietinum* L.) and Mung beans (*V. radiata* L.) was done by combining the data from PDA and MS to allow facile and efficient identification. Three screening strategies were used as follows. Firstly, where reference compounds were available as standards, the presence or absence of a particular biophenol was assessed by comparison of retention time and UV–visible spectra, with that of the reference. The presence was further confirmed by molecular mass data. Secondly, when reference compounds were not available, the TIC traces in both negative and positive mode at soft ionisation and strong ionisation conditions were scanned for appropriate pseudomolecular ions $[M+H]^+$ Confirmation was performed by examining UV–visible spectra and mass spectral fragmentation data at the expected elution time, depending on pre-identified compounds in the sample, the structure of the target compound, and the literature data. Thirdly, major peaks in TIC and UV chromatograms that were not identified by either of the other screening processes were screened for novel compounds by generating mass spectra. In all, screening was conducted for approximately 100 biophenols from different classes. The main aim was to demonstrate the power of combined use of HPLC–PDA and ESI-MS in screening of legume extracts.

Samples were injected into an LC-MS system, allowing spectral analysis followed by mass determination to identify the moieties attached to the aglycone. For this reason, MS scans were made up to 1415 mass/charge (m/z), because most diglycosides have an m/z of 431 to 700 and single acyl moieties add between 269 and 474 m/z. Parent peaks were identified at 700, 650, 641, 627, 611, 610, 595, 474, 449 and 431 m/z with daughter peaks at 474, 448, 432, 303, 312, 287, 271 and 269 m/z. A subsequent direct injection MS-electron scan detected these peaks as well as several others in lesser quantities. These masses were compared with all combinations of known flavones and anthocyanidins.

Results and Discussion

Identification of compounds

Out of ten isolated compounds from the leaf and eight from the root of *C. arietinum* sevens from the leaf were identified (*C.A. L 2;* Isorhamnetin-3,7-O-diglucoside, *C.A. L 3;* Quercetin 3,4'-O-diglucoside, *C.A. L 4;* Isosakuranetin-5, 7-O-diglucoside, *C.A. L 7;* Isorhamnetin-3-O-glucoside-5-O-arabinoside, *C.A. L 8;* Sakuranetin-5-O-glucoside, *C.A. L 9;* Luteolin 4'-O-glucoside and *C.A. L 11;* Isorhamnetin 3-O-(2)-dirhamnoside) and *C.A. R 5;* formononetin-7-*O*-β-D-glucoside from the root. While from *V. radiata* one compound; V. R. R 6; Apigenin-6, 8-di-C-glucoside out of six isolated attained the required purity and quantity for identification. A summary of the results was tabulated in Table 1.

Table 1: Identified compounds

Compounds	Retention Time (min)	UV Maximum absorbance (λ_{max}) (nm)	ESI-MS fragment ions (% relative intensity)
C. A. Root 5	34.1~34.4	223, 257	*m/z* 454 $[M+Na]^+$ (10) and 431 $[M+H]^+$ (100). *m/z* 269 $[M+H-162]^+$ (<5)
C. A. Leaf 2	19.4~19.7	222, 238, 256, 352	*m/z* 740 $[M+CH_3COOH+OH]^+$ (<5) 663 $[M+Na]^+$ (30) and 641 $[M+H]^+$ (100). *m/z* 623 $[M+H-H_2O]^+$ (<5), 479 $[M+H-162]^+$ (<5), 317 $[M+H-2(162)]^+$ (<5)
C. A. Leaf 3	21.7~22.1	225, 257, 353	*m/z* 650 $[M+Na]^+$ (<10) and 627 $[M+H]^+$ (100). *m/z* 595 $[M+H-O_2]^+$ (<5), 465 $[M+H-162]^+$ (5), 446 $[M+H-(162+H_2O)]^+$ (1), 303 $[M+H-2(162)]^+$ (<5)

C. A. Leaf 4	22.9~23.2	230, 265, 325, 337	*m/z* 633 $[M+Na]^+$ (15) and 611 $[M+H]^+$ (100). *m/z* 449 $[M+H-162]^+$ (15), 430 $[M+H-(162+H_2O)]^+$ (<2), 287 $[M+H-2(162)]^+$ (5)
C. A. Leaf 7	32.6~33.0	238, 254, 353	*m/z* 742 $[M+132]^+$ (10), 633 $[M+Na]^+$ (15) and 610 $[M+H]^+$ (100). *m/z* 478 $[M+H-132]^+$ (30), 461 $[M+H-(132+H_2O)]^+$ (12), 317 $[M+H-(132+162)]^+$ (20)
C. A. Leaf 8	34.6~35.0	230, 265, 347	*m/z* 471 $[M+Na]^+$ (<10) and 449 $[M+H]^+$ (100). *m/z* 287 $[M+H-162]^+$ (5)
C. A. Leaf 9	36.6~36.9	220, 244, 264, 348	*m/z* 533 $[M+H+2CO]^+$ (15), 533 $[M+H+CO]^+$ (65) and 449 $[M+H]^+$ (100). *m/z* 287 $[M+H-162]^+$ (<5)
C. A. Leaf 11	29.4~29.8	218, 224, 348	N/A
V. R. Root 6	15.1~15.4	229, 258, 321	*m/z* 710 $[M+Na+C_6H_5O]^+$ (<5), 617 $[M+Na]^+$ (15) and 595 $[M+H]^+$ (100). *m/z* 432 $[M+H-162]^+$ (<5) and 271 $[M+H-2(162)]^+$ (<5)

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Evaluation of *Girardinia diversifolia* as a potential biopesticide in Kenya

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Abstract

About 60% of fieldand stored crop products are lost to pests and diseases. Community based research has been used for the control ofcrop pests and diseases in many less developed countries. A study with the help of farmers was carried out in Likia (East Mau), Tetu (East Aberdares) and Karumandi (South of Mt Kenya) in Kenya. The objective of the study was to investigat e the effectiveness of *Girardinia diversifolia* as a bio-pesticide. Training workshops were carried out to train farmers on sustainable agriculture practices. The pesticidal effectivenessof *G. diversifolia* was tested by comparing fresh biomass weight, plant growth, pest damage levels and abundance of pest species. Two treatments were arranged in a Randomized Block Design (RBD) with four replications per treatment. *G. diversifolia* biopesticideshowed a significant reduction in pest damage levels on the treated plots in comparison to the control. However, there were no significant differences in biomass weight and plant height between the plots treated with the *G. diversifolia* biopesticide and the control. A total of 9 arthropod species belonging to eight familie s were observed with aphids being the most abundant species noted in the field. Analysis in the laboratory identified the following secondary metabolites: alkaloids, sterols, terpenes, saponins and flavonoids. The laboratory aphid repellency test showed a higher repellency as the concentration of the extract increased.It was concluded that application of *G. diversifolia* extract reduced the crop losses due to pest damage. From this study, it was recommended that further work need to be done on theeffectivene ss of*G. Diversifolia* on different crop pests and in different ecological areas.
Key words: *Girardinia diversifolia,* Biopesticide, Secondary metabolites, on-farm research, Repellency

Introduction

Biopesticides are used either in traditional crude forms or as pure active compunds. A number of insecticidal plants were traditionally used to control different insect pests. Such plants include *Chrysanthemum cinerariifolium* (Trevir) which was used to con trol bedbugs, mosquitoes, cockroaches, domestic flies, mites and spiders. *Nicotiana tobaccum* L. for aphids, leaf hoppers, thrips, white flies and spider mites and *Urtica dioica* L. as an effective aphid repellent (Bozsik, 1996). More studies in Poland showed that water extracts of *U. dioica* was more active against aphids than organic solvents (Achremowicz and Ciiez, 1992). Biopesticides are gaining popularity worldwide because they are environment friendly.Pyrethrins, nicotine and rotenones are common insecticidal properties found in plants (Jensen *et al.*, 1985).

These extracts are plants' secondary metabolites that are not directly utilised by plants, but are useful as defence mechanisms against many herbivory organisms including insects. Most of these extra cts are mixtures of alkaloids or phenols (Tvedten, 2007). These biopesticides may affect the pests in different ways such as antifeedants, sterilants, and repellents or as poisons and causing paralysis. Some extracts may however enhance the crops'vigor, re sistance or compensation ability of the plant under pest attack (Roak, 1942). Secondary metabolites usually have broad spectrum action and therefore pests would not result in developing resistance as commonly found in the application of synthetic pesticide s. The objective of the study was to investigate the effectiveness of *G. diversifolia* as a bio-pesticide.

Literature Summary

Although most farmers use synthetic pesticides, a few use traditional crude forms of botanicals. This practice is worldwide and is in use for centuries for instance *Chrysanthemum cinerariifolium* (trevir) is known for its rapid paralysing effect and toxicity to a wide range of insects but is non -toxic to mammals; its LD 50 to rats is 580mg per kg but to house fly is 15mg per kg. it has keto-esters cinerinn I and II andpyrethrin I and II which is traditionally used to control edbugs, mosquitoes, cockroaches, domestic flies, mites and spiders(Stuart, 1971). *Derris eriptica* and *D. Melaccensis* is used to repel aphids, leaf eating caterp illars and mosquito larvae while

Myrica gale is known to repel fleas.Ngassapa *et al*, (1999) reports that Trichiria emetic Vahl controls mosquitoe larvae while Dale and Greenway (1961) report that Balanitesaegyptica besides controlling Schistosomes also controls water fleas. Kraus and Piteller (1991) found *Urtica* to be effective as aphid repellents while Bozsik(1996) carried out studies on aphicidal efficiency of different stinging nettle extract fermented for six days found it to reduce infestation on plant s although not significantly. In Kenya, a number of indigenous plants have been screened for useful bioactive compounds with quite a number with positive results (Mwangi *et al*, 1999).

Description of Research

Farm level test of the biopesticide

Two treatments were arranged in a Randomized Block Design (RBD) with four replicates per treatment. Each replicate was 3m by 3m with 0.61m wide pathways in between each replicate plot. The two treatments comprised of biopesticide application and control (d istilled water).Leaves of *G. diversifolia* were harvested and dried under shade. They were then ground into powder using a hammer mill. Five kilograms of the powder were put into a plastic tank and 50l of warm water added and left to ferment for 7 days. Wir e mesh sieves were used to remove coarse leaf materials and then a muslin cloth was used to remove small suspended particles. Plants were sprayed with this solution once a week.Growth rate was measuredby use of a ruler on a weekly basis. Fresh biomass was done by use of destructive method. An index was used to record pest damage and a hand lens was used to identify pests into their family and at species level. Analysis of Variance (ANOVA) for RBD and Turkey's Studentised Range (HSD) test was applied to sepa rate means at 0.05 significance level. 95% confidence limit and standard errors were calculated for each treatment mean.

Laboratory Evaluation

Collection of plant materials

Plant leaf materials were collected from the demonstration plotsat Likia (East Mau), Tetu (East Aberdares) and Karumandi (South of Mt Kenya). Leaves were separated from twigs by use of secateurs. They were put in gunny bags and taken to a shed where they were spread on a canvas and left to dry under shade. The dry leaves were then blend in a hammer mill into powder that was stored in dry bags. A concoction was prepared by adding water to the powder in the ratio of 5:1.The mixture was left for seven daysto ferment and theconcoction was sprayed once a week on plants in the field.

Test for Bio-active Plant Products

Some of the dried leaves were taken to the laboratory to test for bio -active compounds. These leaves were ground into a fine powder in an electric blender. About 50g of powder was exhaustively extracted in a soxhlet apparatus using 95% methanol for 48hours. The extract was concentrated in a rotary evaporator in vacuo. This left a thick brown/grey gummy material that was freeze dried at 250 millitor pressure at -40^{0}C and stored in a refrigerator. For alkaloids, 100mg of the extr act was heated with methanol/chloroform (1:1) mixture and the solution was chromatographed on Silica gel plates using the following solvent systems: chloroform/methanol (9:1), chloroform/ethyl acetate (8:2) and methanol/ammonia (100:3). The chromatography was allowed to run for one day and after drying the plates, Dragendroff's spray reagent was applied/ For sterols and terpenes, 0.15g of the extract was stirred with 20 ml petroleum ether (60-80%) to remove the colouring materials. The residue was extracted with 20 ml of chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphate. Then 5 ml of chloroform solution was mixed with 0.5 ml of acetic anhydride followed by two drops of concentrated sulphuric acid. For saponins, 0.5g of the ext ract was shaken with 20 ml water in a test tube. Frothing was checked and compared with a control where water without extract was shaken.For flavonoids, 0.8g of the extract was defatted by several washings with petroleum ether. The defatted residue was dissolved in 30 ml of 95% ethanol and filtered. The filtrate was used for the following tests: to 3 ml of the filtrate in a test tube, 4 ml of 1% aluminum chloride in methanol were added; to 3 ml of the filtrate 4 ml of 1% potassium hydroxide were added, and to 2 ml of the filtrate, 0.5 ml of concentrated hydrochloric acid and a few magnesium turnings were added. For anthraquinones, 2.0g of the extract was shaken with 10 ml of benzene and filtered. A 10% ammonium hydroxide solution (5 ml) was added and the mix ture was shaken.

Anti-aphid Test

Cabbage aphids *Brevicorynebrassicae* were exposed to extracts. About 50 mg of *G. diversifolia* extract was dissolved in 5 ml of water. Then 10, 100, and 500 µl of this solution were made and used to treat filter paper discs. Aphids (50) were placed in the main stem of anolfactometerand the disc in one of the tubes. The other one remained open. The end of the tube where aphids were placed was corked to allow aphids to move in one direction. A control was set where the paper disc was saturated with water. The setup was left for 24 hours and aphids in each tube were counted.

Research Results and Application

On-farm results

Analysis of variance results showed that the mean fresh biomass weight of the treated plants was not significantly different from the control plants(p>0.05). However, the mean number of pests on the treated plants was significantly different from that of the control (p<0.05) (Table 1).

Table 1: ANOVA showing the effectiveness of *G. diversifolia*biopesticide on tomatoes, kales and cabbages

Source of variation	d.f	mean square	F-value	p-value
Species	2	2.505	25.09	<0.0001
Treatment	1	0.005	0.05	0.8530
Treatment*species	2	0.009	0.09	0.9321

Pests that were collected from the three vegetables belonged to two classes: insecta and arachnida. Further identification found that they belonged to eight families. They belonged to two major feeding habits; chewing and sucking groups (Table 2).

Table 2: Pests found on crops under study

Family	Name	Status	Control	Treated
Tetranychidae	*Tetranychustelarius*	Pest	695	462
Coccinellidae	*Hyppodamia variegate*	Predator	4	6
Aphidae	*Brevicorynebrassicae*	Pest	303	295
Aphidae	*Myzuspersicae*	Pest	21	19
Braconidae	*Cotesiaphitellae*	Pest	5	2
Ichinenmonidae	*Diadegmasemiclansum*	Predator	3	1
Plutellide	*Plutellavylostella*	Pest	7	3
Noctoidae	*Agrotisspp*	Pest	4	1
Thripidae	*Thripstabaci*	Pest	3	6
			1182	813

Crop damage and growth assessment

A score index of 1-4 was used to assess damage per crop. From ANOVA results, there was a significant treatment effect (p<0.0001). The pest damage proportion was significantly lower for the treated crops than the control. For the two months weekly observations of the pest infestation, week one had least while week five had the highest.Crop growth in height was recorded weekly in centimetres which showed a nosignificant difference in the study.

Laboratory Results

Bioactive compounds in *G. diversifolia* leaf extract are shown in Table 3. All compounds analysed showed positive results except anthroquinones.

Table 3: Bioactive compounds in *G. diversiflolia* leaf extract

Compound examined	Observation
Alkaloids	+
Sterols	+
Terpenes	+
Saponins	+
Flavonoids	+
Anthraquinones	-

+ Compound present; Compound absent

The aphid repellency test is shown in table 4 where the percentage repellency increased with increased concentration of the extract.The percentage control repellency was very low (less than 1 per cen t).

Table 4: the anti-aphid effect of G. diversifolia leaf extract

Concentration	Extract repellency (%)	Controlrepellency (%)
5 ug/ml	1.5	0.6
50 ug/ml	11.8	0.4
500 ug/ml	63.2	0.9

The results indicate that horticultural crops are damaged more by small insects and other anthropods. Most of the pests recorded composed those with sucking mouth parts, probably due to juicy nature of these plants which caused reduction of fresh biomass. Some of the pests were found on plants but appeared as if they wer e on transit as they could be hardly collected or their damage easily recorded which included grasshoppers.Some of the pests proved difficult to control for instance, cutworms (*Agrotis* spp.) as the devastating larval stage lived undergroundrequiring fumiga tion.*G. diversifolia* proved to be a potential insecticidal plant as laboratory analysis results showed presence secondary metabolytes which are known to be effective in controlling insect pests and other organisms. Such preparations include nicotine which is reported to control aphids and white flies by Bozsik (1996), saponins are reported to control molluscs byVerdcourt and Trump (1969) while terpenes are known to repel aphids (Minja *et al*., 2002). *G. diversifolia* has potential of being a broad spectrum source of biopesticides as the laboratory analysis showed presence of major secondary metabolite and further analysis using GC/MS and NMR revealed presence of carotenoids.The damage by insects was significantly lower on treated plants than the control. This could be attributed to anti-feedant or repellent properties of *G. diversifolia*. Final fresh biomass was not significantly different (p<0.05).

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Symposium 2 Postharvest Plennary

Chemical Composition and Insecticidal Effect of Essential Oils of Some Aromatic Plant of Burkina Faso

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Abstract

The essential oils from leaves of seven aromatic plants, *Laggera oloptera*, *Laggera aurita Cymbopogon schoenanthus, Cymbopogon giganteus*, *Ocimum basilicum, Lippia multiflora* and *Anona senegalensis* were extracted by hydrodistillation, analyzed with GC and GC/MS equipment and tested on the cowpea weevil (*Callosobruchus maculatus*). Results showed that: *Laggera oloptera* presents two chemotypes. In one of them, α-pinene (72.1 %) and limonene (14.8 %) are main components and the secondary components are 4-terpineol (7/4 %), globulol (6/9%) and β-caryophyllene (5.6%), *Laggera aurita* contains, α-cadinol (30/1 %), δ-cadinene (12/3%), τ-muurrorol (8.2%), and terpene-4-ol (7.3%) as major components, *Cymbopogon schoenanthus* shows a large amount of piperitone (57/ 2%), δ-2-carene (18%), and elemol (8%), C*ymbopogon giganteus* oil contains, cis-p-mentha-2, 8-dien-1-ol (17.6%), trans-p-mentha-1(7)-dien-2-ol (15. 7%), limonene (14. 2%), and trans-p-mentha-2, 8-dien-1-ol (14. 2%), *Ocimum basilicum* contains a large amount of linalool (53/6 %), eugenol (18 %), γ-terpinene (4/8%) and trans α-bergamotene (4.3%), *Ocimum basilicum* contains a large amount of linalool (53/6 %), eugenol (18 %), γ-terpinene (4.8%) and trans-α-bergamotene (4.3%), *Lippia multiflora* essential oils major components are thymol (29.8%), P-Cymene (26.1 %) and thymyle acetate (11.8 %), *Anona senegalensis* contains the following essentials oils, α-amorphene (16/8), α-dedrene (12.7), isoamyle octanoate (8%), bicylogermacrene (7/9%) and α-pinene (5.5%). Insecticidal tests against *Callosobruchus maculatus* showed a strong insecticidal effect of the oils extracted from *O. basilicum*, *L. multiflora*, *C. schoenanthus* and *C. giganteus*.
Keywords: essential oils; chemical compositions; insecticidal effects.

Introduction

Synthetic insecticides are very often used against predators for crop protection in the field and post-harvest storage. These insecticides are expensive and therefore increasingly inaccessible to developing countries farmers who have low incomes. In addition, chemical insecticides are harmful to human health and the environment. Enhancement of the use of the essential oils of aromatic plant as natural insecticides in pests control could be an interesting alternative as full or partial substitutes to chemical insecticides. That is why their development is an interesting prospect. Chemical characterization of many species of aromatic plants and their effects insecticides / repellents has been reported in the literature (Gakuru and Foua-Bi, 1995; Ketoh and al., 2004; Bonzi-Coulibaly and al, 1997; Kuiate and al., 2002; Menut and al., 1994; Fekam Boyom and al., 1996; Ekundayo and Oguntinein, 1986; Prakash and Rao, 1997; Adams, 1989; Lamaty and al., 1990; Ashok and al., 1993)

Materials and Methods

Experiment material

The plant materials for essential oils extraction were leaves of the following a romatic species: *Cymbopogon schoenanthus* (Linn.) Spreng, (Poaceae) *C. giganteus* Chiov., (Poaceae) *Lippia multiflora* Mold. (Verbenaceae), *Laggera aurita* (L.f.) Benth. Ex C.B. Clark, (Asteraceae), *L. oloptera* (DC) Adams, (Asteraceae), *Anona senegalensis* Pers (Annonaceae) and *Ocimum basilicum* L., (Lamiaceae). The pest used for testing was cowpea weevil (a cowpea predator) *Callosobruchus maculatus* (Bruchidaea).

Oils extraction
The leaves were air-dried for three days before oil isolation. Four samples of plants material (300g) were water distilled for three (3) hours using a Clevenger-type apparatus. The oils was separated from the water by decantation and dried over anhydrous sodium sulfate.
Analysis techniques
The GC analyses were carried out using a Varian 3800 auto sampler equipped with FID and fused capillary columns: SUPELCO WAX (30m x 0.25mm, 0.25mm, 0.25µm film thickness) and SPB-1 (30m x 0.25mm, 0.25µm film thickness) The oven temperature was programmed (40 - 240°C at 2°C/min) and held isothermal at 240°C for 40min. Injector temperature:230°C; detector temperature: 250°C; carrier gas: helium (20psi); split mode. Quantitative data were obtained from electronic integration of peak areas without the use of correction factors.

The GC/MC was done using Saturn II equipped with an ion trap detector, the electron impact ionization was 70eV and the temperature 220°C. The MS equipment was coupled with Varian 3400 with DB-WAX column (30m x 0.25mm, 0.25µm, film thickness). The operating conditions for oven and detector were as above. Injector temperature was programmed (40°C – 240°C at 180°C/min) and held isothermal at 240°C for 139min.

Bioassays
A sample of 10 weevils is used for each test. Each essential oil is used to coat various parts of the Petri dishes as follows: a) The central portion of the bottom of the box only; b) The rim; c) The central portion of the cover; d) A check Petri dish (without oil). The behaviors of weevil were observed, the survival time in the chamber was noted, and each time the mortality rate is calculated.

Results and Discussion

Extraction Yields
The oils were extracted with the following yields: *L. oloptera*[1]: 0.16%, *L. oloptera*[2]: 0.04 to 0.075%, *L. aurita*: 0.047 to 0.1%, *C. schoenanthus*: 1.28%, *C. giganteus:* from 0.22 to 0.5%, *O. basilicum*: 0.12 to 0.18%, *L. multiflora*: 1.5 to 3%.

Chemical composition of essential oils
Table 1: Chemical composition of essential oils of *Laggera oloptera*

Compounds	***L. oloptera*[1]**	***L. oloptera*[2]**
α-pinene	5,88	72,1
heptadienal	6,51	-
limonene	3,65	14,8
1,8-cineole	3,74	1,5
p-cymene	-	0,4
B-maaliene	-	0,7
iscomene	-	3,5
β-Iscomene	-	1,1
linalool	2,86	0,5
α-ionene	-	0,1
β-cariophyllene	5,57	1
4-terpineol	7,41	0,8
β-senilene	-	1,1
camphre	1,56	-
nerol	2,21	-
thymol	1,22	-
isobutyl propionate	4,67	-
dodecanal	2,14	-
globulol	6,98	-
dodecanol	3,67	-
γ -cadinol	2,78	-
γ-eudesmol	2,92	-
muscone	3,87	-
Total percent	**67,64**	**97,60**

Laggera[1] was harvested at Tenkodogo (southeast region of Burkina) and *Laggera*[2] at Ouagadougou (central region). For this *Laggera*[1], the analyze shows that essential oils of this sample contain several compounds whose proportion is between 5 and 7% (α-pinene, heptadienal, 4-terpineol, globulol, and β-caryophyllene). The second collection site is Ouagadougou. The oil composition of this sample is very different from the first; the oils are rich in α-pinene (72%) and limonene (15%).

Table 2: Chemical composit ion of essential oils of *Laggera aurita*

Compounds	%	Compounds	(%)
α-phellandrene	0,3	α-muurolene	1,7
α-terpinene	1,1	neryle acetate	3,8
β-phellandrene	0,5	γ-cadinene	1,6
γ-terpinene	1,8	δ-cadinene	12,3
p-cimene	0,5	α-cadinene	1,0
terpinolene	0,5	2,5-dimethyl-p-cimene	3,3
linalool	0,8	carophyllene Oxyde	0,5
β-caryophyllene	4,9	$C_{15}H_{22}$	0,7
thymol methyl ether	0,4	germacrene D-4 ol	3,1
terpene-4-ol	7,3	cubenol-1-epi	0,5
α- humulene	0,7	τ-cadinol	5,0
muurola-4(14) ,5-diene	0,4	τ-muurorol	8,2
β-cadinene	1,6	δ-cadinol	1,6
γ-muurolene	0,4	α-cadinol	30,1
$C_{15}H_{24}$	0,4	14-hydroxy-α-muurolene	0,5
germacrene D	0,3		
α-terpineol	0,6	**Total percent**	**97,40**

Laggera aurita was collected in Ouagadougou on several sites. The analysis showed a fairly similar composition of different samples/ Thirty compounds were identified/ The oils are rich in α-and τ-cadinol, muurolol and δ-cadinene. New secondary alcohol and other molecules of the species have been found by Zusthi and al. (1975); Zusthi and Bokodia (1976 a); Zusthi and Bokodia (1976b)

Table 3: Chemical composition of essential oils of *Cymbopogon*

Compounds	*C. schoenanthus*	*C. giganteus*
δ-2-carene	18,04	-
1, 5, 8-p-menthatriene	-	0,58
limonene	2,55	14,25
cis-sabinene	0,77	-
trans-β-sabinene	0,51	-
α-p-dimethylstyrene	-	0,41
trans-p-mentha-2,8-dien-1-ol		14,25
cis-p-mentha-2,8-dien-1-ol		9,76
trans-pinocarveol		0,99
α-terpineol	0,62	-
δ-terpineol		1,27
p-cymen-8-ol		1,17
trans-p-mentha-1(7) ,8-dien-2-ol		15,69
dihydrocarveol		3,90
cis-piperitol		0,79
piperitone	57,18	-
verbenone		2,70
unidentified		1,62
cis-carveol		5,03
cis-p-mentha-1(7) ,8-dien-2-ol		17,62
carvone		4,18
β-elemene	0,94	-
elemol	7,91	-
guaiol	1,39	-

Compounds	*C. schoenanthus*	*C. giganteus*
δ-cadinol	1,99	-
T-muurolol	1,55	-
Total percent	**93,45**	**94,21**

C. schoenanthus is an herbaceous plant very abundant on almost all the territory of Burkina Faso. Samples were collected from three sites Bony, Boromo (southwest) and Rapadama. The species has been studies by several authors (Keita, 1993; Popielas *et al*., 1991; Sidibe *et al*., 2001). The chemical compositions of the oils are almost identical; they are rich in piperitone (57%) and δ-2-carene (18%). These results are consistent with those given by Koumaglo *et al*. (1996) describing the chemical composition of essential oils of the same species at several sites in Togo. The chemical composition of essential oils of *C. giganteus,* naturalized in Burkina Faso, has been described by Samate *et al*. (1992) but on sites other than those where our collections were made. We found almost the same chemical compositions as those already described by the author. But for both species, the concentrations of chemical compounds in essential oils are different probably due to environmental factors.

Table 4: Chemical composition of essential oils of *Ocimum basilicum*

Compounds	Percentage (%)
myrcene	2,62
1, 8-cineole	1,61
limonene	0,68
γ-terpinene	4,78
(E)-β-ocimene	0,8
linalool	53,63
camphre	2,04
citronellal	-
octanoïque acid	0,59
Isopropyle benzoate	0,31
geraniol	0,49
bornyle acetate	1,04
eugenol	18,08
methyleugenol	0,75
β-caryophyllene	0,40
Trans-α-bergamotene	4,34
β-cedrene	0,32
trans-linalool oxyd	0,31
β-farmesene	0,64
citronellyle isobutyrate	0,49
germacrene D	-
α-farnesene	1,14
δ-cadinol	3,42
Total percent	**98,48**

The essential oil analysis of *Ocimum basilicum* showed that the oils are rich in linalool. These results are similar to those given by Moudachirou *et al*. (1999) and Pino *et al*. (1994).

Table 5: Chemical composition of essential oils of *Lippia multiflora*

Compounds	Pourcentage (%)
2-methyl-3-butan-2-ol	2,71
α-pinene	0,3
myrcene	2,21
α-phellandrene	3,01
α-terpinene	1,35
p-cymene	26,18
limonene	1,07
γ-terpinene	4,5
cis-isopulegone	0,82
thymol	29,87

hexenyle acetate	4,51
thymyle acetate	11,76
trans-8-mercapto-p-3-menthanone	0,52
(Z)-isoeugenol	4,02
β-santalene	0,91
Total percent (%)	**94,17**

Lippia multiflora is used in traditional medicine and as a beverage (tea). There are few stands around Ouagadougou. Essential oils were extracted from the flowering buds. Chemical analysis shows that the oil contains mainly thymol (30%), p-cymene (26%) and thymyle acetate (12%). It is similar to the type described by Silou and Ouamba (1992) in Congo, and Koumaglo *et al*. (1996).

Table 6: Chemical composition of essential oils of *Anona senegalensis*

Compounds	%	Compounds	%
α-pinene	5,55	2-dodecanone	1,35
β-pinene	1,31	α-dedrene	12,7
myrcene	1,46	isoamyle octanoate	8,06
α-terpinene	1,95	α-amorphene	16,79
limonene	3,21	4,11- selinadiene	1,96
2-mehylbutyrate de butyle	0,45	undecanoate d'ethyle	1,93
3-mehylbutyrate de butyle	0,71	germacrene D	1,12
Linalool	0,77	bicylogermacrene	7,92
α-cubebene	2,18	γ-bisabolene	1,73
α-bourbonene	0,42	germacrene B	2,44
α-capaene	2,04	γ-eudesmol	0,54
		Total percent (%)	**76,56**

Anona senegalensis (wild custard apple) is a shrub rather widespread throughout the southern part of Burkina. It is much used in traditional medicine. The essential oils of the species collected at three sites in Yalle (southern Burkina Faso) are rich in α- amorphene and α-cedrene.

Insecticidal/repellent properties of essential oils

Samples of essential oils were then used for biological tests on the cowpea weevil and that in order to highlight the insecticidal / insect repellent effects. The following observations were made for each case:

Laggera oloptera

With the oil of this species, we noted that in each case weevils cut across the oil-soaked areas of the Petri dish. After 3 hours, it was observed that 70% of weevils were still alive but most were dying, except in th e check box. The essential oil concentrated *Laggera oloptera* has low insect repellent and insecticide effects.

Laggera aurita

The oil extracted from this species gives similar results like the previous. However, the repellent effect is somewhat higher (50% of weevils fleeing from the Petri dish rubbed with oil) and the insecticidal effect very low (10% of weevils die after 3 hours).

Cymbopogon schoenanthus

The three types of tests have led to the following observations: Oil of the species is not as rep ulsive weevils either cross the regions of the Petri dish rubbed with this oil; After 40 minutes, all weevils are dying; 1 h 40 min, 80% died and 2 h later, mortality was 100%. The oil of this species is not repellent but it has a very effective insecticide property.

Cymbopogon giganteus

For this plant species, all weevils were fleeing oil soaked parts of the Petri dishes. After 20 minutes, 80% of insects were agonizing and 40 minutes later, the mortality of weevils is 100%. The oil of this plant has very interesting repellent and insecticidal virtues.

Ocimum basilicum
No weevil does approach the regions of the Petri dishes rubbed with oil. The oil is 100% repellent. 80% of weevils are dying after 20 minutes of contact with the oil and 30 minutes later, they all died. The oil of this plant has very high repellent and insecticidal properties.

Lippia multiflora
The tests results with the oils of this species show that after 5 minutes 70% of weevils were dying. At 20 minutes 100% are in agony. At 60 minutes, all weevils were dead. The essential oil of *Lippia multiflora* is insect repellent and insecticide on weevils at 100% after one hour.

Anona senegalensis
Most weevils (60%) are not repelled by the oil. Indeed, they pass through regions either Petri dishes rubbed with the oil. And 60 minutes after contact with the oil, 40% are still alive. Insecticide and repellent properties of the oil of the species are low.

Conclusion

The Laboratory investigations on aromatic plants of Burkina Faso showed that some species like *Ocimum basilicum*, have very interesting insecticidal / insects repellent properties, suitable to control the weevil and probably other pests of crops. Following this study, it will be possible to consider formulations of biopesticides based on essential oils for effective biological protection of crops.

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Phytochemistry and Utilization of *Vernonia glabra* (Steetz) Oliv. & Hiern. in Management of Food Spoilage and Food Poisoning Pathogens, in Kenya

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Abstract

Food spoilage is when there is a loss of quality or nutritional value, often linked to food deteriorating in taste, smell, flavour or appearance; while food poisoning is when food ingested has been contaminated with either infectious microbes or toxic substances in it, and causes diseases. Food spoilage pathogens lead to pre-harvest diseases of crops, post-harvest decay of crop produce, and loss of quality or nutritive value of food, leading to high costs of preventing these constraints. Food poisoning cause health issues in humans and animals due to ingested toxins produced in food and feed stuffs. Both food spoilage and poisoning pose a great threat to food security and safety worldwide.

Introduction

Food infection and intoxication are considered as the most common causes of food borne diseases worldwide (Lopez *et al*., 2003). Centres for Disease Control, (2004), estimates that each year 76 million people get sick, 325, 000 are hospitalized, and more than 5, 000 die as a result of foodborne illness. Primarily, the very young, the elderly, and the immune compromised are affected. According to FAO, (2005), nausea, diarrhoea and vomiting are the leading symptoms caused by ingestion of contaminated food with bacterial toxins, such as *Staphylococcus aureus* enterotoxin B and *Escherichia coli* shiga toxin, causing a high incidence of diarrheal diseases in children estimated as 3.3 to 4.1 episodes per child per year in Sub-Saharan African. *Aspergillus niger* is a filamentous fungus which is prevalent in warmer climates, both in field situations and stored foods. It causes pre-harvest losses of onion seedlings caused by a disease known as "black mould", due to its black spores which become systemic manifesting only when conditions are conducive. It is most common species responsible for post-harvest decay of fresh fruits including grapes. It is a common contaminant of cereals such as peanuts, which produce potent mycotoxins called Ochratoxin A, Fumonisin B_2, and aflatoxins which cause kidney necrosis (especially in pigs), kidney failure and death, liver cancer, immunosuppressant, and failure of nervous system (Gautam *et al*., 2010). The contamination with the mycotoxins leads to discolouration of cereals, quality deterioration (loss of nutritional value), and reduction in commercial values (Gautam, 2010).

Literature Summary

Prevention of spoilage and poisoning pathogens in foodstuff is usually achieved by use of chemical preservatives, and also chemical pesticides for management of disease causing pathogens on crops (Lopez *et al*., 2003). These chemical pesticides have negative impacts to humans, animals and the environment, including: health hazards associated with the application of chemicals, chemical residues in food and feed chains, environmental degradation, acquisition of disease and pests resistance to chemicals, emergence of secondary diseases and pests outbreaks and high cost to farmers (Peter, 2002; Yazdani *et al*., 2011). Because of such concerns, there is a great need in applying nonchemical methods such as pesticidal plant extracts with antimicrobial properties in management of food spoilage and poisoning pathogen, which are safe, available, affordable, and easily degradable or environmentally friendly (Adejumo and Kamper, 2012). Plant extracts have long been used to control insects. Dating back as 400 B.C., children were deloused using a powder obtained from the dried flowers of the pyrethrum plant (*Tanacetum cinerariifolium*) (Biopesticide Industry Alliance, n.d). The first botanical insecticide dates back to the 17^{th} Century, when it was shown that nicotine from tobacco leaves killed plum beetles (Biopesticide Industry Alliance, n.d). Biopesticides are generally less toxic to the user, and non-target of organisms, making them desirable and sustainable tools for crop disease management (Chunxue *et al*., 2010). The effects of plant extracts on microbial pathogens have been studied by a very large number of researchers in different parts of the world (Reddy *et al*., 2001; Ateb and ErdoUrul, 2003). Research on antibacterial and antifungal effects of spices and herbs used as seasoning agents in foods and beverages have been done extensively. Garlic onion, Cinnamon, and Clove are some of the subjects of these researches (Lopez *et al*., 2003). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Mohana and Raveesha, 2007). However, increased use of pesticidal plants has not been accompanied by scientific evidence in the efficacy and safety to support its effective claims (WHO, 2000-2005). Therefore, the aim of this project is to investigate the efficacy and phytochemical compounds present in different parts of *V. glabra*; an herb utilized by traditional practitioners in Kenya. A decoction of leaf plus root taken orally is claimed to treat gastrointestinal problems in Kenya (Johns *et al*., 1995). The leaf ash or crushed leaves rubbed into scarification around the snake bite is used as antidote, (Owour and Kisangau, 2006).

Description of Research

Vernonia glabra was selected based on ethnomedicinal information from literature and collected from Kathiiani village in Machakos County, Kenya in January 2010. The specimen was authenticated by a plant taxonomist in University of Nairobi and a voucher specimen (CK 2010/01) deposited at University of Nairobi Herbarium. The flowers, leaves, stem, and roots were air-dried under the shade at room temperature, ground into powder and extracted using Dichloromethane/Methanol in the ratio 1:1, and water, according to standard extraction methods (Harborne, 1998). 20g of powdered plant material was mixed thoroughly with appropriate amount of solvent, left to stand for 24 hours and decanted. The filtrates were combined and filtered using a Buchner funnel. Dry organic crude extracts were obtained after evaporating Dichloromethane and Methanol using a rota evaporator. The aqueous extracts filtrates were dried into powder using a freeze drier. Disc diffusion technique was used as the standard method for antimicrobial activity and minimum inhibitory concentrations (MICs) for active extracts against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger*. 2 ml stock solution at concentration 2000 mg/2 ml (1000 mg/ ml) was prepared for each plant part and entire plant used. Two fold serial dilutions were prepared from each stock solution. Readily manufactured sterile paper discs were used for each concentration prepared by pippeting 100µl onto individual paper discs (0.6cm) drop by drop using a micropipette. The potency for each paper disc per extract for the concentrations prepared was; 100mg/100µl to 0.1953125mg/100µl. Paper discs with crude extracts, were aseptically transferred onto plates inoculated with 1ml of standard inoculum for each test organism. The plates were labelled, sealed with parafilm, and incubated at 37^{o}C for *S. aureus*, *E. coli*, and 25^{o}C for *A. niger*. Streptomycin (for bacteria) and Nystatin (for fungus) were used as standards, while discs with extraction solvents only were used as controls. These were done in duplicates under sterile conditions. The results were recorded after 24, 48, 72 and 96 hours. The antimicrobial activity was determined by measuring clear inhibition zones diameters (including diameter of paper discs) formed using a transparent ruler (in cm). Minimum inhibitory concentrations (MICs) were determined by recording the lowest concentration of the active extracts that inhibited growth of the micro-organisms, Ochei and Kolhatkar, (2000). The organic extracts that were active at low concentrations ($\leq$25 mg/100 µl) were analysed for presence or absence of five classes of compounds namely; alkaloids, Sapogenins, terpenoids, quinones, and flavonoids using Thin Layer Chromatography (TLC) technique according to Harborne (1998). The presence of chemical compounds was displayed by simple scoring (Table 2).

Research Results and Applications

The antimicrobial activity (formation of inhibition zones) of *V. glabra* crude extracts depended on: (a) parts of plant used, (b) extraction solvent, and (c) test-organism factors.

(a) Parts of plant used: In this study, mean inhibition zones were used as the results for figures 1, 2 and 3 below. All the organic crude extracts of *V. glabra* parts used were active on at least one of the three test-organisms used (Fig. 1). It was observed that organic extracts of *V. glabra* leaf had the highest activity (inhibition zone of 1.85) against *S. aureus*, followed by organic extract of flower with inhibition zone of 1.78 against only *S. aureus*. The two extracts showed no significant difference in activity from each other, but were significantly different in activity from streptomycin (inhibition zone of 1.30). Also organic extract of leaf recorded significant activity (inhibition zone of 1.43) against *A. niger*, compared to nystatin (inhibition zone of 0.83), while the organic extract of whole plant (all parts mixed) showed significant activity (inhibition zone of 1.50) against *S. aureus* compared to streptomycin's (inhibition zone of 1/30) low activity and low activity against *A. niger* (Fig.1). Aqueous extract of the whole plant of *V. glabra* was the only active aqueous extract against *S. aureus* (Fig.2).

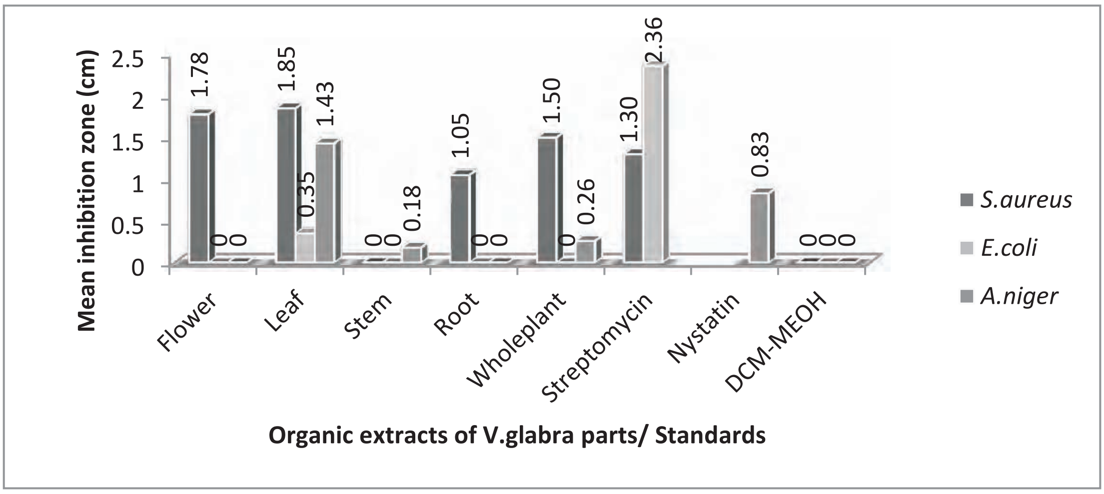

Figure 1: Antimicrobial activity of organic crude extracts of *V. glabra* parts compared to streptomycin and nystatin at 100 mg/100 µl

Note: CH_2Cl_2:CH_3OH- Dichloromethane and Methanol in the ratio 1:1(Control) was not active against any test organism.

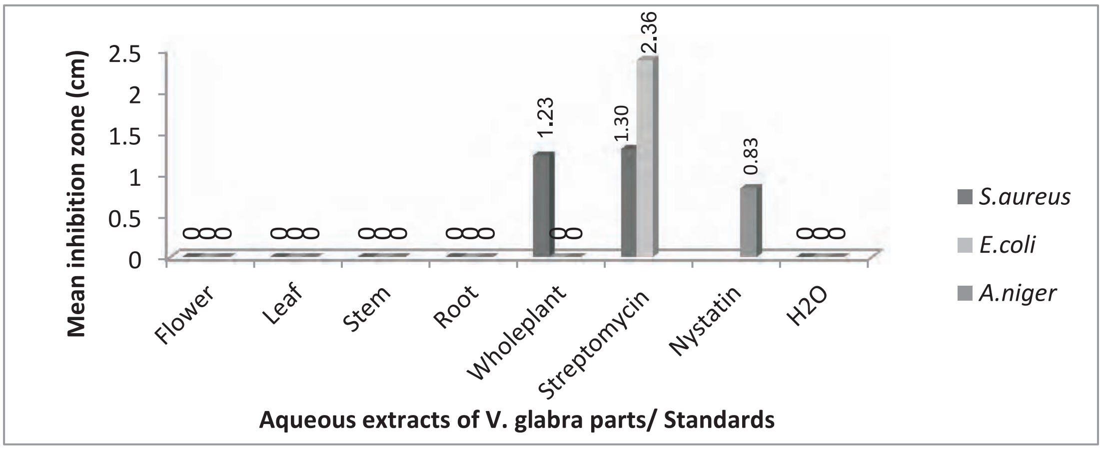

Figure 2: Antimicrobial activity of aqueous crude extracts of *V. glabra* parts compared to streptomycin and nystatin at 100 mg/100 µl

The control, sterile distilled water (H_2O) was not active against any test-organism.

(b) Extraction Solvent: The organic extracts of *V. glabra* were generally more active than the aqueous extracts most of which had lower activity or no activity against at least one of the three micro-organisms tested (Fig.1 & 2). **(c).Test-organism:** *S. aureus* was the most susceptible to organic and aqueous extracts compared to *A. niger, and E. coli* (Figures 1 & 2).

Minimum inhibitory concentration: Organic extract of flower showed highest activity recording the lowest MIC of 1.5625 mg/100 µl against *S. aureus*, lower than the standard (streptomycin) with MIC of 6.25 mg/100 µl. The organic extracts of root, whole plant against *S. aureus*, and leaf against *A. niger and S. aureus* had low activity. The organic extract of whole plant against *A. niger*, organic extract of stem against *A. niger*, and aqueous extract of whole plant against *S. aureus*, and organic extract of leaf against *E. coli* were less effective at higher concentrations (Table 1).

Table 1: MICs (mg/100µl) and Inverse of MICs (mg/100µl) of organic and aqueous extracts of *V. glabra*/Standards against *S. aureus*, *E.coli*, and *A. niger*

Extract/Antibiotic	Test-organism	MICs of Extracts/Antibiotics (mg/100µl)	Inverse of MICs (mg/100µl)	Remarks
Organic extracts				
Flower	*S. aureus*	1.5625	0.64	High activity
Streptomycin	*E. coli*	0.02+1	0.98	High activity
(Standard)	*S. aureus*	6.25	0.16	Moderate activity
Nystatin (Stanndard)	*A. niger*	3.125	0.32	Moderate activity
Leaf	*S. aureus*	12.5	0.08	Low active
	E. coli	100	0.01	Low active
	A. niger	25	0.04	Low active
Stem	*A. niger*	100	0.01	Low active
Root	*S. aureus*	25	0.04	Low active
Whole plant	*S. aureus*	25	0.04	Low active
Aqueous extract				
Whole plant	*S. aureus*	100	0.01	Low active

Note*: (0.02+1)- 1 was added to 0.02 in order to get a value that will minimize the big range among the inverse numbers, because values without whole numbers tend to have high inverse numbers. Extracts/ standards with high inverse of MICs indicate higher activity (MIC 0.64-0.98 mg/100 µl) at low concentrations; extracts/standards with inverse of MICs of 0.16-0.32 mg/100 µl indicate moderate activity, while the extracts with low inverse of MICs display low activity at higher concentrations (Table 1).

Table 2: Five classes of compounds screened present in *V. glabra* extracts (flower, leaf, and root)

	Five Classes of Compounds Screened Present				
Extracts	Alkaloids	Sapogenins	Terpenoids	Quinones	Flavonoids
Flower	+ + +	+	+ + +	+ +	+ + +
Leaf	_	+ + +	+ +	+ +	+ + +
Root	+ +	+ + +	+	+ + +	+ + +

Key: + + + = high or greatly present; + + = moderately or fairly present; + = less present (trace amounts); - = Not present.

Flavonoids were highly present in all the three extracts: flower, leaf, and root. Sapogenins, terpenoids, and quinones sufficiently present, while alkaloids were least present because they were found only in a few extracts; flower, and root extracts. Alkaloids were greatly present in flower extract, while the root extract had moderate number of alkaloids. The root and leaf extracts showed high presence of sapogenins, while flower had less sapogenins. Terpenoids were highly present in flower extract, moderately present in leaf extract and less present in root extract. Root extract showed high presence of quinones, while flower and leaf extracts showed moderate number of quinones.

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Insecticidal characteristics of *Maerua edulis* (DeWolf) against *Sitophilus zeamais* Motschulsky in stored maize grain

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Abstract

In Zimbabwe and Africa as a whole, search for cheaper and effective stored pest control methods has resulted in the testing of plants with perceived pesticidal properties. *Maerua edulis*, a locally available pesticidal plant prioritized as a grain protectant by smallholder farmers in Zimbabwe was evaluated singly for its repellency, toxicity, oviposition deterrence and fumigation potential, and its effects on progeny development against *S. zeamais.* The results show that *M. edulis* uses all modes of action synergistically to result in enhanced insecticidal activity to protect stored maize against insect pest damage. The results are discussed in the context of sustainable use by smallholder farmers.

Introduction

The maize weevil *Sitophilus zeamais* Motschulsky remains one of the most ubiquitous and damaging insect pests of stored maize in Sub Saharan Africa (SSA). The insect pest infests maize starting from the field, through to storage and transportation (FAO, 1999). In maize, this insect can cause weight losses as high as 19% (Giga *et al.,* 1991) and 100% damage in an 8-9 month storage season if left untreated (Mvumi *et al.,* 2002). In Zimbabwe and Africa as a whole, the control of stored-product pests at farm level has largely depended on the application of synthetic chemicals and these have come at a cost for the resource - poor farmer. Widespread and indiscriminate use of synthetic pesticides for storage pest control can cause serious problems, pest resistance build up, environmental and health concerns. There is need for widening pest management options for controlling insect pests of stored products. Pesticidal plants can be an effective alternative for resource - poor farmers. One of the most important factors that make pesticidal plants an attractive alternative for resource-poor farmers is their local availability, ease of use and minimal cost. One of the plants that have been reported as a grain protectant in some parts of Zimbabwe is *Maerua edulis* DeWolf.

Literature Summary

Smallholder farmers throughout SSA have serious problems in protecting their harvested crops from insect pest infestation during storage. Successful storage of grain commodities throughout a storage season has often been hampered by insect pests, the principal ones being *Sitophilus* spp, *Sitotroga cerealella*, *Tribolium* spp (Mvumi *et al.,* 2003)and of late *Prostephanus truncatus* (Nyagwaya *et al.,* 2010). Storage losses due to storage insects are a serious threat to food security and household incomes (Belmain and Stevenson, 2001). Farmers tend to over rely on synthetic pesticides for controlling storage insect pests. However, extensive and indiscriminate use of pesticides can result in build-up of insect resistance against a certain insecticide because of insect evolution (Nauen *et al*., 2001). There is then the need to develop safer and cheaper options that are benign in the environment and one such option is the use of pesticidal plants. There is a long tradition of subsistence farmers using whole plants or parts of plants to protect stored products from insect attack (Golob *et al*., 1999). Promising pesticidal plants for the control of *S. zeamais* have been reported like *Tephrosia vogelii* (Kamanula *et al.,* 2011), *Lippia javanica* (Chikukura *et al.,* 2011), *Securidaca longepedunculata* (Stevenson *et al.,* 2009) among other plants that are being used by farmers in Africa. Most of these plants have been investigated for their mode of action on the insects. Some plants have been found to be repellent or insecticidal to stored-product insects (Golob *et al*., 1999). Other plant powders and essential oils act as fumigants, as contact insecticides (Tripathi *et al*., 2001; Suthisut, 2011), as antifeedants, or as repellents (Parugrug and Roxas, 2008; Suthisut, 2011). The plant *Maerua edulis* (Gilg and Bened) De Wolf 'soswe', is a shrub growing up to three meters in height, and have flowers that are pale yellow with white tentacles and gives green to blue fruit when ripe, has been applied in a number of ways in grain as a pesticidal plant (Stathers *et al.,* 2002). There is scant scientific information on the bioactivity and effectiveness of *M. edulis* when applied against storage insect pests.

Description of Research

In our experiment, leaf powder from the plant *M. edulis* was evaluated for its repellency, toxicity, oviposition deterrence and fumigation potential against *S. zeamais* in stored maize grain. The leaf powder was evaluated at varying application rates and at different time points. Laboratory reared *S. zeamais* insects were used on clean, chemical free maize grain in 300ml glass jars and replicated 3 times in all the experiments. The following specific tests were carried out: (a) ***Toxicity assays*** - 50g of maize grain was thoroughly admixed with powdered leaves of *M. edulis* (dosage - 0, 0.5 1, 2, 4, 6, 8 and 10% *w/w*) in glass jars. A synthetic pesticide was applied at label rate (0.05% *w/w*) as the positive treatment. Twenty, 7-day old unsexed adult maize weevils were introduced in each jar which was covered with filter paper and a screw on top. Mortality assessments were done at seven day intervals from the start of the experiment for 21 days**.** Parameters measured included: total live and dead adult insects and progeny that emerged thereafter. **(b)** ***Repellency assays -*** *M. edulis* was evaluated for repellency, using the same application rates as used in the toxicity bioassays per 20 g of maize. Treated and untreated grains (20 g each) were placed randomly in eight 50ml -glass jars connected to the base of a closed circular glass arena. The numbers of insects found within each jar connected to the circular arena were counted after 1hr, 4 hr and 24 hr, respectively. Data were converted to percentage repulsion (PR) using the following formula: **PR (%) = (Nc – 50) x 2,** Where **Nc** is the percentage of weevils present in the untreated control. (c) ***Anti-oviposition/ growth inhibition test-*** The tests were carried out following the method by

Parugrug and Roxas (2008) with some adjustments. Same application rates as in the repellency assays were used. The total development period (TDP), and weight of adult insect data were collected to determine the growth inhibitory action of *M edulis*. (d) ***Fumigant toxicity assay -*** The tests were done following the method by Tripathi *et al.,* (2009) with adjustments. The plant powders were tested for fumigant toxicity in a space trial test. Plant powders at different concentrations (0, 2, 4, 6, 8, 10% *w/w*) were placed at the bottom of 0.5 l glass bottles. The negative control had no leaf powders whereas in the positive control a fumigant (Phosphine) was used at label rate. Three replicates were set up for each treatment. Mortality of adults was observed at 2 day intervals for 12 days.

Research Results and Application

Results from the toxicity assays show insect mortalities for plant treatments being ≥80% in the first 7 days with ASD having 100% mortality in the first week. Mortality reached a 100% in the second week of exposure for all plant treatments. There was no significant difference between ASD and all plant treatments by the second week (*P*<0.05). There was however significant difference between all plant based treatments and the untreated control (*P*?0/05)/ The untreated control had below ≤30% mortality by week 3/ Insect mortality increased largely with exposure period and also with concentration. There was significant difference (P<0.05) between the F1 generation that emerged from the plant leaf treated grain and the untreated control. An average of 2 insects emerged from the treated grain compared to more than 7 in the untreated control. There was however no F1 emergence from grain treated with ASD.

In the repellency tests, the leaf powders of *M. edulis* 8%*w/w* had the highest repellency of 62% after a 4 hr exposure period. *M. edulis* at concentrations from 4 to 10%*w/w* were classified as IV since they all had a repellence rating of between 60.1–80%. The untreated control attracted insects after a 4 and 24 hr exposure period. There were significant differences between the untreated control and all the plant treatments (*P*<0.05). There was no set trend in repellency in the first hour of exposure (Table 1). Repellency of the plant material was higher in the 4^{th} hr than after 24hrs. Repellency generally increased steadily with an increase in concentration except were *M. edulis* 6%*w/w* was outperformed by the 4%*w/w.* Generally there were no significant differences in repellency between the plant leaf treatments after 24hrs (*P*<0.05).

Table 1: Percentage repellency of *S. zeamais* in maize grain treated with varying concentrations of *M. edulis* (n=3)

Dose (% w/w)	[2]PR (± SEM)			[3]RC
	1hr	4hrs	24hrs	
0[1]	3.0 (±0.66)	0.3(±1.34)	0.9 (±1.36)	0
0.5	49.0(±13.80)	25.5(±3.31)	37.2 (±5.36)	ii
1	51.6(±7.58)	31.71(±2.10)	41.6 (±9.58)	iii
2	47.6(±23.63)	28.3(±3.61)	38.0(±9.69)	ii
4	56.8(±6.05)	56.8(±6.05)	56.8(±5.06)	iv
6	51.9(±9.80)	52.0(±2.54)	52.0(±6.12)	iv
8	51.0(±11.43)	61.8(±11.86)	56.4 (±9.59)	iv
10	50.3(±17.75)	50.3 (±3.47)	50.3(±1.77)	iv

[1]Untreated control, [2]Percent repellency after 24 hours, n=3; [3]Repellency class after 24hrs.

The plant material showed some anti-ovipositional action. The leaf powder was able to deter the insects from burrowing larval tunnels and laying their eggs. From all the treated grain, the average highest number of larval tunnels from all plant treated samples was ≤2/33/ There were no significant differences between the plant treatments (*P*<0.05) and ASD. From the larval tunnels, insects only emerged from the untreated control, 0.5 and 2% w/w treatments. The untreated control had the least number of days for insect TDP which was 22 ± 2 days. Average weight of the insects that emerged from the grain was between 25.8 and 26.9 mg. The insects that emerged from the untreated control had a slightly higher average weight when compared to those that emerged from the treated grain.

Fumigant toxicity assay results showed that *M. edulis* at all application rates having no noteworthy fumigant activity. Insect mortality started in week 8 where the highest was 15%, effected by the highest concentration from the plant leaf powder (Fig 1). There was significant differences between the chemical fumigant and the plant leaf powders (*P*?0/05)/ The chemical fumigant had ≥95% mortality in the first 2 days of exposure and by day 4 had a 100% kill. There was significant difference between all the plant leaf powder and the untreated control. Mortality followed a somewhat dose dependency trend though there was no difference in fumigant potential of the plant leaf powders at concentrations of 4, 8 and 10%.

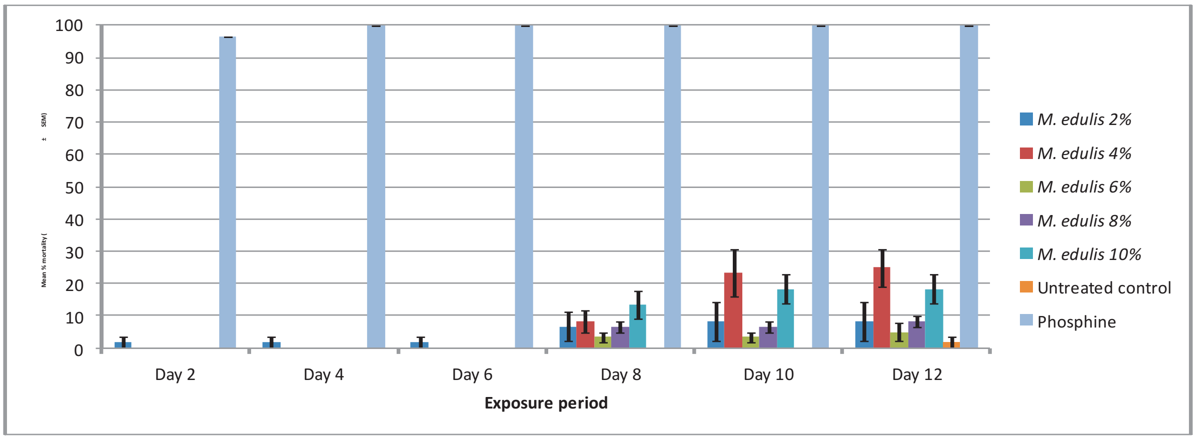

Figure 1: Activity of *M. edulis* against *S. zeamais* in fumigant assays (n=3)

From the assays it shows that the plant *M. edulis* uses all modes of action synergistically to result in enhanced insecticidal activity to protect stored maize against insect pest damage. However further experiments need to focus on determining toxicity of the essential oils of the plant as they may be more active than the leaf powders. Use of such a plant as an alternative to synthetic pesticides is a welcome development for resource poor farmers as the plant effectively controls *S. zeamais* and already occurs naturally in their localities. Farmers can use the plant as an affordable treatment option against storage insect pests.

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Evaluation of Ethanolic plant extracts for the control of *Balanogastris kolae* and *Sophrorhinus* spp (Coleoptera: Curculionidae) infesting stored kolanut

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Abstract

The kola weevils *Balanogastris kolae* and *Sophrorhinus* spp are the most destructive of all kola pests. This study was designed to evaluate the protective properties of ethanolic extracts of 5 plant materials each at 1000ppm, 2500ppm, 5000ppm and 10000ppm, against the weevils on stored kolanuts. The plant materials are *Azadirachata indica, Chrysophyllum albidum, Khaya sp, Cedrela odorata* and *Chromolaena odorata.* Parameters assessed were adult emergence, number of weevil exit holes and the number of kolanuts w ith colour change. The mean number of adult *B. kolae* emergence from the various treatments did not differ significantly from one another. However, some of the treatments compared well with the standard treatment (1.38 ± 0.25) in terms of emergence. A similar trend was observed for *Sophorhinus spp*, but emergence of adult weevils was extremely low. All the ethanolic extracts differed significantly from their control treatment (101.16 ± 11.26) in terms of number of weevil exit holes. Some compared effectively with the standard treatment (8.16 ± 0.75). The mean number of kolanuts with colour changes did not follow a particular trend.
Key words: Kolanuts, weevils, plant extracts, treatments, emergence, exit holes, colour change.

Introduction

Cola acuminata and *Cola nitida* (Schott and Endl) are the only edible species of kolanuts grown in commercial scale in Nigeria (Jacob, 1973). The kola weevils *Balanogastris kolae* and *Sophrorhinus* spp are the most destructive of all kola pests (Daramola, 1983). According to Daramola and Taylor (1975), the havoc caused by these insect pests approximately claims 60% of the total kolanut production in Nigeria. The weevils feed, oviposits and completes their life cycles entirely within the kolanuts thereby exposing the kolanuts to secondary invasion by micro-organisms, especially fungi (Odebode, 1990). The use of physical and synthetic chemical means was suggested in the past however, the deleterious impacts of chemicals have necessitated the search for alternative control measures against the kola weevils (Anikwe and Ojelade, 2005). Moreover, kolanuts do not require further processing after skinning and curing before consumption, so the use of synthetic insecticides no matter how minimal should be discouraged. These alternative bio-pesticides have been adjudged safe, biodegradable and environmental/user friendly (Ogunwolu and Idowu, 1994; Asogwa and

Osisanya, 2003). The present study was designed to evaluate the protective properties of ethanolic extracts of five plant materials against the kola weevils *B. kolae* and *Sophrorhinus* spp on stored kolanuts.

Materials and Methods

The following plant materials: *Azadirachta indica (stem bark)*; *Cedrela odorata (stem bark); Chrysophyllum albidum (stem bark), Khaya spp (stem bark)* and *Chromolaena odorata (leaf)* were chopped into bits and air-dried for one week and oven-dried at 60^{o}C for 6 h. Then the samples were pulverized with a high-speed mill and extraction was carried out using the the Soxhlet apparatus according to the method of Ofuy a *et al*., (1992). 25 cured kolanuts were randomly sorted into transparent plastic 1 litre bowls each containing four concentrations (1000, 2500, 5000 and 10000 ppm) of each plant extract and left to soak for 12 hours. The control kolanuts were soaked in di stilled water (0 ppm) for the same period of time. The standard treatment kolanuts for comparism were soaked in Cypermethrin 10 EC for 1 hour. Each of the treatments was replicated 4 times in a completely randomized design. The nuts were thereafter cured f or 72 hours in order to reduce the moisture content to a minimal level. The 25 cured kolanuts were each stored in black light gauge polythene bag of dimension 42.5 x 21.0cm for 112 days. Parameter measured forthnightly were direct counting of newly emerged adult weevils, number of weevil exit holes and the number of kolanuts with colour change. Data obtained were subjected to the analysis of variance (ANOVA) at P <0.05 and differences between means were tested with Turkey's HSD using PC -SAS.

Results and Discussion

The rate of development and emergence of the adult weevils (*B. kolae* and *Sophrorhinus* spp) generally decreased with increased concentrations of the various extracts (Tables 1 and 2). The development and emergence of *B. kolae* from the stored kolanuts treated with the various extracts were significantly lower compared to the control treatment with a mean adult emergence of 71.28 ± 9.29. The mean number of adult *B. kolae* emergence from the various extract treatments did not diffe r significantly from one another (Table 1). However, some of the treatments compared effectively with the standard treatment with a mean record of 1.38 ± 0.25. A similar trend was observed for *Sophrorhinus* spp but emergence of adult weevils was extremely low (ranging from 0.03± 0.13 to 0.34± 0.10. The plant extracts effectively suppressed the development and emergence of *B. kolae* within the first 42 days, which coincided with the second generation emergence of the weevils. This was followed by an unprecedented high population and increased number of exit holes created by the emergence of the subsequent generation of weevils from the 56th day (Tables 1 and 3), due to the breakdown of the protective properties of the extracts. There was no significant differen ce in the mean number of weevil exit holes recorded for the extracts at 2500 ppm, 5000 ppm, 10000 ppm treatment levels. However, they all differed significantly from their control with a mean weevil exit holes of 101.16 ± 11.26. Among the various extracts, only *A. indica and Chromolaena odorata* both at 5000ppm and 10000ppm were not significantly different from the reference standard treatment (8.16 ± 0.75) (Table 3). The colour changes on the nuts did not increase progressively with the increased concentra tion of the extracts applied. The few colour changes observed on the kolanuts might have been as a result of physiological factors associated with storing fresh kolanuts with polythene materials and not from the plant extracts or standard insecticide used. The kola weevils are said to be "field to store pests" as their infestation is initiated in the field and persists in storage (Daramola and Ivbijaro, 1975). Plant extracts are slow acting and degrade easily in the environment. Earlier research findings therefore recommended their application at high rates and at an increased frequency to achieve effective pest control (Ofuya *et al*., 1992) There are vast types of secondary metabolites in higher plants which confer pesticidal activity on them (Dales, 1996). Although this aspect was not investigated in the present study, it is logical to suggest that the reduction in population of kola weevils with these plant extracts may have been impacted by their bioactive chemical components. And in addition, due to their significant effects over the no-treatment controls, the potentials of these plant materials as suitable alternatives should be further explored.

Table 1: Effect of various ethanol plant extract treatments on the progress of *Balanogastris kolae* development and emergence from stored kola nuts

Conc. of plant extracts (ppm)	Exposure periods (Days Post Experimental Period (DPEP)								
	14	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**Mean ± SE**
				Total number of adult *B. kolae* emergence					
Cederela odorata									
$1x10^3$	8	104	117	232	269	143	9	7	27.78 ^{b} ± 4.31
$2.5x10^3$	7	89	106	198	218	134	11	2	23.91 ^{bc} ± 3.60
$5x10^3$	0	58	101	164	189	120	7	0	19.94 ^{bc} ± 3.19
$1x10^4$	0	46	67	157	176	112	5	0	17.5 ^{bc}± 3.01
***Khaya* spp.**									
$1x10^3$	0	118	125	231	217	130	13	7	26.28 ^{b}± 3.88
$2.5x10^3$	0	97	98	224	162	119	11	5	22.38 ^{bc} ±3.47
$5x10^3$	0	71	89	215	143	109	3	1	19.72 ^{bc}±3.29
$1x10^4$	0	48	64	139	94	85	2	0	13.5 ^{bc}± 2.19
Azadirachta indica									
$1x10^3$	5	69	117	266	134	79	13	4	21.47 ^{bc}± 3.73
$2.5x10^3$	2	56	94	167	119	70	1	3	16.0 ^{bc}± 2.61
$5x10^3$	0	46	81	147	109	53	1	2	13.72 ^{bc}± 2.34
$1x10^4$	0	29	74	109	102	47	1	1	11.34 ^{bc}± 1.95
Chromolena odorata									
$1x10^3$	3	93	105	240	250	58	11	4	23.88 ^{bc}± 4.23
$2.5x10^3$	1	64	88	229	185	53	7	3	19.69 ^{bc}± 3.64
$5x10^3$	0	51	77	214	151	47	2	1	16.97 ^{bc}± 3.32
$1x10^4$	0	37	58	201	130	40	2	0	14.63 ^{bc}± 3.08
Chrysophyllum albidum									
$1x10^3$	4	87	113	286	229	86	4	9	25.56 ^{b}± 4.48
$2.5x10^3$	1	66	103	279	166	65	3	9	21.63 ^{bc}± 4.11
$5x10^3$	0	53	85	249	150	54	1	3	18.59 ^{bc}± 3.70
$1x10^4$	0	27	74	213	105	41	1	1	14.44 ^{bc}± 3.11
Control (0)	9	140	295	545	628	385	185	94	71.28 ^{a}± 9.29
Standard ($1x10^2$)	0	4	8	10	14	8	0	0	1.38^c± 0.25

*Means with the same superscript are not significantly different (P ? 0/05) by Tukey's test

**Each value represents a total of four replicates

Table 2: Effect of various ethanol plant extract treatments on the progress of *Sophorhinus* spp. development and emergence from stored kolanuts

Conc. of plant extract (ppm)	Exposure periods (Days Post Experimental Period (DPEP)								
	14	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**Mean± SE**
				Total number of adult *Sophorhinus* spp. emergence**					
Cederela odorata									
$1x10^3$	2	3	2	4	0	0	0	0	0.34^b ± 0.10
$2.5x10^3$	2	2	2	3	0	0	0	0	0.28^b ± 0.19
$5x10^3$	0	2	2	3	0	0	0	0	0.22 ^{b}± 0.19
$1x10^4$	0	0	0	1	0	0	0	0	0.03 ^{b}± 0.13
***Khaya* spp.**									
$1x10^3$	0	0	3	2	0	0	0	0	0.16 ^{b}± 0.07
$2.5x10^3$	0	1	2	1	0	0	0	0	0.13 ^{b}± 0.06
$5x10^3$	0	1	1	2	0	0	0	0	0.13 ^{b}± 0.06
$1x10^4$	0	0	1	3	0	0	0	0	0.13 ^{b}± 0.06
Azadirachta indica									
$1x10^3$	0	2	2	3	0	0	0	0	0.22 ^{b}± 0.11
$2.5x10^3$	0	3	2	1	0	0	0	0	0.19 ^{b}± 0.08
$5x10^3$	2	0	1	1	0	0	0	0	0.13 ^{b}± 0.06
$1x10^4$	0	0	1	1	0	0	0	0	0.06 ^{b}± 0.04
Chromolena odorata									
$1x10^3$	2	3	2	1	0	0	0	0	0.25 ^{b}± 0.10
$2.5x10^3$	1	1	2	3	0	0	0	0	0.22 ^{b}± 0.87
$5x10^3$	0	3	1	1	0	0	0	0	0.16 ^{b}± 0.07
$1x10^4$	0	0	2	1	0	0	0	0	0.09 ^{b}± 0.05
Chrysophyllum albidum									
$1x10^3$	2	1	2	2	0	0	0	0	0.22 ^{b}± 0.09
$2.5x10^3$	1	1	2	1	0	0	0	0	0.16 ^{b}± 0.07

$5x10^3$	0	0	1	1	0	0	0	0	$0.06\ ^{b}\pm 0.04$
$1x10^4$	0	0	1	1	0	0	0	0	$0.06\ ^{b}\pm 0.04$
Control (0)	2	3	7	6	4	3	0	0	$0.78\ ^{a}\pm 0.14$
Standard ($1x10^2$) 0	0	0	0	0	0	0	0	$0.00^{b}\pm 0$	

*Means with the same superscript are not significantly different (P ? 0/05) by Tukey's test
**Each value represents a total of four replicates

Table 3: Effect of various ethanol plant extracts on the number of weevil exit holes on stored kolanuts

Conc. of plant extracts (ppm)			Exposure periods (days post experimental period (DPEP)						
	14	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**Mean ± SE***
			Total number of weevil exit holes on the kolanu t**						
Cederela odorata									
$1x10^3$	8	77	108	230	512	512	512	512	$77.22^{ab}\pm 9.75$
$2.5x10^3$	7	65	82	190	387	387	387	387	$59.13^{bc}\pm 7.29$
$5x10^3$	0	49	78	183	364	364	364	364	$55.19^{bc}\pm 6.89$
$1x10^4$	0	39	73	173	283	283	283	283	$44.28^{bcd}\pm 5.33$
***Khaya* spp**									
$1x10^3$	0	101	121	242	469	469	469	469	$73.13^{abc}\pm 8.51$
$2.5x10^3$	0	80	113	220	349	349	349	349	$56.53^{bc}\pm 6.13$
$5x10^3$	0	57	82	208	316	316	316	316	$50.34^{bc}\pm 5.74$
$1x10^4$	0	40	63	158	265	265	265	265	$41.28^{bc}\pm 4.90$
Azadirachta indica									
$1x10^3$	4	72	97	285	460	460	460	460	$71.81^{abc}\pm 8.54$
$2.5x10^3$	2	58	86	173	320	320	320	320	$49.34^{bc}\pm 5.84$
$5x10^3$	0	30	46	165	280	280	280	280	$42.53^{bcd}\pm 5.43$
$1x10^4$	0	20	39	130	239	239	239	239	$35.78^{cd}\pm 4.69$
Chromolena odorata									
$1x10^3$	2	35	70	226	389	389	389	389	$59.03^{bc}\pm 7.60$
$2.5x10^3$	1	29	69	201	294	294	294	294	$46.13^{bc}\pm 5.55$
$5x10^3$	0	21	65	180	270	270	270	270	$42.06^{bcd}\pm 5.16$
$1x10^4$	0	18	61	175	260	260	260	260	$40.44^{bcd}\pm 5.04$
Chrysophyllum albidum									
$1x10^3$	5	29	86	304	428	428	428	428	$66.75^{abc}\pm 8.23$
$2.5x10^3$	2	28	54	294	425	425	425	425	$64.94^{abc}\pm 8.42$
$5x10^3$	0	18	33	267	375	375	375	375	$56.81^{bc}\pm 7.56$
$1x10^4$	0	21	31	226	309	309	309	309	$47.31^{bc}\pm 6.20$
Control (0)	10	72	254	385	629	629	629	629	$101.16^{a}\pm 11.26$
Standard ($1x10^2$) 2	23	23	38	45	45	45	45	$8.16^{d}\pm 0.75$	

*Means with the same superscript are not significantly different (P ? 0/05) by Turkey's test
**Each value represents a total of four replicates

Table 4: Effect of various ethanol plant extracts on the number of stored kolanuts with colour change

Conc. of plant extracts (ppm)			**Exposure periods (Days Post Experimental Period (DPEP)**						
	14	**28**	**42**	**56**	**70**	**84**	**98**	112	Mean± SE*
			Total number of kolanut with colour change*						
Cederela odorata									
$1x10^3$	10	25	27	27	27	27	27	27	$6.16^{a}\pm 0.39$
$2.5x10^3$	10	20	26	26	26	26	26	26	$5.81^{ab}\pm 0.38$
$5x10^3$	11	21	22	23	23	23	23	23	$5.28^{abcd}\pm 0.30$
$1x10^4$	12	21	21	21	21	21	21	21	$4.97^{abcde}\pm 0.16$
***Khaya* spp**									
$1x10^3$	2	8	18	18	18	18	18	18	$3.69^{def}\pm 0.42$
$2.5x10^3$	0	17	17	17	17	17	17	17	$3.72^{def}\pm 0.37$
$5x10^3$	0	17	17	17	17	17	17	17	$3.72^{def}\pm 0.29$
$1x10^4$	0	13	15	15	15	15	15	15	$3.22^{ef}\pm 0.29$
Azadirachta indica									
$1x10^3$	2	15	23	23	23	23	23	23	$4.84^{abcde}\pm 0.48$
$2.5x10^3$	0	17	17	17	17	17	17	17	$3.72^{def}\pm 0.26$
$5x10^3$	2	8	17	17	17	17	17	17	$3.50^{def}\pm 0.37$
$1x10^4$	3	14	14	14	14	14	14	14	$3.16^{ef}\pm 0.22$
Chromolena odorata									
$1x10^3$	1	22	26	26	26	26	26	26	$5.59\ ^{abc}\pm 0.44$
$2.5x10^3$	1	21	23	23	23	23	23	23	$5.0^{abcde}\pm 0.41$

$5x10^3$	1	18	18	18	18	18	18	18	3.97^{bcdef}± 0.27
$1x10^4$	0	11	20	20	20	20	20	20	4.09^{bcdef}± 0.49
Chrysophyllum albidum									
$1x10^3$	7	11	25	25	25	25	25	25	5.25^{abcd}± 0.44
$2.5x10^3$	4	11	16	22	22	22	22	22	4.38^{abcde}± 0.46
$5x10^3$	2	17	18	19	19	19	19	19	4.13^{bcdef}± 0.28
$1x10^4$	2	17	17	17	17	17	17	17	3.78^{cdef}± 0.36
Control (0)	5	11	23	24	24	24	24	24	4.97^{abcde}± 0.41
Standard ($1x10^2$)	2	7	9	11	11	11	11	11	2.28^{f}± 0.18

*Means with the same superscript are not significantly different (P ? 0/05) by Tukey's test/
**Each value represents a total of four replicates.

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Calpurnia aurea essential oil and its components as repellents against maize weevil *Sitophilus zeamais*

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Abstract

Plants volatiles have been shown to exhibit bioactivity; neat oil and the identified components present in the leaves of *C. aurea* have been tested against *S.zeamais*, a stored grain pest, with a view to develop new effective environmentally friendly pests repel. Essential oil was extracted from the fresh leaves of *C.aurea* through hydro-distillation. The individual components of the essential oils were identified through GC-MS and

GC-FID co injection with the authentic standards. The major components identified include: Alpha pinene 0.5%, Linalool 2.9%, Limonene 1.3%, Eucalyptol 1.4%, Terpinen-4-ol, 0.4%, Beta ionone 3.7%, p-Cymenol 0.7% and Phytol 4.6%. The repellent activity of the essential oil was also evaluated by a choice bioassay using a Y-shaped Olfactometer. The data obtained was analyzed using statistical analysis system (SAS). The repellent ability of the components of the essential oil from *C. aurea* was also determined. At $P < 0.005$ level of significance the neat oil showed 62% repellence, Eucalyptol showed 71% repellence while Linalool exhibited 65% repellence. The oil and its components may be used for a repellant formulation.

Introduction

S. zeamais causes heavy economic loss to farmers in the tropics. The pest destroys the grains by tunneling through the grains; this causes loss of quality and viability of the seeds (Hill and Waller, 1990). There has been a need to come up with novel solutions to take care of this problem since the synthetic chemical products used currently are expensive for the subsistence farmer and hence they are not widely used by resource poor farmers. There are also environmental and health concerns associated with the handling and used of synthetic chemicals. This has made it necessary for researchers to investigate plant materials that have been seen to exhibit insecticidal or repellent activity for new and safer ingredients to replace the products in the market (Peterson and Coats, 2001).

Literature Summary

Insect repellents are an alternative to the use of insecticides. Plant extract from *Tephrosia vogelii* has shown repellent activity (Koona *et al*., 2007). Neat oils obtained from plant by hydro distillation have also been used as repels (Ukeh *et al*., 2009; Kouninki *et al*., 2007). Specific components of essential oils like Menthone and Pulegone (Liu *et al*., 2011), Ascaridole (Chu *et al*., 2011), Cis-ocimene (Mossi *et al*., 2011), Phytol, n-octacosane, + Cedrol (Ndung'u *et al*., 1999) and Calamusenone (Huang *et al*., 2011) have been isolated and tested for their repellent activity. This shows that plant volatiles are a viable source of repellent that can be used by farmers. The reason for this study was to analyze the chemical composition of the oil present in the leaves of *C. aurea* and to identify individual chemical constituents of the essential oils as well as to evaluate the effect of the essential oil and its constituents on repellency of *S. zeamais.* The information obtained will be useful in development of an efficient and cost effective plant-derived repellent for *S. zeamais* control.

Description of Research

S. zeamais was obtained from the Kenya Agricultural Research Institute (KARI), Laboratories. The maize was placed in large plastic containers and moisturized. Maize seeds were obtained from small scale holdings in Nairobi and Subukia, in Kenya. *C.aurea* leaves were collected from Limuru and Subukia Forest Hill. The identity of the plant materials was confirmed at the East African Herbarium, Nairobi by Mr. S. Mathenge. The plant materials were packed in Khaki wood pulp based bags and taken to the laboratory for extraction within the shortest time possible to avoid loss of essential oils through evaporation. Any materials left were placed in a freezer to avoid rotting and attack by mould. 400grams of fresh materials were macerated and steam distilled using a Clevenger type apparatus for 3 hours. The steam distillate was collected every one hour, and extracted in 5mls of hexane. The oil-solvent extract was dried with anhydrous sodium sulphate. Hexane was then removed by distillation at 60°C using a micro distillation apparatus. When condensation stopped, the oil was collected and weighed in small amber colored vials and stored at 0°C (Ndung'u, 1993)/ The reagents and solvents used were analar grade purchased from Sigma Aldrich England and Ranchem Ltd. India.
Characterization, identification and determination of relative amounts of the components of the essential oil was done through Gas Chromatography (GC), Gas Chromatography – Mass Spectrometry (GC-MS), and GC Co injection of the essential oils with authentic standards.

GC Analysis of the essential oil was carried out on a Shimadzu GC-14B, fitted with a Flame ionization detector (FID) and with a carbowax capillary column (50m x 0.2mm x 0.33µm film thickness) was used for the separation of the essential oil components. Nitrogen was used as the carrier gas at a flow rate of 2ml/min. The injector and detector temperature was maintained at 250 °C and 270 °C respectively. The temperature program was starting at 70 °C, which was raised at a rate of 10 °C/min to 230 °C where it was maintained for 15 minutes.

GC - MS analysis were carried out on an Angilent technologies GC 7890A System coupled to a 5975C Inert XLEI/CI MSD with triple axis detector mass spectrometer. The spectrometer was operated in the EI mode at 70eV with temperature of the source held at 180 °C, multiplier voltage at 1350, scan cycle at 1.5 s, and scan range of m/z 38-650. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$. The pressure of the ion source and MS detector were held at 9.4×10^{-6} and 9.4×10^{-6} mbar respectively. Identities of the essential oils were confirmed by GC co-injections with authentic standards. Identification of other compounds that were not available was based on detailed comparison of their mass spectra with those in the libraries. The preliminary identification of the constituents was based on the computer matching of mass spectral data of the components against the standard Wiley, Chemecol and N IST library spectra constituted from spectra of pure substances and components of the known essential oils, and literature MS data.
The attractiveness or repellency of volatiles was assessed using a glass Y-tube Olfactometer. The set-up was in a dark room at 22°C. A tripod holds the Y- tube in an inclining position (angle 25^0 between Y tube and horizontal plane). The Y-tube was placed at the centre of a black box covered inside with black paper in order to avoid visual stimuli. A halogen lamp illuminates one arm of the Olfactometer. The remaining free ends of the Y-tube are connected to the sources of the volatile oil and the standard respectively. Twenty micro liters of the volatile component diluted in hexane at the control side was applied on the filter p aper pieces 30 minutes before the first maize weevil was released, in order to allow the odor to reach a constant release rate. The air flows over the oil loaded filter paper and the control filter paper that has hexane as the control. At the base of the Y tube the air is sucked off by means of a membrane pump, producing an air flow that ensures uniform distribution of the essential oil in the chambers. (Kogel *et al.,* 1999).

For each assay 30 randomly selected adult maize weevils of mixed sex and age were introduced into compartment A. The assay was run for 1 hour and then the number of weevils in the control arm (Nc) and in the treated arm (Nt) of the Olfactometer were counted. After each test the Olfactometer was thoroughly cleaned and dried at 100°C. The assay for each dose of the test material was replicated 5 times, and the same for the standard *N,N*-diethyl-m-toluamide (DEET). Percentage repellency (PR) values were computed using the formula:

$$PR = \left[\frac{Nc - Nt}{Nc + Nt}\right] \times 100$$

Percentage Repellency data were analysed using ANOVA. The GLM Procedure was applied Tukey's Studentized Range (HSD) Test for repellency was used to show interactions between the dose and repellence.

Research Results and Application

The analysis of the oil revealed complex mixture of constituents. A total of 8 c ompounds were identified in the essential oil of *C. aurea* by GC - MS and GC (Table.1). The constituents of the essential oil were confirmed by co injection with authentic samples of oils. The essential oils identified by co-injection were Alpha pinene 0.5%, Linalool 2.9%, Limonene 1.3%, Eucalyptol 1.4%. The others were Terpinen-4-ol 0.4%, p-cymene-ol 0.7%, Beta ionone 3.7% and Phytol with the highest quantity at 4.6%.

Table 1: Major identified constituents of *Calpurnia aurea* essential oil and their relative proportion in the oils

	Compound identified	RT (min)	*% comp	Method of Identification
1	Alpha–pinene	9.4250	0.508	RT,MS,CI
2	Limonene	11.396	1.298	RT,MS,CI
3	1,8 cineole	11.441	1.400	RT,MS,CI
4	Linalool	12.673	2.888	RT,MS,CI
5	Terpinen-4-ol	13.950	0.351	RT,MS
6	p-cymene-ol	17.914	0.729	RT,MS
7	Beta ionone	18.227	3.726	RT,MS
8	Phytol	24.857	4.557	RT,MS

* Percentage composition of the oils; RT = Retention Time, MS = Mass spectrum, CI = Co injection

The essential oil of C. aurea and the authentic samples that were available were used for repellence bioassay. Table 2 represents the mean repellency values of the essential oil extract and authentic samples at different dose levels against *S. zeamais*. The repellence of the *C.aurea* oil has shown a constant increase in repellence as the dose increases.

Table 2: Mean percentage repellencies (±s.e.) of some compounds identified from *C. aurea*, the oil of *C. aurea*, and DEET to *S. zeamais*

Compound	2µl	0.2µl	0.02µl	0.002µl
C. aurea	62.404±2.505 (a,b)	47.540±3.680(a,b)	45.124±2.166(a)	39.160±3.670(a)
Eucalyptol	70.874±2.637(a)	58.293±9.917(a)	29.000±4.00(a,b)	24.080±10.576(a,b)
Linalool	63.634±5.948(a,b)	26.082±8.786(a,b,c)	14.273±5.401(a,b)	6.602±4.443(a,b)
Alpha pinene	41.830±8.747(a,b,c,d,e)	22.446±5.103(a,b,c)	11.034±3.271(a,b)	17.540±7.039(a,b)
Limonene	18.756±7.198(d,e)	37.124±10.263(a,b,c)	21.918±8.756(a,b)	22.842±9.300(a,b)
DEET	25.000±0.00(e,d)	20.000±0.00(b,c)	6.700±0.00(b)	4.000±0.00(a,b)

*Means with the same letters are not significantly different at the 5% level

The repellence activity against the dosage is increasing in all the cases. The repellent action of the essential oil extract of *C. aurea* was dosage dependent. At the highest dose 2µl the repellence was 62% which is comparable to the activity of linalool 64% which is one of the components of the neat oil. Eucalyptol also produced the highest repellence, at the highest dose 2µl the repellence wa s 71%, which compares favorably with the commercial insect repellent DEET. Limonene showed the least repellent activity even though it activity was comparable to that of DEET. The other essential oils had repellent activities that were not significantly different at the different dosage levels. The repellence of the crude oil may be due to the presence of Eucalyptol and linalool which have shown high repellence at the highest dose level. These essential oils are key in the production of an effective repel . Essential oils have been reported to be highly lipophilic and hence they have a high ability to penetrate the cuticle of insects (Richards, 1978). This may explain the effectiveness of the essential oils as repellents. Medicinal plants have been tried ou t as repellents like *Allium sativum* and it displayed antifeedant and repellent activity (Arannilewa *et al.,* 2006). The plant extracts are therefore of tremendous importance in dealing with insect pest. It is therefore important to assess the viability of the essential oil obtained from *C. aurea* for its efficacy in the field trials; this should include mode of application and its production. The plant is also widely distributed in areas with adequate rainfall hence its availability to farmer is adequate to m eet their demands. The Four essential oil components that were studied could also be used to come up with a formulation that is based on the amounts of the components in the essential oil extract. The essential oils samples that gave the highest repellence may be used for the blend.

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Assessing Effectiveness of Botanical Extracts from Garlic and Neem on Controlling Potato Soft Rot Pathogens

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Abstract

Soft rot of potatoes [(*Solanum tuberosum* (L.)] caused by *Pectobacterium* and *Dickeya* subspecies leads to huge economic losses in agriculture worldwide including Zimbabwe. The aim of the present study was to evaluate efficacy of botanical plant extracts in controlling soft rot pathogens. A laboratory experiment was carried out using potato tuber maceration test to screen the effect of two botanicals extract: neem (*Azadirachta indica*) leaf and garlic (*Allium sativum*) cloves in controlling soft rot disease caused by *Pectobacterium carotovorum* subspecies *carotovorum* (*Pcc*)*, Pectobacterium atrosepticum* (*Pa*) and *Dickeya dadantii* (*Dd*)*.* Preparations of two concentrations [(10 and 25% (w/v)] of the aqueous extracts of garlic and neem plants were selected and used in dip and spray applications. Sterile distilled water was used as a negative control. Three tuber halves were used for each treatment/ Five filter paper discs (Whatman's No/ 1) pre-soaked in 1 x 10^6 cfu/ml bacterial cell suspensions of *Pcc, Pa* and *Dd*, respectively were placed on each tuber half and incubated for 48 h at 25 °C. After 48 h the filter paper discs were removed and the rotting zone diameter was measured. The experiment was a 3x2x2+1 factorial laid out in a Completely Randomised Design, each treatment replicated three times. Botanicals significantly reduced the maceration effect of the bacteria (P<0.01). There were some significant interactions for bacteria x botanical x concentration and bacteria x botanical x m ethod (P< 0.01). Method of application and concentration had no significant effect on the rotting zone diameters. The botanicals were effective in reducing the maceration effect of *Pa* and *Dd* but did not provide control against *Pcc*. Therefore the botanicals can be used for controlling soft rots caused by *Pa* and *Dd*.

Introduction

Bacterial soft rot of potato has become a very important disease world-wide due to the losses it causes during the various stages of crop development and in storage (Reeves *et al*., 1999; Bdiliya and Dahiru, 2006). *Pectobacterium* and *Dickeya* species are known to cause soft rot, blackleg and wilting diseases that cause economic losses (Baghaee-Ravari *et al*., 2011). Sub-species described under *Pectobacterium*: *atroseptica*, *carotovora*, *betavasculorum*, and *wasabie* (Duarte *et al*., 2004; Baghaee-Ravari *et al*., 2011) and sub-species under *Dickeya*: *dadantii*, *zea* and *dianthicola* (van der Merwe *et al*., 2010) are known to cause serious disease on potato. In Zimbabwe, losses due to *Dickeya dadantii* have been reported to be around 20-60% (Ngadze *et al*., 2010). Under bad handling conditions, losses can reach to about 100% (CAB International, 2000; Manzira, 2010). Chemical control is not effective in controlling soft rots which occur either in the field or in storage (Abo-Elyousr *et al*., 2010). However, there have been various reports on the use of plant extracts against some plant pathogens and botanical extracts are considered a better alternative to fungicides because they are safer on the environment (Bdliya and Dahiru, 2006; Bianchi *et al*., 1997). According to Locke (2006), plant extracts should be effective at very low rates, be easy to grow and its preparation should be simple, not time consuming or requiring excessive technical input. Two such plants are garlic (*Allium sativum*) and neem (*Azadirachta indica*) (Koul and Walia, 2009; Slusarenko *et al*., 2008; Bdliya and Dahiru, 2006).

Literature Summary

Botanical extracts contain various compounds with antimicrobial properties and can be effective in controlling pathogens. The antimicrobial substance, allicin, which is produced in garlic is active against a wide range of pathogens both *in vitro* and *in vivo* (Portz *et al*., 2008). Other constituents of garlic include alliin, saponins and flavonoids (Locke, 2006). Garlic has been shown to be effective against *Phytophthora infestans*-for the first time and *Pseudoperonospora cubbensis* on tomato and cucumber seedlings respectively. Mycelia growth of *Fusarium solani* and *Rhizoctonia solani* was also inhibited by garlic extracts (Bianchi *et al*., 1997). Flavonoids and saponins of red garlic exhibited anti-bacterial properties against *Baccillus subtilis* (Locke, 2006). Neem products have been used mainly in insect pest management because of their pesticidal and anti-feedant activities (Bdliya and Dahiru, 2006; Slusarenko *et al*., 2008). The most active substance in neem preparations is azadirachtin (Slusarenko *et al*., 2008) and this is active against a wide range of pests (Koul and Walia, 2009). Neem has also known to have fungicidal (Bankole, 1997; Govindachari *et al*., 1998) and bactericidal (Mahfuzul-Hoque *et al*., 2007; Slusarenko *et al*., 2008) properties.

It is important that more botanicals are explored and evaluated for their effectiveness against plant pathogens and the knowledge shared amongst researchers, people in the agro-chemicals industry and farmers. In this study, the efficacy of clove and leaf extracts of garlic and neem on controlling of potato soft rot was assessed on three soft rot causing bacteria, *Pcc*, *Pa* and *Dd*.

Description of Research

The aqueous plant extracts of neem leaves and garlic cloves were prepared separately, in two concentrations: 25% and 10% (w/v) by blending 1 kg of garlic cloves and neem leaves in four litres and ten litres of water respectively. The mixture suspension was filtered through a 1 ml sieve. The plant extracts were then stored in a refrigerator at 4°C for approximately one month/ The pure culture strains of *Pcc*, *Pa* and *Dd* were obtained from the Plant Pathology Laboratory, Crop Science Department, University of Zimbabwe. The isolates were re-initiated in nutrient broth (NB) and re-streaked on NA solid media for purity. The cultures were transferred into Luria Bertani (LB) liquid. Potato tubers were washed using tap water, surface sterilized in 10.0% sodium hypochlorite solution for 5 minutes and then rinsed in sterile water. Tubers were allowed to air dry and cut longitudinally into halves. Botanical treatments were applied on the cut surfaces using dip and spray treatment applications of neem and garlic extracts at two concentrations [25% (w/v) and 10% (w/v)]. Treatment applications were done approximately for 5 seconds for each tuber half. Tuber halves treated with the same volume of sterilized distilled water were used as a negative control/ Filter papers (Whatman's No/ 1) discs, 10 mm diameter, were soaked in the bacterial suspensions of *Pcc*, *Pa* ana *Dd* were adjusted to a concentration of 1×10^6 cfu/ml using a spectrophotometer ($OD_{600} = 0.1$). Soon after dipping or spraying application of botanical extracts, five inoculated filter paper discs were placed on each tuber half and then incubated at 25°C for 48 hours. Humid conditions were maintained. After 48 h, the filter papers discs were removed and the rotting zone diameter was measured in mm using a ruler. The experiment was laid out in a Completely Randomised Design with a 3x2x2x2+1 factorial treatment structure with a control. Each treatment was replicated three times. Observed data were managed using excel spreadsheets. Data were explored for normality and homogeneity using Anderson-Darling test and Bartlett's and Levene's tests at $P<0.05$, respectively. The plot of residuals was done using Minitab Release 12.22 (1998) to check assumptions for the analysis of variance (independence, normality, homogeneity). Data were analysed with Genstat Version 13 as a 3x2x2x2+1 factorial using the control as a dummy variable. When F test was significant ($P<0.05$), the means of different treatments were compared to the control using LSD at 5% level.

Research Results and Application

The analysis of variance showed that the following factors were significant: dummy (P=0.008), bacteria (P<0.001) and botanicals (P=0.006). In addition, the interactions bacteria x botanical x concentration and bacteria x botanical x methods were significant with respective P values of 0.003 and 0.008. The factors botanical concentration and application methods were not significant. The comparison of the different treatment means with the control is presented in Figures 1 and 2. The combination *Pcc*-Garlic was not different from the control. In other treatments different from the control, the combination Pcc -Neem exhibited higher rotting diameter than the control and the remaining combinations lower rotting diameter. The experiment results showed that the use of the botanicals led to a general reduction in the severity of soft rot on two bacterial species, *Pa* and *Dd*. The different sub-species of the soft rot bacteria had a marked effect on the severity of soft rot. Pectolytic bacteria cause rotting by producing enzymes such as pectinases, cellulases and proteases which cause tissue maceration (Pitman *et al*., 2008). Variation in pathogenicity of different sub-species of soft rot bacteria is related to the different amounts of pectic enzymes (Pérombelon (2002). Their pathogenicity is also temperature dependent (Pérombelon, 2002). The pectin enzyme, endopolygalacturonic transeliminase (PGTE) production by *Pa* is higher at lower temperatures (? 15°C)/ In contrast, production of the enzyme in *Pcc* is high at both high and low temperatures. In *Dd*, strains that would have lost ability to produce large quantities of PGTE during the course of the experiment lose virulence (Peltzer and Sivasithamparam, 1985). Since all the bacteria were exposed to the same temperature of 25°C, this might have favoured pectic enzyme production in *Pcc* better than in *Pa* and *Dd*. Resultantly, *Pcc* gave the highest tissue maceration followed by *Pa* and *Dd*. Similar work that has been done on botanicals has shown that plant extracts from garlic and neem have anti-microbial properties against a wide range of plant pathogens (Alkhail, 2005).

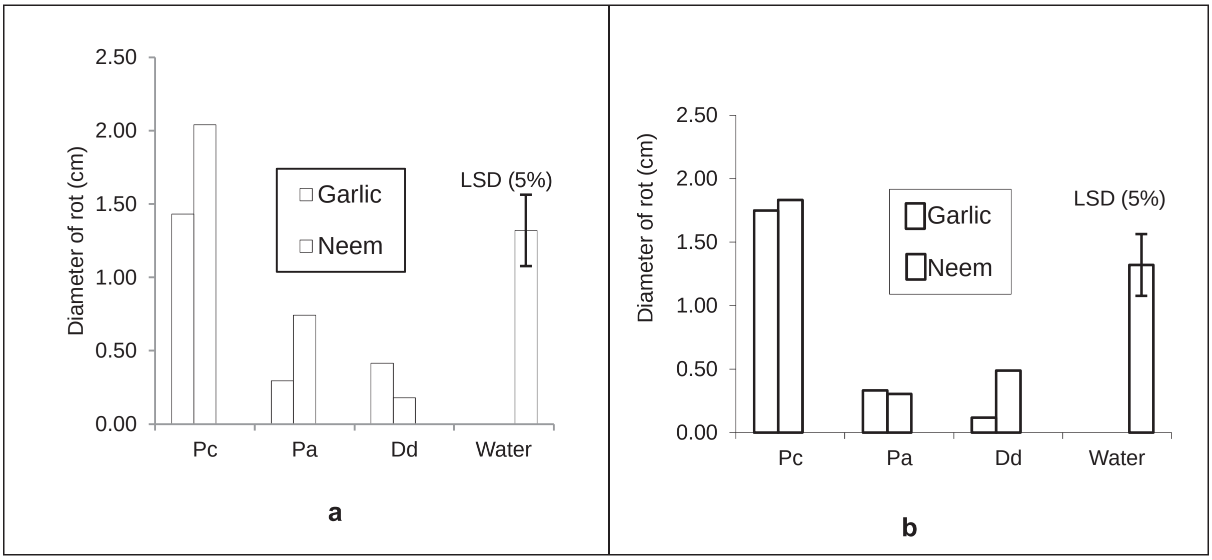

Figure 1: Efficacy of garlic (clove) and neem (leaf) extracts at 25% (a) and 10% (b) concentration in (dip/spray) treatment application against *Pcc*, *Pa* and *Dd* pathogens. Sterilized distilled water used as a control treatment

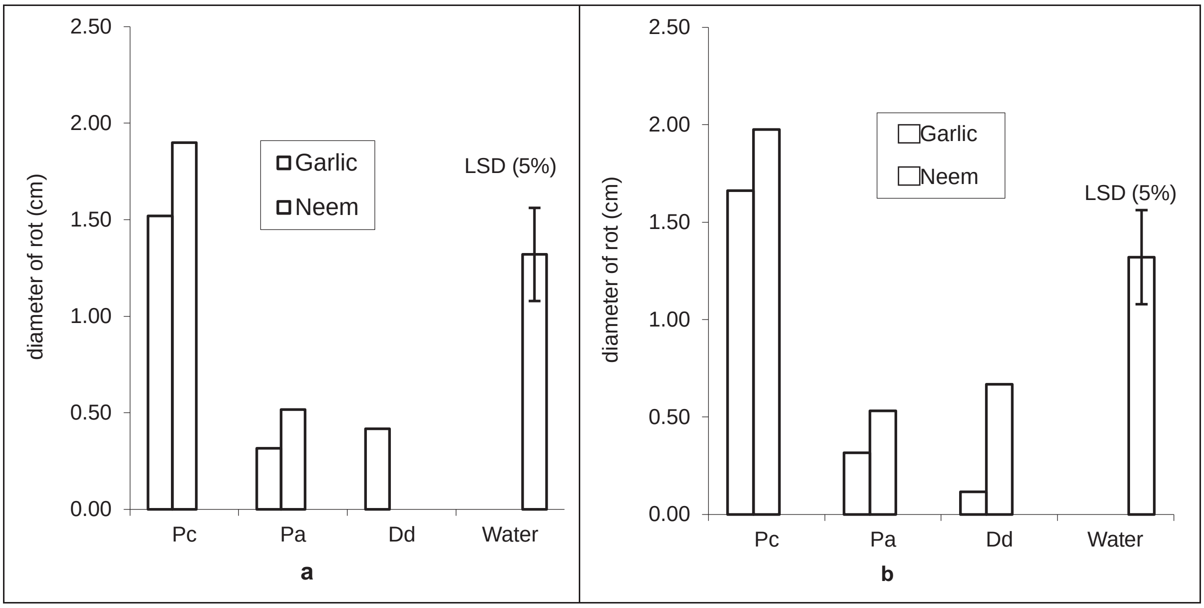

Figure 2: Efficacy of garlic (clove) and neem (leaf) extracts at 25% (a) and 10% (b) concentration in (dip/spray) treatment application against Pcc, Pa and Dd pathogens. Sterilized dis tilled water used as a control treatment

Acknowledgements

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Bio-insecticide formulations with organic flours as carriers in management of cowpea brucids *Callosobruchus maculatus* Fabricius (Coleoptera:Bruchidae) in stored cowpea grains

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Introduction

Stored cowpea *Vigna unguiculata* (L.) Walpers, seeds in Nigeria is infested and damage by bruchids, especially *Callosobruchus maculatus* Fabricius (Jackai and Daoust, 1986). Cowpea seeds are very important in human nutrition in tropical Africa, being the major affordable source of protein in the diet of the people. However, damaged seeds have reduced weight, poor nutritional and market values; and low viability (Caswell and Akibu, 1980). Stored product insect pest control is based mainly on the use of highly effective fumigants and protectants. However, increased health and environmental hazards, social concerns and frequent deaths associated with the use of synthetic pesticides in stored cowpeas (Egwuatu, 1987) culminated in the ban in Nigeria by the National Food Drug Agency and Control (NAFDAC) in 2006. The demand for pesticide-free food necessitates the need to develop integrated strategies for monitoring pesticide abuse in grain storage and stored pest management.

Materials and Methods

The insecticidal powders from dried *D. tripetala* seeds and *P. guineense* fruits were processed according to the method described by Okonkwo and Okoye (1996), while the insects *C. maculatus* used for the experiment were cultured according to the method described by Jackai and Daoust (1986). The tubers (yam *Dioscorea rotudata* L, cassava *Manihot esculenta* L., sweet potato *Ipomea batatus* L. and cocoyam *Colocasia esculenta* L.) for organic flours were processed according to the method of Okonkwo *et al*. (2009). Each formulated dust was tested at effective rate of 2%, i.e. 0.4g/20g grain or 20g/kg of grain, for plant powders (Lale, 1995). 20g of cowpea was mixed with 0.2, 0.4, 0.6, 0.8, 1.0 and 2 % of each active material (Ofuya *et al*., 2010). Ten unsexed 36h old adult *C. maculatus* were introduced into each vial and allowed to lay eggs. Adult mortality of test insect was determined for 72hr; F_1 progeny, seed damage and germination were assessed.

Results and Discussion

D. tripeala and *P. guineense* formulated with each carrier at 0.8% caused 100% mortality of adults of *C. maculatus* in 72hr (Figs 1 & 2), suppressed oviposition and F_1 progeny 40 days post-treatment compared to 0% mortality and 100% seed damage recorded in the untreated control. Mean percentage of *C. maculatus* F_1 adult emergence was significantly lower in 0.2 and 0.4% active material than the untreated control.

The formulated *D. tripeala* and *P. guineense* dusts effective against *C. maculatus* are safe for human and animal consumption while the flours are sources of carbohydrate in diets of Nigerians compared to synthetic insecticides. The formulation should be produced commercially by private enterprise and packaged into ready-to-use form on-shelf by farmers and traders.

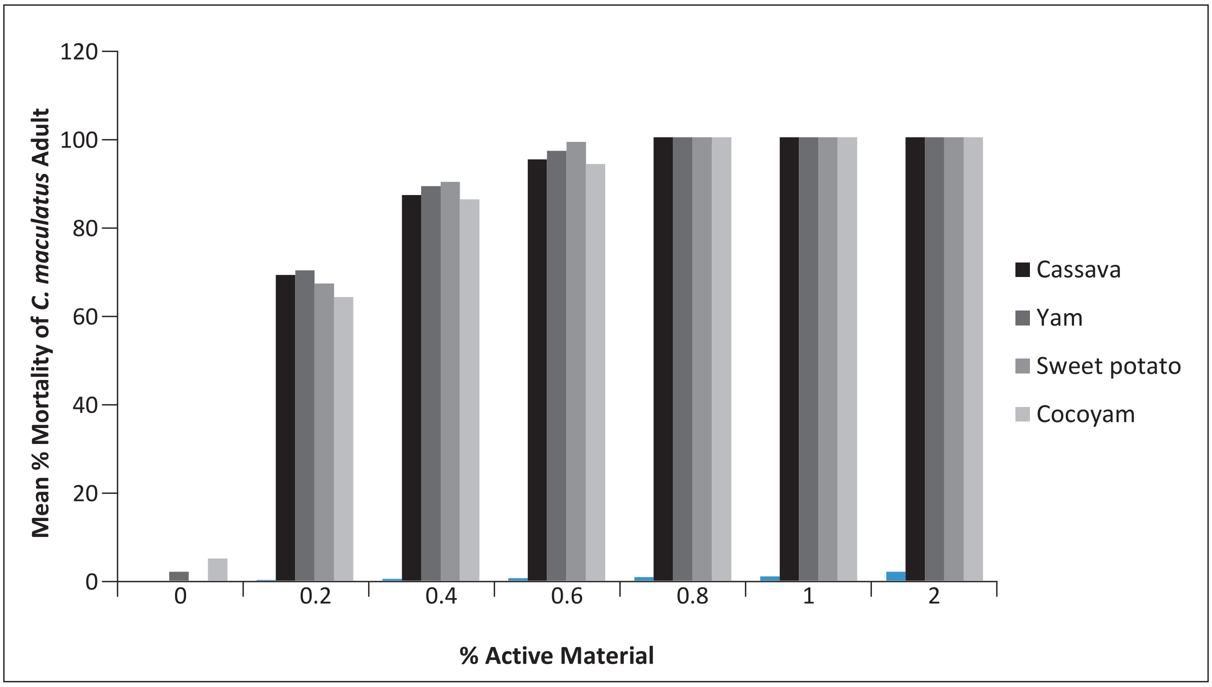

Figure 1: Mean percentage mortality of adult *C. maculatus* in cowpea seeds treated with *Dennettia tripetala* dust formulated with cassava, yam, sweet potato or cocoyam at 0.8% active material after 72hr

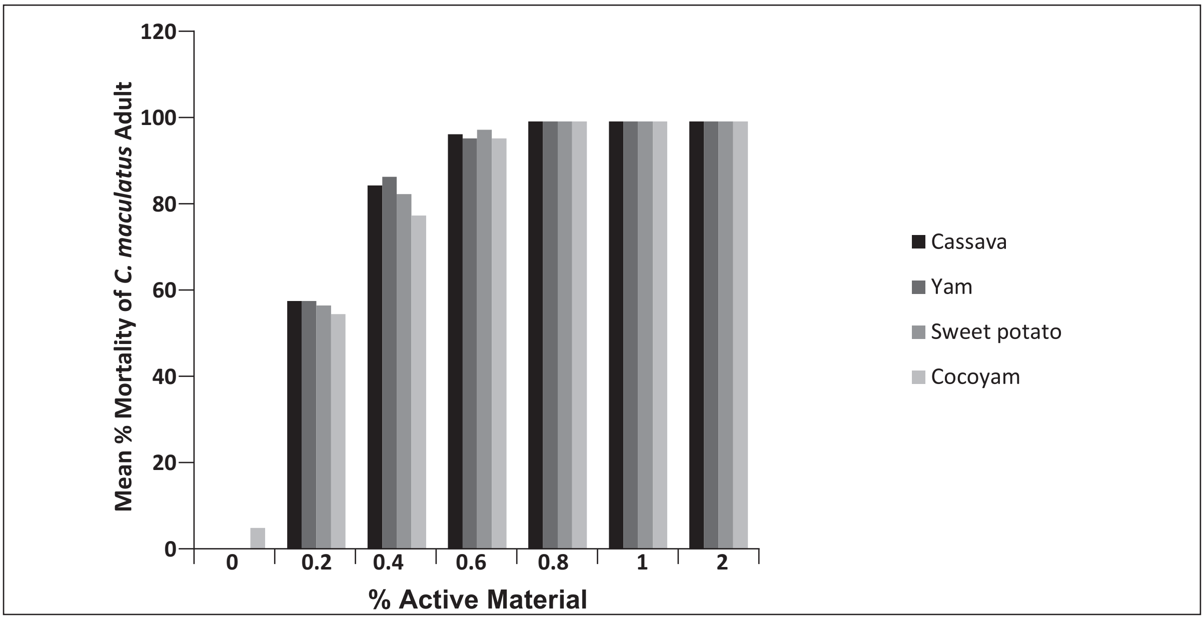

Figure 2: Mean percentage mortality of adult *C. maculatus* in cowpea seeds treated with *Piper guineense* dust formulated with cassava, yam, sweet potato or cocoyam at 0.8 % active material after 72hr

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Efficacy of Selected Botanical Insecticides for Reduced Post Harvest Losses of Common Beans by Bruchid Species in Farmers' Households in Tanzania

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Abstract

Six pesticide plant products reported by farmers from various bean producing regions were collected in Tanzania and used to evaluate for their ability to control bruchid infestation in stored beans. Crude products were prepared by grounding into fine powders of each of the barks and roots from plant species: *Zanha. Africana* (Mjui Magome), Neorautanenia *mitis* (Tupa daka), *Z. african* (Livanga roots Njombe), Actellic Super Dust, *Z. africana* (Mjui Roots Morogoro), *N. Mitis* (Lidupala) and *Schinus molle* (Mpilipili) and *Chenopodium ambrosioides* (Ikanganyishe)/ Fields and laboratory experiments were conducted in different farmers' households and at Sokoine University Seed laboratory. Bean samples of 1kg were thoroughly mixed with crude extracts at a dosage of 500g/100kg of bean seeds. Furthermore, a preparation of various combinations with three botanical mixtures from each of the six plant species was tested to determine the best combination for use. *Z. african* (Livanga roots njombe), *Z. africana* (Mjui Roots Morogoro), and *N. Mitis* (Lidupala) provided higher inhibition in bruchid emergence. On the other hand, *Chenopodium ambrosioides* (Ikanganyishe) demonstrated high mortality of insects when tested under laboratory conditions but was not effective under household bruchid trials. This work has demonstrated botanical pesticides as alternative products with comparative efficacy to Actellic super dust a commercial product used for storage pest management.
Key words: Bean bruchids, botanical pesticides, postharvest losses, common beans, *Phaseolus vulgaris*

Introduction

Production of common bean in Tanzania is still low due to poor agronomic practices, low soil fertility, lack of improved cultivars, moisture stress and damage caused by pests (Allen *et al.,* 1989; Robinson, 2005). Postharvest losses caused by bean bruchid infestations are among the major constraint to common bean production in Tanzania. A wide range of seed weevils attack the grain of common bean varieties. Bruchids cause heavy post-harvest losses and, consequently, heavy losses of profit because farmers are obliged to sell their beans immediately after harvest when prices are low (Myers *et al.,* 2001; Munyuli, 2003; Kimani *et al.,* 2005; Munyuli, 2009). The two major species of bruchids that are key pests of stored beans around the world are the Mexican bean weevil *Zabrotes subfasciatus* (Boheman), and the bean weevil *Acanthoscelides obtectus* (Say) (Oliveira *et al.,* 2002; Rajapakse, 2006). Rajapakase (2006) reported that, farmers have been using synthetic and botanicals insecticides to control bean storage insect pests for a number of years. The use of botanical pesticides is relatively more beneficial compared to industrial pesticides due to the fact that plants are readily available and can be propagated by farmers also they are safer than industrial insecticides to the consumers and the environment (Rajapakase, 2006).

Farmers use botanicals and inert materials to reduce bruchid infestation. Each Agro -ecological zone has its own plant species with different effectiveness. Also the insecticidal preparation differs from one region to another. However, there is very little information about these botanicals on their effectiveness and application dose for bruchid management. It is believed that botanical insecticides is likely to reduce undesirable side effects observed in synthetic insecticides and can help to preserve the environment for future generations (Tapondjou *et al*., 2002; Papachristos and Stamopoulos, 2003; Munyuli *et al*., 2008). Despite the potential of botanicals as alternatives to synthetic insecticides in bruchid management being high, farmers fail to exploit them due to lack of standard type and method of preparation of a given botanical plant. Farmers rely mostly on estimation and observation based on indigenous experience for appropriate amount of material taken and concentration required for a given botanical pesticide formulation (Mihale *et al.,* 2009). In this study, an attempt was made to evaluate the effectiveness of different bo tanical plants for bruchid management based on local types and preparations used by farmers in selected regions.

Methodology

The plant materials with insecticidal properties were collected from four regions including Mbeya, Iringa, Morogoro, and Kilimanjaro region. Two districts from each selected region were used as source of botanicals plant material collected for the experiment. Names of regions and their respective districts (in brakets) are Mbeya (Mbeya rural and Mbozi), Iringa (Makete and Njombe), Morogoro (Gairo and Kilosa) and Kilimanjaro (Hai). For Kilimanjaro region, only Hai district was included in the study.

In order to investigate the impact of *Acantoscelides obtectus* bruchids in selected villages we collected seeds from the same local villagers few weeks after harvesting and used these seeds to set a bruchid study in the households.

Bruchid screening trials were established in farmer's households to evaluate the efficacy of different botanicals in control of bruchids damage under household storage conditions. Five households from each of the research locations per village were used to determine the presence of bru chids and the efficiency of each botanical pesticide in controlling bruchids. Households were selected by the farmers themselves in for uniform distribution of the trials among households covering the village and household conditions.

Beans samples for this experiment were prepared using 1kg of the two susceptible FPVs beans varieties (Soworo - Kigoma or Soya-Kablanketi). Based on the level of importance for each botanical as presented by farmers from each location we selected a total of six botanical pr eparations that at least represented a botanical plant commonly found in one of the villages in our project groups.

Table 1: Botanical preparations were used in each household

Scientific name	Family name	Local name	Preparation used
1. Zanha africana (Radlk.) Exell	Sapindaceae	Mjui (kaguru)	Tree barks
2.Neorautanenia mitis	Fabaceae	Tupa daka (Sagala)	Ground tuber powder
3.Schinus molle	Anacardiaceae	Mpilipili (Swahili)	Roots powder
4.Zanha africana	Sapindaceae	Livanga (bena)	Roots powder

5. Actellic powder	Commercially available product		
6. Zanha africana (Radlk.) Exell	Sapindaceae	Mjui (kaguru)	Roots powder
7.Neorautanenia mitis (A.rich)Verdc	Fabaceae	Lidupala (bena)	Gound tuber powder
8.Chenopodium ambrosioides (L)	Chenopodiaceae	Ikanganyishe (chaga)	Mature leaves stems and inflorescence

These botanical treatments were applied to 1kg of beans at an adjusted rate of 600g of product/per 100kg bag of beans. Bean samples of the same weight containing Atelic Super Dust a standard chemical used by all farmers for bruchid control was also include d in the experiment and one sample per household without any additive treatment was used. Each of the five households per village received all the treatments. Farmers were advised to store the beans samples in the same way as normal bean storage procedure in their houses. Monitoring of the occurrence and infestation of bruchids were performed in every 60 -70 days following the experiment setting and continued to check for a period of 9 months as total annual possible bean storage time. Beans samples were kept further to one year storage and evaluation in areas with cooler temperatures.

The evaluation and data collection from each household involved bringing farmers together at one common meeting place for each village where examination of samples were perfo rmed in collaboration with researchers and farmers. Bean samples were spilled on a table with a clean plastic mat for easy visualization and sampling of emerging adult bruchids. Bean samples were spread and divided into four quadrants for careful observation of presence of emerging adult bruchids and counting of damaged perforated seeds. When damage or presence of adult bruchids were observed, beans samples were mixed and sampling of approximately 100 seeds was done using a small cup that was to hold an app roximate of 100 bean seeds. Four samplings were taken from the lot and spread for data collection by farmers where the following was scored: Number of damaged or perforated seeds, Seeds with one hole, Seeds with 2 -3 holes, Seeds with 3-5 holes and Seeds with more than 5 holes were recorded for each treatment in each household.

Percent damage over time was estimated based on the increase in number of damaged seeds over storage time for each botanical preparation. For each sample type of bruchid emerging fro m bean sample was identified in order to determine the most prominent bruchid species naturally occurring in different parts of bean growing area over time during the year. On the other hand, a laboratory bioassays and an on -site trials to validate the efficacy of crude botanical extracts in different mixtures for control of two species of bean bruchids, using FPVs was carried out in the laboratory. A no -choice replicated bruchid screening experiment to evaluate the effectiveness of different possible combi nations of insecticidal botanical plant pesticides in control of bruchids. Six pesticide plants and actellic supper dust were used in this experiment. The same pesticide plants used during the preceding on farm trials were used to design the most possible combination that would make a mixture of three pesticides plants preparation for bruchid management with some integrating actellic dust in the mixture. Each treatment was replicated three times where one bruchid susceptible bean variety was placed in glass vials containing the pesticide plant extract in powdery form and well mixed to ensure proper contacts between bean seeds and the insecticide plant preparations. Twenty mature adult bruchids from *A. obtectus* and *Z. subfasciatus* were administered into bean samples in two separate experiments. A dose rate of 600g of the quantity of each crude extract from each botanical preparation) per 100kg of bean seeds for each plant extract was used in the experiment set up of botanical mixtures.

The list of pesticide plant mixtures used as treatments in this experiment is presented in table 2.

Table 2: Combinations of pesticide plant mixtures administered to no -choice bruchid feeding experiments under laboratory conditions

Pesticides combinations		
123	567	267
124	125	157
134	356	237
145	357	256
146	137	167
147	156	127
234	135	456
245	236	457
246	235	467

247	256	Control Actellic with A.O
345	367	control Actellic with Z.S
346	126	Three Control experiments
347	136	Control II

Key: Respective names of pesticide plant species used for the mixtures

1: Mjui Magome *Zanha. african*
2: Tupa daka *Neorautanenia mitis*
3: Livanga roots *Z. african* njombe
4: Actellic Supper Dust
5: Mjui Roots *Z. africana*
6: Lidupala *N. mitis*
7: Ikanganyishe *Chenopodium ambrosioides*

Bruchid inoculated beans vials were kept for 12 days to allow for sufficient oviposition time. On the 13 th day all adult bruchids (live or dead) were carefully removed from each the vial leaving beans samples, botanical preparations and eggs or larvae of bruchids to allow for easy detection of newly emerging F_1 adults of weevils. The experiment was monitored for 60 days in the laboratory under room temperature. Data collected included: Number of emerged adult bruchids (F_1), Number of days to first bruchid emergence (F_1), Number of damaged seeds (perforated by bruchids) and undamaged seeds, Percentage damaged seeds and Number of holes per seed (seeds with 1-3 and seeds with more than 5 holes per seed).

Results and Discussion

During the evaluation of efficacy of botanical plants on bruchid management, data has indicated that bruchid infestation generally increased with the duration of storage (Fig. 1) of beans in each seed samples. The levels of infestation were also different between villages as well as households (Fig. 2) Climatic conditions influences greatly the levels of bruchid infestation. The most dominant bruchid species was *A. obtectus* which was predominantly observed in almost all samples across the study areas. The bean weevil *Z. subfasciatus* was mainly localized in low land areas and was most aggressive during the warm season of the year in the low land areas. Without taking into account on the presence or absence of the botanical plant extracts as strategy for bruchid management, Kisanga and Mtumbatu villages in Morogoro has the highest levels of bruchid infestation and damage on beans samples kept for a year and above. The damage was descriptively increased with time of storage for each village (Fig. 3). When botanical plants are concerned, variation of effectiveness of pesticide botanical plants as monitored during the specified bean storage period in a year showed significant differences in their ability to control bruchids. Samples for control without any pesticide product is included and sample with standard Actellic dust a chemical commonly used for control of storage pests in cereals was included. There was a significant difference in the levels of damage on seeds during the year of bean storage. Botanical plant preparations Mjui Magome, Livanga, Mjui Mizizi and Lidupala indicated better inhibition of bruchid infestation as demonstrated by low quantity of damaged seeds (Fig. 4). Though the number of damaged seeds was higher toward the end of the year when bean seeds have been kept for a period of one year and more, relatively low quantity of damaged seeds was observed in the for botanical plants in comparison to the control treatment. Furthermore, the efficacy of each botanical plant extract can be looked on its performance in each village as it will be easy to link the information with the distribution of adult botanical plant preparations. However, efficacy of botanical was in a declining trend as demonstrated by a systematic increase in levels of infestation or seed damage with time of storage. In the three villages where bruchid infestation was more evident, performance of the chemicals can be linked to the pest pressure (Fig. 5). For instance a clear evidence of the effectiveness of botanical is shown in Munguishi village (Fig. 5) where the same four botanical plant preparations were efficient in reduction of number of seed damage by the adult bruchids. The levels observed in Kisanga and Mtumbatu can be cofounding due to high insect pressure and farmers intersecting the trial with artificially inoculating large quantity of bruchids. However the trend for efficacy indicates that botanicals plant preparations are better when compared to non-treated experiments.

A combined independent analysis of the botanicals in each mixture showed that in any mixture where plant extracts prepared from botanicals # 3, 5 and 6 were the most effective in control of bruchid damage to beans seeds (Fig. 6) by low number of bruchid emergence when compared to the control experiment of bean stored without any of the botanical mixture. This experiment has validated the potential of these plants as useful pesticide effect to bruchid management. However the dosage and understanding the actual time spam for its efficacy. The lowest daily emergence of new adult bruchids from *Z. subfaciatus* was observed in various botanical mixtures containing combinations with botanicals and Supper Actellic Dust (Fig. 7). Any combination containing actellic supper Dust was highly effective in controlling emergence of new adults in *Z. subfasciatus* giving a low mean adult emergence in a period of 48 days of observations. Combinations containing botanicals pesticides # 5, 6, and 7 were highly effective in control of bruchid emergence compared to the controls without any treatment.

The study also demonstrated an interaction of botanical 6 and 7 for effective control of bruchid emergence. The effective of plant extracts from *C. ambrosoides* may be due to its suffocating mechanism as a fumigant. However it works only for few days or hours following application. The other reason for its effectiveness may be ralated to its being used as shredded debris from leaves, stems and inflorescence thus making it a target for adult *Z. subfasciatus* to deposit eggs on these debris. The larvae that could hatch from eggs accidentally glued on the debris of the *Chenopodium ambrosoides* have little chance of penetrating into bean seeds and therefore not completing the insect cycle. The consequence for this is the reduced number of emerging adults in samples from this botanical combination. The effectiveness of plant debris was also reported by farmers and other previous findings (Schoonhoven and Cardona, 1986). In a parallel experiment same botanical mixtures were administered into samples of beans with *A. obtectus* to determine most effective combination of botanical mixture. Results indicated that at any time, the botanical mixtures containing botanical preparations from plant #3 was effective and also those containing plant #5 provided significant reduction in number of bruchid emergence (Fig. 8). Thus application of botanical from plant # 3 in any mixture leads to reduction in bruchid emergence by 63% of the control (untreated bean samples). In the similar manner, botanical plant #5 leads to 62% of the control. However, no interaction was observed to give significant control of the bruchids in these samples.

It is now evident that at least three pesticide plant preparations can be used by farmers in the IPM for bruchid management namely Mjui (*Z. africana* Morogoro provenance from both tree trunk and barks roots), Livanga (*Z. africana* Njombe provenance) roots and Lidupala (*N. mitis* Mbeya provenance). Given a long list of pesticide plants used in this experiment it was invisaged that a botanical combination in a set of three plants can be tested and we therefore ventured into this in order to determine the best combination if any for management of bruchid species. This experiment was conducted in the laboratory and results presented in coming sections. It was also evident that plants that demonstrated lower efficacy to control of bruchids have to be removed from the list of plants to be recommended for use as botanicals in bruchid management. We conducted at the end a participatory ranking of the performance of each of the botanicals based on the levels of control of bruchid damage. Farmers in this respect can use locally available plant materials from the selected botanical pesticide plants for setting a bean seed storage management strategy by using larger quantity of beans in their houses. However, there is still a need to validate the effect of botanical pesticides on cooking and taste quality characteristic changes in beans after storage using these botanical preparations. Expiry time for botanical is also an important factor to investigate in order to understand the decline or durability of antibiosis posed by the crude product. It may also call attention on the determination of the levels of active ingredients in the course of storage.

Summary of data to be presented in this paper

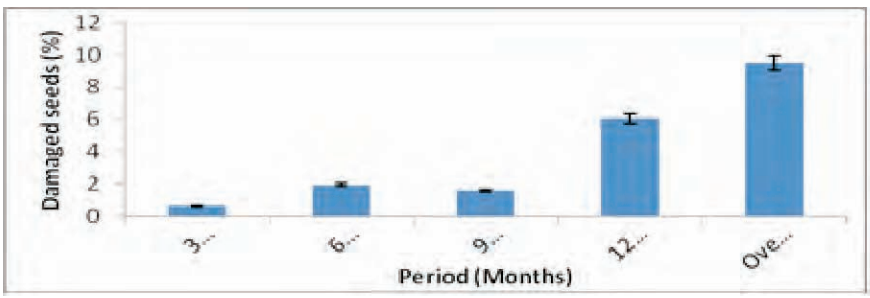

Figure 1: General population increase of bruchids causing seed damage as observed in several study villages during a period of bean storage exceeding one year

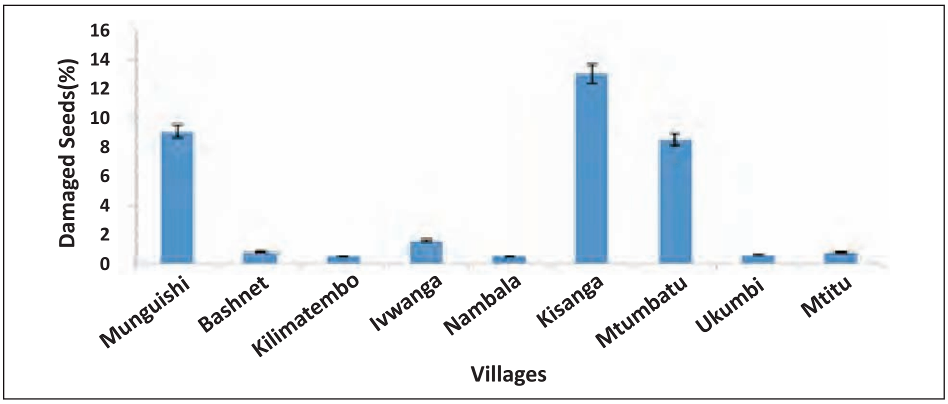

Figure 2: Levels of bruchid damage on bean seeds as cummulatively observed in different bean producing villages of the study area. These levels of bean seed damage by bruchids recoreded over a period exceeding one year

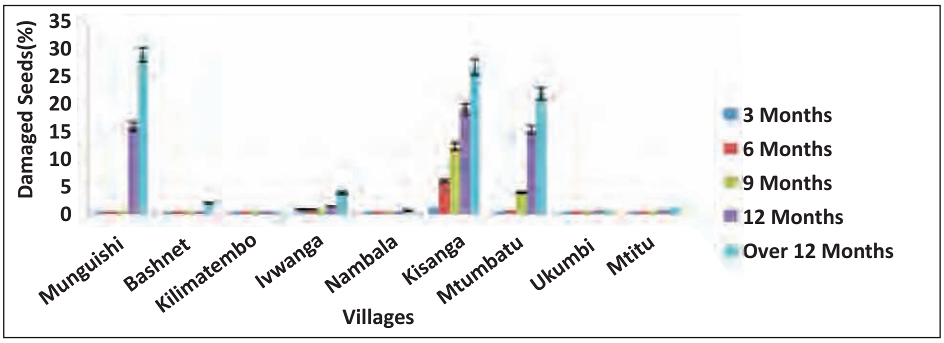

Figure 3: Change in population and levels of bruchid damage on bean seeds as observed in different bean producing villages of the study area. Levels of bean seed damage by bruchids was sequencially recoreded each three months over a period exceeding one year

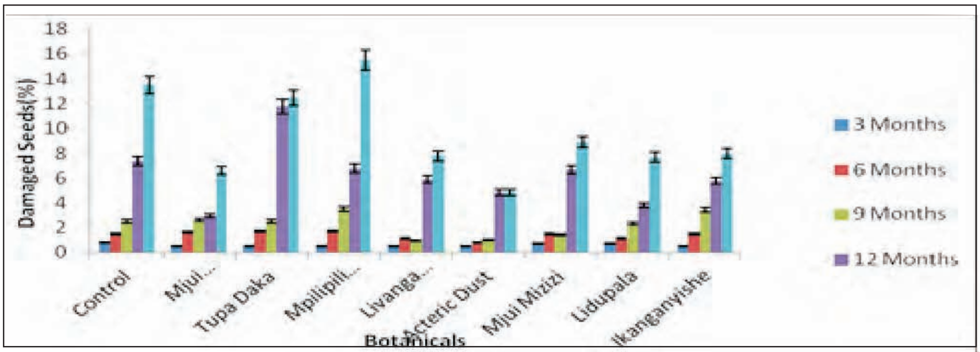

Figure 4: Variation in effectiveness of botanical pesticides as monitored during the specified bean storage period in a year. Samples for control without any pesticide product is included and sample with standard Actellic dust a chemical commonly used for control of storage pests in cereals was included

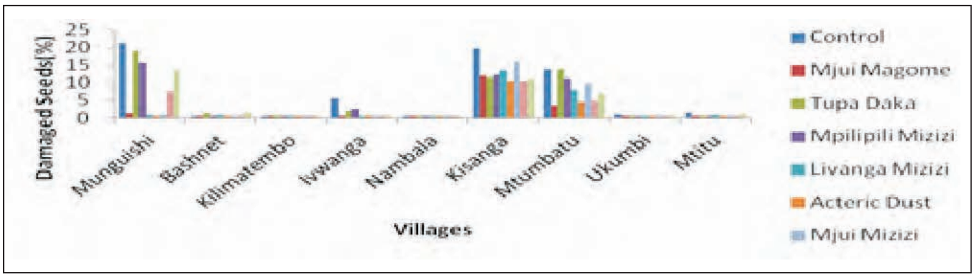

Figure 5: Performance of pesticide plant preparations in different vilages as monitored during the period of more than one year (15 months)

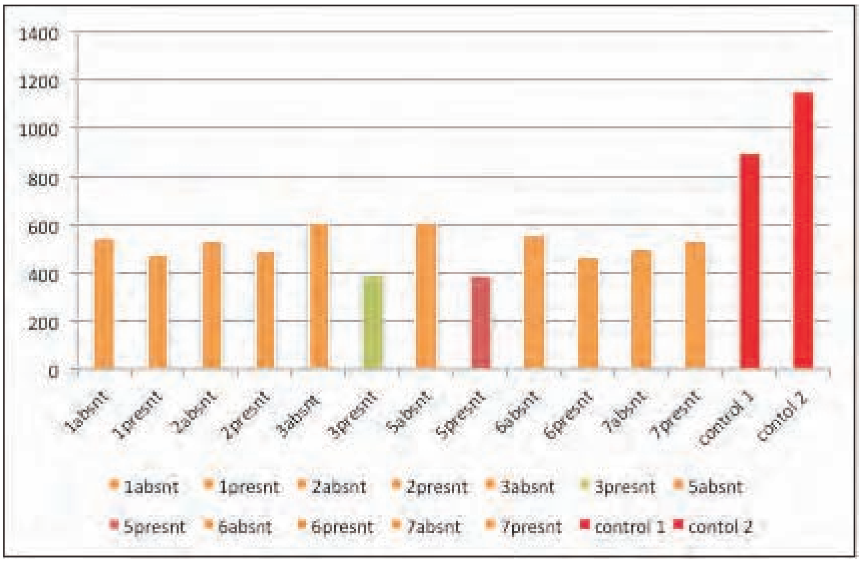

Figure 6: Mean adult emergence of *A. obtectus* among combinations of botanical pesticides

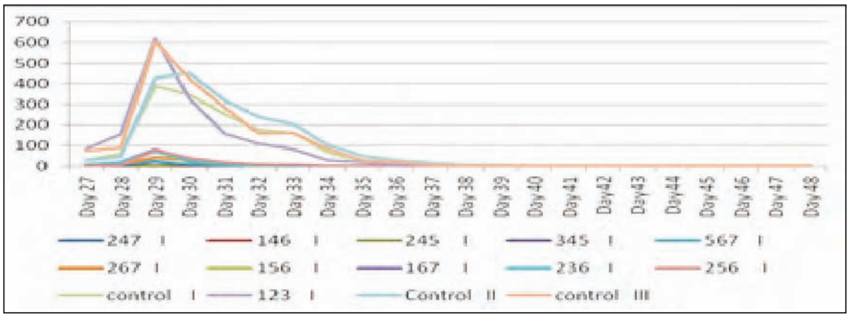

Figure 7: Effective botanical combination for control of *Z.subfaciatus* emergence in a controlled experiment showing daily emergence of adult bruchids from different botanical mixtures

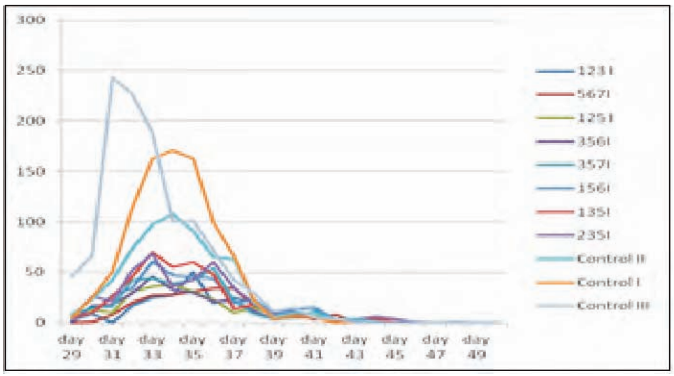

Figure 8: Effectiveness of botanical combination for control of *A. obtectus* showing the daily bruchid emergence from different botanical mixtures and control experimennts without botanical treatemets conducted in a laboratory experiment

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Evaluation of repellant effects of oils from selected Eucalyptus species against *Sitophilus zeamais* (Motschulsky)

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Abstract

The essential oils of Eucalyptus were extracted by steam distillation using a Clevenger -type apparatus from the fresh leaves of various species of Eucalyptus; *Eucalyptus saligna* Sm., *Eucalyptus globulus* Labill., *Eucalyptus camaldulensis* Schlecht and *Eucalyptus citriodora* Hook. A repellency bioassay of the oils with varying concentrations (0.0001, 0.001, 0.01 and 0.1µl/µl)was carried out against maize weevils using a Y-shaped olfactometer. Repeated measures of analysis of variance were applied so as to test the different doses (Dose), different repellents (Repel) and their interactions (Dose*Repel) on the response variable (Percent Repellency).The essential oils of *E. camaldulensis* and *E. citriodora* presented better repellent activity in the 0.1µl/µl concentration (74.35% and 69.15%, respectively) followed by the oils of *E. globulus* (53.68%) and *E. saligna* (40.5%) which presented the lowest percentage repellence. Gas chromatography – mass spectrometry (GC-MS) and GC co-injections with authentic samples showed the presence of the following major constituents in the oils: 1, 8-cineole, α-pinene, β-pinene, limonene, linalool and β-myrcene. The results provide a scientific rationale for the use of Eucalyptus oils in protection of maize against weevils.

Introduction

Weevils (Coleoptera; Curculionidae) are a very large group of beetles, containing more than 60,000 species, worldwide in occurrence, equally well distributed throughout the tropics and temperate regions, and many are pests of cultivated crops and forest trees (Hill, 1987). Food security in Sub Saharan Africa largely depends upon improved food productivity through the use of sustainable good agricultural practices (GAPs) and the reduction of post-harvest losses caused by pests and diseases. For decades, the pest control policy in developing countries has been dependent upon the use of synthetic pesticides. Although synthetic pesticides are known to have undoubted benefits, their adoption rate and use for insect control in grain storage has remained remarkably low in resource-poor farming environments (Ogendo *et al*., 2004).

The subsistence nature of agriculture in developing countries coupled with the high cost, poor information and erratic supply of synthetic pesticides have emerged as reasons for farmers' reluctance to adopt pesticides (Tembo and Murfiti, 1995; Ogendo, 2000). Further, there have been revelations that synthetic insecticides penetrate into stored grain and may be toxic (Lalah and Wandiga, 1996; El Shamy *et al*., 1988) and that pests have become more resistant to synthetic compounds. Therefore affordable and effective methods for reducing pest damage are required.

Literature Summary

Different types of aromatic plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Prakash and Rao, 1997). More research showed that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Singh *et al*., 1989, Shaaya *et al*., 1989, Renault-Roger *et al*., 1993). Major constituents from aromatic plants, mainly monoterpenes, are of special interest to industrial marketers because of other potent biological activities in addition to their toxicity to insects (Kubo *et al*., 1994, Isman, 2000, Weinzierl, 2000).

Previous research done using the various species of Eucalyptus has shown the repellency of the extracted oils. Mossi *et al* (2010) determined that the essential oil of Eucalyptus presented insecticidal repellency against *S. zeamais* using the oils of *E. dunii, E. saligna, E. benthamii, E. globulus* and *E.viminalis*. About 100% of mortality was achieved with doses of 65, 100 and 400 µL for of *E. dunii, E. saligna* and *E. benthamii* respectively. They concluded that although from an economic point of view synthetic chemicals are still more frequently used as repellents; natural products (essential oils) have the potential to provide efficient and safer repellents for humans and the environment. Analysis by gas chromatography coupled to mass spectrometry has also enabled the determination of the chemical composition of *E. saligna* in which the major component was p-cymene (54.2%) and gamma-terpinene (43.8%), in the vegetative state (Sarotelli *et al.,* 2007). The refined form of oil from *E. citriodora* has also been used in insect repellents/ The refined oil's citronellal content is turned into cis- and trans- isomers of p-methane-3,8-diols (PMD) which are active ingredients in insect repellents.

Description of the Research

The various laboratory studies carried out were as follows: *(a) Extraction of the Essential oils:* the fresh leaves of the selected Eucalyptus species were cut into small pieces and the oil extracted by steam distillation using a Clevenger-type apparatus. The steam distillate was collected every hour and extracted with hexane. This prevented possible decomposition of the oil due to heat and exposure to light. The combined oil and solvent extract were dried with anhydrous sodium sulphate. The solvent was then removed using a micro-distillation apparatus to give the essential oils. The oils were stored in sealed amber-coloured vials at about 4°C. *(b)Bioassay tests:* The maize weevils (*S. zeamais*) were obtained from a laboratory colony reared under ambient conditions on insecticide free maize grain. Four concentrations of the oils (0.1, 0.01, 0.001 and 0.0001 µl/µl) were used for the bioassays/ Twenty microliters of the diluted test material was taken for the *S. zeamais* bioassay. The repellent activity of the essential oils against the maize weevils was assessed using a Y-shape olfactometer. It consists of a glass tube with a "Y" shape bifurcation where an insect walks and decides between two choices at the "Y" ends, the volatile oil (treated arm) and the standard (control arm)/ For each assay thirty randomly selected adult maize weevils of mixed sex and age were introduced into the olfactometer. All bioassays were conducted at 25±5°C and 70±5% humidity. The assay was left to run for thirty minutes and then the number of weevils in the control arm (N_C) and the treated arm (N_T) were counted. After each test the olfactometer was thoroughly cleaned and dried. The assay for each dose of the test material was replicated five times and the same for the standard N, N-diethyl-m-toluamide (DEET). Percentage repellence (PR) values were computed using the formula: $PR = \{(N_C - N_T) / (N_C + N_T)\} \times 100$. *(c) Analysis of the Essential oils:* The essential oil samples were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). *Gas Chromatography Analysis:* GC analysis of the oils was performed using a Shimadzu Gas Chromatograph Model GC-14B fitted with a Flame Ionization Detector and a Shimadzu C-R8A integrator. The instrument was equipped with a 60metre carbowax column with an internal diameter of 0.25mm and a film thickness. The Nitrogen carrier gas had a flow rate of 2ml/min. the temperature was programmed from 70°C (2 minutes) then raised at 10°C/min every three minutes to 230°C and held for two five minutes. *Combined Gas Chromatography and Mass Spectrometry (GC-MS):* GC – MS was performed on an Agilent Technologies instrument Model 7890 A (GC System) and Model 5975 C inert XL Electron Impact/ Chemical Ionization MSD with triple – axis detector (MS compartment). The instrument was equipped with a 30metre methyl silicone (HP-5ms capillary column) with an internal diameter of 250µm and a film thickness of 0.25µm. The helium carrier gas had a delivery rate of 4ml/min. The automatic sampling injector (Model 7683 B) operated in the splitless mode and the sample concentration was 250mg/ µl. The column temperature program was 40°C then raised at 10°C/min to 140°C and maintained for 15 minutes, then raised at 10°C/min to 280°C then maintained for 1 minute. For Mass Spectrometry the electron impact was 70eV. The mass analyzer used was QuadrupoleAnalyzer.

The compounds were identified using the program Agilent 5975C MSD Data Analysis by their GC retention time and by comparison of the test compound mass spectra with the National Institute for Standard Technology (NIST), CHEMECOL and Adams2 mass spectral data bases.

The identity of the constituents was established by comparison with retention times of the authentic samples and peak enhancement of the components by co-injection of the essential oil with the standards. *(d) Repellency Bioassay of the pure identified compounds:* The compounds identified from the essential oils were then assayed for their repellent activity against *S. zeamais*. *(e) Data Analysis:* Due to the nature of the sampling technique, repeated measures of analysis of variance was applied so as to test the effects of different doses (DOSE), different repellents (REPEL) and their interactions (DOSE*REPEL) on the response variable (PERCENT REPELLENCY) on *S. zeamais*. The percentrepellency variable was transformed using angular transformation so as to normalize the data and minimize correlation between the mean and variance of the data. Since the level of dosages represent quantitative factor whose effect can often be explained through polynomial relations, hence orthogonal polynomial contrasts were used to investigate repellency trends with concentrations. Mean comparison was performed by use of adjusted treatment (REPEL) means computed by least square method. Statistical Analysis Software (SAS) was used.

Research Results and Application

The chemical composition of the essential oils of *E. globulus, E. camaldulensis* and *E. citriodora* were studied using gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS). Constituents of the essential oils were identified by analysis of their mass spectra, by direct comparison of their mass spectra to the NIST, Chemecol and Adams2 libraries of mass spectra by using computer search routing and by co-injection with the authentic standards in the GC.

Table 2: Constituents of the oils from *E. camaldulensis, E. citriodora* and *E. globulus*

Compound	Relative percentage		
	E. globulus	*E. citriodora*	*E. camaldulensis*
α-thujene	0.132	0.465	
3-carene	0.775		
α-pinene	7.094	2.235	2.500
Camphene	0.445		
trans thujenol	0.093		
β-phellandrene		0.119	2.627
β-pinene	0.343	3.188	
β-myrcene	0.434	1.668	1.654
α-sabinene		1.668	
α-phellandrene	0.490		
2-carene			0.927
Limonene	0.960		
1,8-cineole	17.171	11.203	18.902
γ-terpinene	0.235	1.656	1.185
β-terpineol		0.414	
Terpinolene			0.539
δ-terpinene		0.750	
Linalool	2.113		
p-Menth-2-en-1-ol	0.484		

α-terpineol		0.489	0.316
4-terpineol		3.133	2.295
terpinen-4-ol	2.263		
Cryptone	5.404		
4-carene	0.913		
Verbenone	0.689		
Ciscarveol	0.376		
Citral	0.522		
α-cubebene		0.277	
γ-pyronene		0.272	
Copaene			0.255
Isoledene			0.669
caryophyllene			1.380
α-caryophyllene			1.300
epi-bicyclosesquiphellandrene			1.676
α-cardinol			6.408

Repellency Bioassay

The mean repellency values of the individual compounds, essential oils and the standard DEET at the four dose levels against *S. zeamais* are provided in Table 2. All the dosages were repellent to *S. zeamais* in a dose-response manner. Analysis of variance in dicated significant differences (P < 0.05) between weevil responses to the four dosages tested.

Table 2: The repellent activity of the individuals compounds, the essential oils and the standard DEET against *S. zeamais*

Compound	Dose µl/disc			
	2	0.2	0.02	0.002
α – pinene	41.83±8.75 a,b,c,d	22.45±5.10 b	11.03±3.27 a	17.54±7.04a
β – pinene	25.17±10.66 c,d	32.87±5.87 a,b	28.50±8.25 a	28.65±7.48 a
Citral	25.63±7.14 c,d	21.00±7.76 b	18.58±8.85 a	--------------
1,8 – cineole	70.87±2.64 a,b	58.29±9.92 a	29.00±4.0 a	24.08±10.58 a
Limonene	37.12±10.62 d	18.76±7.20 a,b	21.92±8.76 a	22.84±9.3 a
linalool	63.63±5.95 a,b	26.08±8.79 b	14.27±5.40 a	6.60±5.40 a
DEET	4.00±0 c,d	6.7±0 b	20.00±0 a	25.00±0 a
E. saligna	40.5±4.94 b,c,d	32.25±9.08 a,b	30.95±11.47 a	28.68±11.29 a
E. globulus	53.68±11.75 a,b,c	32.09±4.35 a,b	5.55±1.84 a	--------------
E. citriodora	69.15±3.55 a,b	68.02±8.50 a	24.75±13.00 a	--------------
E. camaldulensis	74.35±6.29 a	41.97±11.40 a,b	12.12±3.42 a	10.51±3.80 a
E. globulus	------	------	-----	-1.76±0.0
E. citriodora	------	------	-----	-12.6±5.70
citral	------	------	-----	- 16.57±0.0

The values are the mean repellency (%) ± Standard Error Margin of five replicates of 30 maize weevils each. The means within the columns followed by the same lower case letters are not significantly different (p<0.05, !NOV! and Tukey's tests)

Table 2 shows that the repellent activity increased with increasing doses. The essential oil of *E. camaldulensis* had the highest activity being more active than DEET or any other oil tested at the highest dose 2µl. The oil of *E. citriodora* had the highest activity at the 0.2µl dose followed by *E. camaldulensis, E. saligna* and *E. globulus*.The essential oils were more active than the standard DEET, except at the 0.02µl dose where *E. globulus* was less active than DEET and at the 0.002µl where *E. globulus* and *E. citriodora* behaved as attractants. This means that there were more maize weevils in the test arm than in the control arm of the olfactometer. The formula: $PR = \{(N_C – N_T) / (N_C + N_T)\} \times 100$ was reversed to become $PR = \{(N_T – N_C) / (N_C + N_T)\} \times 100$. The transformed mean thus calculated for the two doses were called attractance hence the negative sign indicating negative repellence.

In light of the repellence results, the oils with repellent activity greater than 50 percent, that is *E. globulus, E. camaldulensis* and *E. citriodora* were analysed to identify and use the individual constituents. This was done in order to find out which ones are responsible for the high activity.

1,8-cineole, the major constituent of the oils had the highest repellent activity and hence is responsible for the repellence of the oils. Generally all the constituents identified from the oils consistently higher repellent activity than DEET at all the doses tested except for citral at the dose 0.002µl. From the data obtained it was possible to conclude that the different dosages (DOSE) have a definite significant effect on the percentrepellency of the different compounds (REPEL) based on the test statistic computed by the Tukey's tests. The results thus provide a scientific rationale for the use of Eucalyptus oils in protection of maize against weevils.

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Residual bioactivity of *Ocimum americium* L. and *Tephrosia vogelii* Hook. essential oils against coleopteran pests and inhibition of wheat seed germination

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Abstract

Grain pests remain a major threat to food and nutrition security and livelihoods in smallholder agriculture. Although effective synthetic pesticides are available, they are largely incompatible with existing farmer circumstances. In a search for eco-friendly alternatives to synthetic pesticides in grain storage, selected *Ocimum americanum* and *Tephrosia vogelii* essential oils, at five rates (0.0, 0.5, 1.0, 1.5 and 2.0 PL/g grain), were evaluated under semi-hermetic conditions for residual contact toxicity and reproduction inhibition against four coleopteran pests and phytotoxicity on wheat seed germination over a 4 -month grain storage period. GC-MS analysis showed *O. americanum* and *T. vogelii* essential oils were eugenol (49.2%) and germacrene D (19.2%) chemoypes, respectively. Results showed strong dose -, contact time- and storage duration-dependent residual bioactivity and germination inhibitions. At 2.0 PL/g grain and 90-120 d, *O. americanum* and *T. vogelii* fruit oils caused 14-22 and 3-47% kill, respectively, of adult *Sitophilus oryzae, Rhyzopertha dominica,* and *Tribolium castaneum*. Similarly, the leaf oils, respectively achieved 58 -75 and 37-53% kill of *Callosobruchus chinensis*. At the dose range tested, the essential oils reduced adult F_1 progeny by 28-40%. At 2.0 PL/g grain and 120 d, *O. americanum* and *T. vogelii* fruit oils reduced the wheat seed germination by 25-40 and 33-51%, respectively. These results are discussed in the context of the ir relevance as grain protectants and implications for the informal seed industry.

Introduction

The scientific search for botanical pesticides as cost-effective, biodegradable and eco-friendly alternatives to synthetic pesticides in smallholder agriculture has gained momentum in recent years. Botanical pesticides have been used globally for generations as the major control principle in subsistence agriculture (Belmain *et al*., 2001). Higher plants are an excellent source of biologically active natural compo unds, comprising mainly of secondary metabolites, against insect pests of food commodities (Clemente *et al*., 2003). Among the natural plant secondary metabolites, essential oils and their constituents have attracted substantial scientific attention due to their phyto-toxic, repellent, anti-microbial, herbicidal and growth and germination inhibition effects (Batish *et al*., 2004; Liu *et al*., 2007). Plant essential oils are known for their potential to control storage insect pests and preserve food commodities. A study was conducted to evaluate the intra- and inter-plant residual bioactivity of *Ocimum americanum* and *Tephrosia vogelii* essential oils against four major coleopteran pests of stored food commodities and their effects on treated wheat grains over a 4 -month storage.

Literature Summary

Insect pests attacking stored food grains are known to cause av.15% post harvest losses due to their grain damage through feeding, contamination and reduction of nutritional and economic values (Ogendo *et al*., 2003; Ottai *et al*., 2012). The major insect pests attacking stored food grains in the tropics include grain weevils (*Sitophilus* spp.), bostrichid beetles (*Prostephanus truncatus*, *Rhyzopertha dominica*), bruchids (*Callosobruchus* spp., *Acanthoscelides obtectus*) and rust-red flour beetle (*Tribolium castaneum*) (Ogendo *et al*., 2003; Sallam, 2004). Although effective synthetic pesticides are available, they are largely incompatible with smallholder agriculture owing to their high costs and inconsistencies in efficacy (Be lmain *et al*., 2012). The use of plant-derived products for pest control is age-old and remains largely compatible with existing farmer circumstances in smallholder agriculture in sub-Sahara Africa (SSA). The basils (*Ocimum* spp.) and fish poison bean (*Tephrosia vogelii*) are among some of the most widely used plants in aromatherapy/cosmetics, insect control and mosquito repellents. Past studies with *Ocimum* and *Tephrosia* species essential oils have revealed clear insecticidal, repellent and reproduction inhibition effects against various stages of stored product insect pests (Kéita *et al*., 2001; Ogendo *et al*., 2008) and antimicrobial (fungicidal and bactericidal) activity (Isman, 2000).

Description of Research

Hydro-distilled essential oils from *O. americanum* and *T. vogelii* leaves and fruits had their constituents analyzed using GC-MSD apparatus and identified by comparing relative retention indices and mass spectra with authentic samples and GC-MS libraries. The *O. americanum* and *T. vogelii* fruit essential oils were evaluated for residual contact toxicity against laboratory maintained adult *Sitophilus oryzae* L. (5-10 day old), *Tribolium castaneum* Herbst (30-60 d) and *Rhyzopertha dominica* F. (5-10 d) and germination inhibition of treated wheat seeds. The corresponding leaf essential oils were tested residual toxicity against adult *Callosobruchus chinensis* L. (0-3 d) and resultant reduction of F_1 progeny. A 50-g wheat (or 100-g chickpea) grain samples were treated with test essential oils, dissolved in acetone AR (99.8% GC), at five rates (0.00, 0.50, 1.00, 1.50 and 2.00 PL/ g grain), in special self-sealing polythene bags (20 cm x 25 cm; 2 litre capacity) and semi-hermetically stored for 4 months. Untreated and acetone (0.05% v/w) treated grains were used as negative controls whereas Actellic 5EC (1.00 PL/ g grain) and crude soya oil (10.00 PL/ g grain) as positive controls. Grain treatments were arranged in a completely randomized design with 4-8 replicates. Grain sub-samples (10 g for wheat and 20 g for chickpea) were collected from each experimental unit at 30, 60, 90 and 120 days after treatment (DAT) and subjected to contact toxicity and F_1 reduction and wheat seed germination inhibition tests (Ogendo *et al*., 2004, 2008). Adult insect mortality (corrected for natural mortality), percent reductions in F_1 progeny and wheat seed germination computed accordingly (Abbott, 1925; Mao *et al.,* 2004; Ogendo *et al*., 2004; Asawalam *et al*., 2006). All data on mortality, F_1 progeny counts and percent germination and inhibition were first homogenized using arcsine transformation before being subjected to multi-factorial analysis of variance (!NOV!) and treatment means separated by Tukey's HSD test (Mead *et al*., 1994). Concentration-response data (mortality and germination inhibition) were further log-transformed before being subjected to Probit Regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50}/EC_{50} values obtained (Finney, 1971; Mead *et al*., 1994).

Research Results and Application

Results of GC-MS showed clear intra- and inter-plant variability in chemical composition with *O. americanum* and *T. vogelii* leaf essential oils being eugenol (49.2%) and germacrene D (19.2%) chemotypes, respectively (Tables 1). Eight major chemical constituents, comprising monoterpenes and sesquiterpenes are presented. These chemical compositions contrast sharply with documented chemotypes from Africa and Latin America, namely citral, camphor, methylcinnamate, epicamphor and eucalyptol (Golob *et al*., 1999; Kyle *et al*., 2002). This high intra- and inter-plant variations in chemical compositions calls for documentation of chemotypes, their geographical distribution in Kenya and bioactivity against insect pests of stored food commodities.

Table 1: Major chemical constituents in *T. vogelii* and *O. americanum* essential oils

Plant source/ Chemical compound	% Composition		Plant source/ Chemical compound	% Composition	
Tephrosia vogelii	Leaf	Fruit	*Ocimum americanum*	Leaf	Fruit
α-Pinene	5.45	2.30	1,8-Cineole	3.47	6.63
Limonene	2.67	1.74	(Z)-β-Ocimene	9.96	2.89
E-Caryophyllene	6.06	0.94	Linalool	1.42	2.39
α-Humulene	2.01	0.41	Eugenol	49.22	12.59
Germacrene D	19.18	1.50	E-Caryophyllene	2.87	4.23
E-Nerolidol	-	16.10	Germacrene D	3.66	3.02
Caryophyllene oxide	1.64	3.59	σ-Bisabolene	3.81	13.12
(E,Z)-Farnesol	11.22	-	Elemicin	11.05	15.19

The *O. americanum* and *T. vogelii* fruit essential oils produced dose-, insect species- and storage duration-dependent residual contact toxicity and reproduction inhibition effects against adult *S. oryzae, R. dominica* and *T. castaneum* insects and the leaf essential oils of the two plants against adult *C. chinensis* insects (Table 2). Although equally efficacious, results showed 18-79% and 5-38% reductions in efficacy of *O. americanum* and *T. vogelii* fruit essential oils against three major coleopteran pests, *S. oryzae, R. dominica* and *T. castaneum* over a 4-month storage period. The results of leaf essential oils concur with Demissie *et al*. (2008) who reported a 33.0-72.0% mortality of adult *S. zeamais* after 3-month storage on grains treated with sunflower, corn and olive cooking oils (at 0.5% v/w). The observed differential residual contact toxic and reproduction inhibitory effects of *O. americanum* and *T. vogelii* essential oils against four coleopteran pests of stored food grains could be explained by individual and/ or synergistic bioactivity of major chemical constituents and differential responses by test insect species (Arriaga *et al*., 2005). Although not directly investigated the residual contact toxicity and reproduction inhibition could be due the presence of 1,8-cineole, eugenol, methyl eugenol, limonene and α-pinene among other bioactive essential oil constituents (Prates *et al*., 1998; Bekele and Hassanali, 2001; Huang *et al*., 2002). These positive results provide hope for use of essential oils for protection of hermetically stored grains against coleopteran pests for periods consistent with smallholder agriculture.

Table 2: End-point residual mortality and reduction in F_1 progeny of four coleopteran pests in grains treated with *O. americanum* and *T. vogelii* essential oils

Treatment/ Eoil Concentration (PL/ g grain)	Percent adult mortality (Mean ± SE) FRUIT ESSENTIAL OIL			LEAF E-OIL
	S. oryzae[a]	*R. dominica*[a]	*T. castaneum*[b]	*C. chinensis*[b]
O. americanum				
0.00 /Acetone 0.50	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1.00	10.4r 2.4	7.5r 1.5	7.1r 1.7	49.5r 2.2
2.00	18.2r 1.3	15.4r 3.1	13.8r 2.7	64.7r 3.4
T. vogelii				
0.00/ Acetone 0.50	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1.00	19.7r 3.5	10.5r 1.3	1.3r 0.3	24.0r 1.3
2.00	31.1r 2.1	17.7r 1.9	3.3r 1.1	36.6r 2.7

[a,b]End-point mortalities after 120- and 90-d storage, respectively; Actellic 5EC (1.00 PL/g grain) and soya oil (10.00 PL/g grain) caused 100% kill of all test insects after 120-d storage

Wheat seeds treated with *O. americanum* and *T. vogelii* fruit essential oils and positive controls, Actellic 5EC (1.00 PL/ g grain) and soya oil (10.00 PL/ g grain), recorded depressed germination over a 120-d storage period. Actellic 5EC and soya oil caused 27.5 and 56.8%, respectively, reduction (inhibition) of wheat seed germination after 120-d of storage compared to 39.9 and 49.1% for *O. americanum* and *T. vogelii* fruit essential oils.

Table 3: Effects of *O. americanum* and *T. vogelii* fruit essential oil treatments on percent germination of treated wheat seeds after 120-d storage period

Botanical treatment	Percent germination	% Reduction in germination[a]
Untreated control (0.00 PL/ g grain)	81.8±0.9	5.5±0.6
Actellic 5EC (1.00 PL/ g grain)	62.8±1.6	27.5±2.7
Soya oil (10.00 PL/ g grain)	37.4±1.9	56.8±3.3
O. americanum fruit oil (2.00 PL/ g grain)	52.0±1.1	39.9±1.5
T. vogelii fruit oil (2.00 PL/ g grain)	44.0±1.5	49.1±1.7

[a]Reference percent seed germination was 86.6% at 0 days of grain storage

The *T. vogelii* fruit essential oil had a higher (49.1% inhibition) inhibitory effect on wheat seed germination than *O. americanum* fruit essential oil (39.9% inhibition) after 120 d of storage. These results corroborate earlier findings in which essential oils had inhibitory (allelopathic) effects on germination and growth of both crop, including wheat seed, and weed species (Alves *et al*., 2004; Mao *et al*., 2004; Liu *et al*., 2006). Although plant essential oils have broad-spectrum bioactivity, are relatively safe to use and are eco-friendly, their packaging for use in tropical agriculture should consider their compatibility with domestic food preservation and applicability in informal seed sector.

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Evaluation of Botanicals as Maize Protectant against Maize Weevil, *Sitophilus zeamias* motsch

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Abstract

In an attempt to formulate biodegradable and safe insecticides from botanicals materials, essential oil extracts of *Tagetes minuta*, *Chenopodium album*, *Artemisia absinthium* and *Tarchonanthus comphoratus* were screened for insecticidal properties. Essential oil of succulent stem and leaves of *T. minuta*, *C. album*, *A. absinthium* and *T. comphoratus*, obtained from hydrodistillation were analysed by GC and GC-MS. The mortality of essential oil of *T. minuta* and *A. absinthium* against *S. zeamais* were 100% within 24 hours period of exposure at a dose of 1.0%. Thujone present in essential oil of *A. absinthium* (6.9%), d-limonene (8.1%) and eugenol (0.35%) in *T. minuta* have been reported to have bioactivity against *S. zeamais*. The mortality of the formulated product was not significantly different from the actelicsuper ™, a chemical insecticide in the market. The botanicals are therefore promising alternative to the conventional insecticide for the control for the control of stored-product pest, *S. Zeamais*.

Introduction

The food insecurity is a global crisis and FAO has warned of increase in food prices (Stacey *et al.*, 2007). About 35% of crops all over the world are destroyed by insect pests (Shani, 2000). About 60-70% of all grains produced in tropics are stored at farm levels (Golop *et al*., 1999). The chemical insecticides used in stored products, are environmentally unfriendly (Khalequzzaman and Farhana, 2003). There is need for bio-insecticides with greater attention being given to vegetable oils, plant extracts and silica-based inert dusts which are eco-friendly (Khalequzzaman and Farhana, 2003). The use of these is the reason behind active research on the scientific basis for their active constituents and establishment of the best formulations that boost and lengthen their performance.

Literature Summary

Tagetes minuta* L. (**Asteraceae). Volatiles from three species of the genus *Tagetes; minuta, patula* and *erecta* are highly effective toward both larvae and adult mosquitoes (Weaver *et al*., 1997). ***Artemisia absinthium* L. (**Asteraceae) The plant has long been studied for its chemical compositions and insecticidal activity of essential oils (Derwich *et al*., 2009). *A. absinthium* has been the object of several studies especially for its contents of such compounds as thujone isomers and chamazulene with pharmacodynamic properties (Anne *et al*., 2006).Chenopodium album* L.** (Chenopodiaceae). It is reportedly used as traditional protectants for stored beans and groundnuts in Congo (Delobel and Malonga, 1987). ***Tarchonanthus camphoratus* L. (**Asteraceae). The major compounds reported include α-fenchyl alcohol, α-terpineol, 1, 8-cineole and camphor (Mwangi and Achola, 1994).

Description of Research

The essential oils of the plant were extracted by hydro distillation. The experiments conducted were: **a) Effect of essential oil extracts on mortality.** Mortality bioassays were carried out by exposing the *S. zeamais* to the essential oil of *T. minuta*, *C. album*, *A. absinthium* and *T. comphoratus*. Maize grains were mixed with pure acetone containing essential oil so as to give doses of 0.5% (v/w) and 1.0% (v/w). Observation was then made after 24, 48, 72, and 96 hours. A negative control was pure acetone treated maize grains while Actelic Super ™ a positive control. **b) Preparation of insecticide formulations.** For different tests, four doses of the mixture (1.25, 2.5, 5 and 10%) were formulated as powder. The products were DE/AB, Diatomaceous earth and essential oil of *A. absinthium,* and DE/TM, Diatomaceous earth and essential oil of *T. minuta*. The number of dead and live *S. zeamais* was counted daily for five days. **c) Assessment of the weight loss (damage) to the maize grains treated with the formulated product on the field for the period of 12 months.** One (1) kg Maize grains were disinfected and prepared into samples bags for field trials in Kericho, Kenya. The method of Mutambuki *et al*., (1999) was adopted with modification. The grains were treated with the formulated product of diatomaceous earth and *A. absinthum* oil (5%), diatomaceous earth alone (5%), a formulated product of diatomaceous earth and *T. minuta* oil (5%), *T. minuta* oil alone (0.5%) and *A. absinthum* oil alone (0.5%) and the untreated control. Actelic super^{TM} at the recommended dose of 50 g/90 kg was used as a positive control. After every two months, level of damage was determined using the method of FAO (FAO, 2011).

$$\%\ weight\ loss = \frac{(UNd)-(DNu)\times 100}{U(Nd+Nu)}$$, Where U = weight of undamaged grains,

Nu = number of undamaged grains, D = weight of damaged grains, Nd = number of damaged grains.

Research Results and Application

Effect of essential oils on the mortality of *S. zeamais*

The effect of essential oils of four plants; *A. absinthum, T. minuta, C. album* and *T. comphoratus* on the mortality against *S. zeamais* was determined for 72 hours. The results are shown in the table 1

Table 1: Cumulative mean percentage mortality (±SE) for essential oil at 0.5% and 1.0% dose 24, 48 and 72 HAT

Duration	**24 HRS**		**48 HRS**		**72 HRS**	
Dose level	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%
sample (n=5)	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T. minuta	74.00±11.22^{Ac}	100.00±0.00^{Aa}	83.00±7.68^{Ac}	100.00±0.00^{Aa}	85.00±6.12^{Ab}	100.00±0.00^{Aa}
C. album	12.00±4.64^{Aa}	87.00±7.00^{Aa}	32.00±4.36^{Ba}	91.00±6.00^{Aa}	48.00±4.36^{Ca}	93.00±4.36^{Aa}
T.camphoratus	48.00±11.14^{Ab}	88.00±5.83^{Aa}	62.00±8.00^{Ab}	96.00±2.45^{Aa}	81.00±5.34^{Ab}	98.00±1.22^{Aa}
A. absinthum	100.00±0.00^{Ad}	100.00±0.00^{Aa}	100.00±0.00^{Ad}	100.00±0.00^{Aa}	100.00±0.00^{Ac}	100.00±0.00^{Aa}

Mean values with the same capital letters within the same row and same small letters within the same column are not significantly different at 95% confidence level.

A. Absinthum caused 100% mortality 24 hours after treatment at dose level of 0.5%. Mortality of the *S. zeamais* increased with the increase concentration from 0.5% to 1.0%. Toxicity of essential oils is reportedly attributed to effects of different terpene constituents of essential oils to *S. zeamais* (Bekele, 1994).

Mortality of *S. zeamais* as influenced by DE/AB, the Formulated product of Diatomaceous earth and *A. absinthium* oil

The effect of the formulated product, DE/AB, on the mortality of *S. zeamais* at a dose of 1.25% progressively increase from day two with a mortality of 3% to a cumulative mortality of 83% after five days (Table 2).

Table 2: Mortality of *S. zeamais* as influenced by DE/AB, the Formulated product of Diatomaceous earth and *A. absinthium* oil

Sample(n=5)	Dose	Day 1 %Mean±SE	Day 2 %Mean±SE	Day 3 %Mean±SE	day 4 %Mean±SE	day 5 %Mean±SE
DE/AB	1.25%	0.00±0.00	3.00±2.00	19.00±3.32	56.00±6.96	83.00±7.35^{b}
	2.5%	0.00±0.00	6.00±1.87	27.00±2.55	69.00±7.14	97.00±2.00^{c}
	5%	0.00±0.00	12.00±3.39	33.00±6.44	67.00±6.82	99.00±1.00^{c}
	10%	0.00±0.00	15.00±3.54	38.00±5.39	77.00±2.00	99.00±1.00^{c}
DE	5%	0.00±0.00	5.00±3.16	24.00±4.30	62.00±4.36	87.00±2.00^{b}
	10%	0.00±0.00	2.00±1.22	23.00±6.63	63.00±10.32	98.00±1.22^{c}
acetone		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00^{a}
untreated		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00^{a}

Mean values with the same letters within the same row are not significantly different at 95% confidence level

Assessment of the weight loss (damage) to the maize grains treated with the formulated product on the field for the period of 12 months.

Clearly, the low level of damage of the grains treated with DE/AB and DE/TM is shown from the second month to the twelve month of storage. After a period of one year the protecting ability of the DE/AB and DE/T.M was comparable to that of actelic super$^{™}$, the most widely used chemical insecticide in the market. Maize grains treated with DE/AB had weight loss of 4.11%, DE/ T.M had 4.33% and actelic super ™ 4.35%. The untreated control had a weight loss of 18.0% (Table 3). From the results DE/AB had th e lowest weight loss in the 12 months period and therefore a better protectant than the other treatments. There was no significant different (α=0/05) between the !ctelic super, DE, DE/TM and the DE/!B in the same period of time/

Table 3: Mean weight loss of maize grain of formulated products under field trials for a period of 12 months

SAMPLE	2 month Mean± SE	4 Months Mean± SE	6 month Mean± SE	8 months Mean± SE	10 month Mean± SE	12 months Mean± SE
Control	2.83±0.09	5.53±0.42	7.85±0.45	11.05±0.88	16.00±0.41	18.00±0.41[c]
Actelicsuper.	0.20±0.09	1.53±0.19	1.93±0.09	2.63±0.13	3.65±0.06	4.35±0.09[a]
DE(T.M)	0.47±0.27	1.05±0.03	2.03±0.05	2.78±0.09	3.43±0.11	4.33±0.19[a]
DE	0.69±0.06	0.93±0.06	2.33±0.09	2.65±0.13	3.63±0.21	4.25±0.03[a]
DE(AB)	1.20±0.14	1.58±0.09	1.88±0.09	2.60±0.11	3.35±0.06	4.10±0.09[a]
A.a Oil	1.38±0.09	2.15±0.20	2.60±0.04	3.25±0.38	4.18±0.09	6.48±0.26[b]
T.M oil	1.38±0.05	2.40±0.04	2.68±0.06	3.55±0.13	4.13±0.09	6.08±0.31[b]

Mean±SE followed with the same small letter within the same column are not significantly different at α=0/05/The means shows % weight loss/
DE (T.M) =Diatomaceous earth and *T. minuta* essential oil mixture; T.M= *T. minuta*
DE (AB) =Diatomaceous earth and *A. absinthium* essential oil mixture; A.a= *A. absinthium*
DE =Diatomaceous earth alone

Based on the GC profiles and the GC-MS of essential oils of *T. minuta*, *C. album*, *T. comphoratus* and, *A. absinthium*, the following major compounds were identified(Table 5,6, 7 and table 8, respectively).

Table 5: List of identi fied compounds from *T. minuta* essential oil

Compound	% of the total
2,6-dimethyl-, (E)-5,7-Octadien-4-one	10.5
3,7-dimethyl-, (E)-1,3,6-Octatriene	8.5
d-Limonene	8.1
Eugenol	0.35

Table 6: List of the compounds identified from *C. album* essential oil

Compound	% of the total
4-ethenyl-/α/,/α/,4trimethyl-3-(1-methylethenyl)-,[1R-(1/α/,3D.,4.E.)]-Cyclohexanemethanol,	3.0
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	2.9
Caryophyllene	3.5

Table 7: List of the compounds identified from *T. comphoratus* essential oil

Compound	% of the total
α/,/α/4trimethyl-3-Cyclohexene-1-methanol	14.2
4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol	5.0
Borneol	3.9

Table 8: List of the identified compounds from *A. absinthium* essential oil

Compound	% of total
Thujone	6.9
1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-1,6-Cyclodecadiene	5.7
β-Terpineol,	0.6

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Evaluation of the Indigenous Pesticidal Plant, *Maerua Edulis* (De Wolf) Against Four Storage Insect Pests

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Abstract

Insect pests cause severe damage to grain in smallholder stores resulting in loss in weight, seed viability and nutritive quality. *Maerua (decumbens) edulis*, used traditionally as a grain protectant by some smallholder farmers in Zimbabwe and other parts of Africa, was evaluated against *Sitophilus zeamais*, *Prostephanus truncatus* and *Rhyzopertha dominica* in maize and *Callosobruchus rhodesianus* in cowpeas. The results are discussed in the context of how the pesticidal plant can be an effective option for resource-poor farmers to protect their grain against storage insect pests.

Introduction

Insect pests cause severe damage to stored commodity resulting in loss in weight, seed availability and nutritive quality of food stuffs (Maribet *et al,* 2008). Grain losses contribute to food insecurity and low farm incomes in sub-Saharan African countries (Compton, 1992; Azu, 2002). Therefore, efficient post -harvest handling, storage and marketing can tremendously contribute to social economic aspects of rural c ommunities (Compton, 1992). Modern pesticides have helped to control and reduce crop and livestock losses to a remarkable degree. The use of these pesticides has created some of today's major environmental and health problems which includes reduction in the abundance and diversity of wildlife, human health hazards associated with acute or chronic exposure to dangerous products in the workplace, and contaminated air, food and water. Presently many small-scale farmers rely on imported organo -phosphate based pesticides to protect stored grain, but farmers and various authorities are increasingly questioning the safety and efficacy of these chemicals. Subsistence farmers often lack the financial resources to buy good quality commercial insecticides to protect their stored food hence use of locally available traditional protectants like indigenous plants is a rationale decision. However, there is lack of reliable scientific information on the locally available pesticidal plants such as *Maerua edulis.*

Literature Summary

According to a World Bank Report (2011), grain losses after harvests in sub -Saharan Africa (SSA) cost about $4 billion a year and food lost to decay and pest infestation could feed 48 million people. This value of grain losses in the region is out of an estimated annual grain output of $27 billion based on 2005-2007 annual averages, and is roughly equivalent to the value of annual cereal imports in the region (World Bank, 2011). The food security and income opportunities of many rural households in SSA are seriously undermined by storage insect pests. Presently many small-scale farmers rely on imported organo -phosphate-based pesticides to protect stored grain, but farmers and various authorities are increasingly questioning the safety and efficacy o f these chemicals. Being xenobiotics, organophosphate- based pesticides damage the environment. Pesticidal plants can provide a low cost and safer alternative to synthetic pesticides (Stevenson, 2001). Other households, who use traditional materials such as ashes, botanicals and sand to control storage insect pests, are faced with inconsistent and often poor results (Mvumi, *et al*, 2006). However, farmers need reliable information on pesticidal plant to support their decision making with respect to the quality of control they can expect when using a particular plant material (Ary and Perell, 2010). The use of wild plant materials gives an incentive and economic value associated with natural habitats and generates a competitive reason for their maintenance. The economic potential of pesticidal plants can, therefore, be used to enhance environmental management and conservation (Anonymous, 1991). Researchers need to investigate pest control methods that are more compatible with the goals of a sustainable, productive, stable and equitable agriculture. To meet these aims, research should seek to integrate a range of complementary pest control methods in a mutually enhancing fashion.

Description of Research

Two experiments were carried out at the Department of Cr op Science, University of Zimbabwe. One of the experiments was a bioassay which was meant to assess the efficacy and optimum application rate of a pesticidal plant whilst pot experiments were meant to simulate the real storage conditions since most communal farmers' store their grain in solid walled structures/ In the tests, the pesticidal plant material used was *M. edulis* (Sozwe) compared to Actellic Super Gold and Actellic Gold (commercial synthetic pesticides). Shade-dried *M. edulis* were ground and sieved to obtain a finer powder. The plant p owder was then weighed to their relevant quantities for treating maize and cowpea. The insect pests of stored grain used, were *Sitophilus zeamais* (maize weevil), *Prostephanus truncatus* (LGB) *and Rhyzopertha dominica* (Lesser grain borer), in maize and *Callosobruchus rhodesianus* (cowpea weevil) in cowpeas. (a) ***Bioassay***- Plant material was applied to 50g of the grain at concentrations of 0, 0.25, 0.5, 1, 2, 3.5, 4, and 5% w/w. The synthetic pesticide was applied as indicated on the label (0.05% *w/w*). Each treatment was replicated three times (in both maize and cowpeas). Mortality assessments were done at 7 day intervals from the start of the experiment. One experimental period 21days except for *C. rhodesianus* this ran for 14 days. F1 emergence assessments were also made. (b) ***Clay pot experiment***- Plant material was admixed with grain (dosage – 0, 0.5, 1, 2, 4 and 6%w/w) (maize and cowpeas respectively) in claypots having 500g of each grain type. ASG the positive treatment was applied at label rate (0.05% w/w). In maize, 40 unsexed insects (20 *P. truncatus* and 20 *S. zeamais*) were introduced into each 500g of maize. For cowpeas, 20 *C. rhodesianus* insects were introduced per pot. All pots were closed using fine metal wire gauze tightly fitted on the pot mouth. Each treatment was replicated three times (in both maize and cowpeas). Sampling was done after every four weeks for 12 weeks.

Samples were analyzed for insect grain damage and insect population. At the conclusio n of the experiment percentage weight loss was calculated.

Research Results and Application

Bioassay results- Insect mortality of *C. rhodesianus* was above 80% in the first week and by the second week insect mortality ranged between 95 -100%. Untreated control had mortality above 50% by the second week. ASD attained 100% mortality by the first week. In maize, insect mortalities of *S. zeamais* increased steadily from about 80% in the first week to a 100% in the third week for all plant treatments. Untreated control mortality was below 40% mortality in the third week. ASD had 100% mortality on the first week. There was significant difference between ASD and all plant treatments and by the second week (p<0.001). Significant difference was found between all other treatments and the untreated control (*P*<0.05).

R. dominica, mortalities increased with increased exposure period (Fig 1). In the first week in sect mortality were above 20% for all plant based treatments except for the untreated control. ASD resulted in 100% mortality by the end of the first week. *M. edulis* 4%w/w effected the highest mortality (80%) by week 3.

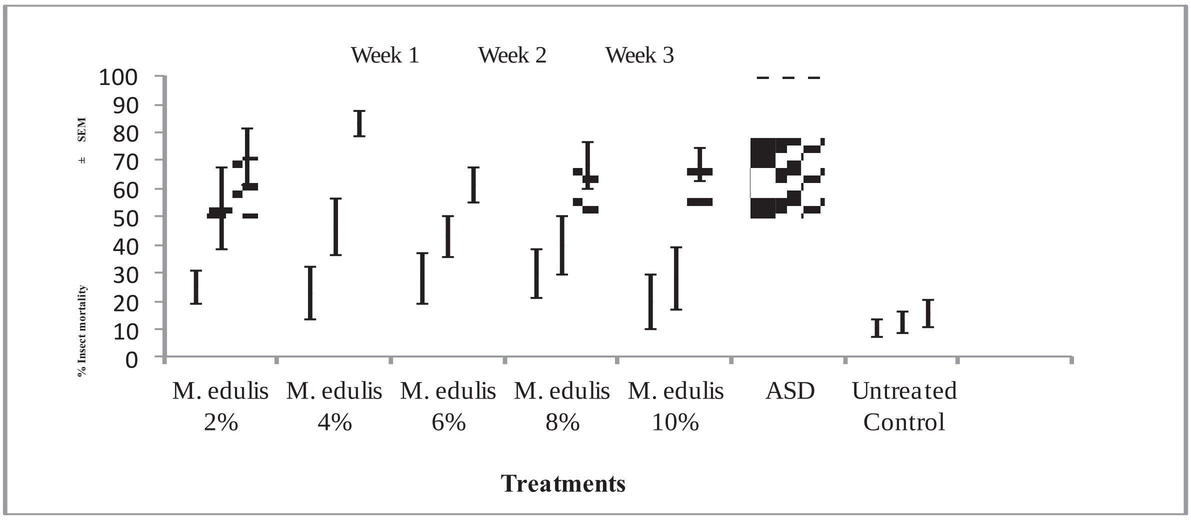

Figure 1: Mean % adult insect mortality of *R. dominica* in maize treated with *Maerua edulis* at different concentrations (n=3)

As shown in Fig 1, 100% mortality of the insect *P. truncatus* was effected by the ASD in the first week. Of the *M. edulis* treatments, 8% and 10%w/w application rates only had mortalities which were above 20% with the 8%w/w application rate having the highest mortality which reached 40% by the third week. For all other treatments, a significant rise in mortality was noticed o n the third week.

The combined results of all the insects' F1 generation (Fig 2) show that no insects emerged from the grain treated with ASD. There are significant differences between the untreated control and the plant based treatments (*P*<0.001). *P. tr uncatus* had the lowest emergence when compared to all the insects. There was a significant difference between ASD and all the other treatments (*P*<0.001).

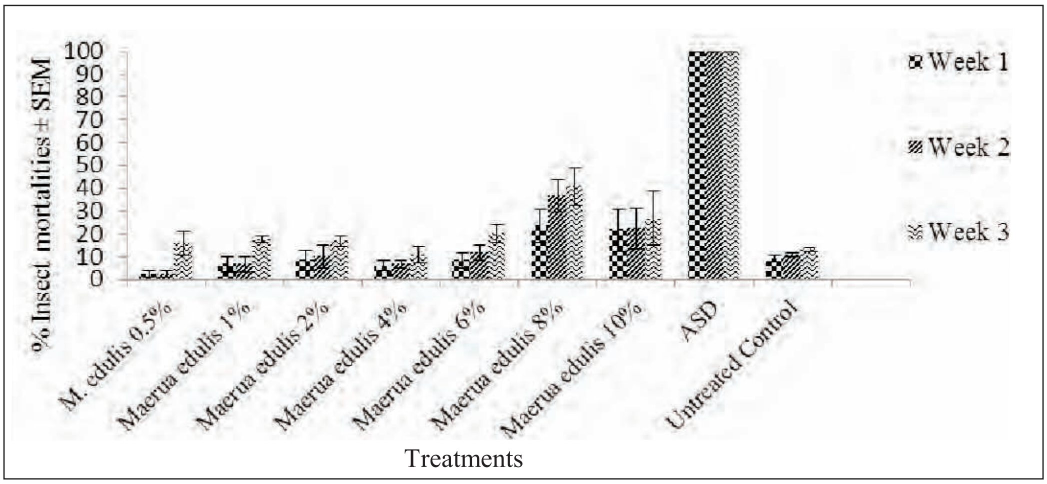

Figure 2: Mean % adult mortality of *P. truncatus* in maize treated with *Maerua edulis* at different concentrations compared to Actellic Super Dust (ASD) (n=3)

Pot experiments results- Cowpea **-**Grain damage on the onset of experiment was around 10% (Fig 3) which then increased to about 15% by week 4. Week 8 the untreated control had above 30% damage with all other treatments having less than 25% grain damage with the *M. edulis* 1% recording the least damage. In the 12^{th} week, *M. edulis* 1% treatment recorded the least damage which was ≤30%/ *M. edulis* 4% had the highest grain damage of 50% amongst the plant treatments. Grain damage increased steadily up to week 24 with the *M. edulis* 4% having the highest grain damage (≤65%) whilst the *M. edulis* 1% had the lowest (≤ 40%) amongst the plant based treatments/ !SG had the lowest grain damage of ≤20%, whilst the untreated control had ≥70%/ Amongst the plant based treatments, 1%w/w recorded the lowest weight loss. Increasing the application rate from 1%w/w to 6% w/w resulted in no significant change in the weight loss (*P*>0.05). Significant difference was noticed between the plant treatments and ASG (*P*<0.05).

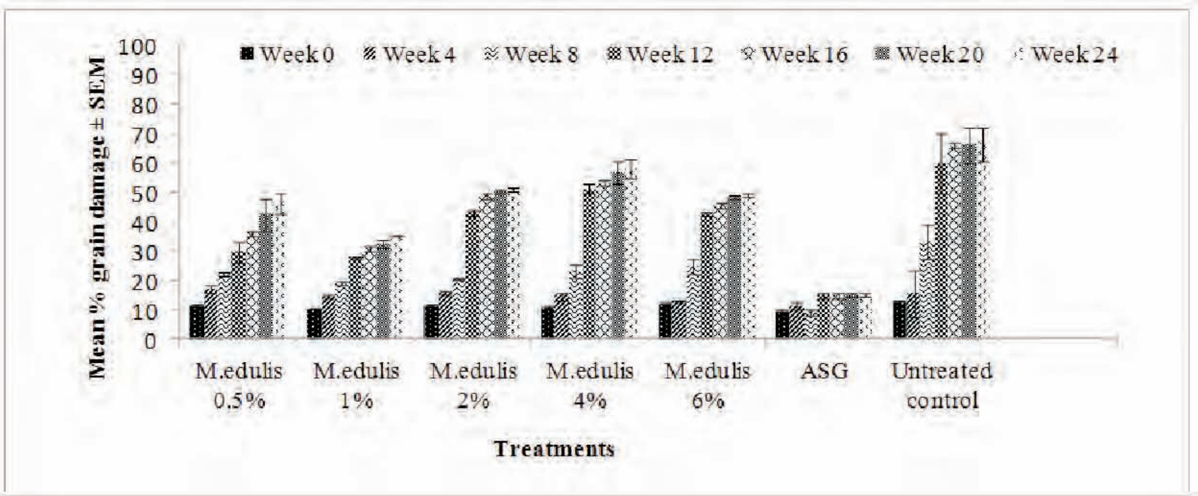

Figure 3: Mean % damaged cowpeas grain treated with *Maerua edulis* at different concentrations compared to and Actellic Super Gold (ASG) (n=3)

Maize- !ll treated grain had ≤10% damage (Fig 4)/ During the first 4 weeks, less than 10% grain damage was recorded on all treatments except for the untreated control. In the 8^{th} week 1% and 2% treatments had ≤20% damage/ Plant treatments 0/5% and 4% had ≥ 20% grain damage/ From the 12^{th} week, there was a gradual increase in grain loss across the treatments/ Week 24, all treatments had grain damage ≤45% except for the ASG. There were significant differences between ASG and all plant based treatments (*P*<0.05) as well as a significant difference between all plant based treatments and the untreated control (*P*<0.05). *M. edulis* 2% w/w amongst the plant based treatments had the lowest damage weight loss after 24 weeks (≤15%)/ There were significant differences between ASG and all plant based treatments (*P*<0.05). *M.edulis* was not as effective as the commercial synthetic pesticide ASG, the plant exhibited knock down effects on *C. rhodesianus,* and *S. zeamais*. However, *M.edulis* takes more time to achieve same results as ASG which allows the pests to oviposit for future infestation. The plant did not exhibit a classical dose dependency and efficacy of *M. edulis* lasts for short periods (12weeks).

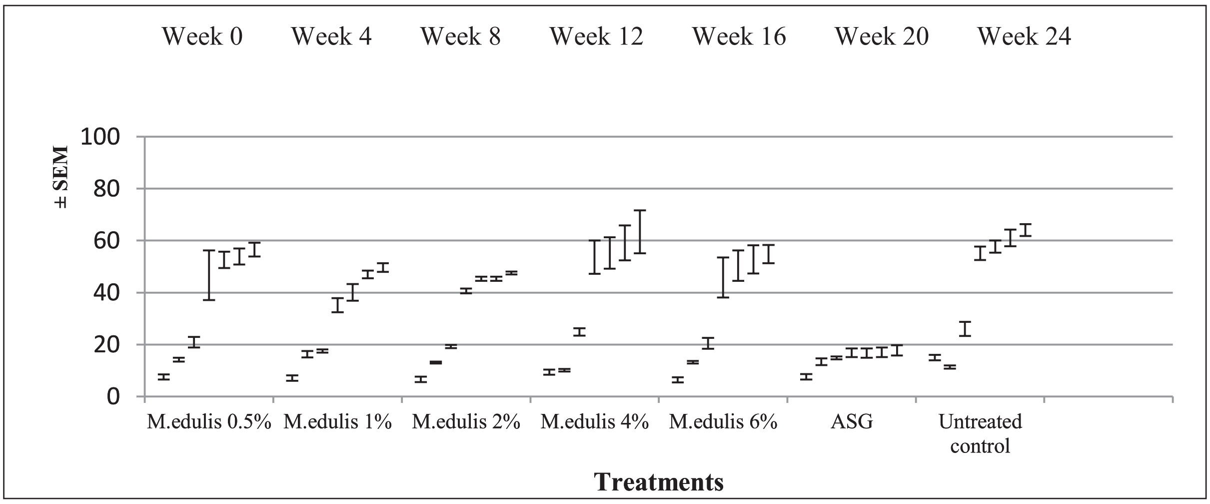

Figure 4: Grain damage of maize grain treated with *Maerua edulis* at different concentrations compared to Actellic Super Gold (ASG) (n=3)

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Evaluation of *Mucuna Pruriens* (Dc) Seed as a Potential Biopesticide Against C*allosobruchus Maculatus* (Fab.) In Stored *Vigna Unguiculata* (Walp.) Seeds

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Abstract

The pesticidal effect of *Mucuna pruriens* (L.) DC. (Fabaceae) was evaluated against *Callosobruchus maculatus* F. (Bruchidae). Plant secondary metabolites such as alkaloids, oils and phenolics are known for their pesticidal properties. This work was carried out to investigate the possible pesticidal effect of *Mucuna pruriens* seed powder when incubated with *Vigna unguiculata* (L.) Walp (Fabaceae) seeds. The antifeedant activity of *Mucuna pruriens* seed powder was determined as 88.35%. *Mucuna pruriens* powder might have caused damages to body systems of the insect by various mechanisms. The seed powder of *Mucuna pruriens* has been found to be effective in causing death of the weevils. The result of this work gave strong evidence that the seed powder of *Mucuna pruriens* has pesticidal properties against *Callosobruchus maculatus*. It may be useful in the conservation/storage of grains and seeds, especially under the subsistent farming system.
Keywords: Antifeedant, Biopesticide, *Callosobruchus maculatus*, *Mucuna pruriens*, *Vigna unguiculata,* Weevils

Introduction

Plants have provided a wide selection of biologically active compounds to man that include insecticidal properties. They are composed of chemical substances of which some are not directly beneficial for the growth and development of the organism. These secondary compounds are usually regarded as a part of the plants' defense against plant-feeding insects and other herbivores (Schmutterer and Ascher, 1984). Plant constituents such as alkaloids, oils and phenolics are also known for their insecticidal properties.

Literature Summary

Mucuna pruriens (L.) DC. (Fabaceae) is an annual, climbing shrub with long vines that can reach over 15 m. Seeds contain high concentrations of levodopa, a direct precursor of the neurotransmitter dopamine. It has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease (Manyam, *et al.*, 2004).

The aim of this study was to investigate pesticidal effect of *Mucuna pruriens* seed powder against *Callosobruchus maculatus* F. (Bruchidae) in stored *Vigna unguiculata* (L.) Walp (Fabaceae) seeds.

Description of Research

Design of the experiment was completely randomized in three replications involving *Mucuna pruriens* seed extract. Twenty cowpea seeds from the rearing cylinder were counted and introduced into each of the sterilized glass vials before the various treatments being investigated were added and spread by gently shaking up every vial. Increase in surface area for activity of powder extract was therefore achieved without harm to the incubated seeds or pest under investigation. The vials for *Mucuna pruriens*, *Azadirachta indica* and Pirimiphos-methyl, each containing 0.5 g of the various powder extracts, were obs erved for mortality with the introduction of weevil-infested cowpea seeds.

Results

Acute toxicity test

The LD_{50} obtained for *Mucuna pruriens* was 1300 mg/kg (LD_{50} range of 501-5000 mg/kg: slightly toxic).

Table 1: Antifeedant property and other physicochemical parameters measured

Powder extract	Antifeedant property (%)	Moisture content of powder extracts (%)	pH of aqueous powder (at 25^{0}C)
Mucuna pruriens seed	88.35±0.08	8.4±0.2	8.76±0.03
Azadirachta indica leaf	90.10±0.17	3.9±0.1	5.81±0.13
Combination of *Mucuna* and *Azadirachta* powders	49.83±0.23	5.0±0.4	5.67±0.18

Moisture content of Cowpea seeds (*Vigna unguiculata*) = 10.93%

Values are expressed as mean ± s.e.m. (standard error of mean)

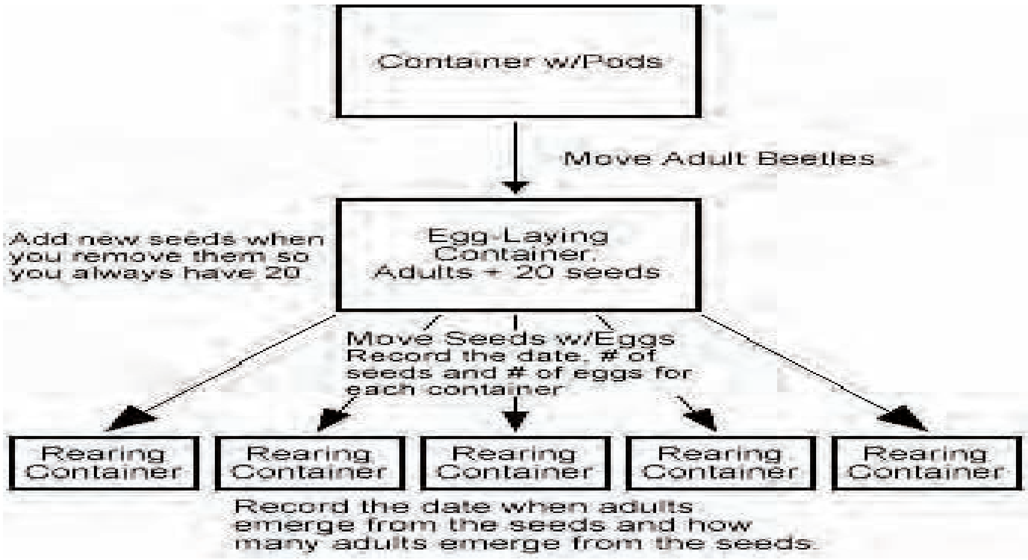

Figure 1: Diagram illustrating the breeding procedure for cowpea weevils (Breeding Weevils, 2008).

*Twenty weevils (ten males and ten females) were harvested and used for each experiment performed on pest mortality studies. Sex identification was determined as described during the breeding procedure.

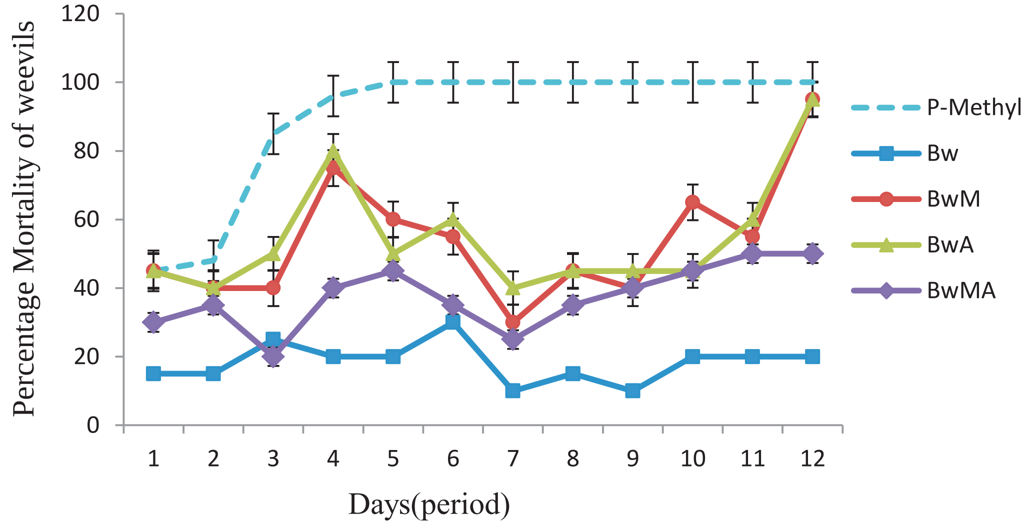

Figure 2: Percentage mortality of *Callosobruchus maculatus* with respect to Duration of Exposure to *Mucuna pruriens* in *Vigna unguiculata* Seeds (n=20 per group)

Key

P-Methyl – Pirimiphos methyl, Bw – untreated weevil-infested *Vigna unguiculata* seeds

BwM – *Mucuna pruriens* seed powder, BwA – *Azadirachta indica* leaf powder, BwMA - *Mucuna pruriens* seed powder *and Azadirachta indica* leaf powder combined.

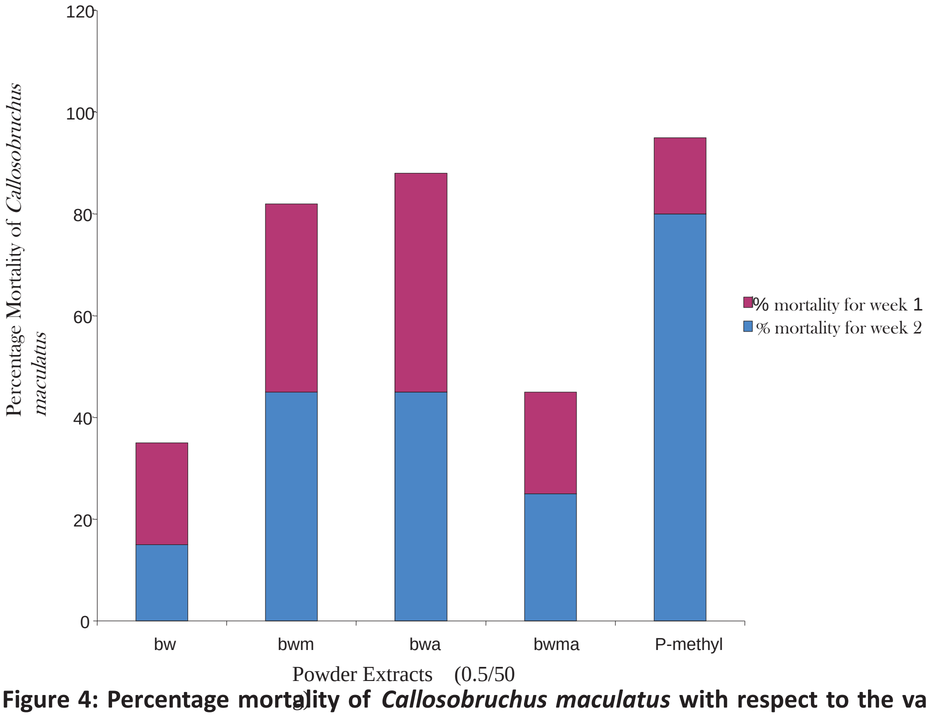

Figure 4: Percentage mortality of *Callosobruchus maculatus* with respect to the various treatments applied to *Vigna unguiculata* seeds

Footnote. Using student's *t*-test ($p<0.05$), for treated versus control (n=20 per group).

Key

bw – untreated weevil-infested *Vigna unguiculata* seeds, bwm – *Mucuna pruriens* seed powder, bwa – *Azadirachta indica* leaf powder, bwma – *Mucuna pruriens* seed powder *and Azadirachta indica* leaf powder combined, P-methyl – Pirimiphos methyl

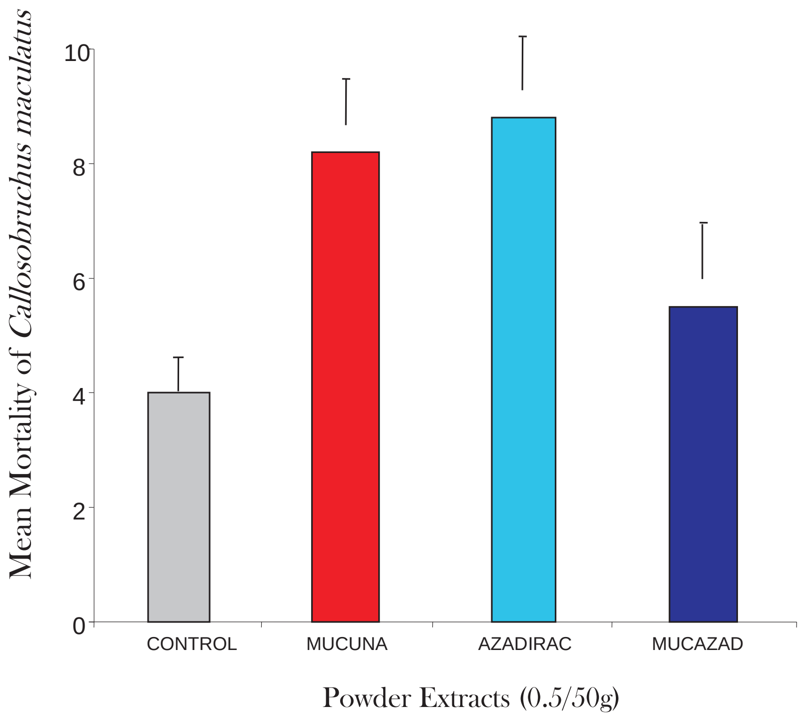

Figure 5: Evolution of Mean Mortality of *Callosobruchus maculatus* adults with respect to the various treatments applied to *Vigna unguiculata* seeds

Footnote: Using student's *t*-test ($p<0.05$), for treated versus control (n=20 per group).

Values are expressed as mean ± S.E.M (┬).

Key: MUCUNA – *Mucuna pruriens* seed powder, AZADIRAC – *Azadirachta indica* leaf powder, MUCAZAD - *Mucuna pruriens* seed powder *and Azadirachta indica* leaf powder combined, CONTROL – Untreated weevil-infested *Vigna unguiculata* seeds

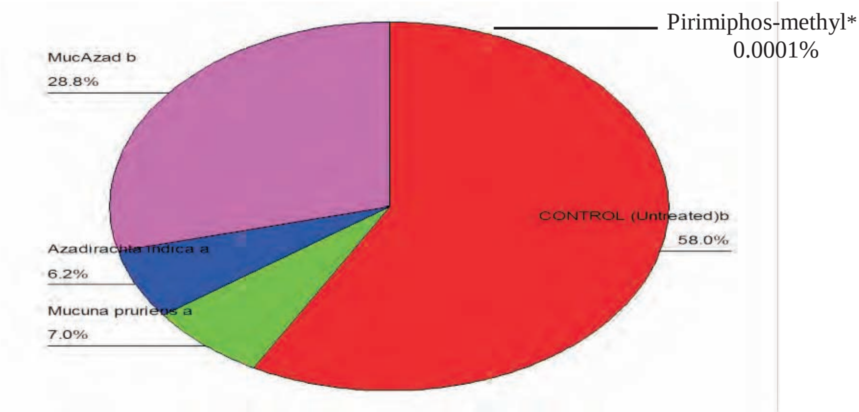

Figure 6: Percentage Average Weight Losses of *Vigna unguiculata* Seeds after Treatment with Various Powder Extracts for Ninety Days †

a- Significant difference (p<0.05)

b- No Significant difference (p>0.05), for treatment versus control, using Student's *t*-test, for treated versus control. Values are expressed in percentages. (n=20 per group)

*Pirimiphos-methyl gave very small values that could not be shown in this chart. The Pie chart revealed the extent of damage the weevils were able to inflict on seeds for each treatment. Adopted and modified after Nakanishi and Kubo (9), as method for assessment of antifeedant property for *Mucuna pruriens*.

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Activity of extractives from *Albizia anthelmintica* Brongu. and *Teclea trichocarpa* Engl. as biorational alternatives to control the maize weevil (*Sitophilus zeamais*)

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Abstract

Organic solvent extractives and isolated compounds of two Kenyan plants *Albizia anthelmintica* and *Teclea trichocarpa* were evaluated for insecticidal activity against maize weevil, *Sitophilus zeamais* Motchulsky, and for brine shrimp lethality. Hexane extract of the leaves of *Teclea trichocarpa* displayed mild brine shrimp toxicity (LD_{50} =153.2 Pg/ml), while the other extracts showed no significant toxicity (LD_{50}! 240 Pg/ml). Both hexane and dichloromethane extracts of leaves of *Teclea trichocarpa* showed the highest mean percentage adulticidal activity at almost all doses. Three-acridone alkaloids melicopicine **1**, normelicopicine **2**, arborinine **3** and the furoquinoline, skimmianine **4** were isolated from *Teclea trichocarpa* and had previously been reported from the other *Teclea* species were noted to have low mortality of between 10% and 22% at 0.1% w/w of the compounds against *Sitophylus zeamais*. The other metabolites were the two terpenoids, β-sitosterol **5** which showed higher activity (12.5±2.5) than D-amyrin **6**. The results are discussed with regard to the use of the two plants as suitable and sustainable alternatives to synthetic insecticide in maize grain storage.

Introduction

Sufficient production of food grains pose a big challenge to man. A variety of techniques have been applied to meet the challenge. One of the strategies is to improve efficiency in grain production and post harvest practices to ensure that food losses are minimized if not eliminated and that the grains produced is of good quality and safe for human consumption. Maize weevil (*Sitophilus zeamais* Motchulsky) is one of the most damaging insect pests of stored maize grains. Prophylactic methods have not constrai ned the pests to acceptable levels. Synthetic pesticides have been used against post-harvest pests. However, the persistence, resistance, prohibitive cost and availability of these conventional insecticide and potential health hazard both to the consumers and to the environment make them unattractive and have necessitated continued use of local plant products (Negahban, *et al*., 2007). Although these botanicals have been in use since time immemorial their efficacy, safety and their active principles deserve more attention (Balandrin *et al*., 1985; Bakkali *et al*., 2008). In present work, two Kenyan native plant extracts and thereof isolated compounds were investigated for their insecticidal property against maize weevil and toxicity, for possible incorporation as a strategy of improving food security in the communities.

Literature Summary

Food quality and security pose a big challenge to agricultural activities and in providing mankind with sufficient food at all times. Tropical countries suffer severe losses of stored food products due to pests. This is partly attributed to conducive climatic conditions. Apart from other causes of food losses like crop diseases and weeds, pre- and post-harvest pests are responsible for ~40% of !frica's food losses (Mandava, 19 85). With the rising concern for environmental safety coupled with the resistance of the maize weevil against many synthetic insecticides. There has been a renewed interest in the use of naturally occurring substances as pesticides, and attention focused on evaluating the efficacy and identifying the active components of ethnobotanical materials used in grain protection. Plants have been screened for repellency and protectants against maize weevil (Lwande *et al*., 1983, Ndungu *et al.,* 1999). This approach has advantages in pest control because natural products are renewable, more selective and less resistant to biological control, biodegradable and may be transformed into products that are not harmful to the environment. Natural products are still a vastly under-utilized resource for practical insecticides, yet we live in a world where most pests can be controlled by these natural products. *Albizia anthelmintica* Brongu. and *Teclea trichocarpa* Eng. belong to the plant families Mimosaceae and Rutaceae, respectively. Traditionally, *A*. *anthelmintica* is widely used in East Africa by resource poor smallholder farmers and pastrolists to treat their livestock against internal parasites and against grain pests (Kokwaro, 1993). Several members of this genus have shown br oad range of activities, ranging from antibacterial to pest control. Water extract of *A. julibrissin* has shown insect feeding deterrence against *Tribolium castana* on leaf disc with a mean deterrence of 50% (Doskotch *et al*., 1977). Essential oil of *A. lebbek* bark showed insect repellant activity (0.125% against *Apii floreas*) (Gupta, 1987), while *A. mollis* bark showed insecticide activity against tobacco beetle (Sievers, 1949). Inhabitants of some arid and semi-arid region particularly from North Rift (Kerio Valley), Kenya are known to use the root bark of *A. anthelmintica* as grain protectant during storage. *Teclea trichocarpa* is reported to be used by traditional healers belonging to the Akamba tribe for malaria treatment and as anthelmintic, while the Giriama tribe steam the leaves and inhale the vapour as a cure for fever (Watt and Breyer-Brandwijk, 1962). The plant bark of the plant showed antifeedant activity against the African armyworm, *Spodoptera exempta* (Lwande *et al*., 1983). The leaves were reported to possess antiprotozoa activities against *Plasmodium falciparum* (Muriithi *et al.,* 2002), *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani* (Mwangi *et al.,* 2010).

Description of Research

(a) Plant materials: The root barks of *A. anthelmintica* were collected from Kiptoro area, Keiyo District, Rift Valley Province in August 2003. Leaves of *T. trichocarpa* were collected from Maina, Marakwet District in August 2004. *(b) Extraction, fractionation and isolation:* The air-dried, powdered root bark (2.7 kg) and leaves, (2.0 kg) of *A. anthelmintica* and *T. trichocarpa*, respectively, were extracted by maceration sequentially with 7.5 litres each of hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH) exhaustively at room temperature. Each extract was concentrated *in vacuo* under reduced pressure at 45^oC using a rotatory evaporator. The hexane extract (25.0 g) and dichloromethane extracts (48.5 g) of *T. trichocarpa* were subjected to vacuum liquid chromatography (VLC) separation on silica gel 60 each at a time, eluted with *n*-hexane with increasing amount of CH_2Cl_2 and later increasing amount of methanol in CH_2Cl_2 up to 1:5, 55 and 65 fractions, respectively, were collected and from TLC analysis similar fractions pooled together. UV active spots on TLC were considered for further separation. From the hexane extract of *Teclea trichocarpa*, chromatographed on sephadex and eluted with a mixture of CH_2Cl_2 and methanol (1:1), fraction 31-32 on crystallization in methanol afforded D-amyrin [**6**] (32.7 mg). Fractions 13-21 was further chromatographed on silica gel and eluted with 2:3 (*n*-hexane: CH_2Cl_2) this yielded E-sitosterol [**5**]. From VLC of the CH_2Cl_2 extract, fraction 34-40 (1014 mg) was loaded onto sephadex column and eluted with CH_2Cl_2: methanol (1:1) this afforded 38.1 mg of melicopicine [**1**]. Fraction 5-18 showed UV active spots, column chromatography of this fraction eluted with CH_2Cl_2: ethyl acetate (2:1) mixture gave 25 fractions from which sub fractions 9-11, 12-15 and 21-25 were followed. Sub fractions 9-11 were subjected to preparative thin layer chromatography, this afforded skimmianine [**4**] (22.2 mg). Sub fractions 21-25 was subjected to preparative thin layer chromatography, this afforded two compounds Melicopicine [**1**] (64.8 mg), and normelicopicine [**2**] (46.9 mg). Fraction 41-42 from VLC was subjected to column chromatography and eluted with ethyl acetate: CH_2Cl_2 (1:9) afforded yellow needle like compound, arborinine [**3**] (99.0 mg) on partitioning between methanol and CH_2Cl_2 (2:1).

(c) Brine shrimp toxicity test. The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer *et al*., 1982). Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Hundred microlitres of suspension of nauplii containing 10 larvae was added into each well of 96-well microtitre plates and incubated for 24 h. Sea salt water and DMSO only served as the drug free control. The number of dead napulii in each well counted and recorded. Lethality concentrations fifties (LC_{50} values) for each assay were calculated. *(d) Sitophilus zeamais* culture. Adult *S. zeamais* was obtained from a laboratory colony reared under ambient conditions with natural photoperiods on untreated (insecticide-free) maize obtained from farmers stock in Keiyo District, maintained at National Agricultural Research Laboratories (NARL), Kenya. Freshly emerged adults of *S. zeamais* were then used for the experiments (Asawalam and Emosairue, 2006). *(e) Adulticidal assessments.* Bioassay test was carried in the laboratory to determine the efficacy of the botanicals under different dosage lev els against

Sitophilus zeamais/ Synthetic insecticide, !ctellic Super™ 2% dust, at 0/05% w/w and untreated grains were included as positive and negative controls, respectively. For pure compounds and blend mixtures the concentrations were double, equal and half that of positive control (Actellic super). The test samples were mixed with talc thoroughly and the dust were admixed with 50 g of maize held in jam jags covered with ventilated lids. Ten pairs of 5-day old *S. zeamais* adults were introduced into treated and untreated maize grains. Percentage mean mortality for *S. zeamais* was recorded after seven days exposure period (Bekele *et al*., (1996). *(f) Statistical analysis*. Data were subjected to analysis of variance (ANOVA) procedure (SAS, 2000) and significantly different (P?0/05) means were separated by using Tukey's studentised range (HSD) test/

Research Results and Application

From the preliminary adulticidal test against maize weevil the methanol and ethyl acetate extracts showed no activity. Therefore, the hexane and dichloromethane crude extracts of *A. anthelmintica* and *T. trichocarpa* were further tested for their toxicity against brine shrimp. The hexane extracts of *T. trichocarpa* with LD_{50} values of 153.2 Pg/ml was considered active, while CH_2Cl_2 extracts of *T. trichocarpa* showed mild toxicity against brine shrimp with LD_{50} values of 279.9 Pg/ml (Table 1). The hexane and CH_2Cl_2 extracts of *A. anthelmintica* showed low toxicity against brine shrimp with LD_{50} values of 693 and 376 Pg/ml, respectively (Table 1). Since a crude is considered active up to a concentration of 240 Pg/ml, an indicator of toxicity, various pharmacological actions, and pesticidal effects (Meyer *et al*., 1982). It was deduced that both hexane and CH_2Cl_2 extracts of *T. trichocarpa* had the better bioactivity against brine shrimp.

Table 1: The mean LD_{99} and LD_{50} values r s.d. for plant extracts screened against brine shrimp (*Artemia salina*, Leach)

Plants extract	A*a*BH	A*a*BD	T*t*LH	T*tLD*
LD_{50}	692.9 ± 0.3	375.5 ± 0.6	153.2 ± 1.0	279.9 ± 0.7

A*a*BH = *A. anthelmintica* bark, hexane extract; A*a*BD = *A. anthelmintica* bark, dichloromethane extract
T*t*LH = *T. trichocarpa* leaves, hexane extract; T*tLD* = *T. trichocarpa* leaves, dichloromethane extract

Table 2: Percent mortality of adult *S. zeamais* on maize grains treated with different concentrations of hexane and CH_2Cl_2 extracts from *A. anthelmintica* and *T. trichocarpa* against maize weevil (*S. zeamais*)

Plants Extract	100 ppm	200 ppm	400 ppm	600 ppm	800 ppm
*Aa*BH	5.0 ± 5.0 c	10.0 ± 5.0 c	35.0 ± 5.0 c	45.0 ± 5.0 c	55.0 ± 5.0 b
*Aa*BD	15.0 ± 5.0 bc	15.0 ± 0.5 c	60.0 ± 0.0 d	65.0 ± 5.0 b	80.0 ± 10.0 a
*Tt*LH	25.0 ± 5.0 b	75.0 ± 5.0 b	75.0 ± 5.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a
*Tt*LD	25.0 ± 5.0 b	40.0 ± 0.0 d	45.0 ± 5.0 c	70.0± 10.0 b	95.0 ± 5.0 a
Actellic super	75.0 ± 5.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Negative control	5.0 ± 5.0 c				

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test)/

From table 2, it is evident that adulticidal activities of hexane and CH_2Cl_2 extracts of *A. anthelmintica* and *T. trichocarpa* on maize weevil are dose dependent. The hexane and CH_2Cl_2 extracts of *T. trichocarpa* were the most active extracts, showing 100% and 95% adulticidal at 600 and 800 ppm, respectively. *A. anthelmintica* extracts (*Aa*BD and *Aa*BH) displayed lower adulticidal activity. However, at 800 ppm *Aa*BD exhibited 80% mortality that was comparable to the actellic super at 95% confidence level. The low adulticidal activity of *A. anthelmintica* could partly be explained by the circumstances that may be it is used in protection, not for their insecticidal effect but may be due to feeding and ovipositional deterrence or even as insect growth regulators. The other reason is that the local communities use a mixture of plants for grain protection, and this mixture could be active due to synergistic effects. The TLC profile of *T. trichocarpa* revealed the presence of several UV active and fluorescing compounds in the crude extracts. Chromatographic separation of the hexane and dichloromethane extracts afforded two terpenoids (D-amyrin and E–sitosterol) and four alkaloids; melicopicine, arborinine, normelicopicine (acridone alkaloids) and skimmianine (furoquinoline alkaloid). The compounds thus isolated were tested against maize weevil (adulticidal) at different doses; results are summarized in Table 3.

Table 3: Mean percentage adulticidal ± s.d. of isolated compounds from *T. trichocarpa* against maize weevil

Compounds	Mean percentage adulticidal at different concentration in w/w	
	0.1 w/w	0.05 w/w
Melicopicine [1]	12.5 ± 2.5 c	2.5 ± 2.5 c
Normelicopicine [2]	15.0 ± 0.0 a	7.5 ± 2.5 ac
Arborinine [3]	22.5 ± 2.5 a	10.0 ± 0.0 a
Skimmianine [4]	17.5 ± 2.5 a	7.5 ± 2.5 ac
β–Sitosterol [5]	20.0 ± 0.0 a	12.5 ± 2.5 a
α–Amyrin [6]	10.0 ± 5.0 ac	5.0 ± 0.0 c
Actellic super	95.0 ± 0.0 b	87.5 ± 2.5 b
Negative control	5.0 ± 0.0 c	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence

From the results in Table 3, all the compounds showed low activities when compared to actellic super at both 0.1 and 0.05 w/w, ranging between 2.5 % to 22 % w/w. The adulticidal activity of the three -acridone alkaloids melicopicine [**1**], normelicopicine [**2**] and arborinine [**3**] were noted to increase with reduction in the number of substituted methoxy group. Comparing the two terpenoids, 3E–sitosterol [**5**] showed higher activity (12.5±2.5) than D-amyrin [**6**] (5.0±0.0) at 0.05 w/w and were significantly different ($p < 0.05$). The isolated compounds were less active than the crude extracts, from which they were isolated, an indication of possible loss of synergism in the isolation process.

Table 4: Mean percentage adulticidal ± s.d. of the blended compounds from *Teclea trichocarpa* against maize weevil

Compounds	Mean percentage adulticidal at different concentration in w/w	
	0.1 w/w	0.05 w/w
Skimmianine/ Arborinine	20.0 ± 0.0 c	12.5 ± 2.5 a
α--Amyrin/ Normelicopicine	17.5 ± 2.5 a	10.0 ± 5.0 a
Arborinine /Melicopicine	20.0 ± 0.0 a	15.0 ± 5.0 a
α---Amyrin/ Arborinine	22.5 ± 2.5 c	17.5 ± 2.5 a
3 β –sitosterol/ Arborinine	17.5 ± 2.5 a	12.5 ± 2.5 a
Actellic super	95.0 ± 0.0 b	87.5 ± 2.5 b
Negative control	5.0 ± 0.0 c	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test)/

In order to ascertain this observation, pure isolated compounds were blended in the same ratio and subjected to adulticidal test. From table 4 mixtures of D-amyrin/ normelicopicine, and skimmianine/ arborinine, at higher concentration showed higher activity than corresponding pure compounds, implying some synergism. Arborinine/ normelicopicine and E–sitosterol/ arborinine mixtures showed lower activity than corresponding pure compounds, implying there was loss of activity (antagonist). Mixture of D-amyrin and arborinine did not show significant change in activity at high concentration but at 0.05% w/w there was increased activity, implying synergism is in play. Although all the test mixtures used were in the ratio of 1: 1, there occurrence in the crude extracts of the plant is not in these ratio hence their effects could differ. Similarly, the isolated compounds were not the only compounds present in the crude extracts as evidenced from TLC analysis and therefore, it is evident adulticidal activity is caused by additive effect of most constituent components with different levels of activity.

The results provide a scientific rationale for the use of *T. trichocarpa* in post-harvest protection. The fact that, the crude extracts at high concentration had significant mean percentage adulticidal against maize weevil is interesting and led support to the traditional use of this plant material as grain protectant against destructive pests. Both extracts represents an attractive candidate for field evaluation as a prot ectant of stored maize. It is expected that, the crude plant extract could offer suitable and ecologically sustainable alternative to synthetic pesticide. However, conclusive recommendation of their use can only be made after exhaustive analysis of the effect of this crude on the quality of grain and safety. Improving grain storage would mean less hunger, improved nutrition for mankind, a higher standard of living and a sounder economy for the nation.

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The feeding deterrence effect of neem oil on the larger grain borer, *Prostephanus truncatus* (HORN)

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Abstract

The larger grain borer (LGB), *Prostephanus truncatus* (HORN), has become one of the most important insect pests of stored maize in tropical Africa where it was introduced in the early 1980s. An experiment was conducted to investigate the feeding deterrence effect of neem oil on the LGB larvae and adults. Maize grains were ground using a hammer mill and treated with neem oil at the rates of 0.5, 1, 2, 3, and 4% (v/w). Larvae were sieved out of cultures and sorted according to age and fed on treated flour. The larvae were removed from the flour and their weights determined using an analytical balance after 7 and 14 days. Adult LGB were rared on "cakes" prepared from flour mixed with Mondamin ® commercial starch at a ratio of 3:1 and treated with neem oil. Treating the flour with neem oil caused a significant re duction (ANOVA, $p < 0.001$) in larval body weight of first, second and third instar larvae at all dosage levels. The effect of neem oil treated flour on survival of the LGB adults was significant ($p<0.05$) at high dosage levels of 3 and 4% only. The effect o f neem oil on the survival of *P. truncatus* on maize grains was significant (ANOVA, $p < 0.001$) at high dosage levels only. The results show that neem oil could be important in checking insect population growth of LGB through feeding deterence.

Key words: larger grain borer, feeding deterence, *Prostephanus truncatus*, neem

Introduction

The larger grain borer, *Prostephanus truncatus* (LGB) is a serious pest of stored maize and cassava with increasing importance in Tropical Africa since it was introduced to this region from its natural habitat of Central America in the early 1980s. The effect of *P. truncat us* on the staple foods posses a serious threat to food security in Africa. Because of limitations associated with synthetic chemical control under small scale such as the farmers' inability to afford effective insecticides, their inaccessibility to them, the possibility of poisoning and environmental hazards, is increased the need for alternative methods of control, especially under small - scale production. Neem tree, *Azadirachta indica*, is one of the plants that have been found to exhibit a wide range of effects on various insects, incl uding repellence, feeding deterrence, mortality and various effects on reproduction. This study was intended to investigate the feeding deterence effect of neem oil as part of the continuing effors to provide alternatives to synthetic chemical control of L GB.

Literature Summary

The use of neem products to control pests of stored products has been practiced since early years, especially in India and the neighbouring countries. The traditional use of neem for control of pests of stored products may differ with the region or cultural background of the farmers involved (Saxena, 1995). The effect of neem products on pests of stored products has been reviewed by several authors (Salakol *et al*., 2008; Biswas *et al*., 2002; Saxena, 1995; Saxena *et al*., 1989;). Almost all pest species on which neem products have been tested have been found to be susceptible, what differs is their individual sensitivity (Saxena, 1995) depending on the species of the insect, the neem product used and the stored product on which it is a pplied. There is little information on the effect of neem oil on *P. truncatus*, at least compared to other insects. The first report seems to have been the one by Maredia *et al.* (1992) who reported a non-profound effect by neem oil at 5-10ml/kg. Further work by Niber (1994, 1995) showed that neem oil, seed powder and slurries significantly reduced insect population increase and reduced grain weight loss. Belmain *et al.* (1999) reported that *P. truncatus* was repelled more than *R. dominica*, *S. zeamais* and *C. maculatus* by stored maize treated with neem plant materials. Boateng *et al.* (2009) significant effect of neem oil on adults, larvae and eggs of LGB. What is the source of the contradiction in the reports? What really happens to *P. truncatus* that leads to the reduction in population that has been reported? How do neem products give the protection that has been reported? What is the effect of neem products on other stages other than adults? This study was intended to address these issues and provide information on issues that were not clearly addressed by previous studies and available literature. The methodologies of the study were designed to ensure maximum capture of missing information. The standardization of neem products used with respect to azadirachtin content was unique to this study and was intended to ease the comparison of different but related results. Special attention was paid to the larval stage of *P. truncatus* since no information was available on the effects of neem products on this stage yet it played a significant role in determining the rate of insect population increase.

Description of Research

Ground maize grains were used to investigate the feeding deterrent effect of neem oil on larvae of *P. truncatus*. Maize grains were ground using a hammer mill and treated with neem oil at the rates of 0.5, 1, 2, 3, and 4% (v/w). Mixing was carried out in 100ml glass jars where the flour was stirred continuously until the oil was uniformly distributed. The treated flour was then divided into 10g portions and placed in glass Petri dishes. Larvae were sieved out of cultures and sorted according to age. The young and delicate larvae were carefully picked using a fine-hair brush. The sorting utilized both the time from hatching and estimation of the size of the head capsules according to procedures used by Bell and Watters (1982) and Detmers (1993) to separate the larvae into three larval stages as shown in Table 3.4. The weight of the larvae was determined using an analytical balance before they were introduced into the treated flour. The samples were kept at 30ºC and 70%r.h. The larvae were removed seven days later from the flour using a fine brush and their weights determined again. They were returned into the treated flour and kept for another seven days. The weighing was repeated after a total of 14 days. Ground maize flour was used to prepare "cakes" for rearing adult *P. truncatus*. The flour was mixed with Mondamin® commercial starch at a ratio of 3:1. Neem oil was then added into the mixture before a small amount of water was added. This resulted into a paste that was then pressed into plastic cells (1.5cm x 1.5cm x 1.5cm). The insects were kept at 30ºC and 70%r.h. for a total of 28 days. The samples of maize grainswere treated with neem oil dosages of 5, 10, 20, 30 and 40ml/kg and kept at 30°C and 70%r.h. for one month. 50 unsexed adult *P. truncatus* of the same age were introduced into the samples and allowed to feed for seven days, after which they were removed and the amount of frass produced was determined by sieving the samples and weighing the resultant frass. The insects were returned into the samples and kept for a total of 28 days. The survival of the beetles to the end of the period was determined by counting the number of live beetles at the end of the experiment.

Results and Application

The results of the study are summarized in the Figures 1-4.below. The effect of neem oil on the survival of the first instar larvae of *P. truncatus* on flour is shown in Figure 1. The effect of the oil was significant (ANOVA, $p < 0.001$); treatment of flour resulted in larvae with significantly lower bodyweight (LSD Bonferroni, $p = 0.05$) than non-treated flour. The samples that contained no flour also resulted in significantly lower weight than in the control but not significantly different from the treated flour samples. Treating the flour with neem oil caused a significant reduction (ANOVA, $p < 0.001$) in larval body weight. Larvae on flour treated with neem oil at 20 and 40ml/kg weighed significantly less (LSD Bonferroni, $p = 0.05$) than those on non-treated flour in the control samples. The larvae in the control and in 5 and 10ml/kg dosage samples gained weight while those in all the other treatments lost. In the third instar larvae, the change in weight could not be clearly related to the different treatments used most likely because of the decline in body weight at the onset of pupation. Because of this inconsistency only weights after a seven-day exposure period were recorded (Fig. 4). The effect of the oil treatment for this exposure period was however significant (ANOVA, $p < 0.05$).

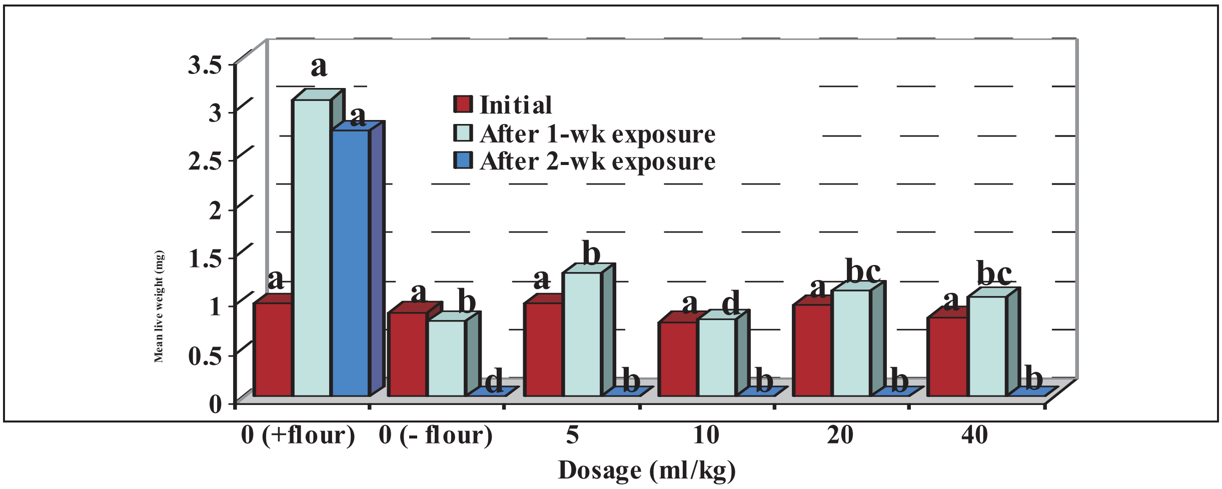

Figure 1: Effect of neem oil on first instar larvae of *P. truncatus* kept on maize flour. Means denoted with the same letter in different treatments are not significantly diffe rent (LSD Bonferroni, p = 0.05)

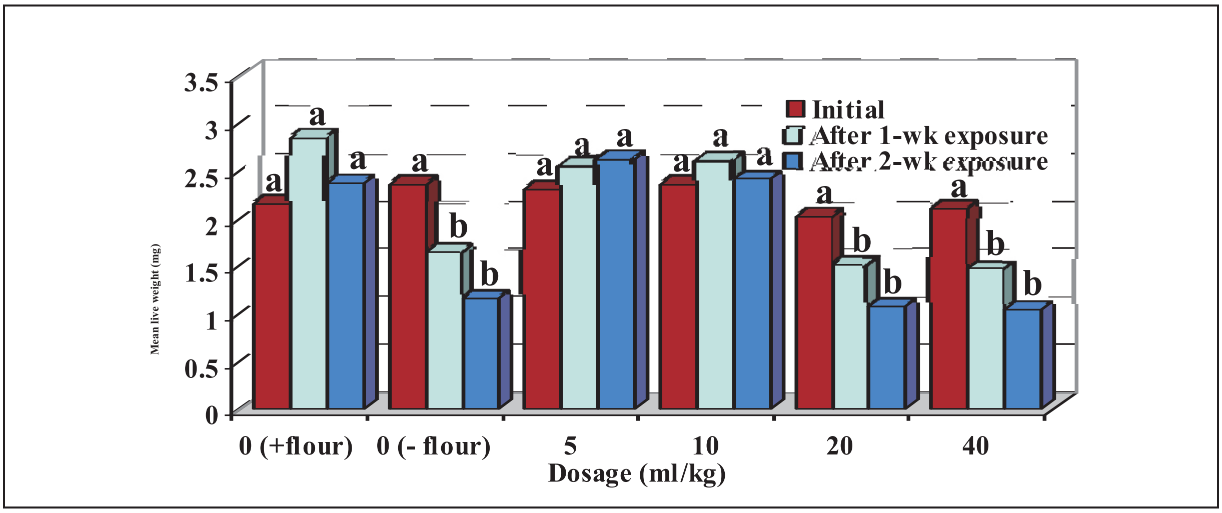

Figure 2: Effect of neem oil on second instar larvae of *P. truncatus* Means denoted with the same letter in different treatments are not significantly diffe rent (LSD Bonferroni, p = 0.05)

Treatment of flour with neem oil caused a significant effect on the survival of *P. truncatus* on maize (ANOVA, $p < 0.001$). However, only the effects of 30 and 40ml/kg treatments were significantly different (LSD Bonferroni, $p = 0.05$) from the control, resulting in lower percentage survival than in the control samples. The effect of neem oil on the survival of *P. truncatus* on maize grains was significant (ANOVA, $p < 0.001$) (Fig. 4) In the case of grain weight loss, the effect of neem oil was significant (ANOVA, $p < 0.001$), but only 40ml/kg dosage treatment values were significantly different from values for all the other treatments including the control (LSD Bonferroni, $p = 0.05$). Maize grain weight loss was highly positively correlated to survival (Pearsons' correlation coefficient = 0.9558, $p < 0.001$).

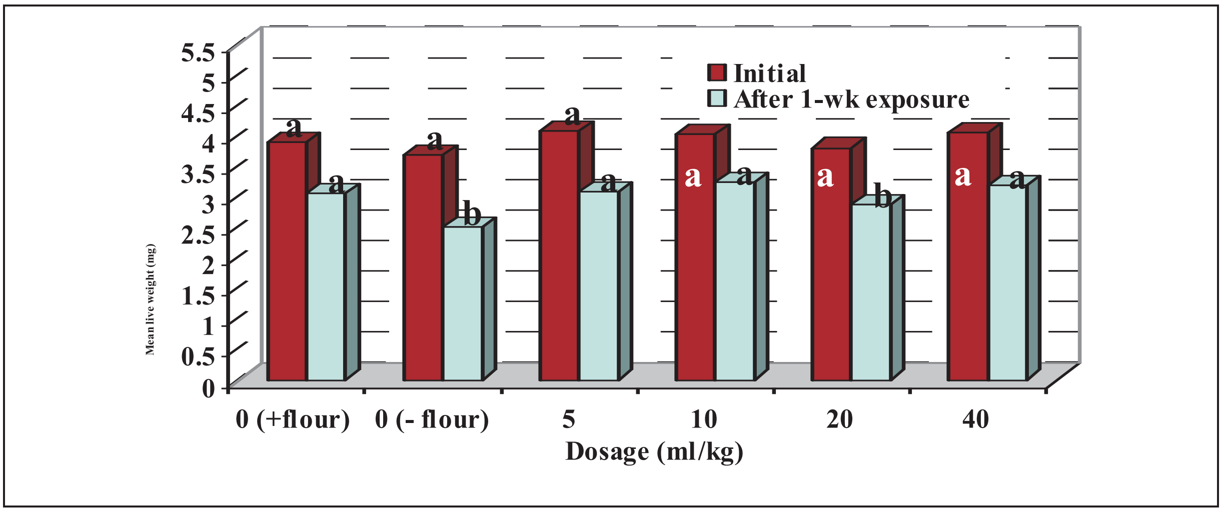

Figure 3: Effect of neem oil on third instar larvae of *P. truncatus* Means denoted with the same letter in different treatments are not significantly diffe rent (LSD Bonferroni, p = 0.05)

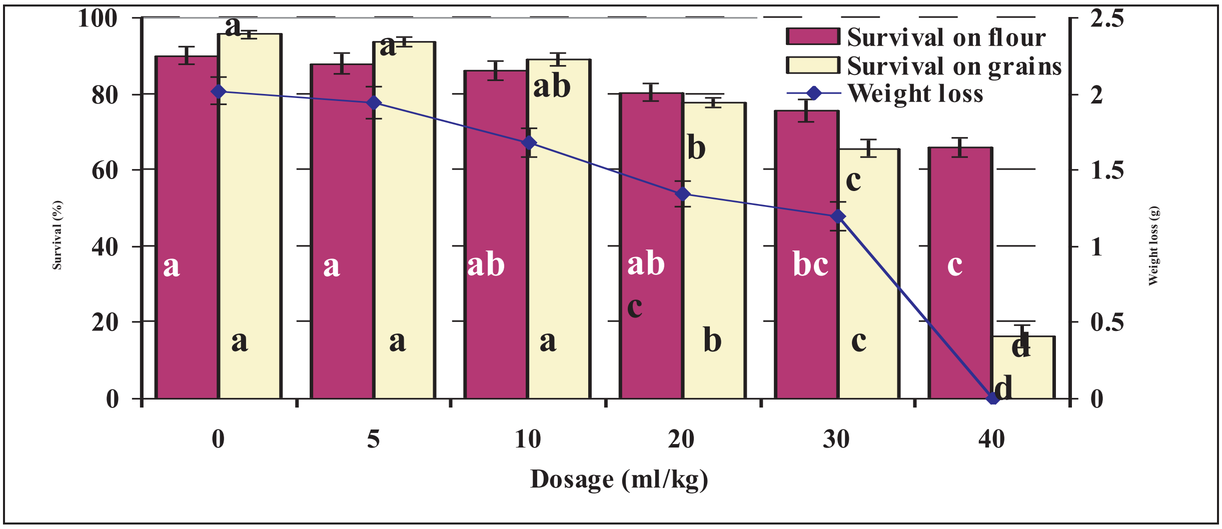

Figure 4: Survival of *P. truncatus* adults on maize grains and on flour after 28 days, and grain weight loss by *P. truncatus* after seven days Means denoted by the same letter at different dosages are not significantly different (LSD, Bonferroni, p = 0.05)

From the results of this study, neem oil exhibited antifeedant properties against both larval and adult *P. truncatus*. Feeding deterrence on the larvae was maximum for first instar larvae and declined with age. The results point to a possibility of control insect population increas e by controlling larval growth at the first instar with very low dosages of neem oil.

Acknowledgement

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Phytochemical Studies of Some Indigenous Plants as Grain Protectants against *Sitophilus Zeamais* (Maize Weevil)

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Abstract

Maize farming, though successful in some parts of Africa is greatly hampered by the post harvest handling procedures. Resource farmers in developing countries like Kenya use different plant materials to protect stored grains against pest infestation by mixing grains wit h protectants made up of plant products. Dry ground plant material, were tested for their activity against the maize weevil; *Sitophilus zeamais*; (Coleoptera: Curculionidae). All showed no reasonable protectant ability. Essential oils extracted were analyzed by gas chromatography–mass spectrometry (GC–MS) and evaluated for repellent and insecticidal activities against *Sitophilus zeamais* using the area preference and contact methods. Most oil components identified by analyzing their mass spectra to the NBS library of mass spectra using computer routing were oxygenated monoterpenoids or phenolic compounds and the oils were active in repelling at doses between 0.005 and 0.125 ppm. The mean mortality values for *Lippia kituiensis* and *Chrysanthemum cinerariaefolium* (pyrethrum) was not significantly different after 24 hrs of exposure with an average activity of 79 %. *Chenopodium chenopoides*, and *Ajuga remota* were not significantly different after 24 and 48 hrs of exposure with a mean of 18 and 20% activity, respectively. The mortality rate of *S. zeamais* by contact increased with the exposure time and the concentrations of the essential oils. The most active oil extracts of plants were those of *Eucalyptus globulus*, *Rosemarinus officinallis*, *Lantana camara*, *Lippia kituiensis*, and *Azadirachta indica* with LD_{50} values as 0.609, 0.199, 0.171, 0.220, 0.399 and 0.333, respectively. These oil extracts were reasonably comparable to the already known adulticidal and repellent pesticides such as pyrethrum with its LD_{50} being 0.245. The constituents which may be more responsible for the maize grain protectant ability includes 1,8 -cineole, limonene, α–pinene, β-pinene, α-terpineol, terpinen–4-ol, and globulol. This implies that a conclusive research carried out using standard conditions could reveal all the responsible components for such high activities. Blends of plants whose activity is known were made and tested against the maize weevil. A blend of *Eucalyptus globulus* and *Rosemarinus officinallis* at 1.0 % concentration gave a PD and WPI of 3.5 and 7.2,

respectively. These values are far much lower than their individual PD and WPI values of 15.3 and 12.3, 25.2 and 21.3, respectively. This therefore gives the impression that blend effects in the bioactivity of plant products may well be the norm to follow. Mortality tests by inert dusts were also carried out. Wood ash, Kaolin and Bentonite showed high activities of upto 70 % at 5 % concentration after 168 hours. Gypsum Whiting dusts was not comparatively active since they only exhibited activities below 40 % even at 10 % concentrations after 168 hours.

Introduction

Insect pests control in stored grain products relies heavily on the use of gaseous fumigants and residual contact insecticides. The implications of their use include toxic residues, health and environmental hazards, development of insect strains resistant to insecticides. Plant derived substances hav e been used as botanical pesticides since ancient times. Botanical pesticides are plant natural products that belongs the group of secondary metabolites, which include thousands of alkaloids, terpenoids, and phenolics (Jacobson, 1989). The biological activity of these products on insects, nematodes and phytopathogenic fungi among other organisms is well documented in the recent explosion of literature in chemical ecology. Apparently, every plant species has developed a unique chemical complex that protects it from pests. Thus the plant kingdom provides us with a vast cornucopia of defense chemicals comprising repellents, feeding and oviposition deterrents, growth inhibitors, sterilants as well as toxicants. Research in the use of repellents as a method of pr otecting humans, plants and animals is experiencing a renewed interest.

Maize, *Zea mais*

Maize is the most important stable food in Africa and provides over 50% of stable food calories. About 70% of Africans in rural areas and 50 million families derive th eir livelihood on farming. The population increases at 3.5% while food production at 2.5%.The high population growth has resulted to decline in unit of land per family and thereby putting pressure on ecosystems and loss of biodiversity. The average maize y ield in Africa is 1.7 compared global 4 tones per hectare. Efforts to increase production are extremely hampered by the pre and post-harvest loses due to pest and weeds. Grain storage pests are most important biotic constraints to maize production in Africa. The yield losses due to insect damage range from 25 to 40% reaching 80% in serious infestations and 40-80% in stored products (Chhabra, 2007). In Kenya, the maize yield loss estimated at 12.9% amounting to 0.39 million tones of maize, with estimated val ue of US$ 76million. The yield loss caused by insect pests to maize varies widely in different regions and range from 20 to 40% according to the pest population density (Fatope *et al*., 1996).

Maize Weevil, *Sitophilus zeamais*

This is the most common storage food grain pest in Kenya/ It’s a very serious major (primary) pest/ Its infestation starts in the field and is later carried into the stores. Both larval and adult stages cause heavy damage. The larva feeds on the starchy interior of the grain wher eas the adult hatches and eats their way out of the grain and continues to feed voraciously on the grain (Ndungu, 1993). It predominates in bag stacks since it can penetrate beyond a depth of several centimeters. Storing the grain in a dry and clean condit ion in insect proof containers has been used as a control method. If the grain moisture is below 9%, then the insect is unable to breed. This method is impracticable. Its control in stored grains is a serious problem in developing countries within the tropics due to favourable climatic conditions and poor storage structures other than fumigation (Bekele, 2006).

Repellents

Repellents are chemicals which cause organisms to make oriental movements away from the source. Critical evidence suggests that repellents act directly on chemosensory systems. Chemoreception is the physiological process occurring in chemo receptors as a result of their contact with certain chemicals. Vapour repellents are chemicals which act in the vapour phase and are most often stimul i of the olfactory receptors. Contact repellents are those compounds with which the insect must come in direct contact and which act upon receptors not normally sensitive to vapours. Essential oils known to show insect repellency acting on their vapour phase due to their volatility. However, to date, the mode of action of repellents is still unresolved (Verlag M. 2000).

Essential oils

Essential oils are complex mixtures of odorous and steam volatile compounds which are deposited by plants in the subcuticular space of glandular hairs, in cell organelles in idioblasts, in excretory cavities and canals, or exceptionally in heart woods. Essential oils may have no specific biological function implants, but some oil bearing plants are attractive to certain animals and insects whereas others are repellent. Essential oils have wide and varied industrial applications they are used in the manufacture of perfumes, cosmetics and toilet soaps, as flavouring material in candy, chewing gum, ice–cream and for flavouring alcoholic and non –alcoholic beverages. Others have antibacterial, antifungal, insecticidal and antiseptic properties and are valuable in medicine and dentistry (Chhabra *et al*., 1990).

Combined Gas Chromatography and Mass Spectrometry (GC-MS)
This is a technique in which a mass spectrometry is directly linked to a gas chromatograph so that the eluted compounds may have their mass spectra recorded in the chromatograph.GC-MS is one of the most recent highly specific analytical methods, and could be used as a confirmatory technique in the identification of the various components in the essentials oils analyzed. This is because the mass spectra of the various components eluting during GC separation are recorded, and the mass spectrum of a compound is characteristic of the identity of the compound. So long as there is a library equipped with the mass spectra of all compounds, any compound in the essentials oils can be identified. Hence identification by this technique is not limited to the availability of authentic sample. One of the disadvantages of this method is that quantitative analysis cannot be achieved using this technique, since peak sizes depend on the extent of ionization of compounds thus a GLC as a separate technique has also to be used (Rose and Johnstone, 1982).

Literature Summary

***Plectranthus sylvestris* Guerke**
Plectranthus sylvestris belongs to the Lamiaceae (Labiatae) family and has the following names: Country borage, Cuban oregano, Indian borage, French thyme, Spanish thyme, Mexican mint and so up mint. An infusion of the aerial parts is drunk against intestinal worms while the leaves are for stomach diseases and as a purgative. They are boiled in water and the vapours inhaled in case of psychiatric problems (Kokwaro, 1994). Few studies have been conducted on the chemotype and activity of the essential oils from different species of Plectranthus. The phytochemical analyses of extracts of Plectranthus spp. have revealed the presence of abietene diterpenoids and sesquiterpenes. The essential oil of Plectranthus spp. contains carvacrol, thymol, α-terpinolene, β-caryophyllene, 1,8-cineole, p-cymene and patchoulane (Marwah *et al*., 2007).

***Lippia kituiensis* Vatke**
It is commonly called the Frogfruit and synonymously as *L. ukambensis* belonging to the Verbenaceae family. It is purposely used for ornamentals, fences and hedges (Kokwaro, 1994). It is used also in treatment of gastrointestinal and respiratory disorders and as seasoning. Some of these species has shown antimalarial, spasmolitic, sedative, hypotensive and anti-inflamatory activities (Pascual, 2001). The essential oil contains camphor, 4-thujanol, cineole, monoterpene alcohol, camphene, limonene and several other terpenoid substances. Extracts of leaves contains common fatty acids, stigmasterol, phytol, ursolic acid and camphene glycol (Chogo and Crank, 1982).

Description of Research

Ground Powder Material Activity Tests on Maize Weevil
The fresh plant materials comprising of aerial parts, inflorescence stems, succulent stems and leaves were air dried for four weeks after which they were ground into fine powder using a laboratory blender. These were then weighed into 5.0 and 10.0 g portion concentrations and put into sterilized glass jars in readiness for 5 replicate tests. Twenty adult maize weevils were released into each of the glass jars containing these ground powder after which a perforated lid was replaced back. These were then kept at room temperature of 22 ^{o}C. The numbers of live and dead maize weevils were counted after 12, 24, 48, 72 and 96 hours. Insects were considered dead if they were immobile and did not react to three probings with a blunt dissecting probe.

Maize Weevil Repellency

The repellency was based on the area preference described by Mc Donald *et al*. (1970) and has since been modified (Lale and Alaga, 2000). Test arenas consisted of a 9 cm Whatman No.1 filter paper cut into two halves. One part was treated with 50 mg of plant sample as uniformly as possible by means of a teat pipette while the other half was left untreated. For the control experiment, half of the filter paper was treated with the solvent (pure acetone) and the other half remained untreated. Another control experiment was set up without any treatment of plant oil. All the half discs treated and untreated were air dried at room temperature to evaporate the solvent completely for 30 minutes. The treated and untreated half discs were rejoined together using clear adhesive tape and placed in a glass Petri dish of 9 cm diameter. The 20 adult *Sitophilus zeamais* were released at the center of each repellency chamber and then covered. Each treatment and control was replicated 5 times. The number of insects present on treated and untreated discs were counted and recorded after 30 minutes. This procedure was then repeated for 75, 100 and 125 mg concentrations.

Maize Weevil Mortality

Toxicity by contact was assessed as described by Adjoudji *et al.* (2007) and Bekele., (2006). 100 g of maize was mixed with 3 ml of pure acetone containing 0.5 ml of the essential oil at a final concentration of 0.5 % (v/w) of essential oil/maize ratio (0.5 ml/100 g) (Bekele, 2001). Acetone was then allowed to evaporate completely for 30 minutes. Batches of 20 g treated maize were then weighed and sampled into sterilized glass jars so as to have 5 replicate. The 20 adult *Sitophilus zeamais* were introduced into each glass jar and then covered with their plastic lids that were perforated. A control containing pure acetone treated maize was set up. Observations were made after 12, 24, 48, 72 and 96 hours where the number of dead and alive maize weevils was recorded. This procedure was repeated at 1.0 % (v/w) of essential oil/maize ratio (1.0 ml/100 g).

Adult Emergence and Grain Damage Tests

These tests were performed with the infested and treated maize, where 100 g of the grains were treated with 3 ml of pure acetone containing 0.5 % (v/w) of essential oil/maize ratio (Bekele, 2006) and left open for 30 minutes to completely allow the solvent to evaporate at room temperature. This was then weighed in batches of 20 g each and put into clean 5 replicate glass jars for every oil sample being tested, covered with their perforated plastic lids and left for observation after 49 days (7 weeks). The extent of weevil damage to the grains was assessed using the exit-holes counted as a measure of damage to the grains. Grains that were riddled with exit-holes were counted. This procedure was also repeated at 1.0 % (v/w) of essential oil/maize ratio (1.0 ml/100 g). The grain Percentage Damage (PD) and Weevil Perforation Index (WPI) of the weevils to the grains were calculated using the Adedire and Ajayi (1996).

Mortality Tests by Inert Dusts

The use of inert materials, for instance, activated silica, sand, crushed limestone and wood ash fills up the granular spaces and therefore hinders any insect activity. Furthermore, diatomaceous earth, for instance, Dolomite; functions through its physical properties by abrading the surface of the insects thus leading to their desiccation. Very fine dusts with minute particles size (0.012 µm) are the most effective. However, dusts seem to be more effective when applied in aqueous solutions as surface treatments. The use of abrasive ash from paddy husks and mixed with grain dehydrates the insect pests leading to desiccation and death (Verlag, 2000). These strategies have contributed important attributes of Integrated Pest Management (IPM) programmes since they are focused on the host plant resistance (Bekele, 2006).

Repellency, Mortality and Adult Emergence and Grain Damage Bioassay

Maize weevils, *Sitophilus zeamais* were initially obtained from ICIPE laboratories colony and thus reared under ambient conditions so as to obtain a stock of homogeneous maize weevils. Maize grains which had not been treated with any pesticide was purchased locally from the field and disinfected in an oven at 40 ^{o}C for 4 hours and kept in an open jar before use (Adjoudji *et al.,* 2007; Bekele, 1995).

GC – MS Analysis

A VG analytical 12-250 GC – MS equipped with a Hewlett Packard 5890A GC and a data system was used. The column and temperature programme that were used for the GC analysis were also used for GC – MS determinations. The injector was used in the split less mode. Library – MS searches using the Wiley NBS data base in the GC – MS data system was carried out. The identity of the constituents of the essential oils was established by comparison with retention times of the authentic standards and peak enhancements of the components by co-injection of the essential oil with the standards.

Applications

The essential oils of the three extracts tested were toxic to *Sitophillus zemais* unlike the ground material whose activities were very minimal. The insecticidal activities of the compounds suggest structure-activity relationships and a mode of action that may be useful in the design of synthetic analogs. Chemical components such as camphor, limonene and even 1,8-cineole offer an irresistible insecticidal activities. Toxicity of the essential oils is mainly attributed to these components (Bekele, 2001). The repellency of these plant oils against *Sitophillus zemais* show that most of the oils are comparatively active and hence may easil**y** be compared to pyrethrum. As reported by other researchers, Pyrethrum oil may act as a substitute for methyl bromide to control the maize weevils. This activity may be very useful in postharvest handling of maize grains under the experimental conditions carried out. Organophosphate and carbamate based pesticides are the commonly used maize stores. These compounds are relatively more toxic than the essential oils.

The compounds identified from the plants seem to be responsible for the high activity, although not all were identified. Specifically, *iso*-eugenol and 1,8-cineole provides strong toxicity towards the *Sitophillus zeamais*. This implies that a conclusive research carried out using standard conditions could reveal all the responsible components for such high activities.

On mortality tests, though results are given, it was done with the assumption that an insect was dead if they became immobile and did not react to three probing with a blunt object. Two of the inert dusts showed reasonable activities. However, full investigations should be done to fully identify and isolate all the constituents of these plant essential oils and bioassay carried out to establish all the active compounds against the *Sitophillus zeamais*.

The practice by farmers to mix their harvests with these plant materials can be a significant act if this was done carefully in blends and may be three times within a given storage period. This will ensure no re-infestation occurs.

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Toxic and repellent properties of *Cupressus lusitanica* and *Eucalyptus saligna* essential oils against *Callosbrochus chinesis* and *Sitophilus zeamais*

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Abstract

Essential oils of *C. lusitanica and, E. saligna* were evaluated forcontact and fumigant toxicityand repellence against adult *S. zeamais and C. chinensis*. Essential oils were separately applied at rates of 0, 5, 10, 15 and 20 μlL^{-1} air in fumigation and 0.05, 0.1, 0.15 and 0.2 % v/w in contact toxicity and repellence tests. Treatments were laid out in a completely randomized design with four replicates. Results indicatedboth plants were effective fumigants causing mortality of 65-100% at 10-20 μlL^{-1}post fumigation in both insects. Test plants oil at 0.2% v/w post exposure caused 62 and 91 % mortality in *C. chinensis.* Plants showed negative repellence in *C chinesis*but moderate (27-37%) positive repellency in *S. zeamais.* Information is discussed in the context of its relevance for inclusionin pest control options in small scale farming.

Introduction

The quantitative and qualitative post-harvest losses of stored cereal and legume grains varying in magnitude from 10 to 100% in tropical countries is attributed to insect pests. These substantial losses are caused by *Sitophilus* spp., *Sitotrogacerealella* and *Prostephanustruncatus* on cereals, *Acanthoscelides* and *Callosobruchus* spp. on legumes (Mugisha-Kamatenesi *et al*., 2008, Ogendo *et al.,* 2012). Current recommended control measures for stored product insect pests rely on synthetic insecticides which pose health and environmental hazards. Scientists have now shifted focus to botanical pesticides, which are locally accessible and available, relatively inexpensive, biodegradable, less toxic to mammals and less prone to resistance by insect species (Ogendo *et al.,* 2012). In the current study fumigant and contact toxicity of essential oils obtained from aerial parts of *C. lusitanica*and *E. saligna*were evaluated against *C. chinensis* and *T. castaneum.*

Literature Summary

Plant derived materials, with insecticidal properties, have been used traditionally for generations throughout the world and remain the major control principle in subsistence agric ulture (Ogendo *et al.,* 2003, chebet *et al*., 2013). In last few decades, several plant essential oils, powders and other extracts have been evaluated against several insect pests of cereals and legumes and found to have contact toxic, repellent, fumigant to xic and antifeedant properties (Rosman *et al*., 2007, Ogendo *et al.,* 2008). For instance,powders, crude aqueous extracts, essential oils and their constituents from plants in the families, Lamiacae, Verbenaceae, Fabaceae , Leguminosae among others, have shown good bioactivity against a wide range of field and stored-product insect pests (Asawalam *et al.,* 2006; Ogendo *et al.,* 2011; Wambua *et al*., 2011; Ogendo *et al.,* 2012). Essential oils extracted from aerial parts of *Ocimum americana,Lantana camara* and *Teph rosia vogelli* and monoterpene constituent, eugenol, are known to have concentration, exposure time, species (plant and insect) and plant part-dependent contact, fumigant and repellent potency against adult *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Callosobruchus chinensis* (Ogendo *et al.,* 2008).

Description of Research

Bioassays were conducted to evaluate the toxic (contact and fumigant) and repellent effects of leaf essential oils of *Cupressus lusitanica,* and *Eucalyptus saligna*against *Callosobrochus chinesis* and *Sitophilus zeamais*. Modified Clevenger apparatus' hydro-distilled essential oils from the two tropical plants were tested against above insects under controlled temperatures (28±2 ^{o}C) and relative humidity (65±5%) and arranged in completely randomized design with four replicates each. The fumigant toxicity experiment was conducted in space fumigation chambers consisting of a 3.4L flask and suspended metallic cages carrying 20 adult insects and food. Essential oils were separately applied at a rate of 0, 5, 10,15 and 20 µlL^{-1} air on filter papers and suspended in the fumigation chamber and number of dead insects recorded 24, 72, 120 and 168 h post fumigation.Incontact toxicity test, essential oil was also appliedat rates of 0.05, 0.1, 0.15 and 0.2 % v/win 100ml glass jars, containing 10 or 20g of grains depending on size.Number of dead insects recorded 1, 3, 5, 7 and 10 days post-treatment. In the repellency bioassays, similar doses of essential oil were used in an alternate untreated (control)-treated arrangement. Number of insects in the control (N_C) and treated (N_T) grains recorded after 1, 3, 5 and 24 h after exposure and percent repellency subsequently computed.

Results and Application

Results of fumigant and contact toxicity, and repellence bioassays revealed that *C. lusitanica,*and *E. saligna*leavesessential oils at the end of 168h were significantly (*P*<0.05) influenced by plant species, concentration of essential oil, duration of exposure and corresponding factor interactions. Leaf essential oils of *E. saligna* and *C. lusitanica*at a low dose of 10µlL^{-1} air evoked 100% and 85% kill, respectively, against adult *C. chinesis* after168 h exposure in space fumigation chamber/ However, the two plants' oils induced a weak fumigant toxicity of 65% and 67%, respectively against adult *S. zeamais*even at a higherdose of 20 µlL^{-1}**.**

In the contact toxicity test on the other hand, and at a concentration of 0.2% w/w and 168h exposure, *E. saligna*and *C. lusitanica,* leaf essential oils caused mortality of 62%, and 91%, respectively of adult *C. chinesis*.In the repellency bioassays, the *C. lusitanica* and *E. saligna* essential oils were generally attractive (negative repellence) to *C. chinesis*. However, they (oils) evoked a moderate repellency with highest of 37% for *C. lusitanica* and 27% for *E. saligna* against *S. zemais.*

The observed differentail responses by test insect species to *C. Lusitanica* and *E. saligna* essential oils could be explained by individual/or synergistic fumigant and contact toxicity and repellent properties of their chemical constituents. The fact that the essential oil of *C. lustanica* and *E saligna*, at concentrations of 10-20 µlL^{-1}air, was effective to cause a mortality of more than 60% in both *S. zeamais* and *C. chinensis* offers practical potential as fumigants. Results are comparable to those of Ogendo *et al.* (2008) who found *O. americanum* leaf essential oil at 50ulL^{-1} air, 7d exposure and 120 hour post fumigation time to cause 95 -100 and 66% kill of *S*. *oryzae* and *R. dominica* and *T. castaneum*, respectively. The standard of fumigant efficacy recorded in this stu dy could be comparable to methyl bromide (30-50gM^{-3} grain), Labiatae species oil ZP51(50ulL^{-1}air) andallyl acetate(50-150mgL^{-1}) to achieve 94-100% mortality of all insect pests of stored cereals and legume grains(Busvine,1980, Rajendran and Muralidharan, 2005).

In the contact toxicity studies the essential oil of *C lusitanica* and *E. saligna* showed strong species-specific toxicity against *C. chinensis* highly dependent upon the dosage and time after treatment. The mortality of 62-91 % achieved in *C. chinesis*, at concentrations of 0.2 % v/w, 68hrs post exposure is comparable to that of previous studies.Plant powders from *T. vogelii* have shown a 93.7% reduction in insect damage by bruchids (Koona and Dorn, 2005), caused 85.0-93.7% mortality on storage insects (Ogendo *et al.,* 2003: Ogendo *et al*., 2008) whereas its essential oils have been shown to kill up to 83% of storage insects.

It is evident from results of this study that *C lusitanica* and *E saligna* EO are weak repellents; PR 27-37 % in *S. zeamais* and negative PR (-10 %) in *C. chinesis.*This provides nothing to ride home about as far as an effective repellent is concerned. However, negative repellency in *C. chinesis* could scientifically be exciting especially in the push-pull strategy in integrated pest management where a protected source(crop) is unsuitable to pest (Push) while luring the towards an attractive source (Pull) from where the pests are subsequently removed (Cook *et al*., 2007) .

Results point to *E. saligna*and *C. lusitanica*essential oils as candidate substances for further bioactivity studies to determine their individual and combined effects on more insect pests as fumigants and contact toxicants and possible integration into pest management options in subsistence agricultu re.

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Can the biofuel crop, *Jatropha curcas*, be used as a locally-grown botanical pesticide? A lab and field study in Zambia

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Abstract

Jatropha curcas is grown as a biofuel crop in the tropics, and in many parts of Africa it also has a number of domestic uses, e.g. it is frequently grown as a hedge. The collapse of the biofuels market in Zambia has inspired a search for alternative uses for this plant. Previous laboratory studies suggested that Jatropha exhibits a range of beneficial properties, including pesticidal properties. In this paper, we report a series of studies aimed at testing whether formulations of Jatropha powder or oil are effective against storage pests infesting cowpeas and maize. These include laboratory experiments in the UK and field studies conducted with village farmers in Zambia. We report these findings, and discuss the role of participatory research in aiding the adoption of locally-grown botanical pesticides amongst resource-poor farmers in rural Zambia.

Introduction

Jatropha curcas is a pan-tropical, non-edible shrub within the family Euphorbiaceae (Carels, 2009) that is commonly grown as a hedge plant in many regions of Africa. There have been several studies reporting anti-arthropod activities of *Jatropha curcas* plant parts, most likely due to the toxic protein curcin and/or phorbol esters (Devappa *et al*., 2010). Recently, there has been an increase in the cultivation of Jatropha in some parts of Africa, due to its potential value as a biofuel crop. However, in Zambia, the Jatropha biofuel market has collapsed and many farmers are left with a worthless crop that is either left unattended or is replanted. As a consequence, alternative uses for the plant are sought.

Recent literature suggests that Jatropha oil, seed or leaf extracts have a range of pesticidal and antimicrobial properties. For example, Jatropha oil acts as a repellent against termites (Acda 2009); Jatropha seed extracts kill the larvae of mosquito species that vector infectious diseases such as malaria (Sakthivadivel and Daniel 2008); Jatropha leaf extracts significantly reduce fungal pathogen growth (brown blotch disease, *Colletotrichum* spp.) in both cowpea (Onuh *et al*., 2008) and banana (Thangavelu *et al*., 2004); and the sap of Jatropha inhibits the growth of several common bacteria (*Staphylococcus*, *Bacillus* and *Micrococcus* spp.), and nematode parasite species (*Ascaris lumbricoides* and *Necator americanus*) (Fagbenro-Beyioku *et al*., 1998). Moreover, extracts from Jatropha leaves exhibit insecticidal activity against a range of Lepidopteran species (Ratnadass *et al*., 1997; Valencia *et al*., 2006; Phowichit *et al*., 2008), and Jatropha seed extracts are reported to cause 97 - 100% mortality against stored product pests, such as *Sitophilus* weevils (Asmanizar *et al*., 2008) and *Callosobruchus* seed beetles (Adebowale and Adedire, 2006). However, most of these previous studies were laboratory-based studies using a range of extraction methods that are not readily available to resource-poor farmers and so the relevance of these findings to field conditions is unclear.

As part of a much larger study exploring the nature of knowledge exchange between farmers and scientists called *Bridging Knowledge Systems for Pro-Poor Management of Ecosystem Services*, the aim of the current project is to determine whether more readily available Jatropha products (seed oil and powdered leaf) is effective against stored product pests affecting stored maize and cowpeas. Inevitably, when determining the efficacy of a new pesticide, there is an inherent trade-off between the realism typified by farmer-led field evaluations ('farmer experiments') and the degree of control over extraneous influences typified by well-designed and replicated laboratory-based studies ('lab experiments')/ ! half-way house along this trade-off curve is to design well-controlled experiments that are conducted in collaboration with farmers in the field ('field experiments')/ This paper describes preliminary results using each of the approaches.

Methodology

(a) *Farmer experiments*: These experiments were designed by a consortium of individuals from Lancaster University, UK, the Green Living Movement (GLM), a Zambian based NGO that with local farmers to experiment and test alternative agricultural practices and innovations, and a farmer from Chibobo in Central Zambia – "Professor" Spider !llan/ These experiments are conducted on an ad hoc basis using a limited supply of Jatropha oil, leaf powder and seed cake, and were part of a wider study exploring the uses of locally-available plants as botanical pesticides (see Vermeylen and Wilson, this volume, for more details.

(b) *Lab experiments*: These studies were conducted at Lancaster University in the UK as part of an MSc project. They main experiments used Jatropha oil (diluted with sunflower oil to make concentrations of 0%, 0.1%, 1%, 10%, 50% and 100% Jatropha) on cowpeas to determine their effects on egg-laying behaviour and larval survival of *Callosobruchus maculatus* beetles, compared to non-treated controls. The experiments involved 'no choice' and 'choice' assays in which mated females were given access to cowpeas lightly coated in the oil formulation for 7 days and the number of eggs laid after 24 hours and 7 days was determined and their survival to adult emergence recorded.

(c) *Field experiments:* This experiment involved a collaboration between the Kasisi Agricultural Training College (KATC) in Lusaka, Lancaster University, UK, and ten farmers living near KATC. The ai m of these studies was to test efficacy of Jatropha leaf powder, both as a dry formulation and diluted in cold water, against the storage pests of maize, mainly *Sitophilus* beetles. The experimental design was aimed at maximising statistical power within the constraints imposed by large numbers of treatments and replicates. There were 5 treatments: a dry control (maize seeds); a wet control (maize seeds that had been soaked in cold water for 2 minutes before being dried to 0% humidity as determined by a grai n moisture probe); a synthetic chemical pesticide treatment (*Sumba Super Dust*, used at the manufacturers recommended rate); a Jatropha powder treatment (dried leaves crushed to a fine powder with a mortar and pestle and applied at a rate of 2% w/w); and a Jatropha solution treatment (dried leaf powder soaked in cold water overnight to make up a 10% solution w/v and maize seeds dipped in the solution for 2 mins before being dried in the shade to 0% humidity and re-bagged). The 5 treatments were replicated 4 times with each of the farmers using 20kg bags of maize. Bags of the same treatment were stacked together to maximise treatment effects and the normal storage mechanism, but different treatments were kept apart within a single store room. Immediately after treatment, and at monthly intervals thereafter for 6 months, 4 separate samples (each weighing approximately 250g was taken from each bag and the following metrics determined: (i) number of insects per weighed sample and their identity; number of holes per seed for each of 250 seeds, replicated 4 times; and (iii) weight of 1000 seeds.

Results

(a) *Farmer experiments*: There was anecdotal evidence for an effect of Jatropha seed cake on the incidence of attacks on stored maize by rodents, but as there was no ade quate control, it was difficult to establish its robustness. Jatropha leaf powder was not effective. Jatropha oil was also reported by the village "professor" to be effective against stalk borer/

(b) *Lab experiments*: In the no-choice assay, there was no effect of the different oil treatments on the number of eggs laid after 24 h. However, after 7 days, there were significantly fewer eggs laid on the cowpeas that received the 50% and 100% Jatropha oil treatments, compared to those treated with oil comprising 10% Jatropha or less. There was a significant impact of oil treatment on the number of adult beetles to emerge: untreated cowpeas = 27.6 + 7.5; 0% Jatropha oil (i.e. 100% sunflower oil) = 0.8 + 0.2; 0.1% Jatropha oil = 0.4 + 0.1; 1% - 100% Jatropha oil = zero emergence. In choice tests, after 24 hours there was significant avoidance of all the oil-treated seeds, with 47% more eggs being laid on the untreated seeds than the treated ones. However, amongst the oil -treated seeds, the oil treatment (% Jatropha) did not significant influence the number of eggs laid per seed. Results after 7 days were broadly similar.

(c) *Field experiments:* Preliminary analysis, conducted on sampling data from 3 months after the experiment started revealed the following patterns (Table 1) : (i) As expected, according to most metrics, the synthetic chemical (Sumba Super Dust) reduced the incidence and impact of stored product pests. (ii) The number of insects (mainly *Sitophilus* beetles) infesting the maize did not differ between the control group and the group treated with Jatropha powder, but in the maize treated with Jatropha solution, the number of insects was approximately 3 times greater than in the dry untreated maize and 25% greater than in the wet control maize (i.e. maize that had be en pre-treated by soaking in cold water for 2 minutes); wetting the maize like this results in a doubling of the number of insects compared to the dry controls. (iii) The number of maize seeds attacked and the total number of holes made by exiting insects was not affected by treating with Jatropha powder, but both were significantly increased in seeds that had been soaked in Jatropha solution. Soaking in water enhanced the number of seeds attacked but not the number of exit holes recorded. (iv) The mass of seeds was significantly reduced in both water treatments (control and Jatropha solution), but adding Jatropha to the solution did not affect the weight loss.

Conclusion

There are pros and cons to different methods for testing the efficacy of botanical pe sticides. Field experiments provide a suitable compromise between well-controlled lab experiments with limited realism to the field setting and farmer-led observations that are not well controlled, randomized or replicated. The studies reported here highlight these constraints. The field experiments suggest that Jatropha leaf powder has limited utility as a botanical pesticide against storage pests. Likewise, Jatropha leaf solution fails to protect stored maize. Indeed, soaking maize in water for just two m inutes and then drying significantly enhances susceptibility to *Sitophilus* weevils, and adding Jatropha leaf powder to the solution appears to enhance its attractiveness to insects and the damage they inflict. Further lab studies will be required to determ ine the cause of this, but it is possible that this could be exploited in a form of “push-pull” approach, with the Jatropha solution pulling insects away the main storage crop, which could be protected by a different botanical pesticide or repellent.

Table 1: Effects of Jatropha curcas leaf powder and solution on infestation of maize seeds with stored product pests

Treatment: **Metric:**	**Control (dry)**	**Jatropha powder**	**Control (wet)**	**Jatropha solution**	**Sumba chemical**	**F** (df = 4,195)	**P**
Number of insects per kg seeds (mean + SE)	29.50 + 3.55a	38.40 + 3.26a	71.25 + 6.60b	100.87 + 8.55c	4.47 +0.45d	145.92	<0.0001
Number of seeds attacked per 1000 (mean + SE)	48.70 + 4.08a	47.50 + 2.91a	65.20 + 5.81b	124.70 + 9.48b	26.40 + 2.04c	45.33	<0.0001
Number of exit holes per 1000 seeds (mean + SE)	92.60 + 9.36a	93.30 + 6.09a	96.40+ 7.81a	223.60+ 17.12b	43.90+3.38 c	46.00	<0.0001
Mass of 1000 seeds (g) (mean + SE)	363.60 + 2.08a	365.10 + 1.61a	353.40 +1.68b	357.00 + 2.33b	362.40 + 2.00a	6.30	<0.0001

Means that are not significantly different from each other share a common letter, as determined by Bonferonni tests. F and P values are more an ANOVA model.

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Symposium 3 Farming Systems, Veterinary and General Session Plenary

Potential and Uncertainties in Utilization of Pesticidal Plants in Pest Management among Small-scale Farmers in Nakuru, Kenya

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Abstract

The domestication of crops has required farmers to devise ways of managing pests in order to increase yields, improve quality of produce and ensure profitability. After the World War II, the use of synthetic pesticides made impressive impacts on pest control and resulted in pest management becoming synonymous with pesticide use. However, it was not long before the limitations of synthetic pesticides became evident as pest problems persisted and also negative effects on human beings and the environment. A study was conducted in Nakuru to determine pest management practices among vegetable farmers. The objective was to find out the extent to which farmers used alternatives to pesticides in pest management. The farmers acknowledged that less dependence on synthetic pesticides was safe for their health and the environment. The plant based pesticides were viewed as a cheap alternative of pest management since the materials used were locally available. The major limitation in the use of the botanical methods was lack of specificity in dosages and methods of application which the farmers said limited their effectiveness in pest management. The farmers reported that although the botanical methods provided protection for their crops it was more of trial and error because they were not sure of the correct dosage and spraying interval. The farmers indicated the need for more research and education on ingredients, dosages, methods of preparation and application and target pests. Pesticidal plants can thus provide a much needed alternative in pest management especially for small-scale farmers but with more research and education on preparation and utilization methods.
Key words: Small-scale farmers, Plant based pesticides, Pest management, Pesticidal Plants

Introduction

Since the first domestication of crops, people have devised ways of managing pests with the aim of increasing yields, improving quality of produce and ensuring profitability in farming. The period following the end of the World War II was especially significant in the use of synthetic pesticides which made impressive impacts on pest control and resulted in pest management becoming synonymous with pesticide use (Flint and Gouveia, 2001). However, it was not long before the limitations of reliance on synthetic pesticides became evident as problems of pests persisted and negative side effects on human beings and the environment resulted. Hence, worldwide there is increasing concern on the negative effect of pesticides used in agricultural production. Pesticide residues accumulate in soils, air, and water causing alarm about the health of the environment (Carson, 1962).

In Kenya, agriculture is a major occupation for over 70% of the people living in rural areas with smallholder farmers constituting 81 percent of the farmers in the country (Gautam, 2000). The horticultural sector, in particular, has grown fast as a commercial enterprise for both local and export market for many farmers. Many farmers depend on synthetic pesticides in the production of vegetables despite social and economic problems as well as environmental issues that challenge the sustainability of synthetic chemical-based approaches to pest management. Brookfield (1991) points out that, a sustainable future in agriculture is more likely if farmers shift to biologically based and diversified approaches that are more stable and of less damage to human health and the environment. The purpose of the study was to determine pest management practices among vegetable farmers in Bahati Division, Nakuru.

Objectives of the Study

The objectives of the study were to:
1. Describe the general pest management practices used in vegetable crop production by the smallholder farmers.
2. Identify the kinds of plant based pest management methods used by the smallholder vegetable farmers.

Literature Review

In agriculture, the use of pesticides has particularly raised pertinent questions about their effect on human health and the environment. The use of natural products from plants has thus attracted attention of researchers as potential sources of new pesticides. Pesticidal properties of plants have been explored in many parts of the world. For instance tobacco (*Nicotiana tabacum*) as leaf infusion has been used to kill aphids. Pyrethrum plant from the genus *Chrysanthemum* found in Kenya gives an insecticidal extract, *pyrethrum,* which is an active ingredient of many insecticides (Rajapakse and Ratnasekera, 2008). A study on efficacy of plant oils of different plant species against cowpea weevil *C. maculatas* and bean seed weevil *C. cinensis* revealed that plant oils can completely inhibit oviposition and adult emergence of the weevils. In addition it was observed that only small amounts of the oils were required to attain effectiveness. The oils also showed potential for use in seed dressing (Okwute, 2008). Plants such as *Azadirachta indica* (Neem) and *Tagetes minuta* (Mexican marigold) and *Chrysanthemum* have also been found to be important pesticidal species (Mwine *et al*., 2011).

In a study on pesticidal plants used in South Uganda, Mwine *et al*. (2011) noted that farmers used parts of plants such as leaves to control various pests. Wood ash was also a common addition to many plant extracts used in pest management. It was however observed that the farmers only gave general names for pests controlled and there was lack of specificity in formulation and mode of utilization indicating a need for efficacy evaluation of the plant based pesticides. The potential of plants in pest control can be appreciated in the use of Desmodium (*Desmodium uncinatum*) and Napier grass (*Pennisetum purpurem* in management of stem borers (*Chilo partelus*) and the parasitic weed, *Striga* spp. in cereal crops. A push-pull technique developed by ICIPE utilizing Desmodium as a push factor and Napier grass as the pull factor has led to a significant control of stem borers. The push-pull technique effectively controls the stem borers in maize (Koechlin, 2000). Desmodium has also been found to be effective against Striga or *witch weed.* An evaluation study on IPM in smallholder farming systems in Kenya by Loevinsohn, Meijerink and Salasya (1998) noted that alternatives to synthetic pesticides, such as pesticides from bioactive plant extracts were problematic/ The farmers' main concern was on the time needed to prepare and implement and efficacy of some of the methods. Another observation was that farmers who had undergone training on Integrated Pest Management through Farmer Field Schools in Othaya division in Central Kenya hardly used innovations on botanical pesticides/ The farmers' concern regarding botanicals was the effective dosages needed which neither they nor their trainers could confidently ascertain. There was a feeling by trainers that researchers should provide information on the correct dosages for botanical pesticides.

Methodology

The study was conducted in Bahati Division of Nakuru District, Kenya. Bahati division covers an area of 650 hectares, with a population of about 158,000 people. The study design used was a survey. The population of study was smallholder farmers growing vegetables such as tomatoes, kales, Irish potatoes, spinach and cabbages. Smallholder farmers were considered as practicing farmers owning land of less than 2 hectares which is about 5 acres. Purposive sampling was used to select the farmers to be included in the study. An interview schedule was used to collect the data. The schedule contained both structured and non-structured questions in order to provide in-depth information on pest management from the farmers. Data were collected through face-to-face interviews with the help of research assistants. Data were analyzed using inferential and descriptive statistics applying the Statistical Package for Social Sciences (SPSS).

Results and Discussion

General Pest Management Practices used in Vegetable Crop Production by Smallholder Farmers

The major economic pests for all the vegetables were similar. The most commonly cited pests for Kales and Cabbages were insects, in particular aphids, caterpillars, and cutworms. Aphids in particular were considered a major problem of concern for the farmers. All the farmers used different types of chemicals to manage these pests. The common chemicals used for aphids, caterpillars and cutworms were Karate, Danadim, Ogor, Dimethoate and Diaznon. There were a wide range of other chemicals that the farmers used such as Bestox, Alpha, Sancozeb, Cyclone, Polytrine, Actala and Osho. Kales and cabbages did not have major problems of disease attacks; however, a few farmers indicated encountering blight and stem rot which were controlled using Karate, Danadim, Dimethoate, Pencozeb, Sulphur, Pymarc, Milraz, and Copper. The farmers also used cultural methods of uprooting plants that were affected by root rot. Early blight was the key disease in Tomatoes. Spider mites, white flies and fruit borers were also cited as insect pests of concern in Tomatoes. The results of the study showed a variation in the choice of pesticides for management of blight in tomatoes. The farmers used Ridomil to prevent blight attack and Milraz as a curative measure where the blight had already affected the Tomatoes. Milraz was considered as a stronger and more effective fungicide and a regular choice for the farmers. Other pesticides used for blight were Antracol, Dimethoate and Polytrine. Polytrine , usually recommended for cotton, was applied in the early stages of growth to control blight and other pests such as spider mites, white flies and the fruit borers. The farmers gave the general names of the pesticides and did not have deeper knowledge of the chemical composition, information which if known can guide choice and prevent harmful side effects. The results showed a great dependency on pesticides among the vegetable farmers in Bahati division.

Plant Based Alternatives in Pest Management and Perception of Smallholder Vegetable Farmers regarding their Potential

The study sought to find out the awareness and adoption of non-pesticide alternatives to synthetic pesticides among the vegetable farmers. The results indicated that about 67 percent of farmers had some information about non-chemical alternatives. The alternatives were mostly plant based in particular plants considered to be weeds. Many farmers used the plant extracts in combination with common household detergents and wood ash. Most of the combinations of botanical pesticides were rated from low to moderate effectiveness. Although the farmers knew about the botanical methods and some had tried to use them on their vegetables, opinions varied about their effectiveness and usefulness in vegetable production. It was reported that the alternative botanical methods can help to overcome the harmful effects of synthetic pesticides. Farmers further acknowledged that less dependence on synthetic pesticides was safe for their health and the environment. The botanical methods can also provide a cheap alternative of pest management since the materials used such as the weeds and herbs were locally available on their farms. In spite of the positive views of the farmers towards botanical alternatives to synthetic chemicals, there was a general agreement that they would only use them if their effectiveness can be well proven. The farmers indicated that they could not adopt the botanical methods on a large scale in managing pests on their vegetable crops. The major limitation cited in the use of the botanical methods was lack of specificity in dosages and methods of application which made farmers not to completely trust their effectiveness.

Some of the observations by farmers on use of the botanical methods of pest management were: when wood ash was used to control ants, it prevented the growing of other plants and caused rotting and scotching; the alternatives were slow in giving results posing a risk of crop failure if they did not control the target pests on time; the use of plant based pesticides involved too much time to collect and prepare hence their use was cumbersome and time consuming. The farmers noted that although sometimes the botanical methods provided protection for their crops, the correct dosage and spraying interval posed a problem. The lack of information on rates of application could also lead to side effects on the consumers and the farmers. The farmers indicated that vegetables cannot be grown productively without the use of synthetic pesticides. The findings indicate that the farmers in Bahati were aware of alternatives to synthetic pesticides/ The farmers' suggestion was that more research and education of the farmers was needed. The kind of learning that farmers must be taken through are specific ingredients, dosages, methods of preparation and application and specific pests and vegetables to target.

Conclusions

Farmers in the areas studied in Bahati division depend a great deal on synthetic pesticides in vegetable production. However, the farmers appreciated the need for alternatives to synthetic based pesticides whose negative side effects the farmers were aware. The vegetable farmers had information about pesticidal plants and many explored their use in managing pests. The plant extracts were also combined with others or added detergents or wood ash implying that the farmers considered them ineffective when used alone. The farmers acknowledged the potential of pesticidal plants in pest management when considering their availab ility, less cost and less harm to human beings and the environment. The farmers major concern was the efficacy of the plant based pesticides because even the ratings given on effectiveness given were somewhat general. The active ingredients or the chemical nature of the various plant extracts were unknown hence the farmers were not certain on the precautions to take during their application. The challenge in the use of the plant based alternatives was therefore the uncertainty in methods of preparation and application, quantities or dosages required and frequency of application and possible toxicity from their use.

Recommendations

In order for pesticidal plants to find a niche among farmers especially smallholder farmers with limited resources, further research is needed with emphasis on preparation methods, determining effective doses, and information on human toxicity. The awareness level of farmers in Bahati division about the use of botanical based pesticides can be complimented further by intensive tra ining to increase their knowledge and skills and experimentation at the farm level. The farmers must be led in a systematic way to search, try out and confirm alternative or complementary solutions to pest management that are sustainable and appropriate to their situations.

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The Effectiveness of *Sphenostylis erecta* in controlling blue ticks *(Boophilus decoloratus*) infestation

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Abstract

The efficacy of *Sphenostylis erecta* extracts against *Boophilus decoloratus* ticks prevalent in northern Zimbabwe, was investigated. *B. decoloratus* tick larvae, hardened for 8 to 10 days, were exposed to different concentration levels of *S. erecta* extracts in vitro using a 3*6 factorial experiment in a Completely Randomised Design. The Soberanes, technique was used and tick larvae were incubated at 27 ^{0}C, 85-90 % relative humidity (RH) and photoperiod of 12:12 (Light:Dark). There were three exposure periods of ticks to plant extracts (i.e., 24 hours, 48 hours and 72 hours) at six concentration levels of *S. erecta* extracts (i.e., 10, 20, 50, 80 and 100%) and Tick buster (Amitraz) as a control. Each treatment was replicated six times, with 100 tick larvae per replicate. Mortality was used as an indicator of acaricide efficacy. There was a significant interaction bet ween concentration level and exposure period ($P<0.05$). The 100% concentration level of *S. erecta* extract achieved 94% mortality, while the 10% concentration level had 0% mortality within 24 hours. However, as the concentration increased from 20% to 80%, mo rtality also increased from 10 to 70% within 24 hours. Tick buster had 100% mortality within 24 hours. The conclusion from this study was that *Sphenostylis erecta* root extract is acaricidal and is as potent as tickbuster at high concentration. At lower con centration levels, *S. erecta* requires more than 24 hours to be effective against *Boophilus decoloratus* ticks.
Key words: *Sphenostylis erecta, Boophilus decoloratus*, acaricidal efficacy, tick, mortality

Introduction

The transmission of diseases by external and internal parasites is one of the major constraints to production and improvement of the livestock industry in the tropics (Graf *et al*., 2004). External parasites such as ticks, mites and fleas transmit pathogens that include: bacteria, spirochetes, protozoa, rickettsia, viruses and toxins (Rajput *et al*. 2006). Ticks are the most important ecto-parasites and are responsible for severe economic losses in livestock.

Problems encountered with acaricide use in tick control include: poisoning of treated animals and human staff; pesticide residues in meat and milk; environmental contamination, especially in water bodies; resistance that ticks acquire to acaricides and cost of application (Rajput *et al*., 2006). Ethno-veterinary acaricides offer resource-poor farmers an alternative to conventional acaricides. Several plants have been shown to possess pesticidal properties and are used by some farmers as acaricides. However, such unregistered preparations require careful use to avoid poisoning or skin damage in topical applications (Graf *et al.*, 2004). Plants that have been investigated for pesticidal efficacy include *Lippia javanica, Azadirachta, Tephrosia vogellii* (Madzimure *et al.*, 2011). *Sphenostylis erecta*, a plant found in Mt Darwin, Zimbabwe is used by smallholder farmers as food (seeds and leaves) and as an acaricide (roots). However, *S. erecta* has not been documented as an acaricide, but as a fish poison as reported by Watt and Brandwijk (1962). The objective of this study was, therefore, to determine the efficacy of *Sphenostylis erecta* in controlling blue tick larvae (*Boophilus decoloratus*).

Literature Review

Socio-economic risks from livestock diseases are in the form of losses in production, profitability, cost of treatment, disruptions to markets and zoonosis (Rajput *et al*., 2006). In the tropics and subtropical areas, ticks transmit tick-borne diseases such as anaplasmosis, babesiosis and theileriosis which decrease production and increase morbidity and mortality of animals (de Waal, 2000). Heavy tick infestations lead to anaemia reduced feed intake and emaciation among domestic animals. Inflammation and development of wounds at the bite site may serve as entry points for secondary infection (Hema, 2006).

Various approaches are employed to address the problems of ticks. The most common approach in Southern Africa is centred on intensive vector control using synthetic pesticides. Development of cattle lines or breeds with enhanced genetic resistance is another approach to the control of ticks and tick-borne diseases (Decastro and Newson, 2003). However, poor breeding practices in most smallholder farming communities hamper application of this is approach. Ethno-veterinary acaricides offer resource-poor farmers an alternative to conventional acaricides. Several plants with acaricidal properties are used by resource-poor farmers (Madzimure *et al*., 2011). *Sphenostylis erecta,* a legume species which occurs in Africa is used as food in central Africa (seeds and flowers) and the roots are used medicinally and as a source of dye (Potter and Doyle, 2002). In Zimbabwe, smallholder farmers use its root extracts as an acaricide.

Methodology

Study Site

Sphenostylis erecta and *Boophilus decoloratus* adult ticks were collected from Mt Darwin, Zimbabwe. Mt Darwin lies between is $30^{o}32^{1}$ and 33^{0} 00'N and 16^{0} 42' and 19^{0}15'E, at an altitude of 1 350 metres above sea level. It is characterised by low mean annual rainfall of less than 600mm. Mean temperatures range from 14 ^{0}C in winter to 41^{0}C in summer.

Tick collection and egg incubation

Thirty engorged *Boophilus decoloratus* female ticks were collected from cattle in Mt Darwin district, Zimbabwe. The ticks were given seven days for egg-laying. The eggs were weighed into 1g samples, placed into test tubes and incubated for 28 days at a temperature range of 26-28^{o}C and humidity of 80-90%. Potassium Chloride was used as the culture medium. Twenty eight days were allowed for incubation and hatching. The larvae were hardened for 8 days before being subjected to the *S. erecta* plant extracts.

Experimental Design

The experiment was conducted at the Central Veterinary Laboratory in Harare, Zimbabwe. A 3 * 6 factorial experiment in a Completely Randomised Design (CRD) was used and the factors were concentration of plant extract and duration of exposure of the tick larvae to the treatment. There were 5 plant extract concentration levels and a control, making a total of 6 treatments. Three levels of duration of exposure were used, i.e., 24 hours, 48 hours and 72 hours.

Preparation of plant extracts

Sphenostylis erecta roots were collected from fresh plants specimens and 2 kg of the roots were weighed and ground with pestle and mortar. The extracts were filtered through a mutton cloth, passed through a 10mm sieve. Fifty-two millilitres of the plant extract were drawn and used to make the five treatments, i.e., 10, 20, 50, 80 and 100% concentration from 4 ml preparations of *S. erecta* plant extract and olive mixtures. The plant extracts were prepared 24 hours before testing and stored at room temperature (25 ^{o}C). Tick buster containing 12.5% Amitraz was used as a positive control at 0.03% concentration.

Plant extracts testing

Testing for acaricidal efficacy of *S. erecta* root extracts was done using the Soberanes' technique (Soberanes, Santamaria, Fragoso and Garcia, 2002). Four millilitres of each concentration level were transferred into a 10cm diameter glass Petri-dish containing a 9 cm diameter piece of Whatmann filter paper. Samples of 100 larvae were placed onto each filter paper and it was folded and the edges sealed with paper clips to form a packet. The larvae-containing petri dishes were placed in an incubator at 27^{o}C, 85-90% RH, and photoperiod of 12:12 (Light: Dark). Each concentration level of treatments was replicated six times. The packets were removed from the environmental chamber and numbers of live and dead larvae were recorded after 24, 48 and 72 hours.

Data Analysis

Data were analysed using the Statistical Analysis System Version 9.1.3 (SAS, 2004) and SPSS. Mortality count data were tested for normality with the PROC UNIVARIATE in SAS. The data was normally distributed and a logM transformation was performed and mean logM mortalities were computed using PROC GLM in SPSS. This allowed subsequent pairwise comparison between the means using the least squares test.

Results

Treatment effects

The PROC GLM on rank-transformed data explained 88% of the variation (R^2=0.88); CV=18.80%) on the mortality of *Boophilus decoloratus* larvae, while the PROC GLM on logM transformed data explained 99 % of the variation (R squared = 0.992 (Adjusted R squared = 0.984). Both treatment and duration of exposure had significant effects (P<0.05) on mortality. However, there was a significant interaction of root extract concentration level and duration of exposure (P < 0.05). Tick larvae mortality was both time and concentration-dependent. Concentration levels below 50% plant extract required an exposure period above 48 hours to attain 100% mortality. At 80% plant extract concentration level, mortality was 71% within 24 hours but from 48 hours onwards, 100% mortality was observed. At 100% plant extract concentration level, mortality was 94% within 24 hours and 100% mortality was observed from 48 hours onwards. Therefore, as the plant extract concentration increased, a shorter period of exposure was required to attain 100% mortality. The control, tickbuster (Amitraz) attained 100% mortality across all three durations of exposure.

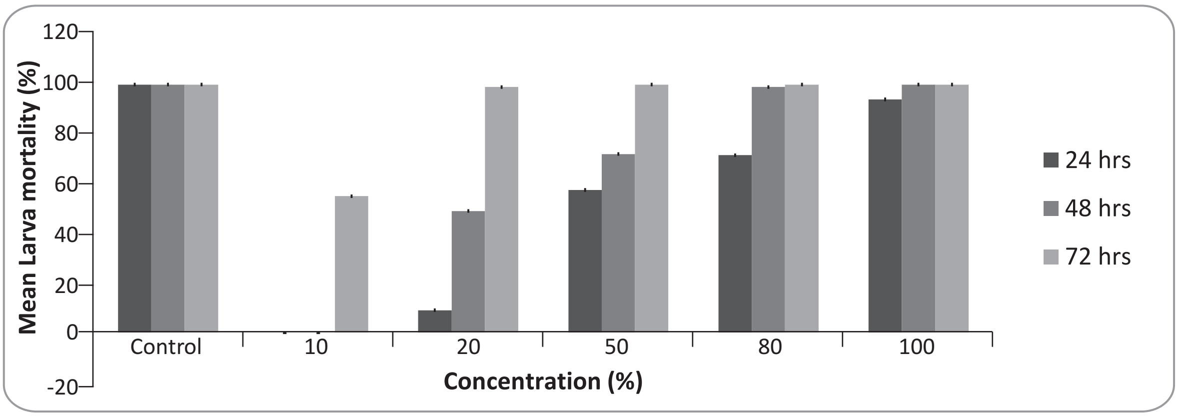

Figure 1: Composite mortality trends from 24 to 72 hours duration of exposure

Discussion

Tick larvae mortality was both concentration and time-dependent as shown by the significant interaction between concentration and duration of exposure. As the concentration of the plant extract increased, the mortality of the tick larvae also increased. This observation concurs with Belmain *et al*. (2001) who also obtained low tick mortality with 5 to 10 % concentration levels in trials with *Lippia javanica.* Similarly, Madzimure, *et al.* (2011), also observed the typical dose-dependency effect when *Lippia javanica* leaf extracts were used as an acaricide against cattle ticks. However, according to Madzimure *et al*. (2011), there was no further increase in efficicacy beyond 10 % concentration level. In this study, lower concentrations below 20% had lower mortalities within 24 hours, while higher concentrations were as effective as the synthetic acaricide, Tickbuster (Amitraz). The type of active ingredient in the botanical extracts determines efficacy. Rajput *et al.* (2006) reported that the performance of an acaricide depends not only on the activity of a product, but on the quality and quantity of the active ingredient. Various compounds with different toxicity levels and effects which include retinoid, sesquiterpene lactones, glycosides, flavonoids and tannins among others have been isolated from different plants. Their effects on ticks vary from general repellent effects to acute toxicity and inhibition of oviposition (Madzimure *et al.,* 2011).

In this study, higher concentration levels of plant extract required less time (i.e., 24 to 48 hours) to kill all tick larvae (100% mortality) while lower concentrations required more than 48 hours to achieve high mortality. Choudhury (2009) also obtained similar results with *Azadirachta indica* against *Boophilus decoloratus*. In Choudhury (2009), 20, 40, 60, 80 and 100 % concentrations of *A. indica* killed 100% of the tick larvae after 27 and 24 hours.

Concentration levels from 50% of *S. erecta* and below attained 100 % mortality after 72 hours. This shows that the acaricide remained active and potent after 72 hours. Madzimure *et al.* (2011) also reported a possibility of residual effect in *Lippia javanica* leaf extracts as the efficacy of the 5% concentration level improved after the seventh week of treatment. It is, therefore, important to establish the length of the residual period of *S. erecta* root extracts to determine frequency of application of the botanical acaricide and also for safety in its use.

Persistency of certain compounds can lead to unacceptable residues in meat and milk. In addition, the acaricide must be highly effective against ticks without injuring the host animal or humans (Graf *et al*., 2004.)

An infective adult tick can infect an animal within two or three days after it attaches. Hence, the strategy i n controlling a one-host species such as *Boophilus* should ensure that females are killed before they can finish engorgement (Junte *et al*., 2008). As such, all concentrations levels above 10% *S. erecta* can be used against *Boophilus* tick larvae. However, con centration levels below 20% should be discouraged to avoid exposing ticks to sub-therapeutic levels. One host ticks such as *Boophilus microplus and B. decoloratus* develop resistance to most chemical acaricides. Acaricide resistance is genetically determine d and its expression can be triggered by various factors (Junte *et al.*, 2008). Recommended concentrations and treatment frequencies must be used to prolong use of the botanical acaricide (Graf *et al*., 2004).

Conclusion and Recommendations

Sphenostylis erecta is effective against *Boophilus decoloratus* tick larvae *in vitro* at concentrations above 10% plant extract. Higher concentrations of plant extract require 24 to 48 hours duration of exposure to attain 100% mortality of tick larvae. The 100% concentration level of plant extract had acute toxicity and had equal potency to Amitraz (Tickbuster). *Sphenostylis erecta* root extracts can be used from concentrations of 50% upwards for controlling *B. decoloratus* ticks in areas where the plant is abundant. Where it is scarce, farmers can use lower concentrations of 20%.

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Effects of land ownership on Land investments: A Case of Central and Southern Malawi

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Abstract

Growth of agricultural sector in Malawi is being undermined by continual loss in crop production plus massive post-harvest losses through poor pest and disease management both in the field and in storage. This threatens food production which is a major income component for the majority of rural households in most parts of Africa. Many authors have indicated the use of pesticidal plant products as a most cost effective traditional way of controlling pest and diseases of crops both in field and in storage structures. This is however constrained by limited population of pesticidal plants in the local communities. The major question is whether growing of pesticidal plants by rural households in Malawi is being affected by tenure security status of their plots. In Malawi, there is information gap on the effects of land ownership on land related investments. The study used a random effects probit econometric model to analyze factors associated with land related investments (Natural trees and Exotic trees (Pesticidal plants)) in central and southern Malawi. A random effect Tobit econometric model was also used to analyze the intensity of investments under a given land tenure security system. Analytical results indicated a weak association between land tenure security and land related investments. It was also noted that land related investments were associated with other factors rather than the land ownership security itself and the investment in question. Hence giving a positive indication those farmers can invest in multiplication of pesticidal plants regardless of tenure status of their plots for future benefits.

Introduction

Malawi still remains one of the poorest countries in the world with an estimated 52 percent of the population still living in poverty/ The major source of employment is agriculture which also supports the country's economy. It accounts for 36 percent of gross domestic product (GDP) and 80 percent of export earnings of which 60 percent comes from tobacco (GoM, 2002). However, the growth of agriculture sector is being undermined by continual loss in crop production through soil erosion as well as the nutrient extraction. In addition, accumulation of pest and diseases as well as massive post – harvest losses emanated from poor pest and disease management practices also contribute significantly to crop production losses both in fields as well as during storage. This is because many farmers are poor hence they cannot afford to procure synthetic pesticides which are costly. This justifies the use of pesticidal plants as a traditional and cost effective way of controlling field and post – harvest losses. However the use of these plants is also undermined by limited population of the pesticidal plants in the rural communities of Malawi. The major question is whether adoption of these technologies by rural households in Malawi is being affected by existing tenure security systems of their plots. It is widely debated that land tenure security induces investments on land (Chand and Yala, 2009, Place and Hazell, 1993, Place and Swallow, 2000, Feder and Noronha, 1987). However this paper found a weak relationship between land ownership security and land investment using random effects probit and tobit models/ This means there is potential for multiplying these plants since they don't rely on tenure security of farmers' plots/

Literature Summary

Customary land in Malawi claims a larger proportion of land in terms of area and the number of workers (Lunduka *et al*., 2008). Land in the system is acquired through traditional leaders (custodians of the land). Under customary system, land cannot be sold since it is considered a property of the community and can only be sub-divided by members of the family (Chirwa, 2008). In addition, land tenure is categorized depending on descent (Patrilineal and Matrilineal) as well as residency (Patrilocal, Matrilocal and Uxililocal) practices (Lunduka *et al.*, 2008, Place and Otsuka, 2001, Mbaya, 2002). In matrilocal system, the land descends through mothers and daughters which Peters (2002) called matriline. However in patrilocal system, land is owned through men who have powers to pass the land to children (Place and Otsuka, 2001). Land transfers and tenure arrangements are also linked to investment in land in terms of farm and tree management i.e. they may either strengthen or weaken the incentives to invest on the available land (Besley, 1995). Many researchers have argued on the importance of secure property rights for agriculture on investment, improved credit access and facilitating functions of land sales and rental markets (Feder and Feeny, 1991, Schweigert, 2006, Besley, 1995, De Soto, 2000, Chand and Yala, 2009). Incentive to invest in more productive inputs and efficient land use by farmers is weakened by absence of secure ownership of property thus according to economic theory (Feder *et al.*, 1988, Hayes *et al.*, 1997, Roth and Haase, 1998). The tenure system has also a greater influence on the organization and efficiency of a gricultural production and the efficient allocation of productive resources. Economic theory states that farmers with secure legal rights will have more incentives and better ability to invest due to lower perceived risk Feder (1987). In another similar st udy in Ethiopia by Deininger and Jin (2006), it was also revealed that where households undertake investments to increase their tenure security and transferability of land rights, rural development and productivity can be enhanced. In Malawi very little empirical evidence has been sighted with no conclusive results on the land tenure impact on investments and productivity. The studies found that customary tenure still dominates a greater portion of land in Malawi e (Mbaya, 2002). In addition, customary land tenure claims over 90% of total available land in most parts of Africa according to World Bank (2003) despite limited legal status. Place and Otsuka (2001) also found five different categories of acquiring land under the matrilineal and patrilineal system s, which have different tenure security implications. According to the same Place and Otsuka (2001), agricultural development can be hindered by the existing customary land tenure institutions. This may be due to limited incentives given to farmers to emba rk on land investments. Holden et al (2006) further noted the possibility of increasing marginalization of women over rights and access to land in Malawi. This may not only be in family lineage and negotiations, but also in bargaining policies related to i mplementation of any land reform policies and programmes (ibid). This may also act as a disincentive to investment by women in the existing land ownership security regimes (Place and Swallow, 2000). However, the authors further suggests establishment of special mechanisms that protects women against direct discrimination and more indirect processes of marginalization in Malawi as means of encountering the problems. Lack of well -defined property rights under traditional land ownership systems has also been a rgued by many authors (Sjaastad and Bromley, 1996, Gavian and Fafchamps, 1996, Feder and Feeny, 1993), which makes land insecure to invest. Studies in Malawi have on the contrary found different results i.e. according to findings by place and Otsuka (2001) and Chirwa (2004), Land was found to be secure under the customary system. There was also no any evidence of any disincentives to investments.

Description of Research

This paper is about the effects of land ownership security on land investments. It is believed that, in many parts of Africa, adoption of technologies by rural households is being affected by existing tenure security systems of their plots. It is widely debated that land tenure security induces investments on land . This is supported by three major hypotheses by Feder et al (1988) which depict three important economic relationships to be considered while trying to link land tenure security and productivity relationships; thus title can be used as collateral when getting credit for agricultural i nvestment. In addition, title increase farmers tenure security and enhance their willingness to make medium to long -term investments. Finally, title facilitates the transfer of land resources to more productive farmers. This paper therefore dwells much on the second hypothesis where investment on land is a function of title. In addition, this paper is trying to explore potential of reducing both pre and post -harvest losses through investing in botanicals pesticides in situations where land ownership security has no direct linkage with land investments, or where a weak relationship exists.

Results and Discussion

The pragmatic discussions focus mainly on the effects of land ownership security on land investments particularly planting of exotic trees and Nat ural trees (pesticidal plants)

Probability of Investing in Natural and Exotic Trees

This section explores the probability of farmers investing in both exotic and natural exotic trees (pesticidal plants). To analyze this, a random effects probit model was used in a two stage analytical procedure to predict location of residence (patrilocal – dummy) which is used in a second stage as a proxy for land tenure security. The results of the model show that there is an association between land ownership system (pa trilineal – dummy) and the location of residence. Thus, patrilineal system is positively and significantly correlated with the patrilocal residence which means, most households in the patrilineal system are very likely to be in the patrilocal residence after marriage. Then the variable was predicted and used in the second stage to analyze if it has any significant influence on land investments

The results show that patrilocal residence is not significant in all two investment models. This means that there is no association between choice of patrilocal residence and the probability of planting exotic and natural trees (pesticidal plants). This implies that there is no significant effect of land ownership security on land investment choices in central and southern Malawi.

Level of Investing in Natural and Exotic Trees

The predicted variable was also used in the investment model to analyze the intensity of investments. The results in the table below show that there is a slight correlation between patrilocal residence and levels of investment in both exotic and natural trees (pesticidal plants). However, the significance level is very low to make any conclusive results. This means there is a very weak association between land ownership security level of investment in both natural and exotic trees (pesticidal plants).

Random effects probit model on probability of investing in Exotic Trees and Natural trees

Variables	Exotic Trees Model		Natural Trees Model	
	Estimated coefficient	Std. Err.	Estimated coefficient	Std. Err.
Patrilocal	0.043	(0.12)	-0.064	(0.13)
Patrilocal Error	-0.001	(0.01)	0.004	(0.01)
Loam (dummy)	0.240**	(0.11)	0.394****	(0.12)
Average fertile (dummy)	0.548****	(0.13)	0.544****	(0.13)
Not fertile (dummy)	0.364**	(0.14)	0.338**	(0.15)
Zomba (dummy)	0.127	(0.16)	0.340**	(0.17)
Kasungu (dummy)	0.080	(0.24)	0.892****	(0.26)
Lilongwe (dummy)	0.377	(0.25)	1.141****	(0.28)
constant	-1.176***	(0.44)	-0.083	(0.48)
Insig2u constant	-2.758**	(1.11)	-4.870	(9.12)
Probability>chi2	0.004		0.000	
Number of Observations	951		951	

*Significance level, *10%, **5%, ***1% and ****0.1%.

*Matrilocal residence is used as a reference point in the estimated model. Other variables included in the model were tropical livestock units, quality of house, distance from home to plot, household labour, number of plots, years in marriage, land endowments, number of years in school, age of household head, age^2, number of years in school, age of household head, age^2, soil characteristics, district dummies and year dummies.

Random Effects Tobit Model on Level of Investment in Exotic Trees and Natural Trees

Variables	Exotic Trees Model		Natural Trees Model	
	Estimated coefficient	Std. Err.	Estimated coefficient	Std. Err.
Patrilocal	0.391*	(0.22)	0.443*	(0.26)
Patrilocal Error	-0.011	(0.03)	0.014	(0.03)
Tropical Livestock Units	0.065*	(0.04)	-0.063	(0.05)
Number of plots	0.040	(0.06)	0.239****	(0.07)
Land endowments	-0.004	(0.07)	0.174**	(0.08)
Loam (dummy)	0.446**	(0.21)	0.619**	(0.24)
Slight (dummy)	-0.404**	(0.19)	-0.550**	(0.23)
Average fertile (dummy)	0.980****	(0.24)	0.736**	(0.29)
Kasungu (dummy)	-0.245	(0.45)	1.341**	(0.53)
Year 2007 (dummy)	0.294	(0.20)	1.696****	(0.25)
Year 2009 (dummy)	0.148	(0.20)	1.904****	(0.25)
constant	-1.317	(0.83)	-2.935***	(1.01)
Sigma_u constant	0.000	(0.57)	0.000	(0.57)
sigma_e constant	2.286****	(0.08)	2.519****	(0.10)
probability>chi2	0.000		0.000	
Number of Observations	951		951	

Significance level, *10%, **5%, ***1% and ****0.1%.

Other variables included in the model were quality of house, distance from home to plot, household labour, years in marriage, number of years in school, age of household head, age^2, number of years in school, age of household head, age^2, soil characteristics, district dummies and year dummies.

Conclusion and Application

From both models analyzed above, land ownership security in central and southern Malawi has a very weak influence on investment in both exotic and natural trees (pesticidal plants). This means growing of pesticidal plants and multiplication in Malawi cannot be affected by land ownership security of farmer's plots. Hence there is need for scientists to lobby for better policies that encourage farmers to invest in the pesticidal plant products. In addition, there is need to sensitize farmers on how to process and utilize pesticidal products that are locally available so that farmers understand the importance of having them in stock. Furthermore, there is need to use multi-disciplinary approach i.e. social scientist need to play a role in training farmers the socio and economic costs and benefits of using pesticidal plants and there role in reducing pre and post-harvest losses.

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Farmers' Knowledge and Perceptions on the use of pesticidal plants for rodent control in maize farming system in Tanzania

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Abstract

A study was conducted in three districts in Eastern Tanzania from May to September 2012 in three villages selected per district/ The aim of the study was to assess farmers' knowledge on the use of pesticidal plants for controlling rodent both in maize fields and storage structures. A total of 270 farmers were randomly selected from 9 villages (30 respondents from each village) for individual interview. Farmers (56%) ranked higher the rodent pests than the other pests. The crop loss due to rodents estimated by farmers was 64% and 36% in fields and stores, respectively. The most common used rodent control measures were ro denticide (30%), traditional methods (snap and wire traps, pitfall trap, digging rodent burrows, bush -fire and cat) (18%) while some farmers (52%) used both rodenticide and other traditional methods at the same time. Rodenticide use showed statistical significant difference ($p < 0.00$) among districts where Handeni district was the most of all. However, farmers use rodenticides with inadequate knowledge of their effects to environment and health. Only 15% of respondents reported that they attained training o n how to apply rodenticides. With regard to the use of pesticidal plants only 4% of respondents reported to use them for rodent control. This study revealed that there are some farmers with knowledge on application of pesticidal plants for controlling rode nts. Therefore, evaluation of pesticidal plants would come up with plant product which can reduce chemical risks to poor resource farmers in Tanzania.

Introduction

Maize is cultivated in the tropical, subtropical and temperate climatic regions of the world (Flavio *et al.,* 2004). It is the predominant crop which is used in a multitude of ways varying from region to region (Flavio *et al.,* 2004). About 30% of world production is used for direct human consumption and as an industrial input, while 70% is used as animal feed (Flavio *et al.,* 2004). In Tanzania, maize is grown throughout the country with major producing areas being those in the southern and North-Eastern part (Njau, 2001). It is a staple food crop grown almost in all villages in Tanzania (Rugumamu, 2009). As human food, maize grains are prepared by boiling or roasting or milled into flower for preparation of stiff porridge ('' *ugali*" in swahili) and it is ranked as number one food crop (Macha, 2001). Maize husks are used for livestock feeding (Ajiboso, 2007), and the maize cobs after shelling may be used as fuel in small scale farms for cooking food. In other parts of the world, for example, in German, maize is used as a feedstock for biogas plants (Plöchl, 2009).

In Tanzania, over the years maize has been produced by small holder farmers who produce 95% from farms and 5% from estate farms (Macha, 2001). However, its yields are hampered by problems such as soil infertility, drought, weed infestation, crop diseases and pest attack (Sanchez *et al*., 1997). Although arthropod pests cause damage to crops, rodents are more than serious pests in agriculture and affect most crops at sowing, seedling stage and at physiological maturity as well as in storage and therefore cause reduction in both quality and quantity of harvested crops which lead to widespread of food shortage. To reduce the rodent infestation, farmers have been trying to adopt different rodent control metho ds (Makundi *et al*., 2006). According to Mulungu *et al*. (2010) the rodent control measures include bounty schemes, burning of houses and vegetation, trapping and poisoning. In modern times, rodenticides have been an important element in managing rodent pests. However, the use of synthetic rodenticides may be associated with; develop rodent bait shyness (toxiphobia), require surplus baiting to ensure that most of the rodents have access to the bait and could feed as much as possible (Mbise, 2006), hazardous to environment, risks to non-targeted organisms including human (Makundi *et al.,* 2006), expensive to farmers (Mbise, 2006). Therefore, rodent control programs should rely on ecological based management which reduces synthetic chemical dependence and hence safety to environment and less cost to small scale farmers/ These have drawn researcher's interest in plant and plant products as source of pesticide. Pesticidal plants have long been used in Tanzania for various field and storage pests (Antonio, 2009). However, little has been documented on their use for rod ent control. This study therefore, investigated the knowledge of small scale farmers on the use of pesticidal plants for rodent control.

Literature Summary

Rodents are a key mammalian group, and are highly successful in many environments throughout the world. Tanzania, like most other tropical African countries, is highly and widely populated with different rodent pest species. Multimammate rat, *Mastomys natalensis* and *Arvicanthis niloticus* are some of the commonest field rodent pest species in the country and they are semi-domestic and found in all types of fallow and cultivated land (Massawe *et al*., 2003). However, *M. natalensis* is a pest of primary importance which is widely distributed in the country (Makundi *et al*., 1999). In rural communities, it inflicts heavy damage to maize crop in fields and during storage. The types of damage most often caused by rodents to crops in fields are destruction of seeds after sowing and damage to the mature crop on the stem (Mulungu *et al*., 2003). In Tanzania, rodent damage is estimated to an average 15% of maize in fields (Makundi, 1991), corresponding to 400,000 tonnes, the amount which could feed 2.3 million people for a whole year and a financial loss of US$ 40 million (Leirs 2003). In most part of Tanzania, farmer s struggle to avoid crop loss by using traditional methods as well as synthetic chemical such as rodenticides for reducing rodent population size (Makundi *et al*., 1999). These methods, however, provide only a short-term solution because the removal of a rodent simply provides a space into which individuals from the surrounding areas may be drawn and the colony soon recovers of which the problems posed by the rodent persist. This calls for an alternative way of rodent control using pesticidal plants.

Description of Research

The study was carried out in Handeni, Mvomero and Kilosa districts in Tanzania between May and September 2012. A multistage sampling technique was employed and the sampling frame was district, village, and finally a household. Purposive sampling was used to select the districts and villages with rodent infestation where three districts were selected. In each district three villages were selected for survey making a total of 9 villages. Within a village the list of farming households growing maize was used as a sampling frame from which respondents were selected randomly. Thirty famers per village were sampled, giving a total sample size of 270 (90 farmers in each district). The aim of this study was to determine the knowledge of farmers on the use of pesticidal plants for rodent control. Techniques that were used to capture information in the study areas included individual interviews using semi-structured questionnaire comprised of both closed and open ended questions. The questionnaires were designed to seek information on household socio-economic characteristics (age, education, experience on farming, major source of income and farm size); rodent control measures and perception on the use of pesticidal plants for rodent control.

The questionnaires were checked and edited for completeness and internal consistency. Questionnaires were then sorted, numbered and data coded before entry into access software. Quantitative data processing involved categorization, reorganization, editing, codi ng and entered in a computer by using Statistical Package for Social Sciences (SPSS) program. A substantial part of the analysis in this study was based on descriptive statistics analysis by using SPSS computer software based on t-test, frequency analysis and percentages. Data were presented using texts, graphs and tables to illustrate findings.

Results and Application

Out of the 270 respondents interviewed, 63% were males and 37% females. Most respondents (96%) had the age of above 25 years and very few (4%) aged below 25 years. In all districts most of the respondents (36% in Handeni, 32% in Mvomero and 32% in Kilosa) had primary school education. The proportion of respondent with secondary school education was higher (75%) in Mvomero district than in Handeni (17%) and Kilosa (8%) districts. The major source of income in the study areas was Agriculture; as mentioned by 99% of the respondents from all study districts. The minority (42%) of the respondents reported that they have their income obtained from non-agricultural activities (small business and salaries) where as 58% obtained income from only farming. Some respondents (45%) reported that they have been in farming for more than 20 years while 12% said that they had 6 – 10 years of experience in farming.

The cultivated farm size of majority of farmers (80%) was more than 3 acres and only 20% of farmers cultivated fields of less than 3 acres. Significant difference ($p<0.00$) between districts was recorded for the size of farms cultivated. Handeni had greater number (42%) of framers who cultivated more than 3 acres followed by Kilosa (24%) and Mvomero (13%). Most respondents in Mvomero (10%) cultivated small fields of less than 3 acres.

Majority of farmers (80%) differentiate rodent species by color while minority (20%) differentiated them by appealing to body size, mouth structure and place of living (house rats and field rats). Results show that in all districts; rodents (both in the field and store), storage insects and lack of farm inputs were the major constrains to maize production/ The study further revealed that rodents in both people's homes and fields caused great damage to maize. It was revealed that in all districts rodent damage maize at the period just after sowing (55%). Damage in this stage forced farmers to re-sow the seeds something that doubled the farming costs to the farmers. This was followed by germination stages (35%) at which more crops were damaged. Only few respondents (8%) in all districts claimed that their crops were being damaged during flowering stage. At maturity stages (2%) crops were less damaged compared to other stages of crop development. Poor soil fertility, droughts, and crop diseases were not terrifying problems for maize among respondent farmers in Mvomero district. However, crop diseases and weeds were not a problem in Handeni and Kilosa, respectively.

To overcome rodent infestation farmers (83%) used different control measures. Rodent control methods mostly mentioned by respondents were rodenticides (30%), traditional methods (snap and wire traps, pitfall trap, digging rodent burrows, bush fire and cat (18%), and use of rodenticide in combination with other traditional methods (52%). With regard to when implementation of the rodent control measure using rodenticide starts, majority of respondents (73%) reported that they start after they observe rodent movement in their fields while 27% of farmers start after being instructed by extension officer as well as part of routine farming practices.

Of all respondents interviewed majority of farmers (53, 55 and 62 %) in Kilosa, Handeni and Mvomero, respectively had used synthetic rodenticides to protect their crops from being damaged by rodent pests. Fourty three per cent of respondents in all districts claimed to have applied rodenticide both in field and store. Farmers applying rodenticide in fields was 37% more than those applying only in stores 20%. The frequency of rodenticide application by farmers per farming season was once (30%), Twice (46%) and thrice (24%). However, most of the farmers (85%) reported that they had never attended any training on how to apply the rodenticide. They apply the chemical basing on their own experience. Most of farmers (63%) said that they provide information to their neighbour prior application of rodenticides. With regard to availability of rodenticide, 51% of respondents obtain rodenticides from local markets.

In regard with effectiveness of rodenticides, farmers had different perceptions. It was revealed that farmers 36, 24, 21 and 18 % perceived that the rodenticides they use are excellent, very good, good and fair, respectively. While only 1% replied that rodenticides do not work at all.
The study aimed to determine whether the respondents used pesticidal plants for rodent control . When asked if they had ever used plants for rodent control, majority of farmers (96%) replied directly that they had never used pesticidal plants for rodent control. Most of the pesticidal plants were, however, reported to be used for

insect-pest control. When farmers asked on which types of pesticidal plants they use for insect pests, some names were mentioned in their vernacular languages which were later translated to English. Such plants mentioned by farmers were aloe, neem, pepper, Mimosa and tobacco. When asked if they had any information on some plants which can be used for rodent control, majority of respondents (90%) contended that they were not aware while the remaining (10%) of farmers reported to know the pesticidal plants for rodent control. However, most of farmers (97%) responded that they were willing to use pesticidal plants whenever they became aware of plants capable of rodent control.

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Evaluation of in vivo acaricidal effect of soap containing essential oil of *Chenopodium ambrosioides* leaves in the western highland of Cameroun

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Abstract

Study on acaricidal properties of foam soap containing the essential oil of *Chenopodium ambrosioides* leaves was carried out on *Rhipicephalus lunulatus*. Four doses of essential oil (0.03, 0.06, 0.09 and 0.12µl/g) and a control with four replications for each treatment were used for *in vitro* trial. Each replication consisted of 10 ticks in a petri dish with filter paper impregnated uniformly with the foam soap on the bottom. Following *in vitro* trials three doses (0.06, 0.09 and 0.12µl/g) and the control in three replications were selected for *in vivo* test Based on mortality rate recorded from *in vitro* trial. Each replication was made up of 10 goats naturally infested with ticks. Results of this study indicate that foam soap containing essential oil of *Chenopodium ambrosioides* leaves is toxic to *R. lunulatus*. The *in vitro* mortality rate was observed vary from 0 to 33.33% on day 8 after treatment with the control as compared to 79.16% with the lowest dose (0.03 µl/g) on day 8 and 100% With the highest dose (0.12 µl/g) on day 4. Meanwhile, the *in vivo* mortality rate was observed to be 22.69% with the control on day 8 after treatments wher eas the highest (0.12 µl/g) dose killed 96.29% of the ticks within the same day as control. LD50 of the foam soap containing essential oil of this plant were 0.037 and 0.059 µl/g on day 2 after treatment, in the laboratory and on the farm respectively. Thi s indicates the potentially high efficiency of this medicated soap on this parasite. In conclusion, the result indicate that the medicated soap containing essential oil of *Chenopodium ambrosioides* leaves can be used as antiparasitic agent in the treatment of ticks in infested goats.
Keys words: soap foam; essentials oils; *Chenopodium ambrosioides*; *Rhipicephalus lunilatus*; LD_{50}

Introduction

Ticks are one of the main causes of mortality in animals (IEMVT, 1989). They are also responsible for secondary infections which could be bacterial, viral or protozoa related (Soulsby, 1982). Furthermore, each of the conventional methods of tick control are quite costly and environmentally unfriendly (Pamo *et al.,* 2005). Attention is then shifted towards natural su bstances with therapeutic properties like essential oils extracted from some plants. In fact, a substantial part of plants contain in their leaves, fruits, flowers, stems and roots, antiseptic, anti-inflammatory, insecticidal, bactericidal healing substanc es (Kuiate, 1993). *Chenopodium ambrosoides* leaves contain essential oils which make them to be sometimes irritating and toxic (Daget and Godron, 1995; Tapondjou *et al*., 2002; Pamo *et al*., 2002). The acaricidal effect of essential oils of many other plants has been demonstrated (Pamo *et al*., 2002; Pamo *et al*., 2003; Pamo *et al.,* 2005b). This study is therefore aimed at finding an efficient, cheap and easily applicable method of using essential oils to fight against ectoparasites in general and ticks in part icular.

Material and Methods

Study Area

The study was carried out in the Western High Lands of Cameroon, between 25°6' North Latitude and between 10° and 11° East Longitude. The mean altitude of the region is 1420 m. The climate is equatorial. In this zone, rainfall varies between 1500 and 2000m m/year. Annual temperatures vary between 10°C in July and 25°C in February. There are two main seasons in the region: a short dry season running from mid -November to mid-March and a long rainy season (corresponding to cultural season) from mid -March to mid-November (Pamo *et al.,* 2005a). Subsistence agriculture, together with breeding and trade are the main economic activities of the region. The vegetation is the savannah with shrub, and sparse forests in some areas (Pamo *et al.,* 2004).

Extraction of Essential Oils of *C. ambrosoides*
Leaves of the plant were harvested, taken to the Laboratory and dehydrated for three days at room temperature before extraction. Oil was extracted by the Hydro -Distillation technique (Kuiate, (1993) cited by pamo *et al.* (2005b) using modified Clevenger type apparatus. The technique consisted of placing the mixture of plant and water on a hot plate for eight hours. The essential oil was obtained through evaporation. The mixture of oil and water vapour condensed in the distillation apparatus where cool water circulated permanently. We obtained in a graduated tube associated to the apparatus, a two -layered distillate, with an upper part being the essential oil. After allowing water to flow out through a tap, the oil was then collecte d in a container and dehydrated using anhydrous sodium sulfate. The calculation of the yield was done using the following formula:

$$\textbf{Yield (\%)} = \frac{\text{Weight of the essential oil}}{\text{Weight of the plant material}} \times 100$$

The Production of soap
Solutions of soda and sikalite were mixed in a container and allowed to stand for 15 minutes. After this, palm oil was added and mixed by turning the preparation in the same direction for 15 minutes without resting during which essential oil was added. The final mixture was then put into chosen moulds left on the g round in darkness for seven days for solidification.

***In vitro* tests**
Ticks used for *in vitro* tests were collected from African dwarf goats in villages of the Western High Lands of Cameroon. The harvesting was done manually without any distinction of sexes and with great care avoiding the destruction of their rostrum. To work with a homogenous population, ticks were weighed on a scale of the type “Denver Instrument” with a capacity of 210 g and a sensitivity of 0/001g/ Their length was also measured using a millimeter paper. The average weight and height were 0.05 ± 0.01g and 6.5 ± 0.04 mm respectively. Selected ticks were now fixed with ethyl acetate and identified as *R. lunulatus* according to Walker *et al.* (2002). Once identified, ticks were ready for th e tests.

The soap produced for bioassays had a weight of 450 g and contained 900 Pl of essential oils, i.e. a dose of 2 Pl/g. To obtain applicable concentrations, dilutions were made so as to obtain the following doses: 0.03; 0.06; 0.09 and 0.12 Pl/g of soap. Final doses were obtained after many i*n vitro* tests and three of the*m* (0.06, 0.09 and 0.12 Pl/g) were used for *in vivo* tests.

Tests consisted of the evaluation of *in vitro* toxicity by contact of soap foam on ticks. The doses obtained above were applied to ticks. With a 10 ml pipette, the solution was uniformly distributed in Petri dishes with an area of 63.61 cm^2, in which a round filter paper (Type Whatman N° 1 with a diameter of 9cm) had already been placed. Each treatment had four replicates made of 10 ticks introduced in one of the above Petri dishes. Counting of dead ticks was done every 24 hours for eight days. Using the method of Abott (1925), the mortality rate in each dish was calculated using the following formula:

$$\mathbf{M_c = M_o\text{-}M_e / 100\text{-}M_e}$$

M_o: mortality of ticks registered in treated replicate
M_e: mortality of ticks in the control
M_c: corrected mortality of ticks (%)
The LC_{50} was determined by the Bliss Method (1938) cited by Valette (1972) based on the regression of mortality depending on the logarithm of essential oil doses. The method was used for *in vitro* and *in vivo* tests.

***In vivo* Tests**
Host animals chosen based on the infestation with ticks, w ere grouped into four sets of 10 goats each (i.e. 40 goats) in which three were treated and one was the control. Each replicate representing a dose of oil was done in two replicates. Ticks were collected and counted before onset of the tests. The test cons isted of applying lather on the animal, insisting on points where ticks were susceptible to group. After the treatment, mortalities were evaluated every 24 hours.

Statistical Analysis

Data obtained were analyzed using ANOVA (Mc Clave and Dietrich, 1979) after correction of observed mortalities in relation to those of the control and the differences between treatments when they existed, were separated at 5% significant level by Student "t" test.

Results and Discussion

In vitro* effects of the essential oil-based soap from *C. ambrosoides* leaves on *R. lunulatus

The yield of the oil extraction was 0.024 %. This was lower than the results obtained by Martines *et al.* cited by Quarles (1992) and Tapondjou *et al.* (2002) which were 0.4 % and 0.8 % respectively. This difference can be explained by many factors including method of distillation and the period during which the plant was harvested. The evolution of these mortalities in time depending on the doses of the essential oils incorporated in the soap is illustrated in Figure 1.

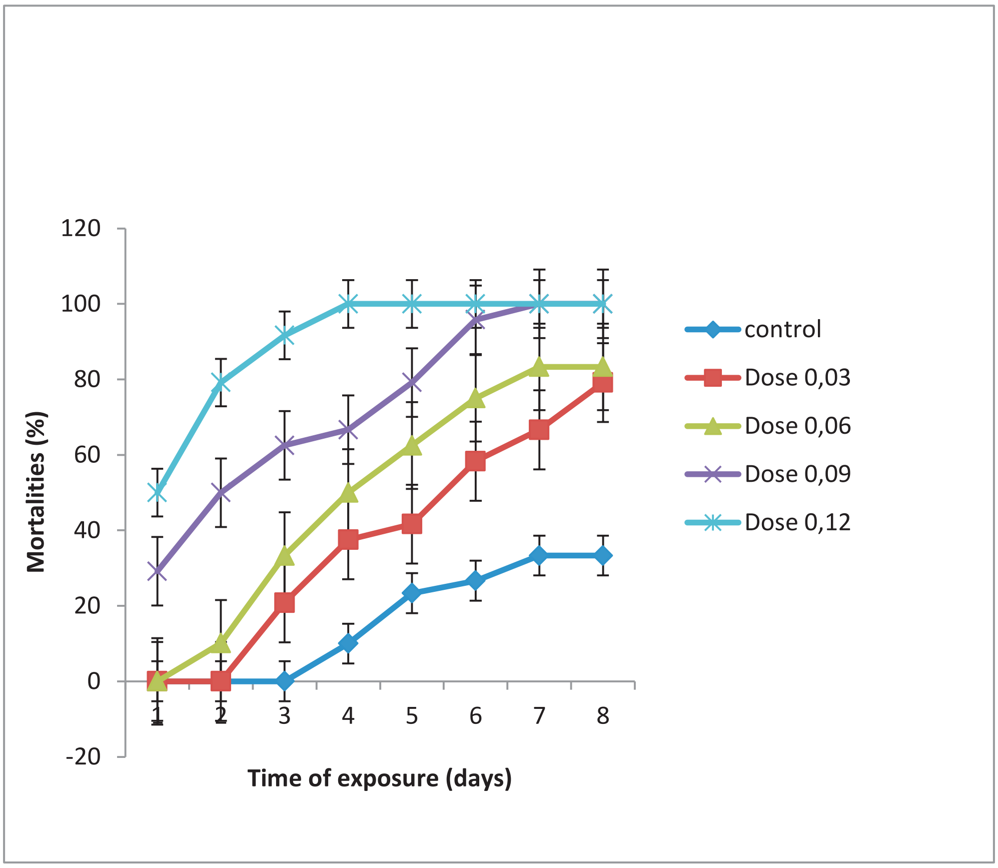

Figure 1: Evolution of *in vitro* mortalities according to time and doses of essential oils of *C. ambrosoides* leaves contained in the soap

From this figure, the mortality rate of *R lunulatus* increased with the doses of essential oils in the soap (Pl/g) and according to time. This mortality was maximal (100%) on the fourth and the seventh days respectively for the doses 0.12 and 0.09 Pl/g. For the doses 0.03 and 0.06 Pl/g, the highest mortality on the 8th day was 79.16% and 83.33 % respectively. This mortality remained relatively weak in the controls (33.33% on the 8th day). The mortalities observed in controls may be attributed to soap ingredients and particularly soda. The difference in mortality between treated replicates and the control showed the toxicity of essential oils of *C. ambrosoides* leaves contained in soap on *R. lunulatus.*

The toxicity of essential oils of *C. ambrosoides* may have been partly due to a local irritation of the digestive tube of the parasite and even more by the direct repressive action on its cardiovascular and respiratory systems (Quarles, 1992). This toxicity is generally attributed to ascaridole (Pollac *et al.,* 1990 cited by Tapondjou *et al.,* 2002). Symptoms of ascaridole poisoning include nausea, depression and extreme fatigue in man (Quarles, 1992) which could be due to possible nervous system attack. Therefore, based on this, the substance may have had effects on the nervous system of *R. lunulatus*. According to Tapondjou *et al.* (2002), toxicity of these oils can also be attributed to other major compounds such as thymol and α-terpinen. These compounds are known for their insecticide and acaricide effect (Tapondjou *et al.*, 2003; Tapondjou *et al.*, 2005; Ndomo *et al.*, 2009) The high mortality rate observed in controls may be attributed to others chemical contents of the soap not yet known and reported.

The adjustment of cumulated mortality rates and corrected means in terms of doses of the essential oils of *C. ambrosoides* leaves contained in the soap with time were used to draw the regression line:

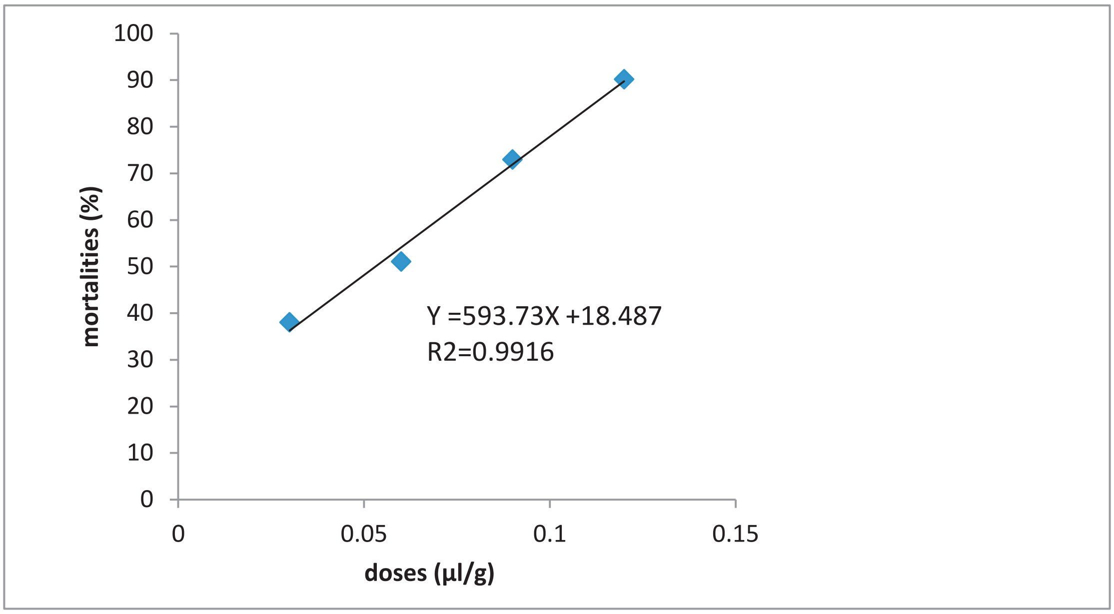

Figure 2: Evolution of mean cumulated percentages of *in vitro* mortality in terms of doses of the essential oils of *C. ambrosoides* contained in the soap

The high value of determination coefficient (0.99) showed that a large proportion (99.10 %) of cumulated mortality rates could be attributed to the treatment. The transformation of mortality rates into probits at the end of the second day in view of evaluating the LC_{50} permitted us to obtain the probit regression line in terms of logarithm of doses of essential oils of *C. ambrosoides* in soap with the equation:

$Y = 9.622\ X + 15.081\ (R^2 = 0.9449)$

From this regression line, it would appear that on the second day of exposure, LC_{50} is 0.037Pl/g thus confirming the high toxicity of essential oils of *C. ambrosoides* on *R. lunulatus.*

In vivo* effect of the soap made from essential oils of *C. ambrosoides

The cumulated mortality rate increased with the dose of the oil with time to reach its peak on day 8 i.e. 76.12; 90.27 and 96.29 % respectively for the doses 0.06; 0.09 and 0.12 Pl/g. Meanwhile, these mortalities were absent in the control for the first four days and have reached a peak of 22.69% on day 8.

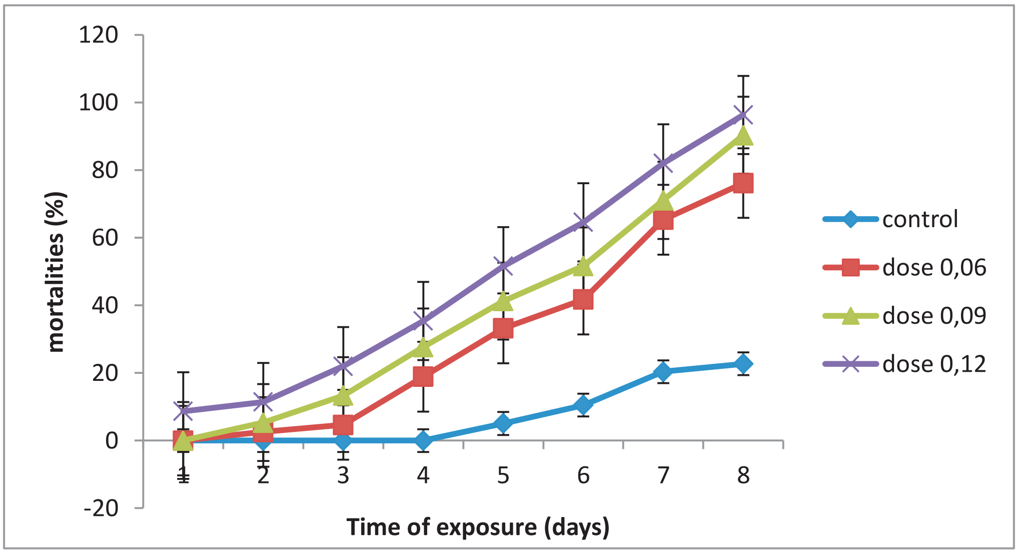

Figure 3: Variation of *in vivo* mortalities of *R. lunulatus* in terms of time and doses of the oil of *C. ambrosoides* leaves in soap

These differences observed among treated replicates with varied doses and between treated replicates and controls showed the increasing acaricidal effects of soap with time and depending on the doses of the essential oils of *C. ambrosoides* leaves in the soap.

The adjustment of the mean cumulated mortality rates *in vivo* depending on doses of the essential oils of *C. ambrosoides* leaves in soap with time gave a regression line with the equation:

$Y = 269.67 X + 13.847$ ($R^2 = 0.9963$). This regression line indicated that a high proportion (99.63 %) of the variation of cumulated mortality, corrected with time is only due to the effects of different doses of essential oils contained in the soap. The transformation of mortality rates into probits at the end of the 2nd day in order to evaluate the LC_{50} helped us to obtain a regression line of probits of *in vivo* mortalities depending on the logarithm of doses of essential oil of *C. ambrosoides* leaves in the soap with the equation: $Y = 1.97 X + 5.4448$ ($R^2 = 1$). The LC_{50} derived from this equation was 0.55 Pl/g on the 2nd day of exposure, showing the *in vivo* toxicity of the soap made from essential oils of *C. ambrosoides* on *R. lunulatus.*
During this study, no undesirable effects were observed neither in the behavior nor the physiology of the animal.

Compared to the *in vitro* study, mortality rates obtained *in vivo* were quite low. This difference may be due to fluctuations of experimental conditions. In this study the undesirable effect of rain during treatments contributed to the washing off of chemicals applied on the animal. Despite these remarks, it is obvious that soaps made from essential oils of *C. ambrosoides* leaves have toxic acaricidal effects on *R. lunulatus* in natural conditions.

Conclusion

In conclusion, we observed that soaps from essential oil of *C. ambrosoides* leaves were toxic on R*. lunulatus* both *in vitro* and *in vivo*. Mortality rates of ticks increased gradually in terms of doses and time. This toxicity is not the same *in vitro* as *in vivo*. Low LC_{50} obtained (0.037 Pl *in vitro* and 0.059 Pl *in vivo*) showed the high toxicity of applied treatment. The analysis of the chemical composition and the study of the acaricidal effects of the different fractions of the essential oil of this plant will permit us to improve on our knowledge of the toxic effects of our treatment/ It'll be equally important to determine with accuracy the active ingredient of the applied treatments.

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Effect of pounded dawadawa (Parkia biglobosa) pod husk extract on strongyle in West African Dwarf (WAD) goats

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Abstract

Apart from resistances to antihelmintics, poor availability and affordability of antihelmintics by resource -poor farmers in developing countries have compounded the problem of worms in small ruminants. Additionally, there is growing concern over drug residues in the food chain and the environment. Search for novel antihelmintics that are both more sustainable and environmentally friendly is undoubtedly a sensible approach to the control of parasitic infections in animals. Ethnoveterinary knowledge is thus b eing vigorous pursued as an apt alternative. A series of *Parkia biglobosa* pod husks trials on strongyles were conducted in sheep and goats. This experiment looks at the particular case of WAD goats.

Introduction

Livestock production is a source of employment and livelihood in Ghanaian agriculture (FAO, 1996). However the menace of ill-health is a threat to animal production and development in rural and peri -urban communities (Martin, 1996). Germs and worms and other low forms of life pick close to a billion dollars a year from the pockets of livestock producers, often so expertly that the producer does not even realize his loss (Gove, 2004). Cole (1986) reported that worms cost the Australian sheep industry $369M/yr which could increase to $700M by 2010. The case of sub-Saharan Africa including Ghana is no exception.

Ethnoveterinary medicines are available for the treatment of internal parasites but are often neglected in favour of conventional dewormers. Plants such as Parkia which grow naturally in West Africa are one of the most important economic trees in the northern part of Ghana and have been reported to be growing in 18 sub-Saharan African countries including Ghana and Nigeria (Abbiw, 1990). Parkia biglobosa plays numerous roles in the treatment of many diseases (Campbell-Platt, 1980; Abbiw, 1990). A series of trials of *Parkia biglobosa* pod extracts (DPHE) against strongyles proved positive because ova levels of strongyles fell in djallonke sheep with a dosage of 0.4ml/kg live body weight (Iddrisu, 2009). Hamidu (2010) found that repeated doses of 0.6ml/kg live body weight of these pods extract at intervals of 28 days could clear almost all strongyle by the third administration. However any likely residual effects were not investigated. Also, of the helminthes affecting small ruminants in the West African sub -region, strongyles have been identified as one of the most prevalent worms (Ockling, 1989; Fakae, 1990; Agyei *et al*., 2004). Given that goats and sheep are similar in many respects, this study therefore sought to, first ascertain the chance of DPHE having an effect on strongyle (one of the most prevalent helminthes) in West African Dwarf (WAD) goats and also to examine whether DPHE had any residual effect.

Literature Summary

According to Ingale *et al.* (2010), most parasitic infections in domestic animals are caused by gastro -intestinal (GI) nematodes, which may result in depression in appetite, impairment in GI functions, alteration in protein, energy and mineral metabolism and changes in water b alance. Many parasites are harmful to their host, but in most cases these effects are not of such importance that the host is being killed. Such effects comprise; wasting, super infections and production of toxic compounds (Poulin, 2000). According to Hunt er (1994), worms develop resistance to anthelmintics if they are administered regularly at below recommended doses, used indiscriminately or if only one drug is used continue for prolonged periods. Thus, veterinary supervision is essential wherever anthelmintics treatment is on the lambs and the yearlings (Liverkusen, 1979). Ethnoveterinary medicine has existed with human being as a practice that helps people to solve health related problems (Martin, 1996). Wanyama, (1997) reported that livestock owners hav e excellent knowledge of ethno botany, which formed the basis for screening plants materials as potential sources of medicinal drug. The bark and husk of the pods of *P. biglobosa* contains tannins (Campbell-Platt, 1980). Tannins are usually classified either as hydrolysable tannins (HT) or proanthocyanidins (CT) based on their molecular structure (Haslem, 1989). The CT is the most common type of tannin found in forage legumes, trees, and shrubs (Barry and McNabb, 1999). Condensed tannin-containing forages have the potential to help cont rol anthelmintic-resistant gastrointestinal parasites. They have been shown to decrease faecal egg counts in sheep and goats. This reduces pasture contamination and ingestion of infective larvae and by itself might provide adequate control of gastrointestinal parasites (Min and Hart, 2003). There exists a huge indigenous knowledge base on preventive and curative herbal medicinal practices, as reported by Yidana *et al.,* (2006). Naandam and Turkson (2008) also reported that some farmers in East Mamprusi Distr ict of Ghana, used P*. biglobosa* pods in treating their animals but the extent of efficacy was not ascertained.

Description of Research

This study was undertaken on the livestock production farm of the Animal Science Department, University for Development Studies – Nyankpala in the Tolon/Kunbungu District of the Northern Region – Ghana. It was carried out in December 2011. The study area lies within the Guinea Savanna zone, characterized by large area of low grassland interspersed with trees. The area has single rainfall pattern which starts from May and ends in October/ Nyankpala lies on altitude 183m, latitude 09°25"N and longitude 00°58"W with a mean annual rainfall of 1043.60mm and temperature of 28.30℃. Mean annual day time relative humidity is 50% (SARI, 2009). The goats were housed semi – intensively, where animals were provided with a structured pen and were allowed to graze freely during the day on their own outside the pen. Supplementary feed such as cassava peels, yam peels, cotton seeds and wat er were provided after grazing. Eight (8) nanny West African Dwarf (WAD) goats aged between 1-2 years with mean weights of 15.3kg for treatment one (T1) and 15.4kg for treatment two (T2) were used in the experiment. Animals were tagged for purposes of clea r identification. A Completely Randomized Design (CRD) was used in the experiment. Clean matured dry dawadawa pod husks were collected from farmers in Nyankpala. Husks were well dried in the sun for easy pounding. About 3kg of dried dawadawa pod husks were pounded using well clean mortar and pestle. Pounded husks were soaked in distilled water (1kg of the pounded husks were put into 1.5liters of distilled water). The mixture was left overnight (12hours) for maximum extraction of the water soluble active ing redient and then decanted and filtered to obtain a clean extract. The extract was stored in a clean plastic container for use.

There were two treatments viz Treatment one (T1): Nanny goats under this treatment received no dawadawa pod husk extract or any conventional dewormer during the period of study. Treatment two (T2): Nanny goats under this treatment received 0.6mls/kg live body weight of the DPHE. Faecal samples were taken from the animals directly from the rectum through the anus by fingers covered with gloves. Baseline faecal samples were taken by restraining the animals and 3 grams of faeces taken before administering the DPHE. These baseline samples helped to know the total worm ova counts of the experimental animals before administering the DPHE. The gloves were changed after each faecal sample was collected to avoid contamination. Faecal samples were put in a clean container, sealed and sent to the Veterinary College a t Pong Tamale for laboratory analysis. Seventy two (72) hours later and subsequently on a 3-day basis until 18days after the administration of the DPHE, faecal samples were similarly collected for analysis. Faecal samples were stored in a refrigerator at 5°C when it was not possible to carry out analysis immediately. The McMaster Egg Counting Technique espoused by the University of Pennsylvania School of Veterinary Medicine (2006) was used. The strongyle ova were identified by the morphology (colour, shape and size) of eggs with aid of microscope and with a guide from a helminthological chart. A two-tail t test was used for the data analysis.

Research Results and Application

Mean ova count observed in the faecal sample from day 12 to 18 were significantly different ($p<0.05$) between treatments after the administration of the DPHE i.e. the DPHE appeared more effective after day 9 to day 18. However, whereas DPHE showed some activity as registered in the mean ova count for treatment by 18th day, this activity in absolute terms seemed to be on the decline as ova count for day 18 was greater than day 15 (Table 1), suggesting that DPHE as a drug may be acting over a reasonable number of days and confirming the statement by (Birkett, 1995) that after a single dose, drug concentration falls in an exponential manner with time.

Table 1: Mean strongyle ova counts

Day	Control (epg)	Treatment (epg)	SED	Significance
0	3033	2467	430.8	NS
3	3500	2633	837.3	NS
6	3667	2667	903.1	NS
9	3000	1967	841.3	NS
12	3600	1267	397.2	*
15	3367	1267	495.5	*
18	3367	1633	481.9	*

NB: NS- Not significant   *- Significant ($p<0.05$)   SED- Standard Error of Difference

Given that any heterogeneity in factors like body weight and composition, age and sex were reduced to the barest minimum as possible for the treatment groups, other factors that could possibly have affected individual DPHE response and cause variation still remained, among which was a complex array including variability in absorption, bioavailability, physiological condition, starvation, feeding status and pharmacogenetics (Franconi *et al.*, 2006; Galbraith *et al.*, 2007; Severino and Del Zompo, 2004; and Wilkinson, 2005).The random source of error in the control group (Fig. 1) may also be found in the treatment group. But in addition, the treatment group had a second source of error due to individual DPHE response variation that has been alluded to above, which translated into the longer error bars for the treatment group (Fig. 1).

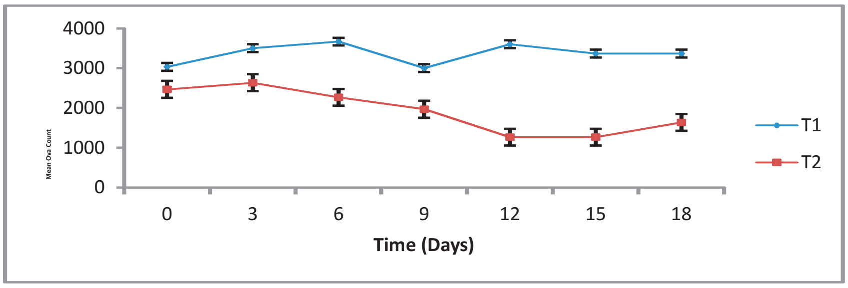

Figure 1: Mean ova count over time with standard error bars

It was generally observed that the treatment (0.6mls/kg BW) of DPHE had an effect of reducing strongyle worm ova count over time. The percent change in mean strongyle ova count is captured in table 2.

Table 2: Percent change in mean strongyle ova count over time

Treatment	Before treatment (epg)	After treatment (epg)	% change
T1	3033	3362	(10.8)
T2	2467	1986	19.5

NB: T1 – Control   T2- Treatment

There was a decrease in the ova count in the treatment group of approximately 20% over the entire study period (Table 2). This could probably be in consonance with what some researchers have alluded to as being the result of the presence of tannins in the bark and husk which killed some of the worms. Campbell-Platt (1980) reported that, the bark and husk of the pods *Parkia biglobosa* contains tannins. Condensed tannins-containing forages have the potential to help control anthelmintics resistant gastrointest inal parasites. This study shows that DPHE may have some residual effect as well being able to control strongyle in goats to some appreciable level.

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Effect of *Crotalaria falcata* in crop rotation options on potato bacterial wilt incidence, disease severity and latent infection in tubers and field soil

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Abstract

Potato in tropical highlands, among other constraints, is threatened by bacterial wilt (BW) caused by *Ralstonia solanacearum*. Recent upsurge in BW in tropical highlands may be related to poor cultural practices, declining soil fertility, short or no fallowing, improper crop rotation, use of low quality seed and possibly global warming. Technologies aimed at reducing soil-borne *R. solanacearum* are imperative to maintain supply of BW-free seed for sustained potato production. Thus, field experiments were conducted in a field endemically infested with BW in south western Uganda at 2200 m above sea level to test the effect *Crotalaria falcata* as an improved fallow or green manure crop in as a component of crop rotation regime to improve soil fertility and reduce or eliminate soil-borne *R. solanacearum*. A one-year *C. falcata* fallow reduced BW incidence by more than 85% compared to beans, maize, and natural grass fallow where final BW incidence was reduced by 43.9, 37.6 and 27.0%, respectively in two seasons from the original incidence. In plots continuously planted with potato, > 50% of harvested tubers were visibly infected with BW compared to < 3% in plots where *C. falcata* fallow was used for one year. Apparently healthy tubers collected from plots where *C. falcata* was used free from latent BW infection compared to tubers from plots previously with maize, beans or natural grass fallow treatments. A natural grass fallow was minimally beneficial in reducing both visible and latent BW infection. The residual effects of *C. falcata* on BW incidence, severity and latent infection were evident even after one season when it was used as green manure for maize or beans before the plots were replanted with potato. This legume demonstrated having more beneficial effects on potato beyond soil fertility improvement. It showed potential to improve soil health favouring growth of potato in fields previously infested by *R. solanacearum*. However, the mechanism of *C. falcata* in improving soil health for production of BW-free potato tubers requires further investigation.
Keywords: bacterial antagonists, bio-pesticide, bio-disinfectants, pyrrolizidine alkaloids, soil health

Introduction

Bacterial wilt (BW) of potato and tomato caused by *Ralstonia solanacearum* (EF Smith) Yabuuchi *et al*. yr) has long been known as a disease of warm agro-ecologies and little known in cool tropical highlands and temperate regions. Global warming, short fallow periods, inappropriate crop rotation, use of low quality seed and growth in potato international trade have resulted in appearance of BW in areas hitherto u nknown (Priou *et al.*, 1999). The disease currently occurs globally in tropical highlands at more than 2400 metre above sea level (a.s.l.) and higher latitudes as in the Netherlands, UK, Sweden, Northern USA and Canada (Priou, *et al*., 2006; Breukers *et al*., 2006; Anon., 2010). Bacterial wilt is estimated to affect more than 1.5 million hectares of potato in eighty countries with global damage exceeding US$ 950 million per year (Floyd, 2007; Anon., 2010). The high occurrence of *R. solanacearum* (race 3, biovar 2) in tropical highlands and temperate regions is putting production of most susceptible crops particularly potato, that depends on vegetatively produced planting material, in great danger. If it is not properly controlled, the farmers who depend on this crop may face hunger and economic hardships in future. Bacterial wilt particularly negatively impact seed potato enterprises where it is not tolerated. It is a quarantine disease not only in Europe and America (Priou, *et al*., 1999; Breukers *et al*., 2006; Anon., 2010) but in most countries in sub-Saharan Africa (SSA) as well. The disease will occur at different intensities depending on eco-climate, management practices, seed production and supply system, quarantine regulations and potato-trade related policies (Breukers, *et al*., 2006).

Ralstonia solanacearum occurs in five races or five biovars with different agro-ecology and host adaptation (Priou *et al*., 1999; Denny, 2006). It is a soil-borne where it can survive for a long time in symptomatic and symptomless host plants, plant debris or volunteer potato or even in non-host plants as a saprophyte or in the root rhizosphere (Messiha *et al*., 2007a). The pathogen has also been detected in vegetation-free fields after three years (Messiha *et al*., 2007a), indicating potential its to survive facultatively (Hayward, 1991; Ong *et al*.; 2007). Crop rotation, which is commonly recommended for BW control, requires long periods without host crops in order to have a significant impact on field populations of the BW pathogen. In south western Uganda, a two seasons' rotation with non-host crops reduced BW incidence from 81% to 22% when healthy seed of a relatively BW resistant cultivar was used (Lemaga, *et al*., 2001b). However, this is not adequate if tubers from such a crop are to be kept for sale as ware potato or be used as seed. It is difficult to reclaim BW-infested fields considering its wide host range including weeds where the pathogen can live as a saprophyte. Therefore, strategic selection of crops for rotation with potato combined with other cultural practices particularly those that improve soil health and fertility is paramount if BW soil inoculum is to be drastically reduced or completely eliminated.

There is insufficient evidence in SSA where smallholder potato farmers deliberately practice crop rotation as a tool for controlling BW because even in cases where farmers appear to know the importance of this and other BW management practices, they are rarely used considering the high incidence of the disease in most potato fields (Low, 2000). This is probably partly due to limited knowledge of the disease and its negative impact on potato production and, small and highly fragmented land holdings that do not favour planned long term BW management, declining soil fertility and ignoring strict field sanitation (Lemaga, 2001b). The value most small scale farmers attach to volunteer potato as food during the time of scarcity, latent BW infection in planting material and infrequent seed renewal result in accumulation of BW pathogen in soil and planting stocks. Considering the large scale occurrence of BW in major potato growing areas and its appearance in zones hitherto unknown, novel technologies need to be developed and promoted reduce or halt spatial pathogen spread in order to sustain potato production particularly in the tropical highlands where potato is a key source of livelihood. In zones where BW has been introduced, disease control may be probably possible by availing disease-free seed, improving soil fertility and soil health where these practices would retard or halt build up and dissemination of *R. solanacearum*.

Alternating potato with non-host crops and use of disease-free seed of relatively resistant varieties in BW-free soil would reduce future incidence and severity of BW (Tusiime *et al*., 1997, Lemaga, *et al*., 2001b). Similarly, soil amendment with green manure and mineral fertilizers decrease the incidence and severity of BW while promoting both total and marketable tuber yields (Akew *et al*., 1996; Lemaga *et al*., 2001a; Fontem and N'tchorere, 2009-)/ Improvement in yield due to soil amendments is achieved through improving plant nutrition and vigour but will not prevent *R. solanacearum* infection. While mineral fertilizers can be accessed in sufficient quantities for planting large potato acreage, green organic amendments for similar purposes may not be easily available unless they are deliberately produced prepare and easily delivered in amounts that would have similar effects as mineral fertilizers in fields larger than research-size experimental plots.

Organic amendments particularly green manures if available in sufficient quantities would be appropriate for poor farmers because of their potential to improve soil fertility and possible suppression of soil-borne pests either through physical decomposition by producing toxic ammonia or release of toxic plant metabolites. Crude Extracts from certain legumes and crucifers have been demonstrated to have *in-vitro* suppression of bacterial growth (Akew, *et al.*, 1996- Fontem and N'tchorere, 2009- Wagura *et al*., 2011). However, these authors did not adequately quantify the impact of such green manures directly applied or grown as improved fallows in fields infested with *R. solanacearum* would have on the BW pathogen in soil when a susceptible host is replanted. Plant species with biocide or biostatic effects on soil-borne pests can be applied as soil amendments green cover crops and manures or form part of crop rotation and fallowing practice. Different plant species have varying potential in suppressing the BW pathogen in both *in-vitro* and *in-vivo* while others may have no effect at all (Fontem and N'tchorere, 2009- Wagura, *et al*., 2010). The choice of the bio-pesticidal plant and how it would be effectively and cheaply delivered on large scale beyond laboratory and research experiments and demonstrate its benefits to potential users still remains a question. The impact of organic amendments, crop rotation and green cover crops on BW suppression and soil fertility enhancement is usually expressed fresh yield improvement (Hayward, 1991; Lemaga *et al*., 2001a). In seed potato production where BW is not a quarantine disease that will affect the use of the product, yield improvement is not adequate without addressing latent infection in both seed tubers and field soil.

Consequently, field and laboratory experiments were conducted in south western Uganda at Kachwekano Zonal Agricultural Research and Development Institute (ZARDI) to test and quantify the effect of crop rotation and green cover crops on incidence, severity and latent infection of BW in soil and progeny tubers. In the field experiment, a block with a long history of BW infestation was used between 2009 and 2011 to develop crop rotation and fallowing options for sustainable production of quality potato in a BW-endemic environment.

Materials and Methods

Field and laboratory experiments were conducted at Kachwekano ZARDI between 2009 and 2011 to test a crop rotation and post-potato field management practices for controlling potato bacterial wilt infestation in both tubers and field soil. Kachwekano ZARDI is located in south western Uganda, 01° 16'S 29° 57'E at 2200 m above sea level. The soil at this location is isomeric typic palehumult, generally deficient in nitrogen and has high acidity (Lemaga, *et al*., 2001a). Most fields at this location have a long history of potato bacterial wilt infestation.

A field, 75 m by 15 m on a terrace that had severe bacterial wilt infestation in 2007 was fenced off to limit human and animal interference in December 2008. After primary land cultivation, this field was divided into numbered 3 m x 3 m quadrants. From each quadrant, one kilogram of soil to a depth 25 cm was collected from five spots per plot and thoroughly mixed in a plastic bag. Each soil sample per quadrant was transferred to 30 cm diameter, 15 cm deep plastic basins placed under 1000G translucent plastic tunnel. The basins were accordingly labelled with numbers corresponding to the quadrants where the soil samples were collected. In each soil sample, 10 tomato seedlings at five-leaf stage, raised in steam-sterilized sand were transplanted and regularly irrigated with clean rain water. The seedlings were monitored twice a week for 30 days and assessed for wilt symptom development. The number of wilting tomato seedlings per assessment was counted and recorded. Tomato seedlings that showed no BW symptoms after 35 days were serologically tested for latent infection with *R. Solanacearum* using direct double antibody sandwich immusorbent assay (DAS-ELISA).

To determine the chemical and physical characteristics of the soil in the experimental field, about one kilogram of soil was collected from each of six randomly selected spots in the field, thoroughly mixed and then one kilogram composite sample was taken for analysis. Thereafter, the field was partitioned into 36, 4.5 m x 3.0 m plots. In each plot, 60 BW-indexed seed tubers of the potato cultivar, Victoria (CIP 381381.20) were planted in September 2009. The seed tubers in each plot were planted at 75 cm between rows and 30 cm between seed tubers in a row. At the time of planting 80 Kg ha^{-1} of N:P:K: 17:17:17 fertilizer was applied as a side-dressing. This crop was used to further confirm presence and distribution of BW in the experimental and further build the pathogen inoculum by artificial inoculation in plots where disease incidence was not uniform. In plots where BW incidence was low by potato flower-bud initiation stage, five plants per row were artificially inoculated by injecting 30µl of race 3 biovar 2 of *R. solanacearum* suspension containing approximately 1×10^7 cfu ml^{-1} in a leaf axil of one of the main stems per plant mid-way the stem using an insulin syringe (Tusiime *et al*., 1997; Lemaga, 2001). The experimental treatments (Table 1) were then randomly assigned to the different plots at this time to avoid future bias. The plots were protected with trench barriers to avoid cross-contamination and labelled permanently for the duration of the study. The experimental crop was effectively protected against late blight attack with fungicide sprays of Agrozeb (contact) and Ridomil Gold (systemic) fungicides. This experimental crop was assessed for foliage BW incidence and severity during growth and in tubers at harvesting.

In the March-July and Sept-Dec. 2010 cropping seasons, the respective plots were planted with assigned crops in the crop succession treatments (Table 1). In three of the experimental plots, one per replication were planted with BW-tested seed tubers of variety Victoria obtained from the potato program at Kachwekano ZARDI, to represent continuous potato planting, a common practice among potato farmers in SW Uganda as a control. Three other plots were kept as crop-free grass fallow dominated by fox-tail grass (*Setaria* sp.) as natural fallow. Ten crop rotation and *Crotalaria falcata* fallow or green manure. *Crotalaria falcata* is a leguminous herbaceous plant with no immediate use as food or fodder because its roots, foliage and seeds contain pyrrolizidine alkaloids that are toxic to mammals, birds and fish (Joosten and van Veen, 2011). It occasionally grows in some natural fallows with other plant species, but is rarely a dominant in natural systems unless it is deliberately supported to establish. The crops used in rotat ion in various succession orders were beans, maize, cabbage and onion before potato was reintroduce in all the plots after one year (Table 1). A randomised complete blocks design with three replications was adopted.

Table 1: Crop[1] succession sequences tested for control of potato bacterial wilt at Kachwekano ZARDI, south western Uganda for two years between 2010 and 2011

Code	Treatment	Description
1	P-B-O-P	Potato→Beans→Onion→Potato
2	P-M-Ca-P	Potato→Maize→Cabbage→Potato
3	P-B-M-P	Potato→Beans→Maize→Potato
4	P-M-B-P	Potato→Maize→Beans→Potato
5*	P-(B+Co)-M-P	Potato→(Beans+Crotalaria intercrop)→Maize→Potato
6*	P-(M+Co)-B-P	Potato→(Maize+Crotalaria intercrop)→Beans→Potato
7	P-(B+Co)-Co-P	Potato→(Beans+Crotalaria intercrop) →Crotalaria→Potato
8	P-(M+Co)-Co-P	Potato→(Maize+Crotalaria intercrop)→Clotoraria→Potato
9	P-P-P-P	Potato→Potato →Potato →Potato
10	P-Co-Co-P	Potato→Crotalaria →Crotalaria →Potato
11	P-F-B-P	Potato→Fallow→Beans→Potato
12	P-F-F-P	Potato→ Fallow →Fallow →Potato

[1]Crops rotation treatments: B = Beans, Ca = Cabbage, Co = Crotalaria, F = grass fallow, M = Maize and P = Potato. A plus sign (+) between two crops in parenthesis means intercropping.

*In treatments 5 and 6, P-(B+Co)-M-P and P-(M+Co)-B-P, *C. falcata* was ploughed-in after harvesting the intercrop and before planting either maize or beans during the third rainy season basing on the previous crop.

Data Collection and Analyses

Germinated potato hills were counted every four days sta rting from two weeks after planting. After full crop emergence, about one month after planting, the crop was assessed every week to determine the date of BW onset per experimental treatment. Wilting potato plants at every assessment were counted, recorded and pegged to avoid multiple counting. The number of wilted potato plants per plot at every assessment was expressed as a percent of germinated hills per plot. Visual BW assessment per plots was halted when the plants started to senesce, about 85 DAP or wh en all the plants in a given plots had shown wilt symptoms. Average BW incidence (%) per treatment per assessment date was used to plot disease progress curves and compute integral BW severity as relative area under disease progress curve (Messiha *et al*., 2007b).
Basing on surviving apparently healthy potato plants, the experimental crop was dehaulmed at full maturity, approximately 95 DAP and harvested two weeks later. At harvesting, two, 35 -45 mm in diameter tubers from each plant in the middle two rows of each plot that showed no BW infection symptom, were collected for indexing against latent *R. solanacearum* infection. Soil samples were also collected from the experimental plots for physical and chemical analysis and, determining latent BW in field soil .

Latent BW infection in tubers was tested using nitrocellulose membrane, indirect, triple antibody sandwich immunosorbent assay (NCM-TAS-ELISA). Latent BW infection was considered on a plot basis than treatment averages in order to identify plots whose t ubers or soil were probably free from latent *R. solanacearum* infection. The significance of treatments among quantitative data was tested using analysis of variance. Means of significant treatments were compared using Fisher's protected least significance (LSD) test at 5% probability. Data were analysed with relevant procedures in Genstat Release 11.1 for Windows® statistical computer program while MS-Excel was used to generate pertinent graphs.

Results

Experimental site soil physical and chemical charac teristics

Laboratory soil analysis for physical and chemical properties revealed a relatively low pH, 4.0 and 5.2, in the experimental field. Similar results at this site were reported by other workers (Lemaga *et al*., 2001b). The natural fallow before setting the experiment in 2009 did not greatly differ in soil properties in 2011 plots (Table 2). However, it had a high proportion of organic matter and extractable nitrogen than other treatments.

The various crop succession patterns did not differ greatly in the quantity of organic matter, extractable nitrogen and other soil variables except in the P-B-M-P succession which had visibly low organic matter (Table 2).

Plant nutrient content of *Crotalaria falcata*

Plant tissue analysis of oven dried samples of *C. falcata* indicated a high concentration of nutrients in leaves than roots, particularly calcium (Table 3). This indicates a high contribution to plant nutrients from above than below ground parts of this legume (Table 3). However, when nutrient proportions from both sources are combined, substantial amounts of plant nutrient requirements can be supplied by *C. falcata* as a green manure.

Table 2: Soil chemical and physical analyses at Kachwekano ZARDI study site in a natural fallow before establishing a crop[1] rotation experiment, 2009 and after the experiment in 2011

Treatment	pH	OM %	N %	Av. P (ppm)	K	Na	Ca	Mg	Sand %	Clay %	Silt %
					Cmoles/kg(me/100g)				**Texture**		
Natural fallow	4.5	6.45	0.32	9.2	0.25	0.07	5.6	0.96	76	11	13
P-B-O-P	4.0	5.62	0.28	10.1	0.35	0.08	3.4	1.25	70	10	20
P-M-Ca-P	4.5	4.87	0.20	6.32	0.30	0.08	4.3	1.22	53	29	18
P-B-M-P	5.1	3.27	0.20	6.39	0.35	0.09	3.1	1.31	70	15	15
P-M-B-P	4.0	4.85	0.19	8.35	0.28	0.08	6.4	1.10	69	19	12
P-(B+Co)-M-P	4.1	5.37	0.23	9.54	0.25	0.04	4.8	1.24	70	19	11
P-(M+Co)-B-P	4.0	4.46	0.20	6.58	0.21	0.06	3.2	0.96	72	21	07
P-(B+Co)-Co-P	4.2	4.87	0.19	7.32	0.21	0.06	5.2	1.04	73	14	13
P-(M+Co)-Co-P	4.2	4.14	0.21	8.39	0.23	0.07	4.1	0.68	71	10	19
P-P-P-P	4.1	4.79	0.25	10.24	0.21	0.05	5.3	1.02	74	10	16
P-Co-Co-P	4.2	4.56	0.22	9.25	0.25	0.09	5.5	0.95	73	11	16
P-F-B-P	4.1	4.62	0.21	7.28	0.18	0.05	4.4	1.35	69	14	17
P-F-F-P	4.2	3.47	0.15	8.25	0.35	0.09	4.5	1.22	70	12	18

Table 3: Basic plant nutrients available from *Crotalaria falcata*

	Percentage composition in oven-dried samples				
Plant part	Nitrogen	Phosphorous	Potassium	Calcium	Magnesium
Roots	1.31	0.17	1.26	0.01	0.33
Leaves	2.90	0.22	1.30	1.12	0.94
Leaves:roots ratio	2.21	1.26	1.03	112.0	2.84

Initial distribution of bacterial wilt inoculum in the experimental field

The occurrence of BW in the experimental field based on tomato bioassay and DAS-ELISA indicated a random distribution of the pathogen inoculum (Diag. 1) and was estimated at 60%. Similarly, the incidence of BW in the initial potato crop among trial plots before imposing treatments in 2009 showed no prior significant (P ≤0/05) difference in disease incidence and proportion of BW infected tubers among the proposed treatments. Incidence ranged between 20 and 40% with no pattern among proposed treatments (Table 4). This indicated that plot treatment assignment was not biased.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A	X		X		X	X		X		X	Y	X		X		X		X	X		X		Y		X
B	X	X		X	X		Y		X		X		X		Y		X			X		X		X	
C			X	X		X		X	X	Y	X	X	X		X	X		X	X		Y		X		Y
D	X		X		X		X	X	X		X	X		X	X		X	Y	X		X	X		X	X

Diagram 1: Distribution of wilting of tomato seedlings grown in soil samples collected from 3m x 3m experimental plots for developing technologies for bacterial wilt control in 2009

There were no significant (P≤05) differences in final BW incidence (%) among plots before treatments were imposed in 2009 (Table 4). After re-planting all the plots with potato in 2011 there was significantly (P≤05 lower final BW incidence where *Crotalaria* sp was previously planted than two successive seasons natural grass fallow (P-F-F-P), or P-M-B-P and P-B-M-P cropping patterns. The lowest final BW incidence was obtained where *C. falcata* was used as a fallow for two successive seasons particularly where it was first intercropped with either beans or maize for one season than in pure stands after removing the first potato crop. The final wilt incidence did not significantly (P≤05) differ between using *C. falcata* as a green manure after one season or as an improved fallow for two consecutive (Table 4).

Bacterial wilt incidence in revealed no pattern in the occurrence of rotten tubers (%) due to BW infection before imposing the crop rotation treatments in 2009 (Table 4). In fact, plots that were planted with potato for four successive seasons initially had the lowest incidence of BW infected tubers. At the end of the trial in 2011, *Crotalaria* fallow had lowest BW incidence in tubers (Table 4). A one year g rass fallow did not significantly reduce the incidence of BW in tubers (Table 4).

Seasonal rainfall, quantity and distribution

Half yearly rainfall (mm) and rain days' assessment reflected adequate moisture supply for optimal potato growth in the period under consideration (Table 4). The number of rain days per month shows a fair distribution of rainfall in each cropping season to allow potato growth and BW development in infested fields (Table 5).

Table 4: Effect of crop rotation and fallowing options on final bacterial wilt (BW) incidence (%) before (2009) and after (2011) imposing treatments at Kachwekano ZARDI, Kabale, Uganda

Crop rotation pattern	Final wilt incidence (%)		BW infected tubers (%)	
	2009	2011	2009	2011
P-(B+Co)-Co-P	38.3	3.3	9.9	0.4
P-(M+Co)-Co-P	25.6	3.3	8.5	2.7
P-Co-Co-P	30.0	4.4	4.7	0.2
P-(B+Co)-M-P	27.8	5.0	16.5	4.4
P-(M+Co)-B-P	22.8	5.6	4.4	1.3
P-F-B-P	32.2	8.3	5.4	3.6
P-B-O-P	27.8	12.2	24.4	5.3
P-M-Ca-P	36.7	13.9	11.1	3.9
P-M-B-P	25.0	15.6	9.4	4.6
P-B-M-P	27.8	15.6	17.2	5.2
P-F-F-P	24.4	17.8	4.7	12.2
P-P-P-P	30.6	68.9	2.7	54.4
$LSD_{0.05}$	21.3	10.2	9.8	8.2

Table 5: Seasonal rainfall and rain days at Kachwekano Zonal Agricultural Research and Development Institute between 2009 and 2011

	2009		2010		2011	
Month	Rainfall (mm)	Rain days	Rainfall (mm)	Rain days	Rainfall (mm)	Rain days
January	35.3	6	102.2	6	25	1
February	100.8	4	58.4	4	47.5	4
March	72.2	5	204.2	10	90.2	8
April	69.4	4	76.5	7	44.8	4
May	53.3	5	72.7	5	33.2	5
June	0	0	8.2	1	72.6	5
Sub-total	**331**	**24**	**522.2**	**33**	**313.3**	**27**
July	0	0	0	0	0	0
August	47.4	2	0	0	78.8	8
September	42.8	5	141.4	11	44.1	4
October	75.0	5	129.9	10		
November	102.2	9	33.9	5		
December	33.7	4	93.6	6		
Sub-total	**301.1**	**25**	**398.8**	**32**	**122.9**	**12**
Total	**632.1**	**49**	**921.0**	**63**	**436.2**	**39**

Effect of crop succession on potato bacterial wilt development and progress

Successive potato planting in the same plots revealed BW onset barely 34 DAP or 6-10 days after full emergence in the Sep-Dec 2010 or third consecutive cropping season (Figure 1). By 60 DAP, visible BW infection had approached 80%, then 90% by 71 DAP and levelled-off at about 80 DAP with more than 95% of the plants showing BW symptoms (Figure 1).

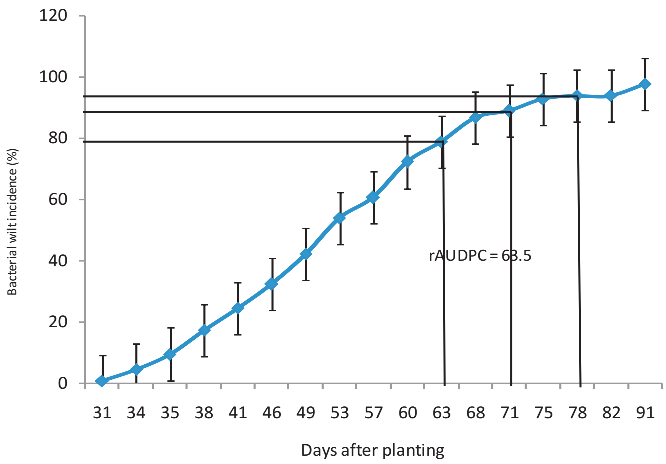

Figure 1: Disease progress of bacterial wilt after three consecutive cropping seasons of potato planting at Kachwekano ZARDI, Uganda in Sep-Dec 2010

When the other experimental plots with the controls were re-planted with potato in the March-July 2011 cropping season, BW infection symptoms did not appear where *C. falcata* was grown for one or two successive cropping seasons until 66 DAP (Fig. 2A). Bacterial wilt was evident in plots successively planted with potato by 39 DAP (Fig. 2C). The disease symptoms appeared in plots previously planted with two successive food crops about one week later. However, the former crop succession regimes revealed slow disease progress and lower final BW incidence than the later and the natural fallow (Fig. 2B). A crop succession treatment involving potato-fallow-beans-potato (P-F-B-P) delayed disease onset, but equally had rapid disease progress once symptoms appeared (Fig. 2B). Bacterial wilt symptoms appeared in plots with a natural fallow (PFFP) about a week after it had been seen in plots continuously planted with potato (Fig. 2C). The final BW incidence in treatments involving *Crotalaria* as a green cover crop and used as a green manure or improved fallow did not exceed 6% compared to succession treatments involving food crops, a natural fallow or continuous potato planting (Fig. 2).

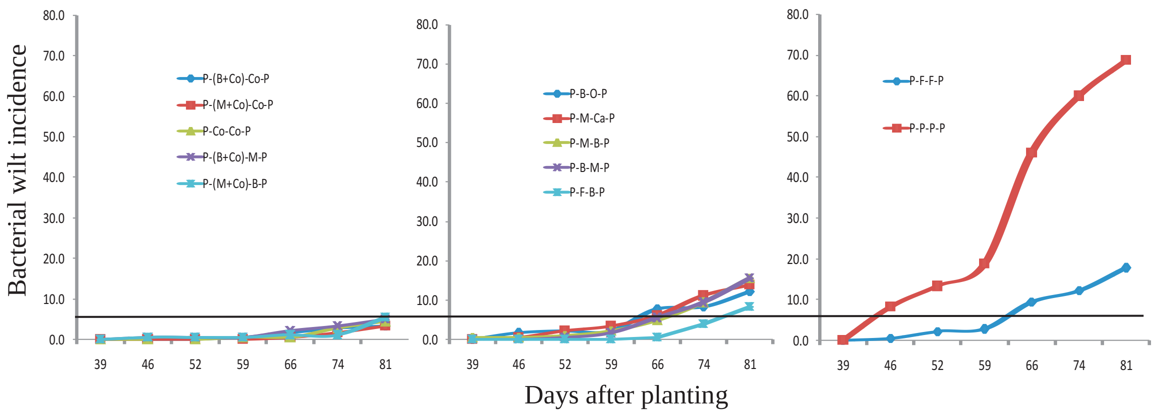

Figure 2: Disease development and progress among various crop succession and fallowing regimes for bacterial wilt management at Kachwekano ZARDI in March-July 2011 cropping season

Integral disease severity as relative area under disease progress curve before imposing experimental treatments showed no pattern in the data in 2009 among test factors (Fig. 3A). After various crop succession treatments, three groups in disease severity are evident in 2011 (Fig. 3B). One group consists of treatments where *Crotalaria* sp. was used either as in pure stand, intercropped wit beans or maize and either ploughed -in as green manure after one season or used as an improved fallow (3B). A second group consisted of treatments where two BW non-host food crops were used and a continuous fallow while the third group is where potato was planted for four consecutive seasons (Fig. 3B).

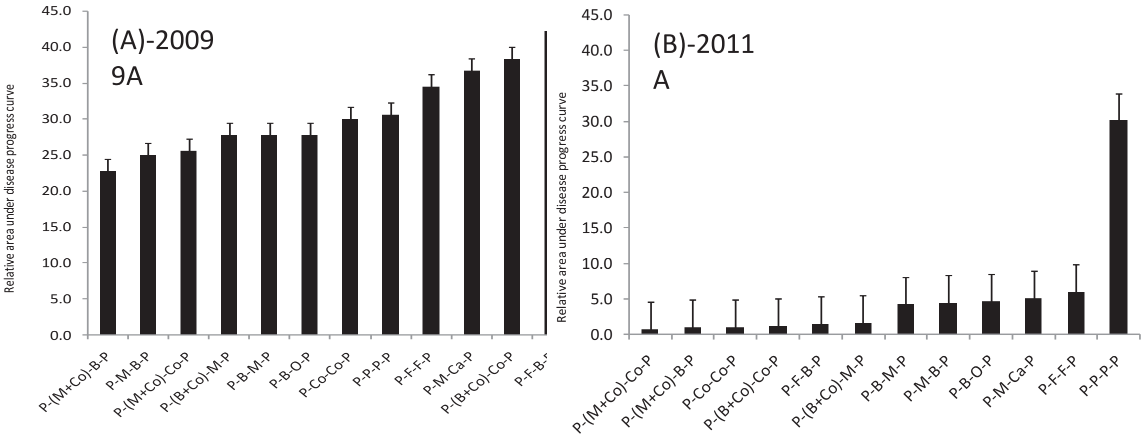

Figure 3: Effect of crop[1] rotation on integral bacterial wilt severity as relative area under disease progress curve at Kachwekano, Uganda before (A) and after imposing treatments (B)

Bacterial wilt severity (rAUDPC) and final BW incidence (%) between 2009 and 2011 was reduced by more than 85% in plots where *C. falcata* was used as an improved fallow for one year and was independent of whether it was initially grown in pure stands or intercropped with beans or maize (Fig. 4). Bacterial wilt severity increased by more than 120% after four season of successive potato planting (Fig. 4B). Crop succession options; P -F-B-P, P-M-Ca-P, P -B-O-P, P -B-M-P and P-M-B-P in decreasing order were better than one year grass fallow (P -F-F-P) in reducing BW severity (Fig. 4B).

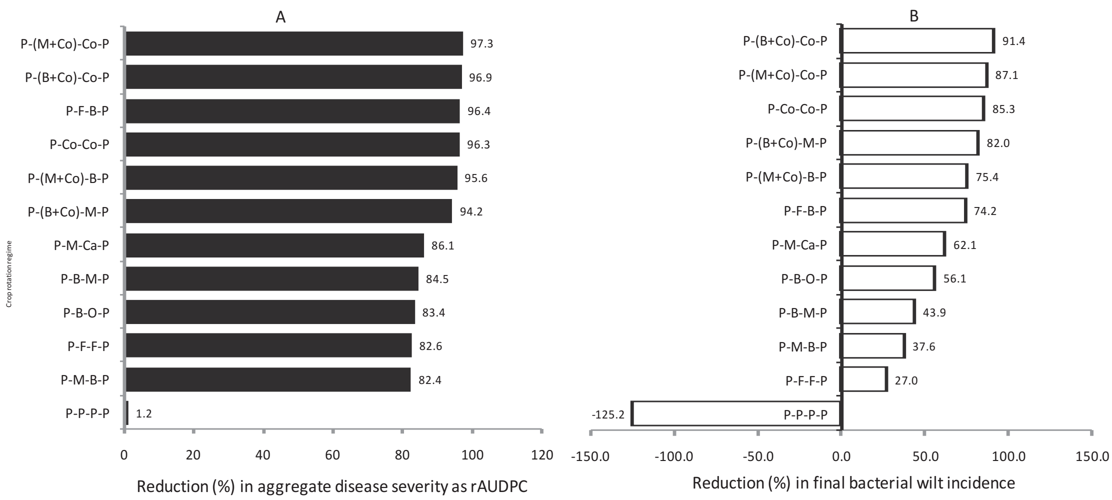

Figure 4: Reduction in disease severity as relative area under disease progress curve (rAUDPC) and final wilt incidence (%) at Kachwekano ZARDI between 2009 and 2011 among various crop rotation regimes

Occurrence of latent bacterial wilt infection in experimental field soil and potato tubers

Replanting all plots with potato after two seasons of crop rotation or fallowing revealed that BW latent infection in tubers was associated with plots where beans and maize, irrespective of succession order without *Clotalaria* were used (Dig. 2). Similarly, tubers obtained from plots with continuous potato or one or two seasons with natural fallow were latently infected with *R. solanacearum*. Apparently healthy tubers obtained from plots planted with *C. falcata*, as an improved fallow or ploughed-in as a green manure after one season were free from latent BW infection, except in plot 20 (Diag. 2). Similar results were obtained from soil samples collected from the experimental plots and tested with DAS-ELISA.

Diagram 2: Occurrence of latently infected tubers after two seasons of crop rotation in an endemically bacterial wilt infested field at Kachwekano, Kabale, Uganda during the first cropping season of 2011

Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Treatment	P-B-O-P	P-M-Ca-P	P-B-M-P	P-M-B-P	P-(B+Co)-M-P	P-(M+Co) -B-P	P-(B+Co)-Co-P	P-(M+Co) -Co-P	P-P-P-P	P-Co-Co-P	P-F-B-P	P-F-F-P
Status	H	H	X	X	H	H	H	H	X	H	X	X
Plot	24	23	22	21	20	19	18	17	16	15	14	13
Treatment	P-(M+Co) -B-P	P-(B+Co) - M-P	P-B-M-P	P-B-O-P	P-(M+Co) -Co-P	P-Co-Co-P	P-M-B-P	P-P-P-P	P-M-Ca-P	P-(B+Co)-Co-P	P-F-B-P	P-F-F-P
Status	H	H	X	H	X	H	H	X	H	**HS**	X	X
Plot	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Treatment	P-(M+Co) - Co-P	P-M-B-P	P-Co-Co-P	P-(B+Co)-Co-P	P-P-P-P	P-M-Ca-P	P-B-O-P	P-B-M-P	P-(M+Co) - B-P	P-(B+Co)-M-P	P-F-B-P	P-F-F-P
Status	H	X	H	H	X	H	X	H	H	H	H	X

X Infested H Healthy

Discussion

Bacterial wilt infestation and soil characteristics before crop rotation

Initial bacterial wilt infestation in the experimental field revealed a fairly random distribution basing tomato bioassay. At least 60% showed visible BW symptoms while the remained tested positive in DAS ELISA. This indicated presence of the BW inoculum in every plot that was used in the study before imposing treatments. This shows that that the results that were obtained after the experiment were not biased by incipient BW soil inoculum at the beginning of the experiment.

There was little difference in soil physical and chemical properties in the experimental field before and after the rotation treatments except the natural fallow. The observed differences in BW incidence and severity may not be attributed, to a large extent, to improvement in fertility, although rich soils are known to reduce the incidence and severity of BW in potato (Fontem and N'tchorere, 2009, Messiha *et al*., 2007, Lemaga, 2001, Lemaga *et al*., 2005). The soils in the experimental field had high acidity probably affecting availability and uptake of some nutrients. Future studies in soil fertility and soil health improvement should consider reducing inherent soil acidity and examine response of potato nutrient uptake and BW suppression.

Successive potato planting for four consecutive seasons in the same plots resulted in early disease onset compared to plots where the crops were alternated before replanting potato. This may be due to build-up of BW inoculum and possible loss of plant basic nutrients with successive potato cropping.

Plots planted with *C. falcata* either in pure stands or intercropped with beans or maize, or ploughed-in as a green cover crop and plots planted with beans or maize before replanting potato showed delayed BW onset, slow rate of disease increase, and lowest aggregate and final disease severity compared to plots where beans, maize cabbage or onion or natural grass fallow were involved potato without *C. falcata*. This may be due to toxic metabolites produced by *C. falcata* that probably reduced base populations of *R. solanacearum* to cause early disease epidemics. *Crotalaria falcata* and related species is known to produce pyrrolizidine alkaloids that have bactericidal properties probably reduce population growth of *R. solanacearum* in soil adequately to cause severe BW in potato before it matures. Alternatively, *Clotalaria* metabolites might have totally destroyed the pathogen in the previously infested soil as a bio-disinfectant (Joosten and van Veen, 2011, Waguru *et al*., 2011). The reduction in BW incidence and severity may alternatively be due to production of toxic alcohols and ammonia, during anaerobic fermentation of the vegetable matter from Crotalaria, reducing soil inoculum density or destroying soil-borne *R. solanacearum* (Messiha *et al*., 2007a). A low BW inoculum load would probably slow the rate of disease development, as observed from this data while total destruction of the soil-borne inoculum will result in BW-free soil unless it is re-introduced otherwise.

Soil chemical analysis in this study did not reveal large differences in organic matter content among crop succession regimes to adequately support the high reduction in BW incidence and severity. Improvement in soil organic matter promotes growth of facultative bacteria such as *Clostradium* sp. and *Bacillus* sp. that would compete with *R. solanacearum* (Messiha *et al*., 2007b, Nguyen and Ranamukhaarachchi, 2010). However, there were not great differences in soil fertility improvement across the various crop rotation options to explain the observed difference in disease incidence and severity to better organic matter supply. The high reduction incidence and severity in plots previously planted with *C. falcata* may be due to soil bio-disinfection trough antimicrobial plant metabolites or production of toxic ammonia and alcohols due to anaerobic fermentation than soil fertility improvement.

The incidence and severity of BW in plots planted successively with maize, beans, or onion in various patterns was higher than where *C. falcata* was used. This is possible since *Clotalaria* may destroy *R. solanacearum* reducing soil inoculum adequately to delay or prevent disease infection. Additionally, some crops such as beans are symptomless carriers of *R. solanacearum* and are likely to act as inoculum reserviours until the susceptible host is re-planted (Lemaga, 2001). Nevertheless, maize, beans, cabbage or onions in various crop succession patterns reduced the incidence and severity of BW more than one or two seasons of natural grass fallow.

Fallowing is often recommended in integrated BW management, however, this study revealed that it is next to repeated potato planting in reducing BW and its effects. It was more beneficial to use the land for another non-host crop before fallowing. This is because volunteer potato may be removed as weed in the non-host crop reducing the density of susceptible hosts where the pathogen can multiply and consequently reduce the soil-borne BW inoculum. Therefore the quality of the follow for BW management should be judge on the nature of species composition otherwise it may not be effective if the plant species diversity includes susceptible and symptomless carriers. The limited impact of natural fallows, even including bare-land to control BW compared to fields planted with a cover or any other crop is similarly reported in Ong *et al*., 2007.

Though a food or green cover crop may have little biomass that is returned to the land adequate to improve fertility or soil health, these crop are likely to reduce the host abundance of *R. solanacearum* during weeding where volunteers are treated as weeds. Some may produce small quantities of antimicrobial metabolites or denying the pathogen appropriate hosts (Ong *et al*., 2007). Therefore, a green cover or rotation crop may do more than simply starving the pathogen or improving the nutrient content of the soil, but may possess antimicrobial properties affecting pathogen population development. In this study, the possible impact of manure *C. falcata* which produce pyrrolizidine alkaloids, was demonstrated in plots where it was used. It was equally effective in reducing BW incidence, severity and latent infection in where it used a cover crop green manure for beans or maize before replanting potato. This means that *C. falcata* does not have to be grown in pure stands to have a significant impact on BW pathogen. Similarly it can be ploughed-in as green manure and it was able to effectively suppress BW even after one cropping season with a non-host crop before the land was replanted with potato. The means that *C. falcata* can be made attractive to small holder farmers when it used as inter-cropped in food production with higher benefits in yield improvement and BW suppression in subsequent potato crops.

Latent BW assays showed that apparently healthy tubers obtained from plots continuously planted with potato were latently infected with *R. Solanacearum*. Similarly, tubers from naturally fallowed plots had latent BW infection indicating better survival of the pathogen in a fallow with high species diversity. Basing on data from this study, natural fallowing particularly after potato without an intermediate crop was not beneficial in reducing both visible and latent BW infection. The growing alternative non-host crops in rotation after potato reduces ground keepers and other alternate hosts during weeding that would sustain the pathogen in the absence of a susceptible host. Data showed that it was more beneficial to include a plant species with bio-pesticide properties in crop succession regime where it can be used either as a green manure or improved fallow than fallowing land after potato harvesting with a natural fallow due to reduction of latent infection. Legume species with potential to improve soil fertility and soil health but lacking immediate human use, can be made attractive in competing land use alternatives particularly in highlands as a component of the farming system, by growing them as inter-crops with compatible food crops where food production outweighs other land use options. Data revealed that such beneficial cover crops can be ploughed-in as green manure after harvesting the inter-crop or used as an improved fallow for long term soil productivity and soil health improvement.

Conclusion and Recommendations

This study demonstrated the impact of crop rotation on suppressing of visible and latent potato bacterial wilt in progeny tubers and field soil where *C. falcata* was used. Crop rotation involving beans and maize irrespective of order of succession reduced BW incidence and severity improved fresh tuber yields more than a natural fallow but had little or no effect on latent BW infection. *Crotalaria falcata* grown in pure stand or intercropped with maize or beans; and used as green manure after one season or cover crop for two seasons greatly reduced BW incidence and severity. Tubers collected from plots planted with *C. falcata* from apparently healthy hills were free from latent BW infection. A natural grass fallow neither improved potato yields nor reduced BW incidence, disease severity nor latent infection than any crop rotation options. A natural grass fallow immediately after potato therefore should not be strongly recommended as a tool for soil fertility improvement and for reducing soil-borne BW inoculum without assessing the plant species composition in the fallow. *Crotalaria falcata* strongly improved potato fresh tuber yield and seed tuber health. Considering land shortage and competing land use options in highlands, it is recommended that for ware potato producers can rotate potato with maize and or beans but with destruction of volunteers however, this may not prevent latent infection. In seed potato production where BW is not tolerated, *C. falcata* can be integrated in the crop rotation and fallowing practice with very high potential to destroy R. soil-borne *R. solanacearum* inoculum and consequent latent infection in seed tubers. This notwithstanding, the mechanism soil BW inoculum suppression or destruction needs further investigation. Future assessment of such cover crops for bio-pesticide effects should endeavour to quantify the effects of such plant species on population densities and species diversity of soil-borne fauna and flora community as well as allelopathic effects on other plant species.

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Above-ground Competitive and Complimentary effects of Artemisia (*Artemisia annua* L.) and Maize (*Zea mays* L.) intercropping in a sub-humid Ecozone of Maseno, Kenya

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Abstract

An intercropping study at Maseno, western Kenya investigated some above -ground competitive effects of Artemisia (*Artemisia annua* L.,) and Maize (*Zea Mays* L.) intercropping in two consecutive rainfed seasons, by evaluating growth habits and yield patterns from among different spacing regimes in a shrub -based agroforestry (AF) system. The Competitive Ratio (CR) was used in the evaluation of competitive effects of the intercrops. Artemisia as an *Asteraceae* was found to be 1.3 (or 30%) more competitive than maize, a member of the grass family (*Poaceae*) when the two component crops are grown in association with optimal spacing. It is concluded that by varying spacing regimes and hence plant densities for variable costs of Maize and Artemisia, profitable Artemisia+Maize intercropping require that farmers apply spatial arrangements in which complementary effects on net output exceed competitive abilities of Artemisia. These attributes may provide critical lessons for exploiting the allelopathic potential of Artemisia in controlling annual weeds of food crops in AF systems. From a botanical perspective, the potential use of several planting patterns of Artemisia and Maize intercrops are discussed in this paper.

Introduction

Intercropping practices include agroforestry systems (AF) as multifunctional agriculture in which food crops are cultivated on the same land management unit as shrubs in either in some form of temporal se quence or spatial arrangement (FAO, 2005). Shrub based AF may possess higher rates of return than tree based AF on account of the respective length of fallow periods before realizing harvestable yields. For purposes of on -farm biodiversity conservation (Ghosh, 2004), crop diversification and value addition to subsistence farming, Maize (*Zea mays* L.) is a staple food crop in Kenya; While Artemisia (*Artemisia annua* L.) is a medicinal shrub species recently introduced for commercial cultivation in the region (EABL, 2005). In addition, *Artemisia* has aromatic properties, and qualifies as an enriched fallow plant species that also exhibits a potential for short season ratooning (Chumba *et al*., 2012). The benefits accruing from intercropping Artemisia and Maize on yield of both crop components in regard to competitive abilities have not been documented. Understanding the above-ground competitive and complimentary effects of *Artemisia* and Maize intercrops may therefore offer enormous capacity to optimise maize grain yield and artemisia leaf yield, while minimising field preparations and lowering production costs to enhance food security and higher farm incomes.

Literature Summary

Intercropping creates component interactions between any two or more individual plants that may either reduce or increase the vigour of one or all of the component crops. According to Dhima *et al.,* (2007), this may have a significant impact on the growth rate and yield of the different species used in intercropping. Crop yield variability comes from complex interactions between the environment, spacing, management, progenitors and abiotic factors that occur across a field (Baumann *et al.,* 2001) of intercrops. For example, Artemisia secondary metabolites have significant phytotoxic activity, even on the artemisia plant itself, making it a suitable candidate for natural herbicide through allelopathic e ffects (Lydon *et al.,* 1997). Sunwar, (2003) also reported the use of *Artemisia spp.* as a natural pesticide in home gardens which consists of vegetables, fruits and fodder.

According to Cadisch *et al.* (2002) mixing species with compatible and complimentary root and/or shoot growth patterns leads to a more diverse system and may also maximise above and below ground growth resources' utilisation/ In this respect, Sobkowiez (2006) further reports that two commonly used intercropping strategies entail planting a deep-rooted crop with a shallow-rooted crop, or planting a tall crop with a shorter crop that requires partial shade. In sub humid zones on acid soils competition between shrub -crop intercrops for light, nutrients and moisture can be very severe due to the effect of shading (Lawson and Kang, 1990), where crop yield reductions with increase in distance from the shrub rows have been reported (Chirwa *et al.,* 2007). Since water and nitrogen are critical nutrients for plant growth and productivity in fallow systems for both the *Artemisia* (Ferreira and Janick, 2009) and Maize components (Hartemink *et al*.,2000), competition can also be more severe in degraded sloppy areas of western Kenya with erratic rainfall or flat land with poorly drained soils and vulnerable to water logging.

Competition occurs between the same plant species, called intraspecific competition, or between different species, called interspecific competition (Van der Meer, 1989). Ultimately, any sustainable multifunctional intercropping system involving maize as a food crop and artemisia as a medicinal shrub will entail effective application of good agriculture practices for minimum pesticide usage. This will ensure the least possible impact on the environment and yielding a product that can be accurately traced from the field where it is grown to the consumer (WHO, 2003). Planting shrub-based AF intercropping components should be designed in such a way as to minimise competition for growth resources, and manipulating spatial arrangement is one way of attaining this. Willey and Rao (1980) demonstrated that the competitive ratio (CR) could be useful in comparing the competitive ability of different crops, measure competitive changes within a given combination and determine what competitive balance between crop components is most likely to give maximum yield advantages. Furthermore, the CR represents the ratio of individual LERs of component crops and takes into account the proportion of the crops in which they are initially sown (Putnam *et al.,* 1985). Since the CR targets a range of growth resources for competition, it may hence be more applicable interchangeably in '!dditive Series' and 'Replacement Series' of intercropping (Fukai and Trenbath, 1993) targeting interplant competition for one specific growth resource. However, there is still scarce scientific evidence from sub humid regions on interactions between inter-plant competition with positive effects of shrubs and food crops; But the biological merit of intercropping makes it an important conservation farm practice for smallholder farmers, since the system permits nutrient recycling and reduces the need for herbicides in most cases (Van Noordwijk *et al.,* 1999).

Description of Research

The experiment was carried out at Maseno University field station, Kenya during the period from September 2009 to August 2010, relying on rainfall precipitation of two consecutive seasons interspersed with a short fallow period of 45 days. The soils in the area are classified as Acrisols, well drained, deep reddish brown clay, fairly acidic with pH ranging between 4.6 and 5.4, and are deficient of P and N (Jaetzold *et al.,* 2005). Land was prepared to fine tilth before planting of certified Maize seed from Kenya Seed Company, variety H *513*. After the first weeding of Maize at knee high height, Artemisia was transplanted when the young plants had uniformly grown to 50 cm in height with average of 15 true leaves, in accordance with the practices of Ferreira *et al.*, (2005). Di-ammonium phosphate (DAP) fertilizer was applied at planting of Maize in all the plots at the recommended rate of 50 kg ha^{-1} while Calcium ammonium nitrate (CAN) was used for localized top dressing of Maize (Okalebo *et al.,* 1999) only after 1st weeding also at the rate of 50 kg ha^{-1}. The plant spatial arrangements were in '!dditive series' (Fukai and Trenbath, 1993) to result in a constant Maize plant density in all treatments but varying Artemisia population. Each plot replica size measured 6m x 4m ($24m^2$) including two control plots of pure stands. The experiment had eight treatments, laid out as a randomized complete block design (RCBD) in 3 replicates, with three different intrarow spacings of the Artemisia. The treatments were designed as follows (Chumba *et al*., 2012):

- T_1 *= Artemisia 1m X 1m; Maize 0.90m X 0.75m;*
- T_2 *= Artemisia 1m X 0.75m ; Maize 0.90m X 0.75m ;*
- T_3 *= Artemisia 1m X0.9m ; Maize 0.90m X 0.75m;*
- T_4 *= Artemisia 0.75m X 0.75m ; Maize 0.9m X 0.75m ;*
- T_5 *= Artemisia 0.9m X 0.75m ; Maize 0.9m X 0.75m ;*
- T_6 *= Artemisia 0.9m X 0.9m ; Maize 0.9m X 0.75m ;*
- T_7 *= Maize 0.90m X 0.75m (Pure Stand);*
- T_8 *= Artemisia 1m X 1m (Pure Stand);*

Above ground plant biomass of both Maize and Artemisia was determined at harvest to mimic farmers' practice, from an area of $24m^2$ at harvest and extrapolated to production ha^{-1}.

The competitive ratio (CR)

Measurements to demonstrate the existence or not of competition by comparing the CR among the intercrops in each treatment was calculated following the method of Willey and Rao (1980) :

$$CR_{maize} = (LER_{Maize} / LER_{Artemisia}) \times (Za / Zm), \quad (1a)$$

$$CR_{Artemisia} = (LER_{Artemisia} / LER_{Maize}) \times (Zm / Za) \quad (1b)$$

Where, LER_{Maize} is the partial land equivalent ratio (LER) for Maize, $LER_{Artemisia}$ is the partial LER for Artemisia in the crop mixture. Zm and Za are the proportions of Maize and Artemisia in the mixture respectively. The LER was computed by the equation suggested for simple AF experiments by Rao and Coe [1992]:

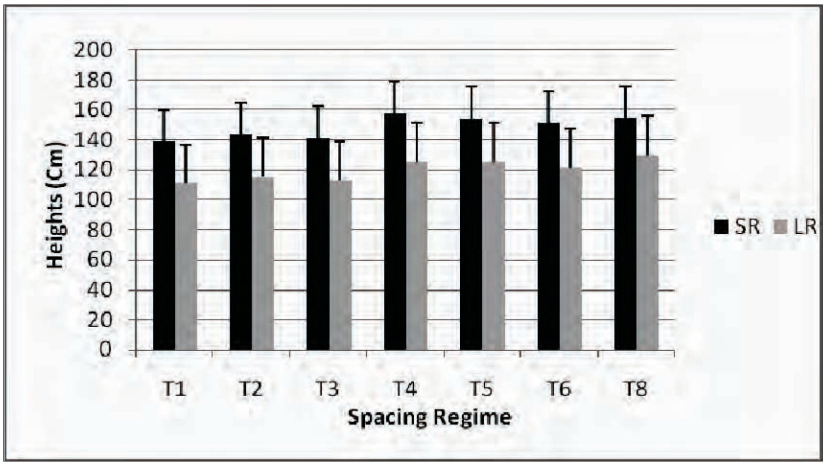
$$LER = C_i/C_s + T_i/T_s \quad (2)$$

Where, C_i = Crop yield under intercropping; C_s = Crop yield under sole cropping; T_i = Shrub yield under intercropping; and T_s = Shrub yield under sole system.

The data collected on CR, plant heights, biomass, grain yield of Maize and leaf yield of Artemisia was subjected to analysis of variance for RCBD using the COSTAT version 6.4 statistical computer package. The treatment means were separated using the least significant differences (LSD) test at 0.05%, while homogeneity of variances was verified by Bartlett's test/

Research Results and Application

The treatments had no significant effect (P>0.05) on plant height of both Artemisia (Fig.1) and Maize during both seasons, suggesting that the spacing regimes tested did not constitute crowded conditions to effect negative competition for light and other growth resources. The two component crops of maize and artemisia can therefore be successfully grown in association.



+Figure 1: The effect of spacing Artemisia and Maize on Artemisia Plant Heights. Data points are the mean of three replications and bars represent standard error

+LEGEND:

T_1 = Artemisia 1m X 1m ; Maize 0.90m X 0.75m; **T_2** = Artemisia 1m X 0.75m ; Maize 0.90m X 0.75m
T_3 = Artemisia 1m X0.9m ; Maize 0.90m X 0.75m; **T_4** = Artemisia 0.75m X 0.75m ; Maize 0.9m X 0.75m
T_5 = Artemisia 0.9m X 0.75m ; Maize 0.9m X 0.75m; **T_6** = Artemisia 0.9m X 0.9m ; Maize 0.9m X 0.75m
T_7 = Maize 0.90m X 0.75m (Pure Stand) **T_8** = Artemisia 1m X 1m Artemisia (Pure Stand)

The different spacing regimes had a significant effect ($P<0.05$) on biomass yields and the Competitive Ratio (CR) of artemisia against Maize among the intercrops during both seasons (Table 1).T_3 artemisia exhibited the highest mean CR at 1.75 while T_1 maize had the Lowest CR at 0.85 and were statistically different from the control. T_1 maize (*CR=1.2*) had a higher CR than Artemisia (*CR=0.85*).

On average, Artemisia was 1.3 (or 30%) more competitive than Maize du ring both cropping seasons in this study. If CR < 1, there is a positive benefit for maize relative to artemisia; and if CR > 1, there is a negative benefit to the secondary crop relative to the main crop (Ghosh *et al.*, 2004). Since CR represents the proportion of the crops in which they were initially sown (Putnam *et al.,* 1984), higher CR values for artemisia suggest that the crop utilised the growth resources more aggressively than Maize, despite having been planted sequentially. In general, the more a co mpetitive ratio of each treatment approached unit value, the more the Maize+Artemisia intercrops balanced the competition between both species. This suggests an advantage in Maize intercropped with Artemisia in single hedgerows of each plant species from such treatments as T_1, T_4, T_5 and T_6. This yield advantage is probably due to different above -ground growth habits occasioned by different plant densities for causing optimal use of growth resources or factors. This argument corroborates that of Awal *et al*., (2007), who reported that as CR approaches unit values, intercrop associations in Barley (*Hordeum vulgare* L) and Peanut (*Arachis hypogaea*) effectively counterbalance the competition for growth resources between these species.

Table 1: Effect of spacing Maize and Artemisia on Biomass Yields and Competitive Ratios of Artemisia + Maize Intercrops

Treatment+	Intercrop Population ($24m^2$)	Biomass yields(t/ha)		Competitive Ratios	
		Maize	Artemisia	$CR_{Artemisia}$	CR_{Maize}
T_1	85	2.38bc	7.36bc	0.85c	1.20a
T_2	78	2.10c	7.29bc	1.47b	0.69bc
T_3	74	2.78bc	5.39d	1.75a	0.52bd
T_4	90	2.35bc	9.67a	1.30b	0.76bc

T_5	90	2.25bc	8.975a	1.16b	0.95b
T_6	85	2.38bc	7.08c	1.24b	0.80c
T_7	50	3.85a	-	-	0e
T_8	35	-	8.75ab	0d	-
CV%	-	19.96	16.45	19.3	26.49
$LSD_{0.05}$ -		0.31	0.81	0.385	0.29
Spacing -	*	*	*	*	

{Mean values in a column followed by dissimilar letter(s) indicate differences at 0.05 (*) level of significance}

[+]**LEGEND:**

T_1 = Artemisia 1m X 1m ; Maize 0.90m X 0.75m; **T_2** = Artemisia 1m X 0.75m ; Maize 0.90m X 0.75m

T_3 = Artemisia 1m X0.9m ; Maize 0.90m X 0.75m; **T_4** = Artemisia 0.75m X 0.75m ; Maize 0.9m X 0.75m

T_5 = Artemisia 0.9m X 0.75m ; Maize 0.9m X 0.75m; **T_6** = Artemisia 0.9m X 0.9m ; Maize 0.9m X 0.75m

T_7 = Maize 0.90m X 0.75m (Pure Stand) **T_8** = Artemisia 1m X 1m Artemisia (Pure Stand)

Plate 1: SR artemisia + maize intercrop T_4 at physiological maturity

The effect of positive component interaction between the intercrops was more pronounced in T_3 Maize, to result in high biomass yields of 2.78t ha^{-1}. The highest biomass yield from T_7 Maize at 3.85t ha^{-1} is attributable to the effect of 'Niche differentiation', or lack of competition in favour of Maize as was reported by Mkamilo, (2004). A comparative Maize and Beans (*Phaseolus vulgaries*) system in western Kenya was also found by Woomer *et al.,* (2004) to allow larger light penetration, which likely benefits the

Maize as well as the legume through partitioning of resources. This process allows two species to partition certain resources so that one species does not out-compete the other as dictated by the 'competitive exclusion principle' in short term agroecological terms. The high biomass yields of Artemisia obtained from T_5 (8.98t ha^{-1}), T_4 (9.67t ha^{-1}) and T_6 (7.03t ha^{-1}) apart from the control plot of T_8 with 9.65t ha^{-1} may thus have been possibly due to greater spatial complementarity. These spacing regimes represent plant proportions and densities that optimise total biomass yield through facilitative component interactions that occur in Maize +Artemisia intercropping systems. Complementarity of resource use may also have occurred through synergistic effect of applying commercial fertilizer to Maize intercrops, as demonstrated by the significant effect of spacing (P<0.05) on biomass yields of Artemisia (Table 1). This implies that when intercropping Maize and Artemisia, single localised application of fertilizer on Maize is sufficient to guarantee optimal yields from both crop components.

Even though Artemisia proved a better competitor than Maize, facilitative component interactions was exhibited in T_1 by resulting in significantly higher Maize biomass yields (Table 1). T_1 intercropping arrangement could hence be more desirable for plant architectural arrangements if Maize is to be considered as the main crop in the mixture for optimal yields. This is the opposite of T_3, T_4, T_5 and T_6 when Artemisia is targeted as the main crop. Shahid and Saeed (1997) also used CR values >1.0 to report that lentil (*Lens esculentum*) was a better competitor when sown in association with Wheat (*Triticum* *aestivum*). Completely no pests and/or diseases of economic importance affected both intercrops (Plate 1) and Artemisia pure stand throughout the two growing seasons, after canopy closure of artemisia. Only the Maize monocrop T_7 had a pronounced incidence of weed infestation including Black Jack (*Bidens pilosa*), *Oxalis latifolia*, Wondering Jew (*Commelina bengalensis*), *Cynodon dactylon* and *Chloris gayana*, while a few cobs had isolated incidence of fungal head smuts (*Ustilago maydis*) observed during the LR season. Chumba *et al.,* (2012) also demonstrated over 50% reduction in variable costs associated with weeding in Artemisia compared to Maize monocrops crops; where no weed emergence and fungal infestation (*Ustilago maydis*) was observed after first weeding and canopy closure of Artemisia stands compared to Maize. This further supports an allelopathic potential of Artemisia as earlier reported by Mekky, (2008) and Lydon *et al*., (1997) for natural control of resident weeds. Artemisia aqueous extracts or crude powders after value addition could thus provide suitable biopesticides for the agriculture industry in sub-humid regions of western Kenya.

By varying costs of Maize and Artemisia production through manipulation of plant densities, successful intercropping requires that farmers design systems in which complementary effects of intercropping on net returns exceed competitive effects. This is in agreement with Alabi and Esobhawan (2005) from studies on Okra (*Abelmoschus esculentus*) and Maize mixtures, that any strategy which reduces cost of production in intercrops will increase its attractiveness to farmers. Maize monocrops are significantly more vulnerable to weed infestation and disease incidence than all Maize+Artemisia intercrops and the Artemisia monocrop. A production system that entails intercropping maize and artemisia, with optimal component interactions can hence provide a viable food security intervention through cost saving on labour input for weeding and promote land use efficiency. Furthermore, AF systems incorporating maize and Artemisia intercrops could provide an eco-friendly approach for controlling weed incidence through minimal use of commercial synthetic herbicides.

The CR index provides an improved estimate of the cumulative effects of above-ground component interactions for relative advantage of a shrub based AF intercropping system employing short fallow periods. The CR can thus aid researchers/extension agents in selecting intercropping practices that are most suitable on basis of optimal component interactions for recommendation to farmers. Based on CR and biomass yields in this study, farmers with a preference for Maize a spacing regime of T_1 (*Artemisia 1m X 1m; Maize 0.90m X 0.75m*) will be ideal; while for farmers with a preference for Artemisia T_5 (*Artemisia 0.9m X 0.75m; Maize 0.9m X 0.75m)* is recommended. For farmers with more intensified forms of intercropping with a preference for both intercrops, a spacing regime of T_4 (*Artemisia 0.75m X 0.75m; Maize 0.9m X 0.75m*) or T_6 (*Artemisia 0.9m X 0.9m; Maize 0.9m X 0.75m*) is recommended.

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Acaricidal Efficacy of Pesticidal Plants Used for Smallholder Tick Control

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Abstract

As part of two projects namely, the Southern African Pesticidal Plants (SAPP) (2007 – 2009) and African Dryland Alliance for Pesticidal Plants Technologies (ADAPPT) (2010 – 2012), a number of plants with acaricidal properties have been identified in Zimbabwe from interaction with farmers and tested. Simple water extracts of the plants showed a decline in total tick counts in initial efficacy experiments. In this report we report several optimization strategies that were evaluated including: use of a surfactant; pure plant compounds; mixing different plant materials; hot water extraction; and organic solvent extraction using the plants *Solanum panduriforme*, *Strychnos spinosa*, *Lippia javanica* and *Maerua edulis* in trying to increase the efficacy of the plant extracts against cattle ticks. The findings showed that using hot water and liquid soap (surfactant) increased acaricidal activity of the plant materials understudy. Methanol also showed high extractive ability evidenced by the higher activity of *M. edulis* extracted using the solvent against tick larvae. Pure compounds from *L. javanica* and *S. spinosa* did not cause any tick mortalities. Overall, it can be deduced that there is scope to improve acaricidal activity by simple low-cost interventions like using liquid soap and use of hot water.

Introduction

One of the greatest challenges to smallholder communal livestock production is the inaccessibility of commercial acaricides to comprehensively deal with the problems of ticks and tick borne diseases (TTBD). Most communal farmers live in remote areas which are not well serviced by modern veterinary services prompting the search for alternatives that may help in the control of TTBD. Engaging farmers has shown that there are plants that have been used from time immemorial in the control of ticks. The SAPP and ADAPPT projects have facilitated scientific research of some of these plants. Preliminary results confirmed acaricidal properties in some plants but one of the limitations has been that the activity has not been able to match that of commercially available synthetic acaricides. In the experiments by Madzimure *et al.* (2011) the animals used in the studies under the plant extracts treatments were still classified “tick infested” by Zimbabwe veterinary standards. These observations have motivated further studies to find low cost ways of increasing the acaricidal properties to levels perhaps closer or even better than the commercial acaricides and to identify ways of reducing application rates to conserve the plants and prevent over-harvesting and eventual extinction.

Literature Summary

TTBD control remains a key component of profitable livestock husbandry practice the world over (Jongejan and Uilenberg, 2004). Intensive commercial livestock systems have relied heavily on synthetic acaricides and several other high–technologies that include use of vaccines and antibodies (Godfray *et al*., 2010). This has generated challenges with development of tick resistance, environmental concerns in effective disposal, residual contamination of animal products and the ever increasing costs of the acaricides (Madzimure *et al*., 2011). In Zimbabwe for example, the recommendation by veterinary authorities is that cattle should be dipped once every week in summer and once every fortnight in the dry season but in the recent past communal cattle could go for several months without being dipped because of the unavailability of acaricides (Gunjal *et al.*, 2009). The use of plants with acaricidal properties is gaining worldwide recognition as a viable alternative tick control method (Isman, 2008). In Zimbabwe a few plants have been identified and tested for acaricidal efficacy, toxicity and phytochemistry. Madzimure *et al.*, (2011) reported considerable efficacy of *L. javanica* at 10% w/v inclusion level in on station trials with Mashona cattle. Oral toxicity studies of the same plant concluded that there are possible health dangers in consuming fresh *L. javanica* water extracts in large concentrations. Other plant materials that have been also studied and showed acaricidal properties include *S. panduriforme and S. spinosa* at 10% w/v inclusion levels (Madzimure, 2008). While *S. panduriforme* is a known toxic veterinary plant, Nyahangare *et al.*, (2012) also reported that water extracts of unripe fruits of *S. spinosa* could cause oral toxicity. A critical observation from these studies was that the commercial acaricide used as a positive control out performed crude water extracts of these pesticidal plants leaving a research gap of ways of increasing activity.

The use of hot water for extraction is useful in many ethnomedicine programmes and is a prominent feature in countries with strong ethnomedicine programmes like China (Huei, 2002). The yield of bioactive compounds from hot water extracted plants can be as high as that obtained from using organic solvents (Katsvanga *et al.*, 2006). Surfactants use often increase pesticidal properties and several researchers have used it for purposes of increasing emulsification, dipersibility, spreading and wetting properties of the extracts with positive results (Czarnota and Thomas, 2010). The combining of different plant materials can possibly increase acaricidal efficacy if the active compounds have a positive synergistic effects and increase tick mortality (Warnock and Cloyd, 2005). A mixture of pesticides also help delaying onset of resistance developing in arthropod pest populations (Bielza *et al.*. 2009) which is a real issue in pesticide research and development (Cloyd, 2009). Traditionally organic solvents like methanol have been used for extraction of plant material for better results than water. Methanol is used because the hydroxyl (-OH) group contains a greater negative charge than the methane group making it a very effective solvent (Esteban *et al.*, 2006). Afify *et al.* (2011) extracted *Syzygium cumini* using methanol, hexane and ethyl acetate, in experiments to determine acaricidal activity of the plant against *Tetranychus urticae* (Koch) and the methanol extracts showed the highest acaricidal activity with 95.5% mortalities, whilst hexane and ethyl acetate had 94% and 90% mortalities respectively. However solvent extraction is not a viable option in the context of most smallholder farmers because of access and affordability issues.

Description of Research

A total of five laboratory bioassay experiments were carried out at the Central Veterinary Laboratory (CVL) of Zimbabwe in Harare as described: (a) *Effect of hot water and surfactant on efficacy*: Hot water (80^0C) + 1% *v/v* surfactant extracted *L. javanica*, *S.panduriforme* and *S. spinosa* at 5% *w/v* was incubated with *Boophilus* spp. tick larvae using the Soberanes technique (Soberanes *et al.*, 2002). Treatments were incubated in an incubator set at 27^0C and relative humidity of 85-95%. Mortality of tick larvae was recorded after 24 and 48h. (b) *Effect of using hot water and different levels of surfactant:* In this study, hot water extracted *L. javanica* at different concentrations (5 % and 10 % *w/v)* with different inclusion levels of liquid soap of (0, 0.1, 0.5, 0.8 and 1% *v/v*) was tested for efficacy in the same conditions as described in (a) above. (c) *Mixing different plant materials:* The effect of mixing different plant materials on efficacy was tested in laboratory bioassay with tick larvae. Plant extracts at 5% (*w/v)* dilutions were mixed with liquid soap (surfactant) at 1% w*/v*)and mixed in a 1:1 ratio as *L. javanica / S. panduriforme; L. javanica / S. spinosa; S. spinosa / S. panduriforme;* and *L. javanica / S. panduriforme /S. spinosa.* (d) *Effect of using isolated pure plant compounds against tick larvae:* In this study, pure compounds, kingicide, loganin (from *S. spinosa*) and chlorogenic acid and xanthine (*L. javanica)* were tested against tick larvae. Methanol was used as a negative control and Tickbuster as the positive control. (e) *Effect of extraction method on acaricidal efficacy of* Maerua edulis *against* Rhipicephalus (Boophilus) decoloratus *tick larvae*: In this study, the effect of different extraction solvents and methods (cold and hot water, surfactant and organic solvents) on efficacy against cattle ticks was tested against tick larvae in laboratory bioassays. Several concentrations of the plant material (5, 10, 20 and 25%) were used. The extraction was done in several ways with; cold water only; cold water + liquid soap, hot water + liquid soap; hexane extracted and methanol extracted *M. edulis*. Amitraz based commercial acaricide (Tickbuster®) was the positive control.

Research Results and Application

The results overall show that the use of hot water and addition of a surfactant (liquid soap) are low cost available measures which farmers can use to increase acaricidal activity of the plant materials under investigation. There was no significant difference between the positive control (Tickbuster) and 5% *w/v* plant material extracted with hot water and 1% v/v liquid soap after 48h. (Tables 1). Mixing different plant combinations showed significant comparable acaricidal activity with Tickbuster only after 48h, suggesting a delayed effect There was no significant difference between higher surfactant and lower inclusion levels in the 5% w/v *L. javanica* treatments so 0.1% v/v can be recommended for use. Besides its reported use as a grain protectant, these experiments demonstrated that *M.edulis* can also be used to reduce tick infestations. While hot water increases efficacy, methanol extraction proved to be the best extraction method for this plant with no difference in activity with Tickbuster (Fig 1). However methanol use will be limited by availability and affordability for smallholder farmers. In the experiment on the effectiveness of pure compounds, there was no observed effect of the isolated pure compounds on tick larvae. While further studies are still essential to probe that observation, it is possible that the pure compounds do not work in isolation hence the inactivity.

Table 1: LSM mortalities of hot water extracted plant materials and 1% v/v surfactant

Treatment	Mortality (%) 24h	48h
5% w/v L.javanica	56.8[a]	98.5[a]
5% w/v S. panduriforme	46.0[a]	92.6[a]
5% w/v S.spinosa	47.7[a]	90.5[a]
Water + liquid soap (1%v/v)	22.0[d]	54.1[b]
Tickbuster®	98.0[c]	100.0[a]

[a, b, c &d] represent within column least squares means where there is significant difference and where they are the same (p<0.05). Comparison is within column (*Mutama, 2011)*

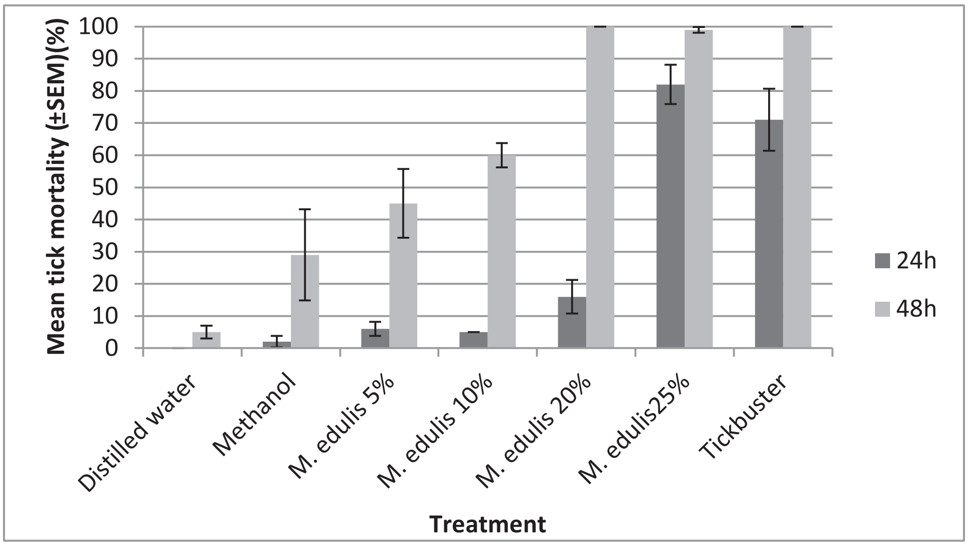

Figure 1: Effect of methanol-extracted *M. edulis* on mortality of *Rhipicephalus* (*Boophilus) decoloratus* tick larvae *(Maramba, 2012)*

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Larvicidal Compounds from *Fagaropsis angolensis* against *Anopheles gambiae*

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Abstract

The struggle to eradicate or control malaria over the past years has not been successful especially in the developing countries. This is clearly indicated by the alarming number of death and infection cases being reported annually in the sub-saharan parts of Africa. The use of synthetic insecticides to kill adult mosquitoes has been rendered unsafe due to development of mosquito resistance, creation of problems such as adverse environmental effects and high operational costsof the insecticides. Bioassay studies have in the recent past been conducted on different plant species as they are known to be easily biodegradable and environmentally friendly.This study explores the use of extracted compounds (volatile and non-volatile) from the plant *Fagaropsis angolensis* (Rutaceae) against the third instar larvae of *Anopheles gambiae.* The observed mortality activities from these compounds are reported in this paper.

Introduction

Malaria is one of the world's most common and severe tropical diseases transmitted by the female *Anopheles* mosquitoes of which the *Anopheles gambiae* is considered the most virulent(Gutie' rreza *et al*., 2008). According to Breman *et al.*, (2004) and Snow *et al.*, (2005), more than 300 millions of people are infected with malaria annually in the world. These infections are estimated to cause approximately 1.2 to 2.7 million deaths globally each year with about 90% of all infections occurring in Africa, south of the Sahara. The most vulnerable group to malaria infections are often pregnant women and children under 5 years of age (Steketee *et al.,* 2001). A lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes (Ansari and Razdan, 2000) and this has propelled the search for and use of eco-friendly plant based products (Navneet*et al*., 2011). The *Fagaropsis angolensis* (Rutaceae) is ethno botanically known for its ability to treat malaria (stem bark) in Kenya while in Malawi and Zimbabwe, its root powder taken in drinks to treat male sterility (Lemmens, 2008). Therefore, the aim of this study is to investigate the larvicidal activity of *F. angolensis* which could inturn stem the spread of malaria through reduction of mosquito population.

Literature Summary

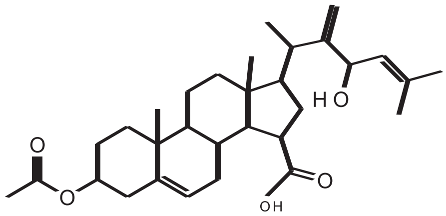
Mosquito insects are known not only totransmitmalaria but alsoseveral other diseases such as yellow fever, dengue fever, filariasis and Japanese encephalitis (Vatandoost and Vaziri, 2001; Radhika *et al*., 2011).Vector control is so far the most successful method for reducing incidences of mosquito borne diseases, however, there is emergence of widespread insecticide resistance and the potential environmental issues associated with some synthetic insecticides (ICMR, 2003) and there is no effective malaria vaccine is yet available (CDC, 2008; Matasyoh *et al*., 2008).These vector control strategies include methods aimed at reducing human - vector contact such as insecticide-treated bed nets, use of repellants, indoor residual spraying and methods aimed at reducing vector density such as space spraying, sterile male, source reduction and larviciding.Larval control measures are intended to reduce malaria transmission indirectly by reducing the vector population density near human habitations sincethe larvae are localized in their breeding sites and cannot easily avoid control measures (Killeen *et al*., 2002). Ethnobotanical and laboratory based studies have revealed the existence of insecticidal plants belonging to different families. Crude solvent extracts of these plant parts, essential oils (Harve and Kamath, 2004) or chromatographic fractions are shown to have various levels of bioactivity against different developmental stages of malaria vector mosquitoes (ICMR, 2003).Due to the concern over the quality and safety of life and the environment, the emphasis on controlling mosquito vectors has shifted steadily from the use of conventional chemicals towards alternative insecticides that are target - specific, biodegradable, and environmentally safe, and these are generally botanicals in origin(Navneet *et al*., 2011).

Description of Research

Fresh leaves of *F. angolensis* were collected from Kakamega Forest after its identification. The leaves were air dried in the shade and ground into fine powder in readiness for extraction with methanol solvent. Methanol crude was then suspended in water and extracted with chloroform. Bioassay guided fractionation using chromatographic techniques was carried out on the chloroform crude extract which finally led to three larvicidal compounds (**1, 2** and **3**).The NMR spectra were recorded on aBruker Advance 500MHz NMR spectrometer at the Technical University of Berlin,Germany. All the readings were done in Deuterated chloroform solvent with tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on silica gel (70-230 mesh) and Rf values were measured on analytical aluminium TLC plateswith fluorescence indicator.For the larvicidal assays, exactly 0.06 g of **1, 2** and **3** and 0.1g of the essential oil (hydro-distilled)were weighed and dissolved in ?1% DMSO to make a concentration of 1000 mg/L as stock solutions. Serial dilutions of 750, 500, 250, 125 upto 3.9 mg/L were prepared from the each of the three stock solutions. All the bioassays were conducted according to standard WHO procedure (2005) with slight modifications at (KEMRI-CGHR), Kisumu, Kenya, where the insects were reared in enamel trays using spring river water. The experimental temperatures were maintained at 26±3°C and humidity between 70% and 75%. The bioassays were performed with third instar larvae of *An. gambiae* and carried out in triplicate using 20 larvae for each replicate assay. The larvae were placed in 50 ml disposable plastic cups containing 15 ml of test solution and fed on tetramin fish feed (TetraMin®) during all testing. Mortality and survival was established after 24 hours of exposure. The number of the dead larvae in the three replicates was expressed as the percentage mortality for each concentration. The negative control was 1% DMSO in spring river water while positive control was 100 mg/L of pylarvex. For the essential oil the procedures followed.The Lethal concentrations (LC_{50} and LC_{90}), upper and lower limits at 95% confidence levels of the mortalities observed were calculated according to the probit regression methods (Finney, 1971)using IBM SPSS software version 19. GC-MS was carried out onan Agilent GC-MSD apparatus.Identification of oil compounds was done by comparison of their retention times and mass spectra with those found in literature (Adams, 2007) and supplemented by Wiley 7N.l, HPCH1607.L and QuadLib 1607 GC-MS libraries.

Research Results and Application

The bio-assay guided fractionation of the chloroform crude extract gave rise to three larvicidal compounds phenanthrene carboxylic acid derivative (**1)** and aliphatic esters (**2**and **3**).



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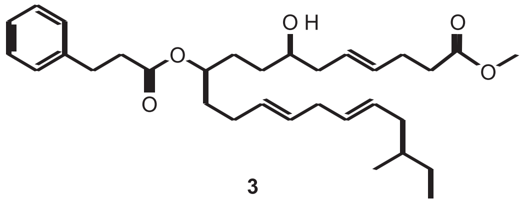

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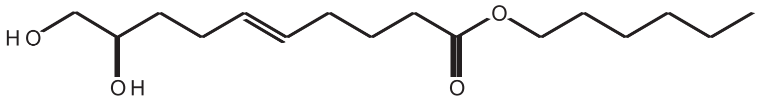

2

To evaluate the mosquito larvicidal activities of these compounds, third instar larvae of the malaria vector *An. gambiae* were used. All the isolated compounds showed strong larvicidal activity. At a dosage of 750mg/L, compounds **2** and **3** exhibited 100% mortalities while compound **1** had 96.7%. The lethal concentrations at 50% and 90% larval mortalities were calculated and reported as shown in table 1. Compounds **1, 2** and **3** had LC_{50} values of 245.5mg/L, 147.6 mg/L and 144.4 mg/L respectively. Likewise, they possessed LC_{90} values of 471.6 mg/L, 292.1 mg/L and 259.4 mg/L in the same order. Their percentage mortalities were noted to be concentration dependent. In all the experiments, positive control (pylarvex) was used and showed 100% mortality at 100 mg/L while the negative control which was spring water with less than 1% Dimethylsulfoxide exhibited no larvicidal activity.

Table 1: Larvicidal activity and LC values associated with compounds 1, 2 and 3

Concentrations (mg/L)	**Percentage Mortality ± SD**		
	1	**2**	**3**
3.9	0.0±0.0	0.0±0.0	0.0±0.0
7.8	0.0±0.0	1.7±2.9	0.0±0.0
15.6	0.0±0.0	0.0±0.0	0.0±0.0
31.3	0.0±0.0	3.3±2.9	0.0±0.0
62.5	0.0±0.0	5.0±5.0	6.7±5.8
125.0	11.7±7.6	21.7±7.6	30.0±8.7
250.0	46.7±34.0	91.7±10.4	91.7±2.9
500.0	96.7±5.8	100.0±0.0	100.0±0.0
750.0	96.7±5.8	100.0±0.0	100.0±0.0
1000.0	100.0±0.0	-	-
Pylarvex (100 mg/L)[a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Spring water DMSO[b]	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
	LCvalues (mg/L)		
LC_{50} (mg/L)	245.5 (199.9-297.3)	147.6 (97.0-233.5)	144.4 (118.9-176.4)
LC_{90} (mg/L)	471.6 (379.0-662.5)	292.1 (195.1-870.9)	259.4 (206.4-389.2)

[a]Positive control, [b]Negative control.

The yellow essential oil of *F. angolensis* leaves had a yieldof 0.037% (w/w) and its density was determined to be 0.88 g/ml. It was evaluated for larvicidal activity against the third instar mosquito larvae (*An. gambiae)* andfound to be active. At a dosage of 500 mg/L, the leaf essential oil induced 100% larval mortality towards *An. gambiae* larvae within 24 hours. When the dosage was decreased to 250 mg/L, the larval mortalities of *An. gambiae* larvae against leaf essential oil were 76.7% and at a concentration of 3.9 ppm no larval mortalities were observed (table 2).The bioactivity of the *F. angolensis* oil was comparable with many essential oils reported recently as mosquito larvicides (Cavalcanti *et al*., 2004).

Table 2: Larvicidal activity of *F. angolensis* oils

Concentration (mg/L)	% Mortality ± SD	LC_{50} (mg/L)	LC_{90} (mg/L)
3.9	0.0±0.0		
7.8	3.3±5.8		
15.6	3.3±5.8		
31.3	13.3±10.4		
62.5	55.0±17.3	83.7 (63.4 -108.8)	324.0 (236.2 - 497.7)
125.0	55.0±18.0		
250.0	76.7±2.9		
400.0	90.0±13.2		
500.0	100.0±0.0		
650.0	100.0±0.0		
1000.0	100.0±0.0		
Pylarvex (100 ppm)[a]	100.0 ± 0.0		
Spring water + DMSO[b]	0.0 ± 0.0		

[a]Positive control, [b]Negative control.

The chemical composition of the essential oil was done using GC-MS. From the databases of the libraries used, only eight components were positively identified accounting for 2.64 % of the total oil. The remaining 67.83% were partially identified and 29.0% were classified as unknowns.Therefore, the oil contained mainly new compounds whose mass spectra could not be found in the GC – MS databases used. The isolated compounds and the oils on conclusion can be used in the development of natural mosquito larvicides.

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Room for plant derived pesticides in agroecological management of crop pests? Experience from some African cropping systems

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Abstract

Plant-derived pesticides are part of the toolbox of IPM and organic agriculture, as substitutes to synthetic chemical pesticides. On the other hand, for the very same reason (substitution rather than re-design), they are less in line with the agroecological approach.We however provide examples of the potential role of such plant-derived pesticides (with emphasis on extracts of neem and Jatropha), in a strategy of pest management via agroecological pathways.These encompass seed-dressing for black-beetle management on upland rice in Madagascar, and sprays for manageme nt of sorghum stem-borers in Cameroon, of sorghum panicle-feeding bugs in Mali and of cowpea pestsin Niger.The way the plant -derived pesticide-based tactics can be synergistic rather than conflicting with the agroecological pest management strategy is disc ussed mainly along two lines: i) Potential integration of botanical pesticide-producing plants in the cropping system (e.g. Jatropha live-hedges around vegetable market-gardens inthe Sahel)- ii) Pro's and con's of the use of such repellent, deterrent, and/or biocidal extracts in an "assisted" push-pull strategy.

Introduction

Plant-derived pesticides have been part of the toolbox of IPM and organic agriculture, as substitutes to synthetic chemical pesticides.It should however be noted that their plant orig in is not necessarily a guarantee of safety vis-à-vis humans and natural enemies. Nicotine from tobacco, and rotenone from derris roots, for instance,are highly toxic and non-selective (broad-spectrum) insecticides. Also, although more renewable than synthetic chemical pesticides, they are not necessarily in line with the agroecological approach, since they rely on "substitution" rather than cropping system re-design.We however provide examples drawn from African experiences, of the potential role of such plant-derived pesticides in a strategy of pest management via agroecological pathways. We thus studied the potential of neem (*Azadirachtaindica*) extracts on black beetles (Coleoptera: Scarabaeidae) damaging upland rice in Madagascar and on stem borers (Lepi doptera: Noctuidae) damaging sorghum in Cameroon, of extracts of neem and physic nut (*Jatrophacurcas*) on panicle-feeding bugs (Hemiptera: Miridae) damaging sorghum in Mali, and on a physic nut extract on the spectrum of insect pests damaging cowpea in Niger.

Literature Summary

The use of plants or plant extracts with pesticidal properties is a way of utilizing natural or cultivated vegetational diversity for regulation of crop pests, that has been used since the Antiquity in China, India, Egypt and Greece (Isman, 2006; Maia and Moore, 2011). Even in Europe, this use has expanded in the mid nineteenth century before being overthrown a century later by that of synthetic chemical pesticides. However, they have been subject to a renewed interest in the past quarter c entury, due to the growing societal awareness of the harmful effects of synthetic chemical pesticides on human and environmental health (Isman, 2006).Among the more than 2,000 recordedplant species with known pesticidal properties (Jacobson,1989), there are at present four major types of botanical products used for insect control(pyrethrum, rotenone, neem, and essential oils)(Isman, 2006).Azadirachtin and other terpenoids from plants of the Meliaceaefamily, particularly the neem tree *A.indica*, and to a lesser extent *M.azederach*, are among the most popular (Schmutterer, 1995; Carpinella *et al.*, 2003). Among the botanicalswhich have shown promise for wider use, are the extracts of the physic nut shrub *J.curcas* (Ratnadass and Wink, 2012).

Description of Research

Black-beetle management on upland rice with neem extract in Madagascar

In 2006–2007, we conducted at Ambohitsilaozana(Lake Alaotra region,Mid -East of Madagascar), a teston the B22 upland rice cultivar, in a randomized complete block design with four rep licates, to compare neonicotinoids (imidacloprid, clothianidin and thiametoxam) with an organic seed -dressing treatment.The imidacloprid and clothianidin formulationstested were Gaucho® T45WS (insecticide/fungicide:350 gkg^{-1}imidacloprid + 100 gkg^{-1}thiram)and Poncho® 600 FS (insecticide: 600 g L^{-1}clothianidin)respectively, while the thiamethoxamformulation tested was Cruiser® 350 FS (insecticide:350 g L^{-1}thiamethoxam). Locally purchasedCalthir® (fungicide, 800 gkg^{-1}thiram) was testedboth as a fungicide-only control and combinedwith clothianidin, while the fungicide tested incombination with thiamethoxam was Maxim®XL 035FS (25 g L^{-1}fludioxonil + 10 g L^{-1}mefenoxam(Metalaxyl -M)). International suppliers wereSyngenta -Switzerland for Cruiser® and Maxim®and Bayer CropScience, South Africa, for Gaucho®and Poncho®.The organic seed -dressing involved products supplied by Elvisem-Europe, Chiavari, Italy: SS3®,a natural elicitor of plant defences against fungal infection at 5 gkg^{-1}, Umisan TY10®, a neem–tree seedextract at 5 mlkg^{-1},and a drop (approximately 1 mlkg^{-1}) of Liquid Humus®, a fertilizer derived from fermented organic materials. On the 96 central hills of experimental plots, damage to rice by white grubs/black beetles was scored at tillering on a 1-5 scale, and paddy yield was measured at harvest (Ratnadass *et al.*, 2012).

Stem borer management on transplanted sorghum with neemextract in northern Cameroon

! trial was conducted infarmers' fields at two sites (Balaza and Djarengol) of the *Extrême-Nord* province of Cameroon in 2003-04. Sorghumcultivars were *Safraari* at Balaza and *Majeeri* at Djarengol,two of the most popular landraces of *muskuwaari*(transplanted recession cropped sorghum) cultivatedin Northern Cameroon.There were nine treatments, corresponding to two "levels" (based on the number of sprays) for the control (namely no spray and seven bi-monthly sprays with plainwell water from 30 to 105 days after transplanting: DAT), three levels for the synthetic neonicotinoid insecticide Acetamip rid (Matador®)(resp. one spray at transplanting; two sprays at 45 and 75 DAT; and the combination of both, each time at the dosage of 50 g ai ha^{-1}), and four levels for the neem-seed extract (the first two like those of Acetamiprid, the third one with two additional sprays at resp. 30 and 105 DAT, the fourth with two more sprays at 60 and 90 DAT, resulting in a total of seven sprays for the latter).Leaf application was performed using knapsack sprayers. For treatments with neem, kernels were ground to a fin e meal. The meal was soaked in water for 12 h (overnight) at the dose of 200 g L^{-1} and this was filtered before spraying. At harvest, the stems damaged by noctuid borers (mainly *Sesamiacretica*), namely those showing borer holes externally, and, after disse ction, stem tunneling, were separated before harvest (120 days after transplanting), which made it possible to calculate percentage damaged stems. Grain weight was taken in each plot and converted to grain yield per ha(Aboubakary *et al.*, 2008).

Sorghum panicle-feeding bug management with neem and physic nut extracts in Mali

In 1996 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Samanko, Mali located about20 km southwest of Bamako,six treatments were compared for the mana gement of sorghum panicle-feeding bugs (Hemiptera: Miridae), particularly *Eurystylusoldi*, on four sorghum cultivars (two susceptible and two resistant to panicle-feeding bugs), in two dates of sowing, in split-plot designs with three replications, namely:

- Neem and Jatropha aqueous extracts, obtained by filtration of 100 gL^{-1} of ground seeds and applied after one night of soaking, with a knapsack sprayer at the dose of 900 Lha^{-1};
- 2.4 Lha^{-1} of an EC formulation with a methanolic extract of physic nut with 30% Phorbol Ester content;
- a "blank" consisting in a treatment at 2/4 Lha^{-1} with only the adjuvants of the EC formulation, particularly methanol;
- an unprotected control (tap water);
- an EC formulation of deltamethrin (12.5 g aiha^{-1}).

All treatments consisted of three sprayings, at weekly intervals, starting at end of flowering.Observations weremade on head-bug visual damage score on a 1–9 scale(Ratnadass *et al.*, 2009).

Cowpea pest management with physic nut extract in Niger
The insecticidal properties of *J. curcas* oil were assessed against cowpea insect pests (leafhoppers, aphids, thrips and pod-sucking bugs) at the ICRISAT research station,Sadoré, Nigerlocated about 40 km southwest of Niamey. In 2002, four concentrations of physic nut oil extract, formulated as an emulsifiable concentrate (EC) (namely 2.5%, 5%, 7.5% and 10%), were evaluated as field sprays along with an untreated control (water spraying) and a conventional insecticide (DeltamethrinDecis® EC) treatment. In 2009, these latter two checks were evaluated alongside four concentrations of a "blank" formulation consisting of only the adjuvants of the 2002 EC formulation (namely 1.25%, 2.5%, 3.75% and 5%)(Katoune *et al.*, 2011).

Research Results and Application

Upland rice black-beetle management in Madagascar
Damage on seedlings from seeds treated organically (which involvedneem extract) was similar to those treated with imidacloprid and thiamethoxam, namely not significantly more or less damaged than those from seeds treated with clothianidin on the one hand, and both controls on the other.The organic treatment produced significantly better yield than both controls, and, although lower than all four insecticide treatments, this was statistically significant only for imidacloprid at 0.875 g/kg (Fig.1)(Ratnadass *et al.*, 2012).

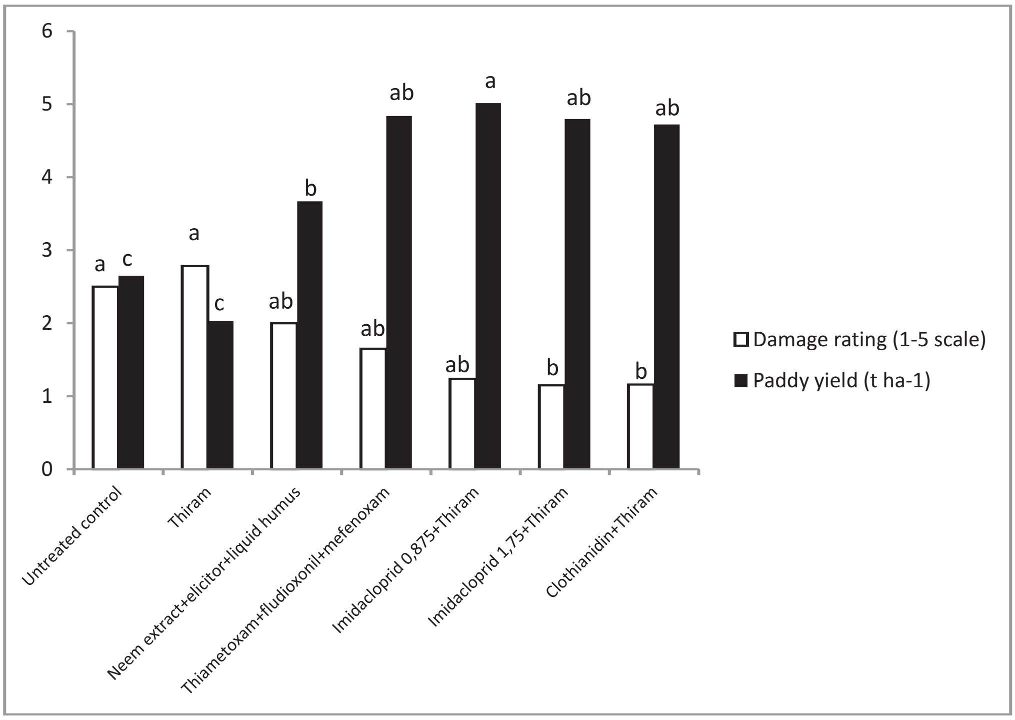

Figure 1: Effect of different seed dressing treatments on black-beetle damage to paddy rice and paddy yield (Ambohitsilaozana, Madagascar, 2006–2007)
Bars with the same letters are not significantly different at P<0.05, according toNemenyi test following Friedman test for damage rating, andNewman-Keuls test following F-test for paddy yield

Sorghum stem borer management in Cameroon
Under high infestation by *S. cretica* (average of 60% damaged stems at harvest on the controls), Acetamiprid with three foliar sprays at 50 g ai ha^{-1} per application, at transplanting, and at 45 and 75 DAT, or filtrate from ground neem kernels at 200 g L^{-1} in seven foliar sprays at transplanting, and at 30, 45, 60, 75, 90 and 105 DAT, considerably reduced losses caused by Sesamia stem borers. Thus acetamiprid, from level 2, and *a fortiori* level 3, gave better results than neem at levels 3 and 4, with a yield gain of less than 10% though. On the other hand, from level 2, neemtreatment did better (namely more than 50% yield gain) than the controls (Aboubakary *et al.*, 2008).

Sorghum panicle-feeding bug management in Mali

Jatropha oil application on sorghum panicles showed some effect on panicle-feeding bugs when damage level was high, better than Jatropha and neem aqueous extracts. However, it did not compete with pyrethroid protection level (Fig.2) (Ratnadass *et al.*, 2009).

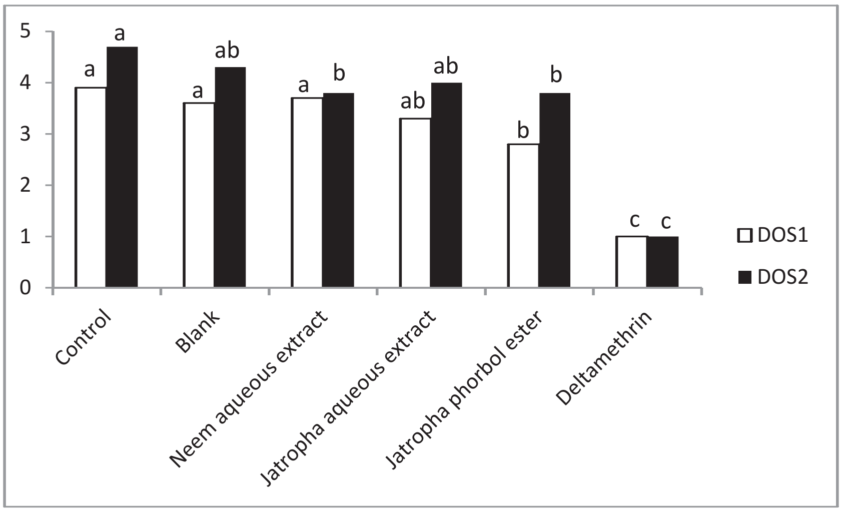

Figure 2: Effect of neem and Jatropha extract applicationson sorghum panicles on mirid bug damage ratings (1-9 scale) (Samanko, Mali, 1996, 2 dates of sowing: DOS)

Bars with the same letters are not significantly different according to LSD test at P <0.05

Cowpea Pest Management in Niger

In 2002, application of Deltamethrin and physic nut oil at 7.5% gave the highest seed yields, with more than 1000 kg ha^{-1}. Both treatments, alongside the one with 10% oil, sustained significantly lower thrips (*Megalurothripssjostedti*) infestation than the water-sprayed control. All oil extract treatments and the Deltamethrin treatment sustained significantly lower infestation by *Clavigrallatomentosicollis* bugs than the untreated control, with the lowest infestation occurring with 7.5% oil. Furthermore, correlations between oil concentration and thrips and bug infestation were negative and significant, while correlation between oil concentration and seed yield was not significant, due to a phytotoxic effect of oil at high concentrations . The follow-up studies in 2009 confirmed that effects of Jatropha oil on cowpea insect infestation and seed yield observed in 2002 could be ascribed to the physic nut oil fraction alone (Katouné *et al.*, 2011).

Discussion

Partial effects of neem and physic nut extracts on pests of several crops were thus highlighted. In the case of stem borers of transplanted sorghum in northern Cameroon, neem extract was as efficient as the synthetic insecticide. However, when used as mere substitutes to synthetic pesticides, rather than calling for agroecosystem re-design, plant-derived pesticides are not so much in line with the agroecological approach. Still, in an agroecological perspective, the physic nut (*J.curcas*) is exemplary because it can be planted as live fences/hedges for other purposes (such as protection of market gardens from damage by domestic animals, or for erosion control in fields of annual crops)(Kumar and Sharma, 2008).For instance, cowpea is a major crop in the Dryland Eco-Farm (DEF) system, and fences of Jatropha are used for fencing African Market Gardens (AMG), both systems being promoted by Icrisat in the Sahel (Ratnadass *et al.*, 2011).). In Niger, neem tree is also commonly grown as a windbreak around mango orchards/ On the other hand, in an “assisted push-pull” perspective, it could also be helpful to separate repellent from pesticidal *per se* (= biocidal) compounds in a single plant extract, since their use in combination might be counter-productive.For instance, an early infestation of the vegetative stages of the crop with little-damaging pests might be beneficial overall if it results in early colonization by generalist predators that may control later occurring and more damaging pests, e.g. fruitworms or fruitflies.However, the limitations of such an approach, due to the instability of individual components as compared to that of raw extracts, have also been discussed (Ratnadass and Wink, 2012).

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Effect of herbal plant extracts on *Trypanosoma brucei rhodesiense* infected mice

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Abstract

Water extracts of *Solanum nigrum* (SNE) were tested for their anti-inflammatory and hepatoprotective activity in *Trypanosoma brucei rhodesiense* infected mice. The results obtained indicate that SNE had a better anti-inflammatory activity than dexamethasone in addition to reducing the pathogenicity of the disease. The parasitaemia, PCV, albumin and bodyweights in SNE treated infected mice were dose dependent. *S.nigrum* is therefore a potential adjunct in prevention of trypanosome-induced liver damage. To the best of our knowledge this is the first study of the on the anti-inflammatory and hepatoprotective activity of *S. nigrum* in trypanosomosis.

Introduction

Sleeping sickness (Human African trypanosomosis) caused by the flagellated parasites *Trypanosoma b. rhodesiense* and *T. b. gambiense* and transmitted by tsetse flies of the *Glossinaspp* is responsible for many deaths in Africa. Available treatments for the disease have poor efficacy and safety outcomes especially in late stages of the disease with the parasite in the central nervous system. The pathology of this disease is mediated by host inflammatory responses. These responses cause tissue damage through production of reactive oxygen species and consequently increase the severity of the infection. Tissue inflammatory damage during trypanosomosis significantly affects the treatment and prognosis. *S. nigrum* has been shown to have antioxidant and anti-inflammatory activity. Considering the potential anti-inflammatory effects of *S. nigrum,* we evaluated the hepatoprotective activity of water extracts of *S. nigrum*and its effects on the pathogenesis in a mouse model of trypanosomosis and the consequent lengthening of survival time in *T. b. rhodesiense* infected mice.

Literature Summary

The WHO estimates of the number of people in Sub Saharan Africa at risk of Human African trypanosomosis (HAT) at 66 million (WHO, 2004). This is a serious challenge to the health sector and the society in Africa since trypanosomosis causes a lot of suffering and loss of the labour force. Although the disease has been under control in Kenya (Kagira *et al*., 2011), recent cases of infections among tourists visiting Maasai Mara have been reported (Wolf et al., 2012). The search for vaccines against trypanosomes has been elusive yet the drugs used to treat HAT are not ideal with long duration of treatment and severe side effects such as post treatment reactive encephalopathy observed in 10% of the patients treated with melarsoprol (Pepin and Milord, 1994). During disease progression several tissues are normally damaged including the liver, heart, kidneys and the gut due to enhanced host inflammatory responses stimulated by the endotoxin-like molecules produced by the trypanosomes (Ngure *et al*., 2009) which can be enhanced in the presence of divalent metal ions such as Fe from haemoglobin breakdown (Novo and Parola, 2008). Considering its crucial role of the liver in synthesis, regulation of biomolecules, detoxification and metabolism of drugs, damage to this organ greatly affects the outcome of the disease progression and treatment due to impaired drug metabolism and waste accumulation.Due to the toxicity of the synthetic anti-trypanosomal drugs it is important to reduce inflammation during trypanosomosis especially in the encephalitic syndrome by use of synthetic anti-inflammatory drugs such as dexamethasone and diazepam (Jennings, 1993). *S. nigrum,*has been shown to have antioxidant and anti-inflammatory activity in different studies including carbon tetrachloride ($CCl_{4)}$induced liver damage (Lin *et al.,* 2008)and thioacetamide induced fibrosis (Hsieh *et al.,* 2008) with the possible mechanism of action being interruption of the lipid peroxidation chain reaction thereby preventing depletion of endogenous antioxidants glutathione depletion (Umar *et al.,* 1999).

Description of Research

S. nigrum leaves were collected from Kapchuriai in Nandi County located in the Rift Valley of Kenya, cleaned with water, dried and the plant fluid extracted according to the method described by Lin *et al.,*(2008) after which the resulting extract was freeze dried. The toxicity of the extract was tested for a period of 10 days with various doses ranging from 250mg/kg to 2000mg/kg and the mice monitored closely throughout the 10 day period of toxicity study and a further 4 days without treatment for evident toxic responses. Male Swiss white mice were obtained from Kenya medical Research (KEMRI) small animal breeding unit and each mouse treated with Ivermectin® and acclimatized for a period of two weeks prior to infection. Trypanosome strain KETRI 2537 was propagated and maintained in healthy Swiss white mice a few days before the commencement of the experimental infections. The experimental mice were infected 10^4 trypanosomes intraperitoneallyand randomly divided into 6 groups of 6 mice per group, marked with picric acid, and each group of mice housed separately.The infected groups of mice were treated orally with decreasing concentrations of SNE of 10g/l (group 1), 6.7g/l (group 2) and3.3g/l (group 3). One group of the infected mice were also treated with 0.02mg of dexamethasone every two days (group 4) while another infected untreated (group 5) and an uninfected untreated group (group 6) acted as controls.The mice were maintained on commercial mice pellets(Unga Company, Kenya) and water provided *ad libitum* with or without the plant extracts. The mice were then monitored for parasitaemia, PCV, albumin and body weights through the experimental period. The liver tissues of experimental mice were also collected at intervals of 10 days and observed for gross pathology. The data obtained for albumin, parasitaemia levels, body weight and packed cell volume in the test mice treated with SNE or dexamethasone and the untreated control mice at each sampling time were presented as a mean ±

standard error of the mean (mean ± SEM). The Kaplan-Meier method was used for determination of survival distribution functions of the experimental and control mice. The data was analysed using the GenStat® statistical programme and the significance of difference between means determined by ANOVA and were considered significantly different at $p < 0.05$.

Research Results and Application

The prepatent period of the parasite ranged from 3-4 days with the first peak of parasitaemia of 1.3 x 10^8 trypanosomes/ml observed by 6 dpi in all the groups of mice except in those treated with 10g/l where there was a peak parasitaemia of 1.3 x 10^8 trypanosomes/ml recorded by18dpi (Figure 1).This showed that although the extract did not clear the parasitaemia it reduced the levels of parasitaemia observed dose dependently. There was also dose dependent decline in the PCV levels with the group infected and treated with the highest dose of SNE showing a slower decline in PCV and the infected untreated group showing the highest decline in PCV levels. The body weights as a general measure of the health state of the mice showed that the mice treated with higher concentrations of the extract had slight decline in body weights. The infected untreated mice showed gradual decline in body weights after the peak of the first parasitaemia on 9 dpi till death of all the mice. The degree of anaemia in trypanosomosis is proportional to the severity of the disease in the absence of other factors causing anaemia (Murray *et al.*, 1990). Analysis of the data between control and experimental mice showed that the extract reduced the levels of anaemia in the infected mice as depicted by the PCV levels. This could be attributed to the polyphenols present in the extract which react with free radicals (Harborne and Williams, 2000) thus preventing the peroxidation of erythrocytes andhave been known to have anti-trypanosomal activity against *T. cruzi*(Chataing *et al.*, 1998).

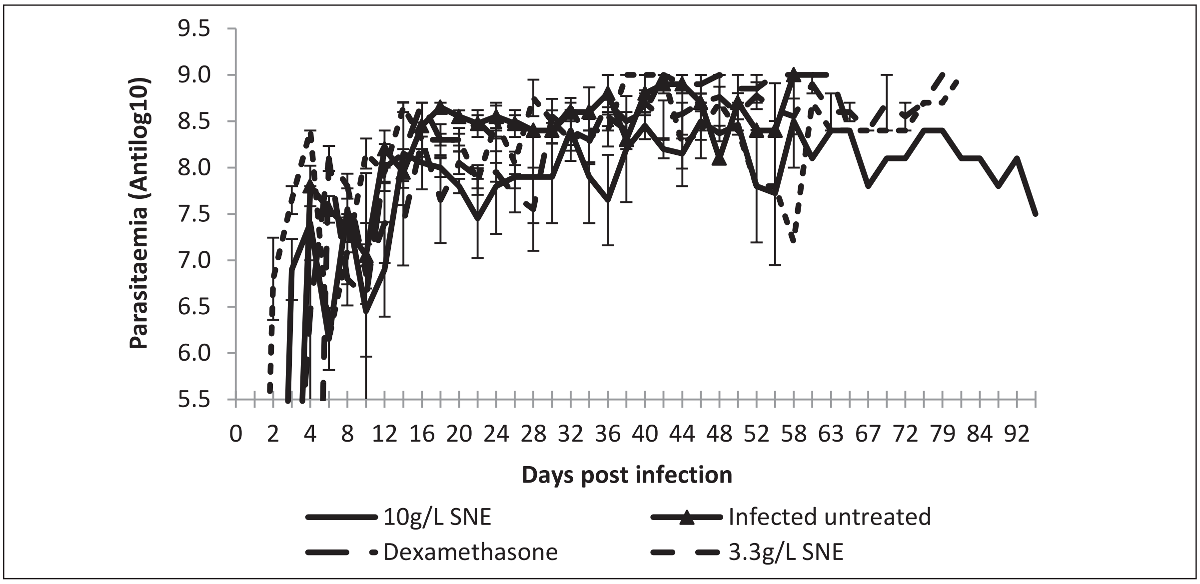

Figure 1: Mean parasitaemia levels (antilog$_{10}$ ± S.E.M) of *T. b. rhodesiense* infected male mice untreated or treated with either SNE or dexamethasone

There was also dose dependent decrease in the levels of the negative acute phase protein albumin (Figure 2). There was a significant difference between the levels of albumin amongst different treatments groups ($p < 0.05$) and day of sampling ($p < 0.05$). By 42 dpi there was a significant difference in albumin concentration in all the infected mice and between the treatment groups with the decline in albumin concentrations in dexamethasone treated mice being generally higher compared to those treated with SNE. However there was no significant difference in the albumin concentrations of dexamethasone and 3.3g/l SNE treated mice by 42 dpi.

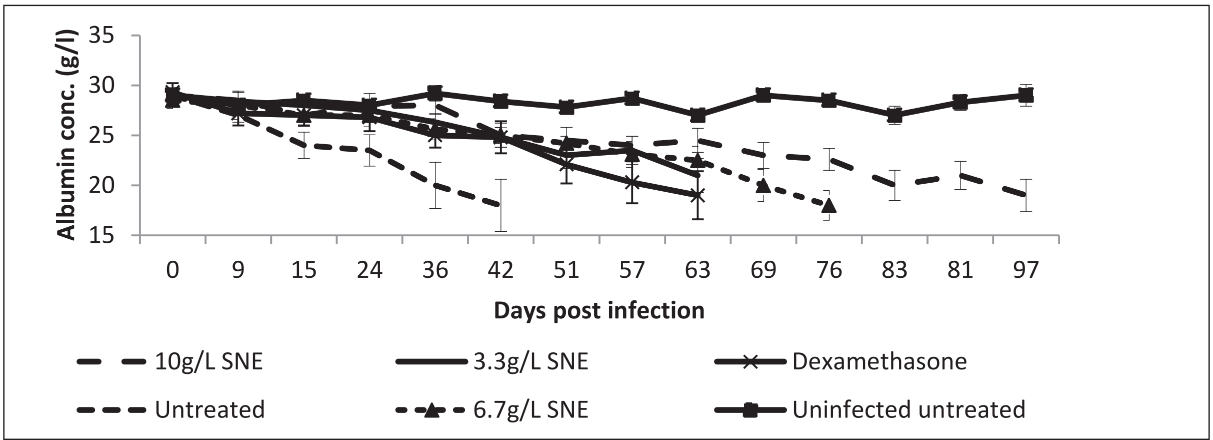

Figure 2: Changes in albumin concentrations of uninfected and *T. b. rhodesiense* infected mice untreated or treated with different concent rations of SNE or dexamethasone

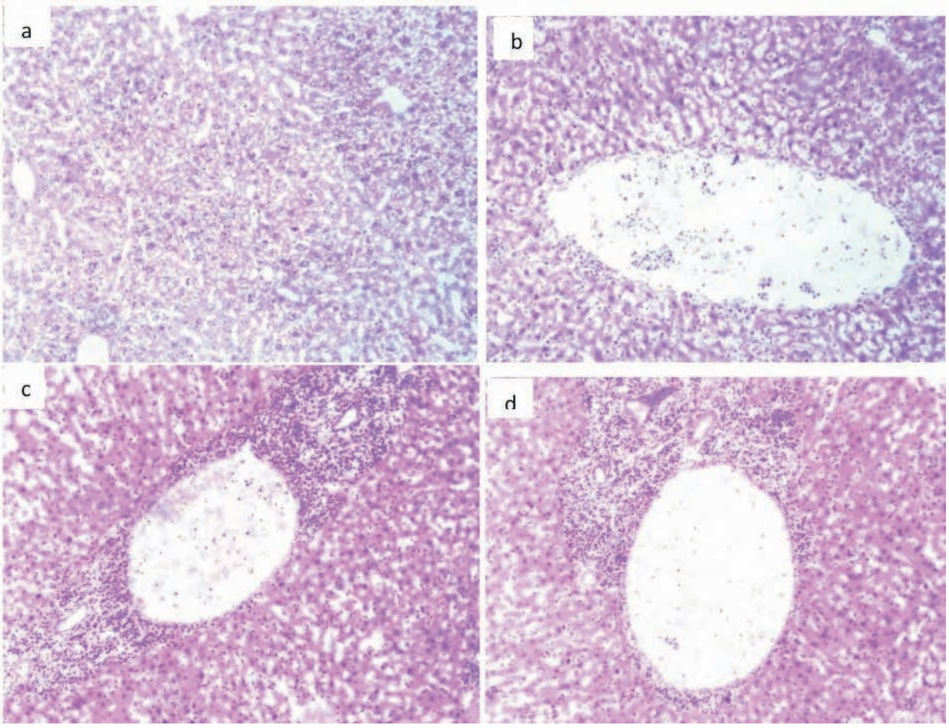

Figure 3: Liver tissue of uninfected untreated control mouse; b) Liver tissue of infe cted SNE treated mouse showing minimal infiltration on 50 dpi; c) Liver tissue of infected dexamethasone treated mouse showing massive infiltration of macrophages on 50 dpi. d) Liver tissue of infected untreated mouse showing massive infiltration (Haematox ylin and Eosin Mag =×200).

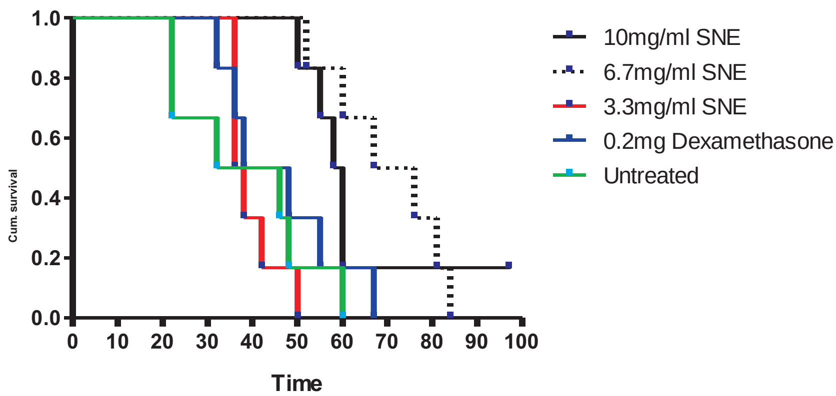

Figure 4: Survival distribution function of *T. b. rhodesiense* infected male mice treated with different concentrations of SNE or dexamethasone and infected untreated controls

The treatment of the trypanosome infected mice led to decreased liver pathology. This was mediated by decreasing the level of inflammation as observed by higher albumin levels in the mice treated with higher concentrations of SNE. The concentrations of albumin decreased during inflammation because of decreased synthesis (Limdi and Hyde, 2003) in addition to acting as buffer in sustained anti-inflammatory responses such as trypanosomosis due to the presence of a free thiol group (Pupim *et al*., 2004). The treatment of the mice with SNE therefore reduced inflammation and thus prevented the peroxidation of the host tissues resulting in reduced liver pathology compared to the untreated groups. Dexamethasone which is an anti-inflammatory drug was used as a control for inflammation and the results show that SNE at 10g/L had significant anti-inflammatory activity than dexamethasone. The decreased inflammation observed in SNE treated mice led to decreased pathology in the infected mice and the resultant increase in survival time for the SNE treated mice as shown in figure 4.In conclusion this study showed that water extracts of *S. nigrum*modulated the pathogenesis of trypanosomosis in mice with more enhanced anti-inflammatory activity than dexamethasone and is a candidate for use in prevention of trypanosome-induced liver damage. Further studies should be conducted to determine the anti-inflammatory effects of extracts from *S. nigrum* in controlling the post treatment encephalopathy caused by melarsoprol during treatment of second stage of HAT.To the best of our knowledge this is the first study of the anti-inflammatory and hepatoprotective activity of *S. nigrum* in trypanosomosis.

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Symposium 4 Biospesticides Technologies Session

Biopesticides and their commercialisation in Africa

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Abstract

The road to the widespread use of biopesticides is a long and complex journey. Many promising biopesticide candidates never reach the final stage when they are commercialised and adopted legally for use by the farmer. The Real IPM Company (K) Ltd produces and commercialises biopesticides in different countries in Africa. The aim of the paper is to highlight the challenges and country differences of commercialising biopesticides in various parts of Africa. Unlike Europe where the process of crop protection agent registration has been harmonised across the EU, most African countries currently have national approval schemes. However there are moves to develop regional registration systems. Examples will be taken from a range of countries and regions including West Africa, and East Africa. In any crop protection product two key crite ria that should be assessed are a) is the product safe to the operator, the consumer and the environment, and b) is the product effective. When commercialising crop protection products including biopesticides there is a legal requirement to obtain regulat ory approval for their use. The process of regulatory approval will be described and examples drawn from different countries. In addition the challenges that biopesticides face in obtaining regulatory approval will be discussed. The paper will conclude with highlighting the key stages and decision processes involved in commercialising biopesticides and give pointers on how to successfully commercialise a biopesticide.

Introduction

The main method of pest and disease control has been through the use of conventional synthetic pesticides over many decades. The use of these pesticides has served the world and their benefits are well reviewed by Cooper and Dobson (2007). However they have drawbacks including development of pest and disease resistance and the safety of the operator, consumer and environment. As a consequence scientists, input suppliers and farmers have looked for alternative control strategies which included Integrated Pest Management (IPM). For example entomologists were the first to observe that a chemical treatment against one pest could unbalance the population regulation occurring within trophic chains and cause outbreaks of new pests. They proposed to manipulate these regulations in order to restore the natural biological control of potential pest populations, instead of directly destroying them. In 1978, the catastrophic outbreak of the brown rice plant hopper in Indonesia demonstrated the unsustainability of in tensive systems exclusively relying on chemical control. This event led FAO to promote IPM. This concept stresses the need to use a wide array of convergent control methods and recognizes the importance of the choice of cultivar and fertilisation rate to prevent build-up of pest populations. (Labussière *et al*, 2010)

What are Biopesticides?

A major component of IPM has been the development and use of biopesticides. Biopesticides come in different categories being derived from natural materials, primar ily microbes but also originally from animals and plants. They fall into four main types:

Microbial Pesticides

Microbial pesticides containing bacterium, fungi, virus or protozoan as active ingredients. These can control many different kinds of pests, a lthough each separate active ingredient is relatively specific for its target pest(s). For example there are fungi that kill specific species of weeds and other fungi that kill insects. The most widely used microbial pesticides are sub species and strain s of Bacillus thuringiensis, or Bt. Each strain of this bacterium produces a different mix of toxic proteins and specifically kills one or a relatively few related species of insect larvae. Some Bts kill moth larvae whilst others are specific for flies a nd mosquitoes.

Biochemical Pesticides

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest . Biochemical pesticides include substances like sex pheromones that interfere with mating as well as various scented plant extracts that attract insects to traps.

Plant extracts

There is a large spectrum of plant extracts i.e. unprocessed extracts representing a "cluster of substances" or highly refined products containing one active substance. In addition the risk associated with the use of plant extracts may vary between low and very high risk, depending upon the toxicityof the chemical components of the extract.

Plant incorporated protectants

Where genetically modified plant material is allowed, such as the USA, then Plant Incorporated Protectants (PIPs) are considered Biopesticides. PIPs are pesticidal substances that plants produce from genetic material that has been added to the plant. For instance a gene for the Btpesticidal protein can be introduced into the plant's own genetic material/ The plant, instead of the Bt bacterium manufactures the substance that destroys the pest.

The advantages of Biopesticides are they usually are:

- inherently less toxic that conventional pesticides,
- specific to the target and closely related organisms in contrast to broad spectrum conventional pesticides,
- active in small quantities and often decompose resulting in lower exposure and pollution,
- compatible with IPM programmes.

Biopesticide registration and approval for use

Biocontrol may be a good alternative to pesticide use. Like all other products used to control pests and diseases, they need to undergo a comprehensive risk assessment. Although the procedure has been adapted to better meet the characteristics of micro-organisms, the registration is based on rules originally developed for synthetic pesticides. These strict rules are often considered as an economic barrier to the use of alternative pest and disease control solutions (IBMA, 2005).

Retailers are perceived as major drivers of European agri-food systems. Relying on certification schemes, not only are they determining price and food quality attributes, but they started, in recent years, to implement private agri-food standards that are also related to the environment or animal welfare. A reduced use of plant protection products meets consumer demand, but some of these standards go beyond the scope of existing regulations by forbidding the use of some authorised molecules and requiring the use of biocontrol tools. This can be challenging for farmers, which are often requested to have zero-residues in their products, as well as no damages at all (Endure, 2012).

The International Biocontrol Manufacturers Association (IBMA) outline their Basic principles on the Regulation of Biologicals and their position is that the registration of Biological cannot be extrapolated and considered similar to the regulation of chemical pesticides, since their nature, properties and characteristics, their mode of action, their dose and use differ fundamentally from conventional synthetic pesticides (IBMA, 2005).

In Africa two countries have published specific biopesticide procedures, these being Ghana (EPA 2012) and Kenya (PCPB, 2012). However the issues still remain a challenge for organisation wishing to register their Biopesticides, these being:

- Every country is different in their requirements
- There are differing degrees of transparency
- Clarification is need on what is required,
- How the regulations are interpreted and
- The need for consistency in risk assessment.

Commercialisation – key factors

In the commercialisation of any product, whether a biopesticide or not there are some key factors that must be considered. For Biopesticides these include:

- The size of the market for the product. Which crops, what is the area grown, and what are the target pests.
- The cost of bringing the product to market. This includes the costs of registration and marketing.
- Identification of the competitive products and their position in the market place.
- What are the drivers for the farmer to adopt the biopesticide.
- What should be the price, especially important for the small scale farmer.
- What is the efficacy of the product.
- Availability, distribution and shelf life constraints
- Compatibility in existing crop protection programmes
- Can the product be reliably produced and maintained at a consistent quality and
- Finally – what will our return on investment be?

Conclusions

In the commercialisation of biopesticides, the regulations and approval is a major consideration. Understanding and knowledge of this procedure is essential. Also all concerned must be confident that the products are safe and efficient. For biopesticides to be sustainable they must be commercial viable. We believe in them but we must get the end user to repeatedly use them and value their use. Finally crop protection is an always changing and dynamic situation and the development of biopesticides as commercial products needs to be flexible, rapid and responsive.

Seed treatment with a binary pesticide and aqueous extract of *Eclipta alba* (L.) Hassk for improving sorghum yield in Burkina Faso

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Abstract

Seed health testing and field trials were carried out in Burkina Faso, to evaluate the effects of seed treatment with aqueous extract of *Eclipta alba* and a binary pesticide on seed-borne fungi, seedling emergence and yield of sorghum. *E. alba* extract and the pesticide caused significant inhibition of *Leptosphaeria sacchari in vitro* and a stimulatory effect on seedling emergence and yield increases of 7 to 38% in field. In this paper, results on the first large scale field testing of *E. alba* in seed treatment of sorghum and the first direct comparison with a thiram-based pesticide and seed priming are discussed.

Introduction

In West Africa, the *Fusarium*, *Curvularia* and *Phoma* are among the fungi most common mycoflora on sorghum seeds (Zida *et al*., 2008a). These pathogens are transmitted and disseminated by seeds. They affect seed germination, cause diseases in seedlings and plants and produce mycotoxins that pose health risks to humans and animals. In farm saved-seeds from Burkina Faso, *Leptosphaeria sacchari* (syn. *Phoma sorghina*) infection often exceeds 30% and this fungus is one of several species having the ability to cause grain mould and seed rot in sorghum (Girish *et al*., 2004a).The use of fungicides is an effective strategy to control seed-borne fungal diseases in crops. Treatment of sorghum seeds with both fungicide and insecticide is in generally recommended (FAO, 2011). In Western Africa, a binary pesticide, Calthio C, containing the fungicide, thiram, and the insecticide, chlorpyrifos-ethyl, is widely available for seed treatment as dry powder. But, the resource poor African sorghum farmers usually find it difficult to apply commercial pesticides. Hazardous effects on the environment and induction of genetic resistance due to a repeated use of synthetic pesticides are other major problems associated with the use of such chemicals. In many instances, botanical extracts are reported as effective and cheaper alternatives to chemicals in combating insect pests and fungal diseases in plants (Tegene and Pretorious, 2007; Zida *et al*., 2008a). Recently, a significant reduction of seed infection by *Phoma* sp. and a positive impact on sorghum grain yield were observed by applying an aqueous extract of a local plant, *E. alba*, for seed treatment in a small-scale field trial (Zida *et al*., 2008b). The use of *E. alba* in seed treatment represents a low risk activity compared to the use of synthetic pesticides. In addition, this plant has the potential to provide a low-cost alternative to synthetic fungicides. The objective of this study is to evaluate the potential of applying *E. alba* extract or the chemical Calthio C to combat fungal infection in seeds of sorghum and to obtain a substantial yield increase in a large scale field study.

Description of Research

Bioassays were carried out at Kamboinse Research Centre (Ouagadougou) and field experiments in two agro ecological zones of Burkina Faso, West Africa. Nine farm saved-seed samples of sorghum, naturally infected by fungal pathogens (*Curvularia*, *Fusarium*, *Phoma*) were used in 2009 for testing dose response of seed treatment with *E. alba* extract. Two large seed samples were used in 2010 and 2011 for field trials. In 2011, five other seed samples were used in separate experiments. The plant extract was obtained from leaves and stems of *E. alba*. The plant material collected was air-dried and ground. The powder was mixed with distilled water at 6.25-25% concentrations, on a W/V basis, and incubated at 25-30°C for 20 h. Aqueous extracts were obtained by filtering the mixtures with a piece of cloth (hand filtering). Seed treatment with *E. alba* extract consisted of soaking seeds in the extract for 10 to 20 h. The soaked seeds were dried for 20 h before use.

Before initiation of field trials, in 2009, nine seed samples treated with *E. alba* extract at different concentrations (6.25%, 12.5% and 25%), seeds treated with Calthio C (4 g kg^{-1} of seeds) and non-treated seeds were tested for health, using the standard blotter method (Mathur and Kongsdal, 2003). For each of the nine seed samples used and for each treatment, 200 seeds were germinated on moistened blotters in Petri -dishes and incubated for 7 days at 22°C under alternating cycles of 12 h near-ultraviolet light and 12 h darkness. Incubated seeds were examined individually in stereo and compound microscopes and the number of seeds infected by *L. sacchari* was recorded. Seeds were also tested for emergence and grain yield in a small scale trial. Seeds were sown in six fields in 6 m-long rows at distances of 0.80 m between rows and 0.40 m between holes in the same row. At 15 days after sowing, the number holes with emerging seedlings were counted. At harvest, grain weight was recorded two weeks after drying.

In 2010 and 2011, seedling emergence and grain yield were evaluated in three large scale trials. Treatments included: soaking seeds in *E. alba* extract (10 and 12.5% concentrations), soaking seeds in water, dusting seeds with Calthio C (4 g kg^{-1} of seeds) and no treatment/ Twenty three (23), 17 and 13 farmers' fields were included in the three trials, respectively. Data on emergence and yield were recorded as described above.

Research Results and Application

In non-treated seeds, the average infection level of 40% was found for *L. sacchari* and a significant and dose dependent inhibition of this fungus was observed for the *E. alba* extract (Fig. 1A). For the 12.5% *E. alba* extract, fungal inhibition close to 80% was observed ($p < 0.001$). However, stronger fungal inhibition (97%) was observed for treatment with Calthio C ($p < 0.001$). With respect to crop yield, average yields obtained varied from 749 to 1082 kg ha^{-1} (Fig. 1B). Overall increases in ranged from 17% (*E. alba* high dose) to 44% (Calthio C). The highest concentration of *E. alba* extract (25%) appeared to have an unfavorable effect on yield compared to the lower doses. However, the results obtained for both antifungal effect and yield prompted us to test concentrations of *E. alba* extract close to 10% (W/V) in the subsequent field trials.

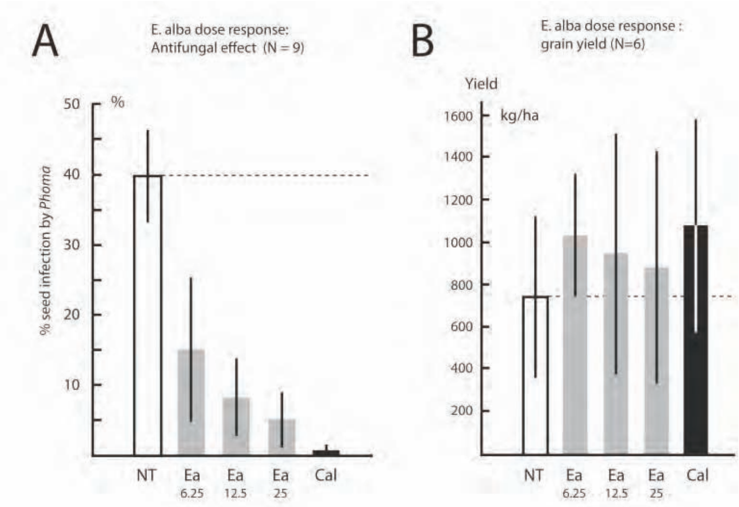

Figure 1: Effects of seed treatment with three concentrations of *E. alba* extract (6.25% ; 12.5% ; 25%) and a soaking time of 20h tested against non-treated seeds (NT) and Calthio C (Cal). A) Antifungal effect. B) Grain yield of Sorghum obtained in six farmers fields. Small bars indicate 95% confidence interval of mean

In all trials, crop emergence rates were higher on *E. alba* extract treated seed (77 to 83%) and Calthio C (82 to 97%) than seeds treated with pure water or no treatment (71 to 77%) (Fig. 2). Thiram-based fungicides have been reported to increase germination in sorghum seeds and yield increases up to 30% were reported (Girish *et al*., 2004b). However, this is the first report on an extension of these findings to a large-scale field testing, comparing a more specific control (water treatment) and a relevant pesticide containing the antifungal compound, thiram.

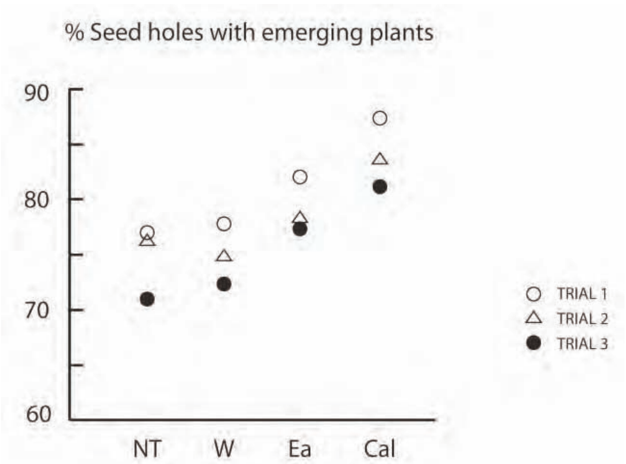

Figure 2: Crop emergence in field trials in Burkina Faso shown for non-treated seeds (NT), water (W), *E. alba* extract (Ea) and Calthio C (Cal) treatments

With respect to effect on crop yield, the lowest yields were obtained for non-treated seeds and those treated with distilled water and the highest yields for seeds treated with *E. alba* extract and Calthio C (Table 1; Fig. 3). Seed treatments with *E. alba* extract and Calthio C significantly ($p < 0.01$) increased yield compared to non-treated seeds. Normalized yields (relative to non-treated seeds) were 117% for *E. alba* and 125% for Calthio C (Table 1). Significant differences were also found between *E. alba* and water and between Calthio C and water. Antifungal effect of plants and positive effects on yield and/or germination following application of botanical extracts have been demonstrated earlier in sorghum (Tegene and Pretorious, 2007; Raghavendra *et al*., 2007; Wulff *et al*., 2012). Consistent and significant improvement of the emergence rate and yield was observed for both pesticide and plant extract, when tested in two different growing seasons. With regard to antifungal effects and yield increases, Calthio C appeared to be the most promising fungal inhibitor and also the most efficient promoter of yield.

Table 1: Levels of infection of seed samples and normalized yield of sorghum recorded in three large scale trials in 2010 and 2011

TRIAL	Fields (N), year	Experimental conditions	% Fungal infection (non-treated seeds)			Normalized Yield (%[N])			
			Phoma	Curv.	Fus.	NT	W	Ea	Cal
1	N=23, 2010	Ea 12.5% 20h soaking	17	42	7	100[a]	98[a]	122[b]	130[b]
2	N=17, 2011	Ea 12.5% 10h soaking	25	38	37	100[a]	111[ab]	116[b]	138[c]
3*	N=13, 2011	Ea 10% 10h soaking	38	8	24	100[ab]	87[b]	113[a]	107[a]
Mean			26	29	23	100	99	117	125

Ea = *Eclipta alba*; NT = non-treated seeds; W = soaking seeds in water; Cal = dusting seeds with pesticide (4 g kg^{-1} of seeds).
*For this trial figures of fungal infection are the average of five seed samples used.
Means on the same row followed by the same letter are not significantly different, using *t*-test at 5% level.

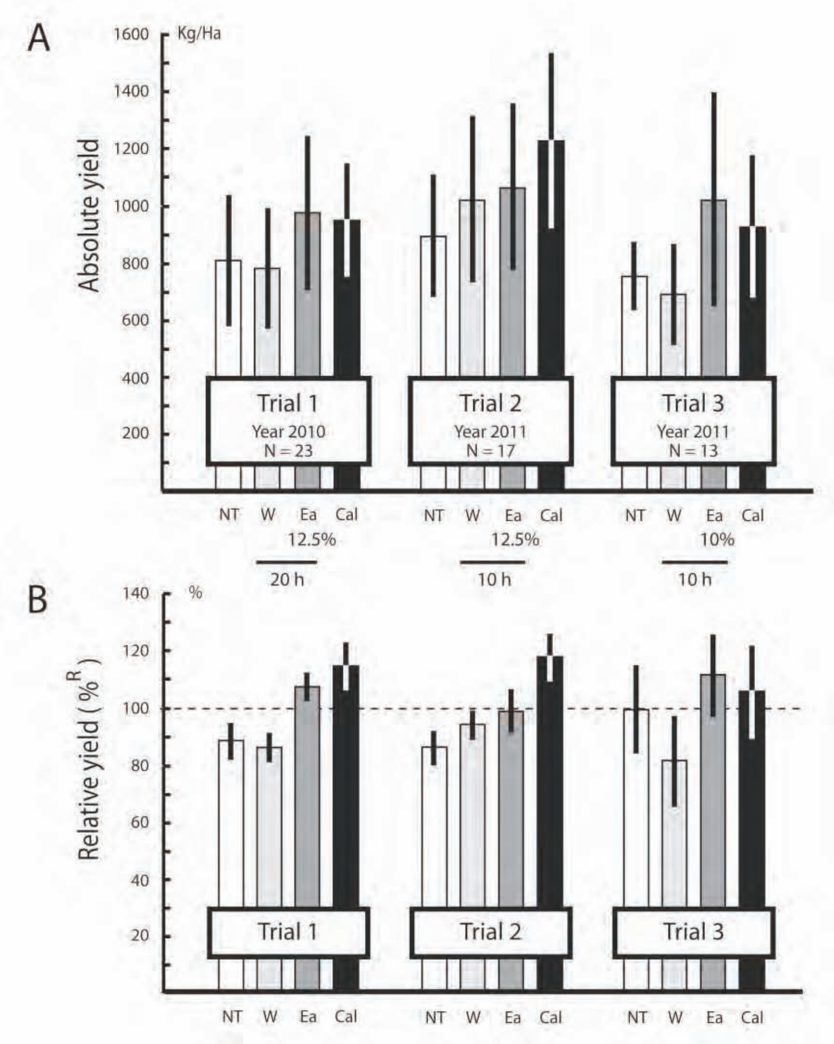

Figure 3: Effect of four seed treatments on grain yield. In all trials non-treated seeds (NT) were compared to seeds treated with pure water (W), *E. alba* extract (Ea) and Calthio C (Cal). A) Absolute yield (kg/ha). B) Relative yield (%[R]). Small bars indicate 95% confidence interval of mean.

The use of an aqueous plant extract implies exposure of seeds to water and several previous studies reported significant yield increases (> 20%) following seed priming, using a short exposure time (8-10 h). In the present study, an increase of yield (+11% normalized yield) was observed in one of the two trials employing the short soaking time (10 h), whereas a marked decrease of yield (-13% normalized yield) was observed in the other. Differences between the seed material used in different trials seems a plausible explanation of this inconsistency. Nevertheless, the effect of plant extract on yield and emergence cannot be explained as a simple exposure of seeds to water (seed priming) and a dose dependent anti fungal activity was demonstrated against *L. sacchari*, commonly found on seeds of sorghum in Burkina Faso. It emerges from the present study that the potential of antifungal seed treatment in Sahel exists and a simple aqueous extract of a local plant, *E. alba*, is capable of increasing sorghum yields when tested on a larger number of fields and when applied to farm-saved seeds carrying a natural inoculum of mycoflora.

Acknowledgments

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Symposium 6 Propagation and conservation session

Prospects for a rapid *in vitro* regeneration system for propagation of the pesticidal tree *Melia volkensii*, Gurke

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Abstract

An *in vitro* system for regeneration of *Melia volkensii* Gurke shoots is described. *M. volkensii,* a drought-tolerant tree native to East Africa, has under-utilized potential as a source of botanical pesticides. Extracts of its fruits and seeds have larvicidal, growth retarding and anti-feedant effects on mosquito larvae and locusts. The species has been over-exploited for its timber. Production of adequate planting material for replenishment of declining stock is constrained by difficulties in propagation via seed and cuttings. The objective of this study was to develop an *in vitro* culture protocol for rapid and efficient shoot regeneration in two ecotypes of *M. volkensii.* Mature zygotic embryos were aseptically cultured on Gamborg *et al*., (yr)'s B5 medium containing the plant growth regulator Thidiazuron. High frequency callus induction and regeneration were achieved. Callus subcultured to hormone-free ½ MS or B5 medium formed multiple somatic embryos which grew into micro shoots. ½ MS was superior to B5 medium for induction of somatic embryos. Thidiazuron concentration had no significant effect on callus induction but significant effects were observed on fresh mass of callus and somatic embryo induction ($F_{test,}$ $p < 0.001$). Microshoots elongated well on B5 medium containing 0.1 mg/l Benzylaminopurine plus 5 or 10% (v/v) coconut water. Frequent multiple shoot induction, with 4 to 12 shoots per initial shoot, was also observed on the elongation medium. This protocol produced phenotypically normal shoots of height ≥5/0 cm in 3/5 months/ Further work is in progress to attain rooting of the shoots. This study offers a simple protocol that could be optimized for exploitation in large scale *in vitro* cloning of *M. volkensii* mother trees with elite genotypes and phenotypes.

Key words: *In vitro*, regeneration, *Melia volkensii*, Thidiazuron.

Introduction

Melia volkensii Gurke (Meliaceae; mahogany family) is an important multipurpose tree native to the arid and semi arid lands of Kenya, Ethiopia, Somalia and Tanzania (Stewart and Blomley, 1994). It has received considerable attention in the search for botanical pesticides owing to the presence of limonoid triterpenes (Arnason *et al.,* 1987; Champaigne *et al.,* 1992). However, commercial exploitation of these botanical pecticides is yet to be realized. The wider adoption and commercialization of botanical pesticides has been limited by three drawbacks: low sustainability of source material, difficulties in standardization of botanical extracts and lack of regulatory approvals (Isman, 1997; 2006). For production of botanical pesticides on a commercial scale, the source plant biomass must be obtainable on an agricultural scale, which requires that the plant be either abundant in nature or amenable to cultivation (Isman, 2006). Natural variations in the chemistry of the active principles across geographical zones, genotypes and seasons also makes the standardization of the extracts difficult (Isman, 2006). These sustainability and standardization problems could be addressed through *in vitro* mass propagation. An efficient *in vitro* regeneration system could increase the availability of planting material and the uniformity of extracts by allowing mass cloning of elite trees. Natural populations of *M*. *volkensii* have been over exploited for supply of valuable mahogany timber, wood fuel and termite resistant poles (Runo *et al.,* 2004). Currently, large-scale cultivation of *M. volkensii* is severely constrained by lack of adequate planting material due to difficulties in seed extraction, a complex mechanical seed dormancy and high post germination mortality (Indieka *et al.,* 2007). Vegetative propagation is also difficult due to poor rooting of cuttings (Stewart and Blomley, 1994). Tissue culture may offer the solution to these problems. The objective of the present study was to determine the TDZ-mediated *in vitro* culture responses of *M. volkensii* zygotic embryo explants from two agro-climatic zones of Eastern Kenya, as a prelude to a larger study on the effect of ecotype on regeneration in this species.

Literature Summary

Melia volkensii fruit and seed extracts contain several anti-insect limonoids including volkensin, meliavolkenin and salannin (Isman, 2006; Akhtar *et al.,* 2008). The fruit extract of *M. volkensii* is toxic to a broad range of insects (Mwangi and Rembold, 1988; Akhtar and Isman, 2004). Seed extracts have potent antifeedant and growth inhibitory properties against the army worm, locusts, larvae of cabbage looper and mosquito larvae. (Rajab *et al.,* 1988; Mwangi and Rembold, 1988; Kabaru and Mwangi, 2002; Akhtar and Isman, 2004; Akhtar *et al.,* 2008). There is a dearth of studies on *in vitro* culture of *M. volkensii.* To our knowledge, the only published works in this area are by Indieka *et al.,* (2007) and the authors of this paper (Mulanda *et al.,* 2012). Indieka *et al.,* (2007) obtained plant regeneration from cotyledon explants via direct somatic embryogenesis using Murashige and Skoog (MS) (1962) medium supplemented with combinations of the cytokinin 6-Benzylaminopurine (BAP) and the auxin 2,4-Dichloro- phenoxyacteic acid (2,4-D). However, the shoot regeneration and rooting frequency attained were suboptimal for large-scale production of planting material. Mulanda *et al.,* (2012) reported high frequency plant regeneration (up to 96.67%) from mature zygotic embryo explants on Gamborg *et al*/'s (1968) B5 medium supplemented with 0/05 to 4 mg/l of low-cost agrochemical Thidiazuron (Phenyl-1,2,3-thidiazol-5-ylurea; or simply TDZ), as the sole plant growth regulator. However, both studies relied on plant materials from a single agro-climatic zone in eastern Kenya hence the need for a study of the effect of ecotype.

Description of Research

Seeds were obtained from mature fruits collected from wild trees in two agro-climatic regions of eastern Kenya, Mavuria provenance in Mbeere District and Nguutani provenance in Mwingi West District. Zygotic embryos were surface sterilized for 15 minutes in 10% Jik® commercial bleach with 2 drops of Teepol® detergent added as a surfactant and rinsed with sterile water. Callus was induced on Gamborg *et al*'s (1968) B5 salts supplemented with Murashige and Skoog (1962) MS vitamins and organics, 20 g/l sucrose and 12 g/l Oxoid® agar. TDZ was added at concentrations of 0, 0.05, 0.125, 0.25, 0.5, 1, 2 and 4 mg/l and pH adjusted to 5.80 ± 0.2. Media was dispensed into culture bottles and autoclaved for 20 minutes at 1.06 kg cm^{-2} steam pressure (121^{o} C). Five zygotic embryos were placed in each bottle, with three replicate bottles for each concentration of TDZ and ecotype, in a complete randomized design. The experiment was repeated three times. Callused zygotic embryos were subcultured to two types of hormone-free medium, ½ MS and B5, for induction of somatic embryos and microshoots, then to ½ MS plus 0.1 mg/l BAP ± 5 or 10% (v/v) coconut water for shoot elongation. Incubation temperatures were 29.8 ± 0.8 and 25.5 ± 0.1 ^{o}C max/min respectively (mean ± S.E). Other conditions were fluorescent light ~ 60 µmol photons m^{-2} s^{-1} and 16 hours light: 8 hours dark photoperiod. Images were taken using a Keyence® (Z35) VHX Digital Scanning Microscope and a Sony digital camera (Model DSC-W390). Data were subjected to one-way Anova using SPSS version 17.0.

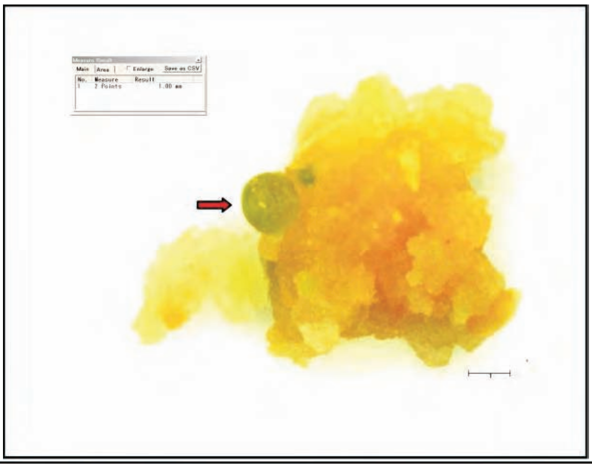
Research Results and Application

A high frequency of callus induction was observed within 4 to 10 days in all TDZ concentrations and on hormone-free medium. TDZ concentration had no significant effect on percent callus induction in both ecotypes. Endogenous plant growth regulator (PGR) levels in the zygotic embryos may be sufficient for callus induction/ TDZ concentration had significant effect on fresh mass of callus in both ecotypes (Table 1)/ Tukey's HSD located the significance at 0.05 mg/l TDZ. It appears very low TDZ concentration is favorable for callogenesis as reported in other plant species (Guo *et al.,* 2011). Callus masses were significantly larger in Mbeere than Mwingi ecotype but more explant-level variability in callus fresh mass was seen in Mbeere ecotype than Mwingi (Table 1). This explant-level variability may be attributed to the asynchronous flowering and fruit setting in *M. volkensii* which causes fruits on the same branch to be at different stages of development (Orwa *et al.,* 2009). Callus had to be transferred to hormone-free B5 or ½ MS medium to form somatic embryos. TDZ was crucial for formation of somatic embryos as these only formed in calluses from media with TDZ. This agrees with findings in a number of plant species (Guo *et al.,* 2011). TDZ modulates both auxin and cytokinin effects in plant tissues, removing the need for application of combinations of PGRs (Huetteman and Preece, 1993). It appears that presence of TDZ in the medium confers embryogenic competence, but its depletion or withdrawal is essential for actual development of the embryos. The somatic embryos were induced as green globular structures (Figures 1a; b), which conforms to the findings of Ammirato (1987) in other species. The globular embryos developed leaf primordia and formed well defined microshoots (Figure 1c). ½ MS + 0 PGR may be superior to B5 + 0 PGR in percentage induction and vigor of somatic embryos. These results also suggest the existence of ecotypic differences in regeneration ability. Microshoots elongated on ½ MS medium with either 0.1 mg/l BAP ± 5 or 10 % coconut water (Figure 1d). Media having coconut water allowed multiple shoot development in the range of 4 to 12 shoots. Similar results were obtained by Indieka *et al.,* 2007 on MS medium with 0.5 mg/l BAP and 0.2 mg/l IAA. This study shows the amenability of *M. volkensii* to *in vitro* propagation from mature zygotic embryos. This protocol gave multiple phenotypically normal shoots of ≥5cm height in 105 days (3/5 months)/ It is a rapid and fairly simple protocol that could be optimized for application in clonal forestry for commercial supply of timber and botanical pesticides, and in other tissue culture related technologies such as *in vitro* conservation and genetic modification of the species. The only remaining obstacle is the apparent difficulty in rooting, for which further work is in progress.

Table 1: Summary of the effects of TDZ concentration on % callus induction and fresh mass.

	% callus induction		Fresh mass per callus/mg	
Component	Mbeere	Mwingi	Mbeere	Mwingi
Range of means	95.56 - 100	88.88 - 100	107.89 - 301.98	45.17 – 167.33
Optimal TDZ (mg/l)	N/S+	N/S+	0/05	0/05
F-value	1.317	2.158	4.725	5.560
d.f	(7, 64)	(7, 54)	(7, 64)	(7, 54)
F test Significance	*p >0.05*	*p >0.05*	*p < 0.001*	*p < 0.001*
R^2 value for regression upon TDZ concentration	0.768	0.157	0.252	0.252

+ = Optimal *concentration was Not specific (N/S) as 100% callus induction was attained in most of the treatments.*



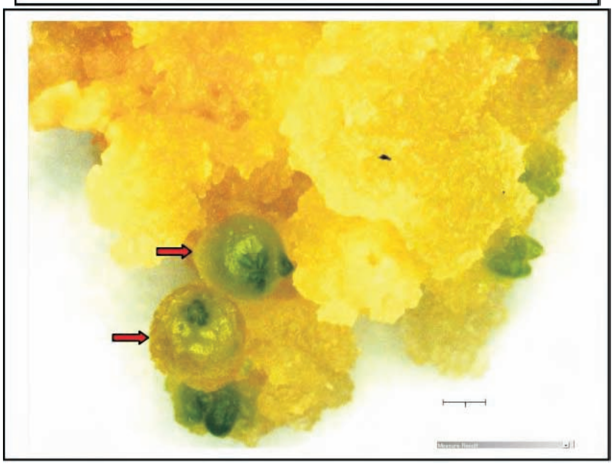


Figure 1: **A**. Scanning photomicrograph; globular stage somatic embryo (arrow) forming from callus 14 days after transfer to B5 + 0 PGR medium; cumulative age = 35 days. Thin scale = 1mm. **B.** Scanning photomicrograph; Initial stages of shoot morphogenesis (arrows) after 15 days on B5+0 PGR medium; cumulative age = 36; Thin scale = 1mm. **C & D.** Multiple microshoots after 34 days on ½ MS + 0 PGR medium; cumulative age = 55 days **(C)** & Shoot elongation after 37 days on ½ MS + 0.1 mg/l BAP + 10% coconut water, cumulative age = 92 days **(D)**.

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Increasing Stewardship of Ethno systems of Zambia

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Abstract

This ethnobotanical diagnosis was generated by applying multiple tools involving literature review, observations and user consultations with sample communities, practitioners and FBO's[8] in Lusaka and Copperbelt provinces on plant medicine knowledge pool. The synthesis of ethno systems in Zambia observed knowledge degradation and deprivation of indigenous plant medicine use. These underlining factors inhibit application of folk knowledge which is further exacerbated by depreciating knowledge base, thereby inhibiting necessary opportunities essential to support knowledge and ecosystem integrity. Evidently communities have remained ill-informed or completely ignorant of commercial beneficial traits of indigenous practices. Lack of

[2] Zambia Climate Change Network
[3] Community Based Enterprise regional working group
[4] Association of Taxonomists
[5] Africa Dryland Alliance for Plant Pesticide Technologies
[6] Ecosystems and Livelihoods Adaptation Network
[7] Africa Adaptation Knowledge Network
[8] Faith Based Organisation

deliberate efforts to collect, collate and promote ITK[9] threaten its perpetuity. Consequently indigenous knowledge in Zambia remains relic to rural communities or simply enthusiast. It is absent in the formal health delivery system and confined to the traditional medicine practitioners. Invariably innovative actions to promote natural plant medicines in Zambia and beyond constitute plausible efforts to enhance use of indigenous knowledge for sustainable natural resource management. This is consistent with efforts enshrined in the national guideline for traditional medicine research in Zambia finalised in 2010. Increasingly indigenous knowledge pool can support opportunities that enhance commercilaisation of traditional systems and niche folk products. Indigenous knowledge integration can provide incentives for tradit ional systems integrity in Zambia.

Introduction

Although many cultures have used plants to manage different ailments worldwide as documented by WHO[10] which has estimated that even today more than two thirds (⅔) of the world populations still rely on p lant derived drugs (O. Akerele etal, 1991), a great pool of knowledge remains un documented. At local level there is insufficient appreciation of this valuable knowledge pool among communities. It has been appreciated that the absence of well-documented profiles of folk knowledge remains major limitation to improvement and integration of indigenous knowledge into formal health system in Zambia.

This state of affairs has continued to date despite the fact that nearly all cultures have used various plants t o manage different ailments at different levels. It has been argued by many enthusiasts in the past that as many as 80% of the rural populations in Zambia still depend on traditional medicine man for their health care (MOH[11], guidelines on traditional medicine research , 2010). This status remains true in Zambia even today where evidence of traditional medicine man has continued to manifest across the country in one form or the other.

Despite this important reality the paradox of knowledge loss of ITK among communities continue to be on the increase as evident by information has remained oral without formal documentation of this important relic ITK is lacking due to failure to put this practice into written record. As part of emerging evidence during this st udy it was clear that the quality of ITK was steadily diminishing with loss of older generation.

Literature Summary

According to the review conducted by World Health Organisation in 2001 on Legal status of traditional medicine and complementary/alternative medicine, it was observed that various types of traditional medicine and medicinal practices referred to as complimentary or alternative medicine have been increasingly used in both developing and developed countries. Further that national policies and regulations on traditional medicine could ensure the safety, quality and efficacy of application of these therapies and products, and function as important steps towards integrative health care systems.

However, relatively few Afric an countries have developed policies and regulations on traditional medicines thereby creating gaps in creating infrastructure necessary to support knowledge generation on ITK. It is in this context that Zambia finalized national guidelines of traditional medicines research in 2010 as way of accelerating knowledge based integration of traditional medicine in the mainstream health care system (Guidelines in Traditional medicine Research of Zambia, Ministry of Health, 2010)

Almost a century ago John Dewey u nderlined concerns that suggested that in all seriousness the part played by custom in shaping the behavior of the individual as over against any way in which man can affect traditional custom, is as proportion of the total vocabulary of their mother tongu e over against those words of his own baby talk that are taken up into the vernacular of his family. It is thus necessary that ITK is documented as submitted by holders of this knowledge in their own language.

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[9] Indigenous Traditional Knowledge
[10] World Health Organisation
[11] Ministry of Health of Zambia

Cunningham further observed in his book published under the work on people, wild plants and conservation initiative that despite the great importance of indigenous medicinal plants plays in !frican people's lives, far more attention has been given to plant use in Asia or Latin America than Africa. H e further observed that knowledge of most resource users is usually focused within a home range which varies rapidly from large spatial scales to smaller scale among sedimentary communities. This landscape threatens the security of ITK in many African countries unless urgent measures are accelerated.

Description of Research

The study was delivered using multiple tools comprising of review of literature including on line materials, personal interview of traditional medicine practitioners and users in Zambia. Direct observation on the status of the community of practice was also employed to triangulate the reviews. 47 % of the study participants included women while the rest were men of mixed ages.

The study was motivated by the need to enquire on the status of documentation of ITK among communities in Zambia while at the same time increase understanding of value and benefits of ITK broadly. It directly responds to the growing crisis of degradation and deprivation of knowledge pool of ITK in Zambia and beyond among the young and educated societies.

As indicated this synthesis is anchored on review of not only of available literature but also the community of practice of ITK in Zambia. It is an attempt to contribute to the continuing scientific dialogue on the incentives preservation of relic knowledge on plant uses. It has rema ined ironic that people and conservation interface has been neglected in the past at human peril. Part of this neglect has been due to lack of appreciation of the relevance that knowledge, institution and cultural perspective of local societies have in res ource management and conservation.

This paper therefore serves as a contribution to the call for urgency for affirmative action to profile and document indigenous knowledge in plant medicines. It provides a brief synopsis of the status of knowledge profiling and documentation of ITK among communities in Zambia.

Research Results and Application

It has emerged that unless societies can tap into their heritage of traditional knowledge, generations will continue to lose useful pool of knowledge. The pool of ITK provides a unique experimental landscape with important outcomes that in turn informs human actions. These unique experiential lessons would be costly to undertake in formal research.

There are clear trends of loss of ITK among both rural and urban societies in Zambia while at the same time experiencing an infusion of other alternative medicines options especially from Asia. On account of the character of the material the problem of cultural life presents itself often as that of the interrelation bet ween various aspects of culture. This desire to grasp the meaning of traditional knowledge and culture as a whole compels societies to consider descriptions of standardized behavior merely as a stepping -stone leading to other problems.

Importantly this study has illuminated the sad reality that there is increasing degradation of pool of ITK due to lack of documentation of these sets of knowledge. Undoubtedly this deprives societies of beneficial knowledge pool necessary to address various health burdens.

Despite the great importance of plant medicines play in Zambia in people's lives in meeting health care, it has been noted that there is limited attention being given to profile and document the knowledge pool. This has led to rapid loss of relic ITK with the loss of holder of such knowledge.

This study appreciates the intensity or lack of profiling and documentation of ITK that has led to limited integration of this pool of knowledge into our scientific body mass.

Use of folk medicine knowledge is not restricted within the society to those who have served an apprenticeship, undergone some sort of training or testing, or have achieved a specific social status. Theories and practices of folk medicine do influence, or be influenced by, the formalized medi cine systems of the same culture

Indigenous knowledge in Zambia remains relic mainly in rural communities or simply enthusiast. It is absent in the formal health delivery system and confined to the traditional medicine practitioners.

Invariably innovative actions to promote natural plant products in Zambia and beyond constitute plausible efforts to enhance use of indigenous knowledge for sustainable natural resource management

This paper has concluded that it is not a question of imperialism, or of race prejudice, or of a comparison between faiths, we are preoccupied with the uniqueness, not of the scientific body of the world at large, which has been less attended but of necessity to our scientific achievements and civilization.

Acknowledgments

This synthesis of stewardship of ethnosystems of Zambia is a dedication to holders of the relic ITK in Zambia and beyond for they have distinguished themselves in rescuing this knowledge pool from total loss. It is a reflection that is not just based on literatur e review but also targeted field work and product of discussions with colleagues over some sustained time.

I wish to thank Dr Phosiso Sola for providing her professional input into the work in the course of its compilation. Finally I extend my thanks the ADAPPT network for providing the space to continue to advance scientific knowledge in natural systems on the biomes of Africa. My dear wife Gertrude is honoured for being patient with me in my course of enquiry as I explored into the often controversial la ndscapes and field work.

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Posters

In vitro and greenhouse evaluation of fungicidal properties of botanical extracts against *Rhizoctonia solani* and *Phythopthora infestans*

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Abstract

The aim of the study was to evaluate plant extracts for antifungal activities against *Rhizoctonia solani* and *Phytophthora infestans,* important pathogens of potato which cause stem lesions and late blight diseases respectively. Acetone, ethyl acetate and water extracts of garlic (*Allium sativum*), Pawpaw (*Carica papaya*), Neem (*Azadirachta indica*), Mexican marigold (*Tagetes minuta*) and peri –winkle (*Vinca rosea*) were screened in vitro for their antifungal activities against *Phytophthora infestans* and *Rhizoctonia infestans* using the disc agar infusion and microtitre double-dilution techniques. The same extracts were then tested for antifungal activity in vivo in the greenhouse on inoculated potato plants. *Allium sativum* and *Azadirachta indica* were effective both in vitro and in vivo in controlling late blight and stem lesions in potato and have a potential to be used as fungicides against these diseases. The plants are readily available and the extraction method is also simple and could lead to high adoption as fungicides by resource poor farmers.

Introduction

The soilborne fungal pathogen *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk), pathogenic to potato (*Solanum tuberosum* L.), is ubiquitous in potato production worldwide (Agrios, 2005). The pathogen can cause lesions (canker) on below-ground plant tissues, and produces sclerotia on daughter tubers (black scurf). Both diseases reduce marketable tuber yields. Fungicide control is not always effective, especially when initial inoculum levels are high (Tsror & Peretz-Alon 2005). The late blight disease of potatoes is the most devastating disease of potatoes in the world. The disease is caused by the pathogen *Phytophthora infestans* and the pathogen also infects solanaceous plants. Late blight may kill the foliage and stems during the growing season. It also attacks potato tubers in the field, which may rot either in the field or in storage. Late blight may cause total destruction of all plants in the field within a week or two (Agrios, 2005).

Literature Summary

Chemical control can be effective in controlling diseases caused by *Phytophthora infestans* and Rhizoctonia solani. Despite the effectiveness of synthetic fungicides, there are potential harmful effects on human health and the environment (Demoz and Korsten, 2006). There is then a need to examine possible non-synthetic chemical approaches for disease management. Research has demonstrated that biological control (Widyastuti *et al*., 2003; Jacobsen *et al*., 2004) is a potentially feasible alternative to the use of pesticides (Madi *et al*., 1997).

Description of Research

The plant materials used in the present study were Peri-winkle (*Vinca rosea*) leaves, Mexican marigold (*Tagetes minuta*) leaves, Neem (*Azadirachta indica*) leaves, garlic (*Allium sativum* L) bulbs and pawpaw (*Carica papaya* L) leaves. All the plant materials were air dried in the shade and ground to powder using a pestle and mortar. Sequential extractions were performed on 1 kg of each plant powder by soaking them in 2 litres of 27·06 moles acetone, 20·30 moles ethyl acetate and sterile distilled water (24 h in each). The organic solvents were removed using a Rotavapor and water plant filtrates were concentrated to powder using a freeze drier. *P. infestans* and *R. solani* were isolated from infected tissues and cultures were morphologically identified using a stereomicroscope, subcultured and stored in a fridge (4 °C) until needed. The antifungal activity of the extracts was determined using the method described by Kritzinger *et al*. (2005) and stock solutions of the crude extracts were prepared. Correct quantities of each crude extract was added to 100 ml of PDA before pouring into Petri dishes to give final concentrations of 0·5, 1·0, 2·5 and 5·0 mg/ml. The PDA containing the different botanical extracts was poured into 65mm Pet ri dishes with preset diametrical lines drawn on the bottom plate to identify the centre of the plate. Fungal plugs (5 mm diameter) of *P. infestans* and *R. solani* from 7-day-old fungal cultures were placed at the centre of the Petri dishes containing PDA am ended with either water extracts, acetone extracts 0·05 ml/ml (v/v), ethyl acetate extracts 0·05 ml/ml (v/v) and the fungicide Ridomil (42.5 g ai/l). The Petri dishes were incubated at 25 °C. Acetone, ethyl acetate and unamended PDA represented the negativ e controls. Ridomil represented the positive control. The diameter of the growth inhibition zones were measured after 3, 6 and 9 days of incubation (DAI). For the microtitre double-dilution assay, Malt extract broth was inoculated with fungi and incubated for 5 days at 25 °C. The broth was adjusted to 0·2 optical density (thus 1×10^5 and 1×10^6 spores/ml for *P. infestans* and *R. solani*, respectively) using before inoculation. In order to yield a series of 50 mg/ml solutions of extracts, 50 mg of each of the plant extracts were dissolved in 1000 µl of 121·7 mg/ml dimethyl sulphoxide (DMSO)/ The 96-well microtitre plates were used and 100 µl of the broth was added to all the wells as follows and different plant extracts were added to different wells. Nutrient bro th was added to as a negative control, while Ridomil (100 µl of 4/2 ml/ml (v/v) was added as a positive control and 100 µl of 121·7 mg/ml DMSO was added as a negative control. Six-day-old malt broth cultures (100 µl) of fungi were added to each well/ The plates were divided into two sets; the first set (six plates) was inoculated with *P. infestans* and the other with *R. solani*. The plates were incubated at 25 °C for 48 h/ Thereafter, 40 µl of 0·2 mg/ml iodonitrotetrazolium (INT) chloride was added to all the wells as a growth indicator to determine the MIC values for the plant extracts. The microtitre plates were incubated for 24 h at 25 °C and evaluated. The final MIC for the extracts was calculated as described by Fawole *et al*. (2009). Steam sterilized soil was thorough mixed with fungal inoculums adjusted to a concentration of 2.5×10^4 spores ml^{-1}. After a 5 day incubation period, soil was treated by incorporating 160 ml of formulated extracts into 5 litres of soil. For the positive control soil was treate d with 160 ml of Ridomil and water was used in the negative control. Treated soil was placed in plastic bags which were closed tightly and incubated for a further 7 days. After 7 days the soil from each treatment was placed in six 20 -cm-diameter plastic and one sprouted tuber of variety Amethyst was planted in each pot. The pots were placed randomly on the greenhouse bench. Plants were assessed for symptoms starting from 2 weeks after crop emergence (WACE) and then weekly thereafter. Each treatment was replicated three times and treatments were arranged in a randomized complete design. Water was applied daily in order to maintain soil moisture at field capacity. Each experiment was repeated twice. Data collected included blight and stem rot disease incidence and severity.

Research Results and Application

Table 2 shows the MIC extracted with acetone, ethyl acetate and water extracts from different plant species. All the *Tagetes* extracts were active against *P. infestans* and *R. solani*. *Allium* extracts were also active against both pathogens. However, the water extract failed to inhibit the growth of *P. infestans* . *Azadirachta* acetone and ethyl acetate extracts were active against both fungi with MICs of 1·56, 3·13 and 6·25 mg/ml, respectively, but the range of concentrations of water extracts did not reveal a MIC. *Carica* and *Vinca* water extracts were active against *P. infestans* , but their organic extracts were inactive against both Pathogens. DMSO was inactive against both *P. infestans* and *R. solani*. Ridomil had a MIC of 0·09 mg/ml, the highest inhibitory activity when compared with all the plant extracts.

Table 2: The MIC of selected plants extracts on *Phythopthora infestans* and *Rhizoctonia solani*

Plant Name	Solvent					
	Acetone		Ethyl Acetate		Water	
	PI (mg/ml)	RS (mg/ml)	PI (mg/ml)	RS (mg/ml)	PI (mg/ml)	RS (mg/ml)
A. sativum	0.78	0.78	0.78	3.13	0	6.25
A. indica	6.25	3.13	3.13	1.56	0	0
T. minuta	3.13	6.25	0.78	1.56	1.56	3.13
C. Papaya	0	0	0	0	1.56	0
V. rosea	0	0	0	0	12.5	12.5
Ridomil	0.09	0.09	0.09	0.09	0.09	0.09
DMSO	0	0	0	0	0	0

PI= *P. infestans* ; RS= *R. solani*; DMSO, dimethyl sulphoxide

Acetone plant extracts showed inhibitory effect on *P. infestans* at 6 DAI (Figure 1a). All concentrations of *Allium sativum* extract completely inhibited growth while *Azadirachta indica* extract completely inhibited *P.*

infestans at 5·0 mg/ml. The general trend was inhibition increased as the concentrations of all extracts increased, with the exception of *Vinca rosea*. *Allium* acetone extracts completely inhibited the growth of *R. solani* at 5·0 mg/ml only, whereas *Carica* and *Azadirachta* extracts inhibited the growth of *R. solani* by more than 90% in both cases (Figure 1b).

P. infestans was completely inhibited by *Allium* ethyl acetate extracts at concentrations of 2·5 and 5·0 mg/ml (Figure 2a), while the ethyl extract of *Tagetes minuta* inhibited *P. infestans* growth by 80% at 5·0 mg/ml compared with the control. All other ethyl acetate plant extracts (*Azadirachta, Carica* and *Vinca rosea*) failed to inhibit *P. infestans* at all concentrations. *Allium* ethyl acetate extract inhibited growth of *R. solani* by more than 80%, although the lower concentrations of same extract were not very effective as they inhibited growth by less than 50% compared with the control (Figure 2b). The *Carica* water extract was effective at 5·0 mg/ml as it completely inhibited the growth of *P. infestans*. This was followed by water extracts of *Vinca* and *Azadirachta* which inhibited growth of *P. infestans* by more than 90% (Figure 3a). *Azadirachta* water extracts effectively inhibited growth of *R. solani* by 80%, while the extracts failed to reach 50% inhibition (Figure 3b).

The potato plants treated with water extracts of *Azadirachta* (5 and 15 mg/ml), acetone extracts from *Carica* (15 mg/ml) and *Tagetes* (5 mg/ml) extracts showed no blight disease incidence (Fig. 4a). The above extracts and 5mg/ml water extracts from *Allium* had low blight disease incidence and compared well with the non-inoculated control and Ridomil. All the acetone plant extracts, 5mg/ml *Allium* and *Tagetes* water extract-treated plants showed significantly ($P < 0{\cdot}05$) lower blight disease severity than water treatments and compared well with Ridomil and the non-inoculated control (Fig. 4b).Water extracts from *Allium*, *Carica* and *Tagetes* and acetone extracts from *Azadirachta, Carica* and *Tagetes* showed no or the lowest disease incidence (Fig. 4c) and compared well with the non-inoculated control and Ridomil. All the plant extracts and the positive controls had stem lesion low disease severity and were not significantly different ($P < 0{\cdot}05$) from each other, except for the plants treated with (15 mg/ml) *Allium* acetone extract and the negative controls (Fig. 4d).
Allium Acetone and ethyl acetate extracts were as effective as Ridomil in inhibiting completely the growth of *P. infestans* and *R. solani* in the agar infusion technique, while *Allium* water extract inhibited the growth of *R. solani* in the microtitre double dilution technique. The performance of *Allium* water extracts on *R. solani* is similar to the findings of Shovan et al. (2008) on *C. dematium*, where *Allium* water extracts inhibited the growth of the pathogen.

(a) (b)

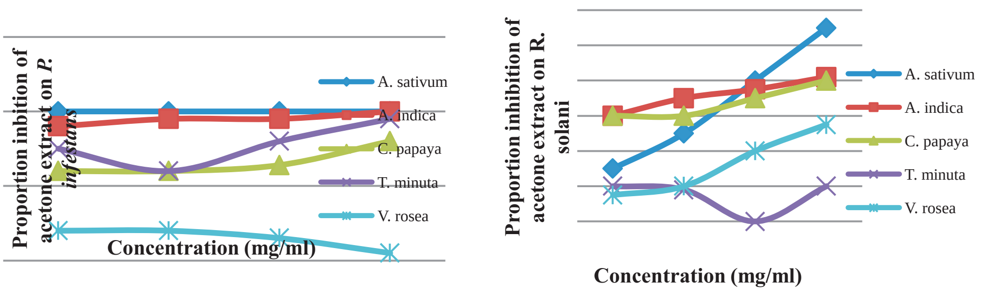

Figure 1: The effect of acetone crude plant extracts on the colony growth of (a) *P. infestans* and (b) *R. solani* at 6 days after inoculation

(a) (b)

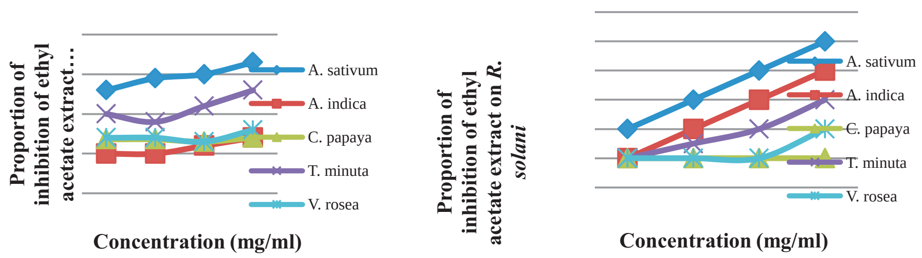

Figure 2: The effect of ethyl acetate crude plant extracts on the colony growth of (a) *P. infestans* and (b) *R. solani* at 6 days after inoculation.

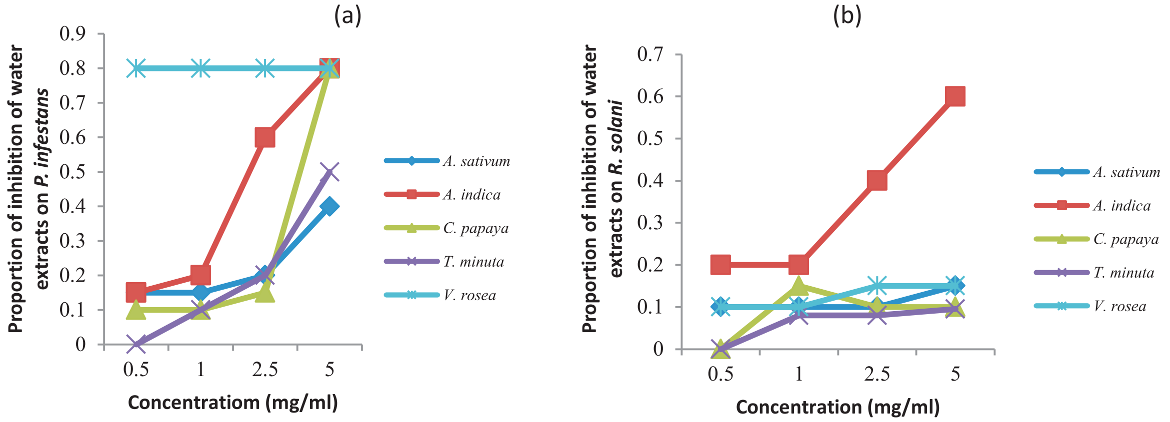

Figure 3: The effect of water crude plant extracts on the colony growth of (a) *P. infestans* and (b) *R. solani* at 6 days after inoculation

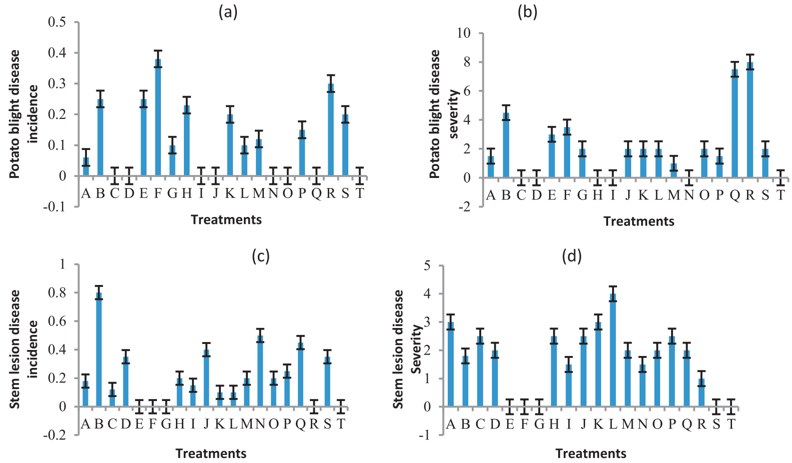

Figure 4: (a) Proportion of potato plants affected with blight: disease incidence. (b) blight disease severity of potato plants treated with plant extracts. (c) Proportion of potato plants affected with stem lesion: disease incidence. (d) stem lesion disease severity of plants treated with plant extracts

Treatments A–H=water extracts and I–P=acetone extracts (A=5 mg/ml garlic extracts; B=15 mg/ml garlic extracts; C=5 mg/ml *Azadirachta* extracts; D=15 mg/ml *Azadirachta* extracts; E=5 mg/ml *Carica* extracts; F=15 mg/ml *Carica* extracts; G=5 mg/ml *Tagetes* extracts; H=15 mg/ml *Tagetes* extracts; I=5 mg/ml garlic extracts; J=15 mg/ml garlic extracts; K=5 mg/ml *Azadirachta* extracts; L=15 mg/ml *Azadirachta* extracts; M=5 mg/ml *Carica* extracts; N=15 mg/ml *Carica* extracts; O=5 mg/ml *Tagetes* extracts; P=15 mg/ml *Tagetes* extracts). Q=non-inoculated control; R=inoculated control; S=DMSO and T=Ridomil.

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Evaluation of the best extraction solvent for *Tithonia diversifolia* biocide for effective pest management

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Abstract

Aqueous, ethanolic, and ethereal solutions of Tithonia *diversifolia* leaf extracts of the same concentration were separately tested against Periplanata *americana* to evaluate the efficacy of the extracts within contact period of sixty minutes. The efficacies of the extracts on the experimental insect pest were measured in terms of percentage mortality within the period. The result showed that all the extracts were fatal to the insect pest. However, the aqueous extract had a very good efficacy causing percentage mortality of 82 % followed by ethanolic extract with 63%, ethereal extract with 45 % and the control (pure distilled water) with 0%. The study therefore concludes that, Tithonia *diversifolia* biocide can best be extracted using water rather than organic solvents for it to be developed into effective environmentally friendly, commercially viable and safe botanical insecticide.

Introduction

To prevent loss of crops during storage and on field, farm ers rely on chemical pesticides as pest control method. In Uganda forty four pesticides are currently registered for use. However, many more found their way into the country and eventually into the environment. These, includes fertilizers, insecticides, f ungicides, herbicides (UBOS), 2012 Report. All pesticides are harmful if incorrectly used and can be linked to adverse human health conditions. Studies have found out that three percent of all agricultural workers in developing countries are affected by pesticides poisoning each year. In Uganda 66% of the working population is engaged in Agriculture and this would translate to about 650,000 pesticide poisoning cases annually (UBOS), 2012 Report. The risks posed by extensive pesticides use in particular have generated concern. There is therefore an increasing need to search for environmentally friendly, cheap safe and plant materials that will not contaminate food products and cause health hazard to humans.

Literature Summary

Tithonia diversifolia is one of those plants that have been extensively researched on. It is a shrub belonging to the family Asteraceas. Many authors across the globe reported about Tithonia diversifolia plant. For instance, it has been reported in Kenya (Niang et al 1996), Nigeria (Aye ri Et al(1997), Zimbabwe (Jiri and Waddington, 1998), Rwanda(Drechsel and Reck,(1998) to mention but a few. In Uganda, it is also present, growing mostly along road sides. Many authors also reported about its uses, which include; Anti malarial (Oyewole et al 2008); manure (Jamia et al 2000), treatment of hepatitis (Lin et al 1993; kuo and chen.1997) crop protection from termites (Adoyo et al 1997; compost (Drenchsel and Reck, 1998) Nginja et al 1998) poultry feed (odunsi et al 1996) to mention but a few. Apart from Tithonia diversifolia,many other plants have also been researched on for different purposes. For instance Aristalochia *ringens,* Allium *sativum* Ficus *easperatalt* and Garania *kola*, were evaluated as grain protectant against the maize weevil, sitop hilus *zeamais* (Arannilewa et al 2006), phyllarithus *amarus,* Acassia *albida* and Tithonia *diversifoli*a leaf extracts were evaluated against the workers of Macrotermes *bellucosus* in vitro (Oyedokun et al 2011).Morinda *Lucida* leaf extract were used against coropea Bruchid (Ajayi O.E 2012). Many authors reported the use of solvents for extraction of these plant materials. For instance, acetone, n-hexane and methanol were used for extraction of morinda l*ucida* leaf extract (Ajayi 2012). Water and methanol for extracting Tithonia diversifolia (Hemsel) leaf extract. Oyewole et -al 2008).

It is reported that for herbal plants to be evaluated for insecticidal activities, they have to be in the right mixture or proportion no matter the concentration in order to hav e an optimum performance when being used in plant protection against insect's pests/ Extracting solvents play an important role in the biocide potency in the plant crude extract (Oyedokun et al (2011). Currently no work has been done to evaluate the best solvent for extracting any plant materials. The objective of this paper is to provide an answer to the recommendation of (Oyedokun et al 2011) by evaluating the best extraction solvent for Tithonia diversifolia.

Description of Research

Fresh leaves of Tithonia diversifolia were collected along Kyambogo University road, Kampala Uganda. Seventy five adult cockroaches were collected from their natural ecological habitat and put in sterilized 250ml beakers covered with perforated Petri -dish to allow air to reach them. Before the experiment, the cockroaches were fed on maize flour for 24 hours. The plant leaf extracts were prepared as described by Oyedokun et al (2011) with some modification. The leaves were washed with clean double distilled water to remove dir t and thereafter air-dried in the laboratory. The leaves were pulverized using hammer milling machine. The powder was kept inside black polythene bag tied with rubber band and kept until needed. Three equal quantities, 240g of the powdered leave were weigh ed and extracted with three different solvents; Ethanol LR (GC) 99% Alpha Chemika Mumbai India), ethoxyethane 99% (Deluxe Scientific Surgico PVT ltd India. A and water respectively.The 240g plant powder weighed was soaked in 240ml of water, Ethanol and Et hoxyethane separately for 24 hours and the mixture was filtered with Muslim cloth. The filtrate were the stock solutions that were each serially diluted (water: extract) in to different concentrations 1 -10(v/v):10%, 3-20(v/v):15%, 4-16(v/v):25%, and 3-10(v/v): 30%. The toxicity of the different concentrations of the extracts was done by carrying out topical application test on the Periplanata *americana* using the different concentration of 10%, 15%, 20%, 255 and 30% of the aqueous, ethanoic ethoxyethane ext ract and pure distilled cold water.

Five adult cockroaches (Periplanata *americana*) were picked randomly and placed into each 250ml beaker with a sterilized forceps. Five treatment were carried out per extract (aqueous ethanolic, ethereal) and pure distilled cold water. 5cm3 of each aqueous, ethanolic and extract concentrations was applied in a clean 250 ml beaker and one cockroach was pick using sterilized forcep and placed in each of the extracts Each treatment was replicated five times. Mortality counts were recorded after every 10 minutes up to 60 minutes. A cockroach was declared dead when it was lying flat on its back and showing no sign of movement of its body after being touched with sterilized forceps.

Research Result and Application

To examine w hether there is any significant relationship between concentration and toxicity and thereafter the best extracting solvent, the row data were exerted to statistical test The mortality counts were converted to percentage mortality rate using a formula:

$$\text{Percent mortality rate} \quad \frac{\text{Total number of dead cockroaches after treatment}}{\text{Time taken for the cockroach to die}} \text{x100}$$

The percentage mortality per treatment was then subjected to analysis of variance (ANOVA). Mean separated by Turkey's test at (*P*=0.05) significant level

Results

Table 1: Showing Mortality Rate recorded after every 10 minutes for 60 minutes of exposure to crude aqueous, ethanolic and ethereal extracts of Tithonia *diversifolia*

Concentration in % (v/v)	Mortality Rates in Different Extracts		
	Aqueous Extract	Ethanolic Extract	Ethereal Extract
10	78.74	63.21	48.97
15	79.46	63.30	46.21
25	82.51	63.02	41.98
30	85.61	63.53	45.33

Table 2: Showing percentage mean mortality of *Periplanata americana* after 60 minutes, exposure to crude aqueous, ethanolic and ethereal extracts of Tithonia *diversifolia a*t difficult concentration

Plant Extracts	Aqueous Extract solution	Ethanolic Extract solution	Ethereal Extract solution	Distilled Water
Mean Percentage Mortality rate	81.5800	63.0400	45.5775	.0000
Std. Error of Mean	±1.57231	±0.26473	±1.40604	±0.00000

The study indicated that crude aqueous, ethanolic and ethereal extracts of Tithonia diversifolia leaf were all fatal to Periplanata Americana the test insect pest. This finding is in consonance with the findings of Oyedokun et al (2011) that aqueous an ethanolic extracts of P. *amaru*s, A.*albida* and T. *diversifolia* showed insecticidal activity via contact toxicity. The results s also indicated that there was no significance difference at (P=0.05) in the time taken for the cockroaches to die in the different concentrations of the extracts. This implies that concentration may not be a factor in causing mortality of the extracts. Aqueous leaf extract of T.d *iversifoli*a however had a very good efficacy causing mean percentage mortality rate of 81.58±1.57 followed by ethanolic extract with 63.04±0.26, ethereal extract with 45.58±1.41and distilled water with 0.00±0.00. The study therefore concludes that T. diversifolia biocide can best be extracted in aqueous state rather than in organic solvents like alcohol and ether. Further research is needed to find out the biocide which is extracted by the different solvents basing on their polarities.

Acknowledgement

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Effects of Botanical Insecticides on the Egg Parasitoid *Trichogramma cacoeciae* Marchal (Hym. Trichogrammatidae)

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Abstract

Laboratory studies were carried to investigate the side effects on *Trichogramma cacoeciae* of two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflor®) and Quassin (alcoholic or water extracts). The results showed that by exposing adults *T. cacoeciae* to residues of Neemazal formulations on glass plates, the tested preparations were either harmful (Neemazal -Blank) or moderately harmful (Celaflor), where the International Biological Control Organisition classification (IOBC -Class) was found

to be 4 or 3 respectively. The two Quassin formulations tested were harmless. When treated host eggs were offered to adults *T. cacoeciae*, all tested chemicals were either slightly toxic (IOBC Class 2) for neem preparation or harmless (IOBC Class 1) for Quassin preparations. In a further test, host eggs parasitized at different time intervals (1-8 days), were sprayed at the same day. The res ults indicated that only Neemazal T/S-Blank formulation was slightly to moderately harmful reducing adult emergence.

Introduction

Parasitoids of the genus *Trichogramma* occure naturaly worldwide and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops. Results of augmentative releases of *Trichogramma* can be affected by the use of broad-spectrum insecticides in or near release plots (Stinners *et al.* 1974, Ables *et al.* 1979, King *et al.* 1984). The search for selective insecticides to be used with Trichogramma releases is of great importance. The recent laboratory studies were carried out to investigate the side effects on *Trichogramma cacoeciae* of two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflor®) and Quassin (alcoholic or water extracts) to study there possible use with Trichogramma releases, since these insecticides are coming from plant origin they are believed also to have the advantage of having the lease impact on the environment..

Materials and Methods

Two formulations of the botanical active ingredient, azadrichtine (Neemazal T/s Blank and Celaflor) as well as two extracts of Quassin (Alcohlic and Water extracts) were included in the tests. The field recommended concentrations were prepared for the tes ts. The study included Exposing adults (susceptible life statgae) of Trichogramma to sprayed glass plates using the method described by Hassan et al. (2000). In other experiments adults *of Trichogramma were Exposed to sprayed host eggs.* The treated host eggs were either offered directly after drying of the spray or the eggs were hold at 15 °C and offered to adults after 6 days. Less susceptible life stage (parasites within their hosts) were also exposed to tested treatments. Thhe method described by Hassan and Abdelgader (2001) was followed. The study included spraying of parasitised host eggs at different interval after parasitisation ranging from 1 – 8 days. The percentage of adult emergence and the reduction in emergence relative to the control were then determind and the pesticides were categorized accordingly.

Results and Discussion

Effects on adults

Results of tested Botanicals on adults are presented in Table (1). The results showed that by exposing adults *T. cacoeciae* to residues of Neemazal formulations on glass plates (standard test method, Hassan et al. 2000), the preparations were either harmful (Neemazal-Blank) or moderately harmful (Celaflor). The two Quassin formulations tested were harmless. In another set of experiments, where treated host eggs were offered to adults *T. cacoeciae,* all tested chemicals were almost harmless.

By exposing adults to treated host eggs both Quassin formulations were harmless. Celaflor was slightly toxic for adults, both when freshly or 6-day old sprayed host eggs were offered to adults of T. cacoeciae. Neemazal - Blank formulation was only slightly toxic when 6 day old sprayed host eggs were offered to the adults.

Table (1): Effects of exposing adult *Trichogramma cacoeciae* to various treatments

Trteatment	PR	Class	FRHES	Class	6 DRHES	Class
Control	18.9^{abc}		28.8^{bc}		36.0^{b}	
Quassin-Alcohol	21.2^{bc}	1	23.1^{ab}	1	31.6^{ab}	1
Quassin-Water	22.0^{c}	1	33.0^{c}	1	33.9^{b}	1
Neemazal-Blank	0.0^{a}	4	24.0^{ab}	1	24.0^{a}	2
Celaflor	1.0^{ab}	3	20.3^{a}	1-2	23.2^{a}	2

PR= parasitism rate (eggs/ female) in glass plate test; FRHES= fresh residue host eggs spraying (eggs/female); 6 DRHES = 6 day day residue host eggs spraying (eggs/ female)

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%); SE = Standard Error; % RC = Percentage Reduction relative to the control; Class = IOBC classification

Effects on immature stages

Spraying parasitized host eggs one day after parasitism resulted in a significantly lower number of black eggs (i.e. lower pupation). All tested insecticides significantly reduced pupation, when host eggs where sprayed two days after parasitism, indicating that *Trichogramma* was very sensitive during this stage. This might have coincided with the hatching of the vulnerable neonate larvae of *Trichogramma* from laid eggs. The pupation rate was not reduced as a result of treatment, when host eggs were sprayed on the third and subsequent days after parasitism (Table 2). This trend can also be seen clearly when the percentage reduction relative to the control and the categorisation according to the IOBC classification was determined (Table 3).

Table 2: Developing Black eggs after treating parasitsed eggs at various days after parasitism

Treatment	1 day	2 days	3 days	5 days	7 days	8 days
Control	427.3[c]	329.0[a]	388.3[ab]	465.2[ab]	440.2[b]	355.5[ab]
Quassin-Alcohol	400.8[c]	189.8[b]	441.7[bc]	464.2[a]	420.7[b]	388.5[bc]
Quassin-Water	401.7[c]	247.8[b]	448.8[c]	506.3[b]	412.0[b]	421.3[c]
Neemazal-Blank	219.0[a]	219.8[b]	357.5[a]	437.5[a]	340.3[b]	325.3[a]
Celaflor	334.3[b]	197.0[b]	466.5[c]	430.2[a]	420.0[b]	323.2[a]
SE	17.5	20.9	19.3	26.0	19.5	20.8

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%); SE = Standard Error

Table 3: Developing Black eggs after treating parasitsed eggs at various days after parasitism (IOBC – Classification)

Treatment	1 day		2 days		3 days		5 days		7 days		8 days	
	% RC	Class	% RC	Class	% RC	Class	%RC	Class	% RC	Class	% RC	Class
Quassin-Alcohol	6.2	1	42.3	2	-13.7	1	0.2	1	4.4	1	-9.3	1
Quassin-Water	6.0	1	24.8	1	-15.6	1	-8.9	1	6.4	1	-18.5	1
Neemazal-Blank	48.8	2	33.2	2	7.9	1	6.0	1	22.7	1	8.5	1
Celaflor	21.8	1	40.1	2	-20.1	1	7.5	1	4.6	1	9.1	1

% RC = Percentage Reduction relative to the control; Class = IOBC classification

Conclusion

The results showed, in general, that both Azadirachtine and Quassin were relatively safe to the tested parasitoid and could therefore be used in combination with *Trichogramma* releases.

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Potential uses of Garneem-FPE for managing whiteflies (Bemisiatabaci) on tomato production under open fields

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Abstract

Excessive use of synthetic chemical insecticides resulted in the development of resistance in whitefly (*Bemisiatabaci,*Homoptera: Aleyrodidae)to a wide range of commercially available insecticides on agrochemical markets. Fermented plant extracts of whole-plant wild garlic (*Tulbaghiaviolacea*) andneem (*Azadirachtaindica*) leaf(GarNeem) are being tested against whiteflies in tomato (*Solanumlycorpersicum*) production in order to develop alternative management strategies for this pest. Weekly application of GarNeemwas tested on various stages of whiteflies over two seasons, with samples being collected weekly just prior to subsequent application. Relative to untreated control, GarNeem reduced larvae and adults, but had no effect on pupae during both seasons. In conclusion, GarNeem could possibly be used to diversify management strategies onwhiteflies; with increasing population densities intermittently reduced using synthetic pesticides in order to reduce excessive use of the materials and therefore, development of resistance.

Introduction

Worldwide, climate change is promoting the resurgent of secondary insect pests to primary economic pests. Whitefly (*Bemisiatabaci*, Homoptera: Aleyrodidae), is one such pest, which has since become a serious economic pest of various crops including tomato (*Solanumlycorpersicum*) under both greenhouse and open field conditions (Tzanetakis, 2004). Whiteflies are viral vectors of the genus *Crinivirus* which reduces the longevity and productivity of crops (Tzanetakis, 2004), with their feeding resulting in the release of honeydew. Development of whitefly resistance to various synthetic insecticides had since increased the focus to alternative tactics in various crop production systems (Kumar and Poehling, 2006).Use of plant extracts is one such alternative. The aim of this study was to investigate if GarNeem would be suitable for use in reducing population densities of whiteflies for at least five weeks and then followed by a normal synthetic chemical spray to knock down the pest in tomato production.

Literature Summary

Neem (*Azadirachtaindica*A. Juss.) and wild garlic (*Tulbaghiaviolacea* L.) have insecticidal properties (Chawla*et al*., 1995; Flint *et al*., 1995). Neem products from various parts of the plant are available on agrochemical markets, especially in India (Schmutterer, 1990; Chawla *et al*., 1995). In contrast, the entire garlic plant contains insecticidal chemicals (Flint*et al.*, 1995). Formulations of botanical insecticides include granules, powders, aqueous extracts and fermented plant extracts.

Description of Research

Tomato seedlings raised in the ZZ2 nursery were transplanted onto 30-cm-high beds with a spacing of 15 cm within the rows. Each plot consisted of two 10-m long and 1.8-m wide adjacent rows, with blocks separated from each other by 2.5-m border rows. Cultural practices, irrigation and fertilisation were as in commercial operations at ZZ2. Fermented crude extracts of garlic and neem leaves at 1 kg each were prepared in one container, with EM culture comprising a mixture of photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, fermenting fungi and other little known fungi (Kyan*et al*., 1999). Treatments consisted of untreated control and 5% dilutions of GarNeem-FPE, arranged in a randomised complete block design, with four replicates. Treatments were initiated two weeks after transplanting using a knapsack sprayer at seven day interval. In this study, there was no positive control. Treatment assessment was started two weeks after planting and carried out weekly, just prior to spraying.Insect samples were collected weekly between 7h30 and 9h30. *In-situ* counts of whitefly adults and nymphs were done on ten randomly selected plants by gently turning the leaf and counting from the top to the bottom parts of the plant. Data were subjected to two-sample Student t-test using SAS software and compared per sampling time at 5% level of probability.

Results and Application

In summer and winter, GarNeem consistently reduced adult whiteflies during all 5 sampling times (Table 1). In summer and winter the material reduced adult whiteflies by 66% (range 58-72%) and 60% (range 43-76%), respectively. In contrast, effects of the material on nymphs were not consistent during both seasons. During summer, the material had no effect on nymph whiteflies during the first two weeks, while during three subsequent weeks the material reduced nymphs by 29% (range 25-31%). During winter, the material was effective only during the second sampling time.During winter, regardless of the treatment, population growth of adult whiteflies over increasing application or non-application frequency (time), was depicted by quadratic relationships (data not shown).

Table 1: Response of adult and nymph whiteflies to GarNeem-FPE at seven day intervals over 35 days on tomato production under open field conditions during summer and winter at ZZ2, Mooketsi

Treatment	Sampling time after weekly treatment (days)									
	7	14	21	28	35	7	14	21	28	35
	Summer: Adult whitefly					Winter: Adult whitefly				
Control	15.95[a]	28.35[a]	35.32[a]	46.07[a]	60.67[a]	5.75[a]	12.65[a]	10.85[a]	13.97[a]	18.35[a]
GarNeem-FPE	6.70[b]	9.52[b]	11.75[b]	15.45[b]	16.97[b]	3.30[b]	3.05[b]	4.10[b]	4.70[b]	8.17[b]
Relative effect (%)	-58**	-66**	-67**	-66**	-72**	-43**	-76**	-62**	-66**	-55**
	Summer: Nymph whitefly					Winter: Nymph whitefly				
Control	4.27[a]	6.77[a]	9.32[a]	11.37[a]	18.82[a]	1.50[a]	4.05[a]	3.60[a]	6.10[a]	7.02[a]
GarNeem-FPE	4.25[a]	5.72[a]	7.02[b]	7.97[b]	13.07[b]	1.05[a]	2.57[b]	3.45[a]	4.87[a]	5.97[a]
Relative effect (%)	-0.50[ns]	-16[ns]	-25**	-30**	-31**	-30[ns]	-37**	-4[ns]	-20[ns]	-15[ns]

Relative effect (%) = [(GarNeem-FPE-FPE/Control) —1) × 100]; [ns] and ** imply that relative effect percentage was not significant and significant, respectively, at 5% level of probability according to two-sample t-test.

Under GarNeem, increase in application frequency explained the suppressed population growth of adult whiteflies by 96%, while under untreated control the increase in non-application frequency explained the non-suppressed population growth of adult whiteflies by 99%. In contrast, during summer, regardless of the treatment, the population growth of adult whiteflies over time was described through linear relationships. In each case, the model described the population growth by 99%.Differences between GarNeem and untreated control for population growth in nymph whiteflies over application or non-application frequency, regardless of season, also had quadratic relationships.Consistent reduction of whiteflies by GarNeem offer new opportunities for the use of this product in tomato production since it confers a broad spectrum of active ingredients, with complementary and supplementary modes of action. Development of insect resistance under such conditions is rare (Cahill *et al.*, 1994). In whiteflies, eggs hatch into non-feeding larvae, which undergo pupation in cocoon, wherein metamorphosis results in non-feeding nymphs, which develop into adult whiteflies (Coudriet*et al.*, 1985). Apparently, GarNeem does not have the capabilities for suppressing nymph whiteflies since this stage neither feeds, mates, ovoposites nor moults. The density-dependent population growth patterns observed in adults during winter and nymphs during both seasons are features of biological entities when they respond to intrinsic and/or extrinsic factors (Salisbury and Ross, 1992). In conclusion,GarNeem-FPE was highly effective in reducing population densities of adult whiteflies during both seasons, but less effective in reducing nymphs. The material could be used to suppress population densities of whiteflies during summer and/or winter, with once a month sprays of highly efficient synthetic insecticide to reduce the slow but increasing population densities of whiteflies during the production cycle. This strategy could extend the application frequency of synthetic chemical insecticides and therefore, minimise the development of resistance to the commercially available insecticides for managing populations of whiteflies.

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A survey of plants with known pesticidal and medicinal values in Malawi: a case of Mkwinda and Malingunde extension planning areas in Lilongwe

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Abstract

A survey was conducted to investigate plants known by indigenous people as having pesticidal and medicinal use in parts of Lilongwe in Malawi aimed at collecting and documenting botanicals locally available that are used to kill pests and cure diseases in plants, livestock and households. A total of 172 structured questionnaires were administered to households in different sections of Mkwinda and Malingunde agricultural extension planning areas (EPAs). Five focus group discussions, comprising 20 to 30 community members each, were also conducted in the sections. Further questionnaires were administered to extension personnel in the sections. The results indicate that communities in Mkwinda and Malingunde EPAs experience pest and disease problems and have indigenous knowledge of plants with pesticide and medicinal values for plant, animal and household protection. Locally available botanicals, parts of the botanical used and the use of botanicals were identified including methods of preparing the botanicals. For example, *Markhamia obtusifolia* (Leguminosae) was used in households as a mosquito repellent while *Vernonia adoensis* (Compositae) and *V. amygdalina* were used to control vegetable insect pests, newcastle disease in chickens and diarrhoea in humans. The limitations of using botanicals as perceived by the farming households in the study area are documented. It was concluded that pesticidal and medicinal plants exist in Lilongwe and therefore scientific investigation on these plants is required.

Keywords: botanical, pests, diseases

Introduction

Crop and livestock protection in Malawi is an essential element of improving food security. The use of botanical insecticides in pest and disease management is considered an ecologically viable proposition (Karnataka, 2008). Most of the indigenous knowledge technologies used in the current forms are unable to suppress pest population densities that have surpassed the damage threshold limits. Herbal medicines have always been a form of therapy for livestock among resource poor smallholder farmers. Furthermore the use of synthetic antimicrobial agents in food-producing animals has recently become a very important public health issue (Jafari *et al.,* 2007). There is scanty literature on indigenous veterinary remedies in Malawi and as in most countries, studies conducted to provide scientific information on the purposes and effectiveness of the botanical plants for pest management in agriculture are limited. The aim of this study was to investigate plants with known pesticide and medicinal use in parts of Lilongwe, Malawi with a main objective to collect and document botanicals locally available that are used to kill pests and cure diseases in plants, livestock and households.

Literature Summary

Many reports have indicated that microbial resistance to these agents may possibly be transferred to human pathogens (Soggard, 1973). Plant protection compounds such as botanical insecticides and antifeedants have been shown to heal animals (El-Sharabasy, 2010). The use of plant products for pest control may impart a selective advantage to plants by inhibiting, repulsing, and even killing non-adapted organisms that feed upon, or compete with the plant (Antonious *et al*., 2006). Dried plants or their extracts have been used by farmers in many developing countries to protect food and fiber from insects (Jacobson, 1986; Kamanula *et. al*, 2011). Karnatana (2008) proved effectiveness of Neem extracts on sap sucking insects to be comparable with conventional insecticide - endosulfan. Botanical insecticides such as azadirachtin are often effective alternatives to organophosphates or other neurotoxins for pest control due to multiple modes of action including toxicity, antifeedant and anti-oviposition effects (Mochizuki, 1993; Sutherland, *et al.,* 2002). Thus plant derived compounds are secondary metabolites (Schmutterer, 1995) affecting insect growth and behaviour (Campagne *et al*., 1992).

Description of Research

With the support from the Department of Science and Technology of the Government of Malawi, a survey was conducted in Lilongwe in Mkwinda and Malingunde EPAs in June, 2012. Out of fourteen sections of Mkwinda EPA (extension planning area), three sections were visited, namely Nanjiri, Vironkho and Msinja. In Malingunde EPA, Kangankhaka and Mphangwe sections were visited out of its twelve sections. Thirty-five (35) structured questionnaires were administered in Vironkho section, forty-three (43) in Msinja, thirty-two (32) in Mphangwe and forty-one (41) in Kangankhaka. In each section, one (1) focus group discussion, comprising of 20 to 30 members from different villages, was conducted and three key info rmants were interviewed namely, agricultural extension officer (AEDO), assistant veterinary officer (AVO) and community health worker (CHW). Data were analysed in SPSS 16.0 (statistical package for social scientists).

Research Results and Application

Table 1: List of crops commonly grown, pests and diseases in Mkwinda and Malingunde EPAs

Common crops	Pests	Diseases
Maize	Stalkborer, termite, wire worms, aphids weevils, witch weed	Headsmut, blights, maize streak virus
Tobacco	Grasshoppers, aphids, nematodes	Wild fire, bushy top, tobacco mosaic virus
Beans	Bean leaf beetles, striped bean weevils, bruchids, bean stem maggots, Caterpillars, aphids, jassids, snails, locusts, coreid bugs	Blights, root rots
Groundnuts	Grasshoppers, aphids, ground beetles, whitegrubs, *mizukila,* coreid bugs, jassids	Rosette virus, fusarium wilts
Cucumber/Pumpkins	Pumpkin beetles, aphids, snails	Powdery mildews
Onions	Red spider mites, termite, cutworms	wilting

Tomatoes	mites, grasshoppers	Blights, leaf spots
Brassicas	bollworms, saw fly, grasshopper, Diamond backmoth, beetles	blights
Cassava	Termites, green spider mites	Cassava mosaic virus
Sweet potatoes	Weevils on potato	Blights, rots
Irish potatoes	Leaf eaters	Blights
Mangoes, guavas,	Fruit flies	Fruit rots

Some similar pesticidal plants mentioned in Tables 2 to 3 are being used by farmers in the Northern region of Malawi e.g *T. vogelii, V. amygdalina, T. diversifolia, E. Tirucali* (Nyirenda et al., 2011). Some of the plant species reported in the present study area are known to be used by farmers elsewhere (Mugisha -Kamatenesi *et al.,* 2008).

Table 2: List of pesticidal plants on crop pests and diseases so far mentioned in Mkwinda and Malingunde EPAs and problem it controls

Local name	Botanical name /family	Crop pest/disease controlled	Part used (botanical)	Preparation method	Method of administration
Adyo	*Allium sativum* (Liliaceae)	Field pests	Bulb	Pond soak in water	spray
Neem	*Azodiracta indica* (Meliaceae)	All field pests	Leaves	Pound, soak, sieve.	spray
Tsabola	*Capsicum* spp. (Solanaceae)	Grain storage	Fruit	Grind to Powder	Mix with grain
Jerejere	*Cassia* spp. (Caesalpiniaceae)	All field & storage pests	Leaves	Pound, soak, sieve	Spray
Nkhadze	*Euphorbia tirucalli* (Euphorbiaceae)	Termites, leaf spots	Stems	Cut in long pieces and plan	place near base of crops, plant near house,
Dema	*Mucuna* spp. (Papilionaceae)	All field pests	Tuber	Dig out, pound, soak, sieve	Spray; Put in drinking water for chickens
Tobacco	*Nicotiana tabacum* (Solanaceae)	Field pests, storage insects	Leaves	Crush dry leaves, soak, sieve; Grind dry leaves mix with ash	Spray; Mix with stored grain
Katupe, mthutu	*Tephrosia vogelii*	All field and storage insect pests	fresh/dry Leaves	Pound, soak 1-2 days, sieve; Pound dry leaves, sieve	Spray on field crops Mix powder with grain
Chingombiro, delia	*Tithornia diversifolia*	All field insect pests on all crops	Fresh leaves	Pound leaves, soak, sieve	Spray on crops
Futsa wa mwamuna	*Vernonia adoensis* (Compositae)	Vegetable insect pests	Leaves	As above	As above
Futsa	*Vernonia amygdalina* (Compositae)	Vegetable insect pests	Leaves, Roots	Pound , soak, sieve	Spray
Kangatsoyo	*Vernonia* spp (Compositae)	Field pests	Leaves Roots	Pound, soak, sieve	Spray,
Maize	*Zea mays* (Poaceae)	Witch weed	Bran, tassel	Maize bran mix with manure	Apply in maize field

The limitations of using botanicals were expressed by 90 % of the respondents as being the tedious process of preparing the botanicals, lack of knowledge, uncertainty of effectiveness of the botanicals, availability of synthetic pesticides and the feeling of undeveloped individual if one uses the botanicals.

For household pest and disease remedies the plants were either soaked and drunk against diarrhoea, or smoked in closed doors against mosquitoes or rubbed on skin or just placed near bed. They included the following: *Cymbopogon citratus* (Poaceae)=Mosquito repellant; *Allium* spp (Liliaceae)=Snake repellent; *Cajanus cajan* (Papilionaceae);= Ear ache; *Cassia* spp. (Caesalpiniaceae)=diarrhoea; *Eucalyptus* spp. (Myrtac.), ARI= mosquitoes; *Euphorbia tirucalli* (Euphorbiaceae)=termites; *Hoslundia opposite* (Lamiaceae)= Mosquito cough; *Lantana camara* (Verbanaceae) = Red ants (*linthumbwi*) and mosquitoes; *Markhamia obtusifolia* (Leguminosae) =Mosquito repellent; *Ocimum cinum* (Labiatae)=Mosquito repellent; *Strychnos spinosa* (Loganiceae)=Small pox; *Thevetia* spp=Mosquito repellant; *Vernonia adoensis* (Compositae)= Mosquito repellent; and *Vernonia amygdalina* (Compositae)= Human diarrhoea;

Table 3: List of pesticidal plants on animal pests and diseases so far mentioned in Mkwinda and Malingunde EPAs and problem it controls

Local name	Botanical name /family	pest/disease controlled	Plant part used	Preparation method	Method of administration
Alovera	*Aloe vera* (Liliaceae)	Newcastle	Leaf	Crush and put in drinking water	drink
Mtangamtanga	*Albizia versicolor* (Mimosacea)	Diarrhoea	Bark	Soak in water	drinking
Chamba	*Cannabis sativa* (Cannabiaceae	African swine fever	leaves	Crush fresh & mix with maize bran	Let animal eat
Tsabola	*Capsicum* spp. (Solanaceae)	Newcastle	Fruit	Grind into powder & mix with *E. trucalli* roots in water	Chickens drink
Jerejere	*Cassia* spp. (Caesalpiniaceae)	Diarrhoea	Leaves,roots	Pound, soak, sieve; Cut, soak in water	drink
Chilambe	*Cissampelos mucronata* (Menisperm.)	Diarrhoea	roots	Cut, soak	drink
Chipwete cha lunda	*Cucumis* spp. (Cucurbitaceae)	Newcastle	Fruit	Cut in half, put water inside	Let chickens drink from the fruit
Mvunguti	*Kigelia Africana* (Bignoniaceae)	Newcastle	Fruit	Crush to powder, soak	drink
Dema	*Mucuna* spp. (Papilionaceae)	Newcastle	tuber	Dig out, pound, soak, sieve	Put in drinking water
Chitedze	*Mucuna* spp. (Papilionaceae)	Newcastle	roots	Chop, soak	drinking
Msolo	*Psueudolachnostylis* spp (Euphorbiaceae)	Various animal diseases	Bark	soak	Animals drink
Chewe	*Sessamum angolense* (Pedaliaceae)	Pig diseases	roots	Cut, soak, mix with bran	Eaten
Maye	*Strychnos spinosa* (Loganiceae)	Diarrhoea, ringworm, goiter	Fruits, roots	ash for smallpox, goiter & ringworm roots cut and soaked	Apply on skin, drinking infusion
Kasokolowe	*Uapaca sansibarica* (Euphorbiaceae)	Pig diseases	Bark	soak	drink
Futsa	*Vernonia amygdalina* (Compositae)	New castle disease	Leaves,roots	Pound , soak, sieve, Cut, soak	drink

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Potentials of locally-available plant materials as protectants of kolanut against *Balanogastris kolae* Desbr. (Coleoptera: Curculionidae) infestation during storage in Nigeria

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Abstract

The kolanut weevil, *Balanogastris kolae* (Desbr) is the most damaging insect pest of kolanut during storage in Nigeria. It is a field to store pest and can cause severe yield loss between 30 -70% in the store. The use of synthetic insecticides to control is usually discouraged due to environmenta l and health hazards. Besides, most farmers cannot afford the high cost of insecticides. In some rural kola -growing communities, locally available plant materials are used for protection of kolanut. These include *Citrus aurantifolia*, *Erythrophleum suaveolens*, *Leea procera, Jatropha curcas* among others. On the other hand, most kolanut traders apply chemical insecticides to store the nuts. However, this has grave health implications since the nuts are usually consumed raw. Against this background, there is need to screen, formulate and package some of the locally available plant materials for their protectant action against *B. kolae* in storage. This paper attempts to appraise the potentials and utilization of botanicals for the control of *B. kolae* in Nigeria.

Key words: kolanut, pesticide, plant materials, protectant, hazard

Introduction

The kola nuts belong to the family Malvaceae made up of 125 species. While the demand is rising, the production remains low due to several limiting factors amongst which are the insect pests. The insect pests of economic importance are the weevils- *Balanogastris kolae* and *Sophrorhinus* spp. They are field-to-store pests which can cause up to 100% loss if left uncontrolled in storage (Asogwa *et al.,* 2008). In most cases, kola nut farmers and traders apply synthetic chemicals; this has grave health implication s as kola nuts are usually consumed fresh without any secondary processing. In Nigeria, many local plant products such as peppers, citrus peels, pawpaw leaves, neem leaves have been identified to provide adequate protection to stored products (Asogwa and Osisanya 2003). Over 2,000 plant species have been reported to possess pesticidal activity but only a handful of pest control products directly obtained from plants are in use (Sundararaj, 2012). This review accentuates indigenous knowledge on locally-available plants with insecticidal properties for safe kola nut storage.

Kola nut production in Nigeria

The cultivated species of *Cola* are widely distributed throughout the Nigeria. Some farmers in the west usually intercrop cocoa with kola nut as a secondary crop where it also serves as a shade crop for cocoa. It is estimated that Nigeria produces about 70% of world kolanut with an annual production of 200,000 MT of fresh nuts mainly from South west Nigeria (CBN, 2002). The tree attains peak production at 15-20 years from planting. Kola fruits usually mature in 4-5 months after pollination, this followed by colour change from deep green to pale tint. Mass harvesting of pods at maturity is usually carried out. This is indicated by dropping of pods. Ideally, the harvesting should be carried out before the pods begin to split and drop to the ground to avoid weevil infestation. Usually fruiting takes place between September and January.

Processing and storage of kola nuts

The harvested follicles are broken to remo ve the seeds which are soaked in water for 18-24 hours or heaped in basket or bare ground with occasional sprinkling of water at regular intervals for 4 or 5 days. Kola nut processing can also be done by burying the seeds in moist soil in layers of 25cm. After a fortnight the nuts are dug up, because the seed coat has decayed sufficiently to be easily removed without damage to the nuts. Another method is by burying the seeds in termite heaps. The termites remove the seed coat and protect the nuts against attacks by insects and loss of moisture. In this case, the termite heap is rebuilt around the kola nuts. This is done to enhance removal of the seed coat. Thereafter, the nuts are skinned and rinsed in clean water, collected in wild flat baskets through w hich excess water drains off; they are then spread thinly on a mat for about 2 – 3 hours. Defective nuts are picked out at this stage. The nuts are graded into sizes for proper storage in unlined baskets, frequently stirred and covered lightly with banana leaves for about five days. The storage baskets are first lined up with thin black polythene sheet followed by a layer of fresh leaf materials like *Musa* spp, *Newbouldia laevis, Tectonia grandis, Marantochola* spp, *Terminalia catapa, Parkia biglobosa* or a host of others depending on which one is readily available in the locality (Asogwa *et al.,* 2012).

Storage insect pest of kola nut

The kola weevils are the most destructive pests of kola nuts in West Africa. There are only five species attacking kola nut in Nigeria, these are: *Sophrorhinus quadicstatus* Faust, *S. inseperatus* Fastus. *S. duvernoyi* Rouzet, *S. gbanjaensis* D & T and *Balanogastris kolae* (Desbr.) (Daramola, 1973). *B. kolae* is widely distributed in Nigeria (Asogwa *et al.,* 2008) and this collaborates earlier studies by Alibert & Mallamaire, 1955, that some of these weevils have a wide geographical distribution and that all the kola trees in Africa are believed to be infested. Weevil infestations of 30 – 70% and in some cases of late harvest 100% have been reported in the Ivory Coast, Guinea and Nigeria (Daramola and Ivbijaro, 1975). The weevils are field-to-store pests of kola nuts. Breeding continues throughout the year on left over nuts and nuts produced in-between the main harvest seasons (Daramola, 1974). The weevil infestation also predisposes the nuts to secondary invasion by other micro organisms, especially fungi which altogether lowers the market value and eventually results in total destruction of the nuts.

Various control strategies for the control of weevils in stored kolanuts

Cultural control

The cultural methods include timely and early harvest of mature pods re duces the level of weevil damage. Removal and proper disposal of fallen and infested pods and nuts at the end of main fruiting season as well as the unripe pods produced between crop seasons (Daramola and Taylor, 1975). Ndubuaku (1989) observed that regular removal of all weevil-infested nuts before and during storage can preserve kola nuts for as long as twelve months if this is done carefully. However, most kola nut farmers and vendors consider this method as laborious.

Chemical Control

Presently, only cultural methods are recommended for the control of kola weevils. The use of chemicals for the preservation of stored kola nut against pest is usually discouraged because it is considered to be unsafe. This is because kola nut is consumed in its raw form without further processing, chemical control of weevils must therefore take into consideration the level of residue of the pesticide in the treated nuts and the possible long-term effect of residue on consumers. However, in the absence of any recommendations on the use of pesticides for storage of kola nuts, the use of solutions of various chemicals in refill bottles has become a common practice among farmers and traders in all kola-producing areas in Nigeria. This is because of the combination of efficacy, ease of use and lost cost of these insecticides make them more attractive to the traders. Besides, in many developing countries, farmers are illiterate or speak only indigenous dialects, whereas pesticide labels are printed only in the official national language

Indigenous approach to control of weevils in stored kola nuts

In Nigeria, there is longstanding indigenous knowledge on the use of plants and plant extracts for preserving stored products against pests. Plant products used as protectants of stored agricultural commodities are normally obtained from leaves, roots, flowers, fruits, seeds, bark and stems. Some of the leaves include *Alchornia cordifolia, A. laxifolia, Artocarpus integrifolia,Citrus aurantifolia, Erythrophleum suavoleons, Rauvolfia vomitoria etc (*Kayode *et al.,* 2009). Other leaves used for the storage of kola nuts include *Thaumatococcus spp., Clinogyne spp., Dorax spp.*, *Sarcophrynium spp.* and *Maranthochloa spp*. (Ndubuaku, 1989). These plant materials are suspected to provide protection against the weevils; preserve the freshness and colour of the stored nuts for long periods. Studies carried out by Anikwe and Ojelade (2005) on the efficacy of *Tetrapleura tetraptera* fruit powder for the protection of kola nuts against the weevils as shown in tables 3 and 4 revealed the insecticidal potentials of the plant material.

Potentials of botanicals against *B.kolae* in kola nut during storage

Botanical insecticides are safe, environment- friendly, biodegradable and host specific (Bishop and Thronton, 1997). Botanical being a renewable natural resource offers better prospects as an integral component of agricultural pest control. It is estimated that only about 10% of the over 250,000 different plant species in the world today have been examined chemically (Farnsworth, 1990). Therefore, potential sources of plant chemicals still exist in the tropical forest which needs exploitation. They offer better compatibility with other biological pest control agents than the synthetics and this has brought them to sudden prominence in pest management programme. While economic benefits from the use of locally prepared botanicals are encouraging, the greatest benefit from the use of these materials may well be in terms of human health (Isman 2008). Furthermore, rural communities in developing countries could benefit financially through involvement in the cultivation and extraction of plants to produce botanical insecticides. In the context of agricultural pest management, botanical insecticides are most suitable for use in organic food production in industrialized countries (Isman, 2006) but they can be a component in integrated pest management (IPM) programs and post-harvest protection of stored products such as kola nuts in developing countries.

Conclusion

Although the potential of various plant species in pest management has been demonstrated, the plants have not been exploited commercially except for the neem tree. Several factors appear to limit the success of botanicals, most notable regulatory barriers and the availability of competing products (newer synthetics, fermentation products, microbials) that are cost-effective and relatively safe compared with their predecessors. As part of the recommendation, there is need to develop holistic efforts at exploiting and integrating botanicals as a central component of pest management in agriculture. Without doubt, some plant – based insecticides can boost the biopesticide industry with support in terms of commercial production and formulation as well as efficient delivery systems that are within reach of the rural farming communities and traders in commodity markets. Consequently, the Government, Private Sector and Non-Governmental organizations should be encouraged by the abundant diverse flora in the forests and savannahs of Nigeria to fund research on botanicals.

Table 1: Some plant materials used in the storage of kola nuts in Nigeria

S/N	Plant materials	Common name	Family	Parts used
1.	*Alchornea cordifolia*		Euphorbiaceae	Leaves
2.	*Alchornea laxiflora*		Euphorbiaceae	Leaves
3.	*Artocarpus integrifolia*		Moraceae	Leaves
4.	*Celtis zenkeri*		Ulmaceae	Bark
5.	*Chrysophylum albidium*	African star apple	Sapotaceae	Leaves
6	*Citrus aurantifolia*	Lime	Rutaceae	Fruit
7.	*Cymbopogon citrates*	Lemon grass	Graminea	Leaves
8.	*Erythrophleum suaveolens*		Caesailiniaceae	Stem bark
9.	*Leea procera*		Leeaceae	leaves
10.	*Jatropha curcas*	Physic nut	Euphorbiaceae	leaves
11.	*Mitragyna stipulosa*		Rubiaceae	Leaves
12.	*Musanga cacropioides*		Moraceae	Leaves
13.	*Raphia hookeri*		Areceae	Leaves
14.	*Rauvolfia vomitoria*		Apocynaceae	Leaves and stem bark
15.	*Sarcophrynium brachystachum*		Marantaceae	Leaves
16	*Senna siamea*		Caesalpiniaceae	Leaves
17.	*Theobroma cacao*	Cocoa	Sterculiaceae	Leaves
18.	*Zea mays*	Maize	Poaceae	Seeds
19.	*Cassia nigricans*	Indian senna	Caesalipiniaceae	leaves
20.	*Hyptis spicigera*	Beni-seed	Euphorbiaceae	leaves
21	*Mentha spicata*	Spear mint	Rubiaceae	Leaves

Source: Kayode *et al.,* 2009 and Ndubuaku 1999

Table 2: Effect of *Tetrapleura tetraptera* fruit powder on the mortality of *B. kolae* during kola nut storage

Concentration (g/kg nuts)	****Period of weevil exposure/weevil mortality**		
	2days	**7days**	**14days**
0	**0^{c}**	**0^{c}**	**0^{c}**
20	1^{c}	6^{c}	15^{b}
40	6^{b}	73^{b}	100^{a}
80	11^{a}	100^{a}	100^{a}
S.E	+1.5	+24.76	+26.88

* Each value represents five replicates; ** Means in columns with same superscripts are not significantly different (P>0.05) by Duncan Multiple Range Test (DMRT)

Source: Anikwe and Ojelade 2005

Table 3: Effect of *Tetrapleura tetraptera* fruit powder on the feeding marks, oviposition and damage caused by *B. kolae* during kola nut storage

Concentration (g/kg nuts)	**number of feeding marks**	**number of oviposition holes**	**%Damage on nuts**
0	**60^{c}**	**50^{c}**	**79.06^{c}**
20	50^{c}	47.5^{c}	77.92^{b}
40	19^{b}	17.5^{b}	12.22^{a}
80	5.5^{a}	5.0^{a}	8.41^{a}
S.E	+12.8	+9.64	+17.06

* Each value represents five replicates; ** Means in columns with same superscripts are not significantly different (P>0.05) by Duncan Multiple Range Test (DMRT)

Source: Anikwe and Ojelade 2005

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Economic Evaluation of Optimized Pest Management Options on Bean Cropping Systems in Malawi

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Abstract

Economic analysis was carried out to determine the economic viability of pesticidal plant products in beans cropping systems. Data for this was collected from agronomic trials carried out in 2010 -2011 cropping season in Malawi. In determining the most economically acceptable treatment, partial budget analysis and Marginal Rate of Return analysis were carried out to estimate the gross value of the component crops using the adjusted yield at 2010-2011 market prices for the crops and inputs. Results showed that higher net returns were obtained from higher net benefit of MK 431, 756.67 and MK 428, 584.47 were obtained from synthetic pesticide and *Vernonia amygdalina* 2% in kasungu, synthetic pesticide and *Tephrosia Vogelli* 2% returned higher net benefits of MK 248, 037.67 and MK 229, 119.97 respectively in Mzimba and In Rumphi higher net benefits of MK 64, 148.30 and MK 62, 537.00 were obtained from *Tephrosia Vogelli* 0.5% and *Tephrosia Vogelli* 2% respectively. However results of the MRR analysis indicated that fa rmers stand to gain better if they change from not using any pesticidal product to using pesticidal plant products especially those treatments diluted with soap.

Introduction

In Malawi apart from the staple foods, common beans (*Phaseolus vulgaris*) are a very important as sources of protein, micronutrients and essential vitamins particularly for poor households. However, they remain low priorities for crop improvement programmes at national and international institutions and government organization despite providing a rare opportunity for farmers to raise themselves out of poverty through the sale of excess product. Legume crop yields are, however, chronically low, often <400kg/Ha, largely attributable to insect pests and disease. Pesticidal plants are an effective but under-exploited alternative, and their promotion with optimized application through well informed extension services would impact on farmers' capacity to manage pests and improve productivity. From these plants, naturally occurring che micals are extracted. These natural pesticidal products are available as an alternative to commercial insecticides but they are not necessarily less toxic to humans hence protective clothing should be worn when spraying. Botanical insecticides break down readily in soil and are not stored in plant or animal tissue. Often their effects are not as long lasting as those of synthetic pesticides and some of these products may be very difficult to find (Nyirenda et al 2011). Malawi government is emphasizing on transforming subsistence agriculture to make farming as a business in rural communities. To achieve this it entails that farmers should be able to produce more from the small land sizes they have and be able to market the surplus. However agricultural produc tion is challenged by pests and diseases of which are the most expensive to control if industrial pesticides are used. There is therefore need of identifying the least costly pesticide that can enable bean farmers not only to be food secure but also generate income for other basic needs. Malawi being a country rich with various indigenous plants has the potential of exploring the most effective botanical plants that can control the pests and disease attacks. This will require clear understanding of the soci oeconomic contexts/constraints of farmers, agro-dealers, private and public sectors across Malawi, and identification of opportunities and constraints for increasing use of pesticidal plant product.

Literature Summary

Research has shown that, pesticidal plants are low costs compared to synthetic pesticides having most of them with multiple uses. For example, *Tephrosia vogelli* as reported by, (Kamanula et,al, 2011 and Nyirenda et.al 2011) is also cultivated for soil improvement. Hence farmers can have it in their fields for soil improvement and harvest the leaves for pesticidal plant products. This provides an opportunity to the farmers who can process more and sell the surplus hence generating farm income in their households. Despite the fact that bean production has been low, many farming families in Malawi rely on bean produce to meet their family requirements, both material and non-material, to achieve maximum economic returns and to reduce the risk in farming (Scott et al, 2003). They continuously judge the costs and benefits of their farming actions against these aims and they base their decisions of whether to change these if the net gain in terms of these set goals is positive (Clayton, 2005). Agricultural research and development organizations are now increasingly under pressure to shift from enhancing productivity of food crops to improving profitability and competitiveness of small-scale farmers.

Description of Research

Data for the economic analysis was corrected from agronomic field trials from Malawi specifically in Kasungu, Mzimba and Rumphi districts. The experiment was laid out in a randomised complete block design replicated four times in each site. The experiment was set to evaluate bean response to application of different pesticidal plant products and to determine application rates of the products. The experiment comprised of ten treatments namely, 1 - untreated Control, 2 - *Tephrosia vogelii* 2%, 3 - *Tephrosia vogelii* 5 %, 4 - *Tephrosia vogelii* 5% without soap, 5 - *Vernonia amygdalina* 2%, 6 - *Vernonia amygdalina* 5% , 7 - *Vernonia amygdalina* 5% without soap, 8 - Synthetic pesticide (seed dressing product or any fungicide then karate), 9 - *Tephrosia vogelii* 0.5%, 10 - *Vernonia amygdalina* 0.5%. All these treatments were planted on a 5m x 0.6m plots. In determining the most economically acceptable treatment, partial budget analysis was carried out to estimate the gross value of the component crops using the adjusted yield (Makinde et. al 2006) at 2010-2011 market prices for the crops and inputs/ The yields were adjusted due to the farmer's inability to reach the level of yield at the experiment. The prevailing rates paid to farm laborers at the location were used to estimate the labour cost that vary. The accruing net benefit and the costs that vary were then compared across the treatments in dominance analysis based on the criterion that any treatment that had net benefit equal to or lower than that of another treatment with lower cost is dominated and as such would not be considered for investment by the farmer (Makinde et al., 2006). Also, marginal analysis was carried out on the un-dominated treatments in a stepwise manner, starting from one treatment with the low est costs that vary to the next. This is to show how the net benefit from a decision to change from one cropping system to another increases with cost. Usually, a minimum rate of return is fixed as the baseline for acceptance of an option in order to accou nt for the cost of capital, inflation and risk. In this regard several authors have established that for the majority of situations, the minimum rate of return acceptable to farmers is between 40 and 100% (CIMMYT 1988; Dillion and Hardaker, 1993; Asumadu et al., 2004). A minimum rate of return criterion of 100% was set for the Marginal Rate of Return (MRR) analysis as the treatments require that farmers change from one cropping system to another without having to learn new skills or acquire new equipment's/ Consequently any treatment that returns MRR above 100% is considered worthy investment by farmers.

Research Results and Application

Grain Yield

There was no significant difference in the bean yield levels across the three . Results in Table 1 show only Va2% and synthetic pesticides recording a yield level of over 2000kgs per ha.

Table 2: Average Bean Yield Results from Kasungu, Mzimba and Rumphi Districts by Treatment Category

Id	Cont	Tv 2%	Tv 5%	Tv 5%wts	Va 2%	Va 5%	Va 5%wts	SP	Tv 0.5%	Va 0.5%	GM	P. value	CVT	CVL
AY	1833	2045	1748	1824	1825	1959	1892	2213	1381	1364	1808	0.156	19.4	35.2

AY- average yield; CVT- CV% treatment; CVL- CV% Location; GM-grand mean; SP-synthetic pesticide;

Partial Budget and Marginal Rate of Return (MRR) Analyses

Experience and empirical evidence have shown that for the majority of the situations, the minimum rate of return acceptable to farmers will be between 50 and 100% (CIMMYT, 1998). Since the technolo gies surrounding use of pesticidal plant products is new and a lot of processing, packaging is involved even procuring of sprayers and intensive training, the minimum acceptable rate of return adopted in the project is 100%, in which case some treatments fell outside the acceptable rate of return. Results indicated that higher net benefit of MK 431, 756.67 and MK 428, 584.47 were obtained from synthetic pesticide and *Vernonia amygdalina* 2%, Table 2.

The dominance analysis in Table 2 did not render any of the treatments unacceptable for investments as all treatments had higher net benefits at lower costs thereby subjecting all treatments for the MRR analysis. The

MRR analysis showed that in Kasungu, MRR for changing from Va 0.5% to Tv 2% was 102.90%, whilst from Tv 5 without soap to Tv 5% was 86,020% and Va 5% to synthetic pesticide was 1839.40. These results indicate that farmers stand to gain in return for every MK 100 invested in changing from no app lication to pesticidal plant products to application of pesticidal plant products at the stated amounts and form of application

Table 3: Dominance and MRR Analysis in Kasungu District

Treatment	Cost (MK/ha)	NB (MK/ha)	Dominance	IC (MK/ha)	IB	MRR%
untreated Control	60 183.33	368 981.67	U			
Tv 0.5%	60 469.91	365 387.59	U	286.58	(3594.08)	(1254.13)
Va 0.5%	60 469.91	303 580.09	U	0	(61807.50)	X
Tv 2%	61 173.03	375 934.47	U	703.12	72354.38	102.90
Va 2%	61 173.03	428 584.47	U	0	52650	X
Va 5% wts	61 355.21	417 602.29	U	182.18	(10982.18)	(6028.20)
Tv 5% wts	61 355.21	358 697.29	U	0	(58905.00)	X
Tv 5 %	61 407.41	403 600.09	U	52.2	44902.80	86020
Va 5%	61 407.41	392 192.59	U	0	(11407.5)	X
Syn. pesticide	63 558.33	431 756.67	U	2150.92	39564.08	1839.40

NB- net benefit; IC- incremental cost; IB-Incremental benefit;

However results from Mzimba showed that, synthetic pesticide and Tephrosia Vogelli 2% returned higher net benefits of MK 248, 037.67 and MK 229, 119.97 respectively Table 3. On the other hand, Dominance analysis results presented in Table 3 show that MRR f or changing from control to Tv 2% was 7866.05%, whilsts from Va 5% to Va 2% was 22,195.97% and Va 0.5% to synthetic pesticides was 1767.79%. Farmers are therefore better off in investing in the application of botanical pesticides of Vernonia amygdalina dil uted at 2%.

Table 4: Dominance and MRR Analysis in Mzimba District

Treatment	Cost	NB	D	IC	IB	MRR%
untreated Control	37,433.33	190,689.67	U			
Tv 2%	38,423.03	229,119.97	U	989.70	77,850.30	7866.05
Tv 5%	38,605.21	186,520.79	U	182.17	(167,274.38)	(91820.7)
Va 5%	38,605.21	186,637.79	U	-	82,258.20	X
Tv 5 %	38,657.41	145,365.59	U	52.20	(40,623.83)	(77823.4)
Va 5%	38,657.41	212,622.59	U	-	92,879.63	X
Va 2%	38,423.03	166,299.97	U	(234.38)	(52,021.80)	22195.97
Tv 0.5%	39,219.91	69,140.09	U	796.88	125,002.88	15686.64
Va 0.5%	39,219.91	83,180.09	U	-	(359,383.58)	X
SP	40,808.33	248,037.67	U	1,588.43	28,080.00	1767.789

NB- net benefit; IC- incremental cost; IB-Incremental benefit;D- dominance; SP- synthetic pesticide

Finally, in Rumphi higher net benefits of MK 64, 148.30 and MK 62, 537.00 were obtained from Tephrosia Vogelli 2% and Tephrosia Vogelli 0.5% respectively. Furthermore, dominance analysis presented in Table 4 indicates that all treatments were un-dominated hence MRR analysis was conducted on all. Results presented in Table 4 show that MRR for changing from control to Tv 2% was 1385.9%, whilst from Tv 5% to Va 2% was 986.87% and Va without soap 5% to Tv without soap 5% was 4951% a nd synthetic pesticides to Tv 0.5% was 549.3%. Farmers are therefore better off in investing in the application of botanical pesticides of Tephrosia vogelli diluted at 5 percent without soap.

Table 5: Dominance and MRR Analysis in Rumphi District

Treatment	Cost	NB	D	IC	IB	MRR%
untreated Control	44,725.00	50,432.00	U			
Tv 2%	45,714.70	64,148.30	U	989.70	13,716.30	1385.905
Tv 5 %	45,714.70	46,328.30	U	-	(17,820.00)	
Va 2%	45,896.88	48,126.13	U	182.17	1,797.83	986.867
Va 5% wts	45,896.88	53,463.13	U	-	5,337.00	
Tv 5% wts	45,949.08	56,047.93	U	52.20	2,584.80	4951.724
Va 5%	45,949.08	50,233.93	U	-	(5,814.00)	
Va 0.5%	46,511.58	47,565.43	U	562.50	(2,668.50)	(474.4)
SP	46,511.58	53,811.43	U	-	6,246.00	
Tv 0.5%	48,100.00	62,537.00	U	1,588.43	8,725.57	549.3224

NB- net benefit; IC- incremental cost; IB-Incremental benefit;D- dominance; SP- synthetic pesticide

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Evaluation of pesticidal plants effectiveness in maize and cowpea storage in Zambia

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Abstract

Pesticidal plants have been used by African farmers for generations and are of particular importance to poor, small-scale farmers for effective, low -cost pest control on field crops, stored products and livestock. A better scientific understanding of how these plants are used and work is crucial for their sustainable use. A research was conducted in Choma Zambia toevaluate the effectiveness of five pesticidal plants in controlling a wide range of pests during storage of maize and cow peas. Field trials were set up to using concentrations of 2% and 5% w/w. Samples were also collected and analysed on a monthly basis for 32 weeks. All the plant materials were effective for 16 weeks. In maize storage *Securidacalongepedunculata*treatments (2%and 5%) had less than 10% damage up to 24 weeks after which all treatments were not effective. In cowpeas pest control lasted for only eight weeks for most treatments. *Cissusquandrangularis*and *Bobgunnia madagascariensis* treatments were effective until week 16 and 32respectively . The study showed that although all the pesticidal plants used had some pesticidal activity but none lasted the whole storage season.

Key Words**:** Pesticidal plants, Sitophilus spp., *Callosobruchus spp., Securidaca, Bobgunnia, Cissus*

Introduction

Pests have been found to pose one of the greatest challenges to food security especially in Africa.Thus grain storage by small holder farmer is of crucial importance in reducing annual food deficits. However, loses in storage remain quite highrangingbetween 20% and 30% which shows that smallholder farmers still have great challenges in post-harvest pest management(Ogendo et al, 2003). Most of the losses are to *Callosobruchus spp.*, *Prostephanus truncates, Sitophilus* spp, and*Tribolium (*Chikukura, *et al.,* 2011). Although most farmers use synthetic pesticides, pesticidal plants remain important to most poorsmall scale farmers for pest control on field crops, stored products and livestock(Nyirenda et al., 2011; Kamanula et al., 2011).There is an argument that botanical pesticides are generally not as powerful as synthetic chemicals; have relatively low, variable and sometimes unknown efficacy levels. In the past two decades, there has been a lot of field research in sub Saharan Africa to evaluate a number of pesticidal plants. These studies have all concluded that evaluated plant species exhibited pesticidal activity. Some of the species studied include *Lippiajavanica*; *Securidacalongepedunculata*and *Tagetesminuta* (Katsvanga et al., 2004; Belmain et al., 2001;Chi kukura, et al., 2011; Muzemu et al., 2011). The objective of the study was to evaluatethe effectiveness of five plants species in controlling storage pests in maize and cowpeas in Choma district of Zambia.

Research Description

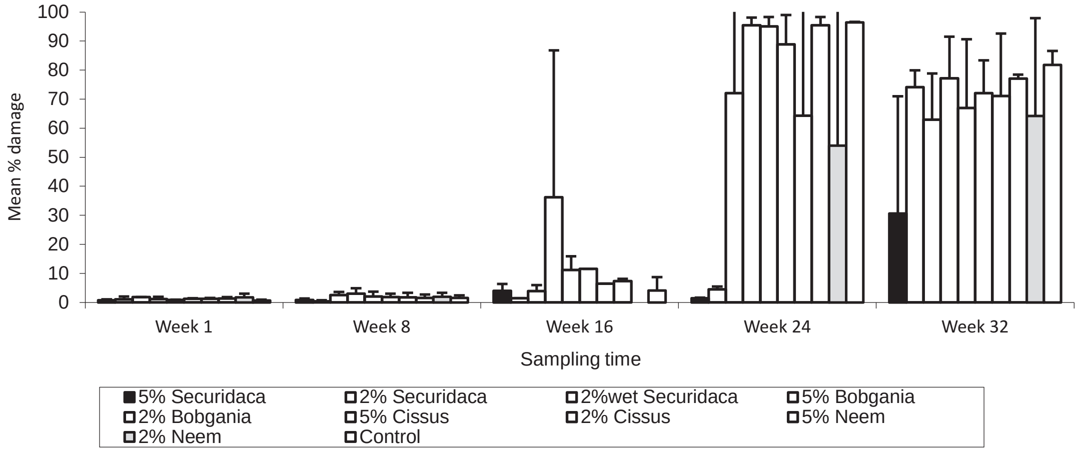
The study was conducted in Nachibanga ward in Choma district of Zambia. The area is characterised by average rainfall of 800mm per year. Vegetation is of typical miombo woodlands dominated by *Brachystegiaspiciformis*and*Julbernadiaglobiflora*. Maize, cowpeas and groundnuts are the major crops grown.Storage trials were established in the 2008/09 seasons. Collection and processing of the plant material was based on the farmer method. This was mainly collecting the material, drying in a shade, pounding to powder using pestle and mortar and then sieving.Two treatments were prepared for each species; 2% w/w and 5%w/w concentrations. These were applied to 20kg of maize and 5kg of cowpeas respectively. Treatments were applied at a central place before being distributed to three organised farmer groups who were used as replicates. Each group had a contact farmer who hosted the trials. The trials ran for 32 weeks which is close to the nine months storage season.

Samples of 1000g and 500g maize and cow peas respectively were collected and analysed for number of pests, and weight loss due to damage on an eight week interval. Farmer groups were also trained in recording observations using a standard format. Community meetings were then held where contact farmers presented monitoring data from the trials and researchers gave feedback on the laboratory analysis.

Results and Application

All the plants evaluated exhibited some form of pesticidal activity; however this did not last to the end of the storage season. Mean grain losses were maintained below 10% for only 25% and 50% of the storage period in cowpeas and maize respectively. However *S. longepedunculata* was effective for an extra 4 weeks in maize while *B. madagascariensis* and *C. quandrangularis* reduced damage for slightly longer in cowpeas (Figure 1).

(a)



(b)

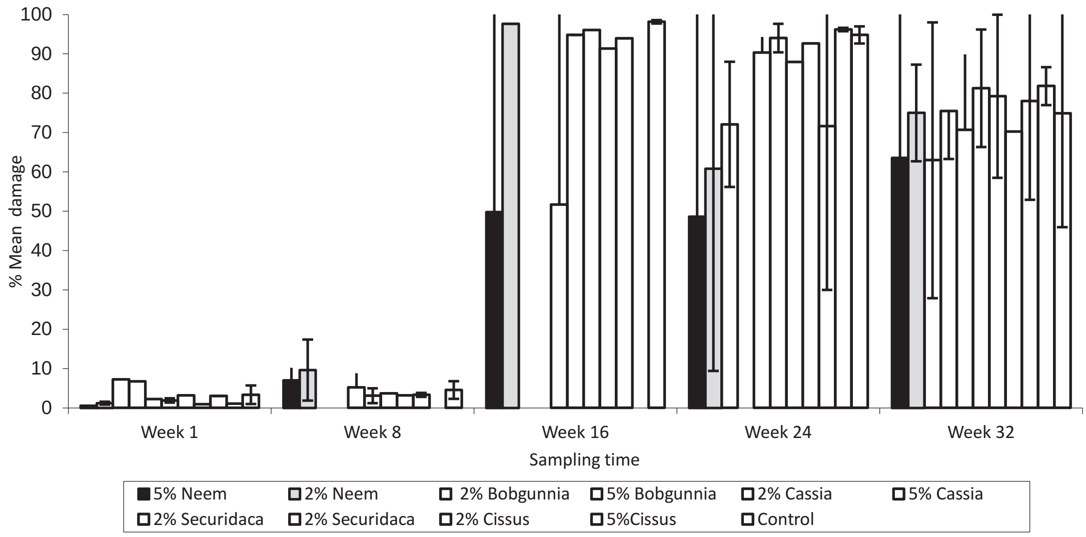


Figure 1: Mean percentage damage (± SEM) in treated and untreated (a) maize grain and (b)cowpeas

Throughout the trials pest count continued to increase almost exponentially in all treatments. However all treatments had fewer pests compared to the control throughout the trial period. *S. longepedunculata* and *B. madagascariensis* had the least number of pests in maize which were dominated by *Sitophilus* whilst in cowpeas *B. madagascariensis and C. quandrangularis* registered fewer pests with *Callosobruchus* accountingfor most of the damage (Figure 2).

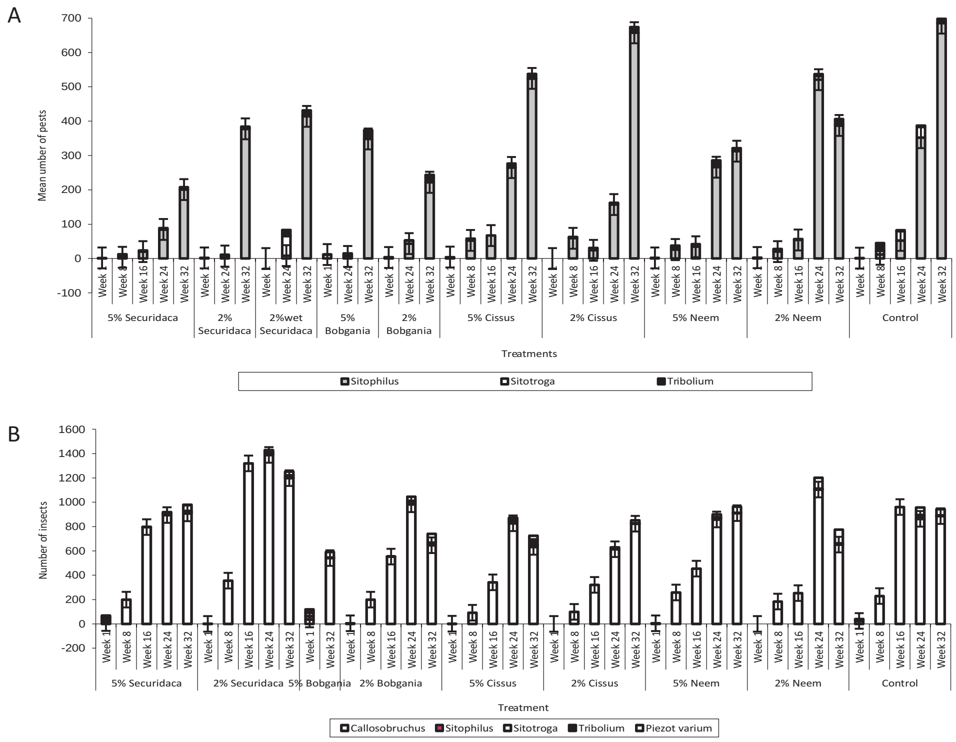


Figure 2: Mean number of insects per kg of treated and untreated (a) 1000g of maize and (b) 500g of cowpeas

The challenge is that most of the active ingredients and their mode of action are not known. Thus, the future of the pesticidal plants lies in the development of consistent and effective products that havea long enough shelf life. However when this happens most often the product is priced out of reach for most resource poor farmers. It is therefore recommended that more research be conducted to improve the farmer method of harvesting, extraction and application in conjunction with the development of commercialproducts. The other challenge is the availability of the pesticidal plantsas most species are sparsely distributed. Coupled with the product research there is great need for exploring propagation options for such species.

We conclude that all evaluated plants have pesticidal properties to varying degrees with *S. longepedunculata, B. madagascariensis* and *C. quandrangularis* being the most effective. The potential and success of pesticidal plants in improving food security by reducing losses in storage will be determined by the investments in further research and development of sustainably harvested, consistent and effective products.

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Farmers' ethno-botanical knowledge of termiticidal plant uses in Zambia

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Abstract

A survey was carried out on ethno-botanical knowledge of termiticidal plants in Chongwe district, Lusaka province, Muswishi area, Central province, and several districts in Southern province of Zambia. The methods used in collecting the data included Focused Group discussion, questionnaires and transect walks among small-holders fields. The study revealed that five plant species in Chongwe district, four plant species in Muswishi district, Central province, and fourteen plants from Southern province were believed to contain putative termiticidal toxicity. The most widely used termiticidal plants in all the study areas were *Tephrosia vogelli* Hook.f, *Bobgunia madascariensis* Desv and *Euphorbia tirucalli* L. The study has shown that indigenous knowledge of termiticidal plants is a novel component that can become part of an Integrated Pest Management (IPM) strategy for use by resource poor farmers. Putative termiticidal properties of selected plants are discussed. Popularization and commercialization of these ethno-botanical termiticides can help increase food security in Zambia.

Introduction

Zambia (15° 0' and 30° 0' E) is a Southern African land-locked country covering 752, 612 km^2. Although the country has 42 million hectares of arable land, less than 10% is cropped annually, and most of the cropped land is under rain-fed agriculture. Zambia has a mixed economy consisting of a modern and urban-oriented sector confined to the line of rail, and a largely rural agricultural set up. Agricultural inputs are expensive and delivered late to the resource-poor farmers that produce 80% of the country's food/ Malnutrition is also rife as close to 40% of children are stunted. Thus, there is an urgent need for agricultural research to avail low-cost inputs including early maturing, high yielding, and pest/disease/drought/acid-resistant crop cultivars.
Due to climate change, termites have emerged as one the major pest problems in Zambian agriculture, leaving resource-poor farmers with few alternative control methods in the face of a world ban on persistent organic pollutants, which were effective against pestiferous termites. In this paper, we report on the farmers's ethno-botanical knowledge of plants used to manage pestiferous termites in crops, forest plantations, and fruit trees found in Chongwe district (Lusaka province) Muswishi district (Central province), and Southern province, Zambia, as alternative termite control methods.

Literature Summary

Although credible information on the economic losses caused by termites is difficult to obtain, in most African countries, losses appear sporadic and localised, but in some countries, it is more widely spread and catastrophic, often causing 100% losses. In Southern province termites were recorded as serious pests with 89% farmers experiencing problems (Nkunika, 1982). Generally termite attacks in Zambia result in pre - and post-harvest maize losses ranging from 20-100%, especially in times of drought. The most important pestiferous termites in Southern province have already been documented (Nkunika, 1982; 1998).
Among rural-poor farmers that produce the lion's share of Zambia's food stock, the insect pest scenario and severity is exacerbated by farmers' inability to afford the expensive insecticides/ Still, widespread use of

chemical insecticides has several health and ecological disadvantages including its harmful effects to the environment, humans, and non-target organisms. Therefore, since the 1990s, research emphasis begun to shift to integrated pest management (IPM) strategies that focussed on less use of inorganic pesticides (Chinsembu and Okech, 1990).

Botanical pesticides are seen as a cheaper and affordable alternative to synthetic chemical pesticides. In Zambia, laboratory and field experiments showed that *Tephrosia vogelii,* had insecticidal properties against maize stem borers and *Cicadulina* leaf hoppers, the vectors of maize streak virus (Mugoya and Chinsembu, 1995). However, data on the ethno-botanical knowledge of plants used to control termites in crops and forest trees in Zambia is still anecdotal. This is despite the fact that termites are major pests of agricultural crops, forest plantations, tea plantations, agroforestry, and wood en timber in Zambia (Nkunika, 1992; Nkunika, 1998; Sileshi et al., 2005).

Description of Research

The study was conducted in Chongwe district (Lusaka province) Muswishi district (Central province), and Southern province, Zambia. Data were collected using three main approaces consisting of focus group discussions, questionnaires and transect walks. Semi- structured questionnaires were used to solicit detailed information on the kind of ethnobotanical methods used to manage pestiferous termites in the study areas. In southern province a total of 120 randomly selected small scale farmers were interviewed, in Chongwe district 100 randomly selected small scale farmers were interviewed and in Muswishi district 20 randomly selected small scale farmers were interviewed giving a total of 240 interviewed farmers. The interviews took place on the farm.

Research Results and Application

Throughout Africa, resource-poor small-holder farmers use their Indigenous knowledge (IK) of plants to help protect field crops, stored grain and livestock from damage caused by pests. This information, which is disappearing very quickly, has been passed down from generation to generation and offers an effective, low cost, sustainable and environmentally friendly pest management strategy. Nkunika (2002) found that farmers in Muswishi district who used indigenous plants to manage maize pests such as termites increased their yields by up to 37%. The collected data in this survey are summarised in tables 1, 2 and 3.

Table 1: Species of indigenous plants with putative termiticidal properties used against pestiferous termites in Chongwe district, Lusaka province, Zambia

Species name	Local name (s)	Method of application	Plant part used
Tephrosia vogelli	Ububa	Leaves are soaked in water; the filtrate is sprayed on affected plants.	leaves
Bobgunnia madagascariensis	Ndale	Dissolve ground pods into water for 24 hours. Spray the filtrate on affected plants.	pods
Azadirachta indica	Neem	Grind the leaves and soak in water for 24 hours. Sieve and spray the filtrate on affected plants.	Leaves
Capsicum frutescens	Impili-mpili	Grind chilly fruits and soak for 24 hours. Sieve the mixture and spray on tree seedlings.	Fruits
Euphorbia tirucalli	Ulunsonga	Soak roots for 24 hours. Sieve and spray the filtrate to affected plants.	Roots

Table 2: Species of indigenous plants with putative termiticidal properties used to control pestiferous termites in Muswishi district, Central province, Zambia

Species name	Local name(s)	Method of application	Plant part used
Tephrosia vogelli	Ububa	Leaves are soaked in water; the filtrate is sprayed on affected plants.	Leaves
Bobgunia madagascariensis	Ndale	Ddissolve ground pods into water for 24 hours. Spray the filtrate on affected plants.	Pods
Euphorbia tirucalli	Mulundungoma/Lunsonga	Roots are soaked in water for 24 hours; the solution is sprinkled on the affected crops.	Roots
Albizia antunesiana	Mukoso	Pods are crushed and mixed in water. The filtrate is sprayed on affected plants.	Pods

Table 3: Species of indigenous plants with putative termiticidal properties used to control pestiferous termites in Southern province, Zambia

Species name	Local name (s)	Method of application	Part used
Bobgunnia madagascariensis	Muyongolo	Dissolve ground pods into water for 24 hours. Spray the filtrate on affected plants.	Pods
Crossopteryx febrifuga	Muleyembezo	Peel of the bark and grind. Soak ground powder into water, and then spray the filtrate on affected plants.	Roots
Julbernardia globiflora	Mumba	Soak leaves in water for 24 hours. Sieve the mixture and spray around the bases of affected fruit trees.	Leaves
Cassia abbreviata	Mululwe	Grind the bark and mix in 5 litres of water together with the dry preparation of *B. madascariensis*. Pour on the affected tree.	Bark
Solanum incanum	Ntuntulwa	Cut, pound and soak the fruits in water for 12-24 hours. Sieve and spray the filtrate on affected crops.	Fruit
Ricinus communis	Mbono	Soak green leaves and water for 24 hours. Filter and spray the filtrate on affected plants.	Seeds and leaves
Euphorbia tirucalli	Mbala	Pound the leaves; soak in water for 24 hours. Spray the mixture on affected plants.	Leaves
Tephrosia vogelli	Ububa	Grind fresh leaves and soak in water for 24 hours. Strain and spray the filtrate on affected plants.	Leaves
Capsicum frutescens	Impili-pili	Grind 500 g of chilly fruits and soak for 24 hours. Sieve the mixture and spray on tree seedlings	Fruits
Strychnos cocculoides	Muwi	Peel bark and pound together with fruits. Soak in water for 24 hours. Sieve the mixture and spray the filtrate around bases of affected plants	Bark and Fruit
Xerodensis stuhmaanii	Mulombe, Muzwamalowa	Strip the bark and soak in water overnight. Sieve and spray the mixture on affected plants.	Bark
Nicotiana tabacum	Tombwe	Crush fresh leaves and soak in water and add powdered soap. Sieve and spray the filtrate on affected plants	Leaves
Strychnos spinosa	Muntamba	Pound fresh fruits and soak in water and leave overnight. Sieve and spray the filtrate on affected plants.	Fruits
Maerua edulis	Sozwe	Spread leaves or roots over and under maize.	Leaves

In this survey, 23 species were used as termiticidal plants in Zambia. Some of these species are already confirmed as pesticidal plants: *T. vogelii* (Stevenson et al., 2012), *B. madagascariensis* (Stevenson *et.al*., 2007), *A. indica* (Isman, 2006), and *C. frutescens* (Gervais et l., 2008). However, other plant species such as *A. antunesiana, E. tirucalli, C. febrifuga, J. globiflora, S. incanum, R. communis, S. cocculoides, X. stuhmaanii, S. spinosa* and *C. glauca* require scientific validation, efficacy evaluation and popularization for use as termiticidal plants.

Elsewhere in Africa, *B. madagascariensis* (syn. *Swartzia madagascariensis*) is reported by farmers to have anti-insect toxicity particularly in stored grain where it is used as a powder and against field pests as a water extract sprinkled onto the plants (Stevenson et al.,, 2007). In Zambia, this plant had repellent effect against termites. Azadirachtin is the most active compound in the neem plant. Neem derivatives do not kill but modify the biological processes of harmful insects in a detrimental way. Their actions include antifeedant or phagodeterrant effect, larval repellent, oviposition deterrent, growth and metamorphosis inhibiting effects, and negative effects on fecundity and egg sterility. *C. frutescens* contains capsaicin, an animal repellent that has also been registered for use as an insecticide, miticide, rodenticide, and feeding depressant (Gervais et al., 2008). *Euphorbia* spp. were also found to have termiticidal activity (Mwine, 2011). *Solanum incanum* and

Ricinus communis were toxicant to termites. Kaposhi *et al.* (1995) showed that extracts of *C. abbreviata* produced low mortality in ticks and insects. *S. incanum* contains solamargine alkaloid as the main active ingredient. This concoction was found to be effective against termites in southern province. Elsewhere, the castor bean *R. communis* contains ricinine, an active compound against the fall armyworm *Spodoptera frugiperda* (Ramos-Lopez et al., 2010). In this survey, concoction made from this plant was found to deter termites. According to the Southampton Centre for Underutilised Crops (SCUC), fresh leaves of the Monkey Orange, *Strychnos cocculoides*, are crushed and soaked in water, and the drained liquid is used for spraying vegetables to repel insects (SCUC, 2006). In this survey, the filtrate from this plant was found to be effective against termites.

In conclusion this study has clearly shown that there is a wealth of indigenous knowledge on plants with termiticidal properties in Zambia. However, further research is needed to standardize termiticidal dosages as well as to isolate active termiticidal chemical compounds.

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Use of *Crotalaria* species in cropping systems as nematode antagonistic plants: a review

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Abstract

Crotalaria species are non-or poor host of plant-parasitic nematodes and when incorporated in various cropping systems as cover crops, green manure for soil amendment, rotation crops or when intercropped with nematode susceptible crops, they can reduce nematode damage below the economic injury levels. Various mechanisms of nematode antagonism involved include production of toxins that directly repel, kill, inhibit growth or immobilize nematode movement. As leguminous crops and in association with rhizobium bacteria , they increase the nutritive status of the plants thus making them more tolerant to nematode infection. Moreover, they attract nematode predators like bacterivorous and fungivorous nematodes that feed on the nematode or help in nutrient recycling which in turn promote plant growth. The objectives of this review are to summarize the knowledge of the efficacy of *Crotalaria* spp. as plant-parasitic nematode antagonistic plants, the mechanisms of nematode suppression, nematode antagonism by different *Crotalaria* application methods in cropping systems and outline the constrains of using the crop in relation to nematode management.

Introduction

Crotalaria species have been known to effectively suppress populations of root knot nematodes (Hooks et al., 2010; Costa et al., 2012) and reduce or even eliminate plant parasitic nematodes, especially root-knot nematodes (Wang and Sipes, 2009) when used in cropping systems. There are several hypotheses on how these crops can enhance nematode-antagonistic activities and a series of events may be involved starting with the decomposition of organic matter that leads to proliferation of bacteria which becomes food base for microbiovorous nematodes. In turn these nematodes serve as food source for nematophagous fungi (Wang et al., 2002). Leguminous crops like *Crotalaria* spp. enhance nematophagous fungi better than other crops making them very useful nematode antagonistic crops. *Crotalaria species* are also known to effectively suppress plant-parasitic nematodes though the production of toxins which either kill or inhibit their development in plant tissues. In some instance, these compounds reduce the ability of the nematodes to sense their hosts which make them starve.

Most plant-parasitic nematodes suppressed by *Crotalaria* species are sedentary endoparasitic nematodes, which are nematodes that remain and feed in one place within the root system. They include root-knot (*Meloidogyne* spp.), soybean cyst (*Heterodera glycines*) and reniform (*Rotylenchulus reniformis*) nematodes. Few migratory nematodes such as sting (*Belonolaimus longicaudatus*), dagger (*Xiphinema americanum*), and burrowing (*Radopholus similis*) nematodes are also suppressed by the genus *Crotalaria.* Most migratory nematodes are not suppressed by *Crotalaria* and these include: spiral (*Helicotylenchus* spp. and *Scutellonema* spp.), stubby root (*Paratrichodorus minor*), ring (*Mesocriconema xenoplax*) and Root-lesion (*Pratylenchus* spp.,) nematodes (Marla et al., 2008; Marahatta et al., 2010).

Literature Summary

Nematode antagonism by different crotalaria application methods

The suppressive effect of *Crotalaria* on nematodes is variable for all the application methods. This variation is due to genetic variation within the nematode species, crop cultivars, cropping season, field history, cover cropping system, concurrent field practices or edaphic factors including various biological components associated with the *Crotalaria* rhizosphere or soil amendment (Wang et al., 2002; McSorley and Frederic, 2002).

Intercropping

Mode of suppression by intercropping with *Crotalaria species* is specifically to attract root nematodes thereby diverting or reducing the nematode population and increasing yield of the companion crops planted (Garrido et al., 2008). In mixed cropping/intercropping, the compounds from *Crotalaria species* may interact with other classes of chemicals from neighbouring plants and these compounds sometimes may have a greater allelopathic effect than the individual compounds alone (Wang and McSorley, 2004). In a yam intercrop where yam planting system were intercropped with *Crotalaria* and with *Crotalaria* combined with pigeon pea, the lowest mean values of yam root infection with *S. bradys* and *R. reniformis* were observed. The conventional yam planting system and the intercropped system with pigeon pea between yam planting rows presented the highest number of nematodes infecting yam fine roots (Garrido et al., 2008).

Crop rotation

The use of rotational crops that are non-hosts, poor hosts, or antagonistic to nematodes is one of the oldest and most effective cultural practices for controlling nematodes (Widmer and Abawi, 2000; McSorley, 2011). *Crotalaria species* are used in crop rotation to decrease nematode population levels and propagate abuscular micorrhizal fungi for subsequent crops (Germani and Plenchette, 2004). The species in a crop rotation can therefore be more effective than synthetic or natural nematicides. Rotating tobacco with *C. fulva, C. grahamiana, C. intermedia, C. juncea*, or *C. spectabilis* has been suggested to suppress damage of root-knot nematode in tobacco (Shepherd and Barker, 1993). Natural enemies associated with *C. juncea* migrated to agricultural crops when the plants were grown in systems of rotation or succession (Gill et al., 2010).

Cover cropping

Modes of nematode suppression by cover crops can be categorized as providing a non-host or a poor host environment for nematodes, (Widmer and Abawi, 2000) producing allelochemicals and enhancing nematode antagonistic flora and fauna or acting as a trap crop to the nematode. Using *Crotalaria* spp. as a cover crop could offer an alternative for managing nematodes (Wang and McSorley, 2010) as they support nematode invasion/ activity but do not permit completion of the life cycle. Germani and Plenchette (2004) reported that the J2 of M. *incognita* and *M. javanica* of inoculated on *Crotalaria* spp. did not evolve into adult females unlike those inoculated to tomatoes. Therefore these plants when used as cover crops, they can act as nematode trap crops hence reducing nematode populations in the soil before a susceptible crop is planted or in subsequent crops.

Green manure for soil amendment

Crotalaria species seem to have a number of desirable agricultural characteristics that suggest that they have a potential to be used as green manure crops and they are extensively used in the tropics (Garrido et al., 2008; Ossom et al., 2010). These plants produces root exudates with nematicide properties and might also produce other compounds with nematicide properties during decomposition in soil. The root tissues when decomposing in soil may release toxic byproducts that kill the nematode juveniles (Marla et al., 2008). When incorporated into the soil the crop produces monocrotaline, (Wang et al., 2004; Marahatta et al., 2010) a compound that inhibits nematode movement and delays nematode development thus it was recommended for managing nematodes before planting pineapple in Hawaii (Wang and Sipes, 2009). Rich and Rahi (1995) reported that soil amended with *C. spectabilis* seeds suppressed *M. incognita* and *M. javanica* better than non-amended soil. In a greenhouse study with soil amended with plant fresh biomass (*Crotalaria*, pigeon pea, and *Crotalaria* combined with pigeon pea) to evaluate the infectivity of *S. bradys* on tomato plants it was observed that the treatments with amendment of fresh *Crotalaria* aerial parts and *Crotalaria* combined with pigeon pea, presented the lowest numbers of nematodes infecting the roots (Garrido et al., 2008). *Crotalaria* beneficial effects are not restricted only to nematicide effects. Studies have demonstrated an increase in nematophagous fungal populations by the incorporation of *C. juncea* aerial parts to soil, and also an increase in nematode predators (Wang et al., 2004). Amendment with C. *juncea* as green manure crop has been repeatedly found to enhance nematode-trapping fungi and has been shown to stimulate population growth of free-living nematodes in soils infested with *Rotylenchulus reniformis* or *Meloidogyne incognita* (Wang et al., 2002, 2003a,b).

Mechanism of Antagonism

The mechanisms by which Crotalaria spp. limit plant-parasitic nematode proliferation are not clearly known, but it may be due to the nematicidal, nematistatic or nematirepulsive compounds contained in t he root system and/or in the aerial vegetative parts (Wang et al., 2004). Nematode mortality might also be due to some nematotoxic compounds released from the roots in to the water. Root exudates may serve as an alternative to chemical nematicides in organic production systems (Marla et al., 2008). These crops inhibit egg masses formation and production of root-knot galls (Mbogoh et al., 2012) with effectiveness comparable to a known resistant plant. The few parasites that penetrated the roots generally do not develop beyond the infesting stages (J2) (Sano, 2005). Some are able to develop genital primordium, but do not reach sexual differentiation. Plant extracts inhibit egg hatching and are lethal to *M. incognita* second stage juveniles and for *R. similis* (Jasy and Koshy, 1992). They also increase nematophagous fungal populations and nematode predators (Widmer et al., 2002). As leguminous crops and in association with rhizobium bacteria, they increase the nutritive status of the companion or subsequent plants thus making them more tolerant to nematode infection.

Constrains of using crotalaria in relation to nematode management

The wide host range of nematodes especially the most common root knot nematodes (*Meloidogyne* spp.) limits the choices of potential crops that may be antagonistic or non-hosts to these important pests when used in a cropping system (Widmer et al., 2002). *Crotalaria* spp. is a poor host to many plant-parasitic nematodes but nematode numbers can resurge to damaging levels on subsequent host crops (Germani and Plenchette, 2004). This is because effects from suppressive rotation crops are typically short -lived, as the residue effects are short term (Wang and McSorley, 2010) with nematodes recovering following a season of a susceptible crop.

Conclusion and Recommendations

Although sequential cropping is recognized as a strategy for nematode control, its adoption especially by small scale farmers is market-driven. A lot of skill is required to design and implement effective crop cycles for the control of such pathogens as nematodes because they have broad host ranges. Suitability of a crop for incorporation into a cropping cycle is not only determined by its efficiency in nematode suppression but also by its economic returns to the farmer. *Crotalaria* is a vegetable that is gaining wide acceptance in the market and therefore can be used in cropping systems as a commercial crop as well as a nematode antagonistic crop. *Crotalaria* produces allelopathic compounds that are toxic to many plant-parasitic nematodes. They also increase nematophagous fungal populations and nematode predators. As leguminous crops and in association with rhizobium bacteria, they increase the nutritive status of the companion or subsequent plants thus making them more tolerant to nematode infection. Due to this multiple nematode suppressive effects, these plants should be incorporated in cropping systems as natural environmental friendly nematode antagonistic plants. As indicated in this review, not all plant parasitic nematode can be managed by *Crotalaria* species and therefore it is important to find a *Crotalaria* species that is antagonistic to the nematode species that need to be controlled.

This scenario strongly suggests that integrating *Crotalaria* in cropping system with other nematode management strategies is necessary. This is supported by the fact that nematode management is rarely successful in the long term with uni-tactic approaches. It is important to integrate multiple-tactics into a strategy. *Crotalaria* offers the potential to be one of the tactics. Among the possibilities for integration are crop resistance, enhanced crop tolerance, selection for fast growing crop varieties, soil solarization, and biological control.

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Effects of antifungal plant extracts on some selected physiological parameters of French beans (*Phaseolus vulgaris*)

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Abstract

Rust (*Uromyces appendiculatus*) is a major foliar disease that reduces yields and pod quality in beans. The field trial of French beans was established at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in a Randomized Complete Block Design replicated 4 times. The plots were 3 × 4 m with 0.5 m paths between plots and 1.5 m between blocks. Variety Amy was planted at a spacing of 30 × 10 cm within and between rows. During the growing period, beans were infected with rust from natural inoculum at the field. Three antifungals belonging to different genera were applied as bio-pesticides to control rust. Physiological responses such as carbon dioxide assimilation, Transpiration (E), Stomatal conductance (gs), and Photosynthetic rate (Pn) of French beans treatments were examined using Infrared gas analyzer (IRGA) in all treatments. *B. angustifolia* - *X. chalybeum* combination and single plant treatment *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants.
Keywords: antifungal, beans, physiological responses, rust.

Introduction

The importance of the French beans is due to their high nutritive value in both energy and protein contents. Therefore, increasing the crop production is one of the most important targets of agricultural policy in several countries. The bean rust fungus (*Uromyces appendiculatus*) is of worldwide importance as a yield-reducing disease of *Phaseolus vulgaris*, potentially causing yield losses up to 50% (Venette and Jones, 1982; Berger *et al.,* 1995; De Jesus Junior *et al.,* 2001). On global scale, studies have shown that some plant species have antifungal compounds (Okemo *et al.,* 2003). Neem cake (*Azadirachta indica*) significantly suppressed population of fungal pathogens such as *Fusarium oxysporum*, *Urtica massaica* L. leaf extracts reduced the severity of potato late blight (*Phytophthora infestans*) and *Maesa lanceolata* has been reported to have antifungal activity (Okemo *et al.,* 2003). A large number of chemicals have been developed for the control of diseases. But due to overgrowing awareness of the hazardous side effects of these chemicals, more and more emphasis is being given to the use of biocontrol agents. Within this context, natural products from plants seem to be a good alternative since numerous plants have the potential to control phytopathogenic fungi, and have much prospect to be used as a fungicide. Despite the many studies performed on biological control, relatively little is known about the role of the plant extracts applied on the physiological parameters of the plants. In this study; we hypothesized that antifungal plant extracts might influence physiological activities of bean plants. Therefore, this study aimed at studying the role of selected plant extracts (added singly or in combination) in influencing photosynthetic activities of bean plants and finding an explanation for the above role based on test attributes.

Literature Summary

The proper use of pesticides takes on significant meaning in Kenya especially with quality regulations and requirements for horticultural imports under review in the European Union (EU). In East Africa, the use of synthetic pesticides has been the major method of pest control. These pesticides have been found to be hazardous to man and environment and are therefore not conducive to support sustainable agriculture. Bean rust, caused by the fungus *Uromyces appendiculatus*, is a common and serious disease of French beans worldwide but is most prevalent in tropical and sub-tropical areas (Robert, 1991). It causes 25 - 100% yield loss depending on the stage of infection and the prevailing weather conditions (Schwartz *et al.,* 2004; Robert, 1991). Kenyan French beans are largely exported to the European markets where consumers demand aesthetic quality products that are disease free. This has generally encouraged excessive use of chemical pesticides in French bean production in Kenya (Mwanthi and Kimani, 1990; Jaffee, 2003; Farina and Reardon, 2000). Chlorothalonil and copper based fungicides have been effective in the control of bean rust (Gerhardson, 2002) but indiscriminate use of these chemicals has often resulted in adverse environmental effects, development of pest resistance and negative effects on human health (Slusarenko, 2008). Concerns over the adverse effects of chemical fungicides on the health of consumers have lead to revision of food safety standards in regard to pesticide residue in fresh produce. A possible alternative is the use of antifungal plant extracts. The plant world comprises a rich storehouse of biochemicals that can be used as biological pesticides which are environmentally safe (Hashim and Devi, 2003). Relatively little is known about the role of the plant extracts applied on the physiological parameters of the plants. Therefore, this study aimed at studying the role of selected plant extracts (*B. angustifolia, Melea volkensii* and *Z. chalybeum*) in influencing photosynthetic activities of bean plants and finding an explanation.

Description of Research

The samples of desired plants (*B. angustifolia, Melea volkensii* and *Z. chalybeum*) from previous experiments (Omwenga, 2009; Kiswii, 2009) for antifungal activity were collected from different parts of the country (Samburu, Mombasa, Mwingi, Kakamega forest and Nakuru) in clean sacks (Table 1).

Table 1: Selected antifungal plant extracts for the study and parts of the plants used

Family	Scientific Name	Common/local name	Parts used
Capparidaceae	*Boscia angustifolia*	Mulule (Kamba)	Leaves, Stem
Rutaceae	*Zanthoxylum chalybeum*	Mjafari (Swahili)	Leaves, Stem
Rutaceae	*Melea volkensii*	Mukau (Kamba)	Leaves, Stem

The plants were identified and verified at Jomo Kenyatta University of Agriculture and Technology (Taxonomy unit, Department of Botany). Voucher specimens were deposited in the Herbarium. The samples were labeled and deposited in the Botany laboratory. The plant leaves and roots were dried separately at room temperature for a period of 1-2 weeks and then ground separately to powder using a grinding mill at 8000rpm (Type 8 lab mill). The powder was stored in plastic bags at room temperature until the time required. Two kilogram of each plant sample was soaked and left overnight to allow extraction of the crude active compounds. The supernatant was filtered in several layers of muslin cloth and volumes adjusted to 20 L. (Stoll, 2000). A combination of *B. angustifolia*, *Melea volkensii* and *Z. chalybeum* extracts. A bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of water extracts. During the growing period, beans were infected with rust from natural inoculum at the field. Seeds were obtained from Regina Seed Company and planted at a spacing of 30cm between rows and 10cm between plants within the rows (Monda *et al.*, 2003). French bean seeds commercially available coated with thiram were used to control root rots. French bean variety Amy seeds were planted in 4×3m plots each separated by a 1m path between the treatments and the replications. Amy is high yielding compared to other varieties therefore it is grown by most farmers. Di-ammonium phosphate was used at planting at a rate of 200kg/ha mixed well before seed placement. Calcium ammonium nitrate was applied at a rate of 100Kg/ha at trifoliate leaf stage. The treatments consisted of six plant extracts, copper hydroxide 61.4% (Kocide DF: metallic copper equivalent 40% formulated as a dry flowable) as a positive control and a negative water control. A spray regime of once a week using a knap sack was employed from the fifteen days after planting until flowering. The extracts used were used as protectants. The fungicide was applied at a rate of 2.5kg ha-1 according to the manufacturers' recommendations. There were a total of seven hundred and sixty plants per replicate. Overhead irrigation twice a week and weeding were done as necessary. Three different types of leaf gas exchange measurements were made on plants from the interior rows of the plots. First, at approximately weekly intervals, measurements of carbon dioxide assimilation rate were made at 0900hrs, 1200hrs, and 1500 hours at the JKUAT farm. Mature, fully illuminated upper canopy leaves were measured at their nominal daytime growth. Daylight patterns of carbon dioxide assimilation rate were measured by the infrared gas analyzer (IRGA). IRGA was used as a null point instrument that allows the flow of carbon dioxide into the system at a rate equivalent to the rate of uptake of the leaf. The amount of carbon dioxide assimilated by the leaf was read directly from the IRGA. French bean leaf tissues from ten selected plants from each treatment were enclosed in the leaf chamber (Leaf chamber =2.5cm2) one at time. The air flow rate through the chamber remained fixed. The carbon dioxide assimilation was monitored for 1 min for each leaf by the IRGA connected in an open gas flow system. During measurement of CO_2 assimilation rate the following parameters were also recorded using IRGA; stomatal conductance, and transpiration.

Research Results and Application

There were significant differences in stomatal conductance (P=0.0173) in the treatments at 9:00am and was rated as *B. angustifolia - X. chalybeum (*77.7 mol/m-2sec-1) combination having the highest stomatal conductance followed by, M. volkensii (46.3 mol/m -2sec-1) and Kocide DF (39.18 mol/m-2sec-1) respectively (Fig 1a). *M. volkensii* plant extract showed lowest stomatal conductance com pared to the other plant extract combination. The stomatal conductance for commercial fungicide (Kocide DF) was significantly lower (25.6 mol/m -2sec-1) than other treatments at 12:00pm. *B. angustifolia - X. chalybeum* (41 mol/m-2sec-1), *M. volkensii* (41.5mol/m-2sec-1) and untreated control (38.3mol/m-2sec-1) were not significantly different from each other at 12:00pm. There were no significant differences in stomatal conductance at 15:00pm of all treatments (P=0.1235). *B. angustifolia - X. chalybeum* combination and single plant treatment *M. volkensii* had a positive effect on stomatal conductance of bean plants. This suggests that these antifungal plant extracts in general may have interfered with any one of the several biosynthetic pathways or energy producti on pathways. Commercial control (Kocide DF) treated plants had the lowest carbon dioxide released compared to all other treatments because of its low stomata conductance.

There were significant differences (P=0.003) in transpiration rates of the treatment s at 9:00am. *B. angustifolia - X. chalybeum* (2.065 mol/m2/s) and *M. volkensii* single plant extracts (1.353 mol/m2/s) had significantly the highest rate of transpiration compared to other treatments (Fig 1b). There were significant differences (P=0.0015) in the rates of transpiration among the treatments at 12:00pm. Kocide DF (0.76 mol/m2/s) had significantly the lowest rate of transpiration while there were no differences in *B. angustifolia - X. chalybeum* (1.12 mol/m2/s), *M. volkensii* (1.135 mol/m2/s) and untreated control (1.067 mol/m2/s). There were significant differences (P<0.05) in transpiration rates of the treatments at 15:00am. *B. angustifolia - X. chalybeum* (0.67 mol/m2/s), *M. volkensii* (0.78 mol/m2/s) and Kocide DF (0.77 mol/m2/s) treated beans had lower rates of transpiration at 15:00pm than the untreated control (1.3 mol/m2/s) at 15:00pm. The untreated control highest transpiration rates might have been caused by high disease severity. Rust caused increased transpiration (E) from infected tissues after sporulation in untreated control. Transpiration before sporulation, which potentially is by a mainly stomatal pathway, is inhibited, probably by stomatal closure; rust is known to inhibit stomatal opening in the light in other diseases, e.g. bean (*Phaseolus vulgaris*) infected by either *Uromyces phaseoli* (Duniway & Durbin, 1971b).

The rate of photosynthesis was rated highest in *M. volkensii* (99.9µmolCO2/m2/s) as compared to the combination *B. angustifolia - X. chalybeum* (72.5µmolCO2/m2/s) and untreated control (53.9µmolCO2/m2/s) respectively ((Fig 1c). There were significant differences (P=0.0132) in the rate of photosynthesis among the treatments at 12:00pm. *M. volkensii* (68.38µmolCO2/m2/s) had significantly the highest photosynthetic rate at 12:00pm followed by *B. angustifolia - X. chalybeum* (59.1µmolCO2/m2/s). However, there were no differences between Kocide DF (23.51µmolCO2/m2/s) and untreated control (24.4µmolCO2/m2/s) at 12:00pm. *M. volkensii* (50.77µmolCO2/m2/s) and Kocide DF (50.7µmolCO2/m2/s) revealed significantly the highest rates of photosynthesis although there were not different from each other at 15:00pm. Untreated control (39.98µmolCO2/m2/s) had the lowest photosynthetic compared to other treatments at 15:00pm. Generally, *M. volkensii* had a positive effect on rate of photosynthesis as compared to other *B. angustifolia - X. chalybeum*. The antifungal plant extract had a positive effect on the rate of photosynthesis than other treatments. Therefore, the results suggest that the photosynthetic capacity of 'commercial control (Kocide DF) treated beans' were constrained at natural condition/

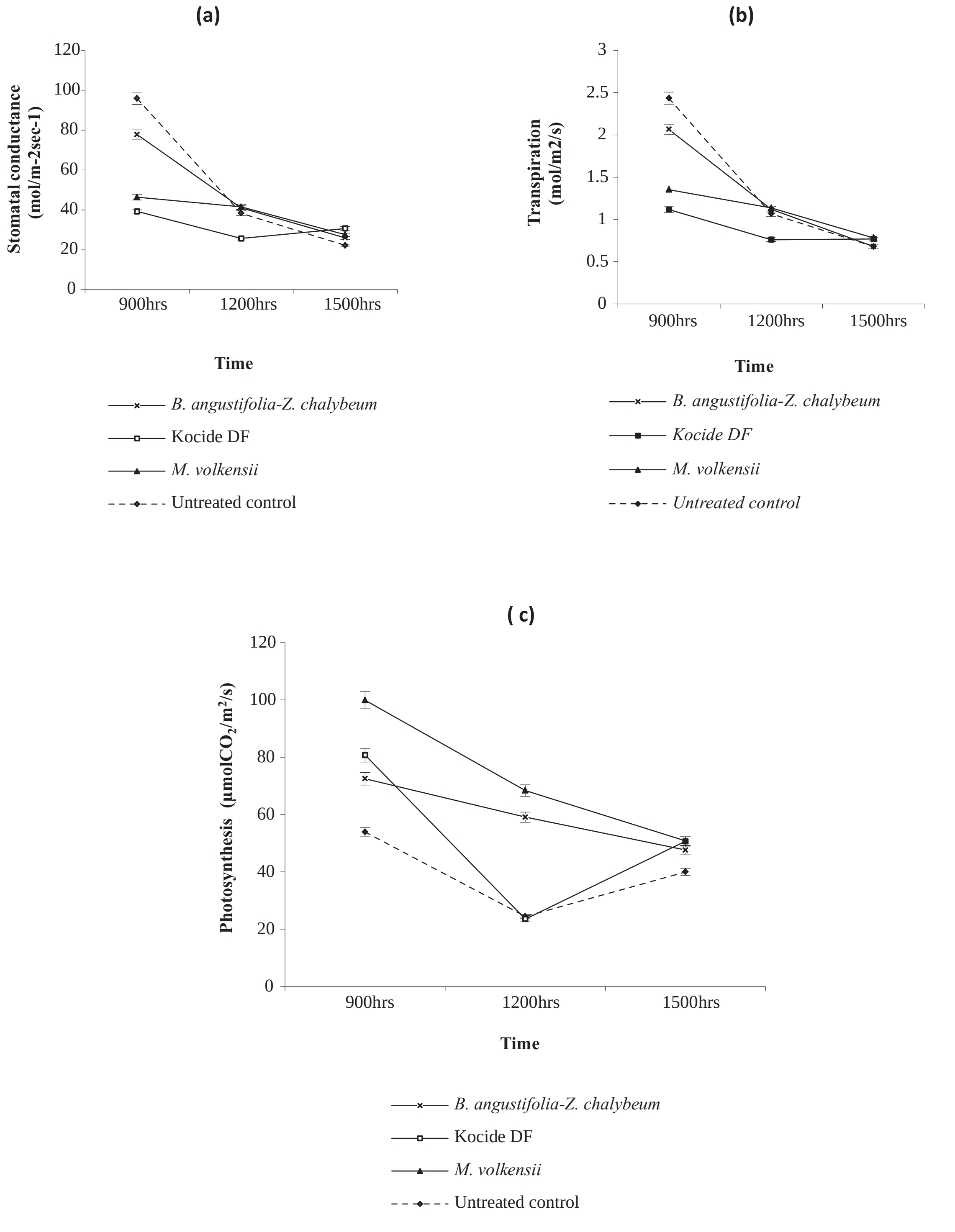

Figure 1: Daily diurnal courses of stomatal conductance (a), rate of transpiration (b) and photosynthetic rate (c) in French beans (Amy variety) exposed to various treatments. Each point represents the mean ± standard error of six replications

The CO2 assimilation reached a peak at 9:00am and decreased sharply at noon and eventually maintained low levels in the afternoon. CO2 assimilation followed the same pattern as that of stomatal conductance. There were significant differences ($P<0.001$) in CO2 assimilation rates among treatments at 9:00am. *B. angustifolia - X. chalybeum* (577.933ppm) treated bean plants had significantly lowest CO2 assimilation rate while there were no differences between *M. volkensii* (679.5ppm), Kocide DF (641.364ppm) and untreated control (651.154ppm) in CO2 assimilation rate at 9:00am. There were no significant differences ($P>0.002$) in CO2 assimilation rate of all treatments at 12:00pm however they ranged from untreated control (362ppm) being the highest then followed by *B. angustifolia - X. chalybeum* (328.33ppm), *M. volkensii* (320.33ppm) and Kocide DF (304.18ppm) respectively. Likewise, at 15:00pm there were no differences (P=0.1425) in CO2 assimilation rates of all treatments.

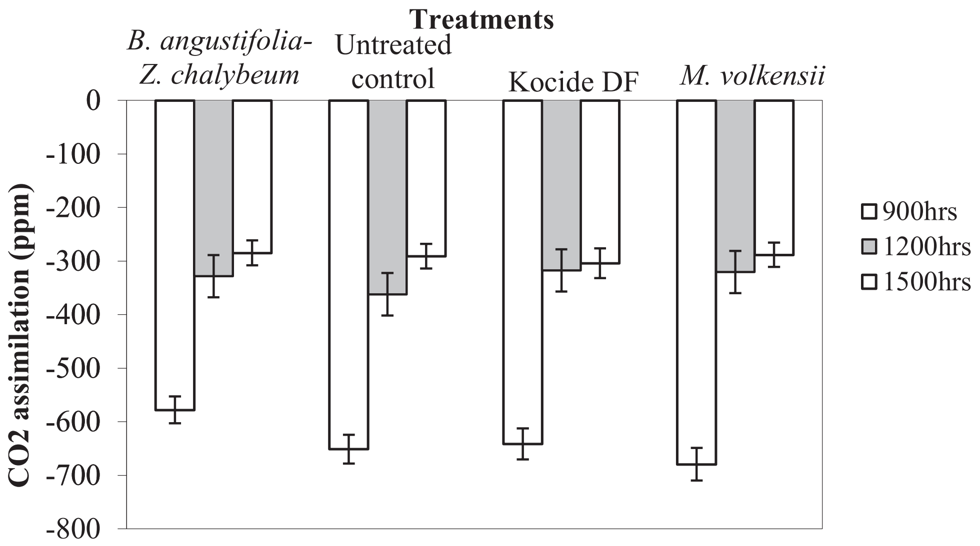

Figure 2:Daily courses of CO2 assimilation in French beans exposed to various antifungal plant extracts and a commercial fungicide under natural conditions

B. angustifolia - X. chalybeum combination and single plant treatment *M. volkensii* had positive effects on enhancing the rate of photosynthesis in bean plants.

Acknowledgments

The financial support obtained from Horticultural Crop Development Authority (HCDA) is hereby acknowledged.

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Climate change and Variability: Experience, coping and adaptation strategies among smallholder organic farmers of Central Kenya

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Abstract

The study to assess smallholder organic farmers' experience, coping and adaptation strategies to climate change and variability (CCV) was conducted in Kajiado, Kiambu and Murang'a counties of central Kenya/ !bout 100 certified organic farmers were interviewed using a semi- structured questionnaire. Experience on CCV was depicted in terms of rising air temperatures (68%), reducing rainfalls (75%), droughts and seasonal fluctuations (50%). Information on CCV was acquired through personal experience (26%) and from both the print and electronic media (69%). Among the leading causes of CCV cited were; emission of green house gases into the atmosphere from industries (34%), deforestation and poor agricultural practices (64%). The most profound effect of climate change was stated as reduced crop yields (85%). Crop failure and flooding of crop fields were reported by 10 and 5% of farmers interviewed respectively. Farmers were using both scientific (74%) and traditional (23%) methods of weather forecasting. The farmers reported that, employing the use of decision support tools alongside modern methods of weather forecasting could provide a noble alternative to the traditional weather forecasting methods and hence better preparedness to combat the effects of C CV. Most (95%) farmers' responded to the effects of CCV through- growing of drought resistant crops, mulching, application of organic inputs and use of innovative organic farming techniques. This is in addition to, agroforestry and rain water harvesting te chniques. The farmers contended that through trainings and exposure, they will be empowered to cope with and reverse the negative impacts of CCV and consequently guarantee food and nutritional security.

Introduction

The issue of climate change and variability (CCV) is a challenge to agricultural production in Kenya as the country's GDP normally mirror rainfall pattern/ Climate change is therefore a major impedance to agricultural production particularly and thus a threat to achieving MDGs of eradicating extreme poverty and hunger (UNDP 2007). The Working Group I of the Intergovernmental Panel on Climate Change (IPCC), Fourth Assessment Report (AR4) (IPCC, 2007) made bold conclusions that global warming is definite. Climate change induced by anthropogenic forces is expected to continue for centuries, even with the current mitigation measures being implemented globally, as it is influenced by timescales associated with climate processes and feedbacks. The surface air warming in the 21st century, by best esti mate, will range from 1/1 to 2/9 °C for a "low scenario" and 2/4 to 6/4 °C for a "high scenario" (IPCC, 2007)/ !s the century progresses, the inter-annual and decadal phenomena will be superimposed on an unprecedented global warming trend. Together these w ill produce rapid climate change, increasing climate variability and more climate extremes (Salinger, 2005).

Literature Summary

A good number of researchers (IPCC, 2007) have concluded that climate change would affect agriculture. This is as a result of increased temperatures, changes in rainfall patterns and increased frequency of extreme events, which could cause changes in pest ecology, ecological disruption in agricultural areas and socio-economic shifts in land-use practices. The changes are expected to be more pronounced in the tropics. For example, crop varieties grown in the Sahel may not be able to withstand the projected warming trends and will certainly be at risk due to projected lower amounts of rainfall as well (Salinger, 2005). Climate varia bility leads to economic and food insecurity throughout the world because of its major impacts on agriculture (IPCC, 2007).To counter these impacts, several approaches are being implemented. For example national policy for climate change adaptation are now being developed and applied, such as the National Adaptation Programmes of Action (NAPAs) agreed on at the Conference of the Parties to the Framework Convention on Climate Change (COP 7). Lenitive measures like confining global climate change by reducing the emissions of greenhouse gases or enhancing their sinks, (IPCC, 2007) are being implemented. Another option being mooted as third-party-policy is compensation for climate impacts, typically conceived as transfer payments (or other assistance) from those countries who disproportionately contributed to climate change to those who disproportionately suffer from it (IPCC, 2007). These transfers are mostly from developed/highly industrialized countries in the West like USA and European countries, to developi ng countries, majority in African and the Asian continent. The adoption of farmer-oriented seasonal climate forecasts (IPCC, 2007) could boost the campaign for armoring farmers. This will enable them downscale the impacts associated with climate risk, especially in small-scale farm systems, by providing information on climate and availing a menu of management options for adjusting farm operations that are sensitive to the prevailing/predicted weather (IPCC, 2007).

Description of Research

The study was carried out in Kajiado, Kiambu and Murang'a counties of central Kenya, between 1st and 4th of February 2012. These are areas where organic cultivation is prevalent due to awareness created there by Kenya Organic Agriculture Network (KOAN). The study involved a survey where semi-structured questionnaires were administered to 100 randomly sampled farmers. A stratified random sampling procedure was used to select respondents with locations forming the stratum. A computer random number generator was employed to select the number of households in each stratum. The questionnaire was pre-tested to check on content and clarity of questions. To ensure the questionnaire ability to accurately measure and capture the intended objectives, it was subjected to review by exper ts, supervisors and peers. The questionnaire was designed to gather information on farmers' experiences, coping and adaptation strategies on climate change and the main organic crops grown. This was in addition to identifying, barriers to, and approaches i n capacitating farmers to better prepare them against the detrimental impacts imposed by climate variability .

Research Results and Application

Farmers' experience

About 93% of the interviewed farmers had heard of and experienced the impacts of climate change, the common aspects being erratic and often inadequate rainfall and raising air temperatures 75% and 68% respectively (figure 1). About 83% of the farmers were of the opinion that the government and research institutions should play a bigger role in assisting the farmers address the impacts/risks associated with the changing weather patterns (figure 5). Over 60% of the farmers mentioned deforestation and poor agricultural practices (PAPs) as the major causes of climate change and about 70% of them acquired information on climate change and variability from both the print and electronic media (figure 3).

Farmers' knowledge

More than 70% of the farmers were familiar with the scientific techniques of weather forecasting which they use to inform their farm management decisions. On the other hand, the use of indigenous techniques is gradually declining due to climate change, complex behavior of animals/plant species and/or fast disappearance of species relied on for weather forecasting. Thus about 25% of farmers reported use of traditional methods of weather forecasting (figure 2).

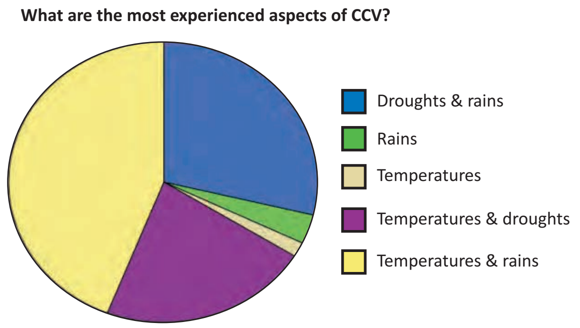

Figure 1:
Aspects of climate change

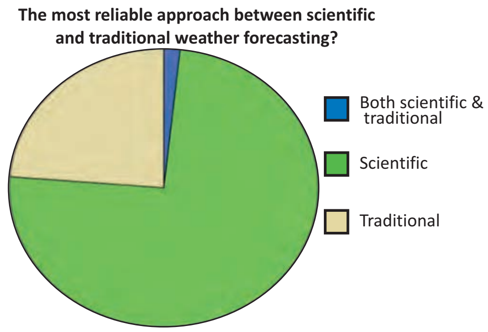

Figure 2:
Rating of weather forecasting methods

Farmer perception

Climate change as an issue to agricultural production was rated high by 85% of the farmers who reported reduced crop yields as the most profound impact climate change and variability (figure 4). A decline in production will therefore affect their incomes and food security. This will exacerbate hunger and poverty, contrary to the aspirations of the MDGs of halving extreme hunger and poverty by 2015 (UNDP 2007; Government of Kenya, 2007).

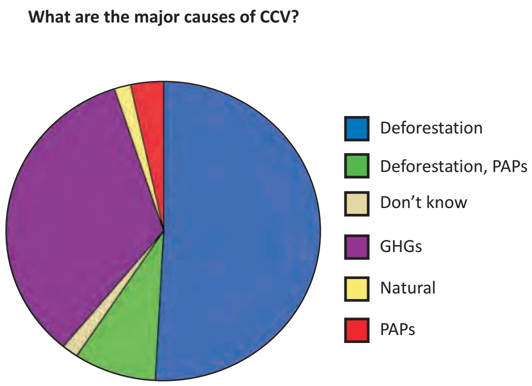

Figure 3:
Causes of climate change

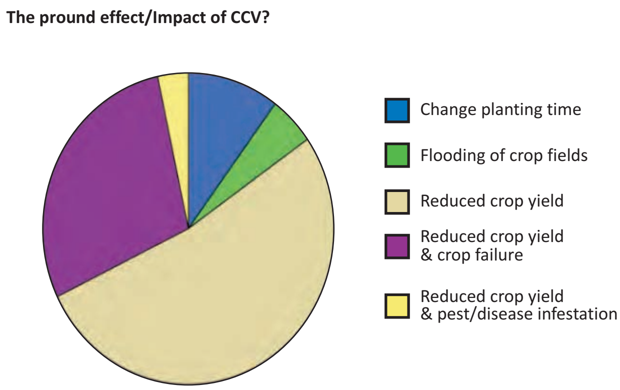

Figure 4:
Impacts of climate change

Adaptation strategies

Good agricultural practices comprising; agro-forestry, mulching, organic inputs, drought tolerant crops and rain water harvesting as reported by about 95% of the farmers were the principal adaptation strategies (figure 6). About 80% of the farmers adduced that through training on farm planning they will effectively cope with the effects of climate change and variability (figure 7).

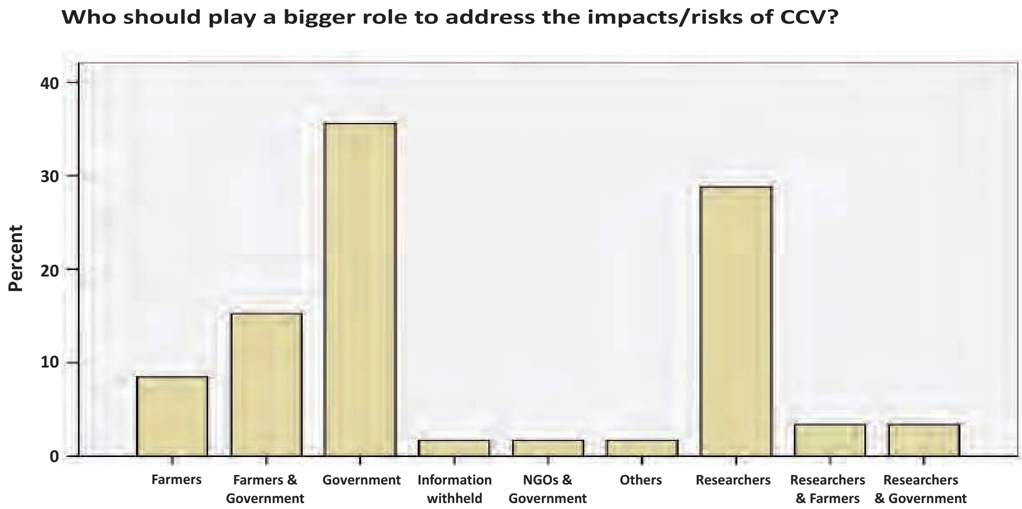

Figure 5: Players in the mitigation of the effects of CCV

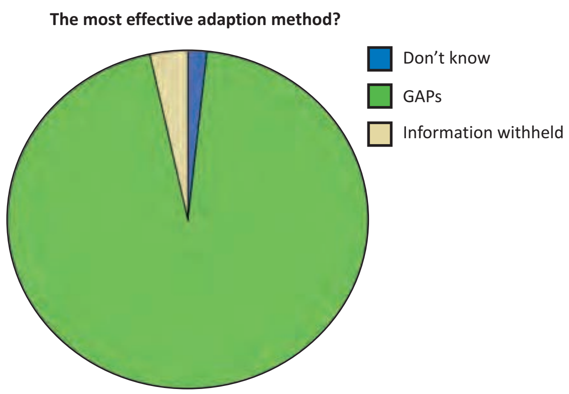

Figure 6: Adaptation methods in response to CCV

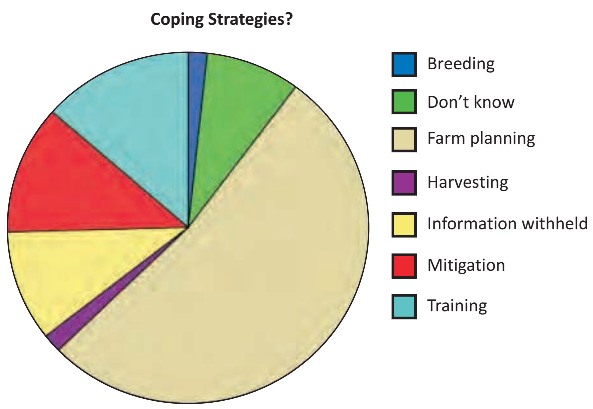

Figure 7: Copping strategies in response to CCV

Farmers will benefit from the accuracy and large scale applicability of scientific methods of weather forecasting while researchers will get insight into farmers' traditional methods thus helps package weather information in a manner comprehensible and usable by farmers. Adaptation/coping strategies involving switching to use of organic inputs create a need to capacity-build on the use of DST in order to tailor production in accordance to the changing weather patterns. Developing appropriate extension packages for the production of the introduced organic crops will require long periods of experimentation, often time and/or resources demanding and consuming. The information gaps highlighted, coupled with farmers experiences/ knowledge captured in the document, will aid in the development of buffering strategies against risks of CCV.

Acknowledgement

We wish to sincerely thank Productivity and Growth in Organic Value Chains (ProGrOV) for funding the project. Our appreciation also goes to University of Nairobi (Departments of; Animal Production and Land Resource Management and Agricultural Technology) for their administrative and technical assistance whenever requested.

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Non Communicable Diseases and their Management in the Lake Victoria Basin, Kenya

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Abstract

Usage of plants as source of medicine remains common despite the current growth and expansion of conventional medicine and health centres in many parts of the country and particularly in the rural areas . Traditional medicine practitioners still remain resource persons to health problems of many especially in cases where the sick have consulted the conventional medicine with no success. The other reason for the popularity of the traditional medicine practitioners is the fact that their services are affordable and payment does not have to be in monetary, but it can be in form of an animal/s or crops as per the agreement. In search for plants used for treatement of microbial related ailments such as malaria, Sexually transmitted illnesses, diarrhoea, pneumonia , a lot of information given by the herbal practitioners interviewde was also on plants used in the management of the non communicable diseases. This paper presents data on the treatment of non-communicable diseases using medicinal and pesticidal plants in the Lake Victoria region.
Keywords: Non communicable ailments, ethnomedicine.

Introduction

Use of plants as medicine remains common in Kenya. Among the reasons are because of its affordability and availability. In areas where poverty index is quite high, herbal practitioners are the resource persons to health problems, especially because there compensation is not only based on money but also in terms of animals, crops and may be done at a later date, unlike in the case of conventional health care systems. In the recent past, there has been an upsurge of a wide range of non commun icable diseases. Herbal practitioners especially in the Lake Victoria Basin use several plants to manage snon communicable ailments, but these plants remain largely undocumented.

Materials and Methods

An ethnomedicinal survey was conducted to document the plant species used medicinally in the Lake Basin. The ethnomedicinal data were based on structured interviews that sought answers to questions about the human ailments treated, local names of plant species, plant parts used, methods of preparation, and administration. In some cases, the interviews were facilitated by translators who were well conversant with the local language. This was done having first obtained verbal informed consent from each traditional healer.

Results and Discussion

Herbal practitioners from the Lake Victoria Basin were found to have a vast knowledge on medicinal plants usage in the management of non communicable diseases. This was evidenced by the result that a wide range of these ailments were reported to be treated using twen ty two medicinal plant species distributed within fifteen botanical families. They were found to play a vital role in the primary healthcare of the local poor people . This may have been due to the inability by the locals to afford modern healthcare costs and the experience from the healers' capability to handle most of their health problems/ Most of the medicinal plants belonged to the plant family Asteraceae, this was followed by Rutaceaea and Leguminosae each having the same number of medicinal plants. This trend is in agreement with the findings of Odhiambo et al., (2011) and Yineger et al., (2008), who both reported the highest number of medicinal plants belonging to the family Asteraceae, however the the second common family differ in both cases. This discrepancy may be due to factors such as ecological, geographical and environmental (Runyoro et al., 2006) which favour the growth of certain plants.

The use of traditional medicinal plants as mixtures by traditional healers to manage one or more hu man ailments was reported. This could be due to perceived synergitic effects. This study reported herbs to be the most used growth form used for remedy prepararation. High use of herbs could be attributed to the fact that they tend to be most available in nearly all climatic conditions, have fast growth and tend to be available in conspicuous places like crop farms, disturbed areas, along the roadsides and along fences where they can easily be accessed by the practitioners. Leaves were the most cited plant parts used by the healers for the preparation of medicine followed by the roots (Odhiambo et al., 2011).

Some healers had high level of secrecy such that they gave the local name of the plants and disease treated only without showing the plant for identification. The secrecy surrounding the ethnomedicinal knowledge among the traditional healers could be attributed to the fact that traditional healers derive income from the treatments they provide. This is in agreement with findings done by Yineger *et al*., (2008) , Yineger & Yewhalaw, (2007) and Kokwaro (1993) that In some cases oath is taken during passage of the knowledge to the younger generation not to share the medicinal information with anybody.

Acknowledgement

The authors wish to thank the InterUniversity Council of East Africa (IUCEA) for sponsoring this research. Traditional healers of Lake Victoria Basin are genuinely acknowledged for their hospitality. Technical assistance by Mr. Simon Mathenge is greatly appreciated, and the University of Nairobi, School of Biological sciences for letting us use the available facilities.

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Beauveria bassiana 5653 could be an alternative to synthetic insecticides against *Plutella xylostella* in Togo

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Abstract

An evaluation of the potential use of the entomopathogen *Beauveria bassiana* strain 5653 for biological control of cabbage major pest, *Plutella xylostella* was carried out under station and farm conditions in the littoral ecosystem in Togo. The experiment was conducted using the sprayed solution of *B. bassiana* 5653 in comparison with synthetic insecticides. The results revealed that the formulation of *B bassiana* 5653 reduced significantly the density of *P. xylostella* populations. The use of *B. bassiana* 5653 increased the cabbage yield by 57% on farm, compared to the control. Contrary to the entomopathogen, the synthetic insecticides CONQUEST PLUS 388 EC. (16 g acetamipride, 300 g triazophos and 72 g cypermethrin) and DECIS 25 EC. (25 g deltamethrin) used as references had any effects on *P. xylostella* densities and cabbage yields compared to the control, indicating that *P. xylostella* was not susceptible to the synthetic insecticides. Thus, the use of the bio insecticide *B. bassiana* 5653 could be an alternative for the management of the insecticide non-susceptible strain of *P. xylostella* in Togo.

Keywords: *Plutella xylostella*, *Beauveria bassiana*, entomopathogen, insecticide, cabbage.

Introduction

Cities extension in Africa is accompanied by the development of urban agriculture, like market-gardening in free spaces with water is accessible. In fact, market-gardening is a mean for small holders to obtain a significant income and to mitigate unemployment in urban areas (Ba Diao, 2004; Schilter, 1991). In Togo, cabbage crop (*Brassica oleraceae* L.) represents a significant part in vegetable production. From 2009 to 2010 it production increase is estimated at 50% (FAOSTAT, 2012). However, the production of cabbage is subjected to serious insect pest damage. Yield losses due to insect attacks vary from 10 to 80%, according to the rate of infestation (Agboyi et al., 2008), in spite of farmers over reliance on chemicals because, the major pest *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has become resistant (Lu, 2000). Many studies conducted through the world revealed that *P. xylostella* could cause a serious damage to cabbage's production (Talekar and Shelton, 1993) because of its resistance to several insecticides (Shelton and Nault, 2004). The problem is amplified by the high pressure of selection due to escalation in the use of insecticides by farmers. In Togo, the situation is critical and therefore, research on alternative strategies to reduce chemicals used in agricultural ecosystem becomes a challenge. *Beauveria bassiana* is one of the best candidates for alternative strategies, as low risk agent. Some studies realized demonstrated it non-infectivity to mammals (Wang et al., 2004) and the absence of major risk to environment (Ward et al., 1998). The aim of this study is to evaluate under field experiments, the potential of *B. bassiana* 5653 to control *P. xylostella* populations in Togo.

Description of Research

Site

The experiments were carried out during the dry season, simultaneously on station and farm (market-gardening site) located in the littoral ecosystem of Togo. The climate is the subequatorial type and characterized by two rain seasons and two dry seasons. The annual average temperature turns around 27 ^{o}C and the annual average pluviometer is 800 mm.

Plant material and Experimental design

The plant material was the most important variety of cabbage cultivated in Togo, var. kk -cross. Field efficacy tests were conducted from October to December 2007. Cabbage seedlings were transplanted to 12 plots in triplicate complete randomized blocks design. Each plot of 12 m^2 (1.2 by 10 m) contained 75 plants ranged on three rows. The distance of 40 cm was observed between plants.

Beauveria bassiana 5653

The entomopathogen *B. bassiana* 5653 is a white powder formulation obtained from International Institute of Tropical Agriculture (IITA). The sprayed solution preparation requires the use of a drinking water and an emulsifier which facilitates the powder-water mixture. The emulsifier used was the oil "tween 80" at the dose of 0.66 ml per liter of water. The powder of *B bassiana* was used at the dose of 1 g per liter of drinking water.

Insecticide treatment and data collection

Each insecticide was applied weekly in the afternoon (16 H to 18 H). The chemicals were recommended to be used every two weeks but in this study they were sprayed weekly in order to respect farmers' practices/

Cabbage plants were sprayed with *B bassiana* 5653 solution at the concentration of 10^{11} spores/l (table 1). Synthetic insecticides, DECIS 25 EC (25 g deltamethrine) and CONQUEST PLUS 388 EC (16 g acetamipride, 72 g cypermethrine and 300 g triazophos) were used as reference. The insecticide application was stopped two weeks before harvest. Evaluations of *P. xylostella* infestations were done weekly on 9 plants sampled on each plot and the number of *P. xylostella* larvae was recorded (number of larvae per m^2). The evaluation was done one day before each treatment and stopped two weeks before harvest. The cabbage head yield was measured at the harvest.

Table 1: Insecticides dose for different treatments

Group of insecticides	Treatment	Dose
Biological insecticide	*Beauveria bassiana* 5653	10^{11} spores/l
Pyrethroid	DECIS 25 EC	0,69 l/ha
Mixture :	CONQUEST PLUS 388 EC	0,56 l/ha
---	Control	---

Statistical Analysis

Data analyses were performed using the STATISTICA (StatSoft, 1995). Insect number was expressed in density (number of larvae per m^2) before running ANOVA test. Yield obtained from different treatments were also analyzed by ANOVA. Means were compared using Newman -Keuls test at *P = 0.05*.

Research Results and Application

Effect of insecticides on density of *Plutella xylostella*

The density of *P. xylostella* was less important in control plots under station conditions than farm. The densities of living larvae per m^2 observed in control plots was 7 ± 3 and 25 ± 4 on station and farm respectively (fig.1). The continuous production of cabbage on farm has created a favorable condition for high proliferation of *P. xylostella* populations. The result obtained after insecticide application revealed that plots treated with synthetic insecticides CONQUEST PLUS and DECIS had more attack of *P. xylostella* than those treated with *B. bassiana* 5653 (F = 6.399, P = 0.009 on station; F = 15.758, P = 0.001 on farm). The use of *B. bassiana* 5653 at the frequency of one treatment per week reduced the density of *P. xylostella* to79% and 73% respectively on station. Contrary to the entomopathogen, the density of *P. xylostella* increase on the synthetic insecticides CONQUEST PLUS and DECIS plots, compared to control. This could be explained by the high selection of a resistant population of *P. xylostella* through an excessive use of chemicals by vegetable farmers in Togo. The same situation was described by Obopile et al. (2008) in Botswana. In addition, the excessive use of synthetic insecticides generated a favorable condition for development of the resistant strain of *P. xylostella* by eliminating of natural enemies and others cabbage pests like aphids competition, indicating that *P. xylostella* is the most serious constraint for cabbage crops in the littoral agro ecosystem of Togo.

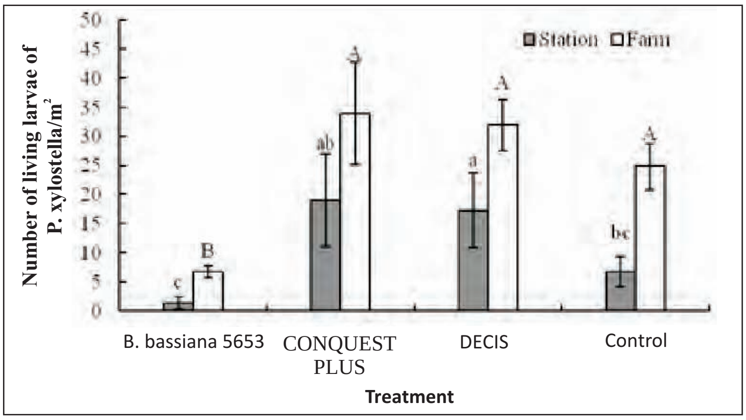

Figure 1: Density of *Plutella xylostella* on cabbage plots per treatment in station and farm

(Mean (± SD); N = 3 replications). Bars with the same letter are not significantly different (Newman-Keuls test, α = 0/05)/

Capacity of *B. bassiana* 5653 to control *P. xylostella*

The assessment of the effectiveness of *B. bassiana* 5653 against *P. xylostella* revealed a great capacity of this biopesticide to control the pest under different conditions. On station conditions, 54% of *P. xylostella* larvae were infested by *B. bassiana* 5653 during the experience (fig. 2). On farm conditions, 57% of dead larvae were recorded on plots treated by *B. bassiana* 5653. His indicate that the mortality rate of weekly application of *B. bassiana* 5653 on field at the concentration of 10^{11} spores/l is almost the same on station and farm.

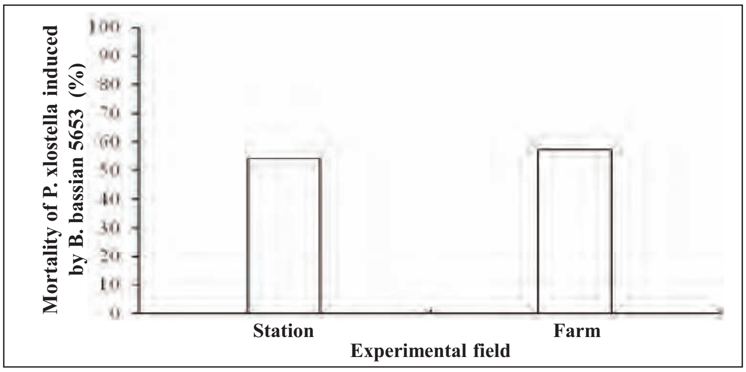

Figure 2: Efficacy of *B. bassiana* 5653 against *Plutella xylostella*
N = 5 data collected

Effect of insecticides on yield
The plots treated by *B bassiana* 5653 under experimental station and farm conditions, had recorded the best cabbage head yield compared to synthetic insecticides and the control (F= 5.492, *P= 0.024* on station; F= 12.318, *P= 0.002* on farm) (fig. 3). Undamaged cabbage yields obtained under experimental station and farm conditions using *B. bassiana* 5653 were respectively 32.81 ± 2.95 $Mg.ha^{-1}$ and 43.58 ± 2.26 $Mg.ha^{-1}$. Compared to the control, the use of *B. bassiana* 5653 on farm could increase the yield to 57%. There was no significant difference in undamaged yields obtained from synthetic insecticides (CONQUEST PLUS and DECIS) and the control, confirming the high cabbage crop losses in farmers practices based on chemicals (fig. 3).

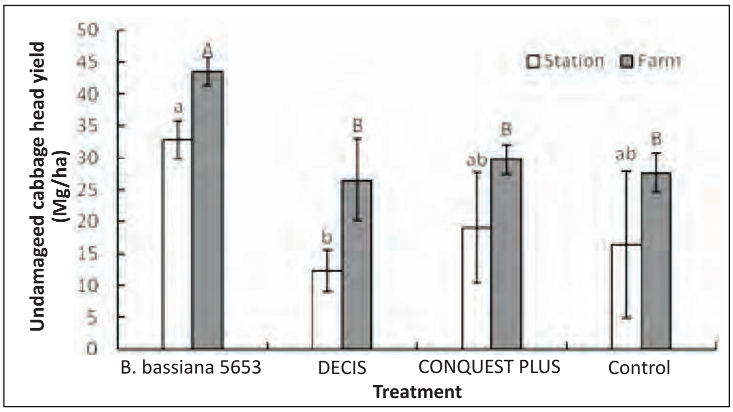

Figure 3: Capacity of treatments to increase cabbage yield
(Mean ± SD). Bars with the same letter are not significantly different (Newman -Keuls test, α = 0/05)/

Our results demonstrated that the continuous use of synthetic insecticides against *P. xylostella* in Togo is not economically profitable for cabbage producers in long term, according to higher level of crop losses. On other hand, an extensive use of synthetic insecticides is prejudicial to the environment, without solving the problem of *P. xylostella* resistance. However, *B. bassiana* 5653 presents a potent capacity to control insecticide resistant populations of *P. xylostella* leading to an increase of yield by 57% on farm. Therefore, *B. bassiana* 5653 seems to be a competitive control agent for the implementation of low risky alternative strategy for cabbage production improvement in Togo.

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Using a computer-based model to determine a botanical nematicide concentration for botinemagationin tomato production

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Abstract

Fermented plant extracts (FPE) of wild watermelon (*Cucumis africanus*) fruitare being tested for botinemagationin managing the root-knot (*Meloidogyne* spp.) nematodes in large-scale commercial tomato (*Solanum lycorpersicum*) farming systems in South Africa. The major drawback FPE through botinemagation is the high level of allelopathy. A computer-based model, namely, the curve-fitting allelochemical response dosage (CARD), was adapted for use to compute tomato plant stimulating concentrations, which should also be suppressive to nematode numbers. Nematode-infested tomato plants were treated with 0, 2, 4, 8, 16, 32 and 64% *C. africanus* fruit-FPE concentrations at 7-day interval. At 56 days, plant and nematode variables were subjected to analysis of variance, with significant variables being further subjected to the CARD model to generate biological indices which allowed computation of an integrated mean concentration stimulation range (IMCSR) – a concentration where plant growth was stimulated. In the validation study, the IMCSR of 2.64% dilution of FPE of *C. africanus* fruit improved growth of tomato plants and suppressed nematode numbers.

Introduction

Production costs in agriculture are high and therefore, prohibit adoption of any product which will not be incorporated into some of the conventional farming activities. Botinemagation –the application of fermented plant extracts (PPE) through drip irrigation for managing plant-parasitic nematodes was introduced with this view in mind. However, the success of botinemagation demands a balance between avoiding phytotoxicity and improving nematode suppression. Traditionally, providing empirical evidence to verify allelopathy is tedious and complex. However, with improved computer-based technologies, verification of allelopathy is much simpler. The objective of this study was to use the curve-fitting allelochemical response dosage (CARD)computer-based model to quantify an integrated mean concentration stimulation range (IMCSR) of fermented dried *C. africanus* fruit for use in suppression of *Meloidogyne* spp. through drip irrigation in tomato (*Solanum lycorpersicum*) production.

Literature Summary

ACARD computer-based model was developed to quantify responses of biological entities toward increasing concentrations of allelochemicals (Liu *et al*., 2003). In the CARD model, responses of biological entities (y-axis) and increasing concentrations of botanical nematicides(x-axis) are characterised by density-dependent growth patterns which are quantified by quadratic relationships and six biological indices (Liu *et al*., 2003). Using twobiological indices, namely, threshold stimulation (D_m)point and saturation point (R_h),IMCSR was computed, which is basically, the non-phytotoxic concentrationthat, incidentally, either stimulates or has no deleterious effects on plant growth (Mashela *et al.*, 2011).Previously, the stimulation of tomato plant growth due to the application of crude extracts from fruit of wild cucumber (*Cucumis myriocarpus*) in the ground leaching technology (GLT) system was referred to as a fertiliser effect (Mashela *et al*., 2011). Others also observed that in animal cells, cucumin ($C_{27}H_{40}O_9$), which is one of the active ingredients from crude extracts of *C. myriocarpus*fruit had stimulatory and inhibitory effects on cell growth at low and high concentrations, respectively (Chen *et al*., 2005). The CARD model quantifies threephases, namely, stimulation, saturation and inhibition,in density-dependent growth patterns of biological entities when exposed to increasing concentrations of allelochemicals(Salisbury and Ross, 1992). The CARD model is being used in botanical nematicides to determine stimulating concentrations (Pelinganga*et al*., 2012).

Description of Research

A greenhouse experiment was conducted in spring (August-October) in 2011 and repeated in autumn (February-April) 2012 at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10''S, 29°44'15''E).Day/night temperatures averaged 29/15 °C and 27/14°C, respectively, with maximum temperatures reduced through automatic thermostat-controlled fans.Crude extracts from *C. africanus* fruit were prepared as described previously (Mashela *et al.*, 2011) and fermented plant extracts (FPE) prepared using the modified method of Kyan *et al.* (1999) and effective micro-organism (Higa, 1993). Uniform four-week-old tomato cv/ 'Floradade' seedlings transplanted in 3.1 (v/v) pasteurised sand and Hygromix/ Treatments, namely, 0, 2, 4, 6, 8, 16, 32 and 64%concentrations were arranged in randomised complete block design, with 6 replications. A day after transplanting, each tomato seedling was inoculated by dispensing ca. 5000 *M. incognita* race 2 eggs and second-stage juveniles (J2s). All other cultural practices, along with data collection and analysis, were as described previously (Pelinganga *et al*., 2012). Interactions between the 2010 and 2011 growing seasons for the variables measured were not significant ($P \leq 0/05$)/ Thus, data were pooled (n = 96) and reanalysed/ Plant variables with significant ($P \leq 0/05$) mean differences were further subjected to the CARD model to generate the related curves and biological indices (Liu *et al*., 2003), where D_m and the adjusted $R_h$were used to compute the IMCSR (Pelinganga *et al*., 2012). Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.The derived IMCSR value was validated under greenhouse conditions.

Results and Application

Treatment effects were different on mean dry shoot mass, dry root mass, plant height, stem diameter of tomato plants and nematodes in root and soil (data not shown). Significant plant variables served as inputs in the CARD model,which resulted in density-dependent growth pattern curves (not shown), with their distinguishing biological indices (Table 1), withthe models accounting for 96%, 97%, 99% and 90% in total treatment variation of dry shoot mass, dry root mass, plant height and stem diameter, respectively.Two biological indices are of importance in quantifying IMCSR,namely, the (1) threshold stimulation (D_m) and (2) saturation point (R_h), but should not be viewed in isolation since the sensitivity index (k) is also an integral part to the IMCSR (Liu *et al*., 2003).

Biological indices, like all other indices, have no units, and therefore, could be accorded the units of the concentrations which were used in the study. Consequently, the IMCSR in this study was approximately 2.64% concentration.

Table 1. Biological indices and mean concentrationstimulation range of dry shoot mass, dry root mass, plant height and stem diameter of tomato exposed to diluted fermented crude extracts of *Cucumis africanus* dried fruit at 56 days after transplanting

Biological index	DSM[x]	DRM	PHT	SDR	Mean
Threshold stimulation (D_m)	2.53	2.20	2.73	1.53	2.25

Saturation point (R_h)	0.71	0.32	1.98	0.08	0.77
0% inhibition (D_0)	11.48	9.21	12.94	5.42	9.76
50% inhibition (D_{50})	164.90	59.00	2899.74	1603.20	1181.71
100% inhibition (D_{100})	703.50	170.30	2901.72	1603.28	1344.70
Coefficient of determination (R^2)	0.96	0.97	0.99	0.90	0.96
Sensitivity index (k)	1	1	1	1	Σ4
Integrated mean concentrationstimulation range (IMCSR)					
Threshold stimulation (D_m)	2.53	2.20	2.73	1.53	2.25
Adjusted saturation point (R_h)[y]	3.24	2.52	4.71	1.61	3.02
IMCSR					2.64

[x]DSM = dry shoot mass, DRM = dry root mass, PHT = plant height, SDR = stem diameter.
Adjusted $R_h = D_m + R_h$, while MDSR = (D_m + adjusted R_h)/2/

Treatment effects were different on mean nematode numbers across the seven increasing concentrations.In root, soil and root + soil the increasing concentrations of the material reduced nematodes by 89 -97%, 45-96% and 78-97%, respectively (Table 2). The mat erial appeared to have been more effective in reducing nematode numbers at lower than at higher concentrations in both root and soil.Growth of tomato subjected to increasing concentrations of dried *C. africanus* fruit-FPE had strong density-dependent growth patterns, which are characteristics of biological entities when exposed to increasing levels of intrinsic and/or extrinsic factors (Liu *et al*., 2003).In tomato plants, assessed organs had similar sensitivities to concentrations of dried *C. africanus* fruit-FPE, as represented by equal k values. Generally, plant sensitivity is indirectly proportional to k values, with low values depicting high sensitivities to the material, while high values suggest low sensitivities (Liu *et al*., 2003). In the study, all sam pled organs had equivalent sensitivities to the test materials, while the overall sensitivity of Σk = 4, suggested that tomato plants were less sensitive to the test material/ Generally, at low concentration cucurbitacins stimulated plant growth, while at high concentration they inhibit plant growth. Incidentally, it should be noted from the biological indices that at IMCSR, inhibition (D_0) is still quite remote, which serves as a cushion for non-phytotoxicity. In addition to concentration, phytotoxicity of a botanical nematicide is also a function of plant growth stage, frequency of application, formulation type and solubility of active ingredients in the FPE (Mashela *et al*., 2011).

Table 2: Relative impact of concentration of fermented crude extracts from *Cucumis africanus* fruit to final nematode numbers of *Meloidogyne incognita* race 2 in roots of tomato alone, soil alone and in roots + soil at 56 days after inoculating each plant with 5000 eggs and J2s

Dil	Nematode in root		Nematode in soil		Total nematode	
(%)	Var	Impact(%)	Var	Impact (%)	Var	Impact (%)
0	1224a[y]	–	401a	–	1623a[x]	–
2	120cd	–90	51e	–87	169bc	–90
4	39d	–97	15e	–96	52c	–97
8	82cd	–93	75cde	–81	155bc	–90
16	184b	–85	177bc	–56	359b	–78
32	67bcd	–95	113bcd	–72	178bc	–89
64	138bc	–89	221ab	–45	357b	–78

[y]Column means in brackets ($\log_{10}(x + 1)$ with same letter were not different (P $\leq$0/05) according to Waller-Duncan multiple range test, while those outside brackets.
[z]Impact (%) = [(treatment/control) —1) × 100.

Using a simple proportion at the IMCSRof 2.64%, dried *C. africanus* fruit-FPE would reduce nematode numbers in root, soil and root + soil to 91 J2s (120 × 2/2.64), 39 J2s (51 × 2/2.64) and 128 J2s (169 × 2/2.64), respectively. Stated differently, relative t o untreated control, IMCSRof 2.64% concentrationdried *C. africanus* fruit-FPE would reduce J2s in root, soil and root + soil by 93%, 90% and 92%, respectively.Generally, the highest percentages on reduction of nematode numbers were at low concentrations, wh ere phytotoxicity was at the lowest. In a validation study on open tomato field systems,3% concentration of *C. africanus* fruit-FPE reduced J2s of *M. incognita* race 2 in roots by 81%, but had no effect on plant variables (unpubl. data).In conclusion, using the CARD computer-based model to quantify various biological indices of *C. africanus* fruit-FPE has provided an opportunity to compute non-phytotoxic concentrations with *M. incognita*-inhibiting capabilities in tomato production.

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Optimised pest management with *Tephrosia* on legume cropping systems in Malawi: A Baseline Study

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Abstract

While new varieties have improved yields, increased productivity will happen with effective pest management over which farmers have some direct control. The study was set out to estab lish baseline indicators against which the project impact will be measured. Using purposeful and simple random sampling techniques, a total of 150 bean farmers were sampled and interviewed. Multiple regression analyses were used to establish the relationship between bean yields and the amount of botanical insecticides used to control pests. Results indicated that many farmers (51%) did not use pest management options in their bean farming systems. Those that used pest management options identified: crop rot ation, early ploughing, timely planting, timely weeding, industrial insecticides and botanical insecticides as possible options. Farmers, who are using the products, indicated that they lacked knowledge on the actual measurements accompanied by lack of pla nt materials to process the pesticides. Regression analyses results indicated that there was a negative relationship between amount of botanical pesticides applied and the resulting bean yield. Furthermore, there was a positive and significant relationship between education level of household heads and bean yield. This therefore calls for intense research on the processing and utilisation of pesticidal plant products and farmer education on the use of botanical pesticides to control pest.

Introduction

The principal requirement for subsistence farmers who constitute the poorest people in Africa and most susceptible to malnutrition is simple: food security, crop productivity and storage. Like in most East and Southern Africa countries, in Malawi, maize pr ovides the staple food but the common bean (*Phaseolus* *vulgaris*) is also very important as a source of protein, micronutrients and essential vitamins particularly for poor households. However, the production levels of the common bean crop remains low, often <400 kg ha-1, largely attributable to various production constraints including insect pests and diseases. While new varieties have improved yields, increasing productivity will only happen with effective pest management and this remains one of a few constraints over which farmers have some direct control. Commercial insecticides are usually effective, but are costly and their use is compromised by poor labeling and adulteration. In addition to incorrect application, these lead to pest resistance and low efficacy. They are also applied without personal protection measures despite known toxicity effects. Commercial insecticide use also result in long term healthy effects since low acute toxicity results in safety complacency. They also have serious impacts on wildlife, pollinators and natural enemies. Therefore many farmers avoid using these products in pest management. Farmers therefore need an intervention they can employ to manage pests in their crops and in the production chain. Pesticidal plants are an effective but under-exploited alternative, and their promotion with optimised application through well informed extension services would impact on farmers' capacity to manage pests and improve crop productivity. Naturally occurring chemicals are extracted from plants to make botanical pesticides, which break down readily in soil and are not stored in plant or animal tissue. Often their effects are not as long lasting as those of synthetic pesticides. Despite the potential impacts of the use of pesticidal plant products, the African continent uses far less botanical pesticides than what is available. And there has been no systematic data collection for baseline information. This paper therefore, provides baseline results reflecting the following specific objectives; (1) to provide baseline information on the current socioeconomic conditions of rural and peri-urban households in communities where the botanical pesticide project is operating (2) to provide specific information on the utilisation, accessibility and availability of botanical insecticides in the study area.

Literature Summary

Pesticidal plants have been used by poor, small-scale African farmers for effective, low-cost pest control. It has to be noted however that natural pesticide products are not necessarily less toxic to humans compared to commercial insecticides. Therefore hence protective clothing should be worn when applying them. For example, *Tephrosia vogelii* is traditionally used as a fish poison and contains the known toxin rotenone which has acute and chronic toxic effects in humans. Its efficacy in the laboratory against stored grain beetles has been evaluated and this work may influence changes in the extension messages that promote the use of *Tephrosia vogelii* for protection of grain stored on-farm. Recent research in Africa by the Natural Resource Institute (NRI) has shown that the use of plant materials is often constrained by a lack of knowledge, which may be limited geographically, generationally or ethnically. Although pesticidal plants are a relevant pest management technology, their use is under threat by a stagnating knowledge base that cannot keep up with contemporary health, safety and reliability directives. If improved, pesticidal plants can enable poor farmers reduce insect pest problems. Scientists have been working to optimize the way pesticidal plants are used, understanding plant chemistry, and the best ways of harvesting and processing plant materials to maximize their efficacy and sustainable use in Malawi (Nyirenda et al, 2011). Economies in Southern African depend heavily on agricultural production, with the sector contributing 20- 60% of Gross Domestic Product (GDP); 30% of exports and to over 60% of all employment for many countries in sub-Saharan Africa (SSA) (Obwona & Chirwa 2006)**.** Despite the dependence and high priority placed on agriculture by SSA governments, agriculture productivity has been steadily declining due to numerous factors including decreasing agricultural production, low access to fertilisers and industrial pesticides, population pressure and poor soil fertility management practices. To sustain food production systems in the region and to increase yields, there is therefore, a need for substantial increases in appropriate use of low cost technologies that can respond to the above mentioned challenges hence offering the most effective means of increasing crop productivity (Wilchens, 2006).

Description of Research

The study focused on smallholder farmers that grow the bean crop in Malawi: Rumphi, Kasungu and Mzimba districts. The study used both secondary and primary data. A two stage stratified sampling procedure was used in the study. The first stage was to purposively select sample districts and farmer groups in specific locations, which were selected based on the districts' bean production capacity and the historical use of pesticidal plant products in crop management. The second stage involved selecting sample households from the farmer groups using simple random sampling. A total of 150 farmers were randomly selected, and pre-tested semi-structured questionnaires were the main instrument for the survey. The collected data were processed and analysed using a statistical package for social science (SPSS) version 18.0 (Shin, 1998) and STATA version 10 (Iachine *et al*., 2004). Descriptive statistics such as frequency, mean and cross tabulations were used to generate baseline information of the sampled households. Statistical tests such as the t-test and the Chi-square test were used to test the significant difference between the socioeconomic characteristics of sampled households by district category. And further a regression analysis was conducted. The rationale for choosing a particular functional form depends on the research questions and the underlying production process to be modelled. Multiple linear regression model was used to analyse quantitative data where the relationship between the dependent variable and independent variables were established. The relationship between use of pecticidal plant products per unit area was computed as follows;

$$Yi = \quad + \; {}_{1}x_{a} + \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where;

Y_i Denotes total bean output in kg and i =1, 2, 3.....150 observations

Is a vector of coefficient estimates for the independent variables

x_a a, a= 1,2,3,4,5 are four explanatory variables explaining the dependent variable

Is a random variable with mean 0 and variance ^{2}

Research Results and Application

Knowledge and use of pesticidal plant products Botanical use

Results indicated that most of the farmers knew and used more than one pest management options these practices include crop rotation which controls pests and diseases, early ploughing, timely planting and timely weeding which are associated with prevention of pests and disease attacks. Even through on a slightly lower side some farmers are using pesticidal plants to control for pests. This shows that if this innovation of using pesticidal plant products is intensified the majority of bean and cowpea farmers could adopt the technology. Taking adequate farm decision by any farmer or group of farmers could be a good farm management practice necessary for increased food production. This ensures that the right things are done in the farm at the right time. Gender of household head has a bearing on the decisions a household makes. There is now a growing recognition of gender-differentiated interactions among welfare, efficiency, and the success of technology transfers. A number of studies have documented difference s in productivity between female and male-headed households (Addis *et al*., 1999). Other studies have shown gender differences in the adoption of improved technologies. The study findings have shown the need to create a gender disaggregated framework for targeting policy and interventions. In many male dominated countries like Malawi, it is the man who makes the ultimate decision on matters that have to do with household development. In such households, it is easy to adopt new farming technologies unlike fem ale headed households. Results from this study revealed that majority of the households were headed by males. This is a positive indication that if a technology is adopted, it can be adopted. Furthermore, literacy levels have a bearing on decision making a t a farm level and bearing on adoption of new technologies. Results from this study indicated that 85% of the respondents knew how to read and write the vernacular language 'chichewa'/ With majority of the farmers being educated, it is assumed that understanding and utilization of pesticidal plant products will not be a challenge to many. The average age 41 – 48 years indicate that farmers are actively involved in bean farming. Furthermore, the age of a farmer can negatively influence levels of production efficiency since the older the farmers, the less willing they are in adopting news skills and technologies. Results have indicated that majority of the, respondents fall within the productive age group of 15-49 in Malawi. As argued by Liu & Zhuang (2000) that as one gets older, the more his/ her physical strength declines. This implies that, although older farmers are more skilful and experienced, the effects of learning by doing diminishes over time hence this is the rightful age group to adopt and implement new technologies.

Frequency in using pest management options

Information on use of botanical pesticides is the core centre for the project as it will enhance understanding of the pesticidal plant products that farmers are using and the existing pest ma nagement options. The results revealed that farmers preferred the use of botanical pesticides to industrial insecticides as they are locally found and could be relatively more cost effective than synthetic pesticides. Results from the study indicated that farmer who use pesticidal plant products in pest control used them every cropping season. Similarly with other pest management options results showed that the majority of the farmers use them almost every cropping season (Table 1).

Table 1: Frequency in using pest management options in Malawi

FREQ	CR		EP		TP		TW		IIS		BIS	
	n	%	n	%	n	%	n	%	n	%	n	%
Every season	105	70.0	110	73.3	120	80.0	123	82.0	67	44.7	59	39.3
Once in two season	11	7.3	9	6.0	5	3.3	6	4.0	7	4.7	6	4.0
Infrequently	14	9.3	17	11.3	12	8.0	9	6.0	21	14.0	33	22.0
Not applicable	20	13.3	14	9.3	13	8.7	12	8.0	55	36.7	52	34.7
Total	**150**	**100.0**	**150**	**100.0**	**150**	**100.0**	**150**	**100.0**	**150**	**100.7**	**150**	**100.0**

Freq- frequency; CR- crop rotation; EP- early ploughing; TP- timely planting; TW- timely weeding; IIS- industrial insecticides; BIS- botanical insecticides

Most of the farmers did not have problems in using both industrial and botanical pesticides products in controlling crop pests. Those who used both products indicated that they had problems in using industrial pesticides especially due to lack of finances (Table 2). Use of botanical pesticides, was associated with labour involvement for processing the products. To overcome the said challenges, farmers proposed that, there is a need for intensive research on the processing and utilisation of the pesticidal plant products. It was further highlighted that agricultural extension workers need to train farmers on the utilisation of these products. Farmers also cited the need to multiply more pesticidal plants which might be at risk of extinction with increasing population pressure on land for agriculture.

Table 2: Proportion of problems respondents face in using industrial and botanical pesticides in Malawi

Problems encountered	IIS		BIS	
	n	%	n	%
Difficult to process	3	2.0	4	2.7
Limited finances	21	14.0	-	-
Not effective	7	4.7	4	2.7
Risky to humans	1	0.7	-	-
Lack of processing knowledge	2	1.3	-	-
Lack of sprayer	1	0.7	2	1.3
Broke sprayer	-	-	7	4.7
Labour intensive	-	-	9	6.0
Not locally found	-	-	1	0.7
None	57	38.0	71	47.3
Not applicable	58	38.7	12	8.0
Total	**150**	**100.0**	**150**	**100.0**

IIS- industrial insecticides; BIS- botanical insecticides

Regression analysis results provide a basis for informing agricultural policy on what needs to be done to improve smallholder bean productivity. The age of household head negatively influenced efficiency in bean production. The negative influence of age on bean production indicates that younger farmers are more efficient than the older farmers. This is because the older the farmer gets, the more their physical strength declines (Table 3). The negative influence of amounts of botanical pesticides used on bean pest control indicates that those farmers who used low amounts of pesticides in crop management were inefficient in bean production. This calls for detailed research on the best utilisation methods of pesticidal plant products so that bean production levels can be improved. There was a significantly positive impact of the number of years spent in school and bean productivity. This implies that more experienced and educated farmers were in a better position of understanding and integrating agricultural instructions and apply technical skills better than the less educated. Thus, the increased education levels may result in efficient input use through effective research and extension information systems.

Table 3: factors affecting bean production

Variable	Coefficient estimate	Std error	P-value	t-value
Constant	129.6	75.7	0.01	1.7
Age of HH head	-2.4	1.4	0.09	-1.7
Schooling years	7.8	4.7	0.1	1.7
HH size	4.1	6.5	0.5	0.6
Amount botanical used	-2.4	0.2	0.05	-2.0
Area	27.2	1.0	0.008	2.7

In conclusion, farmers' education level, age of household head, amount of botanical pesticides applied and area planted to beans were some of the significant factors that influence bean production. In order to improve smallholder bean production, there is a need of exploring the most cost effective ways to use in order to increase bean production. However, there is need for intensive research on safe ways of using botanicals as well as training extension workers on how they can package the research results and deliver to farmers for improved bean production. Policies and strategies that promote rural education will greatly assist smallholder bean farmers to realize the unexploited production gains from beans. It is thus recommended that research on botanical pestcides should target areas related to processing, utilisation and storage of the botanical pesticide products.

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