UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

ASSESSMENT OF FOUR PLANT EXTRACTS AS MAIZE SEED STORAGE PROTECTANTS AGAINST *Sitophilus zeamais* AND *Prostephanus truncatus* IN GHANA.

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WEST AFRICA CENTRE FOR CROP IMPROVEMENT

JULY, 2018.

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BY

GARIBA YAKUBU SAMUEL

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE AWARD OF DEGREE OF MASTER OF PHILOSOPHY IN SEED SCIENCE AND TECHNOLOGY

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

JULY, 2018

DECLARATION

I hereby declare that, this thesis "**Assessment of four plant extracts as maize seed storage protectants against *Sitophilus zeamais* and *Prostephanus truncatus* in Ghana**", is the result of my own original research work under supervision and has neither been presented in whole nor in part for the award of any degree, with the exception of references to the works of other researchers, which have been duly acknowledged.

…… ……..

Samuel Yakubu Gariba (Student) Date

This thesis has been submitted for examination with our approval as supervisors

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Dr. Vincent Eziah (Supervisor) Date

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Dr. Daniel Dzidzienyo (Supervisor) Date

ABSTRACT

Sitophilus zeamais and *Prostephanus truncatus* are two most important storage insect pests of maize in Ghana and Africa as a whole. These insects cause weight loss of about 20 to 90 % of the untreated stored maize seeds. The control of post-harvest pests largely depends on the use of pesticides. Because of the adverse effects of insecticides on humans and the environment, attempts are being made to discover remedies pest management. This study was to assess four plants (*Lantana camara, Moringa oleifera, Citrus sinensis* and *Hyptis suaveolens*) extracts as maize seed storage protectants against *Sitophilus zeamais* and *Prostephanus truncatus* in Ghana. The study was laid out in a Completely Randomized Design (CRD) with three replications. Dried powders at (5 and 10%) and aqueous extracts (0.05 and 0.1 g/mL) of botanicals were prepared and evaluated for their insecticidal activity against *P. truncatus* and *S. zeamais* in treated maize seed. Untreated control and seed maize grains treated with Actellic were included as checks. There was decrease in oviposition and survivorship of insects was lowest when grains were treated with plant extracts at egg stage compared to the control. There was reduction in the number of adult insects that emerged at all developmental stages of the insects in treated maize seeds with methanol extracts of botanicals. All plant extracts at 0.1 g/ mL showed significant ($P<0.001$) difference in repellency compared to the control; however, *H. suaveolens* recorded the highest repellent activity to *P. truncatus* and *S. zeamais* at 93.3 and 96.7% respectively. Maize seeds treated with *L. camara* and *M. oleifera* recorded a percentage germination of 94.0 each followed by Actellic (93.0%), *C. sinensis* and *H. suaveolens* (86.0%) and the control (82.0 %). Maize seeds treated with methanol extracts of the botanicals after 10 weeks in cribs, recorded a reduction in percentage seeds damaged and weight loss caused by the two insects as compared to the untreated seeds which recorded higher number of damaged seeds and percentage weight loss. The phytochemical analysis revealed

that compounds such as alkaloids, saponins, tannins and phenolic, steroids, flavonoids, anthroquinones, phlobatinins, cardiac glycosides and terpenoids were recorded in all the four plant extracts. These compounds may have caused lower progeny emergence, inhibitory effect, repellent action and antifeedant effect to *S. zeamais* and *P. truncatus* in grains treated with the botanicals.The study proposes that the botanicals tested have the potential in the growth of the seed industry to enhance quality seed production. It is therefore recommended to be used by farmers to control *P. truncatus* and *S. zeamais* in stored maize seed.

DEDICATION

This work is dedicated to my parents, my dearest wife Rosemary S. Kaledzi and my children Samuel Sedinam Gariba and Michael Sedem Gariba for their love, understanding, prayers and support that motivated me to do more.

ACKNOWLEDGEMENTS

I wish to thank the Almighty God for His guidance during this research period. I thank all my supervisors, Dr. Vincent Y. Eziah and Dr. Daniel Dzidzienyo for introducing me to this area and providing excellent scientific and personal advice, guidance, critical comments, suggestions and encouragement at every stage of this research that led to the successful completion of this thesis.

I am highly indebted to the West African Center for Crop Improvement (WACCI) for providing financial support for the MPhil Seed Science and Technology programme. My special thanks go to Dr. Agyemang Danquah (WACCI Coordinator, MPhil Seed Science) and Dr. Edmund Darkwa for their useful suggestions and inspiration rendered for the successful completion of this work. I sincerely acknowledge the teaching staff of WACCI and for the Seed Science program for their effort in aiding in my training.

My appreciation goes to the following laboratory technicians at the Departments of Crop Science and Botany of the University of Ghana: Mr. Kurt I. Martey, Mr. William Asante and Mr. George Ashong Akwetey for their enormous assistance throughout this study.

I am indebted to Mrs. Grace Mansa Eshun, headmistress of St. Mary's SHS, Accra for the opportunity and support she gave me during this period and the entire teaching staff of St. Margaret Mary SHS, Dansoman for their numerous encouragements during this period especially tutors in the Science Department. I would want to appreciate my colleagues at WACCI for their diverse support. I am most grateful to Pastor John E. Buami and Mr. Samuel Aryee of ICGC, Triumph temple for prayers, advice and their money to support me. I am also very grateful to Mr. Michael Larbi Ashiquaye and Mr. Justice Nii Aryaa Codjoe for their enormous contribution in diverse ways towards my education. I owe a lot to those who gave me their time, their intellect and their money. God bless all who contributed to this work in one way or another.

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LIST OF ABBREVIATIONS

AFR	Applicable Federal Rate
CABI	Centre for Agricultural Bioscience International
FAO	Food and Agriculture Organization
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ISTA	International Seed Testing Association
LGB	Larger Grain Borer
MoFA	Ministry of Food and Agriculture
NGO	Non-Governmental Organization
SRID	Statistical Research and Information Department
A. indica	*Azadirachta indica*
C. sinensis	*Citrus sinensis*
H. suaveolens	*Hyptis suaveolens*
L. camara	*Lantana camara*
M. oleifera	*Moringa oleifera*
P. truncatus	*Prostephanus truncatus*

S. zeamais	*Sitophilus zeamais*

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L) is a major cereal grain crop in terms of output. World-wide production is predicted to increase by nearly 370 MT through the subsequent 10 years, considering a growth of 15% by 2023 (OECD-FAO, 2014). By 2050, the global call for maize could rise by 50% as reported by Ignaciuk (2014). The increasing demand for maize and its global advance implies that by 2023, maize will account for the greatest share (34%) of the total crop area harvested (OECD-FAO, 2014). Africa produces around 7 % of the total world production (Verheye, 2010; FAOSTAT, 2014). FAO, (2015) also reported that West African countries have experienced an increased total land area cultivated to maize from 3.2 - 8.9 million ha "between" 1961 to 2005. There is a general increase in area put under the cultivation of maize in Ghana according to MoFA as at 2017 stands at 970,000 Ha (SRID, 2017). Maize plays an enormous role in the Sub-region as it has substituted other staple crops such as sorghum and millet in terms of quantity consumed per household and also, has been a main source of income for smallholder farmers (Smith *et al*., 1997). SRID (2017) reported that the trend of productivity in maize in Ghana as at 2016 was 1.99 MT/Ha which increased slightly to 2.05 MT/ Ha in 2017. The Grain and Legume Development Board in view of this, under the national seed support produced 55 tons of foundation seed maize and processed 500 tons of certified maize seed to address the issue of low productivity in maize (ISSER, 2007). Despite this intervention at the production level, there is evidence of seed and food insecurity arising from storage losses. One of the elements contributing to high storage losses is the problem of storage insect pests such as the maize weevil, *Sitophilus zeamais* (Motschulsky); rice weevil, *Sitophilus oryzae* (L.); Angoumois grain moth, *Sitotroga cerealella* (Olivier); and the larger grain borer, *Prostephanus truncatus* (Horn) (Dobie, 1977; Tefera *et al*., 2011). In West

Africa, an estimated 25 - 40% of grain crop is lost in shops every 12 months due to weevil menace (Coasta, 2014).

The insect have the capacity to infest intact kernels and they are known as primary storage pests of maize (Throne and Eubanks, 2002). According to Markham *et al.* (1994) and CABI (2005), both adult weevils and larvae feed on undamaged grains and reduce them to powder. The pest creates holes in whole previously undamaged grains causing the grain to loss its viability and market value. The seed whose germ has been attacked will not germinate. The main effect *S. zeamais* infestation to stored grains is the damage through feeding activities of the adult weevils and the development of egg, larva and pupa stages within the grain (Longstaff, 1981). The use of stored grains as seeds accounts for almost 80 percent of the seeds used by small-scale farmers (Crissman *et al*., 1993; Louwaars and De Boef, 2012).

The LGB is the single most important field and storage pest of dried cassava and maize in Africa (Farrell and Schulten, 2002). LGB causes a wide range of grain losses in maize, which include: weight loss, nutritional loss, loss in grain quality, loss of seed viability, and loss of commercial value (McFarlane, 1989). Postharvest losses in susceptible varieties can range from 40 to 100% (Mushi, 1990; CIMMYT, 1999; Denning *et al*., 2009). However, according to APHLIS (2015), in Africa, between 2003 and 2014 postharvest weight loss of maize grain ranged from 16.8 to 19.9%. Many farmers promote ‘straight away’ after harvest in order to mitigate pest loss. Farmers forfeit prospective earnings that they would have attained if grains stored are sold later (Stephens and Barrett, 2011). Pest infestation starts from the field before crops are harvested and kept in warehouses. Preventive measures employed by farmers include use of pest resistant cultivars, timely harvesting, crop rotation, use of proper cultural practices in growing crops, good store hygiene and appropriate choice of site for cultivation and storage and manipulation of storage environment. Other control methods like traditional biological, chemical, and phytochemicals can

also be used to destroy, repel and inhibit reproduction of insect pest. There is therefore the call to come out with better, dependable, inexpensive options to handle storage pests that attack crops (Dayan *et al*., 2009). Plant based products may provide attractive alternatives to inorganic insecticides for pests control because plant based products possess little danger to the ecosystem. Weinzierl and Henn (1992) justified that, orange peel oil and powder has fumigant action against fleas. Karr and Coats (1988) also accounted that, orange peel powder and oil has fumigant action against house hold insects and rice weevils. Preliminary research showed that the leaves of *L. camara* possess a rich source of bioactive molecules (Sharma *et al*., 1988). The use of botanicals to control pests at storage will have the potential in the growth of the seed industry to enhance quality seed productions. This research aimed at the usage of botanicals to minimize grain damage precipitated by insect pests, in particular *Sitophilus zeamais* and *Prostephanus truncatus*. Specifically, the study sought to;

1. Assess the efficacy of four plant extracts as maize seed storage protectants against *Sitophilus zeamais* and *Prostephanus truncatus.*
2. Evaluate the effects of pre-planting seed treatment on maize seed germination.
3. Evaluate the toxicity and repellency of methanol extract of the best botanical against *S. zeamais* and *P. truncatus* in stored maize.
4. Determine the active compounds present in each of the four plant extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The origin of maize

Maize (*Zea mays* L.) is also called corn in some countries and a member of the grass family Poaceae. The origin of maize still remains indefinite; nonetheless, it is believed to have originated from Central America. It is believed to have been discovered in Cuba by Columbus in 1492 (Lance and Benson, 2002). The crop was domesticated from its wild ancestor, teosinte. Maize was domesticated in Mesoamerica now Mexico some 5600-80,000 years ago (Lance and Benson, 2002). Maize was distributed throughout the world in the fifteenth century (Farnham *et al*., 2003). Globally, North America produces (41%), followed by Asia (28%), Europe (10%), South America (10%) and Sub-Saharan Africa (6%) are the leading producers of maize (FAOSTAT, 2013). Maize was brought to Africa in the 16th century; most likely through Portuguese traders and in 1900, it was a relatively minor food crop in Africa (Obeng-Bio, 2010). It has replaced the local rice, *Oryza glabberima* due to its high yielding capabilities (Tweneboah, 2000). Cultivated maize is probably the outcome of a human selection and domestication process from the annual teosinte *Zea mays L*. subsp. parviglums after careful study of isozyme difference between maize and teosinte, according to Doebley et al., (1987); Matsuoka et al., (2002).

2.2 Economic importance of maize

Maize occupies about 2 times the area occupied by any single crop and hence makes it the most widely spread cereal crop in the Sub-region (Acquah, 2007). Maize plays an enormous role in the Sub-region as it has substituted other staple crops such as sorghum and millet in terms of quantity consumed per household and also, has been a main source of income for smallholder farmers (Smith et al., 1997). The volume of foundation seeds produced in 2017 amounted to 28.8 MT

(GLDB, 2018). In 2017, the quantity of certified maize seed produced by private certified seed growers was 840 MT (GLDB, 2018**).** Akramov and Malek (2012) reported that maize scores for over 50 % of total cereal production in Ghana. The majority of maize produced goes into food consumption and it is arguably the most crucial crop for food security. As reported by IITA (2009), many areas favoured maize as animal feed because it gives a yellow colour to poultry egg yolks. As noted by Sowunmi and Akintola (2010), maize is a principal constituent in babies' food, brewery and poultry feed industries and it is also fermented to produce hydrolyzed dextrin, sugars, and syrup. Maize, together with rice and wheat, command human diets (Ignaciuk, 2014) and furnish at least 30% of the meals energy of more than 4.5 billion people in ninety-four poor regions. Gupta *et al*., 2015 reported that, it serves as the main component for the manufacturing of a lot of industrial products. Maize serves as good source of highly digestible carbohydrates and has higher protein and fat contents as compared to other cereals. It contains vitamin A, C, K and B-complex as well as large amount of beta-carotene and fair amount of selenium (Kumar and Jhariya, 2013). The grains can either be boiled, roasted, fried, ground, pounded or crushed to prepare different kinds of food items like tuozafi, kenkey, akple, banku, etc. (Morris *et al*., 1999). All these food types are readily available among different ethnic groups in various parts of Ghana. Apart from being used as food, maize is used in the preparation of animal diet due to its nutritional composition and health benefits. Maize can be used for the production of cereal baby foods, corn-oil, glucose, gum, starch, pharmaceutical products and alcohol (beer) in processing industries. The starch obtained from maize can also be manufactured into valuable finished products. A watery by-product of maize called steep liquor is widely used as a medium to cultures microorganisms (Manueke, *et al*., 2015). Approximately 40% (130 million tons) of the 332 million metric tons of maize grown annually in the United States of America is used for corn ethanol production (Torres

et al., 2016). The grain, leaves, stalk, tassel and cob can all be used to produce a large variety of food and non-food products.

2.3. Maize production in Ghana

Maize is produced in all the agro-ecological areas of Ghana (Figure 1).

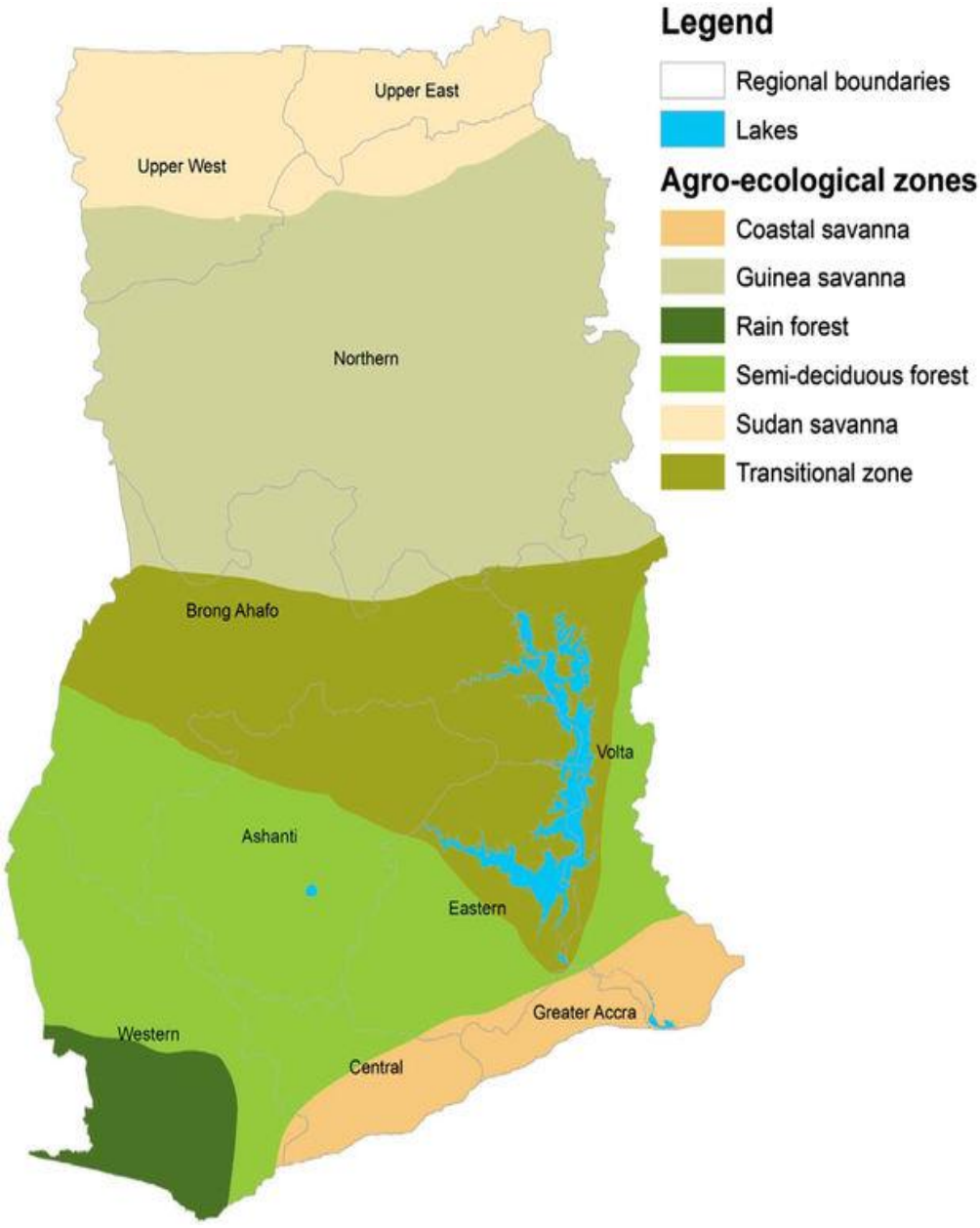

Figure 1: The agro-ecological zones and regions in Ghana (FAO 2005).

The annual rainfall pattern of Ghana is bi-modally distributed, with exception of the northern regions which experience just one season of rainfall. Annual total rainfall amount to only 800 mm. The crop is planted mostly after the beginning of the rainy season (Oteng, 1998). The rainfall pattern in Ghana since 2011 has not been regular and has affected agricultural planning and decision making by key stakeholders especially farmers. The national average rainfall recorded 1080 mm in 2017 and was comparatively better than 2016 which recorded 834 mm (SRID, 2017). The Eastern, Ashanti and Brong Ahafo Regions have two or more growing seasons (major, April – May and minor, September – October). The leading producing areas are mainly in the Brong Ahafo, Eastern and Ashanti regions where 84 % of the maize is grown, with the remaining 16 % being grown in the northern regions of the country (Northern, Upper East and Upper West regions) (SRID, 2013). In Ghana, the Brong Ahafo is the highest production region, which accounted for 27% of national production (IFPRI, 2013).

Maize in forest zone is grown in scattered plots, usually intercropped with cassava, plantain and or cocoyam. There is a general increase in area put under the cultivation of maize in Ghana according to MoFA as at 2017 stands at 970,000 Ha (SRID, 2017). This volume and acreage cultivated are more than triple the next most cultivated cereal, which is rice in Ghana (ISSER, 2016). SRID (2017) reported that the trend of productivity in maize in Ghana as at 2016 was 1.99 MT/Ha which increased slightly to 2.05 MT/ Ha in 2017. Rice (paddy), millet and sorghum were produced on 125,000, 20,000 and 32,000 hectares respectively. These figures show how much maize is important to the economy of Ghana and therefore more much area is allocated to its production.

2.4 Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae)

Plate 1: *Sitophilus zeamais*

2.4.1 Description of *Sitophilus zeamais*

Sitophilus zeamais can be highly destructive to stored maize. It contains three most important species namely *S. zeamais, S. oryzae and S. granarius* (Cornelius *et al.*, 2008). The maize weevil (*S. zeamais*) is a member of the Coleopteran order and Curculionidae family. It varies from dull reddish-brown to almost black with shiny and pitted punctures usually about 3 – 3.5 mm long. The thorax is densely pitted, legs are prominent, and the wings are well developed and can fly readily. *Sitophilus zeamais* usually have four pale reddish-brown or orange-brown oval markings on the elytra. The adult *S. zeamais* has long forward rostrum that carries the mouth parts readily position for perforating plant tissue. The insect has 8 segmented antennae which are often carried in an extended position when the weevil is moving.

The adult males have average rounded aedeagus with two longitudinal channels dorsally and the females with lateral rounded Y-shaped sclerite pointed. The larvae of maize weevils are white, fleshy and legless. The eggs, larvae and pupae stages of the maize weevil are all spent inside the kernel and are not often viewed. The larvae of maize weevils are white, fleshy and legless. The eggs, larvae and pupae stages of the maize weevil are all spent inside the kernel and are rarely seen. The adult weevil emerges by biting a circular hole through external layers of the kernel (CABI, 2010). Adult maize weevil feeds and lives between four to five months (Tefera *et al*., 2010).

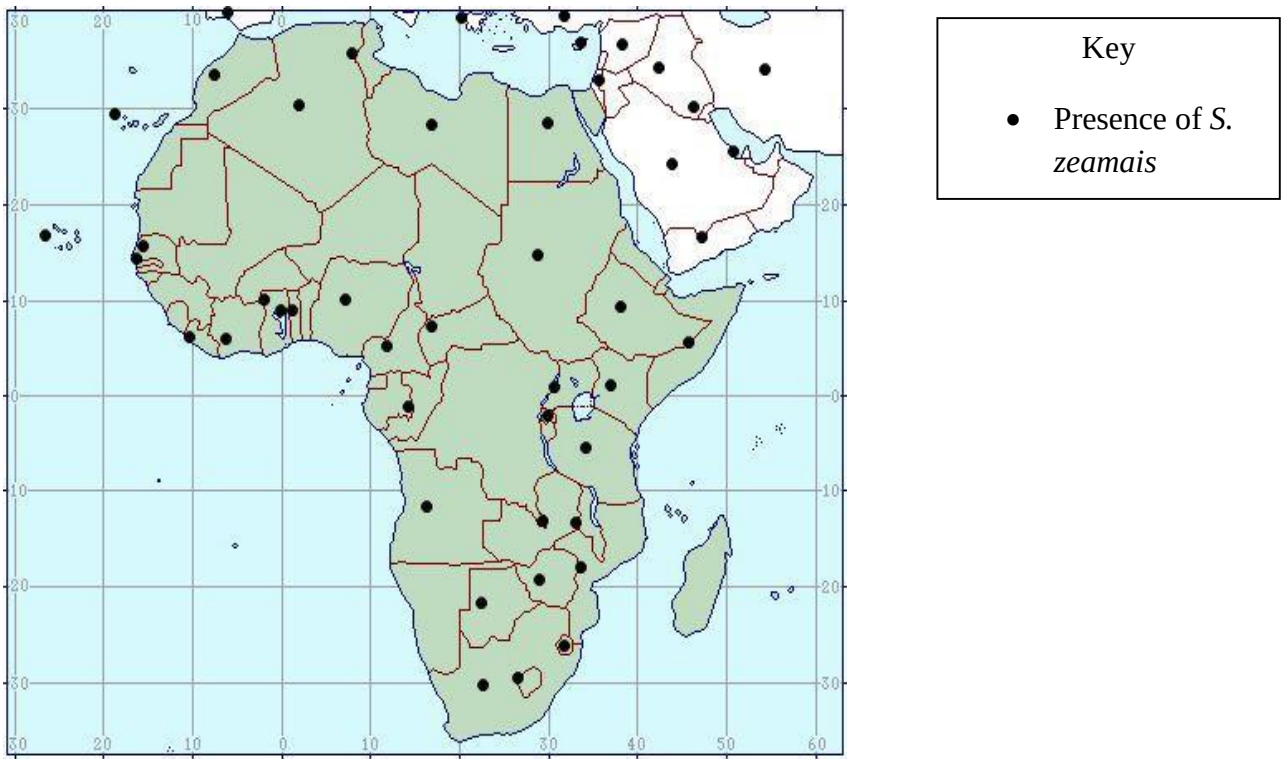

Figure 2: Distribution of *S. zeamais* in Africa (CABI, 2012).

2.4.3 Biology and Ecology of *Sitophilus zeamais*

Sitophilus zeamais is one of the most serious post-harvest pest of stored maize. The pest infestation starts on the field when the grain moisture content is still 50-55% (Adedire, 2001). The adults under suitable environmental conditions can live for several months and are able to lay about 150

eggs in their life time. The female bores through the undamaged grain and deposits eggs into it. The egg is then sealed off by egg plug produced by it. The eggs incubate about six days under suitable temperature of 25°C into larva. The larva feeds inside the grain chambers creating a tunnel as it develops into all the four larval instars. As larval stages feed on the internal parts of the grain, it is difficult to detect infestations early. Pupation takes place after 25 days at 25°C and 70% relative humidity in the maize grain. The pupated (newly developed adult) weevil chews and tunnel out from the maize grain. Not less than 30 days is required for passing through the egg, larval and pupal stages (Mason, 2003). Damage caused by the insect also provides channels for disease and fungal growth in the grain (CGC, 2013b). *S. zeamais* tolerates lower temperatures than *S. oryzae* and can live for 37 days at 0°C (Stoyanova, (1984); Zewar, (1993).

2.4.4 Economic Importance of the Maize Weevil

The insect have the capacity to infest intact kernels and they are known as primary storage pests of maize (Throne and Eubanks, 2002). According to Markham *et al.* (1994) and CABI (2005), *Sitophilus zeamais* have been recognized as an increasingly important constraint to maize production in Africa. Both adult weevils and larvae feed on undamaged grains and reduce them to powder. The pest creates holes in whole previously undamaged grains causing the grain to loss its viability and market value. The seed whose germ has been attacked will not germinate. The main effect *S. zeamais* infestation to stored grains is the damage through feeding activities of the adult weevils and the development of egg, larva and pupa stages within the grain (Longstaff, 1981). The use of stored grains as seeds accounts for almost 80 percent of the seeds used by small-scale farmers (Crissman *et al*., 1993; Louwaars and De Boef, 2012). *Sitophilus zeamais* feeds on separate grains leaving only the hulls. Infested grains show characteristic emergence holes for the adults on the outer layers of the grains. Heavily infested grains by this pest usually become heated at the surface, sometimes to such an extent that germination takes place. The immature life stages

of the weevil are spent inside the kernel, and they are hardly seen outside of the kernel. Internal feeding by this pest reduces both the quality and quantity of the grains (Longstaff, 1981). The metabolic activity of *S. zeamais* accelerates fungal infection and growth (Beti *et al*., 1995).

2.4.5 Damage of seed by the weevils during storage

Sitophilus zeamais causes qualitative and quantitative damage to stored products, with grain weight loss ranging between 20 to 90% for untreated stored maize (Giga *et al*., 1994; Nukenine *et al*., 2002; Muzemu *et al.*, 2013). The direct damage made by weevils is that they may cause the reduction in seed viability, loss in weight; the seed whose germ once attacked will not germinate. Some farmers in Ghana also use saved seed for the next planting season. Therefore, these farmers may not get quality seeds for planting if these seeds are not protected from weevil damage, they may also cause loss in viability and the seeds may not germinate after planting. The indirect damage includes loss of quality of maize which may include nutrient loss, heating and spoilage, production of off flavours, discoloration and predisposition to diseases. There is therefore the need to find an alternate way of treating seeds before, during and after storage to prevent insect damage.

2.5 Larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

Plate 2. *Prostephanus truncatus*

2.5.1 Description of Larger grain borer

The larger grain borer is a serious pest that attack mainly dried cassava and maize cobs through the apex. The adult beetle is cylindrical in shape and is about is 3.2 - 4.7 mm long. The head is curved underneath the thorax to stop the lower back of the head being considered from the above. The eggs of LGB are white to yellow, with no surface features and have an extensive oval shape. The larva is white, fleshy, a thin envelope of hairs and a C-shaped body. It also has a small head and short legs (Anne *et al.*, 2011). Plate 2 shows the Larger Grain Borer (*P. truncatus*).

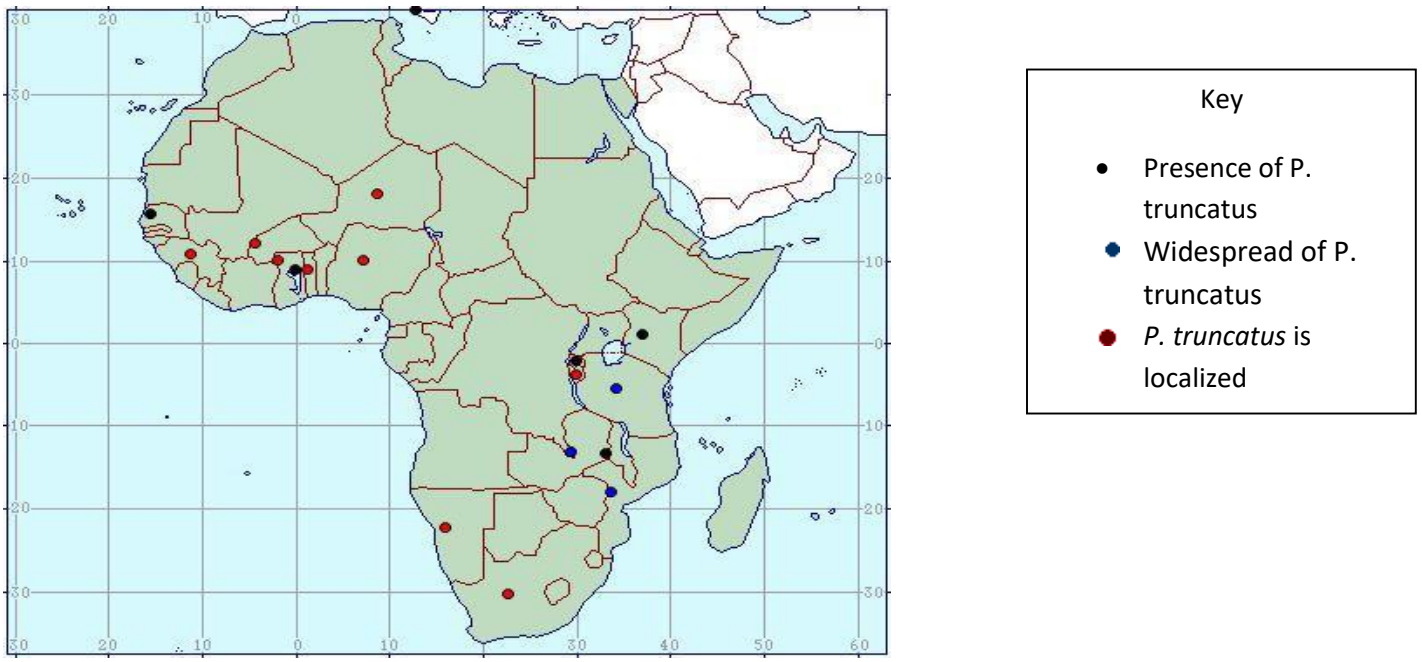

Figure 3: Distribution of *P. truncatus* in Africa (CABI, 2012).

2.5.2 Biology and ecology of larger grain borer (LGB)

The LGB is found in different habitats and ecologies (Hodges, 2002). Hill *et al*., (2002) grouped the ecology of *Prostephanus truncatus* into, ecology outside the storage system and ecology within the storage system. Ecology outside the storage system includes forests, woody frames, grain storage facilities, dry timber, green timber, sap wood and forest branches (Hill *et al.*, 2002). Sixteen tree species belonging to the groups Leguminosea, Burseracea and Anacardiaceae are alternative hosts of LGB. The pest prefers young soft wood to old wood (Nang'ayo *et al*., 1993). Within the storage system, LGB associates with other insect pests that destroy maize, such as predators, parasitoids and ecto-parasites (Hill *et al.*, 2002. The morphology of LGB is described by deflexed head with well-built mandibles and cylindrical body protected by pronotum that give the insect excavation abilities (LI, 1988). LGB has a body length of 2 to 3.5 mm and a width of between 1 to 1.5 mm. The pest is able to reproduce on maize grain and cobs, dry cassava and other stored-

products. Females can lay five to eight eggs in each oviposition chamber and 300 eggs can be produced in its entire lifespan (Tefera et al., 2010).

2.5.3 Economic Importance of larger grain borer

The larger grain borer is a pest of particular significance since its destruction to grains is about 40% in stored maize cob for six months. Its host is mainly maize and cassava but can also be found in minor hosts such as yam, sorghum, triticale and wheat. They bore into several foodstuffs and other materials such as wood causing considerable weight loss. The insect is exploratory hence boring holes into solid substances like wood used to construct farm structures, groundnut, beans, Perspex and polythene not for breeding or nutritional value. These result in increased cost of farming and loss of valuable items (Cornelius *et al.*, 2008).

2.5.4 Damage of seed by the larger grain borer (LGB) at storage.

The LGB is the single most important field and storage pest of dried cassava and maize in Africa (Farrell and Schulten, 2002). LGB causes a wide range of grain losses in maize, which include: weight loss, nutritional loss, loss in grain quality, loss of seed viability, and loss of commercial value (McFarlane, 1989). Postharvest losses in susceptible varieties can range from 40 to 100% (Mushi, 1990; CIMMYT, 1999; Denning *et al*., 2009). However, according to APHLIS (2015), in Africa, between 2003 and 2014 postharvest weight loss of maize grain ranged from 16.8 to 19.9%.

2.6 Control of insect pests of stored products

Pest control is as old as agriculture, as there has always been a need to protect crops free from pests. They cause significant losses to plants and crops throughout the world. As a result, producers have adapted preventive and curative means against pest of stored products (Zehrer, 1980). Pest infestation starts from the field before crops are harvested and kept in warehouses. Preventive measures employed by farmers include use of pest resistant cultivars, timely harvesting, crop

rotation, use of proper cultural practices in growing crops, good store hygiene and appropriate choice of site for cultivation and storage and manipulation of storage environment. Other control methods like traditional biological, chemical, and phytochemicals can also be used to destroy, repel and inhibit reproduction of insect pest.

2.7 Control of *Sitophilus zeamais* and *Prostephanus truncatus*

2.7.1 Cultural methods of storage

The conventional approach might have probably been the first to be employed in food storage, since it is comparatively easy to prevent pest attack from stored grains. It involves the use of methods such as the selective breeding of pest-resistant cultivars, timely harvesting of crops, cleaning stores among others. Poswal and Akpa (1991) reported that ash and sand were among the local materials used by most African countries to protect their grains from insect pest (*S. zeamais* and *P. truncatus*) attack during storage. The protectants do not have any insecticidal value but fill the spaces between grains restricting the movement of adult insects from oviposition. They also act as desiccant dehydrating insects leading to their death. The powders of these protectants in addition may block the respiratory tract of insects leading to eventual suffocation. Golop (1997) reported that some types of sand that contain high amount of quartz cause damage to sensitive cuticle of newly hatched larva by removing waxy layer of the cuticle of the exoskeleton resulting in 80% of mortality in *Callosobruchus maculatus.*

Maize cobs that suspended on bamboo fork sticks in kitchen and smoked completely prevents storage insect pest from damaging the seed (Poswal and Akpa, 1991). Continuous drying of maize grains under the sun may reduce grain moisture content leading to the reduction of the activity of insect pest (*S. zeamais* and *P. truncatus*) in the grain. Granaries should be well cleaned to destroy

hiding places of insects before new grains are filled into the stores. Bins and airtight stores should be closed to prevent *S. zeamais* and *P. truncatus* from getting oxygen.

2.7.2 Biological Control

The use of natural enemies has been one of the key strategies in controlling larger grain borer. One of such biological agent is *Teretrius nigrescens* Lewis. *T. nigrescens* Lewis (Coleoptera: Histeridae) is natural predator of larger grain borer (Paliani and Muwalo, 2001) as it is attracted by aggregation pheromone produced by LGB (Rees *et al.*, 1990). The larvae and adults of *T. nigrescens* feed on eggs and larvae of LGB (Rees, 1987). This was attributed to the fast multiplication of the predator within a short time after release. Rees (1991) reported LGB infestation reduction by 83% after introduction of the natural enemy. Since 1990, *T. nigrescens* has been deployed in selected sites in Malawi (Paliani and Muwalo, 2001). Although, there were reports of reduction in numbers of LGB after introduction of *T.nigrescens*, (Paliani and Muwalo, 2001), the strategy has not been very successful, as the population of the insect pest is on the increase. New Biological control using natural enemies is another management option for maize weevils. Biological control means that useful organisms are used to control harmful organisms. Many insect species that occur in the ecosystem of stored products are potential biocontrol agents and have been studied in the protection of stored product from insect pests. There have been various studies on biological control agents for *S. zeamais*. *Sitophilus zeamais* is commonly parasitized by pteromalids (and occasionally other Hymenopterans). In the case of the maize weevil, common pteromalid parasites found in the tropics include *Anisopteromalus calandrae*, *Lariophagus distinguendus* and *Theocolax elegans*. These biological control agents should be introduced at an early stage in the storage period for effective control of *S. zeamais* (CABI, 2010). Wen and Brower (1994) found that the release of *T. elegans* suppressed the population of *S. zeamais* to over 90%. Also multiple releases of *A. calandrae* suppressed the buildup of weevil

population during long storage periods of maize (Arbogast and Mullen, 1990). The bacterium *Bacillus thuringiensis* can also be used in the control of adult weevil (Anne *et al*., 2011). Brower *et al.* (1995) reported that the use of natural enemies to control insect pest can be classified into different ways; based on their life history, population dynamics and ecology. Hence, the need to develop a more robust system that could effectively contain the spread of LGB in Ghana, and that system should incorporate host resistance.

2.7.3 Use of insecticides

The control of LGB has depended heavily on the use of insecticides mainly organophosphates. Organophosphates such as pirimiphos-methyl, fenitrothion, permethrin and bromophos dilute dust have been used in Tanzania (Golob, 2002). In a trial that was conducted at Tumbi Research Station in Tanzania, only Pirimiphos-methyl was found to be more effective against LGB (Golob *et al.*, 1983). The use of Actellic super by smallholder farmers has been documented in a number of African countries (Kimenju and De Groote, 2010). For example, in Tanzania, Actellic super is overwhelmingly being used by smallholder farmers with an adoption rate of 93% (Kaliba *et al.*, 1998). In Malawi, farmers use Methacrifos 2P, bifenthrin 22 and Actellic super to control LGB (Paliani and Muwalo, 2001; Ching'oma, 2009; Kasambala, 2009). Even though the use of chemical control has largely been effective in mitigating the devastating effects of LGB, there is a possibility of the pest developing resistance to the insecticides due to misuse. For instance, after permethrin was used for 4 years in Tanzania in the form of dust, an increase in adult survival of *P. truncatus* was observed in maize (Golob, 2002). Due to the increasing occurrence of insecticide resistance, possibility of environmental damage, grain contamination and costs, there is need to look for alternative methods to protect maize against LGB (Golob, 2002; Ahmed and Yusuf, 2007; Singano et al., 2009). Among insecticides being used now are organophosphates, carbamates and synergized pyrethroids. Phosphine and Organochlorines like lindane were formerly used as grain

protectants but now banned due to chronic poisoning, high persistence and toxicity to consumers. Organophosphorus like *pirimiphos-methyl* (Actellic) which is broad spectrum (kills both insects and mites), high knockdown effect and prevent re-infestation of pest (like *S. zeamais* and *P. truncatus*) in long storage. Other organophosphorus compounds include Etrimfos (Satisfar), Methacrifos (Damfin) and Chlorpyrifos-methyl has low toxicity and high knockdown effect, high persistent and effective against eggs and larvae of insects in storage. It is in the light of these that prompted the researcher to assess the efficacies of the following plants as maize seed storage protectants against *Sitophilus zeamais* and *Prostephanus truncatus.* The four botanicals identified by the researcher include *Citrus sinensis*, *Hyptis suaveolens*, *Moringa oleifera* and *Lantana camara.*

2. 8 Insecticidal plants used in this study

2.8.1 Orange (*Citrus sinensis* L)

2.8.1 Importance and Uses of Orange Peel

Extracts from the peel had been determined to have a precise total radical antioxidative conceivable potential (TRAP) (Gorinstein *et al.*, 2001). Orange peel powder was able to manage Callosobruchus maculatus in treated cowpea (Don-Pedro, 1985). The orange peel oil in a study showed toxicity towards *Culex pipiens* (Mwaiko and Savael, 1992). Weinzierl and Henn (1992) similarly defined that, orange peel oil and powder has fumigant action towards fleas. Lee *et al*. (2003) also reported fumigant action of orange peel oil against weevils. Odeneyi *et al*. (2000) also put it that, citrus peel powder caused mortality of weevils. Owoade (2008) also confirmed that the use of the powder could have resulted to death in the tendency of the powder to block the spiracle of insects. Okonkwo and Okoye (1996) noted that the powder inhibited adult emergence of maize

weevils. It was also confirmed by Onu and Sulyman, (1997) that the plant volatile essential oils of fruits peels of some citrus species have insecticidal properties against stored grains insect pests. Experiment conducted by Intekhab and Aslam (2009) confirmed that, sweet orange is a medicinal plant prescribed as traditional medicine to treat diverse illness. Han (1998) confirmed through an experiment that sweet orange peel has also been used as insect repellent, antibacterial and larvicide. According to Omomouwajo *et al*. (2005) put it that essential oil of citrus also has fumigant toxicity against mosquitoes.

2.8.2 *Hyptis suaveolens* (L. Poit) (Bush mint/ mint weed)

Plate 4: ***Hyptis suaveolens***

Uses of *Hyptis suaveolens*

Hyptis suaveolens is an importance plant that is regarded as weed in many places (Azevedo *et al*., 2001). Others also use it as medicinal tea in various regions in Asia and as food and source of oil of importance in Mexico and other places. In Ghana, some communities also regard *H. suaveolens* as an important medicinal plant to control mosquitoes in their homes. The plant is harvested or uprooted and then placed in their rooms at any time of the day as it is believed to poses insecticidal properties. The leaves and other parts of the plant are sometimes boiled to be taken as tea and it is considered to cure malaria, colds, cramps, indigestion, enhance virility and other ailment (Olayinka *et al*., 1999; Oliveira *et al*., 2005; Santos *et al*., 2007).

2.8.3 *Lantana camara* (Wild sage)

Plate 5: *Lantana camara* showing seeds and inflorescence.

Uses of *Lantana camara*

Lantana camara is an important plant of research because it is globally distributed. The plant has different medicinal and insecticidal properties (Caroprese *et al*., 2011). Lantana as it is commonly called is listed among one of the important medicinal botanicals globally (Sharma *et al*., 2000). Lantana has an insecticidal action (Abdel *et al*., 2005), nematicidal activity (Oamar *et al*., 2006) and used by people to cure cancers and tumours (Ghisalberti, 2000). Adebayo and Gbolade, (1994) reported that *L. camara* which contains caryophyllene and germacrene D in large quantities exhibited some ovipositional suppression on *Callosobruchus maculatus.* Also Schmutterer (1990) and Ndomo *et al*. (2009) confirmed that that botanical extracts and their essential oils have anti-oviposition and fertility reducing effect on a host of insects.

2.8.4 *Moringa oleifera* (Lam) (Moringa)

Plate 6: Moringa plant and leaves being dried

Uses of Moringa

Moringa seeds contain about 35% oil. This oil is often extracted for cooking and in rare cases, even lubrication purposes. Moringa leaves can be used in salads and soap making (Von Maydell, 1986). Verma *et al*., (2009) assessed the antioxidant properties of *M. oleifera in vivo* and *in vitro* and concluded that *M. oleifera* leaf possessed high phenolic content and potent antioxidant properties, whilst Sulaiman e*t al*., (2008) investigated its anti-inflammatory effect in a rat model, and found it to have anti-inflammatory properties. The use of leaf extracts of *M. oleifera* as indicated by Foidl *et al*., (2001) that growth of plant was enhanced.

The plant also contains numerous secondary metabolites, some of which are of imminent importance due to their medicinal properties. Small proteins separated from the leaves of Moringa possessing antifungal and antibacterial activity (Dahot, 1998). The use of leaf extracts of *M. oleifera* as indicated by Foidl *et al*., (2001) that growth of plant was enhanced. Methanolic extracted from the roots has moringine and moringinine which have been reported to possess analgesic and anticonvulsive properties (Grupt *et al.,* 1999).

CHAPTER THREE

3.0 Materials and methods

3.1 Experimental site

The experiment was conducted in the Entomology Laboratory at the Department of Crop Science, University of Ghana, Legon, from October, 2017 to April, 2018. The final field trial was carried out at the University of Ghana farm, Legon from April, 2018 to June, 2018.

3.2 Collection and culturing of maize weevil & larger grain borer

3.2.1 *Sitophilus zeamais*

Stock of *S. zeamais* used for the experimental set up was collected from the Entomology Laboratory of the Department of Crop Science, University of Ghana. The maize weevils used to set up the culture were obtained from an infested glass jar of maize in the Entomology Laboratory. About 250 unsexed adult *P. truncatus* were introduced into 2L plastic container containing 500 g of maize grain samples and kept in the laboratory of 28 ± 2°C, 65% relative humidity and 12 h light: 12 h dark. The culture was kept on the shelf of the laboratory for one week to allow for oviposition. The adult insects were sieved out and emerging generations were used to set up the experimental cultures. The culturing of *S. zeamais* was replicated four times to get enough insects for the experiment.

3.2.2 *Prostephanus truncatus*

Samples of *P. truncatus* were obtained from infested maize grain stock from the Entomology Laboratory at the Crop Science Department. The grains were first sieved to get rid of dirt and broken particles and sterilized in an oven at 60°C for 3 hours and allowed to cool for 12 hours

before being used for the culture. About 250 unsexed adult *P. truncatus* were introduced into 2L plastic container containing 500 g of maize grain samples and kept in a controlled room of 28 ± 2°C, 65% relative humidity (Osafo, 1998; Weaver *et al*., 1998; Udo *et al.*, 2009). (Plate 7) After one week of oviposition, the adult insects were sieved out and the culture allowed to stand for emerging progeny which were used to set up the research cultures (Udo *et al.*, 2009).

3.2.2 *Prostephanus truncatus*

Samples of *P. truncatus* were obtained from infested maize grain stock from the Entomology Laboratory at the Crop Science Department. The grains were first sieved to get rid of dirt and broken particles and sterilized in an oven at 60°C for 3 hours and allowed to cool for 12 hours before being used for the culture. About 250 unsexed adult *P. truncatus* were introduced into 2L plastic container containing 500 g of maize grain samples and kept in a controlled room of 28 ± 2°C, 65% relative humidity (Osafo, 1998; Weaver *et al*., 1998; Udo *et al.*, 2009). (Plate 7) After one week of oviposition, the adult insects were sieved out and the culture allowed to stand for emerging progeny which were used to set up the research cultures (Udo *et al.*, 2009).

Plate 7: Cultures of maize weevil and larger grain borer used for the experiments.

The picture above (Plate 7) shows maize weevil and larger grain borer cultures used for the research in the Entomology laboratory of the department of crop science. The cultured insects were used throughout the research work.

3.3 Selection of plants.

The selection of botanicals used in the storage of grains in this research was based on the following factors. These factors include previous research carried on the plants, effectiveness of the botanical against stored insects and availability. Four plants of (*L. camara*, *H. suaveolens*, *C. sinensis* and *M. oleifera)* were identified. A reference synthetic pesticide - Actellic was selected based on the fact that it is one of the most commonly used synthetic chemical to store grains in Ghana and a control (untreated grains).

Table 1: Botanicals used for the experiment

Treatment	Family name	Common name	Part used	Stage of collection
Lantana camara	Verbenaceae	Lantana	Leaves	Before/ flowering stage
Moringa oleifera	Moringaceae	Moringa	Leaves	Before / flowering stage
Citrus sinensis	Rutaceae	Orange	Fruit peel	Matured fruit
Hyptis suaveolens	Lamiaceae	Bush mint	Leaves	Before/ flowering stage

Hyptis suaveolens *Moringa oleifera* *Lantana camara* *Citrus sinensis*

3.4 Collection and preparation of plant powders

Sweet oranges (*C. sinensis*) were bought from the Amasaman market and were peeled for use for the experimental work. Fresh leaves of *L. camara*, *Hyptis suaveolens* and *M. oleifera* were collected from bushes at Pokuase and Amasaman all in the Ga – West District in the greater Accra region in clearly labelled manila bags and transported to the experimental site within 24 hours. They were brought into the Crop Science Laboratory of the University of Ghana, Legon where they were prepared for the confirmation of their identity at the Herbarium in the Botany Department of the University. The plant specimens were then washed with tap water to remove sand and other unwanted particles and air dried under room temperature for 14 days (Wambua *et al*. 2011). The selected botanicals were pounded using mortar and pestle after which they were ground to give a fine powder with grinder. The powders were sieved with Impact Test Sieve of a mesh size 70µ to give a uniform size powders. The ground powders were stored in four different air tight containers in a cool place away from sunlight before being used for the treatment of the grains against the insects.

3.5 Preparation of methanol extract of plants

About 100 g each of the plant powders were weighed into six different conical flasks containing 430 mL each of 100% methanol. The flasks were covered with Parafilm and placed in a shaker for 48 hours. The solution was filtered with a net of 2.5µ and concentrated using rotary evaporator at 60°C after which the residues were dissolved in acetone to give a concentration of 0.05 g/mL and 0.1 g/mL for the various bioassays. This was based on preliminary tests carried out.

3.6 Assays on the effect of plant powders on adult insects

Whole grains (5 kg) were kept on metal trays and sterilized in an oven at 60°C for 3 hours. The sterilized grains were equilibrated under a controlled environment at 28 ± 2 °C at 65% relative humidity for 24 hours with the aid of thermohygrometer. Sterilized whole maize grains (100 g) were put in to glass jars and four botanicals powders of two sets (5% and 10%) were admixed to the grains. Actellic 25 EC was applied at 2 ml/L of acetone whiles the control treatment was without any botanical powder. The setups were left to stand for one hour before infesting with 20 adult *S. zeamais* and *P. truncatus* (5-10 days old) in to the treated and untreated maize grains in the bottles. The treatments were replicated three times in a completely randomized design (CRD). Daily mortality of insects was recorded for seven days. Insects were considered dead if they did not respond to probing of by a blunt probe.

3.7 Evaluation of the effect of methanol extracts on adult insect in treated grains

Sterilized maize grains (50 g) were put in kilner glass jars and four different botanicals (*C. sinensis, L. camara, H. suaveolens and M. oleifera*) with two concentrations (0.05 g/ mL and 0.1

g/ mL) and Actellic of 2 ml/ L were applied to the grains in each jar. The control was treated

with only acetone. The treated grains were air dried for one hour to evaporate the solvent following which twenty adult *S. zeamais* and *P. truncatus* (5-10 days old) were introduced into the jars which were then covered with muslin cloth held with rubber bands. The treatments were replicated four times in a completely randomized design and left under controlled room at 28 ± 2° C and 65% relative humidity for one week. Mortality of insects was taken as insects were considered dead if they did not respond to three probing with blunt probe.

3.8 Contact toxicity assays by topical application

In this test, the method adopted by Obeng-Ofori, Reichmuth (1997) was used. Ten adults of *S. zeamais* and *P. truncatus* (5-10 days old) each were placed in a separate petri dish lined with moist filter paper to immobilized insects for three minutes. To the notum of the insects 1 µL each of four botanical extracts, actellic and a control (water) were applied using micro – pipette. The experiment was replicated four times in a completely randomized design. The mortality of insects was taken for five days.

3.9 Evaluation of the effect of methanol extracts on oviposition

Maize grains (50 g) were weighed into glass jars and treated with four different botanicals each. Another jar was treated with Actellic at (2 ml/L) whilst the control was treated with acetone. The treated grains were left for one hour after which the grains were infested with mixed sexes of 20 adult *S. zeamais* and *P. truncatus* (5-10 days old). The jars were covered with muslin cloth held with rubber band and placed under control environment at 28 ± 2° C and 65% relative humidity for seven days to allow for oviposition. The experiment was arranged in completely randomized design and was replicated three times. The adult insects were sieved on the eighth day and the number of eggs laid was determined using the egg plug staining techniques (acid fuchsin method) FAO (2008).

3.10 Evaluation of the effect of methanol extracts of plants on eggs and immature stages of *P. truncatus* and *S. zeamais*.

3.10.1 Evaluation of the effect of methanol extracts on eggs

Sterilized maize grains (100 g) of 12% moisture content were weighed into six glass jars. The grains were infested with 20 adult *S. zeamais* and *P. truncatus* (5-10 days old) of mixed sexes into the two sets of jars respectively to allow for egg laying. The adult insects were removed after seven days of oviposition and the percentage oviposition was determined before grains were treated with methanol extract of *C. sinensis, L. camara, H. suaveolens and M. oleifera* at 0.05 g/ mL and 0.1 g/ mL. The control and the reference were treated with acetone and actellic. Each treatment was replicated three times in a completely randomized design. The emerging adults were counted and recorded.

3.10.2 Evaluation of the effect of methanol extracts on larva.

Twelve glasses jars containing 100 g of sterilized maize each were infested with 20 adult *S. zeamais* and *P. truncatus* (5-10 days old) of mixed sexes and allowed for oviposition for seven days. The adult insects were sieved out on the seventh day and the grains were allowed to stay for extra seven days for the eggs to hatch into larva. The grains were then treated with two levels of concentrations (0.05 g/ mL and 0.1 g/ mL) of the botanicals and actellic whilst the control treated with acetone. The experiment was replicated four times under completely randomized design. The emerging adults were counted and recorded.

3.10.3 Evaluation of the effect of methanol extracts on pupa.

In this experiment, 100 g of sterilized maize grains each was put in twelve glass jar twenty adult *S. zeamais* (5-10 days old) of mixed sexes were introduced into six of the jars whilst the other six jars were also infested with twenty adult *P. truncatus.* The adult insects were sieved on the seven day after oviposition. On the 22nd day, the grains were treated with four different botanicals

extracts and actellic at two concentrations (0.05 g/mL and0.1 g/mL) whilst the control was treated with acetone. Each treatment was replicated three times in a completely randomized design and the adults that emerged were counted and recorded.

3.11 Repellency of methanol extracts assay on *S. zeamais* and *P. truncatus*

The repellency of methanol extract of botanicals on *Sitophilus zeamais and Prostephanus truncatus* was assessed using the method adopted by Obeng-Ofori and Reichmuth (1997) and carried out in the laboratory at 28 ± 2and 68-73% relative humidity. Full disc filter papers were divided into two halves in which one half of the filter papers were treated with the test solutions (0.05 g/ mL and 0.1 g/ mL) whilst the other halves were treated with acetone using micro pipette. The treated filter papers were air dried in the laboratory for three hours. The treated and untreated filter papers of the same dimension were attached to each other to form a full disc using cellotape. Each of the filter paper was placed in a petri dish and 10 adults (5-10 days old) *S. zeamais* and *P. truncatus* of mixed sexes were put at the center of each of the filter paper and covered. The experiment was replicated three times in a completely randomized design. After 30 minutes of the introduction of the insects, the number of insects present on treated (Nt) and control (Nc) were counted and recorded. (Plate 8).

The percentage repellency (PR) values were computed using PR = [(Nc – Nt)/ [(Nc + Nt)] X 100.

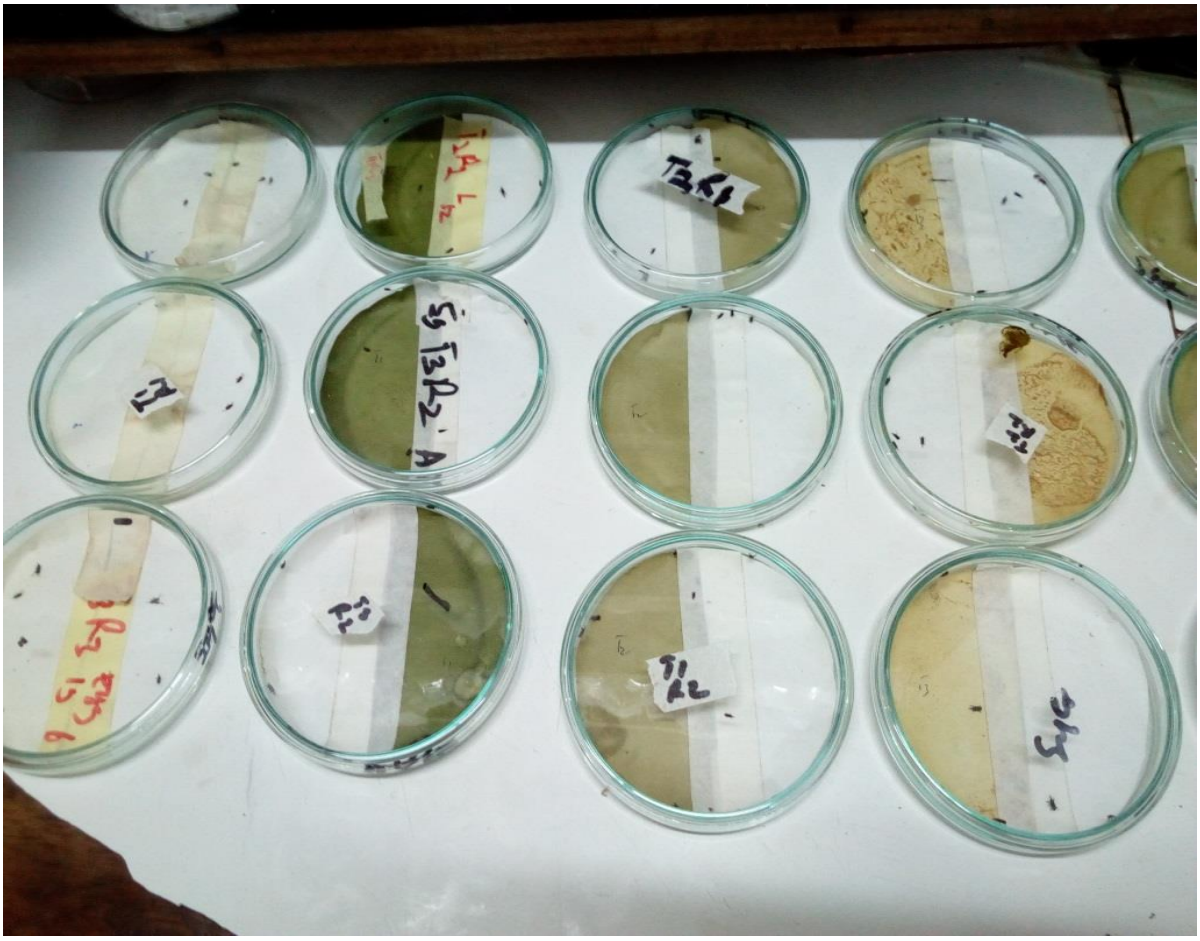

Plate 8: Set up for repellency test.

3.12 Seed Germination Test

Seed germination test was conducted by randomly picking one hundred (100) seeds from the maize before and after the seeds were treated with methanol extracts of four botanicals. The test was conducted with four (4) replicates of twenty-five (25) seeds per replicate. The selected seeds were placed on a wetted blotter paper in Petri dishes. After 7 days, the number of germinated seeds were counted and recorded. The percentage germination (viability index) was calculated using the formula: GP = NSG/TNS ×100; where, GP = germination percentage, NSG = number of seeds germinated from each Petri dish and TNS = total number of seeds tested in each Petri dish (Zibokere, 1994; Ogendo *et al*., 2004).

3.13 Damage assessment of the methanol extracts of plants by *S. zeamais* and *P. truncatus*

Grain damage was assessed using the method adopted by Cornelius *et al.* (2008). Sterilized whole maize grains (2 kg) each was treated with methanol extracts of four botanicals. The control was treated with methanol only. The treated grains were air dried for three hours after which the grains were introduced into 30 x 40 cm sacks. One hundred adult (5-10 days old) *S. zeamais* and *P. truncatus* of mixed sexes were released into the two different bags respectively. Each treatment was replicated three times. The bags were then kept in a crib at the University of Ghana farm for 10 weeks after which loss was assessed using count and weigh method. Samples of 1000 grains were taken from each of the treatments and 500 grains each were counted from the 1000 grains. The 500 grains were separated into damaged and undamaged grains. Each of them was counted and weighed. Percentage weight loss was calculated using the method adopted by FAO (1985) as modified by Cornelius *et al.* (2008) as:

Percent Weight Loss = $(UNd)-(DNu)/ U (Nd + Nu) X 100$

Where *Nu* is the number of undamaged grains

Nd is the number of damaged grains

U is the weight of undamaged grains

D is weight of damaged grains.

3.14 Data Analysis

Data on percentages were arcsine transformed whereas data on counts were square root transformed so as to stabilize the variance. A general analysis of variance (ANOVA) for adult weevil mortality, number of emerged as adults, percentage seed weight loss, percentage seed damage and percentage seed germination were conducted using GenStat statistical package 12th

Edition. Mean separation was done by using Fisher's protected LSD to compare the significant differences between the treatments at 5% level of significance.

3.15 Qualitative and Quantitative Phytochemical analysis of the selected Plant leaves

Chemicals/Reagents

The following reagents were purchased and used for the analysis:

Alcohol, Drangendorff's reagent, Mayer's reagent, Ferric chloride, Benzene, 10% Ammonia solution, Sulphuric acid (aqueous & concentrated), Chloroform, Glacial acetic acid, Aluminium Chloride, Distilled water and Olive oil.

Extraction of plant material

The Methanolic extracts of the various plant materials where prepared by soaking 500g of each in 1000 mL of Methanol in a conical flask, covered and placed on a shaker for 48 hours. The extracts were filtered using a vacuum filtration system and concentrated to dryness using a rotary evaporator. Dry extracts were stored at 4 ^{0}C until analyses. 1 mg / mL of stock solution of each plant sample was prepared and was used for both the quantitative and qualitative analysis.

The whole experiment was conducted in triplicates.

Qualitative Analysis

Test for Phytochemicals.

Test for Alkaloids

Mayer's test: Each sample (0.5-1 mL) was taken into a test tube. A few drops of Mayer's reagent were added; it was shaken well and allowed to settle for some time. Cream colour precipitate indicates the presence of alkaloids in the sample. (Dragendorff's and Mayer's Test).

Tests for Saponins

The method described by Wall *et al*., (1951 and 1954) was used. About 0.5 g of each plant extract was shaken with water in a test tube. Frothing which persisted after heating was taken as a preliminary evidence for the presence of saponins. Few drops of olive oil was added to 0.5 g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of Saponin (Odebiyi and Sofowora, 1978; Ngbede *et al*., 2008).

Test for Tannins and Phenolic compounds.

(Ferric chloride test): Few drops of ferric chloride are added to 0.5 mL of test solution in a test tube. Appearance of blue – green colour confirms the presence of tannins and phenolic compounds in the sample.

Test Phlobatannins

About 0.5g of each plant extract was boiled with 1 per cent aqueous hydrochloric acid. A deposition of a red precipitate was taken as evidence for the presence of phlobatannins (Trease and Evans 1978).

Anthraquinones

(Borntrage's tests)A known quantity of plant extract (0.5 g) was shaken with 5 mL benzene, filtered and 5 mL of 10 per cent ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour indicated the presence of free anthraquinones. (Trease and Evans, 1978)

Tests for cardiac glycosides

(Keller-Killani test): The extracts (5 mL each) were treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. It is treated with concentrated tetraoxosulphate (VI) acid (H_2SO_4). A greenish colour confirms the presence of cardiac glycosides.

Test for Steroids and Terpenoids:

(Salkowski test). About 0.5 -1 mL of test solution was treated with chloroform in a test tube. A few drops of concentrated sulphuric acid were added, shaken well, a red colour will appear at the lower layer indicating the presence of steroids and formation of yellow layer also indicating the presence of steroids and terpenoids.

Test for Flavanoids

(Shinoda Test) Magnesium hydro chloride reduction test – about 1mL of test solution few reagents of magnesium ribbon were added and concentrated hydrochloric acid were added drop wise in after few minutes to confirm the presence of flavonoids in sample.

CHAPTER FOUR

4.0 Results

4.1 Effect of plant powder on survival of *P. truncatus* and *S. zeamais* in treated maize

The response of *P. truncatus* and *S. zeamais* to the powders of the four botanicals used at 5% and 10% of the maize seeds used in the bottles after seven days are illustrated in table 2. Treatments significantly ($P < 0.001$) influenced the survival of the insects.

It was observed from table (2) on the survivorship of insects indicated that maize seeds treated with higher concentrations of powder reduced the survivorship of both *P truncatus* and *S. zeamais* after 7 days. Powders of *Hyptis suaveolens* at 10% reduced the survivorship of *P. truncatus* at percentage mean of 54.8 and *Lantana camara* at 10% also reduced the survivorship of *S. zeamais* at percentage mean of 54.8. All the insects survived in the control after 7 days.

Table 2. Percentage mean survival of *P. truncatus* and *S. zeamais* in treated maize after 7 days.

	Percentage mean(±SE) survival after 7 days	
Treatment	***P. truncates***	***S. zeamais***
5% powder		
Lantana camara	59.0±2.2b	59.0±2.2b
Moringa oleifera	66.1±2.7b	63.4±0.0c
Citrus sinensis	63.4±0.0b	61.2±2.2bc
Hyptis suaveolens	61.7±4.9b	64.4±4.9c
Actellic	00.0±0.0a	00.0±0.0a
Control	90.0±0.0c	90.0±0.0d
LSD (P ≤ 0.05)	7.6	3.9
10% powder		
Lantana camara	48.9±1.9b	54.8±2.0b
Moringa oleifera	66.1±2.7d	61.2±2.2c
Citrus sinensis	61.2±2.2d	59.0±2.2bc
Hyptis suaveolens	54.8±2.0c	56.8±0.0bc
Actellic	00.0±0.0a	0.00±0.0a
Control	90.0±0.0e	90.0±0.0d
LSD (P ≤ 0.05)	5.6	4.7

*Means followed by the same letter within the column are not significantly different (p≤ 0.05).

4.2 Contact toxicity by topical application

It was observed that the toxicity of methanol extract of the various plants were considerably ($p < 0.001$) influenced by type of plant and concentration of extract administered as compared to the control after 96 hours. *M. oleifera* worked effectively at both concentrations (0.05 g/mL and 0.1 g/mL) by increasing the mortality of *S. zeamais* than the other botanicals used for the research (Fig. 4 and 6). However, *H. suaveolens* was also the most effective botanical at both concentrations (0.05 g/mL and 0.1 g/ mL) by increasing the mortality of *P. truncatus* as compared to the other three botanicals (Fig. 5 and 7).

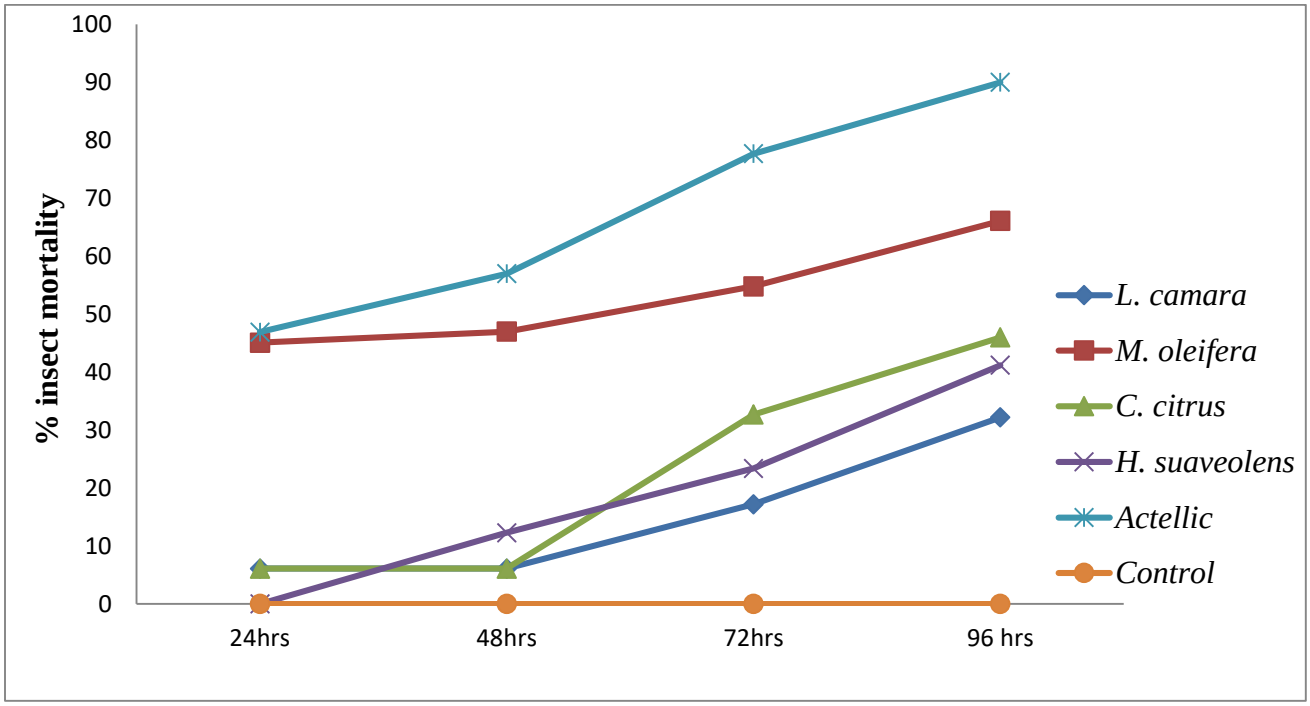

Figure 4. Contact toxicity of methanol extract of four botanicals by topical application to *S. zeamais* at 0.05 g/mL after 96 hours.

It was observed from fig. 4 above that, maize seeds treated with the plant extracts influenced mortality of *S. zeamais*. *M. oleifera* recorded the insect mortality of 66% followed by *C. sinensis* recording (41%). All the insects survived at the control after 96 hours. The reference synthetic Actellic caused morality of almost all the insects after 96 hours. The reference synthetic Actellic

recorded 90 % mortality after 96 hours. All the botanicals also caused insect mortality of between 41 – 48 % after 96 hours. All insects survived at the control fig. 5.

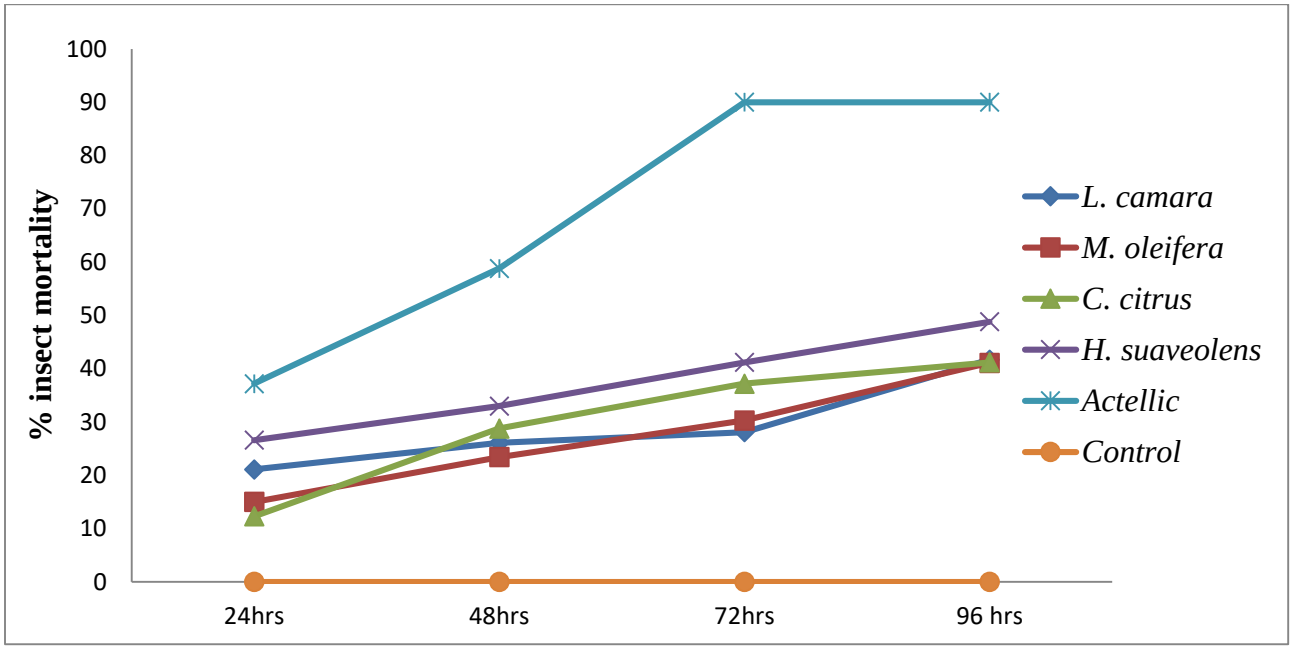

Figure 5. Contact toxicity of methanol extract of four botanicals by topical application to *P. truncatus* at 0.05 g/ mL.

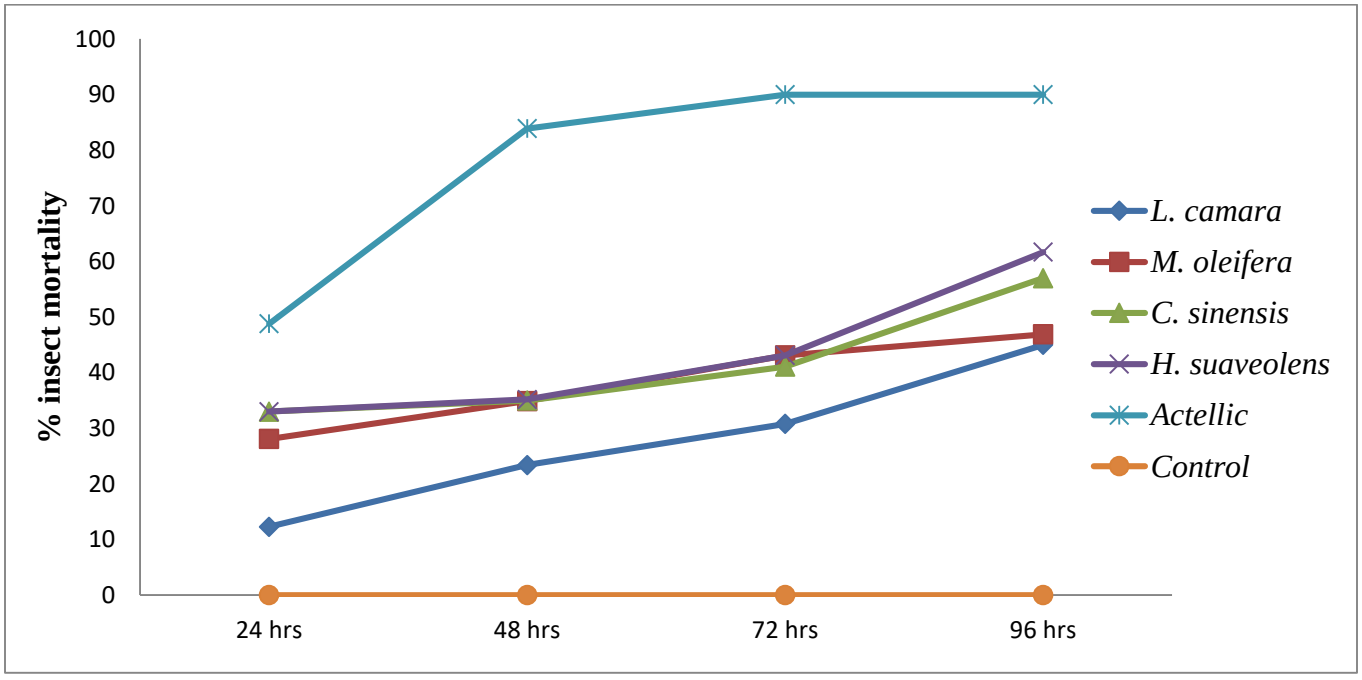

Figure 6. Contact toxicity of methanol extract of four botanicals by topical application to *Prostephanus truncatus* at 0.1 g / mL.

It was observed from Fig. 6 that all *P. truncatus* survived at the control whilst the Actellic recorded 90 % mortality after 96 hours. Maize treated with botanicals caused mortality rate of 45 – 62 % with *H. suaveolens* recording the highest mortality.

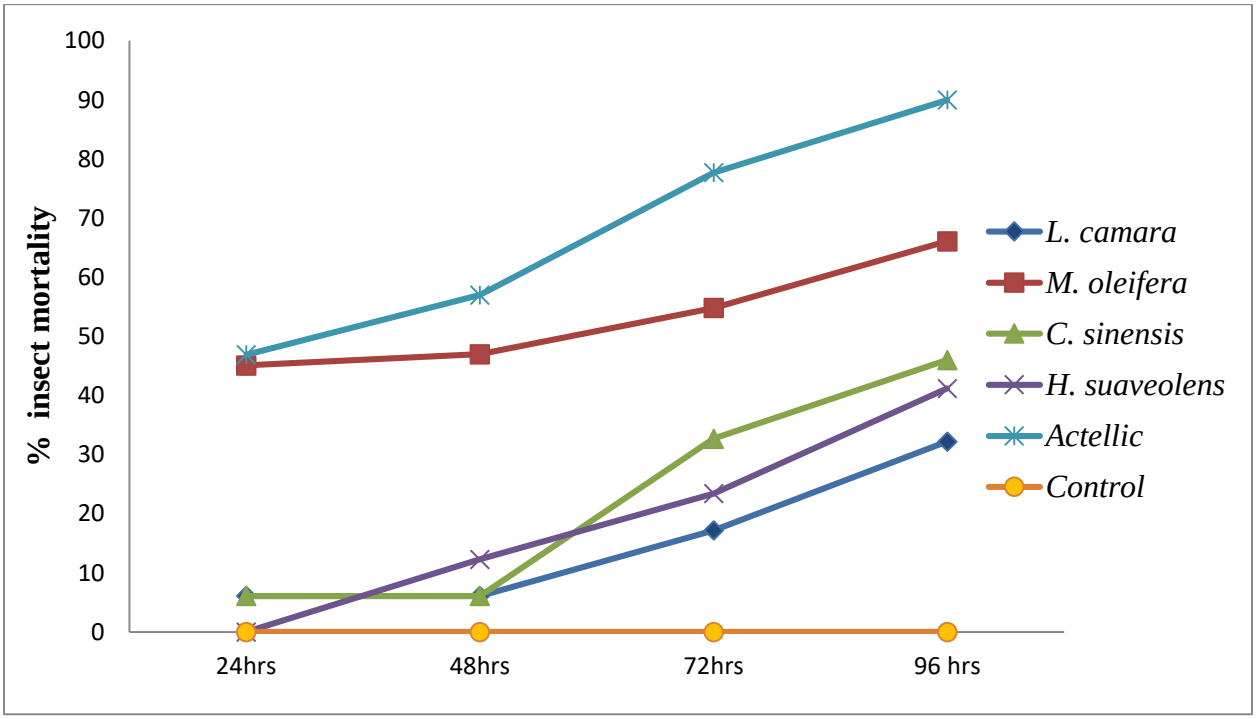

Figure 7. Contact toxicity of methanol extract of four botanicals by topical application to *S. zeamais* at 0.1 g / mL.

It was also observed from Fig. 7 above that the four botanical extract caused insect (*S. zeamais*) mortality of 32 – 66 % with *M. oleifera* recording the highest while *L. camara* recorded the least (32 %) as compared to the control where all the insects survived after 96 hours.

4.3 Effect of methanol extract of botanicals on oviposition of *P. truncatus* and *S. zeamais*

There was a significant ($p<0.05$) difference between the eggs laid on maize seeds treated with botanicals and the control. The number of eggs laid by *P. truncatus* and *S. zeamais* on grains (50 g) treated with *L. camara, M. oleifera, C. sinensis, H. suaveolens* at concentration of (0.05 g/mL and 0.1 g/mL) and Actellic (2 mL/ L) is presented in (table 3). It was observed that the higher

concentration had the least number of eggs (maximum 2.0) laid. *H. suaveolens* extracts recorded the least number of eggs laid by the two insects after 7 days irrespective of the concentrations (0.05 g/mL and 0.1 g/mL) applied as compared to the other botanicals. The reference product (Actellic) was able to reduce the number of eggs laid on grains by the two insects than the botanicals and the control.

Table 3. Mean number of eggs laid by *P. truncatus* and *S. zeamais* in treated maize after 7 days.

	Mean count of eggs laid	
Treatment	***P. truncatus***	***S. zeamais***
0.05 g/mL		
Lantana camara	2.0bc	3.0bc
Moringa oleifera	3.0c	3.0bc
Citrus sinensis	3.0c	3.0bc
Hyptis suaveolens	2.0b	2.0b
Actellic	1.0a	1.0a
Control	3.0d	3.079c
LSD ($P \leq 0.05$)	0.4	0.7

Table 3 Cont'd: **Mean number of eggs laid by *P. truncatus* and *S. zeamais* in treated maize after 7 days.**

0.1 g/ mL		
Lantana camara	2.0b	2.0b
Moringa oleifera	2.0b	2.0b
Citrus sinensis	2.0b	2.0b
Hyptis suaveolens	2.0b	2.0b
Actellic	1.0a	1.0a
Control	3.0c	3.0c
LSD ($P \leq 0.05$)	0.6	0.5

*Means followed by the same letter within the column were not significant ($p \leq 0.05$).

4.4 Effect of methanol extract of four botanicals on eggs and immature stages of *Prostephanus truncatus* and *Sitophilus zeamais*.

4.4.1 Effect of methanol extracts of four botanicals on insect emergence from eggs of *Prostephanus truncatus and Sitophilus zeamais*.

Methanol extracts of *L. camara, M. oleifera, C. sinensis, H. suaveolens* (0.05 g/ mL and 0.1 g/ mL) reduce the emergence of *P. truncatus* and *S. zeamais* in treated grain (Figs. 8 and 9). There was no survivorship of insects from grains treated with Actellic while lower emergence of insects was recorded on grains treated with *H. suaveolens, L. camara* and *C. sinensis* at a higher (0.1 g/mL) concentration.

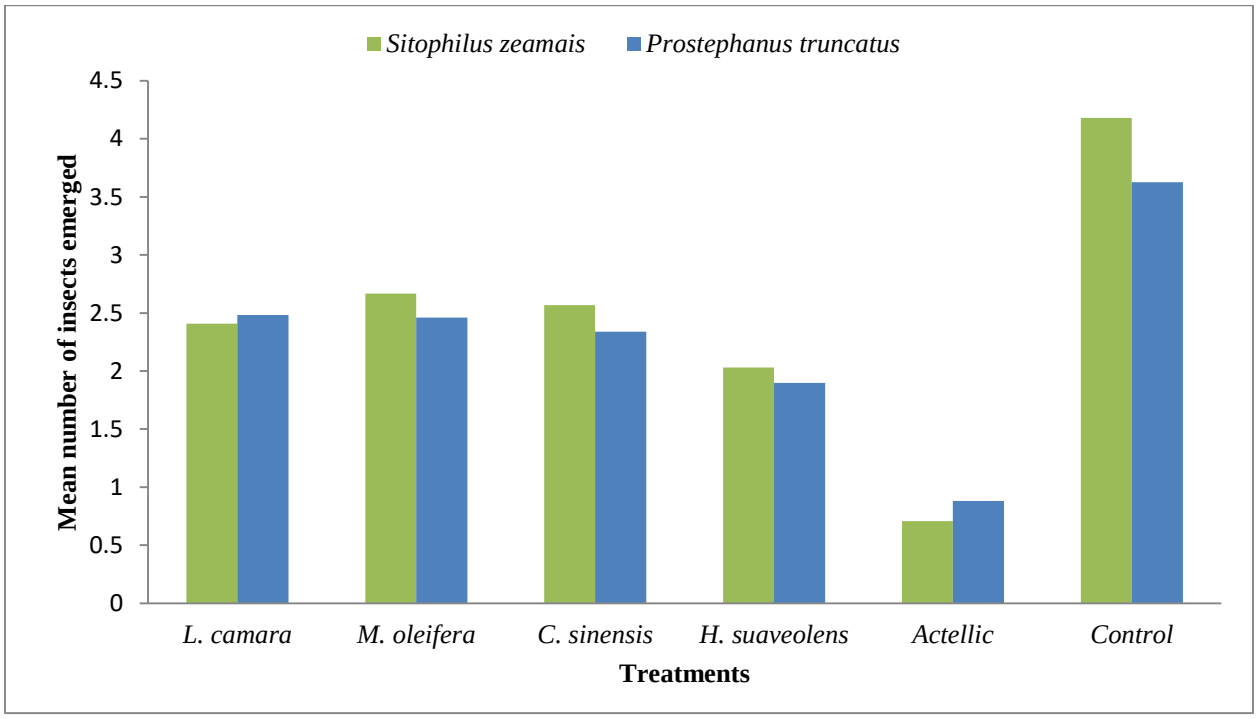

Figure 8. Progeny emergence from larvae of *P. truncatus* and *S. zeamais* in maize seeds treated with four botanicals at 0.05 g/ mL.

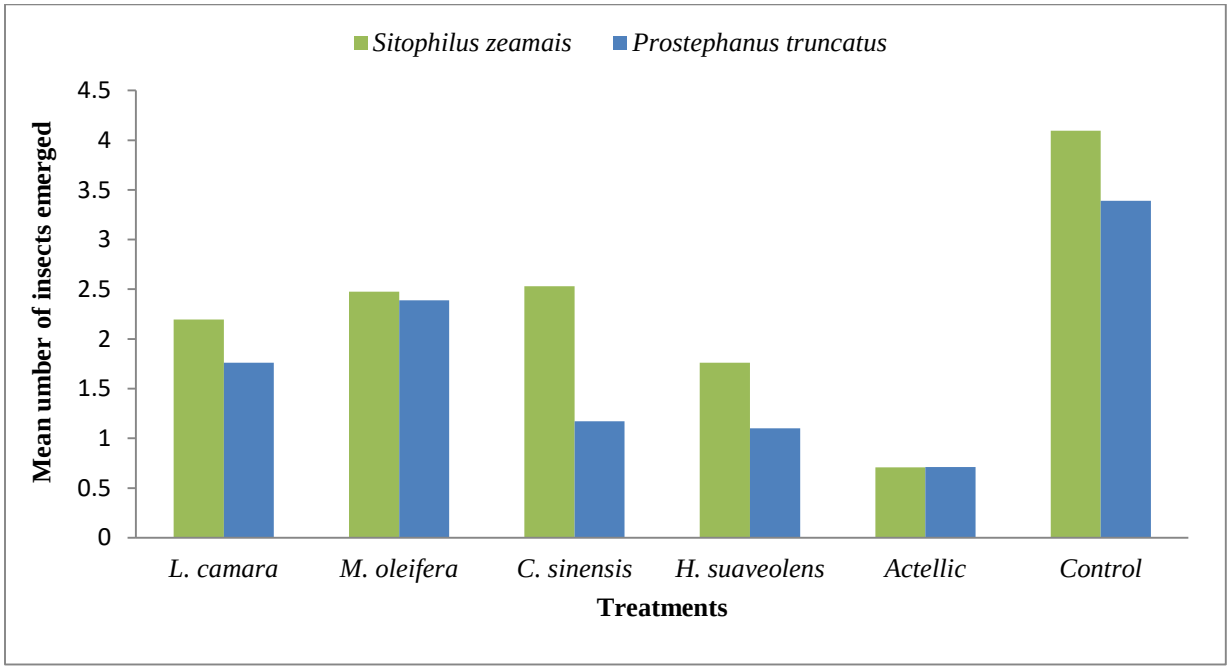

Figure 9. Progeny emergence from egg of *P. truncatus* and *S. zeamais* in maize seeds treated with four botanicals at 0.1 g/mL.

4.4.2 Effect of methanol extract of four botanicals on the larvae of *Prostephanus truncatus and Sitophilus zeamais*.

The botanicals significantly (P<0.001) influenced the emergence of insects in grains treated with extract compared to control. The largest amount of concentration (0.1 g/mL) recorded the least insect emergence (Figure 10 – 11). Least number of *S. zeamais* emerged from grains treated with *H. suaveolens* at 0.05 g/mL than the other botanicals while grains treated with *C. sinensis* at 0.1 g/mL recorded least number of *P. truncatus* that emerged. The reference actellic recorded the least number of insects that emerged from the larval stage compared with the other treatments.

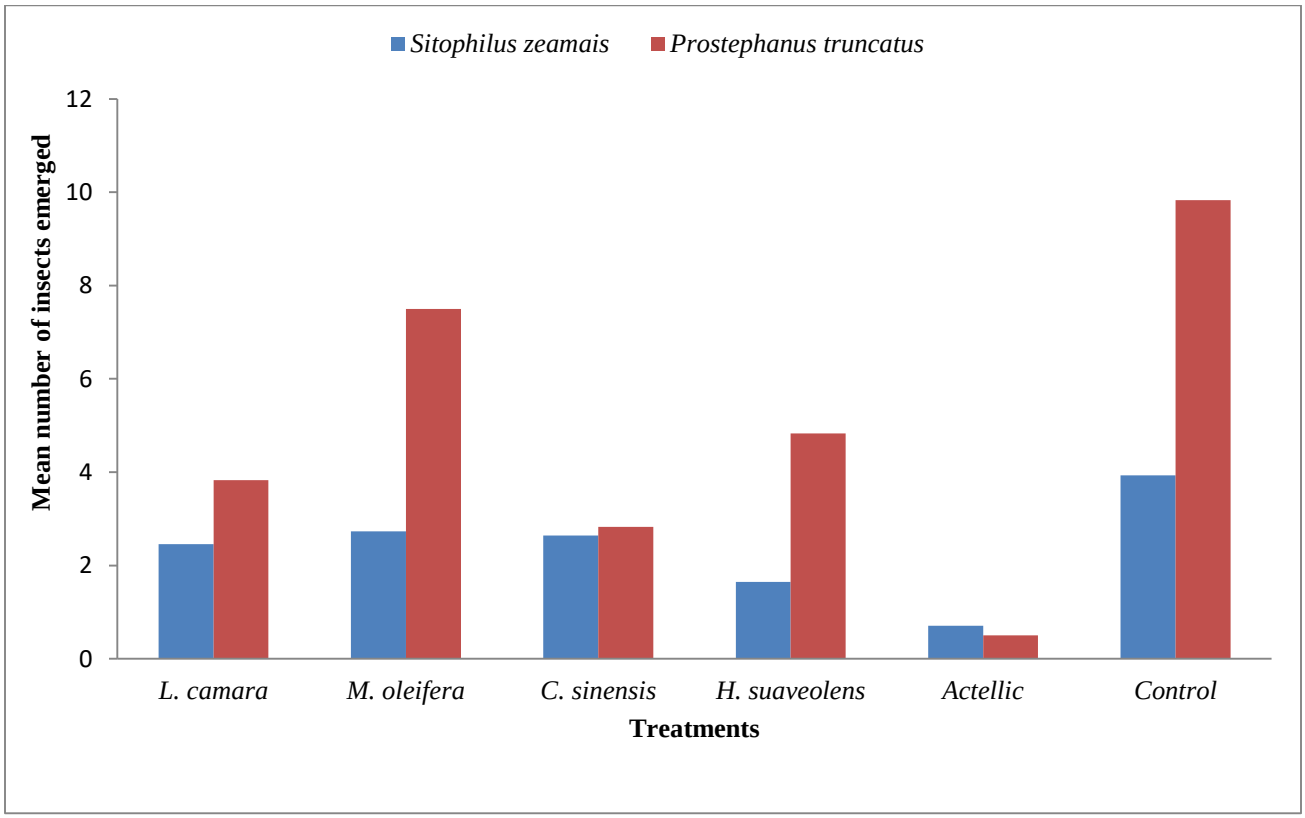

Figure 10. Progeny emergence from larvae of *P. truncatus* and *S. zeamais* in maize seeds treated with four botanicals at 0.05 g/mL.

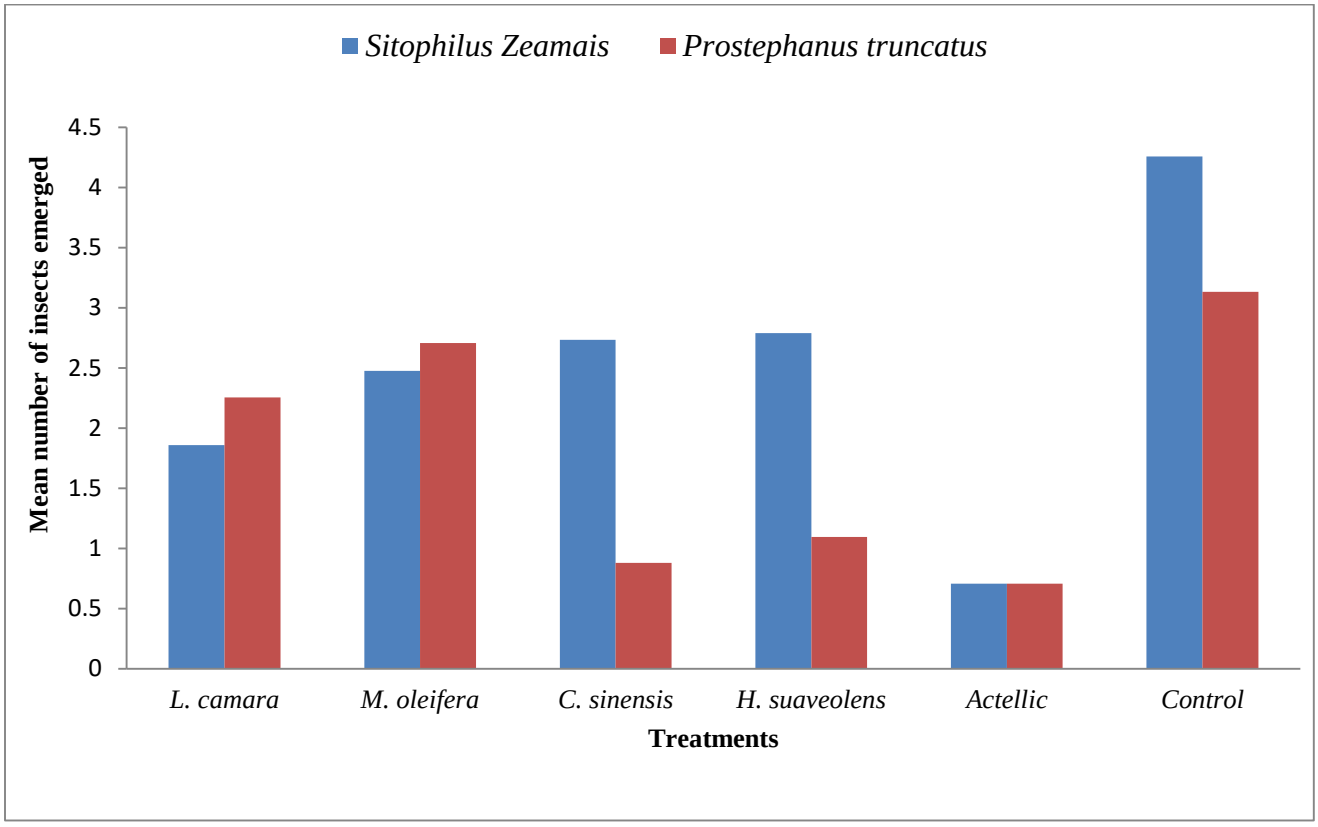

Figure 11. Progeny emergence from larvae in maize seeds treated with four botanicals at 0.1 g/ mL.

4.4.3 Effect of methanol extract of four botanicals on the pupae of *Prostephanus truncatus* and *Sitophilus zeamais*.

The extracts of *L. camara, M. oleifera, C. sinensis, H. suaveolens* were significantly ($P<0.001$) toxic to pupae of the insects in grains administered with botanicals compared to the control (Figure 12 and 13). The differences among botanicals and Actellic at both concentrations were not significant ($P\leq0.05$). Survivorship of both insects was lowest at 0.1 g/ mL after 35 days of storage. It was observed that grains treated with *C. sinensis* recorded the least number of insects that emerged from the pupal stages of both insects followed by *L. camara, M. oleifera* and *H. suaveolens.*

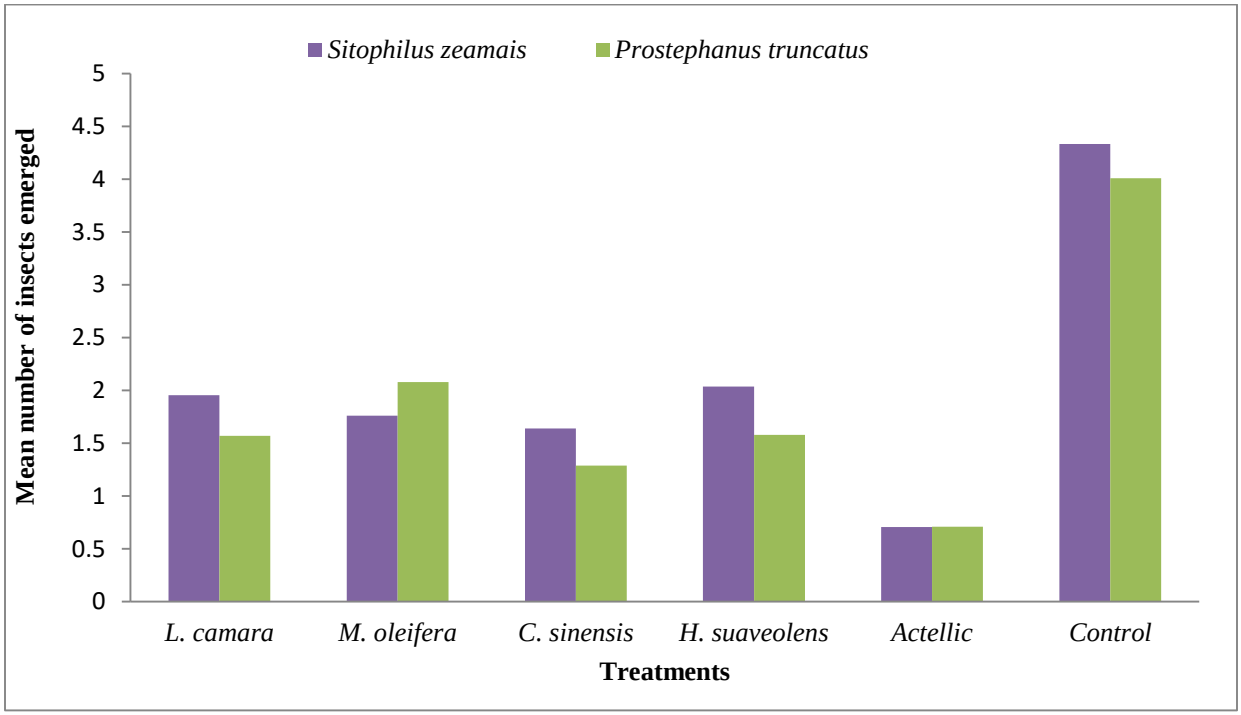

Figure 12. Progeny emergence from pupa of the insects in maize seeds administered with four botanicals at 0.05 g/mL.

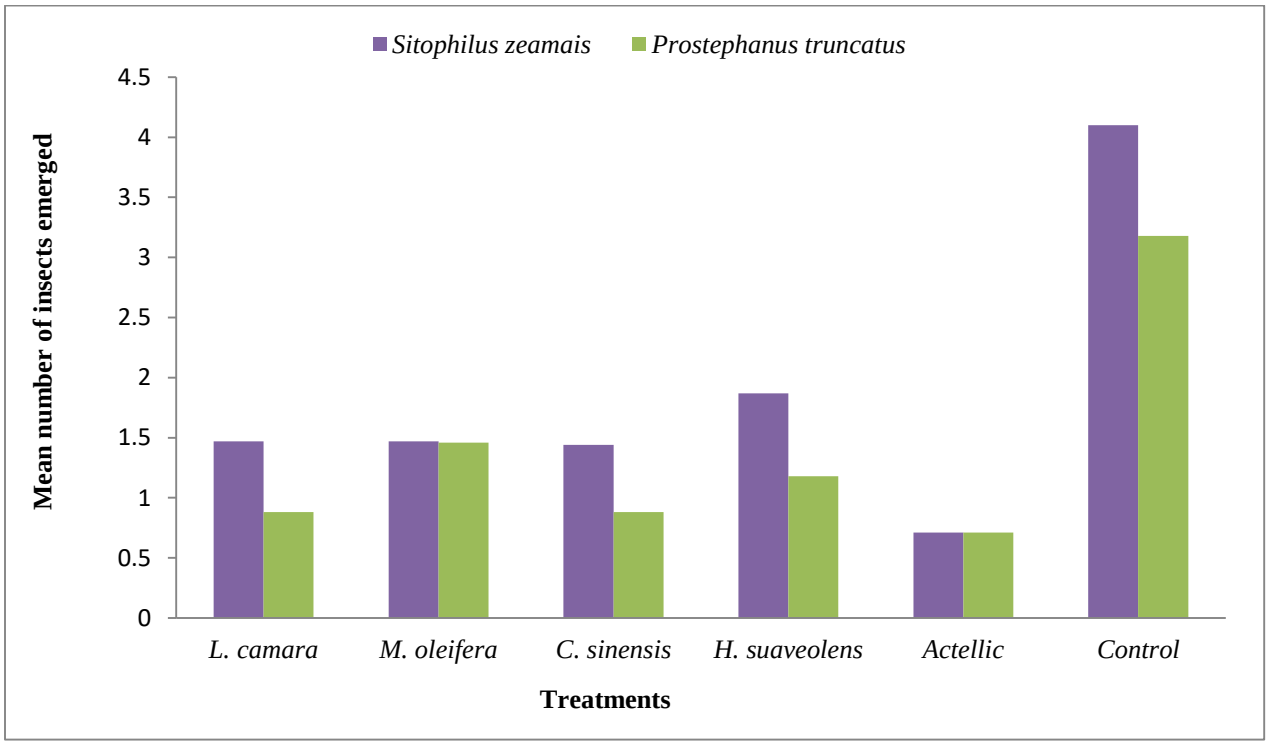

Figure 13. Progeny emergence from pupa of *P. truncatus* and *S. zeamais* in maize seeds administered with four botanicals at 0.1 g/mL.

4.5 Evaluation of effect of methanol extracts assay on repellency of *P. truncatus* and *S. zeamais.*

The botanical extracts were more repulsive to the two insects compared to the control. There was no significant difference between the botanicals and the reference Actellic at both concentrations. *Hyptis suaveolens* recorded 93.3% and 96.7% repulsive action on *P. truncatus and S. zeamais* respectively (Table 5). It was observed that Actellic, *H. suaveolens* and *C. sinensis* recorded (96.7 %) and were more repulsive to *S. zeamais* at 0.1 g/mL more than the other treatments used in the research. Generally, the repellent action of all the treatments at higher concentration (0.1 g/mL) was more than at the lower concentration (0.05 m/mL) to both insects.

4.6 Toxicity of extracts to *Sitophilus zeamais* and *Prostephanus truncatus* in treated grain.

The use of the botanicals was significantly ($p<0.001$) toxic to the both insects than the control. In both treatments, mortality of both insects increased with increasing concentration. The lowest mortality rates of *P. truncatus and S. zeamais* at 0.05 g/mL were 36% and 42% whilst at 0.1 g/mL it further increased to 59% and 61% respectively, after 7 days. The reference product recorded 100% adult mortality in all the treatment (Figs. 14 and 15). It was observed that grains treated with the botanicals were more toxic to *S. zeamais* than *P. truncatus* at both concentrations.

Table 4. Repellency of plant extracts to *Prostephanus truncatus* and *Sitophilus zeamais* after 30 minutes

	Percentage repellency	
Treatment	***P. truncates***	***S. zeamais***
0.05 g/mL		
Lantana camara	90.0	73.3
Moringa oleifera	86.7	63.3
Citrus sinensis	90.0	90.0
Hyptis suaveolens	93.3	86.7
Actellic	96.7	93.3
LSD(P≤0.05)	11.5	16.4
0.10 g/ mL		
Lantana camara	93.3	80.0
Moringa oleifera	90.0	66.7
Citrus sinensis	83.3	96.7
Hyptis suaveolens	93.3	96.7
Actellic	100.0	96.7
LSD(P≤0.05)	23.7	19.2

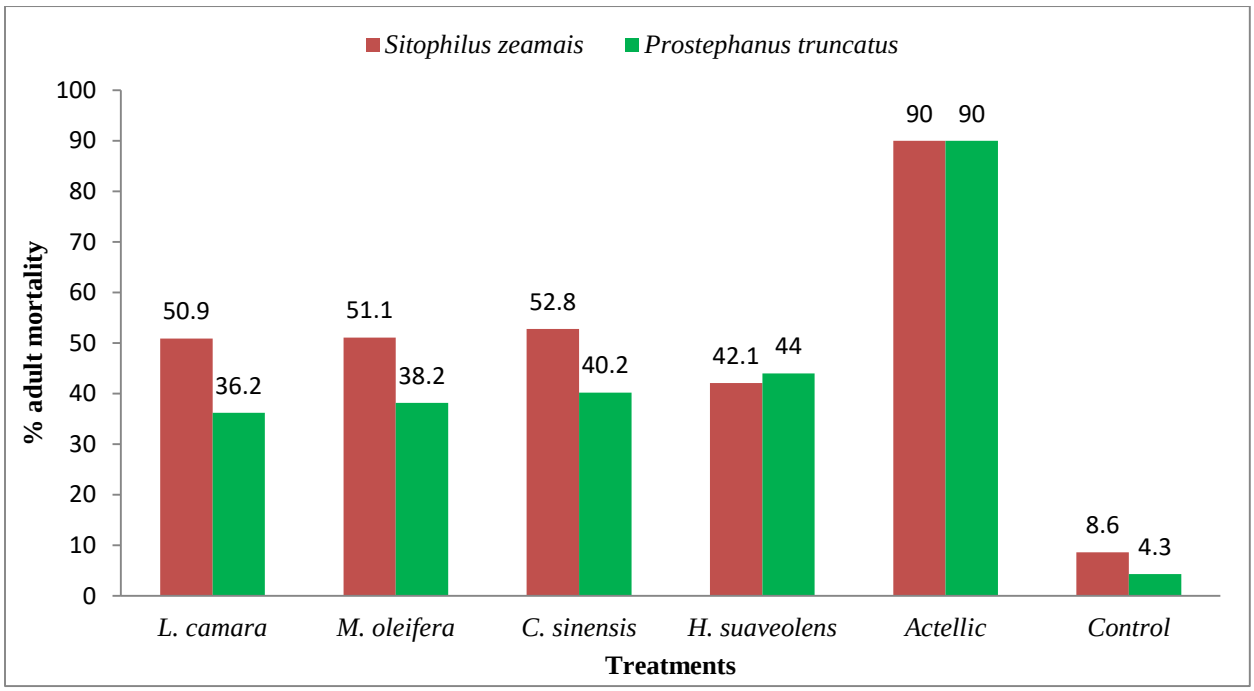

Figure 14. Percentage adult mortality of methanol extracts of four botanicals on *P. truncatus and S. zeamais* at 0.05 g/mL.

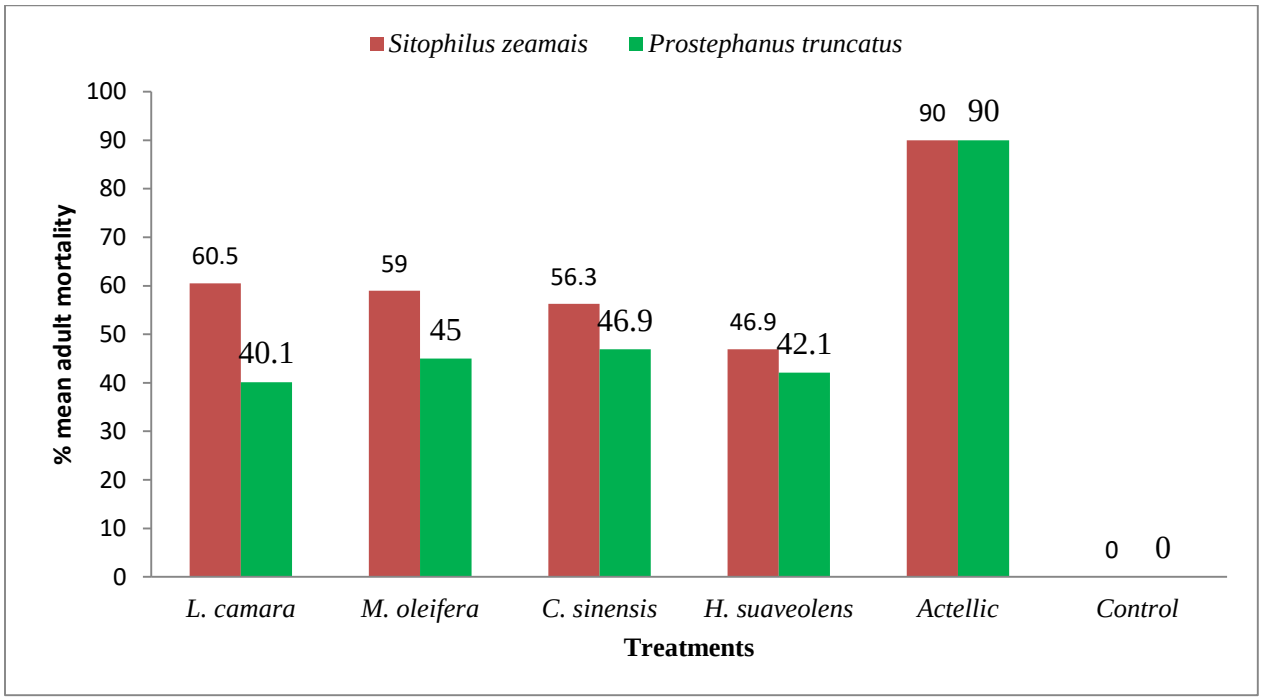

Figure 15. Percentage adult mortality of methanol extracts of four botanicals on *P. truncatus and S. zeamais* at 0.1 g/mL.

4.7 Effect of Pre-Planting Seed Treatment on Maize Seed Germination

There were no significant ($p<0.05$) differences among the treatments in percentage germination. Seeds treated with *L. camara* and *M. oleifera* recorded a percentage germination of 94.0 each followed by Actellic (93.0%), *C. sinensis* and *H. suaveolens* (86.0%) and the control (82.0 %). Seed treatments did not significantly influence germination. It was observed that seeds treated with the botanicals gave a higher germination percentage as compared to the control. However, the outcome generally indicated that seeds were viable and had a good germination percentage which was still in certification limits for seed maize (70%) (ISTA, 2007).

Table 5. Viability of maize seeds pre-treated with plant extracts after 7 days.

Treatment	Percentage (%)
Lantana camara	94.0±2.0
Moringa oleifera	94.0±2.6
Citrus sinensis	86.0±4.2
Hyptis suaveolens	86.0±6.3
Actellic	93.0±2.5
Control	82.0±4.2
LSD($P<0.05$)	11.5

4.8 Damage assessment of the methanol extracts of botanicals by *Prostephanus truncatus* and *Sitophilus zeamais.*

All grains treated with botanicals gave higher protection against insect damage compared to untreated grains. All tested botanicals were more effective at higher (0.1 g/mL) dosage than at lower (0.05 g/mL) dosage in terms of reducing weight loss. Comparatively *P. truncatus* caused more weight loss in grains treated with botanical extracts than *S. zeamais* in both concentrations. The control recorded about 13 % damage weight loss caused to the maize seeds by the two insects. It was observed that from the four botanicals used, maize seeds treated with *H. suaveolens* were least attacked by the two insects and at concentrations of 0.05 g/mL and 0.1 g/mL followed by *L. camara*, *C. sinensis* and *M. oleifera*. The reference Actellic was able to curtail damage caused by the two insects of study than all the other treatments. However, there was no significant difference between the botanicals and the reference Actellic. But there was significant ($p<0.05$) difference between the botanicals and the control.

Table 6. Percentage weight loss caused by *P. truncatus and S. zeamais* to maize seeds treated with methanol extracts of the botanicals after 10 weeks of treatment.

	Mean (±SE) % Weight Loss after 10 weeks	
Treatment	***P. truncatus***	***S. zeamais***
0.05 g/mL		
Lantana camara	3.46±1.05b	2.59±0.5bc
Moringa oleifera	4.01±0.91b	4.17±0.9c
Citrus sinensis	3.01±0.61b	2.82±0.56bc
Hyptis suaveolens	2.32±0.32ab	1.99±0.3b
Actellic	0.13±0.03a	0.12±0.0a
Control	11.80±0.93c	11.07±0.6d
LSD(P<0.05)	2.3	1.7
0.10 g/mL		
Lantana camara	3.03±1.1bc	2.16±0.9ab
Moringa oleifera	4.04±0.9c	3.65±1.0b
Citrus sinensis	2.86±0.6bc	2.34±0.5ab
Hyptis suaveolens	1.82±0.4ab	1.49±0.4ab
Actellic	0.12±0.0a	0.11±0.0a
Control	12.79±0.7d	11.08±1.2c
LSD(P<0.05)	2.7	2.4

***** Means followed by the same letter within the column are not significantly different at (P≤0.05)

4.9 Phytochemical constituents of plants

The results showed that the compounds alkaloids, saponins, tannins and phenolic, steroids and flavonoids were present in all the botanicals used. It was revealed that anthroquinones was present in only *M. oleifera*. Phlobatinins was also present in *L. camara* and *C. sinensis*. Cardiac glycosides and terpenoids were present in all the botanicals except *H. suaveolens*. Table 7, shows the compounds present in the four botanicals.

Table 7. Compounds present in the botanicals after phytochemical analysis.

	Botanicals			
Compounds	*L. camara*	*H. suaveolens*	*M. oleifera*	*C. sinensis*
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins and Phenolic compounds	+	+	+	+
Phlobatinnins	+	-	-	+
Anthraquinones	-	-	+	-
Cardiac glycosides	+	-	+	+
Steroids	+	+	+	+
Terpenoids	+	-	+	+
Flavonoids	+	+	+	+

Key: + = Present; - = Absent

CHAPTER FIVE

5.0 Discussions

5.1 Effect of plant powders on survival of *Prostephanus truncatus* and *Sitophilus zeamais.*

The ground powders of *L. camara, M. oleifera, C. sinensis* and *H. suaveolens* showed different levels of effectiveness against *P. truncatus* and *S. zeamais* in treated grains after seven days. Survival of insects reduced with increasing quantity of powder from 5%- 10%. The survival of *P. truncatus* and *S. zeamais* in grains administered with *L. camara* powder at 10% showed that the botanical is a promising control agent against the two insects since it contained alkaloids. These compounds are toxic (Mithöfer and Boland 2012) to both insects and might have been responsible for the low survivorship of insects in the treated grains. The results of this work have confirmed the protectant potential of *L. camara* and *H. suaveolens* powders against the two insect species that attack stored maize grains, (Ojo and Ogunleye, 2013). Bioactive compounds: tannins, terpenes and steroids might have caused the reduction in survival of the insects in treated grains as reported by Dibua *et al*., (2000). This may explain the efficacy of *L. camara* in this study. The present investigation revealed that the powder has the potential to reduce the survival of *S. zeamais* and *P. truncatus* to 59% when applied at 5% concentration. The survival of insects further reduced to 48.8% as the dosage of the powder increased to 10%. Shifa *et al.* (2010) earlier observed that a higher amount of plant extracts were more effective than lower concentrations in reducing oviposition and increasing the mortality of the target insect pests. The leaf powder of *L. camara* might have also contained other chemical compounds preventing insects from feeding on grains that have been treated with the powder. The use of *M. oleifera* and *C. sinensis* powder as grain protectants against *S. zeamais* and *P. truncatus* caused higher survivorship than other two botanicals discussed above. Although, *M. oleifera* and *C. sinensis* contain alkaloids, terpenoid,

morphine and phenol, they may be in smaller concentration in the plants parts that have been used for the experiment compared to other two botanicals (Akinkurolere, 2012). A suggested possible reason of adult mortality might be the effective adhesion of dust particles to spiracles of pest and their death due to suffocation.

5.2 Toxicity of extracts applied topically to insects

In this study, all the botanical extracts were toxic to insects at different levels compared to the control after 96 hours of treatment by topical application. In all the treatments, the higher concentration (0.1 g/mL) was more effective to both insects than the lower concentration (0.05 g/mL). *Hyptis suaveolens and M. oleifera* was highly toxic to *P. truncatus* (61.7%) and *S. zeamais* (66.1%) respectively. This might be the existence of compounds like flavonoids, saponins, tannins and phenolic in the botanicals (Irvine, 1961). *Sitophilus zeamais* was more predisposed to methanol extract than *P. truncatus* this might be attributed to its more robust nature, high feeding ability and highly sclerotized cuticle which might have decreased the absorption of component of the extract on the skin.

5.3 Effect of methanol extract of botanicals on oviposition of *Prostephanus truncatus* and *Sitophilus zeamais*

All the botanicals were effective at 0.1 g/mL to both insects. This means that the botanicals might possess repellent and/or oviposition deterrent action which might have resulted in the changes prompted by physiology and behaviours in the adult insects as reflected by their egg laying capacity. This confirms the research reported by Adebayo and Gbolade (1994) that *L. camara* which contains caryophyllene and germacrene D in large quantities exhibited some ovipositional suppression on *Callosobruchus maculatus.* Also Schmutterer (1990) and Ndomo *et al*. (2009) confirmed that that botanical extracts and their essential oils have anti-oviposition and fertility

reducing effect on a host of insects. In all the treatments, *S. zeamais* was able to lay more eggs than *P. truncatus*. This might have been as a result of high fecundity level of *S. zeamais* than *P. truncatus.*

5.4 Toxicity of extracts to *Sitophilus zeamais* and *Prostephanus truncatus* in treated maize grain.

The methanol extracts at both concentration applied to adult insects in treated grain after seven days significantly ($P<0.001$) reduced the survival of both insects compared to the control. The toxicity of the extract applied to adult insects in treated grain was influenced by the type of plant, concentration applied and contact duration (days). The most effective botanical on *S. zeamais* was *L. camara*. Earlier study has reported that leaves of *L. camara* is active against insects (Ogendo et al. 2003; Dua *et al*., 2010) whilst the least effective was *H. suaveolens* and the most effective botanical on *P. truncatus* was *C. sinensis*. Although *H. suaveolens* also contains similar chemical components such as saponins, flavonoids, alkaloids, steroids, tannins and phenolic compounds, might have not been potent enough to kill insect pests as compared to *L. camara* and *Citrus sinensis* which contains other additional compounds after the phytochemical analysis. However, they can be used to prevent fungi attack in stored grains (Oudhia, 2008). The higher concentration of the botanical induced lower insect survivorship. *Lantana camara* at 0.1 g/mL recorded mortality of (60%) or (Survivorship of 40%) in *S. zeamais* after 5 days of treatment. Therefore, the extracts were slower in killing insects than the synthetic chemical (Actellic). This confirms earlier report by Obeng- Ofori and Dankwa (2004) that Actellic has rapid knock down action which instantly killed adult insects on contact.

5.5 Effect of methanol extract of botanicals on eggs and immature stages of *Prostephanus truncatus* and *Sitophilus zeamais*

The methanol extract of all botanicals were potent to the eggs, larvae and pupae of *P. truncatus* and *S. zeamais* at both concentrations compared to the control. This agrees with earlier observation by Jayakumar *et al*., (2005) that plant extracts have active effects on the various life cycles of insects' survival which results in the reduction in adult survivorship of insects. Khanam *et al*., (1990) also reported that plant extracts was injurious to insects at their life cycle stages which also significantly, increased the larval and pupal periods. Toxicity of extracts to eggs, larvae and pupae resulted in reducing survivorship of adults from the treated seeds. The potency might be due to bitter anti-nutritive secondary metabolites of the extract on the seed coat acting as a barrier to prevent the eggs from hatching into adults (Tchinda, 2011). *Hyptis suaveolens* and *Lantana camara* was very effective in lowering the emergence of *S. zeamais* at the larval stage.

5.6 Repellency of methanol extracts assay against *Prostephanus truncatus* and *Sitophilus zeamais.*

All the four botanical extracts demonstrated greater repellency against *P. truncatus* and *S. zeamais* just as the synthetic commercial pesticide (Actellic). However, *H. suaveolens* was observed to be rather repellent to the two studied insects, with standard mean repellency of 93.3 and 96.7% respectively to *P. truncatus* and *S. zeamais* at 0.1 g/ mL. Ogendo *et al*., (2003).

5.7 Effect of Pre-Planting Seed Treatment on Maize Seed Germination.

Seed germination was not influenced by the botanical extracts and the control. However, the result generally indicated that seeds were viable and had a good germination percentage which was still in certification limits for seed maize (70%) (ISTA, 2007). The use of plant products by farmers to store their grains does not have any negative influence on germination of the treated grains. The

use of leaf extracts of *M. oleifera* as indicated by Foidl *et al*., (2001) that growth of plant was enhanced.

5.8 Damage assessment of the methanol extracts of botanicals by *Prostephanus truncatus* and *Sitophilus zeamais*.

In all the four botanicals tested, the mean percentage weight loss and grain damage was lower at higher (0.1 g/mL) concentration than in the lower (0.05 g/mL) concentration. This confirms the findings of Parwada *et al*, (2018) that botanicals results in lowering the occurrence in the of weevil attack if the concentration is increased. The research work showed that maize grains treated with *H. suaveolens, L. camara, C. sinensis* and *M. oleifera* at concentrations, prevented emergence and suppressed insect's activities. This also corroborates with the findings of Wahedi (2012) of maize grains treated with neem seed oil was able to decrease the percentage of weight loss caused by the insects. Maize seed without treatment with botanicals experienced higher insects' damage (11.1 – 12.8% weight loss) than those treated with botanicals (1.5 – 4.2% weight loss). The extracts of *L. camara* were used to protect grain against almond moth as the extracts exhibited fumigant and contact activity (Gotyal *et al.*, 2010). Research carried out by Pardawa *et al*., (2018) proved that the efficacy of botanical pesticides decreases with time as shown by the reduced mortality percentages from fortnight three. This suggests that botanical need constant reapplications for them to offer continual protection of the grain against *P. truncatus* and *S. zeamais*.

CHAPTER SIX

6.0 Conclusions and Recommendations

6.1 Conclusions

The experiment conducted was to assess the efficacy of four plant extracts as maize seed storage protectant against *S. zeamais* and *P. truncatus* in Ghana. The present findings showed that the plant extracts exhibited contact toxicity on the insects studied. The powders of the other four botanicals were deleterious to the insects thereby reducing insect survival when applied at 5% and 10% concentration. *Lantana camara* and *hyptis suaveolens* were observed to be the most promising botanicals in protecting maize grains against the two insects.

Maize seeds treated with methanol extracts of the botanicals after 10 weeks, recorded a reduction in percentage seeds damaged and weight loss caused by the two insects as compared to the untreated seeds which recorded higher number of damaged seeds and weight loss.

Apart from exhibiting the anti-oviposition, lowering survivorship and inhibiting reproduction inhibition effects, the methanol extracts of the botanicals demonstrated reduced adult eclosion, ovicidal and repellent properties on the two insects. Methanol extracts of *H. suaveolens and C. sinensis* at 0.1 g/mL exhibited repellency of 96% to *P. truncatus* and *S. zeamais.*

Pre-planting Seed treatment with the four botanicals enhanced good germination and seedling emergence. Maize seed treated with *M. oleifera* recorded the highest with 94% seedling emergence. This suggests that grains treated with *M. oleifera* will enhance good germination of seeds for a good field emergence and establishment. The phytochemical analysis revealed that active compounds such as alkaloids, saponins, tannins and phenolic, flavonoids, anthroquinones, phlobatinins, cardiac glycosides and terpenoids were present in the four botanicals. These

compounds may have caused lower progeny emergence, inhibitory effect, repellent action and antifeedant effect to *S. zeamais* and *P. truncatus* in grains treated with the botanicals. These botanicals demonstrated the effectiveness of decreasing the emergence of progenies of the insects in stored grains. The current results suggest a poisonous nature of *L. camara, M. oleifera, C. sinensis* and *H. suaveolens* on *P. truncatus* and *S. zeamais* that can be utilized in farm stores towards grain pests.

6.2 Recommendation

The reductions in percentage of grain weight loss as well as in damaged seeds have important implications for the use of the botanicals as maize seed storage protectants against *P. truncatus* and *S. zeamais* by farmers. It is recommended that use of these four botanicals can be used as maize seed storage protectants against storage pest.

Further study is needed using other storage insect pests in order to broaden its spectrum of effect.

The use of pre-planting seed treatments with botanicals is recommended especially for commercial seed producers to enhance the production of quality insect -free disease-free seed.

It is also recommended that a further trial should be conducted on the field by planting maize seed treated with these botanicals for definitive conclusions of the effectiveness of the extracts on plant establishment and yield.

However, further work is required to determine the toxicity of these phytochemicals in order to ascertain its potential hazards to consumers and the environment as well. Furthermore, it is also recommended that different solvents should be used for extraction of the chemical component from the plant.

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APPENDICES

APPENDIX 1. Analysis of variance (ANOVA) on effect of plant powder at 5% on *P. truncatus*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	13466.40	2693.28	147.53	<.001
Residual	12	219.06	18.26		
Total	17	13685.46			

APPENDIX 2. Analysis of variance (ANOVA) on effect of plant powder at 5% on *S. zeamais.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	13315.9	2663.18	542.7	<.001
Residual	12	58.9	4.91		
Total	17	13374.8			

APPENDIX 3. Analysis of variance (ANOVA) on effect of plant powder at 10% on *P. truncatus.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	13311.887	2662.377	266.55	<.001
Residual	12	119.859	9.988		
Total	17	13431.747			

APPENDIX 4. Analysis of variance (ANOVA) on effect of plant powder at 10% on *S. zeamais*

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	12890.244	2578.049	372.48	<.001
Residual	12	83.055	6.921		
Total	17	12973.299			

APPENDIX 5. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 24 hours at 0.05 g/mL

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	2446.40	489.28	8.82	0.001
Residual	12	665.35	55.45		
Total	17	3111.75			

APPENDIX 6. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 48 hours at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	4654.51	930.90	28.72	<.001
Residual	12	388.98	32.41		
Total	17	5043.49			

APPENDIX 7. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 78 hours at 0.05 g/mL

Source of variation	d.f.	S.s.	m.s.	v.r.	F pr.
Treatment	5	12948.48	2589.70	62.77	<.001
Residual	12	495.05	41.25		
Total	17	13443.53			

APPENDIX 8. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 96 hours at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	10782.24	2156.45	67.08	<.001
Residual	12	385.77	32.15		
Total	17	11168.01			

APPENDIX 9. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 24 hours at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v. r.	F pr.
Treatment	5	7490.62	1498.12	26.41	<.001
Residual	12	680.83	56.74		
Total	17	8171.44			

APPENDIX 10. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 48 hours at 0.05 g/mL

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	8787.82	1757.56	22.92	<.001
Residual	12	920.15	76.68		
Total	17	9707.97			

APPENDIX 11. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 72 hours at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	11683.6	2336.7	7.86	0.002
Residual	12	3567.4	297.3		
Total	17	15251.0			

APPENDIX 12. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 96 hours at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	14013.8	2802.8	24.00	<.001
Residual	12	1401.3	116.8		
Total	17	15415.1			

APPENDIX 13. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 24 hours at 0.01 g/mL

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	4464.51	892.90	17.03	<.001
Residual	12	629.00	52.42		
Total	17	5093.51			

APPENDIX 14. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 48 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	11239.24	2247.85	39.81	<.001
Residual	12	677.57	56.46		
Total	17	11916.82			

APPENDIX 15. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 72 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12582.78	2516.56	68.07	<.001
Residual	12	443.63	36.97		
Total	17	13026.42			

APPENDIX 16. Analysis of variance (ANOVA) for contact toxicity *P. truncatus* after 96 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12961.78	2592.36	81.62	<.001
Residual	12	381.12	31.76		
Total	17	13342.91			

APPENDIX 17. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 24 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	14293.34	2858.67	44.63	<.001
Residual	12	768.65	64.05		
Total	17	15061.99			

APPENDIX 18. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 48 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	15058.7	3011.7	27.32	<.001
Residual	12	1322.7	110.2		
Total	17	16381.4			

APPENDIX 19. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 78 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	15868.4	3173.7	30.10	<.001
Residual	12	1265.1	105.4		
Total	17	17133.5			

APPENDIX 20. Analysis of variance (ANOVA) for contact toxicity *S. zeamais* after 96 hours at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	16323.98	3264.80	41.70	<.001
Residual	12	939.60	78.30		
Total	17	17263.58			

APPENDIX 21. Analysis of variance (ANOVA) for the effect of methanol on oviposition of *P. truncatus* after 7 days at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	8.21864	1.64373	30.01	<.001
Residual	12	0.65737	0.05478		
Total	17	8.87600			

APPENDIX 21. Analysis of variance (ANOVA) for the effect of methanol on oviposition of *S. zeamais* after 7 days at 0.05 g/mL.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	9.8867	1.9773	13.92	<.001
Residual	12	1.7052	0.1421		
Total	17	11.5919			

APPENDIX 23. Analysis of variance (ANOVA) for the effect of methanol extracts on oviposition of *P. truncatus* after 7 days at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	9.8368	1.9674	15.30	<.001
Residual	12	1.5432	0.1286		
Total	17	11.3800			

APPENDIX 24. Analysis of variance (ANOVA) for the effect of methanol extracts on oviposition of *S. zeamais* after 7 days at 0.01 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	9.84333	1.96867	25.57	<.001
Residual	12	0.92381	0.07698		
Total	17	10.76714			

APPENDIX 25. Analysis of variance (ANOVA) for progeny emergence from egg of *P. truncatus* in maize seeds treated with four botanicals at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	11.9904	2.3981	20.76	<.001
Residual	12	1.3861	0.1155		
Total	17	13.3766			

APPENDIX 26. Analysis of variance (ANOVA) for progeny emergence from egg of *S. zemais* in maize seeds treated with four botanicals at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	18.7983	3.7597	35.85	<.001
Residual	12	1.2586	0.1049		
Total	17	20.0569			

APPENDIX 27. Analysis of variance (ANOVA) for progeny emergence from egg of *P. truncatus* in maize seeds treated with four botanicals at 0.1 g/mL

Source of variation	d.f.	s.s.	m.s.	s.s.	F pr.
Treatment	5	14.8081	2.9616	11.88	<.001
Residual	12	2.9905	0.2492		
Total	17	17.7986			

APPENDIX 28. Analysis of variance (ANOVA) for progeny emergence from egg of *S. zeamais* in maize seeds treated with four botanicals at 0.1 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Treatment	5	18.43389	3.68678	53.07	<.001
Residual	12	0.83360	0.06947		
Total	17	19.26749			

APPENDIX 29. Analysis of Variance (ANOVA) for progeny emergence from larva of *P. truncatus* in maize seeds treated with botanicals at 0.05 g/mL

Source of treatment	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	167.611	33.522	7.36	0.002
Residual	12	54.667	4.556		
Total	17	222.278			

APPENDIX 30. Analysis of Variance (ANOVA) for progeny emergence from larva of *S. zeamais* in maize seeds treated with botanicals at 0.05 g/mL

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	17.7497	3.5499	16.20	<.001
Residual	12	2.6292	0.2191		
Total	17	20.3790			

Variate: Larva_5%_Pt

APPENDIX 31. Analysis of Variance (ANOVA) for progeny emergence from larva of *P. truncatus* in maize seeds treated with botanicals at 0.1 g/mL

Source of variation	d.f.	s.s.	m.s	v.r.	F pr.
Treatment	5	16.0242	3.2048	20.01	<.001
Residual	12	1.9216	0.1601		
Total	17	17.9458			

APPENDIX 32. Analysis of Variance (ANOVA) for progeny emergence from larva of *S. zeamais* in maize seeds treated with botanicals at 0.1 g/mL

Source of variaiton	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	20.55264	4.11053	85.21	<.001
Residual	12	0.57891	0.04824		
Total	17	21.13154			

APPENDIX 33. Analysis of Variance (ANOVA) for progeny emergence from larva of *P. truncatus* in maize seeds treated with four botanicals at 0.05 g/mL

Source of variaiton	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	19.5014	3.9003	20.18	<.001
Residual	12	2.3194	0.1933		

Total	17	21.8208			

APPENDIX 32. Analysis of Variance (ANOVA) for progeny emergence from larva of *S. zeamais* in maize seeds treated with four botanicals at 0.05 g/mL.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	21.8371	4.3674	28.54	<.001
Residual	12	1.8362	0.1530		
Total	17	23.6733			

APPENDIX 35, Analysis of Variance (ANOVA) for progeny emergence from pupa of *S. zeamais* in seeds treated with four botanicals at 0.1 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12.7765	2.5553	10.79	<.001
Residual	12	2.8428	0.2369		
Total	17	15.6192			

APPENDIX 35, Analysis of Variance (ANOVA) for progeny emergence from larva of *P. truncatus* in seeds treated with four botanicals at 0.1 g/mL

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	20.4431	4.0886	20.04	<.001
Residual	12	2.4482	0.2040		
Total	17	22.8913			

APPENDIX 37. Analysis of Variance (ANOVA) for the Effect of plant extracts on repellency of *P. truncatus* at 0.05 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	173.33	43.33	1.08	0.415
Residual	10	400.00	40.00		
Total	14	573.33			

APPENDIX 38. Analysis of Variance (ANOVA) for the Effect of plant extracts on repellency of *S. zeamais* at 0.05 g/mL

Source of variaiton	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1726.67	431.67	5.29	0.015
Residual	10	816.67	81.67		
Total	14	2543.33			

APPENDIX 37. Analysis of Variance (ANOVA) for the Effect of plant extracts on repellency of *P. truncatus* at 0.1 g/mL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	440.0	110.0	0.55	0.704
Residual	10	2000.0	200.0		
Total	14	2440.0			

APPENDIX 40. Analysis of Variance (ANOVA) for the Effect of plant extracts on repellency of *S. zeamais* at 0.05 g/mL.

Source of variaiton	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	4	2226.7	556.7	4.99	0.018
Residual	10	1116.7	111.7		
Total	14	3343.3			

APPENDIX 41. Analysis of Variance (ANOVA) for the Percentage adult mortality of four botanicals on *P. truncatus* at 0.05 g/mL after 7 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	11340.11	2268.02	109.61	<.001
Residual	12	248.30	20.69		
Total	17	11588.41			

APPENDIX 41. Analysis of Variance (ANOVA) for the Percentage adult mortality of four botanicals on *S. zeamais* at 0.05 g/mL after 7 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatment	5	10144.52	2028.90	47.26	<.001
Residual	12	515.18	42.93		
Total	17	10659.69			

APPENDIX 43. Analysis of Variance (ANOVA) for the Percentage adult mortality of four botanicals on *P. truncatus* at 0.1 g/mL after 7 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12240.52	2448.10	70.08	<.001
Residual	12	419.18	34.93		
Total	17	12659.70			

APPENDIX 44. Analysis of Variance (ANOVA) for the Percentage adult mortality of four botanicals on *S. zeamais* 0.1 g/mL after 7 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12938.35	2587.67	78.26	<.001
Residual	12	396.80	33.07		
Total	17	13335.15			

APPENDIX 45. Analysis of Variance (ANOVA) for damage assessment on *P. truncatus* at 0.05 g/mL after 10 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	239.687	47.937	29.27	<.001
Residual	12	19.652	1.638		
Total	17	259.339			

APPENDIX 46. Analysis of Variance (ANOVA) for damage assessment on *S. zeamais* at 0.05 g/mL after 10 weeks

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatment	5	216.8043	43.3609	47.28	<.001
Residual	12	11.0049	0.9171		
Total	17	227.8093			

APPENDIX 47. Analysis of Variance (ANOVA) for damage assessment on *P. truncatus* at 0.1 g/mL after 10 weeks

Source of variation	d.f	s.s	m.s.	v.r.	F pr.
Treatment	5	297.973	59.595	40.16	<.001
Residual	12	17.806	1.484		
Total	17	315.778			

APPENDIX 45. Analysis of Variance (ANOVA) for damage assessment on *S. zeamais* at 0.1 g/mL after 10 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	228.662	45.732	24.96	<.001
Residual	12	21.986	1.832		
Total	17	250.647			

APPENDIX 49. Analysis of Variance (ANOVA) for Germination Percentage 7 DAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	531.33	106.27	1.76	0.171
Residual	18	1084.00	60.22		
Total	23	1615.33			