EFFECT OF TEMPERATURE ON, AND THE EFFICACY OF *EUCALYPTUS* LEAF EXTRACTS AGAINST *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) IN STORED MAIZE

BY

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DECLARATION

I hereby declare that this thesis is the result of the original work personally done by me for the award of a Master of Philosophy Degree in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS) in the University of Ghana. All the references to other people's work have been duly acknowledged and this thesis has not been submitted in part or whole for the award of a degree elsewhere.

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DEDICATION

This work is dedicated to my father Tanka Ivo Sekaa, my mum Nsai Boti Margaret Tanka and to my brothers and sisters.

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LIST OF ABBREVIATIONS

ARPPIS - African Regional Postgraduate Programme in Insect Science

FAO - Food and Agriculture Organization

IPM - Integrated Pest Management

IITA - International Institute of Tropical Agriculture

PHL - Post-harvest loss

SPSS - Statistical Package for the Social Sciences

SSA - Sub-Saharan Africa

USD - United States Dollar

ABSTRACT

This study sought to determine the most effective concentration and favourable temperature at which Methanol and Diethyl ether extracts of *Eucalyptus* sp leaf are most effective in the control of *Sitophilus zeamais* in stored maize.

Bioassay on efficacy of Methanol and Diethyl ether extract of *Eucalyptus* sp leaf was carried out with 4 treatment concentrations of 0.2%, 0.4%, 0.8% and 1.2% dissolved in acetone at 0.5 g/L of solvent. The extract was replicated three times and stored at temperatures of 20ºC, 27ºC and 31ºC from 1 to 168 hours to determine effect of extract on mortality of *S. zeamais*. The efficacy of Methanol *Eucalyptus sp* leaf extract was evaluated over 5 month duration and percentage weight loss was recorded between the 3rd to the 5th month as per the above treatment concentrations and temperatures. The results of the experiment indicated that *Eucalyptus* sp leaf extract was significantly effective (p < 0.001) in controlling *Sitophilus zeamais*. At different temperatures (20ºC, 27ºC and 31ºC), the following mean mortality were recorded 76.67%, 66.67% and 23.33%, respectively by 168 hrs post treatment of maize with 1.2% Methanol extract of *Eucalyptus* sp leaf respectively. Results on efficacy of *Eucalyptus* sp leaf during 5 months storage showed that the extract effectively controlled *S. zeamais* for 3 months but with a steady decrease in mortality. Results on the immature stages indicated that the egg, larval and pupal stages were all susceptible to *Eucalyptus* sp leaf extract at all concentrations compared to acetone treated control and *Azadirachtin* reference control. The larval stage was most susceptible to treatment effects. The study also showed that Eucalyptus leaf extract had significant repellent effect with 100% repellence post exposure after 6hrs and 12hrs at all storage temperatures (20ºC, 27ºC and 31ºC). The differences in grain weight loss were significantly lower with increasing

concentration. At 1.2% of *Eucalyptus* sp leaf extract, percentage weight loss were 0.46% and 4.17% for the 3^{rd} and 5^{th} months respectively during storage at 31ºC.

Percentage mortality was directly proportional to the amount of *Eucalyptus* sp leaf extract used. The present study concluded that *Eucalyptus* sp leaf extract should be used to control *S. zeamais* especially with higher concentration and storage temperature of 27°C to significantly lower percentage weight loss of maize.

CHAPTER 1

1.0 INTRODUCTION

Major cereals produced in Africa are sorghum, pearl millets, teff, finger millets and rice (Macauley *et al.,* 2015). Another major cereal that has taken over the traditional cereals is maize that is widely consumed by people with different food preferences and cultural back ground all over the globe.

Maize contains approximately 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100g (Ranum *et al.*, 2014). Among the most important cereals worldwide, maize is the third in ranking followed by rice and wheat (Golob *et al*., 2004). Maize (*Zea mays*) which remains a very important source of energy and vitamins to man and livestock grows in almost all diverse agro ecological zones of the world, with the US (38%), China (20%), and Brazil (7%) ranking among the top maize producers in the world, producing about 563 of the 717 million metric tons/year (Ranum *et al.*, 2014).

Maize is grown by 46 out of 53 countries in Africa, and of the 22 countries in the world where maize forms the highest percentage of caloric intake, 16 are Africa (Nuss & Tanumihardjo, 2010). Maize accounts virtually for half of the calories intake and protein consumed in eastern and southern Africa (ESA), and 1/5 of the calories intake and protein are consumed in West Africa. Regional average yields are high as 1.7 tons per hectare for West Africa and 1.5 tons per hectare in East Africa, and 1.1 tons per hectare in Southern Africa (Smale *et al.,* 2011).

Maize as a staple food crop has played a vital role in food safety and security of the African continent. Maize production in Nigeria has raised the standard of living, providing income to smallholder farmers and increased foreign exchange earnings (Adiaha, 2017).

In Ghana maize production systems yields above the national average of 1.5 Mt/ha, profitable at private level and contribute to growth of the national economy. It accounts for up to 58% of local cereal production (Scheiterle *et al.*, 2016). According to Coulter (1993), maize is of economic importance and a great source of carbohydrate in most Ghanaian meals playing the role in ensuring food security in Ghana. Maize production however is met with postharvest losses ranging between 5% and 70% especially from insect pests an imperative for meeting Ghana's current developmental goal.

Sub-Saharan Africa so far suffers serious post-harvest losses from more than 20 different species of insect pests including the maize weevil *Sitophilus zeamais* Motschulsky, (Coleoptera: Curculionidae) (Darfour & Kurt, 2016). Studies have shown that about 90% of the postharvest losses worldwide are caused by insect pests, alongside mite infestation; calling for effective as well as efficient control measures (Vachanth *et al.,* 2010). Owusu (1991) and Kabir *et al.* (2011) also indicated that due to infestation from *S. zeamais* and contamination from insect bodies and frass, maize and cowpea faces up to 20% yield loss annually.

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which is a major pest of stored cereals especially rice, sorghum, millet and most importantly maize causes severe qualitative and quantitative damage on stored produces, with grain weight loss ranging between 20 to 90% for untreated stored maize in sub-Saharan Africa (Ojo and Omoloye, 2016).

The severity of damage from *S. zeamais* usually depends on biotic and abiotic factors of the storage environment; factors, such as temperature, atmospheric gases and water, alongside grain conditions and moisture content, determine the rate at which stored product insects develop into large populations capable of inflicting damage on the grain quantity and value (Pedersen, 1992).

Preventing loss of value resulting from insect pests is therefore imperative. In an attempt to preserve stored products from these insect pests, different strategies have been used by scientists but post-harvest losses continue to face serious threats from insect pests (Ahmady *et al.,* 2016).

The use of chemical pesticides on storage produce has so far been effective against insect pests, but associated health hazards to man and environment have become a major hindrance for its continued usage as pesticides (Triantafillou, 1997).

Concerns on the hazardous effects of continued use of chemical control strategies have sparked interest in non-chemical alternatives such as the use of botanicals, exclusion and elevated temperatures and biological control since they do not leave chemical residues on food (Banks *et al.,* 1995).

Temperature for some time now has been used extensively to manage insect pests under different types of methods such as hot air, radio frequencies, microwave and exposure to high temperatures (Mahroof, *et al.,* 2003), but so far, little has been done on the relationship between temperature and botanicals in managing stored product pests.

Some insecticides applied as residual treatments to control most of the insect pests is positively correlated with temperature (Johnson, 1990) especially with temperatures above 37^{o}C and below 10^{o}C where the insecticide becomes less toxic and drop knock-down effect on the pests. The effect of temperature on activity of insecticides for controlling grasshoppers on leafy green vegetables was evaluated and showed significant differences under a range of temperatures. So far, little work has been done on the effect of temperature on the efficacy of botanicals (Amarasekare *et al.,* 2004).

Athanassiou *et al.* (2005), reported that mortality of *Sitophilus oryzae* and *Tribolium confusum* from natural diatomaceous earth was high with extending exposure intervals and increased temperature. The protectant effect of SilicoSec on *S. oryzae* increased with temperature, but for *T. confusum* mortality was lower at 32°C, compared to 30°C, for 24 and 48 h exposure intervals (Athanassiou *et al.,* 2005). Usually, when temperature goes below 20°C, female S. *zeamais* will lay fewer eggs, or nothing at all (Throne, 1994).

The increasing resistance by insect's pests like *Sitophilus zeamais* to some pesticides and destruction of non-target insects and parasitoids couple with health hazards and environmental concerns from the use of synthetic chemicals has made alternative control methods like use of biological control and botanicals inevitable (Cutler, 2013).

So far there are developing programs that are dependent on a safe, cost efficient and locally available alternative materials and strategies that prevent maize grain losses from pests using botanicals (Mulungu *et al.,* 2007). Botanicals such as *Eucalyptus* spp so far have proven to be effective against several insect pests especially storage pest.

Eucalyptus spp a widely used and fast-growing plantation tree with over 500 species (Eldridge *et al.*, 1993) have economic value from its use as timber for furniture and fuel wood beside the leaves being used in the control of insect pests.

Eucalyptus spp have been widely used by local farmers against field and stored product insect pests usually at a lower cost compared to synthetic pesticides that are expensive, not readily available and causes health problems to consumers as they are toxic and many have some residual effect (Jembere *et al.,* 1995).

1.1 Justification

Insect pests cause multiple damages on stored crops primarily through direct feeding. Other species of these insects feed the on endosperm causing severe damage on weight and quality of the product while some of the insects feed on the germ, resulting in poor seed germination and less viability (Malek & Parveen, 1989; Santos *et al*., 1990). As a result of damage caused by insects, grains lose marketing, consumption and or planting value. Populations of storage pests can increase in large numbers within a short period of time upon infestation (Sallam, 2008).

Although synthetic pesticides are developed through very strict regulatory processes to function with reasonable certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from occupational exposure and from residues in food and drinking water (Damalas *et al.,* 2011).

For a considerable period of time now, a significant quantity of pesticide residues found in food crops has been a result of post-harvest application of insecticides and fumigants. Most of the contact insecticides are regularly applied at rates aimed at protecting the products against insect pest attacks for a longer storage period as long as possible. The contact insecticides used at the time are usually either organophosphates of low acute mammalian toxicity, such as fenitrothion, chlorpyrifosmethyl, Malathion, and pirimiphos-methyl, or pyrethroids such as permethrin, deltamethrin and bioresmethrin common in Australia (FAO, 1989).

Usually, most of the insecticide are gradually lost due to breakdown and volatilization and the remaining residue in food crop at the end of the storage period is always considerably lower than that originally applied (Champ & Dyte, 1976) but are still toxic and of great risk to human health. The above facts leave us with no alternative than to effectively engage with botanicals for insect pest control since they have minimal or no residuals that cause health risk.

Using botanicals as a means of managing pests is not something novel but, have mostly been carried out on a local scale. *Eucalyptus* sp leaves alongside some other botanicals are essential medicinal plants, and have repeatedly there have been used grain protectants because of their insecticidal properties for a while now (Muzemu *et al.*, 2013) but just as leaves, the plant will not be at its best consistency as insecticides. To this effect, this study seeks to establish a treatment concentration and temperature at which *Eucalyptus* sp leaf extracts is most effective in suppressing maize weevils by small-scale farmers.

1.2 Main objective

- The aim of the research was to study the temperature effects on the efficacy of *Eucalyptus sp.* leaf extracts against the development of *S. zeamais* on stored maize.

1.3 Specific objectives

- To determine the temperature at which *Eucalyptus sp* leaf extracts are most effective in the control of *S. zeamais* on maize.
- Evaluate the weight loss and viability caused by infestation of *S. zeamais* on maize seeds treated with *Eucalyptus sp* leaf extracts.
- Determine the persistence of *Eucalyptus sp* leaf extract in stored maize against *S. zeamais.*

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Maize and its value

Maize is a word derived from the Spanish form of the indigenous Taíno word for the plant, mahiz; though known by others as corn (en.wikipedia.org/wiki/Maize, 2018). Maize which is an annual grass belongs to the family Gramineae.

Maize reproduces sexually each year; the plant is often 3m in height, but some natural strains can grow to 13m (Karl, 2013). Maize has a stem which is composed of 20 internodes 18cm long (Stevenson & Goodman, 1972). The maize plant has leaves, which grow from each node, usually 9cm in width and 120cm in length but with advance of genetically modified crops the strain may vary (Wellhausen, 1957). Species of maize include the *Zea* genus: *Zea mays* and Zea diploperennis, which belong to the teosinte parent (USDA, 2010).

Before domestication, maize use to grow only to about, 25 millimeters long corn cobs from each plant (Duiker & Spielvogel, 2012). However, many centuries of artificial selection by indigenous Americans brought about hybridize maize plants which were able to produce many cobs per plant, which were several centimeters/inches long (Spielvogel, 2005).

These maize cobs have grains similar to peas in size, arranged in regular rows around a white, chaffy stalk-like substance that forms the ear. The highest possible size of kernels is apparently 2.5 cm (1 in). An ear usually carries about 600 grains. The maize cobs can be of different colors but the most common are white and yellow. Maize have been genetically modified to accumulate more sugar and less starch in the ear which is consumed as a vegetable known as sweet corn. Young ears can be consumed raw, but as the plant matures the cob becomes tougher and the silk dries to inedibility. The kernels also dry up and become difficult to chew without cooking (https://en.wikipedia.org/wiki/Maize, 2018).

There are a number of corn-based human food uses include, grinding the corn into cornmeal or masa, processing into corn oil or into alcoholic beverages like bourbon whiskey (Head, 2016).

Maize is cultivated throughout the world from 58°N latitude to 45°S latitude (Leff, *et al.*, 2004). Worldwide production was 817 million tons, in 2009 more than rice (678 million tons) or wheat (682 million tons) (FAO, 2008). It is estimated that in 2012, the total world production of maize was 875,226,630 tons (FAO, 2012), with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production of maize, respectively. Other world top producing countries included Mexico, Indonesia, India, France and Argentina.

Maize cultivation occupies more than 33 million hectare each year in sub-Saharan Africa (SSA) (FAOSTAT, 2012), occupying more than 17% of the estimated 200 million ha arable land in SSA with more than 300 million people depending on maize as a source of Carbohydrate for their livelihood.

Some of the top maize producing counties in SSA include; Nigeria, Ethiopia, South Africa, Tanzania, Malawi, Kenya, Cameroon, Zambia, Uganda, Ghana, Mozambique, Mali, Burkina Faso, Benin, DRC, Angola, Zimbabwe, Togo, and Cote d'Ivoire, that account for 96% of the maize produced in SSA (FAOSTAT, 2012).

In Nigeria maize was initially used as a crop for sustenance, it has however risen to become an important commercial crop for many agro-based industries in the country (Iken and Amusa, 2004). Maize has the ability to ensure global food security as it is high yielding, easy to process, readily digested and cheaper than other cereals. It is also a versatile crop, allowing it to grow across a range of agro ecological zones (IITA, 2001).

Maize in Nigeria has raised the standard of living, providing income to smallholder farmers and increased foreign exchange earnings (Adiaha, 2017).

In Ghana domestic usage of maize greatly predominates to industrial processing. Besides roasting and boiling of fresh maize cob for food, they have been fermented to produce meals which are delicacies that are usually eaten in most homes. Some examples of food from fermented maize in Ghana include: “koko” (porridge), “banku”, “tuozaafi”, “akple”, “kenkey” etc. Maze are also used to prepare poultry and livestock feeds and this make up a major industrial processing aspect, where limited quantity of maize grains are processed onto shelves (Darfour & Rosentrater, 2016).

2.2 Postharvest Losses of Maize

Post-harvest losses differ substantially depending on the stage from the farmer to the final consumer (Kaminski *&* Christiaensen, 2014). The magnitude of these losses varies greatly among different crops, areas, and economies (Halloran *et al.*, 2014). To quantify the overall ratio of PHL to total production, FAO (2011) considers the losses incurred during each of the five stages of the “farm to fork chain” which are, losses (1) during harvesting such as from mechanical damage and/or spillage, (2) during postharvest handling, such as drying, winnowing, and storage (insect pests, rodents, rotting), (3) during processing, (4) during distribution and marketing, and (5) during consumption.

Globally, one third of the food produced is usually lost or wasted (FAO–World Bank, 2010; Prusky, 2011), ranging to a total of 1.3 billion tons of food per year; a quantity which could feed over 870 million hungry people in the world (Gustavsson *et al.*, 2011).

Report from the World Bank (World Bank, 2011) revealed that, significant quantity of food (as much as 37 percent) is lost after harvest in sub-Saharan Africa (SSA) each year, the value that stands at about USD 4 billion for grains alone, this magnitude of food loss exceeds total value of food aid received in SSA over the last decade (Affognon *et al.,* 2015).

When focused on cereals alone, as opposed to all food losses, it is estimated at 20.5 percent (FAO, 2011). For post-harvest handling and storage loss only, the FAO estimate is 8 percent, and the African Post-Harvest Losses Information System (APHLIS) estimate is 10-12 percent (Kaminski & Christiaensen, 2014).

Costa (2014) also projected losses as high as 59.48% in maize grains after storing for 90 days in traditional storage structures; While Pantenius (1988) reported that in Togo, approximate value of 0.2–11.8% weight loss in maize occurs as a result of infestation by insect, and this usually after some 6 months of storage, using traditional storerooms.

A comprehensive household survey undertaken by Kaminski and Christiansen (2014) to evaluate postharvest losses in maize crop in three countries; Uganda, Tanzania, and Malawi, showed that losses occurring from farm processes were estimated to range between 1.4% and 5.9% of which insects and other pests were implicated as the main cause of losses in stored maize.

Ghana loses about 318,514 tonnes of maize annually to postharvest losses according to Dr Bruno Tran (2016), an expert in postharvest losses information system (APHLIS). This figure amount to 18% of the total maize produced in the country (http://www.ghananewsagency.org). Improving food safety and security by reducing postharvest losses is of great importance for meeting food security objectives, as maize under storage is known to be susceptible to more than 20 insect pests species of such as the maize weevil, *Sitophilus zeamais* (Owusu-Akyaw, 1991).

2.3 Description and Biology of *Sitophilus zeamais*;

Maize weevil, *Sitophilus zeamais* Motschulsky are a small reddish-brown, to black snout beetles (Suleiman & Abdulkarim, 2014) with long thin snout and an elbowed antenna with circular punches on pronotal dorsum. They belong to the family *Curculionidae* and order *Coleoptera* which is the largest order of insects and contains the most common and important stored product pests. The maize weevils are amongst the most destructive stored product pests of grains, cereals, and other processed and unprocessed stored products in sub-Saharan Africa, as well as in all warm and tropical parts of the world (https://keys.lucidcentral.org).

Sitophilus zeamais consist of 5 life stages: egg, larva, pupa, pre-adult, and adult, with the larva instars being one of the destructive stages on stored grains. Usually, infestation gradually starts from the field and continues with the product in the store. An adult female burrows into the grains with the aid of its strong rostrum creating a small hole where they lay eggs and then seal the hole with a mucilaginous secretion (Siwale *et al.*, 2009). The optimum temperature for oviposition is about 25^{o}C with grain moisture contents of above 10% (Brich, 1944).

The eggs of *S. zeamais* are white and ovoid, ranging from around 0.7 mm to 0.3 mm. Gravid femal may produce as many as five eggs per day, laying as many as 150 to 400 eggs during its life span (Nilsa & Bosque-Perez, 1992). Eggs then hatch into tiny grubs after about five to nine days in to legless 4 mm long larva (NRI, 1996).

The larvae of *S. zeamais* are plump, cruciform and creamy-white in appearance and legless. It has 4 larval developmental instars that last for 25 days with moderate temperature of 30^{o}C and relative humidity of 70% but, the larvae might extend up to 98 days under unfavorable environmental conditions (Mattah, 2001).

The pupa stage which last 5 days begins with the pre-pupa which appears as elongated body (3 to 3.5mm) with an elongated head and distinct body segments with a wide thoracic cavity. By the 4th day the pupa can be clearly recognized with 3 distinct body sections and visible appendages (Sharifi & Mills, 1971).

Adults that emerge from the grains can be seen as dark brown or black in color with 4 distinct pale reddish-brown oval pitch on the elytra (Ojumu, 1976). The head is seen with capitate and elbowed antennae with 8 segments. They have well developed metathoracic flight wings and a prothorax with circular punches enabling them to fly to ripen crops in the field and to grains at storage (Longe, 2016).

Under optimal lab conditions of 30^{o}C with 14% moisture, development from eggs to adults of the weevil may take from 30 to 40 days while unfavourable environments such as temperature above 32^{o}C with less than 14% maize moisture level might take up to 110 days (Kiritani, 1965). Adults will live between 6-12 months dependent on the environmental conditions like temperature and relative humidity of the grains (Kuschel, 1961; Giles, 1969; Mound, 1989).

2.3.1 Infestation and Damages by *Sitophilus zeamais*

Sitophilus zeamais is a serious pest that cause severe damage of stored product, and constitute a major pests of maize in the tropics as well as the subtropics (Suleiman & Kurt, 2015). The pest has the ability of multiplying into very dense populations, which cause severe damage to the stored grain (Cosmas *et al.*, 2012).

Predicted extent of damage by *S. zeamais* alone on total grain weight of stored product can range from 5-30 % (Ojo & Omoloye, 2012). Losses of about 80 % have been observed in maze grains that were untreated and stored in traditional storerooms (Tefera *et al.*, 2011).

Infestation by *S. zeamais* on grains begins in the field, but severe damage occurs in stored conditions (Abebe *et al.,* 2009; Suleiman & Kurt, 2015). Both adult and larvae *S. zeamais* feed on grain endosperm and/or germ. Developing larvae feed on maize embryo and on the endosperm, causing the corn seeds to grow with viability loss and declining nutritional quality (Danho *et al.,* 2002). This type of feeding often results in loss in grain weight, reduction in nutritive value and loss in market value. Larvae tunnel in grains and pupation takes place inside the grain where the adults chew their way out through the outer layer of the grain (Sallam & Bothe, 1999). Damages on germs result in reduction seed germination, while both types of feeding decreases seed viability and vigour (Amenga, D. A. 2011).

The developmental activities of *S. zeamais* often lead to severe powdering and tainting of the grains with excrement. The infested grains are then rendered susceptible to caking and mould infection thereby reducing their market value (NRI, 1996; Adedire, 2001; Lale and Ofuya, , 2001). Maize weevils are also known to carry and transmit diseases such as Aspergilus flavus, Fusrium verticillioides and Penicillium islandicum and others to humans (Beti *et al.*, 1995).

Heavy infestations of adults *S. zeamais* have causes postharvest losses and continue to posts increasing constraints to stored product entomologist and food security in the tropics (Markham *et al.,* 1994). The common control methods used so far against this insect pest are chemical insecticides, biological control, and botanical insecticides.

2.3.2 Management of *Sitophilus zeamais;* in Storage

To reduce or eliminate the harm caused by *S. zeamais*, several control strategies are normally carried out. These control strategies including cultural, physical, biological, botanical and chemical.

2.3.2.1 Cultural control

This control options in involves cleaning the granaries, sealing cracks and holes on floors of storage rooms, as well as shelves before and after use. Moreover, filled bags should be properly and neatly stacked to prevent insect infestation and damage of storage rooms.

2.3.2.2 Physical control of insect pests

Physical control involves using chemically inactive materials such as ashes, powders, sand, and other abrasive materials that eradicate or make condition unfavourable for the pest to thrive (Golob & Webley, 1980). These inert chemicals are usually administered in huge doses to occupy the available space in grain bulks so as to restrict insect mobility. Furthermore, the coarse nature of these substances may cause damage to the outer covering of the insect, hence causing rapid water loss and the eventual death of the weevil. Termite mound soil has been reported to cause a significant degree of adult mortality in *S. zeamais* (Firdissa & Abraham, 1999).

Biological control of insect pests has been documented as a significant factor of integrated pest management control options for both field crops and stored product.

2.3.2.3 Parasites and pathogens

Use of parasites and insect pathogens Studies by Kassa (2003) demonstrated the possible successful control for *S. zeamais* on stored and infested cereals using dustable powder formulation of conidia of *Beauveria bassiana* and *Metarhizium anisopliae* isolates. Kassa however indicated the need to evaluate an optimized and economic production system and the most suitable formulation that would optimize its application, efficacy and storage characteristics as well as the persistence after application.

Lariophagus distinguendus is an ectoparasitoid of several beetle species that feed on durable stored products. Its potential for the control of *S. zeamais* was assessed in stored maize. This parasitoid significantly reduced the emergence of *S. zeamais* in stored maize. (Adarkwah *et al.*, 2008).

2.3.2.4 Synthetic insecticides

The use of Synthetic Insecticides as a control measure is common and effective. They are used to repel insect pest and hence prevent infestation and cross infestation. For stored grain, insecticides and fungicides whose mode of action is by contact are the most prevalently used chemicals among small-scale farmers (Gwinner *et al*., 1996).

The destructive activities of insects and other storage pests have been adequately subdued by synthetic chemical control methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with Malathion, carbaryl, pirimiphos methyl or permethrin (Ileke and Oni, 2011).

Other optional touch based insecticides for control of stored-grain are organophosphate, which include: fenitrothion, or pyrethroids which include Pyrethrin/piperonyl butoxide and bioresmethrin (Champ & Highley, 1985). Methyl bromide and phosphine are the solitary fumigants commonly used on a global scale (FAO, 1985).

It was therefore recommended that on mixed infestations by *P. truncatus* and *Sitophilus spp.* a combination of organophosphate and pyrethroids, such as pirimiphos-methyl and deltamethrin, will give an excellent control of both pests. (Makundi, 1986)

Aburto and Garza (1986) reported the control of *Sitophilus spp.* in stored grain with pirimiphos-methyl and trimethacarb; trimethacarb gave good control and had no residual effect after one month.

There are potential health implications to the indiscriminate use of synthetic pesticides against attack by grain weevil (Talukder and Howse, 1994). Problems of pest resistance and resurgence of insect pests and the fact that synthetic pesticides are quite expensive, makes their management a complicated issue for smallholder farmers (Iloba and Ekrakene, 2006).

It is evident that many of these chemicals are hazardous to human health and that of other life forms, as well as have deleterious effect to the environment (Forget, 1993; Igbedioh, 1991; Jeyaratnam, 1985). Pesticides contaminants in the soil, air, water and on non-target organisms in our towns and cities, cause harm to both plants and animals with their effect ranging from beneficial soil microorganisms and insects to non-target plants, fish, birds, as well as other wildlife. No plant and animal is totally safe against being exposed to pesticides and the possible negative implications on health especially in developing countries (WHO, 1990).

Certain chemicals used in the environment, such as those pesticides referred to as endocrine interrupters, evoke their harmful effects by imitating or acting against innate hormones in the pest's body. However, it has been asserted that their effect over time, as well as exposure in low quantities progressively lead to a number of human health defects such as reduced immunity, hormone disorder, reduced intelligence quotient, reproductive anomalies and development of malignant tumours (cancer) (Brouwer *et al*., 1999; Crisp *et al*., 1998; Hurley *et al*., 1998).

Synthetic Pesticides are more and more used by small farmers in an unsustainable way, but the risk of pesticide application to human health and environment is inadequately relied to farmers. Consequent reduction in the use of synthetic pesticides well improves on- and off-farm sustainability, as well as reducing costs to the farmer. IPM systems may also deliver an array of ecosystem goods and services beyond pest control, increasing general resilience at farm and landscape scales (E. Birch *et al.,* 2011).

The use of plant and other local materials in an attempt to protect grains from pest invasion have been used with little or no hazards and remain environmentally friendly. Lajide *et al.* (1998) and Akinneye *et al.* (2006) reported plant materials and other local traditional methods are much safer than chemical insecticides and suggested that their use needed exploitation; especially plant as botanical insecticides.

2.4 Botanicals as stored grain protectant

Plant and plant products have become suitable, alternative tools in most pest management programs because they are effective and non-toxic to activities natural enemies (Schmutterer, 1990; Ascher, 1993). A number of chemicals have been isolated and identified in plants parts (leaves and seeds) which have insecticidal properties and hence can serve as potential pesticides. These active compounds from plant extract act as feeding deterrents and repellents to insects (Eziah *et al.*, 2013). For example, the possible pesticidal activities of neem, pyrethrum and tephrosia products have been reported for the control of several insects in storage (Akhtar and Isman, 2004; Greenberg *et al*., 2005; Mbaiguinam *et al*., 2006; Iloba and Ekrakene, 2006).

Plants are known to have secondary chemical compounds which are used as a form of inate defense against phytophagous insects and other herbivores (Lupina and Cripps, 1987). Some of these plant products are systemic in their mode of action e.g. pyrethrins, nicotine and picrotoxinin. Some are known to attack muscles e.g ryanodine, others affect breathing activities of the pest e.g rotenone and mammein; while another group cause hormonal imbalance; e.g. juvenile and molting hormone analogues and antagonist; and affect reproduction and behavioral patterns e.g. attractants, repellents and anti-feedants (Bell *et al.*, 1990). Pesticides from plant source have become a vital area for integrated pest management programs in economically emerging countries as they require no sophisticated material to implement and use (Bekele *et al.*, 1997). Plant materials with insecticidal attributes, help small scale farmers to adequately manage pest population with chemicals that are cheap, readily available, locally constituted to desired concentrations, possess comparatively low toxicity and cause little damage to the environment (Talukder & Howse, 1995).

Currently, only products from a few plant species have found widespread use as insecticides and in commercial production. These include rotenone from *Derris elliptica* and *Lonchocarpus* species, pyrethrum from *Chrysanthemum cinerariaefolium* and azadirachtin from neem.

Eucalyptus (Myrtaceae) is an extensively cultivated tree group in the world with over 700 species. Eucalyptus leaves contain compounds with an extremely broad range of biochemical effects as well as odor, flavor and functional properties (Ramezani *et al.*, 2002; Silva *et al.*, 2003). Several biological attributes have been associated with the genus *Eucalyptus*, among them insecticidal activity against beetles (Haouel *et al.*, 2010), repellent action against *Phlebotomus papatasi* (Yaghoobi-Ershadi*et al.*, 2006) and larvicidal activity on culicids (Cheng *et al.*, 2009).

Beside, *Eucalyptus* essential oils are used for medicinal and pharmaceutical purposes (Dellacassa*et al.*, 1990; Nicole *et al.*, 1998; Cimanga *et al.*, 2002). Firdissa and Abraham (1999) reported that treatment with leaves from *Eucalyptus globules*, *Schinesemolle*, *Datura stramonium*, *Phytolacca dodecandra* and *Lycopersicum esculentum* caused high adult *S. zeamais* mortality.

2.5 Efficacy of Eucalyptus as a botanical

Recent studies by Simbarash *et al*., 2013 have shown that *Eucalyptus spp* significantly ($p < 0.001$) influence death rate of *S. zeamais*. In this study, mortality of maize weevils in maize treated with *Eucalyptus tereticornis* leaf powder increased with increasing concentrations of 5 g, 10 g and 20 g per 200 g of the powder. Among the botanicals used in the study, *E. tereticornis* leaf powder heavily reduced the number of maize weevils by at least 65%. This then shows that resource poor farmers can use these botanicals for effective control of maize weevils (Mandudz *et al.*, 2016).

Study by Mandudz (2016) also indicated that *Eucalyptus spp* leaf powder is effective in controlling *S. zeamais* in stored maize. After treating with oil of *E. tereticornis* at 5g/kg maize and at 2.5g/kg maize, mortality of *S. zeamais* was 44.3% and 23.3% respectively. This showed that oils extracted from *Eucalyptus tereticornis* have some insecticidal and anti-microbial properties which could be exploited in treating stored grains, depending on concentrations of the oil.

Studies by Isiaka *et al.,* (2011) showed that *Eucalyptus* leaf powder has repulsive consequences on the ability to smell and taste (feed on grains) of *S. zeamais*. The efficacy of *Eucalyptus* sp leaf powder on *S. zeamais* could also be because the weevil picks up large quantity of the powder when feeding on the grains bringing about stomach poisoning.

The results of Mandudz's (2016) findings also indicated that there is positive relationship between concentration leaf powder and insect pest mortality. Thus, high dosage of the powder could result in high mortality of the pest. According to him, this outcome could be attributed to the effects of 1.8-Cineol, constituents of the *Eucalyptus spp leaf* powder.

According to Coats *et al.*, (2003), 1.8-cineole is a key component of the *Eucalyptus* vital oils and in addition, he argued that this constituent is acknowledged to be virulent to *Protephanus truncates* and *S. zeamais*. Knight (2009) also affirms that 1.8-cineole entirely suppresses all developmental stages (eggs, larvae and pupae) of *S. zeamais.* Thus, oviposition and resultant offspring production were suppressed.

Consistent findings by Mbaiguinam *et al*. 2006 indicated that grinding *Eucalyptus saligna* leaves allows them to release their insecticidal effect on weevils more effectively compared to the whole leaf. Wheeler *et al.*, (2001) also reported that dried ground leaves provided greatest protection of maize and sorghum grains against attack by *S. zeamais, Rhyzopertha dominica* and *Sitotroga cerealella*.

The use of the three *Eucalyptus spp* resulted in higher number of insect mortality due to the effect of physical barriers by the leaf powders. This can be ascribed to the fact that the leaf powder has the propensity to block the breathing activities of insects' and as a result lead to the death of the parent and F_1 progeny weevils (Mulungu *et al.*, 2006).

Studies of Araya and Emana, (2009) also indicated of the insecticidal properties of plant powders vary widely and are dependent on different factors like the presence of bioactive chemicals which requires identification, isolation and manufacturing to facilitate use against pest. However, the efficacy of these botanical pesticides decreases with time as proven by the reduced percentages mortality from day 70 after treatment with higher concentration of *E. tereticornis*. The efficacies of botanicals for longer storage duration need constant reapplications for them to offer continual protection of the grain against maize weevils (Golob, 2000).

Designating locally available and effective botanicals will ensure the availability of a sustainable alternative to control storage pests, thus contributing to increased food security in the country.

2.6 Effect of temperature and the efficacy of insecticides

Climatic factors, such as temperature and relative humidity, and grain conditions, such as temperature and moisture content, define the speed at which large enough population of stored product insect, capable of causing severe damage, develop (Pedersen, 1992).

Several fungi and insects that attack stored grains are usually passive at temperatures below 10°C; also at this temperature efficacy of some insecticides may be affected. However, temperatures of about 35°C provides insect with suitable condition to cause significant damage and reduction storage life span of the grain (Bailey, 1992). While temperatures lower than 17°C are sufficient to delay insect growth enough to minimise pest destruction (Burges and Burrell, 1964). Microbial development can also be decreased by two to five folds with a temperature reduction of 10^0 C.

Adults of two species; *T. confusum* and *S. oryzae* were subjected to wheat preserved with diatomaceous earth at four different doses: 0.25, 0.5, 1 and 1.5 g/kg, and placed at 22°C, 25°C, 27°C, 30°C and 32°C. The results showed the effectiveness of SilicoSec against *S. oryzae* was amplified with temperature, but mortality for *T. confusum* was lesser at 32°C, compared to 30°C, for 24 and 48 h exposure intervals.

Tribolium castaneum (Herbst), and *Tribolium confusum* (DuVal), also were exposed to diatomaceous earth (Protect-It) at 22, 27, and 32°C and 40, 57, and 75% RH. After exposure, the beetles were unfed for about 1 week at the same conditions. Mortality of both species was lowest at 22°C but increased as temperature and as exposure interval increased (Arthur, 2000).

Although toxicity responses to pyrethroids with increasing temperature also have been reported, the extent of this response is more or less dependent on the individual species, the insecticide and the method in which it was applied. Insect species exposed by topical application to pyrethroid insecticides document reduced efficacy at temperatures exceeding 25ºC. (Arthur, 1999).

Tyler and Binns (1982), reported on the efficacy of temperature on eight organophosphate insecticides (bromophos, chlorphyrifos-methyl, fenitrothion, jodfenphos, Malathion, phoxim, pirimiphos-methyl and tetrachlorvinphos) at 10, 17.5 and 25ºC. Based upon knockdown and kill, the all insecticides were observed to be more effective at 25ºC than at 17.5ºC, and this was noticeably lower at 10ºC. Knockdown was unavoidably followed by death of the insect although this took longer at lower temperatures.

At 10ºC tetrachlorvinphos, bromophos and jodfenphos were virtually ineffective to *S. granarius* even at 5000 mg/m^2. By contrast at 25ºC, 100 mg/m^2 were sufficient to give tota knockdown of all species with most insecticides, the exceptions being Malathion and tetrachlorvinphos, confirming the positive correlation between temperature and treatment concentrations.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out in the Entomology laboratory at the Department of Crop Science and Bio-Technology laboratory, University of Ghana Legon, Accra from August 2017 to July 2018.

3.2 Sources of Experimental Materials

3.2.1 Maize sample

The untreated maize variety (obatampa) was sourced from the University of Ghana school farm. The grains were then sieved to remove downy particles and other foreign matter before it was subjected to a temperature of 50^{o}C to eliminate any previous infestation after which the moisture content was determined using a Protimeter (Digital Grain master).

3.2.2 Culturing of Insects

The insects cultured were *Sitophilus zeamais* obtained from the Entomology Laboratory Insectary at the Department of Crop Science. The insects were reared in maize at room temperature of 27±2^{o}C at a relative humidity of 55 ± 5%. Three hundred unsexed *S. zeamais* were reared in 5liter jars containing 1kg of untreated maize. The jars were then covered with muslin cloth and fastened with rubber bands to prevent infestation from other insects. *S. zeamais* was allowed to oviposit for eight days after which the adult insects were sieved out from the maize grains. After 31 days the resulting emerged maize weevils were used for the various experiments.

3.2.3 Preparation of *Eucalyptus* sp leaf extract

Healthy and fresh leaves of *Eucalyptus spp.* used for this work were collected from Winneba with authorization from Forestry Commission of Ghana. The plant species identification was done at the Department of Botany University of Ghana before proceeding with the extraction processes. The leaves were dried at room temperature of 27±2°C for three weeks till it was well dried. These leaves were then grinded to powder so as to increase the surface area of the particles using a leaf grinder machine (Thomas Wiley laboratory mill).

Two solvents, methanol and diethyl ether were used for the extraction. 100g of Eucalyptus powder was soaked in 400ml methanol and shaken for 48 hours using a flask shaker. The same procedure was used for the diethyl ether. The extracts were filtered using Whatman No. 1 filter paper. To remove methanol and diethyl ether from the solution, a water evaporator was used to evaporate the solvents. The crude extract was then stored under a temperature of -4^{0}C for later usage.

3.3 Adult mortality test of *S. zeamais* on maize treated with *Eucalyptus sp* leaf extract

Eucalyptus leaf extracted with methanol and diethyl ether were diluted with Acetone at different concentrations, 0.20%, 0.40%, 0.80% and 1.2%. The *Eucalyptus* leaf extracts were mixed in 100g of maize inside 200ml containers. The samples in the containers were thoroughly stirred after every 15 minutes for one hour to ensure a homogenous distribution of the crude extract on the grains. The treated samples were left open in an aerated room for two hours for the solvent to evaporate before 10 unsexed *S. zeamais* were introduced in the containers. The containers were then sealed with muslin cloth. Each sample was replicated three times at the different temperatures of 20°C, 27°C and 31°C. Azadirachtin at 0.40% concentration was used as reference and only acetone was used as the control.

3.4 Effects of extracts on immature stages of *S. zeamais*

To determine the effect of *Eucalyptus* crude extract on the immature stages of *S. zeamais*, 100g each of untreated maize at 12.7% moisture content was measured into 200ml containers with labels indicating the developmental life stages of *S. zeamais* (eggs, larva and pupa). Ten adult *S. zeamais* of about 7 – 10 days old were then placed in these containers which were then sealed with muslin cloths to prevent exit or access by other insects.

The samples were then put in incubators at different temperatures of, 20^0C, 27^0C and 31^0C (Plate 1). After eight days of expected oviposition, the maize weevil was then sieved out and the samples were tested for oviposition. The maize samples were later treated with *Eucalyptus* crude leaf extract depending on expected life stages.

After eight days in storage, (expected egg stage) the first maize samples were treated for eggs using four different volumes of the *Eucalyptus* crude extract 0.20%, 0.40%, 0.80% and 1.2% and then placed back into the incubators at 20^0C, 27^0C and 31^0C until the expected emergence of adults (Plate 1). By day 20 (expected larval stage), *Eucalyptus* crude leaf extract was applied on samples and at day 26 (expected pupa stage), treatments were applied and placed under the required conditions until emergence.

The numbers of adult emergence from all the three life stages (egg, larva and pupa) was recorded.

Plate 1: Setup of treated maize samples in incubators at 20^{o}C, 27^{o}C and 31^{o}C

3.5 Contact toxicity of botanical

To determine the efficacy of *Eucalyptus* crude leaf extract against adult *S. zeamais*, the insects were dipped into *Eucalyptus* leaf extract at different concentrations and then put into Petri dishes containing 100 g of maize. The samples were replicated three times at four different concentrations of 0.20%, 0.40%, 0.80% and 1.2% after dissolving in Acetone at 0.5g/L of solvent. Mortality check was carried out continuously for seven days.

3.6 Persistency of *Eucalyptus* leaf extract

To evaluate the persistency of *Eucalyptus* leaf extract against *S. zeamais* on stored maize grains, observations were made on the number of adult death out of the total number on treated maize for an experimental period of five months at different concentrations 0.20%, 0.40%, 0.80% and 1.2% under three different temperatures of 20^{0}C, 27^{0} C and 31^{0}C. Ten adult maize weevils (7-10 days old) were released into 100g of the treated grains and after seven days, the insects were all sieved to count for the dead and live insects. A new set of weevils were again released on treated maize at 15 day intervals (1, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150) for the five month period to determine percentage mortality of *S. zeamais* during the storage period.

3.7 Repellency

To determine the repellent effect of Eucalyptus crude extract against *S. zeamais*, repellency assays were done according to methods by Jilani (1990). Whatman No. 1 filter paper was cut into two equal halves; the extract was applied on one half as uniformly as possible using a micropipette. Acetone was then applied to the remaining half as a control. The treated half with crude extract and the control half were then attached length wise using adhesive tape and placed in glass Petri dishes. Ten unsexed adult *S. zeamais* (7-10 days old) were then released at the center of the Petri dishes and sealed with Para films. Four different concentrations 0.20%, 0.40%, 0.80% and 1.2% were replicated three times and put in incubators at temperatures of 20^0C, 27^0C and 31^0C. The total number of insects on either side of the treated and the acetone treated halves were recorded for 24 hours at an interval of 1h, 4hrs, 6hrs, 12, and 24hrs. The percentage repellence (PR) was calculated by the formula: PR (%) = [(Nc − Nt) / (Nc + Nt)] × 100 where Nc is the number of insects present in the control half paper and Nt the number of insects present in the treated half.

3.8 Assessment of Damage from infestation

After 90 and 150 days of the maize samples in storage, loss assessment for both treated and untreated maize samples were carried out to determine the proportion by weight of the grain damage by insects and the percentage of damaged grains. From all the containers, a total 100 randomly picked grains was taken and the undamaged grains were then selected from damaged grains, counted and weighed.

The weight of the sample was compared with the weight registered before damage. Percentage grains weight loss was calculated using the base equation of FAO (1985) below;

$$\%\text{Weight loss} = \frac{UNd - DNu \times 100}{U(Nd + Nu)}$$

Where: U= weight of undamaged grains

D= weight of insects damaged grains

Nu= number of undamaged grains

Nd= number of damaged grains.

3.9 Data analysis

Mortality values were corrected with Abbott's formula to eliminate natural mortality of control. Data on mortality and repellency were subjected to Two-way ANOVA using SPSS Statistic Software Version 20.0. Tukey HSD was used to separate the means. Nonparametric test was used to calculate the median emergence of F1 progeny. The relationship between the extract concentration applied, time, and temperature on mortality was determined using multiple regression analysis.

CHAPTER 4

4.0 RESULTS

4.1 Temperature and time effects of methanol and diethyl ether extract of *Eucalyptus sp* leaf on *S. zeamais* mortality

Results on contact toxicity of methanol and diethyl ether extract of *Eucalyptus sp* leaf on adult *S. zeamais* indicated no significant difference in percentage mean mortality (F = 1.549, df =1, p = 0.214) and at temperatures of 20°C, 27°C and 31°C. Although treatment concentrations between methanol and diethyl ether extracts were not significant ($P > 0.05$), there were significant differences within the treated concentrations. As shown in Figure 1: there was a steady increase in mortality of insects with increased treated concentrations of methanol and diethyl extracts. *Eucalyptus* leaf methanol extract showed insecticidal activities when tested against *S. zeamais* (F = 25.811, df = 2, $p < 0.001$) as well as diethyl ether extracts which also showed effectiveness in the control of *S. zeamais* (F = 33.251, df = 2, $p < 0.001$).

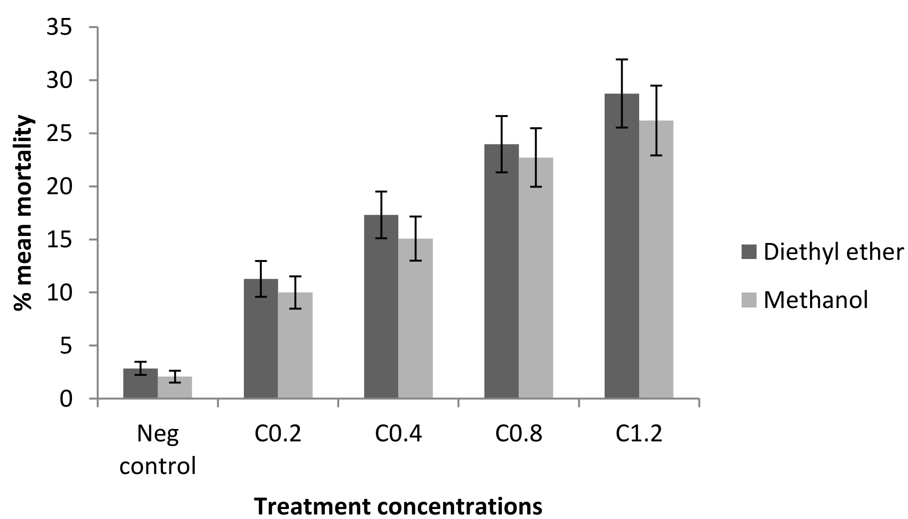

Figure 1: Methanol and diethyl ether extract of Eucalyptus sp leaf on mortality of S. zeamais

The percentage mean mortality at 31ºC and 27ºC for methanol extract of *Eucalyptus* leaf were significantly different compared to 20ºC (F = 135.698, df = 2, p = 0.001). Percentage mean mortality at 27ºC with treatment concentration of 1.2% was higher than that found at 31ºC and 20ºC. The following mean mortality were recorded, 76.67%, 66.67% and 23.33% with temperatures of 27ºC, 31ºC and 20ºC respectively. With higher concentrations at 20ºC, mean mortality was significantly higher than at lower concentrations.

At 20ºC, mean mortality at higher concentrations (1.2% and 0.8%) of *Eucalyptus sp* leaf extract showed significant difference to treatment concentrates only after 144hrs of exposure as shown in figure 2: but was not different from reference control (*Azadirachtin*) with a mean mortality of 30.0%. No mortality was recorded before 48hrs (Figure 2). By 168hrs mean mortality for high concentrations of 1.2% and 0.8 were both at 23.33% indicating no significant difference between them.

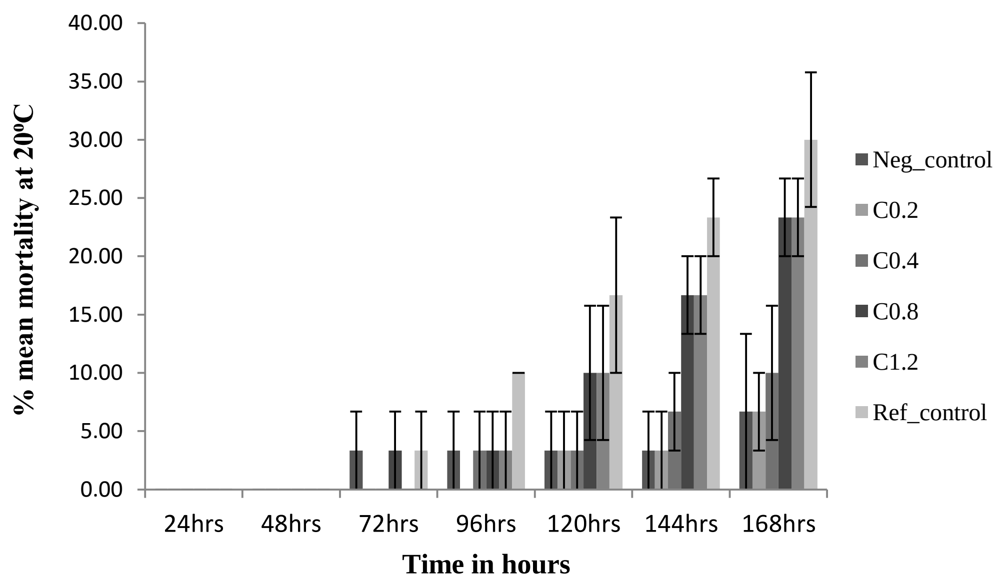

Figure 2: *Eucalyptus sp* leaf extracted with Methanol on mortality of *S. zeamais* at 20ºC.

Effect of *Eucalyptus* sp leaf extracted with methanol also indicated significant difference at 27ºC, with a high concentration of 1.2%, than control and the other concentrations (Figure 3). Mean mortality was higher at the end of 168hrs with concentrations 1.2% and 0.8% reaching mortality rate of 76.67% and 53.33% respectively. There was a significant different between 0.2% and the other concentrations including Acetone treated control and *Azadirachtin* reference control at the end of 168 hrs. Mean mortality in the control (Acetone) and 0.2% eucalyptus leaf extract were 3.37% and 30.00% respectively as shown in figure 3: below.

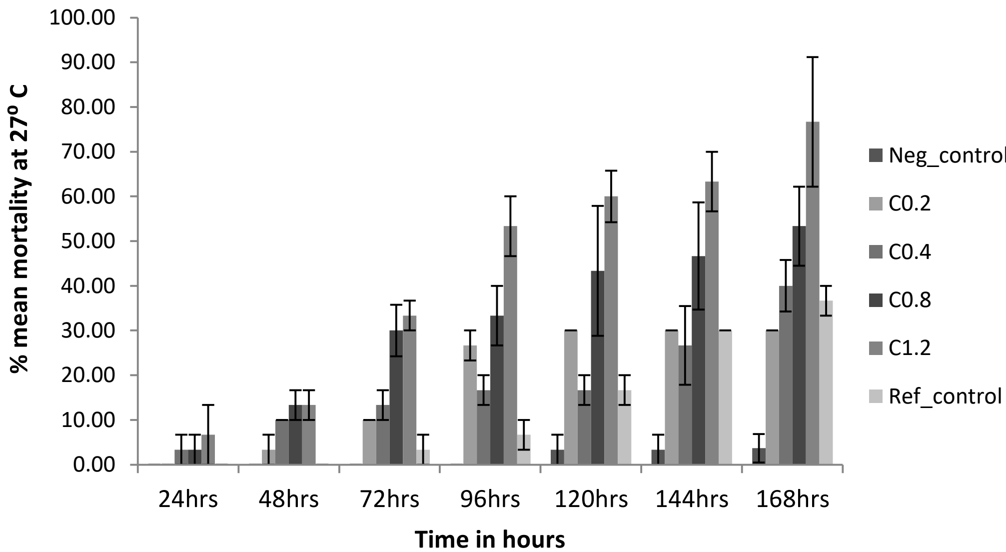

Figure 3: *Eucalyptus sp* leaf extracted with Methanol on mortality of *S. zeamais* at 27ºC

At 31ºC, adult mortality of *S. zeamais* in maize treated with *Eucalyptus* sp leaf extract was higher (66.67%) at treatment concentration of 1.2% and lower with Acetone treated control (6.67%) respectively. There were significant difference between treatment concentrations of Eucalyptus leaf extract and the reference control. However, there was no significant difference with concentration 0.8% of the *Eucalyptus sp* leaf extracts from 168hrs (Figure 4). There was however a significant difference with higher treated concentrates to Acetone treated control during storage at 31ºC.

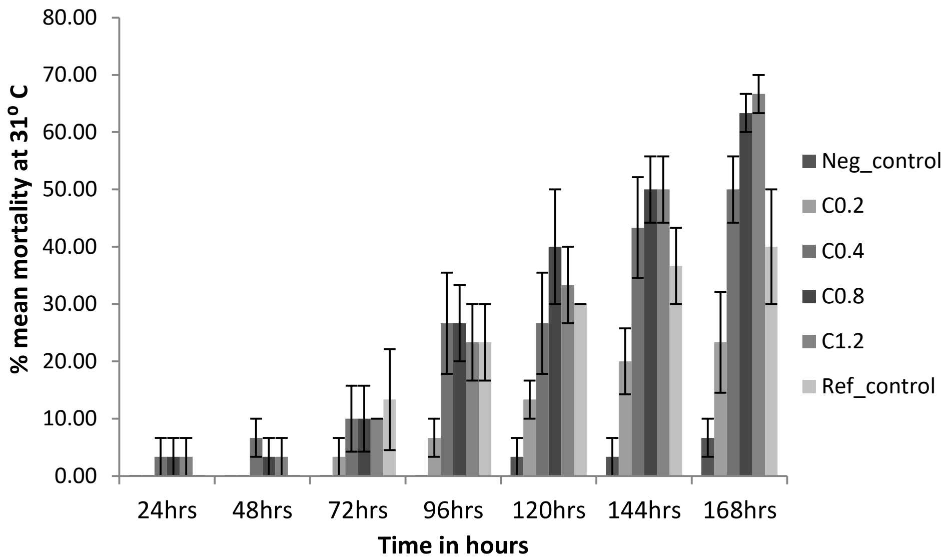

Figure 4: *Eucalyptus sp* leaf extracted with Methanol on mortality of *S. zeamais* at 31ºC

The results also indicated that with high concentrations and at different temperatures, % mean mortality of *S. zeamais* increased with time when exposed to *Eucalyptus* sp leaf extract. Treatment concentrations at 1.2% and 0.8% of the botanical caused high mortality at all temperatures than with lower concentrations and Acetone treated control. Higher concentrations of *Eucalyptus sp* leaf extract proved more effective when compared with reference control (*Azadirchtin*). At 20ºC, mortality started by 72 hrs at higher concentrations of 1.2% and 0.8% and only after 96 hrs for the lower concentrations (0.4% and 0.2%). At 27º C and 31ºC, a steady increase in *S. zeamais* mortality was recorded from 24 hrs up to the end of 168 hrs. Mortality in the control with Acetone was recorded from120hrs at temperature 27º C and 31º C (Figures. 2, 3 and 4). Results also indicated that more than 70% mortality occurred only after 168hrs of exposure compared to less than 30% mortality after 72hrs. The highest mortality was recorded at 27ºC when compared with 31ºC and 20ºC.

Multiple linear regression analysis showed that treatment dosage, incubation temperature and exposure time significantly affected the mortality of *S. zeamais* after a period of 168hrs for diethyl ether extract and for methanol extract of *Eucalyptus* sp leaf (Appendix 1). The different temperatures significantly influenced mean mortality of maize weevil ($F = 135.70$ and $P < 0.001$). Treatment concentrations were also significantly effective ($F = 71.71$ and $P < 0.001$) as while as exposure time in controlling *S. zeamais* in stored maize. The model used indicated that temperature, treatment effect and time of exposure accounted for more than 60% ($R^2 = 0.631$, $F = 4.116$, $p < 0.043$) of the mean mortality (Table 1).

Table 1: Temperature, Treatment effect and exposure time on mortality of S. zeamais

Source	df	Mean Square	*F*	Sig.
Temperature	2	18940.5	135.698	0.001
Treatment concentration	5	10009	71.709	0.001
Exposure Time	6	14708.5	105.378	0.001

a. $R^2 = .631$ (Adjusted $R^2 = .624$)

4.2 Temperature and methanol extracts of *Eucalyptus* leaf against immature stages of *S. zeamais*

Emergence of F_1 generation of *S. zeamais* after treatment with *Eucalyptus* sp leaf extract varied significantly with increasing temperatures and concentrations as shown in the table 1 below. Number of emergence of F_1 generation treated at the various life form stages was different ($p < 0.001$). The larval form was more susceptible than the egg and pupal stages at different temperatures and concentrations. Temperature showed a significant ($p < 0.001$) effect on larval stage compared to the egg and pupa stage at lower concentrations ($p > 0.05$). All life form stages of *S. zeamais* were susceptible to higher concentration of the *Eucalyptus* leaf extract. Emergence of F_1 progeny in all treatments was significantly lower than those observed in Acetone treated control and reference control (*Azadirachtin*). Increasing concentrations of the treatment and increase in temperatures indicated a decrease in emergence rate of F_1 adult *S. zeamais*. The emergence of F_1 adult of *S. zeamais* at 20°C occurred only after week 8 in storage.

Table 2: Temperature and treatment concentrations of *Eucalyptus* leaf extract on immature stages of *S. zeamais* in stored maize.

Source	df	Mean Square	*F*	*P*	R^2
Eggs					
Treatment Concentrations	5	195.94	31.55	< 0.001	0.80
Temperatures	2	97.06	15.63	< 0.001	
Larva					
Treatment Concentrations	5	162.77	42.66	< 0.001	0.85
Temperatures	2	91.13	23.88	< 0.001	
Pupa					
Treatment Concentrations	5	182.96	23.96	< 0.001	0.75
Temperatures	2	63.13	8.27	< 0.001	

The results showed that at 20°C when the different concentrations were applied on the eggs of *S. zeamais*, there was decrease in the number of F_1 progeny emergence (Figure 5). The decrease was significant ($p < 0.025$) for the 1.2%, 0.8% and 0.4% *Eucalyptus* crude leaf extract compared to control (Acetone alone) and reference control (*Azadirachtin*) (Figure 5), however, there were no significant differences within the treatments.

The number of F_1 progeny that emerged after treatment with *Eucalyptus* crude leaf extract on the larval stage was significantly ($p < 0.017$) lower compared to the negative control. But, there was no significant difference between the 0.2% concentration and the negative control. Higher concentrations of 1.2% and 0.8% of the extract indicated a significantly lower emergence of F1 adult maize weevil than the *Azadirachtin* (Figure 6).

Treatment concentrations of 1.2% and 0.8% on the pupal stage revealed a significantly ($p < 0.011$) lower emergence of F1 adult *S. zeamais* than negative control. The reference control showed no significant difference from the different concentrations except at treatment concentration of 0.8% (Figure 7)

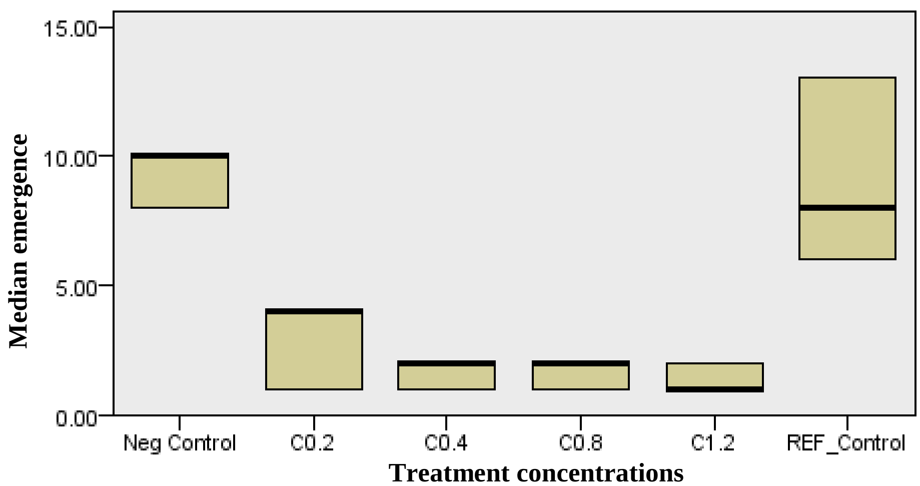

Figure 5: Emergence of F1 progeny after treatment with *Eucalyptus sp* leaf extract during the Egg stage at 20ºC

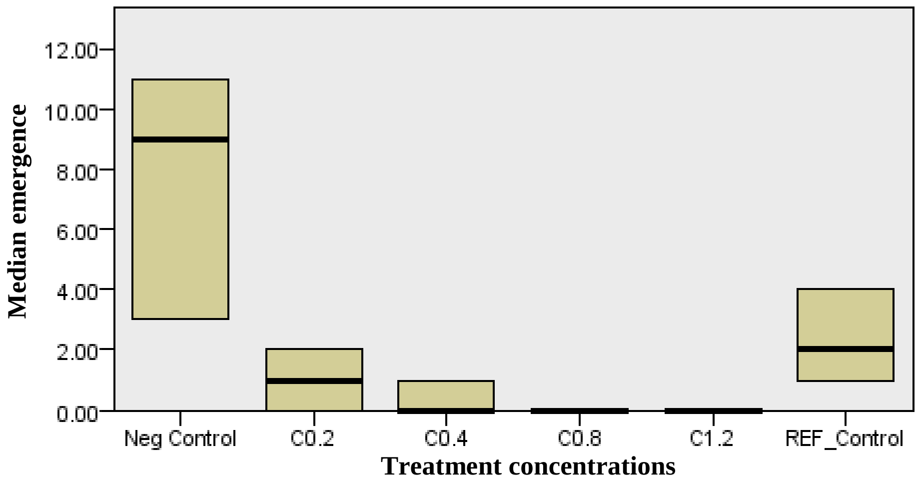

Figure 6: Emergence of F1 progeny after treatment with *Eucalyptus sp* leaf extract during the Larva stage at 20°C

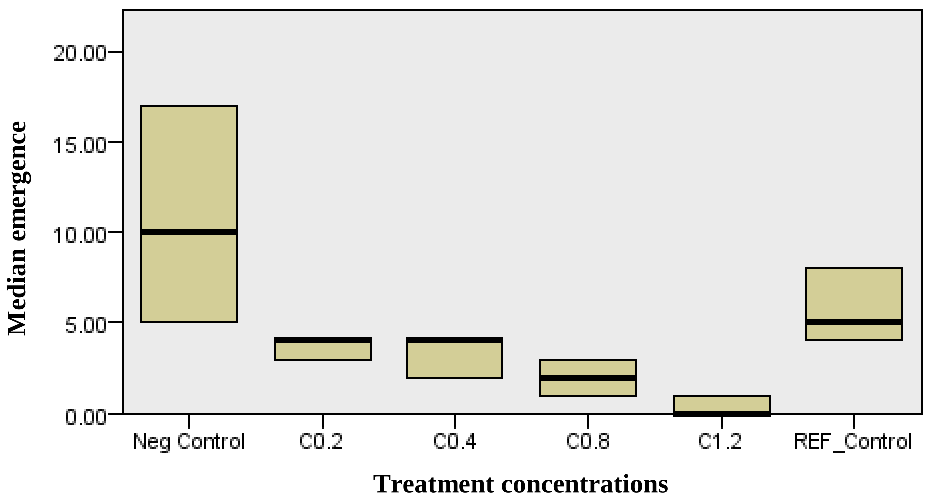

Figure 7: Emergence of F1 progeny after treatment with *Eucalyptus sp* leaf extract during the Pupa stage at 20°C

The treatment at 27°C showed no significant ($p < 0.056$) difference in the emergence of F_1 adult *S. zeamais* when *Eucalyptus* crude leaf extract was applied at varying concentrations on the eggs stage (Fig. 8). However, at the same temperature, treatment with *Eucalyptus sp* crude leaf extract on the Larval stage showed a significantly (0.012) lower emergence of F_1 generation at 1.2%, 0.8% and 0.4% concentrations when compared with negative control (Figure 9). Treatments that were applied on the pupal at 27ºC and concentrations of 1.2% and 0.8% showed a significantly (p < 0.014) lower emergence than the negative control and a low concentration of 0.2%. There was however, no significant difference between various treatments and *Azadirachtin* reference control (Figure 9)

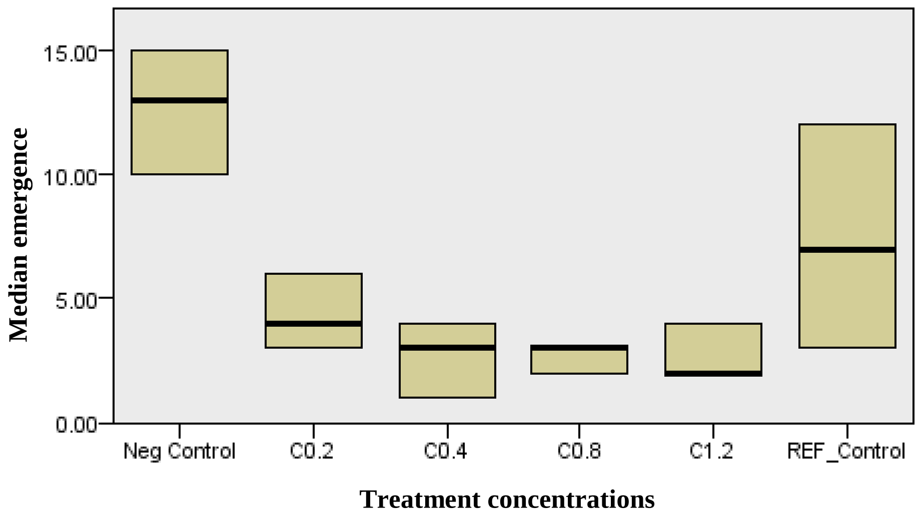

Figure 8: Emergence of F1 progeny after treatment with *Eucalyptus sp* leaf extract during the Egg stage at 27ºC

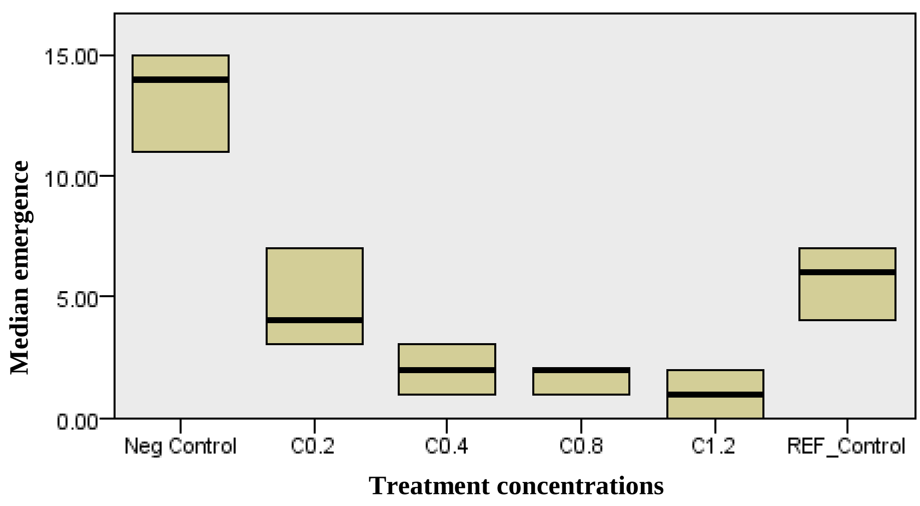

Figure 9: Emergence of F1 progeny after treatment with *Eucalyptus sp.* leaf extract during the Larva stage at 27ºC

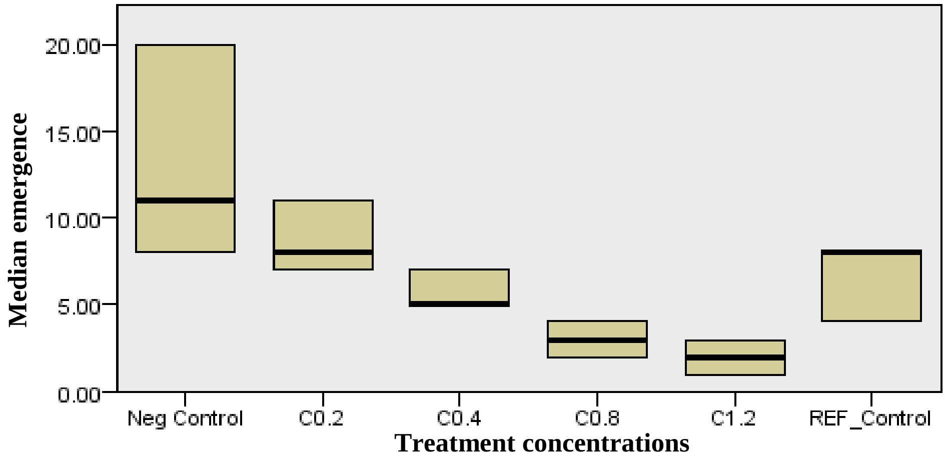

Figure 10: Emergence of F1 progeny after treatment with *Eucalyptus sp* leaf extract during the Pupa stage at 27^{o}C

At 31^{o}C a significantly ($P < 0.011$) lower numbers of F1 *S. zeamais* emerged when *Eucalyptus sp* leaf extract was applied at egg stage compared with high number of emergence from Acetone treated control (neg. control), reference control (*Azadirachtin*), and at lower concentration of 0.2% as seen in the independent-samples Kruskal Wallis bar chats in (Figure 11). The result showed a significant variation between treatment concentrations compared to reference control *Azadirachtin* and Acetone control ($F = 14.86$ and $p < 0.001$) when maize grains were treated at the egg forming stage of *S. zeamais* and stored at 31^{o}C.

F_1 progeny emerged from all concentrations of *Eucalyptus sp* leaf extract treatment, but the number from the of 1.2% and 0.8% concentrations were lower compared to the 0.4% and 0.2% concentrations as shown in fig 11. The highest F_1 progeny (46.67%) emergence were recorded at 0.2% *Eucalyptus sp* leaf extract but low when compared with acetone treated control (88.33). The F_1 progeny weevils which emerged from acetone treated (neg. control) and reference control with *Azadirachtin* was significantly higher than those that emerged from the 1.2% and 0.8%

Eucalyptus leaf concentrations except at 0.4% which showed significant differences from only the acetone treated control.

The effects of the concentrations of *Eucalyptus* sp leaf extract applied on the early stage of larva of *S. zeamais* in maize stored at 31°C significantly ($F = 11.08$ and $P < 0.050$) varied from the acetone treated control and reference control. The highest F_1 progeny emergence (31.58%) was on 0.2% *Eucalyptus* leaf treatment but, this was lower than the acetone treated control with 87.72% emergence. There were emergences from all the concentrations of the *Eucalyptus* extracts however, this was lower than the control (acetone) and reference control, except for the 0.2% concentration which was slightly higher (31.58%) than emergence from reference control (29.82%) (Figure12). The F_1 progeny *S. zeamais* emerged from acetone treated control was significantly higher than the emergence from 1.2%, 0.8% and 0.4% with significant values of $p < 0.002$, $p < 0.028$ and $p < 0.37$ respectively.

There was a significant difference in the emergence of adult from the treated pupa of *S. zeamais* in maize stored at 31° C. Treatment effect also showed significant ($F = 14.48$ and $p < 0.013$) difference compared to Acetone treated control and reference control as presented in fig 13. The number of F_1 progeny that emerged from the higher concentrations of *Eucalyptus* leaf extract (1.2% and 0.8%) was significantly ($P < 0.002$ and $p < 0.003$, respectively) lower to acetone treated control. There was no significant difference between reference control and the treatment concentrations, except at 1.2% with a significantly lower emergence than reference control ($P < 0.038$).

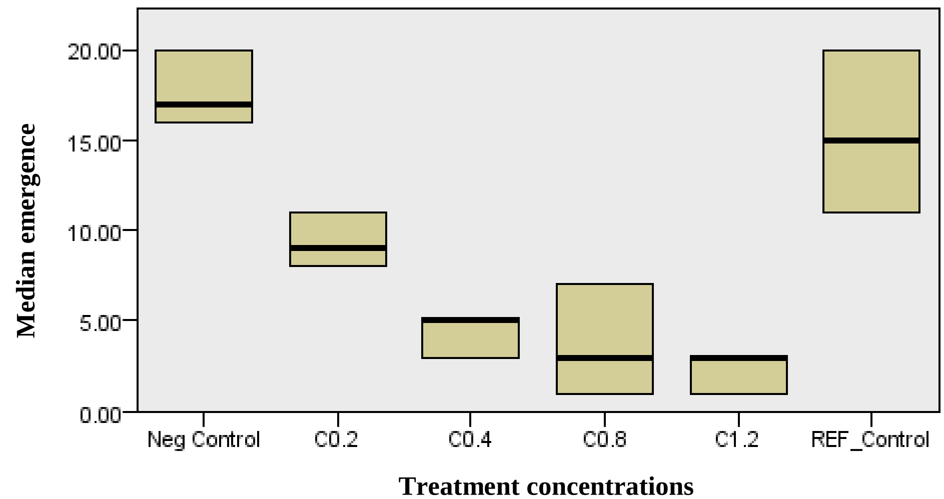

Figure 11: Emergence of F1 progeny after treatment with *Eucalyptus* leaf extract during the Egg stage at 31ºC

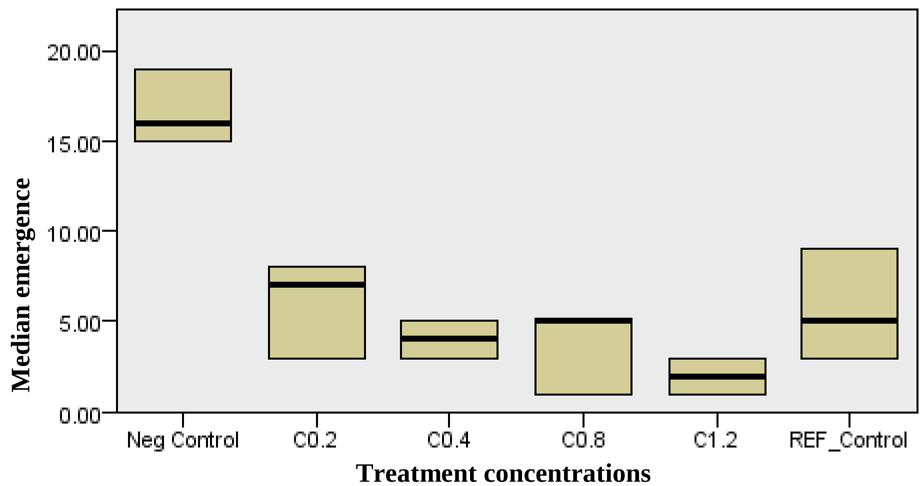

Figure 12: Emergence of F1 progeny after treatment with *Eucalyptus* leaf extract during the Larva stage at 31ºC

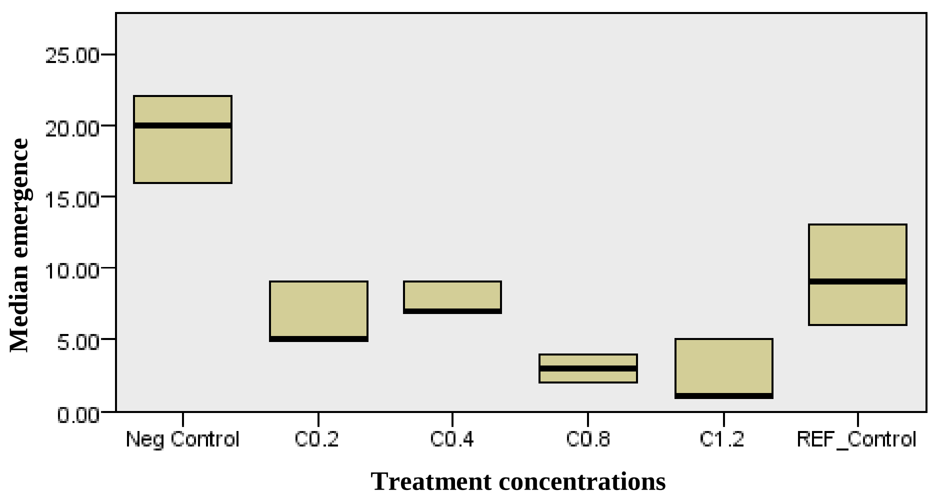

Figure 13: Emergence of F1 progeny after treatment with *Eucalyptus* leaf extract during the Pupa stage at 31ºC

4.3 Persistence of Eucalyptus leaf extract against *S. zeamais* in maize grains

Temperature, botanical insecticide *(Eucalyptus* sp leaf extract) and duration of treatment had significant impact on the control of *S. zeamais*. The results indicate that temperature had significant effect on *S. zeamais* mortality (Table 2). There were significant ($F = 18.16$, $p < 0.001$) differences in mortality among temperatures (20°C, 27°C and 31°C) during the 5 month of maize in storage. The highest percentage mean mortality of 17.6±1.6 was recorded at 27°C. Percentage means mortalities of 17.6±1.6 and 14.1±1.5, respectively at 27°C and 31°C was significantly higher than the 6.7±0.7 mean mortality recorded at 20° C. As temperature increases from 20° C to 27°C insect mortality also decreases from 17.6±1.6 to 6.7±0.7 showing an inverse relationship between temperature and survival of the insects.

Table 3: Percentage means mortality of *S. zeamais* at different temperatures

Temperature	%Mean mortality ±SE
20	6.7±0.7
27	17.6±1.6
31	14.1±1.5
Mean total	12.8±0.8
F	18.16
p	< 0.001

Based on observed means

The mean difference is significant at the 0.05 level.

Percentage mean mortality of *S. zeamais* was higher with increasing treatment concentration of *Eucalyptus sp* leaf extract. The botanical showed high significant difference in mortality of *S. zeamais* ($F = 37.151$, df = 5, $p < 0.001$). Eucalyptus leaf extract at a concentration of 1.2%

recorded the highest percentage mean mortality of 28.5±2.6. With respect to treatment concentrations, there was no significant difference between mortalities recorded at concentrations of 0.2% and 0.4% when compared to *Azadirachtin* (reference control), however, 1.2% and 0.2% indicated a high significant difference from the reference control (Table 3).

Table 4: Treatment effect on mortality of *S. zeamais* on maize for 5 months

Treatments	Concentration	%Means mortality ±SE
Eucalyptus	0.2%	6.3±0.9
	0.4%	12.4±1.7
	0.8%	20.5±2.2
	1.2%	28.5±2.6
Azadirachtin	0.6%	8.7±1.2
Control(Acetone)	1 mL	0.4±0.2
Mean total		12.8±0.8
F		37.151
p		< 0.001

Based on observed means
The mean difference is significant at the 0.05 level.

Effects of treatment duration showed significant ($F = 37.232$, df = 5, $p < 0.001$) difference on insect mortality with extended period. The mortality of *S. zeamais* decreased with increasing storage period (Table 4). From week 0 to Month 1 and 2, the highest mortalities were recorded after treatment with Eucalyptus leaf extract. There was no significant difference in mortality between this storage periods (Week 0 and month 1), however, there were significant differences between mortalities recorded during the first two months and those afterward (Months 3 to 5).

Mortality was significantly lower at Months 4 and 5 with mean mortalities of 3.9±0.7 and 0.5±0.2 respectively (Table 4).

Table 5: Efficacy of *Eucalyptus* leaf extract in maize on mortality of *S. zeamais* for 5 month Storage period

Storage period	Sample size	%Mean mortality ±SE
Baseline	54	23.5±3.5
Month 1	108	24.4±2.2
Month 2	108	19.5±2.0
Month 3	108	10.3±1.3
Month 4	108	3.9±0.7
Month 5	108	0.5±0.2
Mean total		12.8±0.8
F		37.232
p		< 0.001

Based on observed means
The mean difference is significant at the 0.05 level.

Mean mortality at 20ᵒC, 27ᵒC and 31ᵒC with four concentration of *Eucalyptus* leaf extract and *Azadirachtin* (reference control) was significantly higher (p < 0.001) than that of acetone treated control. At 27ᵒC and 31ᵒC, concentrations of 1.2% and 0.8% caused higher total percentage mean mortality (28.5 and 20.5, respectively) than the *Azadirachtin* (reference control) and acetone treated control. There was no significant difference between the 0.4% and 0.2% concentrations from the *Azadirachtin* but were significantly higher than acetone treated control.

Among all temperatures, 27ᵒC was the optimum with the highest total percentage mean mortality of 17.6 compared to those at 31ᵒC (14.1%) and 20ᵒC (6.7%).

The efficacy of *Eucalyptus* extracts with time of exposure at 20ºC (Figure 14) showed a steady decrease in mean mortality. This was observed with decreasing trends on treatment duration irrespective of treatment concentrations. The graph below shows a decrease in mean mortality from 3 to 2.33 from the baseline and 1st month; however, no mortality was recorded from all treatments by the 5th month with temperatures at 20ºC.

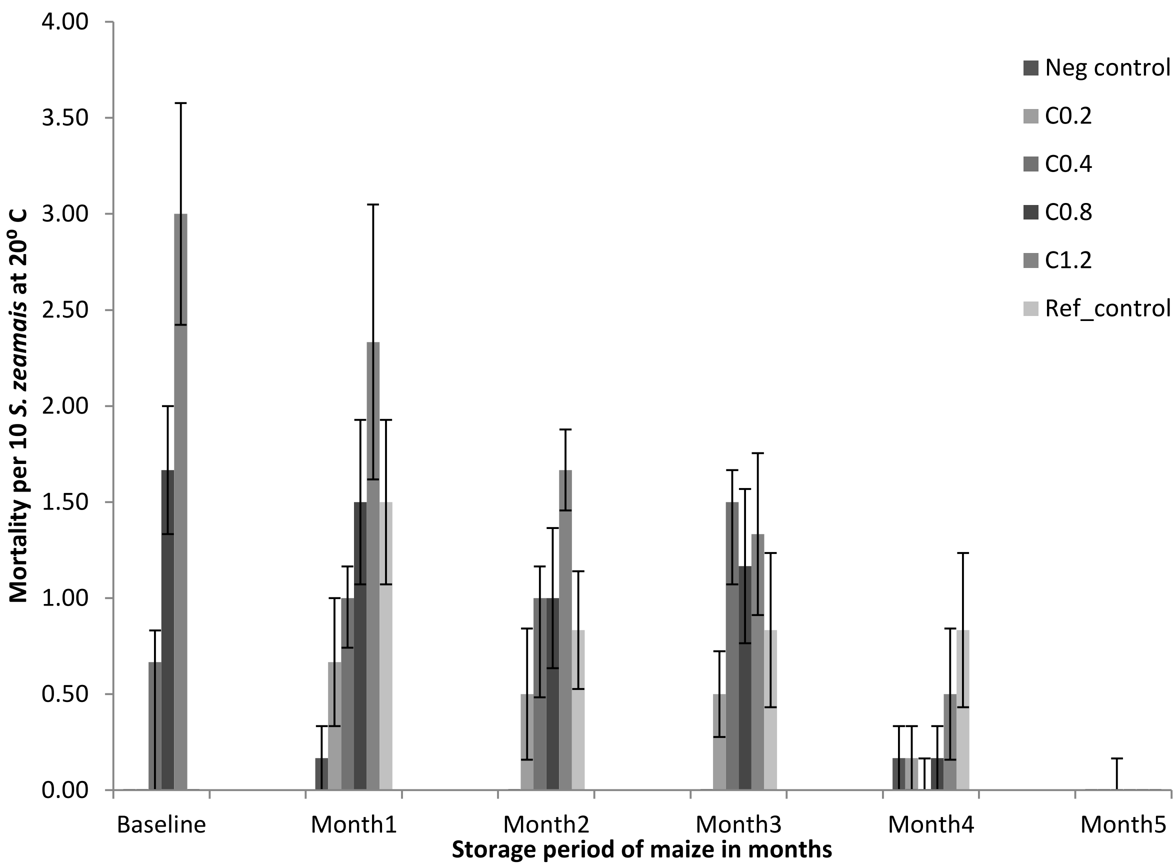

Figure 14: *Eucalyptus* leaf extract on survival of *S. zeamais* in maize grains during 5 months at 20ºC

The graph below (Figure. 15) indicate a steady and decreasing trends in mortality from treated maize with Eucalyptus leaf extract at different concentrations at 27ºC. Baseline data shows mortalities were recorded from all treatment concentrations of *Eucalyptus* leaf extract and *Azadirachtin* (reference control) except with Acetone treated control. Mortality increased per increased concentration with the highest mean mortality from treatment concentrations of 1.2% and 0.8% (6.67 and 5.33) respectively. Reference control recorded the lowest mortality (1.33) compared to treatment concentrations of the botanical. The same trend but with a steady decline was seen from the 1st month to the 4th, however, there were increases in mean mortality from *Azadirachtin* reference control between 1st and 2nd month compared to baseline data. *Azadirachtin* reference control recorded no mortality as of the 4th month and by the 5th month mean mortality (0.50 and 0.17) was recorded only at treated rates of 1.2% and 0.8%.

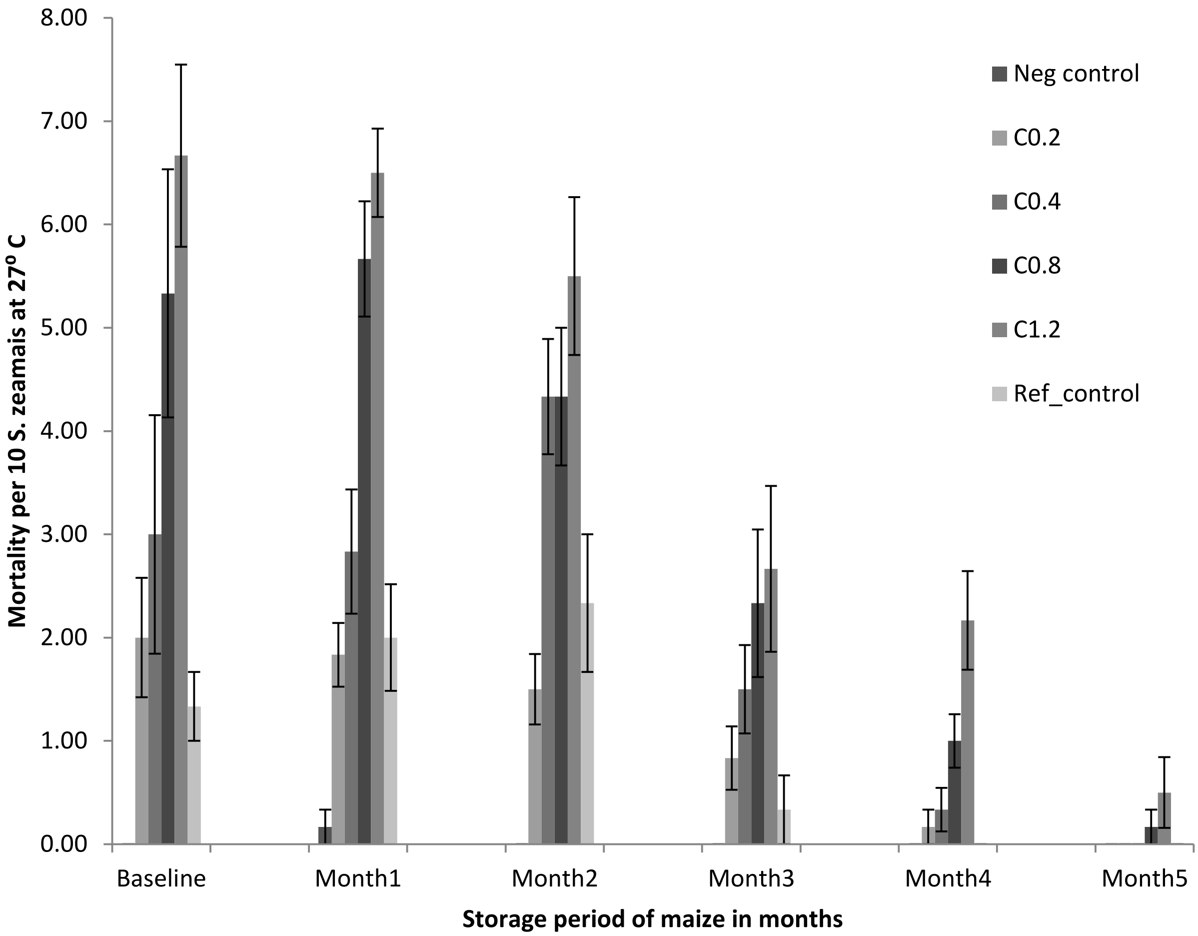

Figure 15: *Eucalyptus* leaf extract on survival of *S. zeamais* in maize grains during 5 months at 27ºC

At 31ᴼC, mean mortality of *S. zeamais* in maize treated with *Eucalyptus* leaf extract during 5 month storage showed a steady decrease with treatment concentrations at 1.2%,0.8% and 0.4% of the botanical (Figure 16). There was a decrease in mean mortality from treatment concentrations of 0.4% compared to baseline data and 1st month (4.33 and 2.33) respectively. Another decrease in mean mortality was indicated with treatment concentration at 0.8% from 4.00 in the 2nd month to 1.00 in the 3rd month. There were variations in mean mortality at lower treatment concentrates of Eucalyptus leaf extract and *Azadirachtin* reference control during storage. No mortality was recorded from the 5th month except treatment rate at 1.2% with mean mortality of 0.17.

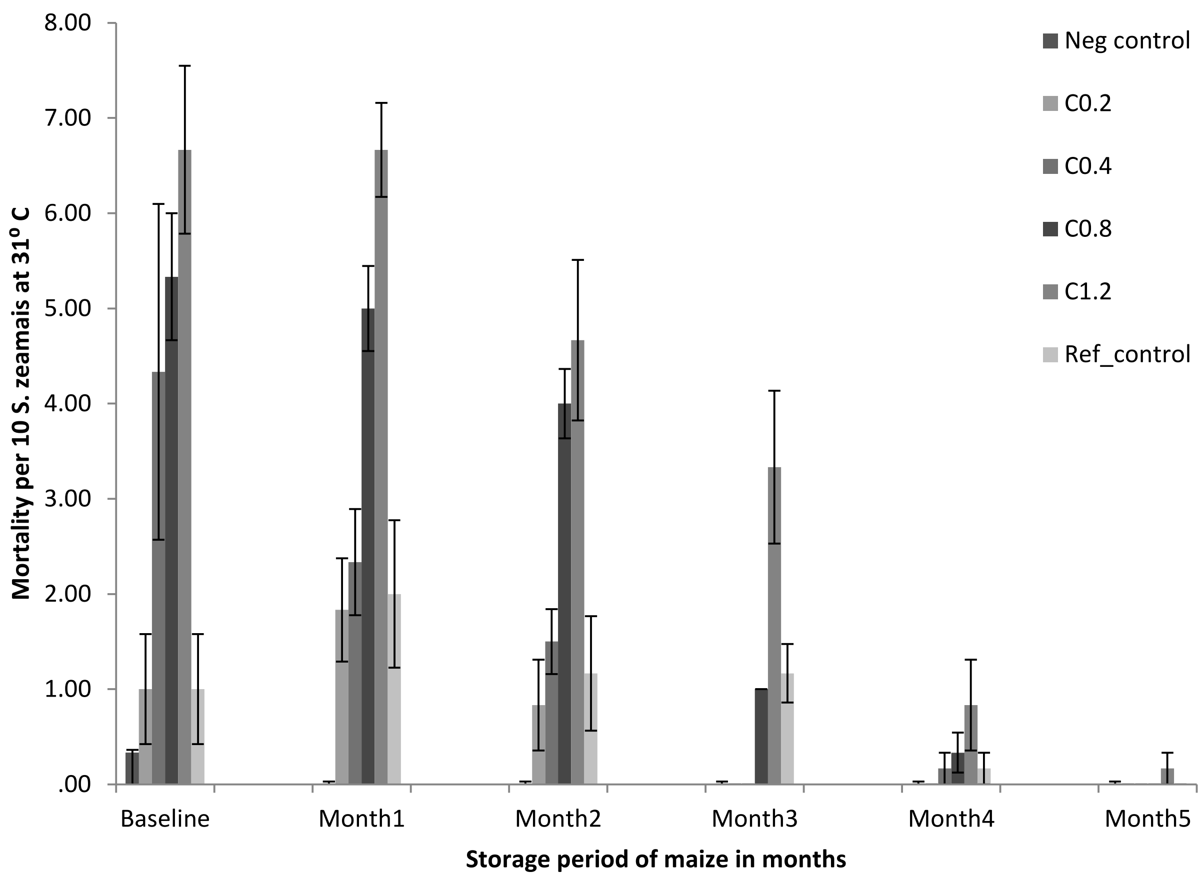

Figure 16: *Eucalyptus* leaf extract on survival of *S. zeamais* in maize grains during 5 months at 31ºC

The results showed that there were significant differences among the treatment concentrations (F = 60.439, df = 5, $p < 0.001$) and storage duration ($F = 60.540$, df = 5, $p < 0.001$) and temperature ($F = 36.447$, df = 2, $p < 0.001$). All temperatures and treatment concentrations showed significant insecticidal activities against *S. zeamais*. Increase in concentrations of *Eucalyptus* sp leaf extract resulted in increased number of insect mortality which decreased with longer storage duration. Increase in temperature from 20^0 C to 27^0 C showed significant increase in mean mortality of *S. zeamais*. Temperature and treatment duration accounted for more than 50% of the variations seen in mortality of *S. zeamais* ($R^2 = 0.538$).

Table 6: Temperature and storage duration on persistency of *Eucalyptus* leaf extract against *S. zeamais*.

Source	Type III Sum of Squares	df	Mean Square	*F*	*P*
Corrected Model	1152.060[a]	12	96.005	56.482	0.001
Intercept	1040.700	1	1040.700	612.270	0.001
Treatments	513.650	5	102.730	60.439	0.001
Storage period	514.508	5	102.902	60.540	0.001
Temperature	123.902	2	61.951	36.447	0.001
Error	987.550	581	1.700		
Total	3112.000	594			
Corrected Total	2139.609	593			

a. R^2= .538 (Adjusted R *2* = .529)

4.4 Repellency of methanol extract of Eucalyptus leaf on *S. zeamais*

Repellency of *S. zeamais* to maize treated with different concentrations of Eucalyptus leaf extract and at different temperatures showed that the repellence of adult maize weevil was not significantly influenced by temperature (F = 1.637, df = 2, $P \leq 0.198$) and exposure time (F = 1.608, df = 3, $P \leq 0.191$) but was significantly influenced by treatment concentrations of *Eucalyptus* leaf extract (F = 47.861, df = 3, $P > 0.001$). Results from multiple regression analysis $R^2 = 0.529$ (Adjusted $R^2 = 0.501$) indicated that treatment concentrations, temperature and exposure time accounted for 53% repellent of *S. zeamais.*

Treatment concentration of *Eucalyptus* leaf extract on *S. zeamais* was significantly effective (F = 21.447, df = 3, $P < 0.001$) at 20°C. The bar chart below (Figure 17) shows a steady trend, increasing with increase concentrations of the botanical at the different exposure intervals, except for 0.2% and 0.8% which were the same (60%) at six hours of exposure. After six and 12 hours of exposure, treatment concentrations at 1.2% caused 100% repellency, significantly higher than all treatment concentrations.

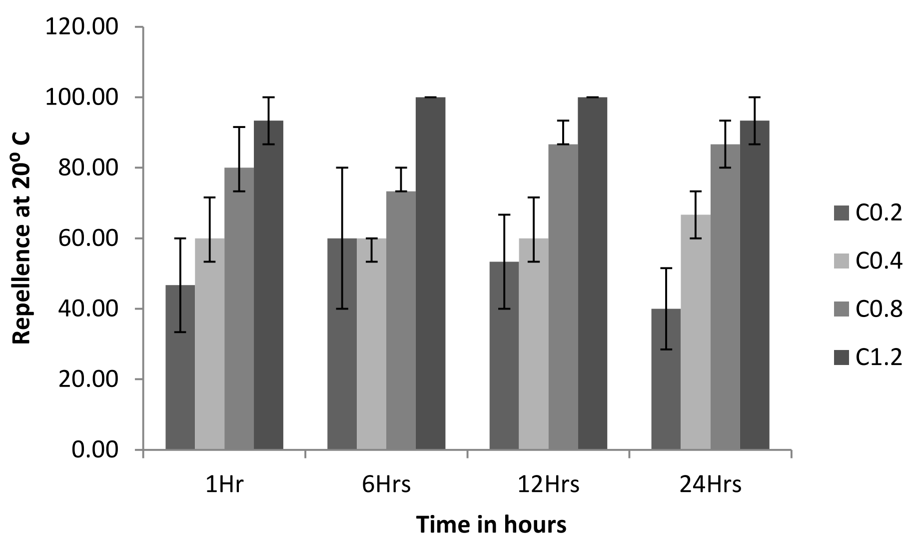

Figure 17: Repellence of different concentrations of *Eucalyptus* leaf extract on *S. zeamais* at 20° C.

At 27°C, treatment concentrations of the botanical also reached highest repellency at 100% with treatment concentration of 1.2% and 0.8% after 6 and 12 hours exposure to *Eucalyptus* leaf extract (Figure: 18). There were no significant differences between the 1.2% and 0.8%, however, the higher concentrations (1.2% and 0.8%) were significantly (F = 19.744, df = 3, p < 0.001) effective compared to lower concentrations of 0.4% and 0.2%. An increase in repellency was observed 1 to 12 hours after exposure to treated rates. A decrease in percentage repellency was observed from 24 hours of exposure at all treatment concentrations.

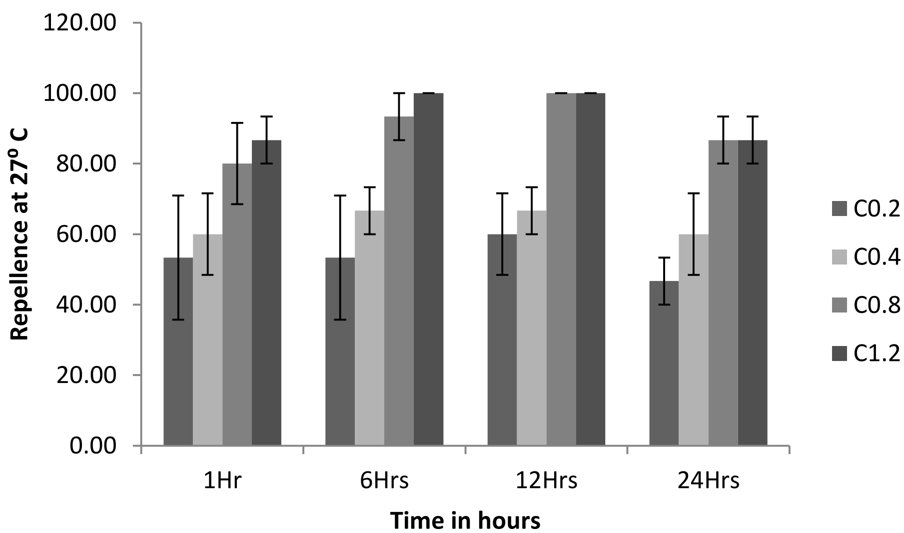

Figure 18: Repellence of different concentrations of *Eucalyptus* leaf extract on *S. zeamais* at 27°C.

At 31°C, considerable differences in repellence of *S. zeamais* to *Eucalyptus* leaf extracts were observed at different concentrations (F = 10.453, df = 3, p < 0.001). From Figure: 19 below, it can be seen that the extracts showed a relatively higher repellence of 93.33%, 86.67%, and 93.33% one hour after treatment for the 0.4%, 0.8% and 1.2% respectively, except for 0.2% with less than 50% repellence. The highest concentration of the extract was able to induce 100% repellency six hours after treatment, however, after 12 and 24 hours of exposure the repellency reduced to about 90% (Figure 19).

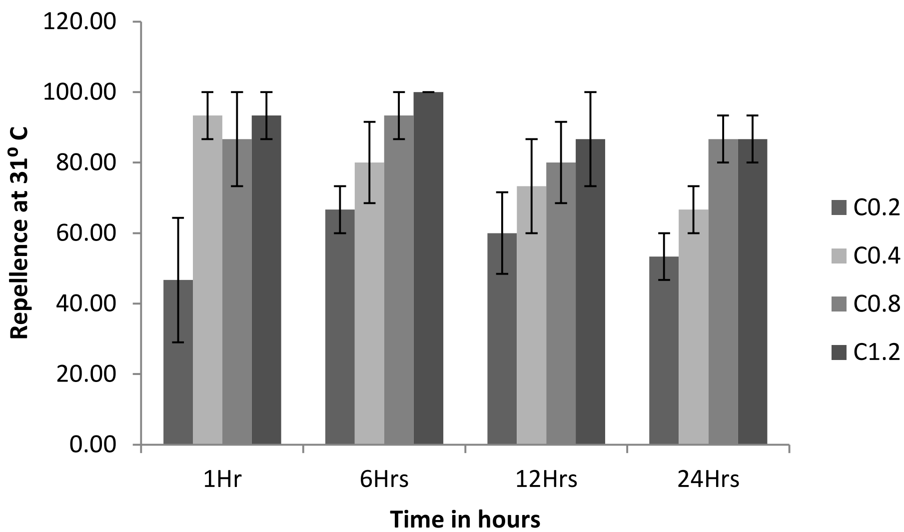

Figure 19: Repellence of different concentrations *Eucalyptus* leaf extract against *S. zeamais* at 31ºC.

4.5 Assessment of damage from infestation

All the treatment concentrations of methanol extract of *Eucalyptus* leaf caused a significant (F = 13.386, df = 5, $p < 0.001$) decrease in percentage weight loss in maize grains. There were significant (F = 27.459, df = 1, $p < 0.001$) differences in grain weight loss for all the concentrations over the 5 months storage period. But, grain weight loss was not significantly influenced by temperature (F = 0.732, df = 2, $p > 0.483$).

After 3 and 5 months post treatment with *Eucalyptus* leaf extract, minimal damage was observed across all the treatment concentrations at 3 months but with a significantly higher increase in the 5th month of storage. Significantly, higher weight losses were recorded with the acetone treated control compared with the reference control (*Azadirachtin*) and *Eucalyptus* treatment concentrations.

Figure 20 shows that percentage weight loss caused by *S. zeamais* in maize treated with *Eucalyptus* leaf extract and stored at 20^{o}C were significantly different depending on treatment concentrations and storage duration (F = 19.886, df = 1, p < 0.001). Acetone treated control showed slightly increased percentage weight loss (4.07%) on the 3rd month but increased to 22.86% by the 5th month. Percentage weight loss decreased as concentration of *Eucalyptus* leaf extract increased, however, percentage weight loss increased as the period of storage increased (Figure 20). At lower concentration of 0.2%, percentage weight was 1.12% after the 3rd month and increased to 7.61% in the 5th month while at a higher concentration of 1.2%, percentage weight loss was 0.14% after the 3rd month and increased to 5.26% in the 5th month respectively.

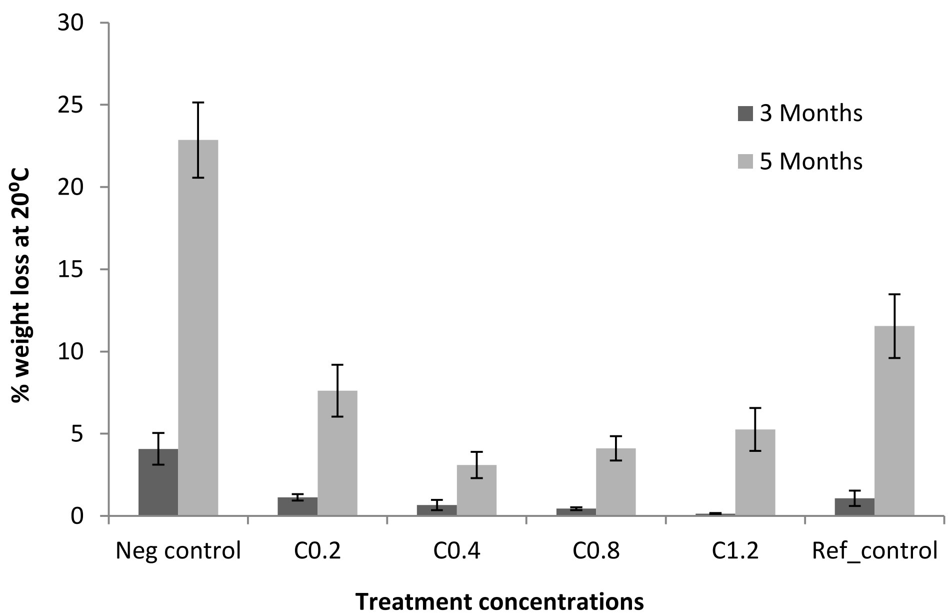

Figure 20: Percentage weight loss of maize grains treated with *Eucalyptus* leaf extracts against *S. zeamais*.

At 27°C, percentage weight loss caused by *S. zeamais* on treated maize grain with *Eucalyptus* leaf extract was significantly ($F = 6.313$, df = 1, $p < 0.017$) affected by treatment concentration and storage duration. Control with acetone indicated increased weight loss in maize grains than treatment concentrations of *Eucalyptus* leaf extract (Figure 21). The protection effect of *Azadirachtin* on maize was better, compared to 0.2% and 0.4% concentration of Eucalyptus leaf extract in the 5th month. A steady decrease in percentage weight loss was realized with every increase in concentration by the 3rd month but, by the 5th month there was increasing variation in weight loss between treatment rates.

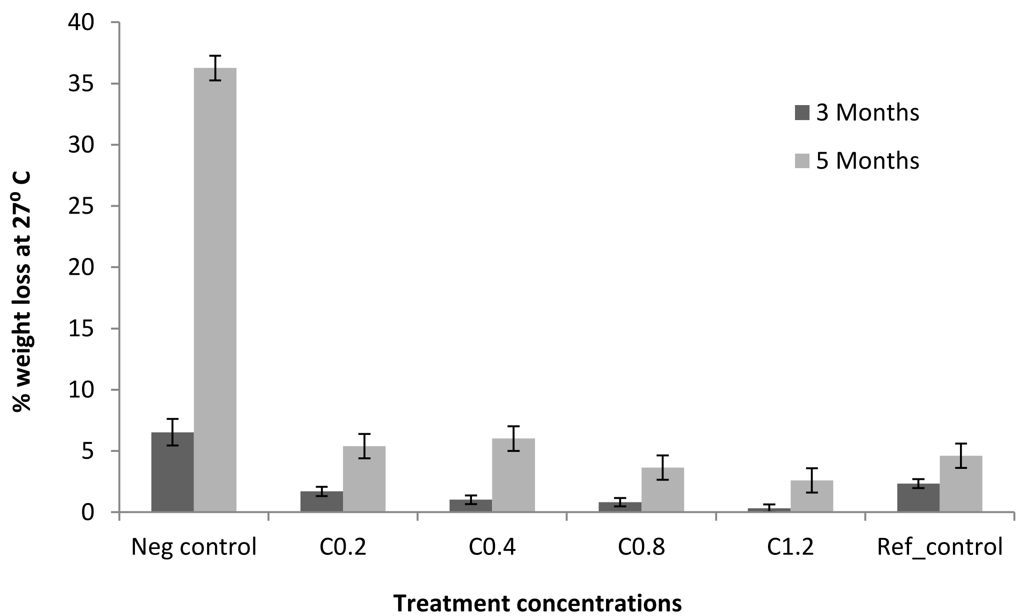

Figure 21: Percentage weight loss of maize grains treated with *Eucalyptus* leaf extracts against *S. zeamais.*

At 31ºC, percentage weight loss of maize treated with *Eucalyptus* leaf extract was also concentration dependent with significant ($F = 9.094$, df = 1, $p < 0.005$) difference between duration of storage. The protectant effect of the *Eucalyptus* sp leaf extract reduced after 3 months specifically with lower concentrations. At 5 months, we see that even at higher concentrations, percentage weight loss was higher; meanwhile it was lower at the lower concentration (Figure 22 below). Similar outcomes as at 27ºC were recorded; percentage weight loss of maize grains was highest at lower concentration than at higher concentration. A significant weight loss was suffered in the 5th month than was in the 3rd month.

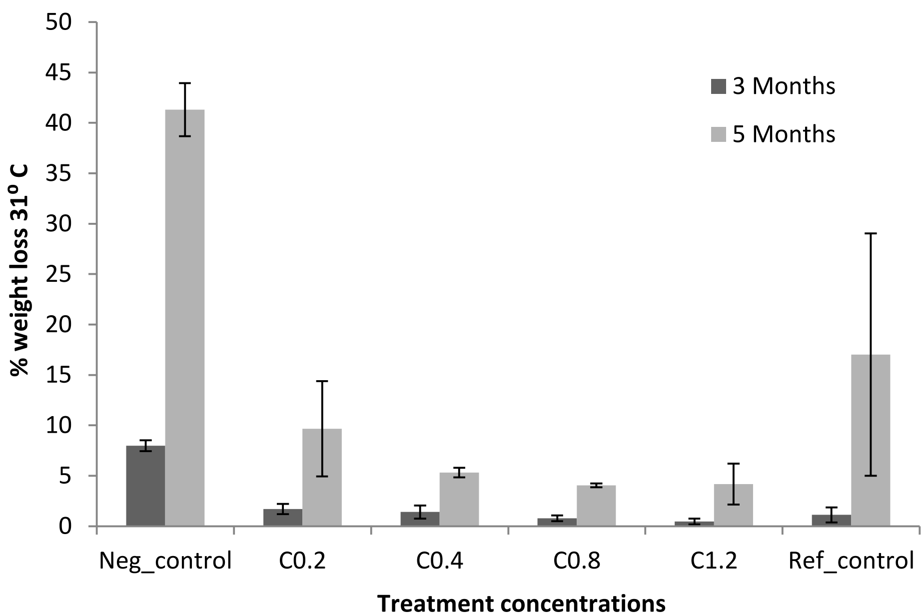

Figure 22: Percentage weight loss of maize grains treated with *Eucalyptus* leaf extracts against *S. zeamais*.

Results from multiple regression analysis showed that there were significant difference among treatment concentrations (F = 20.413, df = 5, p < 0.001) and storage period (F = 53.003, df = 1, p < 0.001), however, temperature showed no significant (F = 1.771, df = 2, p > 0.175) effect on percentage weight loss of maize grains. The model used indicates treatment effects and storage duration accounted for more than 60% (R^2 = 0.616) of the variation in percentage weight loss of maize grains treated with Eucalyptus leaf extracts.

CHAPTER 5

5.0 DISCUSSION

5.1 *Eucalyptus* leaf extracted with methanol and temperature on mortality of *S. zeamais*.

Extract of *Eucalyptus* leaf with methanol showed no significant difference compared to extract of *Eucalyptus* leaf with diethyl ether irrespective of the treatment concentrations and temperature, similar to studies on the effect of *Vitex negundo* leaf extracted with methanol and petroleum ether against *S. zeamais* on relative mortality rates also showed similar response (p = 0.80) between the extracts (Haridasan *et al.,* 2016).

Centered with methanol extract of *Eucalyptus* leaf; the survival of *S. zeamais* to methanol extract of *Eucalyptus* leaf at higher concentrations (0.8% and 1.2%) was less compared with concentrations at 0.2%, 0.4% extract and *Azadirachtin* (reference control) after 168 hours treatment duration. In a similar study by Ouku *et al.,* (2017) *S. zeamais* revealed distinctive effect from methanol extracts of *Ocimun baslicam* with up to 74% mortality at highest applied concentration and 37% mortality at lowest applied concentration. The actual mode of action of *Eucalyptus* leaf extract in suppressing *S. zeamais* populations however has not yet been delineated (Sendi *et al.,* 2013) but, its susceptibility to the Eucalyptus extract might be it acting as a contact and stomach poison judging from slowdown in activities of weevil followed by death at 24 hours from contact with the botanical with respect to treatment concentrations (Mulungu *et al.*, 2007).

Methanol extracts of *Eucalyptus* leaf tested against *S. zeamais* showed effectiveness on mortality with up to 76.67% mortality at higher treatment concentration of 1.2% extract compared to 1.90% mortality with lowest concentration of 0.2% extract. In a similar study by Mandudzi, *et al.* (2016) mortality of *S. zeamais* in maize treated with *Eucalyptus* sp leaf powder increase with concentration when treated with 5g/kg maize and 2.5g reduced the total number of insect pests (44.3% and 23.3%, respectively). This can be explained with relation to quantity and toxicity; the higher the quantity of botanical absorbed the faster toxicity levels are attained enough to interfere with normal metabolic activities of *S. zeamais* (Muzemu *et al.,* 2013). Findings by Ibrahim (2011), showed that, percent mortality from both garlic and chamomile oil extracts against grain insects were dependent on dosage and period of exposure time dependent. All treatment levels of *Eucalyptus* leaf extract recorded higher mean mortality than Acetone treated control and *Azadirachtin* (reference control).

Azadirachtin used as reference control caused very low mortality of *S. zeamais* when compared to methanol extract of *Eucalyptus* leaf. Mortalities recorded in the present study were lower than that found with the study conducted by Tofel *et al.* (2014). Tofel *et al.* (2014) found that after 60 days of exposure, maize grains treated with 5 ml/kg of *Azadirachtin* recorded 88.75% mortality, same as 0 - day but was lower at concentration 2 ml/kg with 26.25% mortality. The low mortality recorded (35.81% at treated rate of 5% w/w) in the studies does not mean *Azadirachtin* is not effective against *S. zeamais* but might have been due to lower treatment concentrations (Erenso *et al.,* 2016).

Treatment effect of *Eucalyptus* leaf extract on *S. zeamais* mortality was significant higher at 27ºC compared to 20ºC and 31ºC irrespective of treatment concentrations and exposure duration. After *S. zeamais* was exposed to *Eucalyptus* leaf extract, the different concentrations showed statistical differences in mortality of maize weevils. Total mortality of *S. zeamais* was lowest at 20ºC (30%) but higher at 27ºC (76%) than at 31ºC (66%) at highest concentrations. Similar findings showed that *B. bassiana* at different treatment rates resulted in high mortality of *S. zeamais* only at moderate temperatures of 15ºC and 25ºC and was not active at a low temperature of 10ᵒC or at a high temperature of 35ᵒC (Amarasekare *et al.*, 2004). This was attributed to the fact that toxicity of the botanical was influenced by temperature. Arthur *et al.* (1999) reported that Pyrethroid toxicity was negatively correlated with temperature but results often vary depending on chemical structure, target species, insecticides, and the temperature range.

5.2 Temperature and methanol extract of *Eucalyptus* leaf against immature stages of *S. zeamais.*

The efficacy of *Eucalyptus* leaf extract reduced the number of emergence of F_1 progeny of *S. zeamais* for all treatment concentrations at egg, larval and pupal stages. This is supported by findings from Tofel *et al.* (2014) which showed that neem oil extracts have compounds that have growth regulatory effects which may block developmental stages of *S. zeamais* or cause mortality of the immature stages. Despite the fact that mortality increased with increasing treatment rate, total inhibition of *S. zeamais* emergence was not achieved even at higher concentration of extract (Ogungbite, 2015). The highest inhibition of *S. zeamais* was reached at rates of 0.8% and 1.2% of the concentrates at different temperatures when compared with acetone treated control and *Azadirachtin* (reference control). At each temperature, F_1 progeny production decreased with increasing treated rates as described by Pizarro *et al.* (2013), who also concluded that different treatment rates had toxic effect against immature stages of *S. zeamais.*

The most effective temperature was 27ᵒC with the lowest number of emergence of F1 progeny compared with 20ᵒC and 31ᵒC irrespective of time of emergence of adult *S. zeamais.* This could be attributed to the fact that at moderate temperature (25ᵒC), *S. zeamais* were more active increasing the contact rate with active ingredient of Eucalyptus leaf extract (Frank, H. *et al.*, 1999). This is confirmed with Findings from Kifle *et al.* (2017) who revealed that *Eucalyptus sp* leaf extract reduces the activity of insects attributing it to deleterious effects on the normal physiology of the insects. Studies from Kaushalya *et al.* (2004) also indicated that temperature affected the efficacy of botanicals against grasshopper nymphs at different ranges of 0°C to 35°C with levels of mortality higher at 25°C and not active at 10°C or at a higher temperature of 35°C.

The larval stage showed significant lower emergence than the egg and pupae at all treatment concentrations and temperature. The larvae are the feeding stages with high respiratory intensity compared to the eggs and pupae of *S. zeamais* (Mahroof *et al.,* 2003). This also complies with other findings that the developmental stages of insects with higher metabolic rate are more susceptible to lethal exchange imbalances incited by an insecticide (Emekci *et al.,* 2001). It was further reported that first instar larvae of *S. zeamais* were more susceptible at third-instar while pupal stage was most resistant to the vapors of mustard essential oil (Soujanya *et al.,* 2016).

5.3 Persistence of Methanol extract of Eucalyptus leaf against *S. zeamais* in maize grains

Methanol extract of *Eucalyptus sp* leaf showed significant effect on the mortality of *S. zeamais* at all treatment concentrations compared to Acetone treated control and reference control *Azadirachtin.* Increase in mortality of *S. zeamais* was directly proportionate with treatment concentration of *Eucalyptus sp* extract used (Shiberu *et al.,* 2017). The botanical showed a significant number of mortalities at 20°C, 27°C and 31°C when exposed to higher concentration (1.2%) of *Eucalyptus sp* leaf extract; however, there was a steady decline in the trend mortality of *S. zeamais* with extended treatment duration from the 3rd to the 5th month irrespective of treatment concentration. The reduction in mortality of maize weevil is in confirmation with findings of Musundire *et al.* (2015) who reported significant reduction in the efficacy of *Eucalyptus sp* leaf powder 128 to 192 days post treatment.

Efficacy of *Eucalyptus sp* leaf extract resulted in lower mortality when maize weevils were exposed to treated grains after the 2^{nd} month; and by the 5^{th} month, no mortality was recorded except at treatment concentration of 1.2% and at 27°C. Previous studies by Bekele and Hassanali (2001) showed that, as storage period of treated grains increases, the efficacy of the botanical also decreases as most of the compounds volatilize and degrade from the plant extract. *Eucalyptus grandis* application rate response between 5 and 2.5g/kg maize against *S. zeamais* only occurred from 96 to 160 days after treatment, with rates of 5g/kg showing significantly lower numbers of live insects (Musundire *et al.*, 2015).

At 27°C, a significantly higher mortality was recorded compared to 31°C though with similar peaks in trend at all treatment concentrations. The results confirm works of Vardeman *et al.* (2006) where the percentage survival of *Sitophilus oryzae* at 27°C was significantly lower at 15.2 cm (above the diatomaceous earth (DE)-treated layer) but showed no difference among DE layer treatments at 32°C. The results from the present study are consistent with works of Collins *et al.* (2001) which showed that the efficacy of DE was less effective against *S. zeamais* at lower temperatures probably due to reduced contact of the insect to lethal effects of the botanical as insect mobility decreases. High temperature increases movement and contact to the botanical resulting in greater cuticle damage from increased metabolic rates (Arthur & Throne, 2003).

5.4 Repellency of Methanol extract of *Eucalyptus* leaf against *S. zeamais*

The efficacy of *Eucalyptus sp* leaf extract resulted in repellence of *S. zeamais* at treatment concentrations of 0.2%, 0.4%, 0.8% and 1.2%. Similar to a report from Mishra *et al.* (2012), *Eucalyptus globulus* at lower and higher concentrations showed significant repellent action against *Tribolium casteneum* and *Sitophilus oryzae*. Each treatment concentration responded with different rates in repellence at various exposure times and temperatures. The present study showed that the repellence effects of *Eucalyptus* sp leaf extract against maize weevil were neither significantly influenced by time or temperature but by treatment rates. This is confirmed by findings of Ouko *et al,* (2017) which showed that, among all tested concentrations of *Ocimum basilicum* extract against *S. zeamais,* the repellent activities showed no significant differences at the 1st to 5th hour after exposure. The highest concentration 1.2% of the *Eucalyptus* leaf extract, however gave the highest repellency (100%) compared to lower concentration of 0.2% extract with 48.89% repellence. The repellent activity of *Eucalyptus globulus* and *O. basilicum* progressively increased with increase in concentration against both stored-grain insect pests. Results on the effect of essential oil of *Ocimum grattissimum* leaves on repellency and toxicity also indicated a moderately repellency to *S. zeamais* at high concentrations (Aswalam *et al.*, 2008).

5.5 Percentage weight loss of maize

The results of the study showed that the efficacy of *Eucalyptus* leaf extract decreased with time and grain weight loss varied with treatment concentration. In confirmation to findings by Musundire *et al*, (2015), the efficacy of *Eucalyptus grandis* and *Tagetes minuta* against maize weevil decreased as storage duration extended from 96 days post treatment to 192 days. Parwada *et al*, (2018) also reported that efficacy of *Eucalyptus tereticonis* against *Sitophilus zeamais* decreases as storage duration increases. Similar studies by Simbarashe *et al.* (2013) showed that mortality of *S. zeamais* at highest application rate of *Eucalyptus tereticornis* extended to 70 days from which a decrease in mortality continued with extending storage period. The differences in grain weight loss were significantly lower with increasing concentration. At 1.2% of *Eucalyptus* leaf extract, percentage weight loss was 0.46% and 4.17% for the 3rd and 5th month respectively during storage at 31^{o}C. From 96 days post treatment with *Eucalyptus grandis*, minimal damage was recorded but, from the 128 days onward weight loss was slightly low at both 2.5 and 5 g/kg compared to untreated control as from 160 days post treatment, a significant higher weight loss was recorded (Musundire *et al.,* 2015). The results showed that *Eucalyptus* leaf extract significantly controlled stored maize against *S. zeamais* for 3 months but by the 5th month mortality of *S. zeamais* significantly decreased in confirmation with findings suggesting that botanicals need constant reapplications for them to offer continual protection of grains against maize weevils (Golob, 2000).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Eucalyptus sp leaf extract can be used as natural grain protectant as it significantly reduced damage caused by *S. zeamais* by either killing or repelling them from stored maize grains as shown by this study. These botanical can be used as an alternative to synthetic pesticides with no or little adverse effects on the human health. The effectiveness of the botanical was most observed at treatment concentration of 1.2% *Eucalyptus* leaf extract. *Eucalyptus* leaf extract was effective for a short storage period (2 months), and reapplying after every 2 months will preserve the quality and quantity of the maize for a longer storage period. At 27ºC *Eucalyptus* leaf extract was most effective against *S. zeamais* compared to 20ºC and 31ºC indicating that temperature and application rates of botanical insecticides and storage duration are factors to consider in stored product environments.

The methanol and diethyl ether extract of *Eucalyptus* sp leaf extracts showed no significant difference on contact toxicity against *S. zeamais.* To this effect, methanol was used as the extraction solvent of *Eucalyptus* sp leaf for this project work. This was because methanol is cheaper compared to diethyl ether and helped reduce cost of extraction of the plant active compounds.

6.2 Recommendations

a. The effect of temperature on contact toxicity of botanicals should be carried out as temperature have a contributory effect on efficacy of plant extracts against insect pests as indicated from current studies.

b. Further research should be done to determine the mode of action of *Eucalyptus* sp against stored product insect pests.
c. The isolates from Eucalyptus leaf should be tested for efficacy as well as side effects to humans.

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APPENDIX

APPENDIX 1: Analysis of variance on effects of Diethyl ether Eucalyptus crude leaf extract on S. zeamais mortality.

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
mortality * Temp_code	Between Groups	(Combined)	21359.259	2	10679.630	33.251	.000
	Within Groups		120442.063	375	321.179		
	Total		141801.323	377			

a. Methods_used = Diethyl ether

Analysis of variance on effects of Methanol Eucalyptus crude leaf extract on S. zeamais mortality.

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
mortality * Temp_code	Between Groups	(Combined)	16674.397	2	8337.198	25.811	.000
	Within Groups		121129.246	375	323.011		
	Total		137803.643	377			

a Methods_used = Methanol

Analysis of variance on temperature effects for Methanol and Diethyl ether Eucalyptus crude leaf extract on S. zeamais mortality.

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
mortality * Methods_used	Between Groups	(Combined)	14.286	1	14.286	.187	.666
	Within Groups		19128.571	250	76.514		
	Total		19142.857	251			

a. Temp_code = T20

ANOVA Tablea

			Sum of Squares	df	Mean Square	F	Sig.
mortality * Methods_used	Between Groups	(Combined)	331.433	1	331.433	.703	.403
	Within Groups		117943.532	250	471.774		
	Total		118274.964	251			

a. Temp_code = T27

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
mortality * Methods_used	Between Groups	(Combined)	381.349	1	381.349	.912	.340
	Within Groups		104499.206	250	417.997		
	Total		104880.556	251			

a. Temp_code = T31

Regression analysis on the effect of temperature, time and treatment concentration of Methanol and Diethyl ether Eucalyptus crude leaf extract on S. zeamais mortality.

Tests of Between-Subjects Effects

Dependent Variable: mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	176751.923[a]	14	12625.137	90.452	.000
Intercept	26439.556	1	26439.556	189.425	.000
Temp_code	37881.034	2	18940.517	135.698	.000
Treatconc_code	50045.245	5	10009.049	71.709	.000
Time	88251.198	6	14708.533	105.378	.000
Methods_used	574.446	1	574.446	4.116	.043
Error	103427.488	741	139.578		
Total	475801.000	756			
Corrected Total	280179.411	755			

a. R Squared = .631 (Adjusted R Squared = .624)

APPENDIX 2: Regression analysis on effects of Eucalyptus leaf extraxt on immature stages of *S. zeamais.*

Tests of Between-Subjects Effects

Dependent Variable: Eggs

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1173.833[a]	7	167.690	27.003	.000
Intercept	2053.500	1	2053.500	330.669	.000
Block_code	194.111	2	97.056	15.629	.000
Trt_con_code	979.722	5	195.944	31.552	.000
Error	285.667	46	6.210		
Total	3513.000	54			
Corrected Total	1459.500	53			

a. R Squared = .804 (Adjusted R Squared = .774)

Tests of Between-Subjects Effects

Dependent Variable: Larva

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	996.130[a]	7	142.304	37.295	.000
Intercept	1005.352	1	1005.352	263.483	.000
Treat_con_cod	813.870	5	162.774	42.660	.000
Block_code	182.259	2	91.130	23.883	.000
Error	175.519	46	3.816		
Total	2177.000	54			
Corrected Total	1171.648	53			

a. R Squared = .850 (Adjusted R Squared = .827)

Tests of Between-Subjects Effects

Dependent Variable: Pupa

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1041.074[a]	7	148.725	19.475	.000
Intercept	2115.630	1	2115.630	277.028	.000
Treat_con_cod	914.815	5	182.963	23.958	.000
Block_code	126.259	2	63.130	8.266	.001
Error	351.296	46	7.637		
Total	3508.000	54			
Corrected Total	1392.370	53			

a. R Squared = .748 (Adjusted R Squared = .709)

APPENDIX 3: Persistence of Eucalyptus leaf extract against S. zeamais in maize.

Analysis of variance on treatment concentrations of Eucalyptus leaf extract against *S. zeamais.*

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Mortality * Trt_conc_grps	Between Groups	(Combined)	513.650	5	102.730	37.151	.000
	Within Groups		1625.960	588	2.765		
	Total		2139.609	593			

Analysis of variance on the Persistence of Eucalyptus leaf extract against *S. zeamais* between 20⁰ C, 27⁰ C and 31⁰ C.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Mortality * Block_grp	Between Groups	(Combined)	123.902	2	61.951	18.164	.000
	Within Groups		2015.707	591	3.411		
	Total		2139.609	593			

Analysis of variance on the Persistence of Eucalyptus leaf extract against *S. zeamais* between the storage duration.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Mortality * Time_code	Between Groups	(Combined)	514.508	5	102.902	37.232	.000
	Within Groups		1625.102	588	2.764		
	Total		2139.609	593			

Regression analysis on persistent of Eucalyptus leaf extract at different concentrations during 5 months storage at temperatures of 20⁰ C, 27⁰ C and 31⁰ C.

Tests of Between-Subjects Effects

Dependent Variable: Mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1152.060[a]	12	96.005	56.482	.000
Intercept	1040.700	1	1040.700	612.270	.000
Trt_conc_grps	513.650	5	102.730	60.439	.000
Time_code	514.508	5	102.902	60.540	.000
Block_grp	123.902	2	61.951	36.447	.000
Error	987.550	581	1.700		
Total	3112.000	594			
Corrected Total	2139.609	593			

a. R Squared = .538 (Adjusted R Squared = .529)

APPENDIX 4: Analysis of variance on the repellent efficacy of Methanol extract of Eucalyptus leaf extract on *S. zeamais.*

Analysis of variance on the effect of temperature on the repellent efficacy of Eucalyptus leaf extract.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Repllence * Temp_code	Between Groups	(Combined)	822.222	2	411.111	.815	.445
	Within Groups		71166.667	141	504.728		
	Total		71988.889	143			

Analysis of Variance on repellent efficacy of Eucalyptus leaf extract against *S. zeamais* at different contrations at 20^{0} C, 27^{0} C and 31^{0} C.

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
Repllence * Treatment_cod	Between Groups	(Combined)	15500.000	3	5166.667	21.447	.000
	Within Groups		10600.000	44	240.909		
	Total		26100.000	47			

a. Temp_code = T20

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
Repllence * Treatment_cod	Between Groups	(Combined)	14000.000	3	4666.667	19.744	.000
	Within Groups		10400.000	44	236.364		
	Total		24400.000	47			

a. Temp_code = T27

ANOVA Table[a]

			Sum of Squares	df	Mean Square	F	Sig.
Repllence * Treatment_cod	Between Groups	(Combined)	8600.000	3	2866.667	10.453	.000
	Within Groups		12066.667	44	274.242		
	Total		20666.667	47			

a. Temp_code = T31

Regression analysis on the repellent effect Eucalyptus leaf extract on S. zeamais with different concentrations and at 20⁰ C, 27⁰ C and 31⁰ C.

Tests of Between-Subjects Effects

Dependent Variable: Repllence

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38088.889[a]	8	4761.111	18.960	.000
Intercept	816011.111	1	816011.111	3249.602	.000
Temp_code	822.222	2	411.111	1.637	.198
Treatment_cod	36055.556	3	12018.519	47.861	.000
Time_code	1211.111	3	403.704	1.608	.191
Error	33900.000	135	251.111		
Total	888000.000	144			
Corrected Total	71988.889	143			

a. R Squared = .529 (Adjusted R Squared = .501)

APPENDIX 5: Analysis of variance on percentage weight loss of infested maize with *S. zeamais* after treatment with Eucalyptus leaf extract between 3rd and 5th months in storage.

Analysis of variance on percentage weight loss at different temperatures.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
weight * Temp_code	Between Groups	(Combined)	144.205	2	72.103	.732	.483
	Within Groups		10341.302	105	98.489		
	Total		10485.507	107			

Analysis of variance on percentage weight loss with different treatment concentration.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
weight * Treat_code	Between Groups	(Combined)	4154.273	5	830.855	13.386	.000
	Within Groups		6331.233	102	62.071		
	Total		10485.507	107			

Regression analysis on percentage weight loss of maize infested with *S. zeamais* after treatment with Eucalyptus leaf extract between 3rd and 5th months in storage at different temperatures.

Tests of Between-Subjects Effects

Dependent Variable: weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6455.887[a]	8	806.986	19.826	.000
Intercept	4337.945	1	4337.945	106.575	.000
Temp_code	144.205	2	72.103	1.771	.175
Treat_code	4154.273	5	830.855	20.413	.000
Time	2157.408	1	2157.408	53.003	.000
Error	4029.620	99	40.703		
Total	14823.452	108			
Corrected Total	10485.507	107			

a. R Squared = .616 (Adjusted R Squared = .585)