KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

BIOACTIVITIES OF *Hyptissuaveolens* AND *Tephrosiavogelii* LEAVES EXTRACTS ON *Prostephanustruncatus*

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M.Sc. THESIS SUBMITTED

BY

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DECLARATION

I hereby declare that this thesis titled (**Bioactivities of *Hyptissuaveolens* and *Tephrosiavogelii* Leaves Extracts on *Prostephanustruncatus***)is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

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ABUBAKAR ABDULLAHIADEOYE **Signature /** Date

APPROVAL PAGE

This is to certify that this thesis by (**AbubakarAbdullahiAdeoye**) has been read and approved as meeting the requirements of the Department ofBiosciences and Biotechnologyfor the award of the degree of Masters (M.Sc.) in Zoology

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Dean, School of Postgraduate Studies (SPGS)	Signature / Date

DEDICATION

I dedicate this work to the Almighty Allah who gave me the opportunity of living till this moment.

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ABSTRACT

The study was carried out to evaluate the bioactivities of *Hyptissuaveolens* and *Tephrosiavogelii*leaves extracts (ethanol and distilled water as solvent) in the control of one of the insect pests of stored maize (*Zea mays*), the larger grain borer LGB (*Prostephanustruncatus*). Parameters studied include; contact toxicity by topical application, repellency, mortality, feeding deterrence (weight loss and seed damage)and F1 emergence. The ethanolic and aqueous extractsof *Hyptissuaveolens* and *Tephrosiavogelii* leaves were applied at the rates of (0.05, 0.1, 0.2, 0.3 and 0.5ml/10g of maize grains) at dosage level and (2.5, 5.0, 7.5, 10.0, and 15.0%) at concentration level and were replicated 3 and 5 times respectively in an experiment laid out in a Complete Randomized Design (CRD). Results obtained showed that all the treatments caused significant ($P<0.05$) toxicity, mortality, deterrence and repellency in adult beetles compared to the untreated control. Qualitative phytochemical screening was carried out and several phytochemicals were present in the extracts such as flavonoids, alkaloids, phenols, glycosides among others, leading to high potency of the extracts. Higher dosages had more effects on the mortality, toxicity and repellency of beetles and also had less weight loss and seed damage while lower dosages had less effects on mortality, toxicity and repellency of beetles and more weight loss and seed damage.Ethanolic extract of *T. vogelii*was observed to cause toxicity leading to 52% mortality while *H. suaveolens*caused 42%. However, the ethanolic extract of *T. vogelii*are the most toxic and produced the best effect with LC_{50} of 11.12% at 7 daysafter exposure and LD_{50} of 0.33ml/10g of maize grains at 7 daysafter exposure. Also, it is shown in the experiment that ethanolic extracts of the plants used are more potent than the aqueous extracts. *T. vogelii*ethanolic extract was more repellent (mean PR value = 70.50%) than *H. suaveolens*ethanolic extract(mean PR value = 59.50%).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction to *Prostephanus truncatus*

The larger grain borer (LGB), *Prostephanus truncatus* (Horn) is one of the major storage pests of maize in Africa. It is now a common pest of stored products in many African countries since its discovery in the late 1970s from the Americas into Tanzania. In West Africa, it was discovered first in Togo in 1984 before it spreads to other neighboring countries like Benin, Nigeria, Ghana, Burkina Faso and Niger (IASPMR, 2019). The host spectrum of this beetle isquite unclear since there are several contradictory reports onthis issue (Muatinte *et al*., 2019).The infested maize and cassava suffer serious losses by the feeding activity of *P. truncatus*(Kavallieratos *et al*., 2017). A very small initial population of *P. truncatus* at the beginning of the storage season was sufficient to cause significant damage by the end (Tefera *et al*., 2011). It has earlier been reported in Tanzania that dry weight losses of maize had increased in excess of 30% over a short storage season after the accidental introduction of *P. truncatus* (Nboyine *et al*., 2015). *P. truncatus* has become a serious pest of stored maize and dry cassava; thereby reducing the storage period of these commodities in granaries of small-scale farmers. The reduction gotten from larval and adult feeding, with consequent shortening of the period these commodities are available for food and income generating sources.

There are different strategies for management of *P. truncatus* in storage. In the past few decades the application of synthetic pesticides to control pests of durable stored food products has been the standard practice.In some countries, a common control measure for infestation of *P. truncatus* is to treat grain with a dust formulation containing a mixture of an organophosphate

(OP) and a pyrethroid insecticide. However, *P. truncatus* is tolerant to OPs and is likely to be resistant to pyrethroids (Nboyine *et al.*, 2015). As seen with evidence that the use of synthetic insecticides poses health hazards to animals, environmental pollution, resistance development by insects and pest resurgence, requirements for effective, affordable and eco-friendly control methods have become important (Rajendran and Sriranjini, 2008). Botanical pesticides, which possess different active constituents and modes of action are target-specific, relatively safe, affordable and readily available. Hence, the readily available botanical pesticide technology for pest management in smallholder agriculture is a viable alternative option. Athanassiou *et al*. (2007) indicated the efficacy of diatomaceous earth formulations, such as a mixture of crystalline silica and abamectin for managing *P. truncatus*.There are insecticidal activities of several plant parts such as essential oils, powders and other extracts which were evaluated against several insect pests of cereals and legumes and found to have contact toxicity, repellence, fumigant toxicity, anti-feedant effects (Chebet *et al*., 2013).

The main causes of stored maize crop losses today are insect pest species belonging to various families including, Curculionidae, Bostrichidae, Gelechiidae, Pyralidae, Tenebrionidae. The most common-pests species are *Sitophilus* spp*., Sitotroga cerealella, Tribolium* sppand *P. truncatus,* causing devastating damage to stored maize (Rugumamu, 2005). However, the larger grain borer, *P. truncatus*, has received little attention in the area of rationalized use of botanical pesticides, especially of the use of leaves powders and extracts of *Hyptis suaveolens* and *Tephrosia vogelii* using water and ethanol for the stored product insect control.

The purpose of the present study is to evaluate the repellent, feeding and oviposition deterrence, emergence inhibition and contact toxicity effects of *H.suaveolens* and *T. vogelii* –

using extractants; water (aqueous) and ethanol on *P. truncatus* with the hope of finding an effective, affordable and environmentally safe product for use by subsistence farmers.

1.2 Statement of theProblem

The pest status of the Larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae), is higher in African countries. This pest has been reported to cause reduction in the storage period of maize grain and cassava chips in granaries of small-scale farmers. This reduced storage period results from larval and adult feeding causing low income generating sources to farmers.Findings from previous studies on LGB reported a yield loss of up to 45 and 100% in maize and cassava chips, when no pest control measures were adopted owing to the high cost of synthetic insecticides. The use of synthetic insecticides has been associated with rising cases of resistant pests, destruction of natural enemies, turning formerly innocuous species into pests and toxicity to non-target organisms including man. These had necessitated the need for evaluation of locally available botanicals pesticides which are effective, eco-friendly and affordable. It is observed that raw leaves of *H. suaveolens* and *T. vogelii* from literature have insecticidal activities, thus the extracts also needs study to evaluate the efficacy. This study seeks to evaluate the efficacy of the two leaves extract in the control of LGB.

1.3 Aim of the Study

The aim of this study is to evaluate the bioactivity of *Hyptis suaveolens* and *Tephrosia vogelii* leaves extract as protectant against larger grain borer, *Prostephanus truncatus* on maize.

1.4Objectives of the Study

The objectives of this study are to;

i.evaluate the phytochemical constituents of *Hyptis suaveolens* and *Tephrosia vogelii*

ii.evaluate the toxicity of *H. suaveolens* and *T. vogelii* leaves extracts against the larger grain borer, *P. truncatus*,

iii.evaluate repellent ability of *H. suaveolens* and *T. vogelii* leaves extracts against the larger grain borer, *P. truncatus*,

iv.assess the damage level that leaves of *H. suaveolens* and *T. vogelii*extracts will prevent against the larger grain borer*, P. truncatus* on maize.

v. assess the effects of*H. suaveolens* and *T. vogelii* leaves extracts on emergence of F1 progeny of *P. truncatus* on maize.

1.5 Justification

InternationalInstituteforTropicalAgriculture(IITA) reported in 2012 that Nigeria is currently the tenth largest producer of maize in the world and the largest maize producer in Africa. Lyon. 2000 reported that maize is the third most grown cereal after wheat and rice. Worldwide, about 25% of all maize is used for human consumption, 66% for feeding livestock, and 9% for industrial purposes. In the developing world, about 50% of all maize is consumed by humans as food while 43% is fed to livestock and the remaining 7% is for industrial purposes (IITA, 2003). The heavy post-harvest losses and quality deterioration caused by storage pests are a major problem facing agriculture in developing countries such as Nigeria (Adedire and Ajayi, 1996).It was reported by Issa *et al*., (2011)that maize is exposed to insect pest attack prior to harvest and in storage. Application of contact insecticides to stored grain is an effective method of control storage insects. Synthetic insecticides have side effects such as resistance to insecticides, toxic residues in food and environmental pollution, adverse effects on beneficial and non-target insects,

increased risk to worker's safety and the high cost of the chemicals. Botanical products have played important roles in traditional storage pest control in the tropics (Belmain *et al.*, 2001). Most of them are non-toxic to consumers and are readily available. This leads to carrying out further researches on leaves extracts as extracts are known to contain more phytochemicals compared to raw botanicals, so *Hyptis suaveolens* and *Tephrosia vogelii* leaves was chosen to carry out the said research as they are both readily available in our environment and were used in controlling other insects types in literatures read before the commencement of this research aside that of controlling *P. truncatus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Prostephanus truncatus*(Larger Grain Borer)

P. truncatus is indigenous in Central America, tropical South America, and the extreme south of the USA as a major, but localized, pest of farm-stored maize. It was introduced into Tanzania, probably in the late 1970s, and has become a serious pest of stored maize and dried cassava in that part of East Africa; it has since spread into other countries in the region. It was first found in West Africa in Togo in 1984 and it has since spread to other West African region. *P. truncatus* known to be a pest of maize and dried cassava roots after harvest in sub-Saharan Africa and also from time to time in Central America (IASPMR, 2019).

Infestations in maize may start on the mature crop in the field, i.e. when moisture content is at or below 18%. Weight losses of up to 40% have been recorded in Nicaragua from maize cobs stored on the farm for 6 months. It was reported in Tanzania that up to 34% losses have been observed after 3 months storage on the farm, with an average loss of 8.7%. *P. truncatus* is a much more damaging pest when compared to other storage insects like *S. zeamais*, under similar conditions; maize losses due to these other species were 2-6, 3-5 and 2-5%, during a storage season in Zambia, Kenya and Malawi, respectively.Losses caused by *P. truncatus* in dried cassava roots can be very high; the dried roots are being reduced to dust by the boring adults and a loss of 70% has been recorded after 4 months in farm storage. It was stated that a group of 25 farmers from five villages in Togo sustained average cumulative losses of 9.7% after 3 months storage, this figure rose to 19.5% after 7 months (IASPMR, 2019).

Not all problems with this pest are restricted to farmers' granaries. In the early days after the arrival of *P. truncatus* in East Africa, countries with the pest found their maize exports banned.

A grain injury model for *P. truncatus* infesting farm-stored maize in West Africa has been developed at the International Institute of Tropical Agriculture. It can be used in conjunction with predictive models of pest population dynamics to guide the development of integrated pest management strategies (Holst *et al*., 2000). Boxall (2002) in his review of *P. truncatus* considered loss of value, nutrition as well as impact at the national level, effect on international trade and modern methods of rapid loss assessment. In the early days after the arrival of *P. truncatus* in East Africa, countries with the pest found their maize exports banned. For example, in 1987-1988, it is estimated that Tanzania lost US $634,000 in export earnings. This situation improved following efforts to upgrade phytosanitary procedures in the region but such procedures, involving fumigation, have their own continuing costs.

2.2 Biology of *P. truncatus*

Maize grain and dried cassava are attracted to *P. truncatus* over short distances. Field studies in both Mexico and Togo suggest that there is no long-range attraction of adult *P. truncatus* to maize grain or cobs, or dried cassava; there is no surprise because wood is the major host of this beetle. Birkinshaw *et al*., 2002 in field studies provided strong evidence that host selection of *P. trunctus* in the case of maize and cassava, occurred by chance (IASPMR, 2019).

Plate1:Image of adult *P.truncatus*

Adults frequently start their attack on stored maize cobs with intact sheaths by boring into the base of the maize cob cores, although they eventually bore into the grain through the tip of the cob by crawling between the sheathing leaves. Adults bore into the maize ‘grains, making neat round holes, they tunnel from grain to grain thereby generating large quantities of maize dust. Adult females lay eggs in chambers bored at right angles to the main tunnels. Egg-laying on stabilized maize grain, like that on the maize cob, is more productive than on loose-shelled grain as the oviposition period is longer, equal in length to the life of the female, and’ the eggs are laid at a greater rate.Larvae hatch from the eggs after three (3) days at 27°C. The larvae are whitish, fleshy and are sparsely covered with hairs. They are parallel-sided, i.e. they do not taper. The legs are short and the head capsule is small relative to the size of the body and they thrive on the dust produced by boring adults. Most of the larvae develop and pupate in dust at the base of dense of the containers in laboratory cultures. The adult then emerges.

The adult is typically cylindrical with bostrichid shape. The declivity is flattened and steep and has many small tubercles over its surface. The limits of the declivity, apically and laterally, are marked by a carina. The antennae have 10 segments with a loose three-segmented club; the 'stem' of the antenna is slender and clothed with long hairs and the apical club segment is as wide as, or wider than, the preceding segments. The body is 3-4.5 mm long.The life cycle of *P. truncatus* has been investigated at a range of temperatures and humidity. Development of the larva through to the adult stage at the optimum conditions of 32°C and 80% RH takes 27 days on a diet of maize grain. Humidity within the range 50-80% RH does not greatly affect the development period or mortality; at 32°C, a drop in RH from 80 to 50% (giving maize with an equilibrium moisture content of about 10.5%) extended the mean development period by just 6 days and increased the mean mortality by only 13.3%. This tolerance of dry conditions was

confirmed during field studies in Nicaragua and Tanzania in which maize at 10.6 and 9% moisture content, respectively, was heavily infested. The success of this pest may be partly due to its ability to develop in grain at low moisture. Many other storage pests are unable to increase in number under low moisture conditions. For example, *Sitophilus oryzae*, a species occurring in the same ecological niche, needs a grain moisture content of at least 10.5% for development. Thus, in dry conditions, *P. truncatus* probably benefits from the absence of any significant competition from other storage pests. *P. truncatus* develops more rapidly on maize grain than on cassava; at 27°C and 70% Relative Humidity, the respective development periods on maize grain and cassava were 32.5 and 40 days, respectively. Under ideal conditions of temperature and humidity on maize cobs or stabilized maize grain, estimates for the intrinsic rate of increase (r) of *P. truncatus* are in the order of 0.7-0.8 per week; this is similar to the rate of increase reported for *Tribolium castaneum* under comparable climatic conditions (IASPMR, 2019).

2.3 Effects of Botanicals on Larger Grain Borers

A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards involved (Khorrami and Soleymanzade, 2016). The insecticidal activities of several plant parts powders, essential oils and other extracts have been evaluated against several insect pests of stored products and are found to have properties such as the contact toxicity (Asawalam *et al*. 2006; Ogendo *et al*., 2008), repellence (Kéita *et al*., 2001; Rosman *et al*., 2007), fumigant toxicity (Lee *et al.,* 2003; Rajendran and Muriladharan, 2005), anti-feedant (Ogemah, 2003).

Plate2:Damagescausedby *P. truncatus*

Source: Culture set-upintheexperiment.

Ogendo *et al*., (2008) reported that essential oils extracted from aerial parts of *Ocimum americanum*, *Lantana camara* and *Tephrosia vogelli* and monoterpene constituent, eugenol, had concentration, exposure time, species (plant and insect) and plant part-dependent instant and residual repellent potency against adult *Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae*, and *Callosobruchus chinensis*. Chebet *et al*. (2013) reported that crude powders of *Azadirachta indica, Lantana camara,* and *Tephrosia vogelii* have strong repellent effects and help in reductions in grain damage and F1 progeny of *P. truncatus*. Crude powdered leaves of *Clausena anisata* and *Plectranthus glandulosus* evaluated against Cameroonian and German strains of *S. zeamais* and *P. truncatus* possess insecticidal activities and effects on progeny production (Nukenine *et al*., 2010). It was reported in Mukui, 2013 that some plants have been found to contain triterpenoids, iridoid glycosides, some of which may be responsible for the observed insecticidal properties. Mukanga *et al,*(2010) also reported that the leaf extracts of Neem, Tephrosia, Water hyacinth and Guava exhibit repellency, anti-feeding, reproduction inhibition effects, vapour and contact toxicity on *P. truncatus*.

2.4 Pest Control

2.4.1 Chemical Control

The most active method of controlling *P. truncatus* in maize is to admix a dilute dust insecticide. *P. truncatus* is highly susceptible to synthetic pyrethroid insecticides such as permethrin and deltamethrin. However, these insecticides are relatively ineffective against other storage pests such as *Sitophilus* spp*., Callosobruchus maculatus, Callosobruchus chinensis* and *Tribolium castaneum*, which occur in the same pest complex and are more susceptible to organophosphorus insecticides. Combinations such as pirimiphos-methyl and permethrin, deltamethrin and pirimiphos-methyl or fenitrothion and fenvalerate have been used successfully

to protect farm-stored grain. Fumigation with phosphine and phostoxin is very effective in large-scale stores.

Recent laboratory and field studies have shown that unless inert dusts are applied at very high rates, they are not particularly effective against *P. truncatus*. However, good control can be achieved when they are mixed with insecticides or soil bacteria metabolites such as Spindeba (Stathers, 2003). However, this method of using synthetic fumigants is harmful because it has also led to increased cost of application, pest resistance, lethal effects on non-target organisms and toxicity to users (Okonkwo and Okoye, 1996).

2.4.2 Botanical Pest Control

With the increased concern about the use of synthetic insecticides, the urge to find suitable alternatives that are readily available, affordable, less poisonous and less detrimental to the environment was evidentas reported by Mukanga *et al,*(2010). Plants in general are able to produce secondary metabolites that are physiologically active in insects and other organisms and that provide the plants with one of the most important defense mechanisms (Strauss and Zangerl, 2002). Although botanical insecticides comprise only a very small portion of the total volume of insecticides used annually, they remain an important component in insect pest management owing to their realized efficacy against insect pests of stored products that have resistance to synthetic insecticides (Weinzierl, 2000). Plant-derived insecticides possess short-lives i.e they can degenerate quickly in the environment, thus pose little risk to non-target organisms. (Isman, 2000; Weinzierl, 2000).

In Nigeria for example, botanical insecticides have been reported and extracted from various plants including Neem (*Azadiracta indica*), Pyrethrum (*Chrysanthemum*

cinararіaefoliun), Tobacco (*Nicotiana tabacum*), Derris (*Derris elliptica*), Pawpaw (*Carica papaya*), Tomato (*Lycopersicon esculentum*), Cashew nut (*Anarcardium occidentale*), Garlic (*Allium sativum*), Aligator pepper (*Aframomum melegueta*), Bushmint leaves (*Hyptis sauvolens*), Fish poison leaves (*Tephrosia vogelii*), Onions (*Allium cepa*), Basil (*Ocimum basilicum*), Bitter gourd (*Momordica charatia*), Ginger (*Zinigiber officinale*), Bitter leaf (*Vernonia amygdalina*), Siam weed (*Chromolaena odorata*), pepper fruit (*Uvaria afzelli*) and many more. Their biological properties have been tested and found to include insecticidal and repellent effects against insect pests. Some have also been found to have antifeedant, growth regulatory, oviposition inhibitory, sterility inducing, antifungal and nematicidal properties.Phytochemicals such as rotenone, nicotine and pyrethrum were all used as pesticides before the advent of synthetic insecticides (Odeyemi *et al*., 2008). Many members of families such asLamiaceae, Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae are known to have various chemical compounds which act as toxicants, antifeedants, repellents or growth inhibitors to many insect species (Odeyemi *et al*., 2008; Ogendo *et al*., 2008).

2.4.3 Biological Control

Initial releases of *T. nigrescens* were in Togo in 1991 and in Kenya in 1992. In both countries it became well established and spread. Subsequently, there have been predator releases in Benin, Ghana, Tanzania, Malawi and Nigeria. Only in the case of Tanzania does it appear that there has been any difficulty in the predator becoming quickly and easily established. However, despite the successful introductions, there are still regular outbreaks of *P. truncatus* and farmers still suffer losses. Nboyine *et al*. (2015) assessed the compatibility of two bio-control agents *Teretrius nigrescens* and *Beauveria bassiana* in the control of *P. truncatus* and found out that the two could be used in the control of the pest. It has been concluded by Holst *et al*. (2000) that *T.*

nigrescens does not offer a good example of classical biological control but as the predator is able to reduce the density of the pest it is considered that it has, nevertheless, a role to play in integrated pest management.

2.4.4 Cultural Control

Good hygiene during storage, mostly by the removal of infested residues and the usage of only good and clean material for storage, can play an important role in reducing infestation by *P. truncatus*. The use of resistant cultivars may also reduce the severity of an infestation, although much work remains to be done on the mechanisms of resistance.

2.5 Extracts of *Hyptis suaveolens*.

Hyptis suaveolens (L) Poit commonly called 'Bush mint plant' belongs to the family Lamiaceae. The plant has been measured as an obnoxious weed, distributed throughout the tropics and subtropics. The whole plant is used in traditional medicine to treat various diseases. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases. Crude leaf extract is also used as a relief to colic and stomach ache. Leaves and twigs are considered to be anti-spasmodic and used in anti-rheumatic and anti-suporific baths, anti-cancer, anti-inflammatory, anti-fertility agents (Kirtikar and Basu, 1991) and also applied as an anti-septic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi, 1997). Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. *H. suaveolens* is used traditionally for the treatment of respiratory tract infections, colds, pain fever, cramps and skin diseases (Iwu, 1993). *H. suaveolens* is used for some ethnobotanicals

applications in rural communities in African countries (Edeoga *et al*., 2006). The phytoconstituents isolated from the plant are hyptadienic acid, suaveolic acid, suaveolol, methyl suaveolate, Beta sitisterol, Oleanolic acid,ursolic acid, rosamarinic acid, dehydroabietionol, 3 Beta hydroxyl lup 12-en-28oic acid, 3 betat hydroxyl lup-20(29)-en-29-oic acid and essential oil. Essential oils isolated from aerial parts of this plant have showed antifungal (Mandal *et al*., 2007; Tonzibo *et al*. 2009), antibacterial (Shenoy *et al*., 2009; Sathish *et al*., 2010) activities.Adda *et al*., (2011) reported that *H. suaveolens* extracts has effect on the population density of pink stalk borer, *Sesamia calamistis*. Conti *et al*. (2012) reported that the essential oil (EO) extracted from fresh leaves of *H. suaveolens* (L), and its main constituents were evaluated for larvicidal and repellent activity against the Asian tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae)

2.6 Extracts of *Tephrosia vogelii* Hook.

Fish poison bean plant, *Tephrosia vogelii* Hook (Fabaceae), is a semi perennial, shrubby plant indigenous to Africa and grows best on depleted light soils where it is a valuable fallow improvement species particularly for the control of *Striga hermonthecaa*s reported by Mukui, (2013). Tephrosia species contain complex mixtures of rotenoids and other flavonoids (Go´mez-Garibay *et al*., 2002). *T. vogelii* only contain compounds such as rotenone, tephrosin, and deguelin which can be economically and commercially exploited as a phytochemical in the pesticide industry as reported by Mukui, (2013). Tephrosine is the poisonous principal and can be gotten from dried crushed leaves which are used as insecticide against lice, fleas and ticks, and as a molluscicide (Orwa *et al*., 2009). The toxic principle compound in the plant is the presence of rotenoids known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insect and mammals. These compounds block the enzymes glutamate and succino dehydrogenase and thus H+transport (Neuwinger, 2004). Anti-

feeding effects of Tephrosia have also been reported on spotted cereal stem borer *Chilo partellus.* There were significant (P = 0.05) increases in grain yield in the sprayed plots and a concomitant improvement in grain quality (Kyamanywa *et al.,* 2001).

2.7 Maize

Maize (*Zea mays* L.) belongs to the family Gramineae and is one of the most important cereal crops in Africa. After wheat and rice, maize is the third most grown cereal (Lyon, 2000).

In sub-Sahara Africa, it is a staple crop for an estimated 50% of the population (IITA, 2003). It is a cereal crop that grows across a range of agro-ecological zones in Nigeria, though it is grown slightly more in the Northern part of the country. Two types of maize are grown in Nigeria, the yellow and white variety and due to their rate of adaptability. The grain is very nutritious, with about 70-72% digestible carbohydrate, 4 - 4.5% fats and oils and 9.5-11% proteins (Larger and Hill, 1991). Worldwide, about 66% of all maize is used for feeding livestock, 25% for human consumption and 9% for industrial purposes. In the developing world, about 50% of all maize is consumed by humans as food while 43% is fed to livestock and the remainder for industrial purposes (IITA, 2003). Almost every part of the maize plant is utilized. While in developed countries, the bulk of maize produced is used as livestock feed and as a raw material for industrial processing in developing countries such as Nigeria, Ghana, it is mainly used for human consumption (Aquino *et al*., 2001). Of the total maize produced in Nigeria between the period 1995 to 1997, 75% and 6% were used for human and livestock consumption respectively (Aquino *et al*., 2001).

2.8 Harmful Effects of Synthetic Insecticides

Insect pest control in stored food products relies heavily on the use of gaseous fumigants and residual-contact insecticides. The implications of these are serious problems of toxic residues, health and environmental hazards, development of insect strains resistant to insecticides, increased cost of application and erratic supply in developing countries due to foreign exchange constraints (Obeng-ofori *et al*., 1997).The current methods for managing stored grain pests depend heavily on synthetic pesticides. However repeated use of certain chemicals in packing houses has led to the appearance of fungicide resistant populations of storage pathogens. In recent years there has been considerable pressure by consumers to reduce or eliminate chemical fungicides in foods. The use of synthetic chemicals to control post-harvest bio-deterioration has been restricted due to their carcinogenicity, high and acute residual toxicity, environmental pollution and their adverse effects on food and side effects on humans (Dubey *et al*., 2007; Kumar *et al*., 2007). The use of synthetic chemicals as anti microbials for the management of plant pathogens has undoubtedly increased crop protection but with some deterioration of environmental quality and human health (Cutler and Cutler, 1991). Their uninterrupted and indiscriminate use has not only led to the development of resistant strains and accumulation of toxic residues on food grains used for human consumption has also led to serious health problems (Sharma and Meshram, 2006).

Another method is the use of synthetic fumigants, which has also led to increased cost of application, pest resistance, lethal effects on non-target organisms and toxicity to users (Okonkwo and Okoye, 1996). There are three types of harmful effects of pesticides: acute effects, delayed effects, and allergic effects. Acute effects are injuries or illnesses that appear immediately after exposure. The effects are usually obvious and reversible if appropriate medical

care is given right away. Delayed effects are illnesses or injuries that do not appear immediately, such as cancer. Pesticides have been known to cause lymphoma, leukemia, breast cancer, asthma, and other immune system disorders. Food safety is receiving increased attention worldwide as the important links between food and health are increasingly recognized. Improving food safety is an important element of improving food security which exists when population have access to sufficient and healthy food. The increasing concern over the level of pesticide residues in food has encouraged researchers to look for alternatives to synthetic pesticides. In the context of agricultural pest management, botanical pesticides play a great role in the post-harvest protection of food products in developing countries. (Dubey *et al*., 2008). This has led to the use of botanicals as grain protectants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the Zoology Unit Laboratory, Department of Bioscience and Biotechnology, Kwara State University, Malete (Longitude 4° 28′02″ E; Latitude 8° 42′58″ N), Kwara State, Nigeria.

3.2 Insect Culture

Two hundred and fifty unsexed adults *Prostephanus truncatus* sourced at Nigeria Stored Products Research Institute were introduced into glass jars with mesh lid containing 1 kilogram of maize grain samples and were kept at 25–30°C and 65–70% R.H. and 12:12 h (light: darkness) in the laboratory. The insects were left on the grains to feed and lay eggs for 14 days and thereafter removed. The jars were left undisturbed until emergent of new progenies. The emergent were used as source of insect for the subsequent bioassay in this study.

3.3 Maize GrainsCollection and Preparation

Maize variety DMRESR purchased from Premier Seed Company in Ilorin, Kwara state, Nigeria, was used in this study. The grains were checked to ensure that they were not infested by visual observation for presence of eggs or any suspicious material. The grains were cold-shocked at 15°C for 14 days to kill all incipient infestations on the grains.

Plate 3: *Hyptis suaveolens* and *Tephrosia vogelii* used in the experiment.

Source: Field collection sites at the premises of Kwara State University, Malete, Nigeria.

3.4 Collection and Preparation of Plant Materials

Hyptis suaveolens and *Tephrosia vogelii* leaves identified by aBotanist in the Department of Botany, University of Ilorin, Nigeria. The two plant materials were collected within the premises of Kwara State University, Malete, Nigeria. They were rinsed with clean water and air dried separately under shade at room temperature 28± 2 °C for 2 weeks. The two dried leaves were ground into powder using universal grinder and kept in an air tight polyethylene bag. The powder was used for extraction and the bioassays.

3.5 Preparation of Plant Extracts

Two solvents used for the extraction were ethanol and water. Two hundred gram (200g) of each leaves powder was soaked in 400 ml of ethanol stored under dark conditions at room temperature for 24 hours with periodic stirring. The solution was then filtered using muslin cloth, then filtered twice using Whatman No.1 filter paper and non-absorbent cotton wool. Ethanolic extracts in the filtrate were concentrated in a vacuum using a rotary evaporator at a temperature of 40°C at 120 tr/min. The aforementioned procedure was done for aqueous extracts of the botanicals except that the extracts were concentrated by freeze drying using the vacuum freeze dryer and distilled water was used for the extraction process. The extracts were stored at 4°C until needed for the bioassays.

3.6 Preparation of Different Concentrations of Extracts

Five different concentrations, namely, 2.5%, 5 %, 7.5 %, 10 % and 15 % of each ethanolic and aqueous extracts of *Hyptis* and *Tephrosia* were prepared by dissolving the stock solution in the acetone. The 2.5%, 5%, 7.5 %, 10 % and 15% concentration of each plant extracts was prepared by diluting 25ml of the extract into 975ml of acetone; 50ml of the extract into

950ml of acetone; 75 ml of the extract into 925 ml of acetone; 100 ml of the extract into 900ml of acetone and 150ml of the extract into 850ml of acetone respectively. The prepared concentrationsof the extracts were used in the bioassay.

3.7 Qualitative Phytochemical Screening of Plant Samples

Phytochemical screening was carried out qualitatively to identify the presence of phytochemicals which includephenols,tannins, saponins, triterpenes, flavonoids, alkaloids and steroids in the extracts of *H. suaveolens* and *T. vogelii*. The activity was conducted following the procedures used by Ajiboye *et al.*, (2015).

3.7.1 Test for Alkaloids

Dragendroff's reagent test was conducted for detection of alkaloids:0.5ml of the extracts each was dissolved in 5 ml of 1% HCl and the mixture was kept for 2 minutes in water bath. 1 ml of the filtrate was treated with dragendroff's reagent. Precipitation was taking as indication for presence of alkaloids(Ajiboye *et al.*, 2015).

3.7.2 Test for Saponin

Saponins are a diverse group of chemicals, which derive their name from their ability to form soap-like foams in aqueous solutions. Five milliltres of distilled water was added to 5ml of the extracts in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. (Ajiboye *et al.*, 2015).

3.7.3 Test for Flavonoids

The alkaline reagent test was used.Extracts were treated with few drops of NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids(Ajiboye *et al.*, 2015).

3.7.4 Test for Steroids

Two milliliters (2 ml) of acetic anhydride was added to 5 ml extracts of sample with 2 ml H_2S04. The colour changed from violet to blue indicating the presence of steroids(Ajiboye *et al.*, 2015).

3.7.5 Test for Tannins

One millilitre of water and 1-2 drops of ferric chloride solution were added in 5 ml of extracted solution. Blue colour was taking as indication for tannins(Ajiboye *et al.*, 2015).

3.7.6 Test for Terpenoids

Salkowski test was used to perform this test. Five milliltres of the extracts were mixed in 2 ml of chloroform, and concentrated H_2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show the presence of terpenoids(Ajiboye *et al.*, 2015).

3.7.7 Cardiac glycosides

Keller-Killani test was used5 ml the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated H_2SO4. A brown ring of the interface indicates the presence of deoxy sugar characteristic of cardenolides(Ajiboye *et al.*, 2015).

3.7.8 Test for Phenolic Compounds

A portion of the extract dissolved in 5 ml of distilled water and to this; 2 ml of a 1% solution of gelatin containing 10% sodium chloride was added. The appearance of white precipitates indicated the presence of phenolic compounds (gelatin test).

Plate 4: PreparedPlants Extracts

Source: Experimental set-upforplantsextraction.

3.8 Bioassays of *H. suaveolens* and *T. vogelli*extracts on *P. truncatus*

3.8.1 Contact Toxicity Assay by Topical Application

Bioassays were conducted to determine the toxicity of the extracts by topical application. Aliquot of 1 µL of each concentration of extract was applied topically on the dorsal surface of the thorax of each*Prostephanus* with the aid of micropipette, while the control received 1 µL of acetone. In order to carry out this bioassay, *Prostephanus* were put inside Petri dishes and kept in the refrigerator for three minutes so as to inactivate the beetles for easy conduct of topical application of the extracts. Each group of ten treated insect individuals was then transferred into 10cm petri dishes. This experiment was replicated four times and arranged in a completely randomized design (CRD). Insect mortality was recorded at 1, 2, 3, 7, 14 and 21 days after treatment. Abbott's formula (Abbott, 1925) was used to correct for mortality. Corrected mortality values were computed as:

$$Pt = [(Po - Pc) / (100 - Pc)] \times 100$$

Where, Pt =corrected mortality

Po = observed mortality

Pc = control mortality

3.8.2 Repellent Activity Bioassay

The repellency was tested according to modified method of Hamouda *et al*., (2014). Half filter paper discs (Whatman number 40, 9 cm diam.) were prepared and a volume of 100 µl of each plant extract concentration was applied separately to one-half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 100 µl of

different solvents - ethanol and distilled water, for ethanolic and aqueous extract respectively. Both the treated and the control halves were air dried at room temperature in the laboratory for 10 minutes. Each treated half disc was then attached lengthwise, edge to edge, to a control half disc with adhesive tape and placed in a petri dish (9 cm diameter). Twenty adult insects were released in the middle of each filter paper circle. Each concentration was replicated four times. Insects that settled on each half of the filter paper disc were counted after 2 hours. The average of the counts was converted to percentage repellency (PR) using the formula of McDonald *et al*. (1970) as follows:

$$PR = [(Nc - Nt) / (Nc + Nt)] \times 100$$

Where Nt = number of insect present on the treated half disc

Nc = Number of insects present on the untreated half disc

3.8.3 Efficacy of *H. suaveolens* and *T. vogelii* Extracts on Mortality of *P. truncatus*

To evaluate the mortality effects of extracts of *H. suaveolens* and *T. vogelii* on *P. truncatus* adults, five different application rates (0.05; 0.1; 0.2; 0.3; 0.5 ml) of the extracts were used. The extracts were mixed thoroughly with 10g maize to ensure uniformity before the introduction of insects. Five pairs of adults of *P. truncatus* were introduced in each treatment, each treatment and control were replicated three times and arranged in Completely Randomized Design (CRD). Mortality of the insects was recorded 1 day, 2 days, 3 days and 7 days after treatment application.

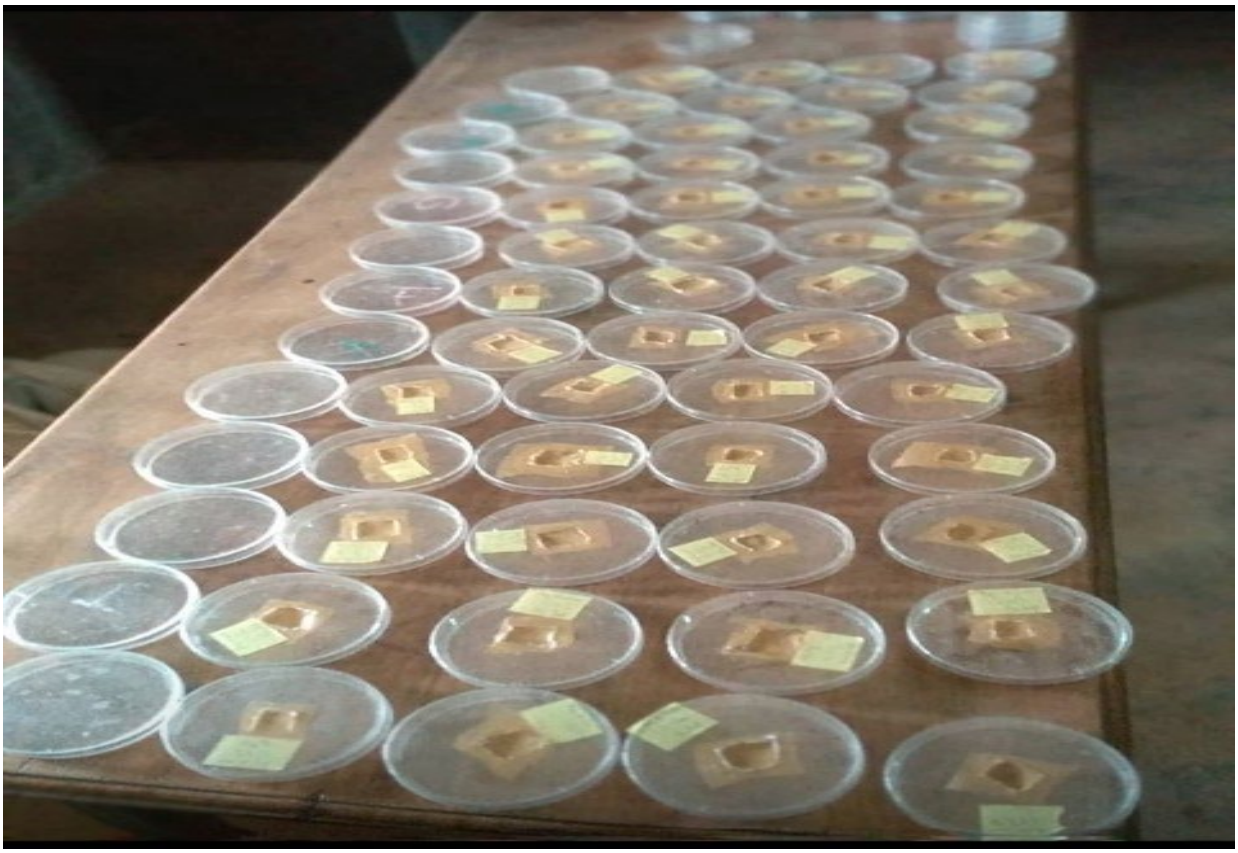

Plate 5: Set-up during the bioassays and taking records from the set-up

Source: Experimental set-upfortopicalapplicationbioassay.

3.8.4 Effects of *H. suaveolens* and *T. vogelii*Extracts on Grain Damage and Weight Loss of Maize Due to Feeding Activities of Larger Grain Borer

To assess the level of damage *P. truncatus* inflicted on maize grains treated with the extracts of *H. suaveolens* and *T. vogelii*, two parameters were used, these include percent grain damage and seed weight loss. Application rates of 0; 0.05; 0.1; 0.2; 0.3; 0.5 ml on 10gmaize was used for the evaluation for each ethanolic and aqueous extracts of the two botanicals. The extracts were mixed thoroughly with 10g maize to ensure uniformity before the introduction of insects. Each treatment and control were replicated three times and arranged in Completely Randomized Design (CRD) design. Five pairs of adults of *P. truncatus* were thereafter introduced into each treatment and monitored for 7 weeks in the laboratory. Damage assessment was measured using % grain damage and grain weight loss. The percentage damage and weight loss of maize were measured after 7 weeks by counting the damaged and undamaged seeds and was calculated using the formula of Compton and Sherington, (1998):

$$\%damage = Nd/Nu + Nd \times 100$$

Where Nd= Number of damaged seeds

Nu= Number of undamaged seeds

$$\% \text{ weight loss} = PW1 - PW2/PW1 \times 100$$

Where, PW1= initial weight of sample before infestation

PW2= final weight of sample after infestation

3.8.5 Effects of *H. suaveolens* and *T. vogelii*Extracts on Emergence of F1 Progeny of *P. truncatus* on Maize

Experiment was set up to determine the efficacy of *H. suaveolens* and *T. vogelii* extracts on emergence of F1 progeny of *P. truncatus* on maize. It was carried out by introducing 5 pairs of newly emerged insects to feed and oviposit for 14 days on 10g maize grains uniformly treated with the two extract types at the rates of 0; 0.05; 0.1; 0.2; 0.3; 0.5 ml. Each treatment and control were replicated three times and monitored for emergence of F1 progenies for 6 weeks in the laboratory. Data on insect emergence was thereafter counted and collected.

3.9Data Analysis

To determine LC_{50} values, the data were analyzed using the probit procedures with IBM SPSS Statistics 21 for Windows®. The percentage data were transformed into arcsin$\sqrt{x}$ before statistical analysis. Data collected was subjected to descriptive statistics and analysis of variance (ANOVA) and treatment means separated using Tukey Honestly Significant Difference (HSD) test at 5% level of probability.

CHAPTER FOUR

4.0 RESULTS

4.1 Evaluation of Phytochemical Constituents of Plant Materials

Phytochemical screening of solvent extracts that was carried out following the method of Ajiboye *et al.*, (2015) showed that their secondary metabolites composition varies with botanical species and solvent used for extraction. The result showed that aqueous extract of *H. suaveolens*contains alkaloids, glycosides, steroids, tannins, flavonoids, poly phenols, terpenoids and partial saponins while ethanolic extract of *H. suaveolens*contains alkaloids, glycosides, steroids, tannins, flavonoids, poly phenols, terpenoids and devoid of saponins. In case of aqueous and ethanolic extracts of *T. vogelii,* glycosides, steroids, flavonoids, poly phenols, terpenoids were present but devoid of tannins and alkaloids.

Table 1: QualitativePhytochemical Screening of Aqueous and Ethanolic Leaf Extract of *H. suaveolens* and *T. vogelii*.

Phytochemicals	Aq. *H. suaveolens*	Eth. *H. suaveolens*	Aq. *T. vogelii*	Eth. *T. vogelii*
Flavonoids	++	++	++	++
Poly phenol	++	++	++	++
Cardiac Glycoside	++	++	++	++
Terpenoids	++	++	++	++
Tannins	++	++	-	-
Steroid	++	++	++	++
Saponin	+	-	++	++
Alkaloids	++	++	-	-

Note; Present: ++; Weakly Present: +; Absent:-.

Key: Aq. = Aqueous, Eth. = Ethanol

4.2 Contact Toxicity by Topical Application

The topical effect of aqueous and ethanolic extracts *H. suaveolens* and *T. vogelii* applied on the thorax of adult *P. truncatus* on mortality at different concentrations and periods is presented in (Table 2).

All the extracts were able to cause weevil mortality over the period of the experiment but at different levels. Ethanolic extract of *T. vogelii*is the most toxic and produced the best effect with LC_{50} of 11.12% (Table 3) at 7 daysafter exposurewith the highest percentage of mortality (15%conc -74%) and lowest mortality (0.05w/v-24%) at 7^{th} day (Table 3). The two *H. suaveolens*extracts (aqueous and ethanol) were the least toxic to the weevils, each killing highest of (15%conc-58%) and (15%conc-64%) respectively at day 7 and lowest (2.5%conc-4.00%) at day 1 and 2 and (2.5%conc-4.00%) at day 2 respectively. These results showed that ethanolic extract of *T. vogelii* exhibited the highest mortality and was more persistent than others.

Table 2: Percentage Topical Application Treated with Different Concentrations of Extracts of *H. suaveolens*and *T. vogelii*

Percentage topical application					
		Duration of exposure (Days)			
Treatment (%)	**Concentration**	**1**	**2**	**3**	**7**
Control		0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.00±1.79^{a}
Aq. *H. suaveolens*	2.5	4.00±2.19^{a}	6.00±2.19^{a}	8.00±1.79^{a}	30.00±2.83^{bc}
	5.0	4.00±2.19^{a}	6.00±3.59^{a}	8.00±4.39^{ab}	34.00±4.59^{c}
	7.5	4.00±2.19^{ab}	6.00±2.19^{a}	6.00±3.35^{ab}	37.50±4.57^{c}
	10.0	6.00±2.19^{abc}	10.00±2.83^{a}	18.00±3.35^{a}	50.00±3.59^{c}
	15.0	6.00±2.19^{c}	10.00±2.83^{bc}	16.00±3.58^{bc}	58.00±3.35^{c}
Eth. *H. suaveolens*	2.5	4.00±2.19^{ab}	4.00±2.19^{ab}	6.00±2.19^{ab}	24.00±2.19^{c}
	5.0	4.00±2.19^{ab}	6.00±2.19^{a}	10.00±4.01^{ab}	30.00±2.83^{c}
	7.5	6.00±2.19^{bc}	6.00±2.19^{bc}	14.00±3.59^{bc}	40.00±2.83^{c}
	10.0	6.00±2.19^{bc}	12.00±3.35^{abc}	20.00±4.91^{bc}	56.00±4.57^{c}
	15.0	8.00±1.79^{bc}	8.00±1.79^{bc}	22.00±3.35^{bc}	64.00±1.79^{c}
Aq. *T. vogelii*	2.5	4.00±2.19^{abc}	6.00±2.19^{bc}	8.00±2.19^{bc}	24.00±2.83^{c}
	5.0	4.00±2.19^{abc}	6.00±1.79^{bc}	10.00±2.19^{bc}	32.00±2.83^{c}
	7.5	4.00±2.19^{abc}	6.00±2.83^{bc}	6.00±1.79^{bc}	40.00±2.19^{c}
	10.0	6.00±2.19^{abc}	8.00±2.19^{c}	16.00±1.79^{c}	50.00±3.34^{c}
	15.0	6.00±2.19^{abc}	10.00±2.19^{c}	22.00±2.19^{cd}	66.00±2.82^{cd}
Eth. *T. vogelii*	2.5	4.00±2.19^{abc}	4.00±1.79^{bc}	8.00±1.79^{c}	24.00±2.19^{c}
	5.0	4.00±2.19^{bc}	2.00±1.79^{bc}	4.00±2.19^{c}	28.00±3.34^{cd}
	7.5	6.00±2.19^{bc}	2.00±1.79^{bc}	4.00±2.19^{c}	32.00±3.34^{cd}
	10.0	6.00±2.19^{cd}	4.00±2.19^{bc}	6.00±2.19^{c}	46.00±3.59^{de}
	15.0	8.00±1.79^{cd}	6.00±2.19^{bc}	8.00±1.79^{c}	74.00±2.19^{de}

Notes: Each value is the mean ± standard error of five replicates, each replicates with 10 adults. Mean followed by the same letters within thesame column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Key: Aq. = Aqueous, Eth. = Ethanol

Table 3: Percentage LC_{50} of 7 Days after Topical Application of Different Concentrations of Extracts of *H. suaveolens* and *T. vogelii* on *P. truncatus.*

Treatment	% LC_{50}after 7 days	95% CL	S.E
Aq. *H. suaveolens*	11.72	5.31-13.86	0.218
Eth. *H. suaveolens*	10.80	11.30-20.19	0.227
Aq. *T. vogelii*	10.40	9.79-18.62	0.225
Eth. *T. vogelii*	11.12	11.73-20.71	0.229

Key: Aq. = Aqueous, Eth. = Ethanol

4.3 Repellent Activity Bioassay

The percent repellence (PR) values obtained from the choice bioassay system are presented (Table 4). Results showed that repellence was significantly ($p<0.05$) influenced by plant species, different concentration of extract applied and types of solvent used in extraction. The PR values for the two tested plants increased with concentration of extract applied and types of solvent used in extraction.

The results further showed significant differences in PR values between and the two plant extracts at all rates except Aqueous extract*H. suaveolens*at 2.5% (w/v) after 2 h exposure. At the highest concentration (15% w/v) evaluated, Eth. *T. vogelii*(PR value: 95%) was more repellent that Eth. *H. suaveolens*(PR value: 80%) against adult *P. truncatus* 2 hours after setup.

Table 4: Percentage Repellency of Different Concentrations of *H. suaveolens* and *T. vogelii*Extracts on *P. truncatus*

Percentage Repellency			
		Repellency after 2hrs of exposure	
Treatment (%)	**Concentration**		**Repellency class**
Aq. *H. suaveolens*	2.5	32.50±2.17^{a}	
	5.0	42.50±4.15^{ab}	
	7.5	50.00±0.00^{abc}	
	10.0	45.00±2.50^{bcd}	
	15.0	55.00±2.50^{cde}	
Mean		45.00±2.26	Class III
Eth. *H. suaveolens*	2.5	40.00±0.00^{defg}	
	5.0	52.50±4.15^{defg}	
	7.5	60.00±3.54^{defg}	
	10.0	65.00±2.50^{defg}	
	15.0	80.00±3.54^{defg}	
Mean		59.50±2.75	Class III
Aq. *T. vogelii*	2.5	35.00±2.50^{cde}	
	5.0	50.00±3.54^{cde}	
	7.5	55.00±2.50^{cde}	
	10.0	57.50±2.17^{cdef}	
	15.0	62.50±2.17^{defg}	
Mean		52.00±2.58	Class III
Eth. *T. vogelii*	2.5	47.50±2.17^{defg}	
	5.0	60.00±3.74^{defg}	
	7.5	62.50±2.17^{efg}	
	10.0	87.50±2.17^{fg}	
	15.0	95.00±2.5^{g}	
Mean		70.50±2.55	Class IV

Notes: Each value is the mean ± standard error of four replicates, each replicates with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Key: Aq. = Aqueous, Eth. = Ethanol

4.4Efficacy of *H. suaveolens* and *T. vogelii*Extracts on Mortality of*P. truncatus*

Mortality increased with ascending dosage levels and time post-exposure for extracts. In general, extracts of *T. vogelii* were more toxic to *P. truncatus* than extracts of *H. suaveolens*. At the same dosage and time of exposure, ethanol extract of *T. vogelii* caused highest mortality of 70.00% at day 7 and ethanol extracts of *H. suaveolens* of 56.67% while lowest mortality at 0.00% at day 1 for two ethanolic extracts. At the low dosages of 0.05ml/10g of maize, aqueous extracts of of *H. suaveolens* and *T. vogelii* caused 0.0% mortality of 1, 2 and 3 days after exposure. Highest mortality was obtained with ethanol *T. vogelii* at the highest tested dosage of 0.5ml/10 g 7 days after treatment (Table 5) and with LD_{50} of 0.33ml/10g of maize (Table 6).

Table 5: Percentage MortalityTreated with Different Dosage Levels of Extracts of *H. suaveolens*and *T. vogelii*

Mortality					
		Duration of exposure (Days)			
Treatment	**Dosage(ml)**	**1**	**2**	**3**	**7**
Control		0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{ab}	0.00±0.00^{ab}
Aq. *H. suaveolens*	0.05	0.00±0.00^{a}	0.00±0.00^{a}	3.33±2.72^{ab}	13.33±2.72^{abc}
	0.1	0.00±0.00^{a}	3.33±2.72^{ab}	6.67±2.72^{ab}	16.67±2.72^{abc}
	0.2	3.33±2.72^{a}	6.67±2.72^{ab}	10.00±0.00^{abc}	20.00±0.00^{cd}
	0.3	0.00±0.00^{a}	6.67±2.72^{ab}	13.33±2.72^{abc}	26.67±0.00^{cd}
	0.5	0.00±0.00^{a}	6.67±2.72^{c}	23.33±2.72^{ab}	46.67±2.72^{cd}
Eth. *H. suaveolens*	0.05	0.00±0.00^{a}	0.00±0.00^{a}	6.67±2.72^{cd}	10.00±0.00^{cd}
	0.1	0.00±0.00^{a}	0.00±0.00^{ab}	10.00±0.00^{cd}	16.67±2.72^{cd}
	0.2	0.00±0.00^{ab}	10.00±0.00^{ab}	20.00±0.00^{cd}	30.00±0.00^{cd}
	0.3	3.30±2.72^{abc}	16.67±2.72^{abc}	26.67±2.72^{cd}	40.00±0.00^{cd}
	0.5	3.30±2.72^{abc}	20.00±0.00^{abc}	33.33±2.72^{e}	56.67±2.72^{de}
Aq. *T. vogelii*	0.05	0.00±0.00^{a}	0.00±0.00^{a}	6.67±2.72^{cd}	13.33±2.72^{cd}
	0.1	0.00±0.00^{a}	3.33±2.72^{b}	13.33±2.72^{cd}	23.33±2.72^{cd}
	0.2	0.00±0.00^{a}	6.67±2.72^{bc}	16.67±2.72^{cd}	30.00±0.00^{cd}
	0.3	0.00±0.00^{a}	6.67±2.72^{bc}	23.33±2.72^{cd}	33.33±2.72^{cd}
	0.5	0.00±0.00^{a}	10.00±0.00^{cd}	30.00±0.00^{cd}	43.33±2.72^{cd}
Eth. *T. vogelii*	0.05	0.00±0.00^{a}	3.33±2.72^{bc}	6.67±2.72^{cd}	16.67±0.00^{cd}
	0.1	0.00±0.00^{ab}	3.33±2.72^{bc}	13.33±2.72^{cd}	23.33±0.00^{d}
	0.2	0.00±0.00^{ab}	6.67±2.72^{cd}	16.67±2.72^{cd}	30.00±0.00^{d}
	0.3	3.30±2.72^{bc}	13.33±2.72^{cd}	23.33±2.72^{d}	40.00±0.00^{d}
	0.5	6.70±2.72^{bc}	16.67±2.72^{cd}	33.33±2.72^{d}	70.00±4.72^{d}

Notes: Each value is the mean ± standard error of three replicates, each replicates with 10 adults. Mean followed by the same letters within thesame column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.
Key: Aq. = Aqueous, Eth. = Ethanol

Table 6: Percentage LD_{50} of 7 days of *P. truncatus*Mortality after Treatment with Different Dosages of Extracts of *H. suaveolens* and *T. vogelii.*

Treatment	LD_{50}after 7 days(ml)	95% CL	S.E
Aq. *H. suaveolens*	0.75	0.06-0.14	0.185
Eth. *H. suaveolens*	0.62	0.11-0.18	0.190
Aq. *T. vogelii*	0.35	0.05-0.12	0.179
Eth. *T. vogelii*	0.33	0.10-0.17	0.179

Key: Aq. = Aqueous, Eth. = Ethanol

4.5Effects of *H. suaveolens* and *T. vogelii*Extracts on Grain Damage and Weight Loss of Maize Due to Feeding Activities of Larger Grain Borer

Results showed that the amount of food consumed and feeding deterrence against *P. truncatus* were significantly (P<0.001) influenced by different extracts of the two plants, dosage level of extracts applied, time of exposure and corresponding factor interaction effects (Tables 7). A dose- and exposure time-dependent decrease in amount of food eaten by the weevils was recorded for the two extracts of plant leaves used in the experiment. At the dose range tested (0.05-0.5ml), ethanol *T. vogelii* extract had a higher feeding inhibition of weight loss 0.84% than aqueous*T. vogelii* extract (1.73-100%). Also, ethanol*H. suaveolens* extract had a higher feeding inhibition on weight loss (1.83-100%) than aqueous*H. suaveolens* extract (2.80-100%). Similarly, a dose- and exposure time-dependent decrease in seed damage 0.5ml of ethanol *T. vogelii* extract has the lowest of 8.00% and 0.05ml has 28.07% while aqueous *H. suaveolens* extract has the highest seed damage of 31.57% at dosage level 0.05ml intreatedmaizebut not has high the control which has the highest of 47.10% in the set-up after 5 weeks of the treatment.

Table 7: Percentage Feeding Deterrence (GrainDamage and Weight Loss) of Maize Grains Treated with Different Dosages ml/ 10g of grain.

		Percentage seed damage/weight loss	
		Grain damage	Weight loss
Treatment	**Dosage(ml)**		
Control		47.10±4.63^{a}	10.57±0.28^{a}
Aq. *H. suaveolens*	0.05	31.57±1.14^{b}	3.47±0.20^{b}
	0.1	24.17±1.69^{bc}	3.20±0.00^{b}
	0.2	21.33±2.79^{bc}	3.40±0.17^{b}
	0.3	20.73±2.07^{bc}	3.20±0.09^{b}
	0.5	18.73±2.45^{c}	2.80±0.26^{b}
Eth. *H. suaveolens*	0.05	30.80±2.43^{b}	3.43±0.21^{b}
	0.1	18.33±1.15^{c}	3.40±0.24^{b}
	0.2	18.43±0.94^{c}	3.08±0.72^{b}
	0.3	17.50±3.20^{c}	2.93±0.27^{b}
	0.5	15.77±0.63^{c}	1.83±0.25^{c}
Aq. *T. vogelii*	0.05	29.43±1.98^{b}	3.10±0.31^{b}
	0.1	23.63±1.04^{bc}	3.17±0.14^{b}
	0.2	18.73±1.09^{c}	3.13±0.20^{b}
	0.3	15.67±0.65^{c}	2.10±0.14^{b}
	0.5	10.13±2.15^{d}	1.73±0.14^{c}
Eth. *T. vogelii*	0.05	28.07±2.00^{b}	3.67±0.45^{b}
	0.1	22.73±2.70^{bc}	2.50±0.72^{b}
	0.2	18.13±1.08^{c}	2.41±0.72^{b}
	0.3	15.10±0.82^{c}	1.90±0.27^{c}
	0.5	8.00±1.50^{d}	0.84±0.27^{d}

Notes: Each value is the mean ± standard error of three replicates, each replicates with 10 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Key: Aq. = Aqueous, Eth. = Ethanol

4.6Effects of *H. suaveolens* and *T. vogelii*Extracts on Emergence of F1 Progeny of *P. truncatus* on Maize

The results obtained showed that all the extracts reduced progeny emergence compared to untreated (control). Percentage reduction in progeny emergence offered by the different extracts treatments was significantly different and there was also significant reduction in progeny emergence as the extract dosage increased.

Results have shown a clear dose-dependent reduction in the adult of *P. truncatus* F1 progeny count at concentration between 0.05 to 0.5ml/10g grain of *T. vogelii* and *H. suaveolens* leaves extracts (Aqueous and Ethanol). Ethanol *T. vogelii* extracts at highest dosage level 0.5ml/10g of grain (3.00%) reduced emergence of F1 progeny the most while the least reduction of the emergence was seen in lowest doses but control has the highest progeny emergence(Table 8).

Table 8: F1 Progeny Emergence of *P. truncatus* on Maize Treated with Different Dosage Levels of Extracts of *H. suaveolens* and *T. vogelii*

		Percentage F1 Progeny Emergence	
		F1 Emergence	Reproductioninhibitionrate%
Treatment	**Dosage(ml)**		
Control		50.67±0.94[a]	-
Aq. *H. suaveolens*	0.05	35.67±1.97[ab]	30
	0.1	30.67±2.33[ab]	40
	0.2	19.33±1.25[a]	62
	0.3	10.67±1.70[b]	79
	0.5	9.67±0.72[bc]	81
Eth. *H. suaveolens*	0.05	31.00±2.06[ab]	39
	0.1	28.00±0.98[ab]	45
	0.2	19.33±1.25[b]	62
	0.3	7.00±0.72[bc]	72
	0.5	4.67±0.98[c]	75
Aq. *T. vogelii*	0.05	34.33±1.66[ab]	32
	0.1	29.67±1.97[ab]	41
	0.2	19.67±1.42[b]	61
	0.3	13.67±1.79[ab]	73
	0.5	10.33±0.72[b]	80
Eth. *T. vogelii*	0.05	35.33±1.91[ab]	30
	0.1	27.67±0.47[ab]	45
	0.2	14.67±0.47[ab]	71
	0.3	8.67±0.82[bc]	83
	0.5	3.00±0.27[c]	94

Notes: Each value is the mean ± standard error of three replicates, each replicates with 10 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.
Key: Aq. = Aqueous, Eth. = Ethanol

There was strong relationship between LGB mortality, F1 emergence, weight loss and seed damage in this experiment based on the simple linear correlation analysis (Table 9). Percentage mortality had weak negative correlation with F1 emergence (r=-0.08035), weight loss (r=-0.07305) and seed damage (r=-0.11296) but had a strong positive correlation between F1 emergence, weight loss and seed damage. An increasing mode in mortality leads to decrease in F1 emergence, weight loss and seed damage of the grains.

Table 4.9: Simple Correlation Variables of Maize Grains Infested by *P. truncatus* as Influenced by Different Extracts.

	Mortality	F1 emergence	Weight loss	Seed damage
Mortality	1			
F1 emergence	-0.08035	1		
Weight loss	-0.07305	0.967361	1	
Seed damage	-0.11296	0.967621	0.979132	1

CHAPTER FIVE

5.0 DISCUSSION

There is a renewed interest amongst scientists to study the bioactivity of plant extracts against stored-grain insect pests (Benzi *et al*., 2009). This has led to carrying out different researches to find out plant extracts that are also effective as the synthetic chemicals.This present research revealed that phytochemical screening of solvent extracts had their secondary metabolites composition varying with plant species and solvent used for extraction. In general, ethanolic extracts contained more secondary metabolites than aqueous extracts of the two plants used in the experiment. The alkaloids, flavonoids and phenolic compounds can serve as pesticidal agent. Terpenoid ethers and terpenoid ketones showed juvenile hormone-like activity against *T. castaneum*, but not *T. confusum* that may be resistant to these substances. Terpenes have also been reported to possess anti-feedants, anticarcinogenic, antimalarial, anti-ulcer and antimicrobial activities (Dudareva *et al.,* 2004). Several isoflavonoids have been isolated from *Tephrosia* spp., the compounds isolated had several effects such as anti-feedant, antibacterial, insect-repellent and insecticidal. Thus, all the observed activities could be associated with the presence of these phytochemicals in the bioactive extracts.

The results showed species, dose, types of solvent used in extraction and exposure time dependent efficacy of *H. suaveolens* and *T. vogelii* treatments against larger grain beetle adults. The increased efficacy with exposure time could be attributed to cumulative toxicity against adult beetles. The differences in the efficacies of *H. suaveolens* and *T. vogelii* could be attributed to the varied quantity and quality of chemical compounds/principles responsible for the observed toxic effects against insects (Gökçe *et al.,* 2010). It is shown in the result that all the extracts were able to cause weevil mortality over the period of the experiment but at different levels.

Ethanolic extract of *T. vogelii* had the highest and lowest percentage at Table 2. The two *H. suaveolens*extracts (aqueous and ethanolic) were the least toxic to the weevils, each killing highest at Table 2. The result of this research agreed with previous works in which extracts of botanicals were used in control *of P. truncatus* and other stored product pest (Ileke and Olotuah 2012). The recorded reduction in F1 progeny could be due to the observed adult mortality and potential anti-oviposition, ovicidal and/or larvicidal effects caused by the two plant extracts. The presence of insecticidal properties in *T. vogelii* has been noted previously, even when dried *T. vogelii* had been stored for 27 years (Lambert *et al*., 1993). Various bio-active isoflavonoids isolated from *T. vogelii* and are responsible for the insecticidal properties observed in this study (Ibrahim *et al*., 2000). *H. suaveolens* have been reported to have insecticidal, antioviposition and growth regulating effects against field and storageinsect pests (Mukanga *et al*., 2010).

Results reported in the current study show that leaves extract of *H. suaveolens* and *T. vogelii*have repellent effects on *P. truncatus*.The observed repellent activity could partly be attributed to the presence of volatile constituents such as terpenoids in the two plants extracts. Maize treated with *H. suaveolens* and *T. vogelii*was significantly repellent against adult *P. truncatus*. The degree of repellence was greatly influenced by the plant species, concentration of extracts applied and type of solvent used. *T. vogelii* ethanolic extract was more repellent (mean PR value = 70.50% i.e. Class IV repellence group) than *H. suaveolens* ethanolic extract(mean PR value = 59.50% i.e. Class III repellence group). Although many studies on botanicals have been conducted in the past (Bekele *et al*., 1996), no data are available on the bioactivity of *H. suaveolens* and *T. vogelii* as grain protectants against major stored product insects. The toxic and repellent action of *T. vogelii* and *H. suaveolens* suggests that there exists good potential for the use of the two local plant species as grain protectants in the traditional resource-poor farming

communities in sub-Saharan Africa. It was reported in Mukanga *et al*., (2010) that the strong repellent effect of this leaf extracts and powders may have been largely dependent on olfactory and gustatory sensation of the test insects. Properties like the repellent odour may have caused the insects to be restless (Ogendo *et al*., 2003). The insects were observed to be turning away and settling on the untreated portions. The choice for the untreated portions was because of the repellent chemicals inherent in the leaf extracts and powders. Insect repellents are secondary metabolites which have been identified to be alcohols, alkaloids, phenolics, flavonoids and terpenes.Lamiaceae have traditionally been used in developing countries for their insecticidal and repellent properties against several insect species (Ngamo *et al*., 2007).

The reduction of F1 progeny emergence in the treated grains might be due to the increased adults mortality of the two tested plants extracts. It is seen in the result that the extracts of *T. vogelii* were more toxic to *P. truncatus* than extracts of *H. suaveolens*. At the same dosage and time of exposure, ethanol extract of *T. vogelii* caused highest mortality at day 7 and ethanol extracts of *H. suaveolens* while lowest mortality at day 1 for two ethanolic extracts (Table 5). At the low dosages of 0.05ml/10g of maize, aqueous extracts of *H. suaveolens* and *T. vogelii* caused 0.0% mortality of 1, 2 and 3 days after exposure. Highest mortality was obtained with ethanol *T. vogelii* at the highest tested dosage of 0.5/10 g 7 days after treatment.The high mortality effect of these extract may be due to inability of some of the beetles to feed on the treated grains which had been coated with the extracts thereby leading to starvation (Ashamo *et al*. 2013). The insect mortality may be due to blocking of spiracles of the insect by dust particles and death caused by asphyxia (Fernando and Karunaratne, 2012). Plant product may also penetrate the insect body through the respiratory system (Kedia *et al.,* 2015). *T. vogelii* is traditionally used for its ichthyotoxic, insecticidal and food parasiticidal properties(Ibrahim *et al*., 2000). A leaf extract of

H. suaveolens reduced the population of *Spodoptera litura* and *Aphis craccivora* on groundnut (Jayakumar *et al*. 2004).

The extracts from the leaves of *H. suaveolens* and *T. vogelii* prevented seed damage and weight loss.The higher dosage of the extracts of *H. suaveolens* and *T. vogelii* have resulted in the low grain damage seen. The higher the dosage of ethanolic *T. vogelii* (0.05ml to 0.5ml/ 10g of grains), the lower the grain damage (27.07% to 11.00%) as compared to control (47.10%). Since grain damage and weight loss are related, the higher the grain damage, the higher the grain loss and verse vice. It can be inferred from the results that the low weight loss observed can be attributed to the low grain damage recorded. This is because, as the number of live weevils reduces in the grains in storage, the amount of damage caused to the grains is also reduced as there is less feeding, hence the lower weight loss observed. Similar findings were reported by Mukanga *et al*. (2010) who illustrated that leaf powders of five botanicals (eucalyptus, guava, neem, *Tephrosia*, and water hyacinth) reduce weight loss and clearly suppressed the emergence of *Prostephanus truncatus* populations in dried cassava chips. Based on previous studies, much of the antifeeding of test botanicals could be attributed to their bioactive principles (Abdelgaleil and Nakatani, 2003)

There was least emergence of F1 progeny in the grains treated with highest dosage level of *T. vogelii* ethanolic extracts as compared to aqueous *H. suaveolens* extract and the control. This may be due to the volatile phytochemicals present in the extracts causing the insects to have the inability to lay large quantity of eggs and those with higher dosage level caused more inability of oviposition. Adedire *et al*., 2011 reported that different botanicals effectiveness at higher dosage to various insect pests. Few eggs laid by the beetle were unable to thrive in the grains and this may affect the progeny production (Ileke and Olotuah 2012). Chebet (2013)

reported that the maize grain treated with *L. camara T. vogelii and A. indica* powder recorded 71.6%, 69.7% and 85.6% F1 progeny reduction respectively. However, the efficacy of the plant products is significantly suppressing emergence has largely been attributed to ovicidal properties, which prevent eggs from hatching into larvae.On cowpea, various solvent extracts of *H. suaveolens* were found to be efficient in terms of their oviposition deterrent, ovicidal, or insecticidal effects against *Callosobruchus maculatus* (Jayakumar *et al*., 2005).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

This research work shows that leaves extracts of *H. suaveolens* and *T. vogelii* have toxic, progeny emergence deterrent and repellent effects on *P. truncatus*. Ethanolic extracts are more potent than aqueous extracts in most plant extracts. The protection of grain in feeding deterrence and other bioassays tested was dose dependent, with higher doses offering better protection. However, *H. suaveolens* and *T. vogelii* are effective and readily available to farmers in the rural areas and they can be used for short and long term storage of maize grains, it is advisable to use the two tested plants.

From the study it was established that highest potency was recorded for *T. vogelii* extracts whereby *H. suaveolens* extracts had least potency in toxicity, F1 emergence and feeding deterrence and repellency effects on *P. truncatus*.

It is recommended that further studies should be conducted purifying and separating individual phytochemical in assaying to know the most effective phytochemicals and using higher dosages of *H. suaveolens* and *T. vogelii* extracts and other effective botanicals for long term storage.

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APPENDICES

Appendix 1: ANOVA- Contact Toxicity (Topical Application) of *P. truncatus*Treated with Different Concentrations of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	SS	DF	MS	F	Prob F	Sign. F	C.V. (%)	S.E.M.	S.E.D	L.S.D. (P<0.05)
Treatment	41.67	1	41.66	0.18	0.68			1.39	1.96	4.53
Error							105.5			
Treatment	1851.66	8	231.46				3			
	15038.3		3007.6	25.7						
Conc	3	5	7	7	1.55	**		1.71	2.42	4.88
Treatment x Conc	418.33	5	83.67	0.72	0.61			2.42	3.42	6.9
Error Conc	4668.33	40	116.71				74.94			
	29968.3		9989.4	105.						
Days	3	3	5	8	3.15	**		1.25	1.77	3.52
Treatment x Days	888.33	3	296.11	3.14	0.03	*		1.77	2.51	4.96
Conc x Days	9231.6	15	615.44	6.52	1.76	**		3.07	4.35	8.59
Treatment x Conc x Days	811.66	15	54.11	0.57	0.89			4.35	6.15	12.19
		14								
Residual	13600	4	94.45				67.41			
	76518.3	23								
Total	3	9	320.16							

APPENDIX 2: ANOVA- Repellency of *P. truncatus*Treated with Different Concentrations of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	SS	DF	MS	F	ProbF	Sign.	C.V. (%)	S.E.M.	S.E.D.	L.S.D. (0.05)
Conc	10743	4	2685.6	87.1	3.39	**		1.39	1.96	4.18
Error Conc	462.5	15	30.83				9.78			
Treat	7145	3	2381.7	55.32	3.88	**		1.47	2.07	4.18
Conc x Treat	2068	12	172.29	4	0	**		3.28	4.64	9.35
Residual	1938	45	43.06				11.6			
Total	22355	79	282.97							

APPENDIX 3: ANOVA- Mortality of *P. truncatus*Treated with Different Dosages of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	SS	DF	MS	F	ProbF	Sign. F	C.V. (%)	S.E.M.	S.E.D	L.S.D. (P<0.05)
Treat	1102.78	3	367.59	2.14	0.17			1.55	2.19	5.04
Error Treat	1375	8	171.88				115.11			
Dosage	19281.9	5	3856.4	86.9	2.13	**		0.96	1.36	2.75
Treat x Dosage	1309.72	15	87.31	1.97	0.04	*		1.92	2.72	5.49
Error Dosage	1775	40	44.375				58.49			
Days	23502.8	3	7834.3	448.26	8.31	**		0.49	0.69	1.38
Treat x Days	627.77	9	69.75	3.99	0	**		0.99	1.39	2.75
Dosage x Days	10426.4	15	695.09	39.77	1.36	**		1.21	1.71	3.37
Treat x Dosage x Days	926.38	45	20.59	1.18	0.23			2.41	3.41	6.75
Residual	2516.66	144	17.48				36.71			
Total	62844.4	287	218.97							

APPENDIX 4: ANOVA- Seed Damage of Maize Grains Treated with Different Dosages of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	SS	DF	MS	F	ProbF	Sign.	C.V. (%)	S.E.M.	S.E.D.	L.S.D. (0.05)
Dosage	7033.68	5	1406.7	57.98	5.49	**		1.42	2.01	4.38
Error Dosage	291.16	12	24.26				18.18			
Treat	265.77	3	88.59	14.36	2.59	**		0.58	0.83	1.68
Dosage x Treat	416.21	15	27.75	4.49	0	**		1.43	2.03	4.11
Residual	222.02	36	6.17				9.17			
Total	8228.84	71	115.89							

APPENDIX 5: ANOVA- Weight Loss of Maize Grains Treated with Different Dosages of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	SS	DF	MS	F	ProbF	Sign.	C.V. (%)	S.E.M.	S.E.D.	L.S.D. (0.05)

Dosage	162.61	5	32.5	162.3	1.38	**		0.13	0.18	0.39
Error Dosage	2.405	12	0.2				9.31			
Treat	7.55	3	2.52	13.09	6.19	**		0.1	0.15	0.29
Dosage x Treat	15.92	15	1.06	5.52	0	**		0.25	0.36	0.73
Residual	6.92	36	0.19				9.12			
Total	195.41	71	2.75							

APPENDIX 6: ANOVA- F1 Emergence of *P. truncatus*Treated with Different Dosages of Extracts of *H. suaveolens* and *T. vogelii.*

EFFECT	**SS**	**DF**	**MS**	**F**	**ProbF**	**Sign.**	**C.V. (%)**	**S.E.M.**	**S.E.D.**	**L.S.D. (0.05)**
Dosage	13416.1	5	2683.22	268.32	7.03	**		0.91	1.29	2.81
Error Dosage	120	12	10				12.11			
Treat	82.78	3	27.59	19.35	1.21	**		0.28	0.39	0.81
Dosage x Treat	110.89	15	7.39	5.18	0	**		0.69	0.97	1.98
Residual	51.33	36	1.43				4.57			
Total	1479.7	71	20.84