# PROTECTANT POTENTIAL OF OILS DERIVED FROM SOME INDIGENOUS PLANTS AGAINST THREE STORED PRODUCT BEETLES

By

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# DEDICATION

To the Glory of the Triune God.

To my parents Isaac and Georgina, my Brother and Sisters; Frank, Prosper, Gloria and Evelyn.

Also to Gifty.

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Finally to the wonderful family to which I belong, for their prayer, financial support and encouragement. I say God Bless You for making me come this far. To all those whose names I cannot mention, I say God will surely reward You.

# DECLARATION

I do hereby declare that, except for references to the work of other researchers which have been duly cited, this thesis is my own work and has not been presented for a degree in any other University.

..... Date 8. February 1999

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# ABSTRACT

Toxicity and protectant potential of isoeugenol, limonene and essential oil of *Hyptis spicigera* against *Sitophilus zeamais* (Motsch.), *Callosobruchus maculatus* F. and *Tribolium castaneum* (Herbst.) and the effect of the oils on germination of maize and cowpea were investigated in the laboratory using contact toxicity, grain treatment and repellency assays. The effects of acetone extracts from leaf and seed of *Hyptis spicigera* on oviposition and adult emergence of *C. maculatus* were also investigated in the laboratory.

Isoeugenol, limonene and essential oil extract of *H. spicigera* applied topically could not kill any significant number of the three beetle species. Grains treated with isoeugenol and limonene killed at least 30% of *C. maculatus* and *T. castaneum* but the two compounds and essential oil extract of *H. spicigera* were not toxic to *S. zeamais*.

Isoeugenol and limonene did not cause inhibition of the development of eggs, larvae and pupae of *T. castaneum* and *S. zeamais* but these completely inhibited the development of eggs and larvae of *C. maculatus*.

Maize and cowpea seeds treated with isoeugenol and limonene were less damaged by *S. zeamais* and *C. maculatus* compared to the controls.

The essential oil extracts of *H. spicigera*, isoeugenol and limonene evoked strong repellency against the three beetle species. The repellency was strongest against *S. zeamais* in *H. spicigera* treated grain. All the three oils did not have effect on the viability of cowpea and maize seeds and hence did not affect their germination.

Acetone extracts of both leaf and seed powder of *H. spicigera* caused a decline in the oviposition and adult emergence of *C. maculatus*.

# CHAPTER 1

## INTRODUCTION

The production of food in the world is inadequate for the ever-growing human population. An increase in agricultural production and preservation will therefore continue to be of vital importance. In this context, the need for effective and affordable pre- and post-harvest protection cannot be over-emphasized. A large number of insects including many species of moths and beetles attack stored products in farmers' bins, mills, warehouses, retail stores and homes. Infestation of insects on stored grains causes weight and quality losses, and leads to reduction in commercial value and seed germination.

*Sitophilus, Tribolium and Callosobruchus* species cause considerable damage to stored grains and grain products throughout the world. In areas where grain production is at the subsistence level, grain loss caused by these insect pests can be critical (Golob and Tyler, 1994). For instance, in developing countries and especially Africa, the tropical climate and poor storage conditions are favourable for the rapid growth and development of numerous insect species which cause significant post-harvest losses. At farm levels where financial and technical means are limited, post-harvest losses

in grain legumes can reach 100% in a few months (Lienard *et al.*,1993).

According to Talukder and Howse (1995), over 20,000 species of storage pests annually destroy approximately one-third of the world's food production valued more than $100 billion. Among these, the highest losses which is 43% occur in developing countries. In a similar study, Baba *et al.*, (1992) estimated post-harvest losses in cereals and pulses in tropical countries due to insect pests between 25-45%.

Intensified efforts have been made to develop insecticide-based techniques to protect grains in small traditional farm stores against insect pest infestation, but this has only been partially successful because of the problems associated with the use of insecticides. These problems include high cost of synthetic insecticides, inadequate supply due to foreign exchange constraints, development of resistance, adverse effects on non-target species and the environment as a whole. Many synthetic insecticides used during the last 40 years in agriculture, forestry, households and against vectors of human diseases do not therefore fulfill the requirements of Integrated Pest Management. In developing countries, the poor storage facilities of traditional farmers are unsuitable for

effective conventional chemical control. This is because most storage types are open to re-infestation by arthropod pests, thus the need for alternative pest control agents.

Plants have been known as a source of grain protectants since time immemorial. They were used traditionally by farmers since the 16th century (Regnault-Roger and Hamraoui, 1993; Tiwari, 1994). In recent times, oils obtained from locally available plants had been used for stored grain protection against insect attack by mixing them with grains (Tembo and Murfitt, 1995). Though losses caused by storage insects can be drastically reduced by the use of commercial insecticides, peasant farmers who produce the bulk of Ghana's grains may not have access to them or cannot afford them because of their high cost. In Ghana, only about 1% of small scale farmers use commercial insecticides and other chemicals to protect their stored produce (Baba, 1994 ).

To help resource-poor farmers control storage insect pests at a low cost, attention has been focused on indigenous plants as source of cheap and available insecticides. This has stimulated interest in the re-evaluation of traditional botanical pest control agents (Weaver *et al.*, 1991;Schmutterer, 1990). Botanical insecticides are broad-

spectrum in pest control and many are safe to apply. They are unique in action and can be easily produced by farmers and small- scale industries.

*Ocimum* and *Hyptis* species are medicinal plants widespread in Africa and India and have been used to control various ailments and as insect repellents, particularlly against mosquitoes (Kokwaro,1976). Some local farmers also mix foodstuffs with dry leaves of these plants for protection against pest damage (Abbiw,1990; Hassanali *et al.*, 1990; Obeng-Ofori and Reichmuth,1997). Bekele, (1994) demonstrated the effectiveness of ground leaves and essential oil of *Ocimum kilimandscharicum* Guerke, *O. suave* Willd. and *O. kenyense* Ayobangira for protecting stored maize and sorghum against infestation by *S. zeamais* Motsch., *Rhyzopertha dominica* (Fab) and *Sitotroga cerealella* (Oliv.).

Recently, the constituent compounds in the essential oil of *O. kenyense* and *O. kilimandscharicum* were identified with 1,8 cineole and camphor forming the major components comprising 37 and 70% of the total collection,respectively (Tables 1 and 2)(Bekele, 1994). Isoeugenol and limonene were also identified as minor components of *O. kenyense* and *O. kilimandscharicum*, respectively. More recently, 1,8 cineole

and camphor were shown in laboratory bioassays to be highly repellent and toxic against *S. granarius* (L), *S. zeamais*, *T. castaneum* (Herbst.) and *Prostephanus truncatus* (Horn.) in stored wheat and maize (Obeng-Ofori *et al.*, 1997a & b). The biological activity of the minor components were, however, not investigated.

Table 1. Essential oil components of *O. kenyense* in Kenya

| Common Name | Formula | % Composition |
|---|---|---|
| 1,8 cineole | $C_{10}H_{18}O$ | 36.93 |
| β-selinene | $C_{15}H_{21}$ | 23.57 |
| methyl chavicol | $C_{10}H_{18}O$ | 12.86 |
| isoeugenol | $C_{10}H_{12}O$ | 8.46 |
| methyl isovalerate | $C_7H_{11}O_2$ | 2.99 |
| β-pinene | $C_{10}H_{18}$ | 2.86 |
| α-humulene | $C_{15}H_{24}$ | 2.78 |
| 4-terpineol | $C_{10}H_{18}O$ | 0.79 |
| γ-cardinene | $C_{15}H_{24}O$ | 0.78 |
| trans-caryophyllene | $C_{15}H_{24}$ | 0.66 |
| α-terpineol | $C_{10}H_{16}$ | 0.55 |

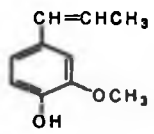
Bekele, (1994).

Table 2. Essential oil components of *O.kilimandscharicum* in Kenya

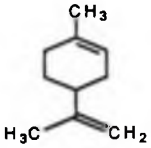
| Common Name | Formula | % Composition |
|---|---|---|
| 1,8 cineole | $C_{10}H_{18}O$ | 5.07 |
| Limonene | $C_{10}H_{16}$ | 6.23 |
| Endo-borneol | $C_{10}H_{18}O$ | 0.60 |
| Myrtenol | $C_{10}H_{16}O$ | 1.30 |
| 4-terpineol | $C_{10}H_{18}O$ | 1.44 |
| trans-caryophyllene | $C_{15}H_{24}$ | 2.80 |
| α-terpineol | $C_{10}H_{16}$ | 0.55 |
| Camphene | $C_{10}H_{15}$ | 5.07 |
| Camphor | $C_{10}H_{16}O$ | 70.43 |
| Linalool | $C_{10}H_{18}$ | 0.47 |

Bekele, (1994).

Isoeugenol and Limonene (98%purity)(Fig. 1 ) used in this work were purchased from Aldrich Limited, Germany. *H. spicigera* Lam is used by local farmers as grain protectants (Abbiw, 1990). However the mode of action and the scientific bases behind this has not been reported.




isoeugenol




limonene

Fig. 1. Chemical Structures of the two essential oils used in the work.

The objectives of the above study were therefore,

1. To evaluate the effect of isoeugenol, limonene and crude essential oil of *Hyptis spicigera* on mortality of *Sitophilus zeamais, Callosobruchus maculatus* and *Tribolium castaneum* and their repellent action against the beetles.
2. To evaluate the effect of isoeugenol and limonene on feeding, reproduction and survival of *Sitophilus zeamais, Callosobruchus maculatus* and *Tribolium castaneum.*
3. To determine the effects of these products on seed viability of maize and cowpea.
4. To determine the effect of acetone extracts of leaves and seeds of *Hyptis spicigera* on the oviposition of *Callosobruchus maculatus*.

# CHAPTER 2

## 2.0 LITERATURE REVIEW

### 2.1 History of Insecticidal Plants.

Plants evolved over some 400 million years, and have developed a number of protective mechanisms to survive attack by insects (Bekele, 1994). These include the action of toxic substances, hormone mimics, anti-hormones, anti-feedants and repellents. Most of the toxic substances are secondary plant metabolites which have been utilized for centuries in crude forms for control of insect infestations. There are about 40,000 secondary metabolites which plants synthesize and store but not more than 20,000 have been identified so far (Tiwari, 1994). Unfortunately, most of these secondary metabolites are distributed in thousands of plant species from which location and identification of insecticidal metabolites are a very tedious exercise .

The number of plants known to possess activity against stored product insect pests are few and only a small number are active against all major stored grain insects (Jacobson, 1983). According to Baba *et al.*, (1992) over 200 plant species have been reported to have insecticidal properties. Some secondary metabolites are toxic to the pests (pyrethrum,

nicotine, rotenone) while others are anti-feedants (azadirachtin, ripe seed extract) and sterilants (extracts from *Acorus calamus* L.)(Ignatowicz and Wesolowska, 1995). The most important botanical insecticides which have been used extensively are nicotine, rotenoids and pyrethrum (Jacobson, 1989) as cited by Bekele, (1994). These have been identified in a large variety of plant species long before the "insecticidal revolution" in the 1930's and the 1940's (Delobel and Malonga, 1987). It is on record that as early as 1690, water extracts of tobacco leaves were being used to kill sucking insects on garden plants (Lodeman, 1903).

**2.2 The use of plant bioproducts for insect pest control**

Botanical insecticides are broad -spectrum in pest control and many are safe to apply. They are unique in action and can be easily produced by farmers and small-scale industries. Because they are readily biodegradable, it is feasible to meet the demand of integrating plant derivatives with pest suppressing properties into pest control programmes (Sarac and Tunc, 1995). Unfortunately, these traditional practices have been largely neglected by farmers on the advent of synthetic and petroleum based insecticides. Synthetic pesticides are currently the method of choice to protect grain from insect damage. Their widespread use has, however, led to the

development of resistant strains of insects (Champ and Dyte, 1976; Zettler and Cuperus, 1990). At least 447 insect and mite species including storage insects have developed resistance to one or more classes of insecticides as at 1984 (Georghiou, 1986).

Generally, protection of stored products involves mixing grain with protectants made up of plant materials. The strategy used by different communities varies from place to place, depending partly on the type and efficacy of suitable plant materials available (Obeng-Ofori, 1995). Many of these practices are undocumented and the scientific rationale for their continuous use has remained uninvestigated (Hassanali *et al.*, 1990 ; Saxena *et al.*, 1992). The best known compound of this new class of insecticides is azadirachtin which is obtained from the neem tree, *Azadirachta indica* (Juss. ) (Xie and Isman , 1995 ). The leaves of *A. indica* have been used to protect cereal grains against stored product insects (Saim

and Meloan, 1986). Also in India, 13 plant materials thought to have insecticidal properties were obtained locally and their powders incorporated at 15 or 30% (wt./wt.) into a standardized culture medium which contained eggs of *Musca domestica* L. All the 13 species gave 100% control (Ahmed et

al., 1981). In another work by Delobel and Malonga (1987), powders of *Chenopodium ambrosioides* L. and *Tephrosia vogelii* Hook.f. were found to be effective against the stored groundnut beetle *Caryedon serratus* (Oliv.) (Col: Bruchidae).

Repellents, anti-feedants and insecticidal substances had been identified in a large variety of plant species long before the "industrial insecticide revolution" in the 1930's and 40's. During these years, compounds such as nicotine, derris and pyrethrum were the only known effective insecticides (Delobel and Malonga , 1987). Many of these plants with insecticidal properties were cultivated near homes and it is still practised today. Some of these plants are used in the treatment of several ailments. In Rwanda for example, *Tetradenia riparia* (Hochst) is cultivated near homes and it is claimed to be effective against many diseases and ailments such as malaria, diarrhoea and fevers. The leaves are also used to protect foodstuffs by mixing with them in traditional silos (Weaver *et al.*, 1992). Baba (1994), tested powders and slurries prepared from parts of ten indigenous plant species from Ghana under laboratory conditions for their ability to protect stored grain from damage by *S. oryzae* on maize and *P. truncatus*. It was found out that one part or the other of most plants used by indigenous farmers could protect stored

grain depending on the method employed.

### 2.3.1 *Ocimum* species.

Sixteen species of *Ocimum* on the mainland of Africa have been recognized (Bekele,1994). *Ocimum* belongs to the family Labiatae and have medicinal and insecticidal properties and are being used by small- scale farmers as grain protectants. The species of *Ocimum* include *O. americanum* L.(American Basil), *O. basiculum* L., *O. suave* Willd., *O. canum* Sims., *O. gratissimum* Paton, *O. kilimandscharicum* Guerke and *O. kenyense* Ayobangira. *O. basiculum* and *O. canum* are planted near homes and are used as spices. *O. gratissium* is used in Ghana as enema to cure abdominal pains (Abbiw, 1990).

More recently, ground leaves and essential oil extract of *O. kenyense* were shown in laboratory bioassays to be effective protectants of stored maize and sorghum against attack by *Sitophilus zeamais*, *Rhyzopertha dominica* and *Sitotroga cerealella* (Bekele,1994). Ground leaves and essential oil extracts of this plant were also shown in the laboratory to be effective protectants of stored maize and sorghum against attack by *Sitophilus zeamais*, *Rhyzopertha dominica* and *Sitotroga cerealella* in storage (Bekele,1994; Jembere *et al.*, 1995).

**2.3.2 Chemical Composition of essential oil of *Ocimum* species**

The essential oil extract was isolated from the leaves, inflorescences and succulent stems of *O. kenyense* and *O. kilimandscaricum* by steam distillation using Clavenger apparatus (Guenter,1949). The condensing oils were collected in n-hexane solvent (Bekele,1994; Jembere *et al*. 1995). The essential oil was analysed by a VG analytical mass spectrometry, GC-MS (VG 12-250) equipped with data system and Hewlett Packard 5790 gas chromatography with splitless injector and flame ionization detector (Figs. 2 & 3). The constituent compounds were identified by spectral comparison with synthetic standards. A total of 14 compounds with three functional groups (alcohol, aliphatic and cyclic hydrocarbon, ether) were identified(Bekele, 1994).

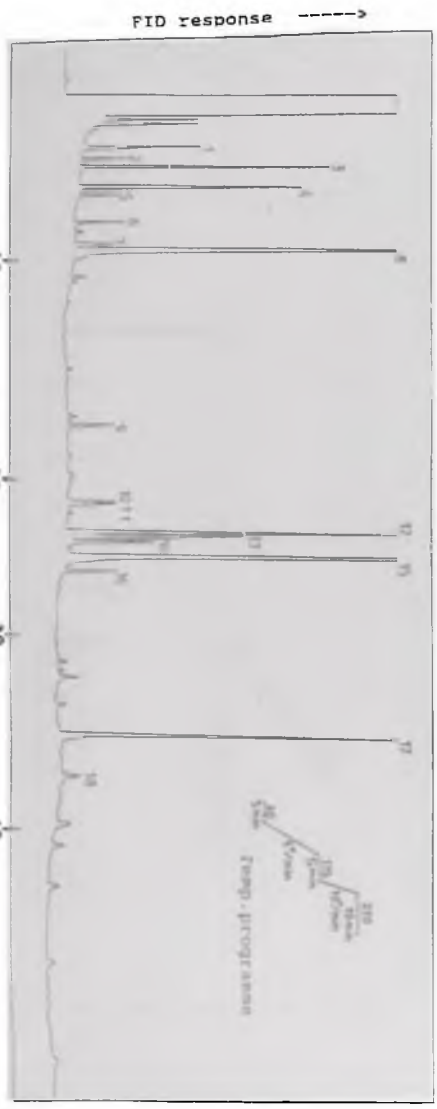



Fig.2 Gas chromatograph profile of essential oil of Ocimum kenyense (Bekele, 1994)

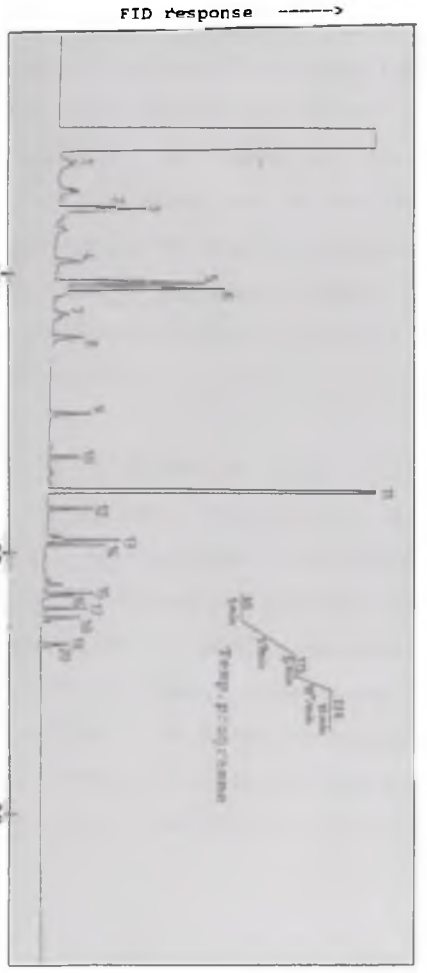



Fig.3 Gas chromatograph profile of essential oil of Ocimum kilimandscharicum (Bekele, 1994)

### 2.3.3 *Hyptis* species

The species include *H. pectinata* (Linn.), *H. suaveolus* Poit, *H. lanceolata* Poir and *H. spicigera* Lam. *Hyptis spicigera* is a tall, erect aromatic herb (Plate 1). It belongs to the family Labiatae. The flowers are very small with the corolla being white with mauve marks on the tip. It is a weed of road sides and cultivated lands. *H. spicigera* often occurs in damp places(Hutchinson and Dalziel,1963). The inflorescence is a dense cylindrical or ovoid-cylindrical spike up to 9 cm long. The herb can grow up to 2.5 m.

Locally, *H. spicigera* Lam. is put in layers below bundles of millet to keep away termites. It is also burned to repel mosquitoes. Crushed leaves of *H. spicigera* is applied to the head as relief of headache, cold and migraine (Abbiw,1990). *H. pectinata* (Linn.) is used as semi cultivated leaf vegetable. It is mixed with *Guiera senegalensis* J.F. Gmelin and indigo plant and boiled for horses to inhale the vapour to control diseases associated with mucous catarrh. Leaf infusions of *H. pectinata* and *H. suaveolus* are also used to treat fevers.

Plate 1. *Hyptis spicigera* in the wild.

Leaf decoction of *H. pectinata* facilitates child-birth and hastens delayed labour (Abbiw,1990). Leaves of *H. suaveolus* are boiled and used as a beverage. It is also used as food flavouring and herbaceous fodder. The leaves of *H. suaveolens* are used to treat boils and eruptions. Juice of pressed leaves of *H. suaveolens* mixed with lime juice when drunk serves as abdominal pain relief. Ellis (1990) has shown that acetone extracts of *H. spicigera* caused a sharp decline in oviposition and adult emergence of *C. maculatus* in laboratory bioassays.

**2.4 Some important food commodities in the tropics.**

Cereal grains, legumes and pulses constitute the most important source of carbohydrate and protein in the tropics. It is thus usually stored to provide a food reserve as well as seed for planting (Baba,1994). In Ghana, annual production of maize and cowpea is about 750,000 and 30,000 tonnes, respectively (Owusu-Akyaw, 1991). The commonly cultivated cereals and legumes in sub-sahelian West Africa are maize, millet, sorghum, soybeans, cowpeas and groundnuts. At farm levels where financial and technical means are limited, post-harvest losses in grain legumes can reach 100% within a few months of storage (Lienard *et al.*, 1993 ). Cowpea is the main protein source for the low income sector in the tropical and

semi-tropical countries. According to Mbata *et al.*, (1992) about 850,000 tonnes of cowpea were produced in the tropics in 1981.

**2.5 Storage insect pests.**

Over 500 bettle species of insects have been found associated with stored products of plant and animal origin eg. cereals, pulses, milled products, manufactured foods, skins, wool, textiles etc. (Halstead,1986). Most storage insect pests belong to the orders Coleoptera and Lepidoptera. One or several species may be found attacking the same commodity at the same time. These may occur on commodities as primary or secondary pests. The most important Coleopteran storage insect pests are among the Bostrichidae, Curculionidae, Bruchidae, Tenebrionidae and Dermestidae families. Most frequently encountered storage pests of cereals and legumes are *Sitophilus oryzae* Linn., *Sitophilus zeamais* Motsch., *Tribolium castaneum* (Herbst.) and *Callosobruchus maculatus* F. (Fatope *et al.*, 1995).

## 2.6 *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae).

### 2.6.1 Taxonomy

The Tenebrionidae are common throughout the temperate and tropical regions of the world. The adults of rust-red beetle, *T. castaneum* are 2.3-4.4 mm long and red-brown in colour. The males are distinguished from the females by the males possessing a hairy puncture on the ventral surface of the anterior femur which is absent in the females (Haines, 1991). In the larvae there are two upwardly curved urogomphi on the 9th abdominal segment.

### 2.6.2 Life history and behaviour

*T. castaneum* is readily cultured in the laboratory. It is active at 25°C but does not fly until approximately 30°C is reached. It cannot climb smooth surfaces and so can be readily confined in batches to a treated surface (Champ and Campbell-Brown, 1970). Cannibalism and predation is often employed in their nutrition. The eggs and pupae are often cannibalized by the adults. The males show a preference for pupae while the females prefer the eggs. *T. castaneum* can penetrate deeply into storage commodities like most storage beetles. Copulation in females may occur many times while they lay their eggs loosely in the commodity throughout their adult lives. The number of eggs laid is dependent on temperature.

At 30°C *T. castaneum* produces approximately 10 eggs/day but only 3 at 25° (Howe, 1962). A single female may lay 350-400 eggs a year. (Haines,1991). Larvae emerge approximately 2.7 days after oviposition. The larvae pupate loosely in the food material. Under optimal conditions, development from egg to adult may take only 20 days (Howe, 1956). Under less favourable conditions development takes about 45 days. The upper and lower temperature limits for development are 40°C and 22°C,respectively. The long life-span and long reproductive period of *T. castaneum* enable this insect to spend a considerable time searching for new food sources. Subsequently, colonization of a new environment which may be inhabited by other species is enhanced by the wide range of diets *T. castaneum* live on and by its ability as a great competitor (Haines,1991).

### 2.6.3 Pest status

*Tribolium castaneum* (Herbst) is cosmopolitan and a serious pest of stored grains and other materials (Champ and Dyte, 1976). It is a major pest of stored food in the tropics and it is the species most frequently found on produce imported from temperate countries such as Canada (Monro, 1969). *T. castaneum* is often the first stored product pest to appear after harvest and shelling of groundnuts (Haines,1991). The

adults and larvae can feed on a wide range of commodities. Apart from feeding, the excreta and exuviae of the insect contaminate the products they infest. The insects also spread an odour in the commodity as they move along. *T. castaneum* is also an important secondary pest on cocoa beans, coffee and cereal products preferring the embryo.

### 2.7 *Sitophilus zeamais* Motsch. (Coleoptera:Curculionidae).

#### 2.7.1 Taxonomy

All adult Curculionidae are characterized by a 'rostrum', which is a forward snout-like extension of the head and which carries the mouthparts in a position that is ideal for penetrating plant tissues. The body size of *S. zeamais* and *S. oryzae* ranges from 2.4-4.5mm but *S. zeamais* is bigger. *Sitophilus oryzae* (Linnaeus) and *S. zeamais* Motchulsky are almost indistinguishable from each other externally. Both have four reddish patches on the elytra but those of *S. zeamais* are more clearly defined (Apert, 1987). They can be distinguished from *S. granarius* (L) by the presence of metathoracic flight wings and by having circular rather than oval punctures on the prothorax. Separation of *S. oryzae* and *S. zeamais* can be done by the examination of the genitalia (Haltstead,1964).

### 2.7.2 Life history and behaviour

*S. zeamais* is found in all tropical and subtropical parts of the world. The adults live from several months to one year. They lay eggs throughout most of the adult life, though 50% may be laid in the first 4-5 weeks and up to 150 eggs may be laid per female (Haines,1991). The eggs are laid individually in small cavities chewed into several grains by the female. Each cavity is sealed by a waxy secretion called an "egg-plug" where the eggs are protected. At 25°C the incubation period of the egg is about 6 days (Howe, 1952). Eggs are laid between 15 and 35°C with the optimum around 25°C at a grain moisture content of over 10%. After hatching the larva begins feeding inside the grain, excavating a tunnel as it develops. Pupation takes place within the grain and the newly developed adult chews its way out leaving a large characteristic emergence hole. Until adult weevil bores its way out, infestation is not evident from outside. Total developmental period ranges from about 35 days under optimal conditions to over 110 days in unfavourable conditions. The length of the life-cycle also depends upon the type and quality of grain being infested. Food preferences of the species is variable, but *S. zeamais* is predominantly associated with maize. *S. zeamais* has a greater ability and tendency to fly than *S. oryzae*. In the field, adults emerge first just before harvest

and emergence continues for sometime later (Giles and Ashman,1971).

### 2.7.3 Pest status

*S.zeamais* attacks all kinds of cereals but it is predominantly found on maize (Longstaff, 1981; Ayertey and Akibu, 1982). Before the accidental introduction of the Larger Grain Borer, the maize weevil was the most important primary pest of stored grain in Ghana, causing estimated weight losses of 7-20% (Hall, 1970) in stored maize, and in some cases up to 35% after 6 months of storage (Wheatly, 1973, cited in Yoshida, 1983). It attacks maize on the cobs or shelled. Since it is a strong flier, it is more likely to fly to the ripening crop in the field and establish an infestation in the grain before harvest. It can fly over distances of 400-800 metres (Chestnut, 1972). If damage is high, the seed for planting will be reduced in quantity and in quality. The species is also a pest of all cereal grains and many other seeds. *Sitophilus zeamais* is able to breed on dried cassava and have been reported as frequent pest of this commodity (Haines,1991).

## 2.8 *Callosobruchus maculatus* (Fab.) (Coleoptera:Bruchidae)

### 2.8.1 Taxonomy

Adult *C. maculatus* has a pair of distinct ridges (inner and outer) on the ventral side of each hind femur which is common with other species of *Callosobruchus*. Both sexes have slightly serrated antennae. In the females, strong markings on the elytra consisting of two large lateral patches mid-way along the elytra and smaller patches at the anterior and posterior ends are found. However, a paler brown cross-shaped area covers the rest of the body.

### 2.8.2 Life history and behaviour

The cowpea beetle *C. maculatus* originated from Africa where it is still the dominant species of *Callosobruchus* (Haines,1991). Presently, it is distributed throughout the tropics and subtropics. The larvae and pupae are normally only found in cells bored within the seeds of pulses. The adults do not feed and are short-lived, usually not more than 12 days under optimum conditions. During the life span, the adult females lay up to 115 eggs, but in the presence of previously infested seeds, oviposition may be depressed. The optimum temperature for oviposition is 30-35$^{o}$C. Eggs are laid firmly glued to the surfaces of the host seed, with preference to smooth seeds than rough seeds. Newly laid eggs are small, grey and

inconspicuous. The eggs hatch in about 3-5 days after laying (Singh and Rachie,1985). Upon hatching, the larva bites through the egg base, the testa of the seed and into the cotyledons. The developing larva feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. During this period, detritus is produced and this is packed into the egg, turning the eggs white and making it clearly visible to the naked eye. The larva moults three times and on reaching the fourth instar, the larva constructs a cell inside the seed and pupates in it. Optimum development conditions are around 32$^{0}$C and 70% r.h. Pupation takes place within the seed of *Vigna unguiculata* (L.)Walp 26 days after oviposition. The total developmental period when breeding on seeds of *V. unguiculata* is about 36 days.

**2.8.3 Pest Status**

Most bruchids feed on the seeds of legumes and all the species that are important pests of stored products are legume feeders. *C. maculatus* is a major pest of *V. unguiculata*(cowpea),*Vigna subterranea*(L.)(Bambara groundnuts), *Lens culinaris* Medik. (lentils) and *Vigna radiata* (L.) (green gram). Since cowpea is an important crop in West Africa and a major source of protein, the beetle has become the most important pest as far as cowpea is concerned (Singh and

Rachie, 1985). According to Fatope *et al.*, (1995), *C. maculatus* is probably the most economically important borer of stored legume seeds in sub-Sahelian West Africa. A lot of damage is done by the larva during feeding inside the seed. Farm storage for 6 months causes a loss of about 30% in weight with up to 70% of the seeds being infested and virtually unfit for consumption (Singh and Rachie, 1985). Infestation can begin in the field when the pods are newly mature. The eggs are laid on the pods but the bruchids prefer to get inside the pods through holes made by other pests and lay their eggs directly on the seeds. As the pods dry, the insect's ability to infest them decreases. This indicates that dry peas stored in pods are quite resistant to attack whereas threshed peas are susceptible to attack throughout storage.

# CHAPTER 3

## 3.0 MATERIALS AND METHODS

### 3.1 Mass rearing of test insects

*S. zeamais, T. castaneum* and *C. maculatus* were obtained from stock cultures maintained at the Crop Science and Zoology Departments of the University of Ghana, Legon. Collected samples were reared at the Zoology Department,using Kilner jars. The insects were reared on maize, wheatbran and cowpea bought from the Madina market about 4 km from the University of Ghana campus. The jars were covered at the open ends with pieces of muslin cloth which were held in place by rubber bands. The temperature ranged in the laboratory between 25 and 27 ± 1$^{0}$C with relative humidities between 70 and 75%. Cultures were under alternating 12 hours dark and 12 hours light cycle. A mixture of wheat bran, finely ground maize and glycerol in the ratio of 8 : 8 : 1 (wt./wt.) (Amoako-Atta & Partida, 1976) was used as food medium for the culturing of *T. castaneum*. *S. zeamais* were reared on whole maize while *C. maculatus* were reared on black-eye cowpea. Cultures were changed from time to time when food source was depleted. The cultures were started with about 100 adult insects and after

the oviposition periods of one week, these adults were removed for the $F_1$ generation to emerge. To avoid cross infestation of mites, other insects and diseases, the food media were sterilized at about 60.0°C for 6 hours before introduction of parent adults. The equipment used in handling insects e.g. scalpel and forceps were also sterilized at about 100.0°C using a Gallenkamp oven. The cultures were placed on Aluminum trays which were surrounded by engine oil as a further precaution to prevent entry of mites and other crawling insects (Goswami, 1988) (Plate 2 ).

### 3.2 Extraction of crude essential oil from *Hyptis spicigera* (Lam.)

Fresh leaves, inflorescence and succulent stems of *H. spicigera* were collected from the University of Ghana campus for oil extraction. About 3.5kg of *H. spicigera* fresh plant materials in batches of 150 g were subjected to heat distillation for three hours to obtain the essential oils using Clavenger type of apparatus (Plate 3a ). The condensing oils were collected in glass vials. The water collected with the oil was removed using a dropping pipette.

Plate 2. Experimental set-up used for culturing test insect species

After a bulk of the oil has been collected and the water removed, 5 g of anhydrous sodium sulphate was added to the oil for 15 minutes to remove the excess water from it. The oil was then filtered using a suction pump and then collected in a glass vial, weighed and the volume measured.

### 3.3 Leaf and seed extracts

The fresh plant materials of *H. spicigera* were dried in the laboratory at room temparature for one week. The seeds were removed and the dried leaves were separated from the stems. The leaves and the seeds were milled separately using a laboratory blender I K A-Universalmuhle M20. The milled samples were then sieved using laboratory test sieve of mesh size 710µm to obtain fine powders. After weighing, a solution of 95% acetone and distilled water was prepared in a ratio of 95:5 (v/v) in 1 litre Kilner bottles and stirred. The powder was added to the solution, stirred and left for three days. The solutions were then poured from the powders and the process was repeated. The acetone solutions poured from the seed and leaf powders were then filtered to remove any particles. A rotary vacuum evaporator (Plate 3a) was used to concentrate the filtrate.

### 3.4 Contact toxicity by topical application

The effect of isoeugenol, limonene and crude essential oil of *H. spicigera* on adults of the three beetle species by topical application was assessed. Aliquots of 3, 6, 9, 12 and 15µl of oils dissolved in 1 ml acetone were prepared. 20 weevils aged between one and seven days were transferred into plastic petri dishes with diameter 11.0 cm. The petri dishes were each lined with Whatman No.1 filter paper. The insects were chilled in batches for five minutes in a freezer to immobilize them. Using a micro syringe, 2 µl each of the solutions were applied to the thorax of each insect. For *Hyptis* oil, concentrations of 0, 10, 20, 30, 40 and 50 µl/ml were used. Control treatments were obtained by applying the same volume of solvent to the thorax of the insects. After the treatments, each batch of insects was placed in 100 g samples of food media. Each treatment was replicated three times and mortality counts were made after 24 hours.

Plate 3. Clavenger apparatus (b) and rotary vacuum evaporator (a) used to extract and concentrate crude oils.

### 3.5 Mortality in grain

The effect of isoeugenol, limonene and crude essential oil of *Hyptis* treated wheat flour, cowpea and maize grain on adult mortality of the three beetle species was assessed in the laboratory by mixing 100 g portions of food media with test solutions at 12, 24, 36, 48 and 60 µl of isoeugenol and limonene dissolved in 4 ml acetone and 40, 80, 120, 160, 200 µl of *Hyptis* oil dissolved in 4 ml acetone in 1 litre jars. These were stirred for five minutes to ensure even spread of the test solution on the grains. Control grains were treated with only solvent. The assays were allowed to stand for 30 minutes to allow the solvent to evaporate completely. 40 adults of mixed sex and aged between one and seven days were introduced into each jar and covered with a piece of muslin cloth held by rubber band. Each treatment was replicated three times. Mortality counts were made 24 hours later.

### 3.6 Effecct of isoeugenol and limonene oils on the development eggs and immature stages of test insects

The effect of isoeugenol and limonene on the development of eggs and immature stages of *S. zeamais, C. maculatus* and *T.*

*castaneum* on or in food media was investigated (Plate 4). Batches of 500 g of cowpea and maize grains were used for *C. maculatus* and *S. zeamais*, respectively. For *T. castaneum* a mixture of wheat bran, finely ground maize and glycerol in the ratio of 8 : 8 : 1 was used. These were held in 1 litre glass jars and 200 unsexed adults aged between one and seven days were confined in the jars and allowed to oviposit. After four and seven days, the parent adults of *C. maculatus* and the other two insect species, were removed, respectively. One day after adult removal, six (100 g) batches of the food media were treated with test solutions at 0, 12, 24, 36, 48 and 60 µl dissolved in 4 ml acetone. These treatments were repeated one, two and three weeks after adult removal. Adults that emerged were counted after six and eight weeks for *T. castaneum* and *S. zeamais*, respectively after adult removal. For *C. maculatus* parent adults were removed after 1 day and treatments were made as indicated above. These were repeated 1, 2 and 3 weeks after adult removal. Each treatment was replicated three times.

Plate 4. Experimental set-up to show the effect of the oils on the development of immature stages of the three beetle species.

**3.7 Effect of isoeugenol and limonene on progeny production**

The effect of isoeugenol and limonene on the $F_1$ progeny production by *S. zeamais* and *T. castaneum* were assessed. Hundred gramme batches of cowpea in 1 litre jars were treated with concentrations of 12, 24, 36, 48 and 60 µl of isouegenol and limonene dissolved in 4 ml of acetone. The control treatments were done with only solvent. 40 one- to- seven days old *S. zeamais* adults were introduced onto each batch of treated and control cowpea after the solvent had evaporated from the treated grains. Pieces of muslin cloth held by rubber bands were used to cover the jars. After three weeks oviposition period, the adults were removed and $F_1$ adults that emerged were counted after eight weeks. This period corresponds to the cycle of one generation. The treatments were replicated three times. For *T. castaneum*, 40 one- to-seven days old adults were bred on wheat flour. After allowing for two weeks oviposition period, the parent adults were removed. Six weeks later, the $F_1$ progeny that emerged were counted.

**3.8 Assessment of grain damage.**

Seed damage caused by *S.zeamais* and *C. maculatus* on maize and cowpea, respectively was assessed by treating batches of 100

g of undamaged grains with 0, 12, 24, 36, 48 and 60 µl concentrations of isoeugenol and limonene. Control grains were treated with solvent only or untreated. 20 pairs of one day old adults of *C. maculatus* and 40 *S. zeamais* adults of mixed sex were introduced onto the samples for two weeks and three weeks, respectively. The parents were then removed and the set-ups were undisturbed for five and eight weeks for *C. maculatus* and *S. zeamais*, respectively. After this period the $F_1$ adults that emerged were sieved out of the grains. The seeds were separated into damaged and undamaged ones. The damaged seeds were those with one or more emergence holes on them while the undamaged ones were those without emergence holes. The damaged and intact grains were weighed and counted separately. Percentage weight loss values were computed following FAO (1985) as

$$\% \text{ wt. loss} = [( UN_d - DN_U ) / U(N_d+N_U)] \times 100 ,$$

where, U = wt. of undamaged seeds

$N_U$ = Number of undamaged seeds

D = wt. of damaged seeds

$N_d$ = Number of damaged seeds.

### 3.9 Repellency bioassay

The repellent action of isoeugenol, limonene and crude essential oil of *H. spicigera* against *S. zeamais*, *C. maculatus* and *T. castaneum* was assessed following the method of Jembere *et al.*,(1995) with few modifications. Repellency was assessed in a choice bioassay system consisting of two half-litre glass jars connected together at their rims by means of a 90 x 1.5 cm transparent tube. A cicular hole was cut at the middle of the tube for the introduction of test insects. The bottles were covered with black polythene bags to prevent entry of light. This was done because the insects are photosensitive (Busvine, 1971). Different concentrations of 6, 12, 18, 24 and 30 µl of isoeugenol or limonene dissolved in 2 ml acetone solution were prepared. Batches of 50 g of cowpea were treated with the test solutions in one jar while the other jar contained untreated grains and acted as the control. 50 one day old adults of *C. maculatus*, one to seven day old *T. castaneum* and *S. zeamais* of mixed sex were introduced separately into the tube through the cicular hole by means of a 5 cm diameter funnel. The hole was then covered with sellotape and the set-up left for 24 hours. After this period, the insects found in the control and treatment sides

were counted. After each bioassay , the glass jars were cleaned and dried at 100°C. For *H. spicigera* oil, concentrations used were 20, 40, 60, 80 and 100 µl dissolved in 2 ml acetone. Blank controls were run periodically to compare insect response to both treated and control grain.

The percentage repellency (PR) values for all tests were computed as $PR = [(N_c-N_t)/(N_c+N_t)] \times 100$, (Hassanali *et al.*, 1990),

where, $N_c$ = no. of insects found on control side

$N_t$ = no. of insects found on treatment side

**3.10 Effect of isoeugenol, limonene and *H. spicigera* crude essential oil on seed viability**

Viability of maize and cowpea grains was assessed after treatment with the oils. 25 g batches of maize and cowpea seeds were treated separately with concentrations of 0, 3, 6, 9, 12 and 15 µl of isoeugenol and limonene solutions, or 10, 20, 30, 40 and 50 µl essential oil of *H. spicigera*. Cowpea and maize seeds were stored for a period of one and two months, corresponding to the life cycle of *C. maculatus* and *S. zeamais*, respectively. 40 seeds were placed in 10 cm diameter

petri-dish lined with Whatman No.1 filter paper and watered with distilled water. After five days the number of seeds that had germinated was recorded. Each treament was replicated three times.

### 3.11 Effect of leaf and seed extract of *H. spicigera* on oviposition and adult emergence of *C. maculatus*

This was determined following the method of Ellis (1990) with few modifications. Fifteen pairs of *C. maculatus* were placed on 25 g cowpea grains treated with 0, 30, 50, 70, 90, 110 and 130 µl seed and leaf extract of *H. spicigera*. These were dissolved in 1 ml acetone. Two weeks after confinement, the adult insects were removed and the number of eggs laid counted for each treatment. Every four days, the adults that emerged were also counted and removed from the assays till no emergence occurred.

### 3.12 Data analysis

The data obtained were analysed using analysis of variance following the method of Gomez and Gomez (1984) and mean comparison was done using the LSD method. Appropriate data transformation methods (arc sine and square root) were applied

where necessary. Abbott's (1925) formula for correction of control mortalities was applied where necessary, as shown below ;

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality \%}} \times 100$$

# CHAPTER 4

## 4.0 RESULTS

### 4.1 Contact toxicity by topical application

Toxicity by topical application of isoeugenol and limonene after 24 hours exposure to the three beetle species is presented in Table 3a. Both compounds were not toxic to *S. zeamais*, irrespective of the concentrations applied. Limonene was also not toxic to *C. maculatus*. Toxicity of both compounds was low because the highest doses killed less than 40% of the beetles exposed. The essential oil of *H. spicigera* was virtually non-toxic to the three insect species as shown in Table 3b.

### 4.2 Adult mortality in grain

Table 4a shows the percentage mortality of the three beetle species in grains treated with different concentrations of isoeugenol and limonene. Both compounds were not toxic to *S. zeamais* within the 24 hours of exposure, irrespective of concentration. Mortality of *C. maculatus* and *T. castaneum* in treated grains was also very low as the highest concentration of both compounds killed about 30% of the beetles exposed. In Table 4b, mortalities were significant in

both *C. maculatus* and *T. castaneum* though very low. However there was no effect on *S. zeamais*.

**Table 3a. Toxicity of different concentrations of isoeugenol and limonene applied topically to the three beetle species**

| Concentration (µl/ml) | Percentage mortality (Mean ±S.E) after 24 h | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| Isoeugenol | | | |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 4 ± 0.5a |
| 3 | 0 ± 0.0a | 0 ± 0.0a | 4 ± 2.0a |
| 6 | 0 ± 0.0a | 0 ± 0.0a | 8 ± 2.5b |
| 9 | 0 ± 0.0a | 0 ± 0.0a | 13 ± 1.5b |
| 12 | 0 ± 0.0a | 23 ± 0.8b | 20 ± 3.5c |
| 15 | 0 ± 0.0a | 30 ± 0.3c | 33 ± 1.5d |
| Limonene | | | |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 4 ± 2.5a |
| 3 | 0 ± 0.0a | 0 ± 0.0a | 10 ± 3.0b |
| 6 | 0 ± 0.0a | 0 ± 0.0a | 20 ± 1.0c |
| 9 | 0 ± 0.0a | 0 ± 0.0a | 20 ± 3.5c |
| 12 | 0 ± 0.0a | 0 ± 0.0a | 20 ± 1.0c |
| 15 | 0 ± 0.0a | 0 ± 0.0a | 37 ± 4.0d |

Mean of three replicates of 20 insects each. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

**Table 3b. Toxicity of different concentrations of *H. spicigera* oil applied topically to the three beetle species**

| Concentration (µl/ml) | Percentage mortality (Mean ±S.E) after 24 h | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 10 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 20 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 30 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 40 | 0 ± 0.0a | 1 ± 0.5a | 0 ± 0.0a |
| 50 | 0 ± 0.0a | 1 ± 0.3a | 1 ± 0.1a |

Mean of three replicates of 20 insects each. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test

**Table 4a. Percentage mortality of isoeugenol and limonene treated grain against the three beetle species**

| Concentration (µl/100g) | Percentage mortality (Mean ±S.E) after 24h | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| Isoeugenol | | | |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 12 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 24 | 0 ± 0.0a | 15 ± 1.5b | 8 ± 2.5b |
| 36 | 0 ± 0.0a | 15 ± 1.5b | 7 ± 2.5b |
| 48 | 0 ± 0.0a | 28 ± 5.0c | 10 ± 5.0b |
| 60 | 0 ± 0.0a | 32 ± 1.5c | 10 ± 2.5b |
| Limonene | | | |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 12 | 0 ± 0.0a | 0 ± 0.0a | 10 ± 1.0b |
| 24 | 0 ± 0.0a | 0 ± 0.0a | 25 ± 1.8c |
| 36 | 0 ± 0.0a | 0 ± 0.0a | 25 ± 1.0c |
| 48 | 0 ± 0.0a | 0 ± 0.0a | 29 ± 1.4c |
| 60 | 0 ± 0.0a | 16 ±1.5b | 30 ± 1.4c |

Mean of three replicates of 40 insects each. Values followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test

**Table 4b. Percentage mortality of *H. spicigera* oil treated grain against the three beetle species**

| Concentration (µl/ml) | Percentage mortality (Mean ±S.E) after 24 h | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| 0 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 10 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 20 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a |
| 30 | 0 ± 0.0a | 1 ± 0.1a | 0 ± 0.0a |
| 40 | 0 ± 0.0a | 4 ± 0.3b | 2 ± 0.2a |
| 50 | 0 ± 0.0a | 4 ± 0.2b | 3 ± 0.1b |

Mean of three replicates of 20 insects each. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

**4.3 Effect of isoeugenol and limonene on development of eggs and immature stages.**

Table 5 shows the number of *C. maculatus* adults that emerged in grains treated at different times after oviposition period with isoeugenol and limonene. Both compounds completely inhibited the developement of eggs and larvae of *C. maculatus* in treated grains up to two weeks. When eggs laid on grains, and first and second larval instars hidden in grains were treated with both compounds, no progeny emerged after six weeks compared with the controls. However, when treatments were applied three weeks after removal of adult *C. maculatus*, emergence occurred in both treatment and control grains suggesting that isoeugenol and limonene did not inhibit the development of pupae hidden within the grains.

Table 6 shows the number of *S. zeamais* adults that emerged from maize treated with isoeugenol and limonene at different times after oviposition. Significant number of eggs died in isoeugenol treated grains. Though there were significant differences in the number of adults produced from the larval and pupal stages eight weeks after oviposition, these were not concentration-dependent. Limonene was not toxic to the eggs, but in the immature stages, toxicity was also not

concentration-dependent especially in the first instar larvae (1 week) and pupae (3 weeks).

**Table 5. Mean number of *C. maculatus* adults that emerged from grains treated with isoeugenol and limonene at different times after oviposition period.**

| Concentration (µl/100g) | Time of treatment 24h | 1 week | 2 weeks | 3 weeks |
|---|---|---|---|---|
| Isoeugenol | | | | |
| 0 | 57 ± 1.5b | 62 ± 1.7c | 63 ± 2.0c | 78 ± 1.4d |
| 12 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 63 ± 12.4c |
| 24 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 71 ± 7.5d |
| 36 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 63 ± 7.8c |
| 48 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 81 ± 11.8e |
| 60 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 68 ± 10.2c |
| Limonene | | | | |
| 0 | 64 ± 1.4c | 73 ± 2.6d | 63 ± 2.0c | 82 ± 7.5e |
| 12 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 69 ± 6.7d |
| 24 | 0 ±0.0a | 0 ± 0.0a | 0 ± 0.0a | 70 ± 11.3d |
| 36 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 72 ± 9.0d |
| 48 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 72 ±3.8d |
| 60 | 0 ± 0.0a | 0 ± 0.0a | 0 ± 0.0a | 84 ± 4.3e |

Mean of three replicates. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

Table 7 also shows the number of *T. castaneum* adults that emerged from flour treated with isoeugenol and limonene at different times after oviposition. Isoeugenol and limonene had no effect on the development of eggs, larvae and pupae of *T. castaneum* in wheat flour. When eggs, first and second larval stages as well as pupae in wheat flour were treated with both compounds at different times after oviposition, adults emerged after five weeks.

**Table 6. Mean number of *S. zeamais* adults that emerged from grains treated with isoeugenol and limonene at different times after oviposition period.**

| Concentration (µl/100g) | Time of treatment 24h | 1 week | 2 weeks | 3 weeks |
|---|---|---|---|---|
| Isoeugenol | | | | |
| 0 | 530 ± 37.0d | 582 ±2 0.0d | 580 ± 88.0d | 651 ± 104.0e |
| 12 | 369 ± 16.0b | 580 ± 100.0d | 478 ± 68.0c | 492 ± 12.0c |
| 24 | 343 ± 37.0b | 686 ± 58.0e | 440 ± 40.0c | 431 ± 33.0c |
| 36 | 377 ± 39.0b | 540 ± 86.0d | 484 ± 44.0c | 587 ± 60.0d |
| 48 | 289 ± 38.0a | 616 ± 111.0e | 576 ± 48.0d | 577 ± 32.0d |
| 60 | 329 ± 22.0b | 549 ± 60.0d | 628 ± 32.0e | 649 ±3 9.0e |
| Limonene | | | | |
| 0 | 55 0± 41.0d | 540 ± 74.0d | 684 ± 60.0e | 621 ±7 4.0e |
| 12 | 435 ± 21.0c | 463 ± 8.0c | 613 ± 11.0e | 585 ± 67.0d |
| 24 | 501 ± 43.0d | 551 ± 38.0d | 552 ± 48.0d | 563 ± 12.0d |
| 36 | 583 ± 20.0d | 436 ± 21.0c | 578 ± 48.0d | 576 ± 60.0d |
| 48 | 559 ± 55.0d | 521 ± 29.0d | 540 ± 71.0d | 704 ± 28.0f |
| 60 | 533 ± 64.0d | 627 ± 75.0e | 562 ± 12.0d | 508 ± 33.0d |

Mean of three replicates. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

**Table 7. Mean number of *T. castaneum* adults that emerged from flour treated with isoeugenol and limonene at different times after oviposition period.**

| Concentration (µl/100g) | Time of treatment 24h | 1 week | 2 weeks | 3 weeks |
|---|---|---|---|---|
| **Isoeugenol** | | | | |
| 0 | 15 ± 1.7d | 12 ± 1.7a | 12 ± 2.0a | 18 ± 2.0e |
| 6 | 11 ± 3.0a | 12 ± 2.0a | 13 ± 2.0b | 17 ± 1.0d |
| 12 | 15 ± 4.0d | 11 ± 1.0a | 10 ± 2.0a | 20 ± 3.0f |
| 18 | 13 ± 3.6b | 9 ± 1.0a | 12 ± 2.0a | 20 ± 1.0f |
| 24 | 12 ± 2.5a | 22 ± 3.0f | 16 ± 3.0d | 19 ± 1.0e |
| 30 | 11 ± 2.6a | 18 ± 0.8d | 10 ± 1.0a | 19 ± 2.0e |
| Limonene | | | | |
| 0 | 15 ± 0.3d | 10 ± 2.0a | 15 ± 1.0d | 12 ± 2.0a |
| 6 | 7 ± 0.5a | 8 ± 1.0a | 8 ± 1.0a | 17 ± 2.0d |
| 12 | 11 ± 2.0d | 12 ± 2.0a | 11 ± 1.0a | 22 ± 2.0f |
| 18 | 15 ± 1.1d | 13 ± 1.0b | 15 ± 1.6d | 16 ± 1.0d |
| 24 | 10 ± 3.0a | 13 ± 1.0b | 14 ± 2.0c | 16 ± 1.0d |
| 30 | 11 ± 2.0a | 13 ± 2.0b | 20 ± 2.0f | 19 ± 2.0e |

Mean of three replicates. Means followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

### 4.4 Effect of isoeugenol and limonene on progeny production

Table 8 shows the number of adults produced in grains treated with isoeugenol and limonene, where parent adults were introduced the same day after treatment and removed at the end of the oviposition periods. There were significant differences among the number of progenies produced by the insects but this was not concentration-dependent.

**Table 8. Number of emerged adults in grains treated with isoeugenol and limonene**

| Concentration (µl/100g) | No. of adults emerged ±S.E | | |
|---|---|---|---|
| | *S. zeamais* | *T. castaneum* | *C.maculatus* |
| Isoeugenol | | | |
| 0 | 163 ± 3.7e | 18 ± 1.7b | 57 ± 6.9e |
| 12 | 57 ± 4.9a | 16 ± 3.8b | 27 ± 0.8b |
| 24 | 107 ± 22.0c | 25 ± 2.4c | 34 ± 4.4b |
| 36 | 89 ± 17.0b | 37 ± 7.9e | 48 ± 7.7d |
| 48 | 66 ± 14.0a | 32 ± 3.0d | 44 ± 6.5d |
| 60 | 85 ± 10.0b | 37 ± 1.3e | 57 ± 6.9e |
| Limonene | | | |
| 0 | 183 ± 2.0d | 10 ± 1.7a | 63 ± 9.7e |
| 12 | 55 ± 5.5a | 15 ± 3.0b | 18 ±2.0a |
| 24 | 164 ± 29.0e | 21 ± 0.5c | 48 ±18.4c |
| 36 | 78 ± 11.0b | 32 ± 5.1d | 56 ±17.0d |
| 48 | 148 ± 12.7d | 21 ± 3.0c | 68 ±14.0e |
| 60 | 64 ± 9.0a | 17 ± 0.0b | 42 ±12.4c |

Mean of three replicates of 40 insects each. Values followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

### 4.5 Effect of isoeugenol and limonene on seed damage caused by *S. zeamais* and *C. maculatus*

Maize seeds treated with isoeugenol and limonene and infested with *S. zeamais* showed variable weight losses and percent number of damaged seeds. The highest weight loss and number of damaged seeds occurred in the control while among the treatments, the highest weight loss and number of damaged seeds occurred in seeds treated with the lowest concentration of 12 µl/100g for both isoeugenol and limonene (Figs. 4 and 5).

Percent weight loss and percent number of damaged seeds in isoeugenol and limonene treated cowpea seeds infested with *C. maculatus* followed a similar pattern (Figs.6 and 7). However, seeds treated with 24 µl/100g of limonene had more seeds damaged compared with seeds treated with 36 µl/100g, but the weight loss in 36 µl/100g was greater.

Fig. 4. Percentage number of isoeugenol and limonene treated maize seeds damaged by *S. zeamais* after 8 weeks of storage

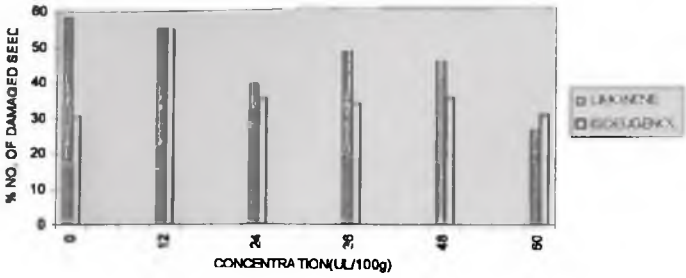



Fig. 5. Percentage weight loss of isoeugenol and limonene treated maize seeds as a result of feeding by *S. zeamais* for eight weeks

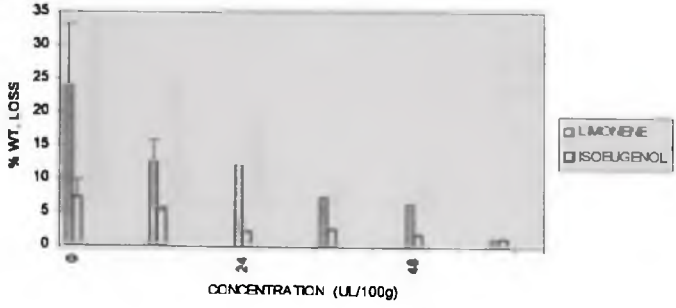


Fig. 6. Percentage number of isoeugenol and limonene treated cowpea seeds damaged by *C. maculatus* after eight weeks of storage

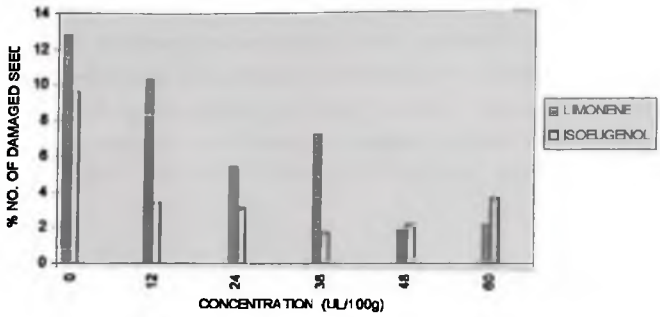



Fig. 7 Percentage weight loss of isoeugenol and limonene treated cowpea seeds as a result of feeding by *C. maculatus* for five weeks

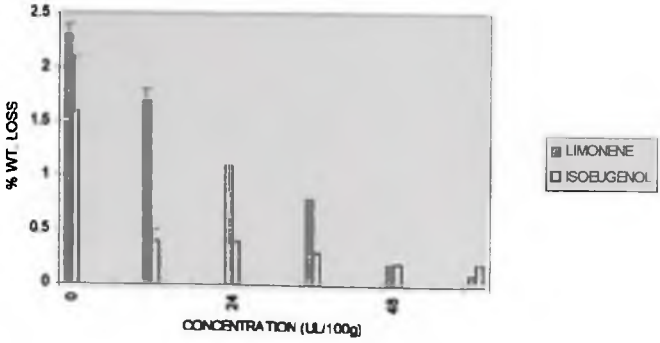


Table 9 shows the weight of dust produced at each treatment by *S. zeamais* as a result of feeding on isoeugenol and limonene treated maize grain for eight weeks. Weights of dust produced in isoeugenol and limonene treated grains were concentration-dependent and significantly different from the controls.

**Table 9. Mean weight (g) of dust produced from isoeugenol and limonene treated maize grain as a result of feeding by *S. zeamais***

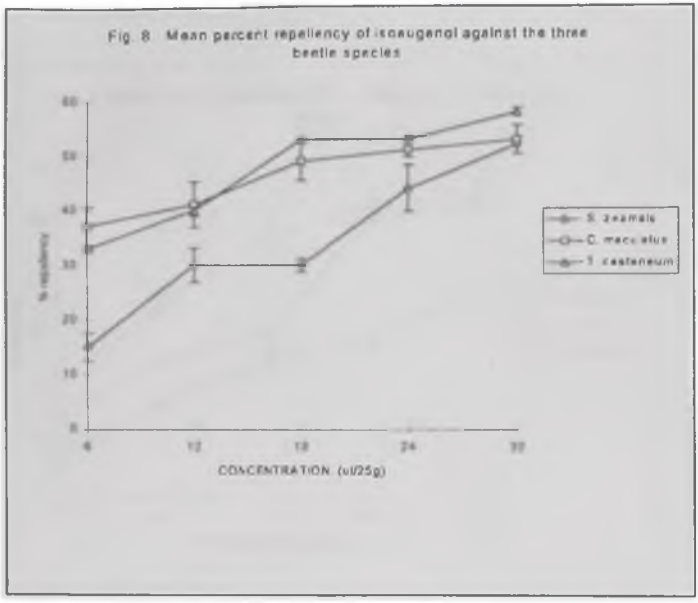
| Concentration (µl/100g) | Mean weight of dust (g) ±S.E |
|---|---|
| Isoeugenol | |
| 0 | 0.8 ± 0.15c |
| 12 | 0.5 ± 0.09b |
| 24 | 0.7 ± 0.15b |
| 36 | 0.5 ± 0.09b |
| 48 | 0.3 ± 0.06a |
| 60 | 0.4 ± 0.12a |
| Limonene | |
| 0 | 1.1 ± 0.58c |
| 12 | 1.0 ± 0.09c |
| 24 | 0.7 ± 0.10b |
| 36 | 0.4 ± 0.12a |
| 48 | 0.9 ± 0.09b |
| 60 | 0.7 ± 0.06b |

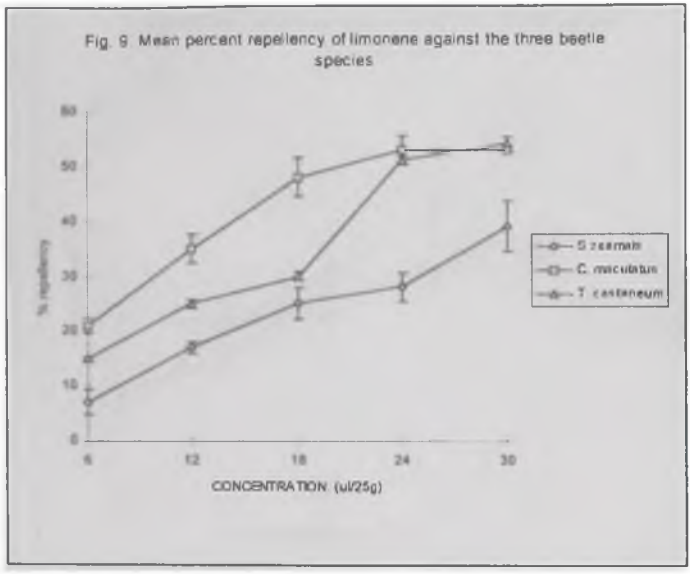
Mean of three replicates of 40 insects each. Values followed by the same letter(s) in each column are not significantly different at the 0.05 level. LSD test.

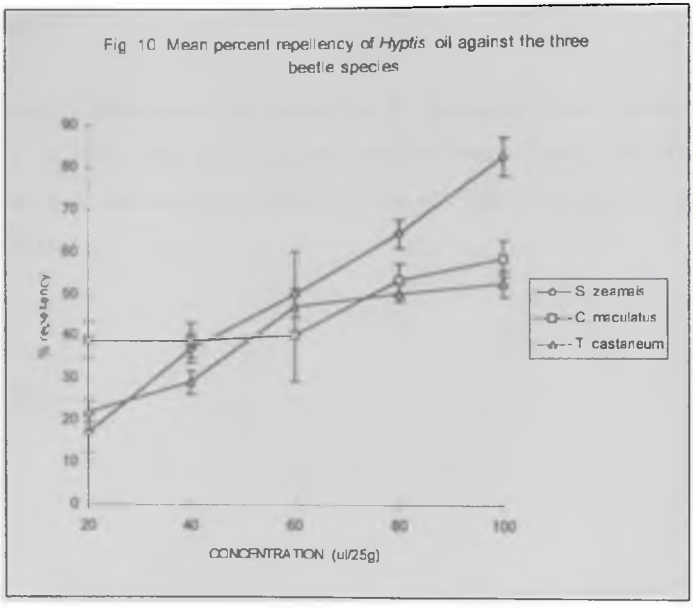
### 4.6 Repellency Tests

Isoeugenol evoked moderate repellency against the three beetle species. The highest repellency was recorded against *T. castaneum* (Fig. 8). Limonene also evoked moderate repellency against *C. maculatus* and *T. castaneum*. Very low repellent action was observed against *S. zeamais* (Fig. 9 ).

The crude essential oil of *H. spicigera* evoked the strongest repellency against *S. zeamais* but was moderately repellent against *T. castaneum* and *C. maculatus*. Repellency was also concentration-dependent ( Fig. 10).







### 4.7 Germination Tests

Table 10 shows the effect of isoeugenol and limonene on the germination of maize and cowpea seeds stored for 8 and 4 weeks, respectively. These two chemicals had no effect on seed viability. All the treated seeds germinated after five days.

Table 11 also shows the effect of *H. spicigera* crude essential oil on viability of maize and cowpea seeds stored for eight and four weeks, respectively. The oil had no effect on seed viability.

**Table 10. Percentage germination of Isoeugenol and Limonene treated maize and cowpea seeds**

| Concentration (µl/25g) | Mean percent germination±S.E | |
|---|---|---|
| | Maize | Cowpea |
| Isoeugenol | | |
| 0 | 100 ± 0.0a | 100 ± 0.0a |
| 3 | 100 ± 0.0a | 100 ± 0.0a |
| 6 | 100 ± 0.0a | 100 ± 0.0a |
| 9 | 100 ± 0.0a | 100 ± 0.0a |
| 12 | 97 ± 0.0a | 100 ± 0.0a |
| 15 | 100 ± 0.0a | 100 ± 0.0a |
| Limonene | | |
| 0 | 100 ± 0.0a | 100 ± 0.0a |
| 3 | 100 ± 0.0a | 100 ± 0.0a |
| 6 | 100 ± 0.0a | 100 ± 0.0a |
| 9 | 100 ± 0.0a | 100 ± 0.0a |
| 12 | 100 ± 0.0a | 100 ± 0.0a |
| 15 | 100 ± 0.0a | 100 ± 0.0a |

Mean of three replicates of 40 seeds each. Means are not significantly different at 0.05 level. LSD test.

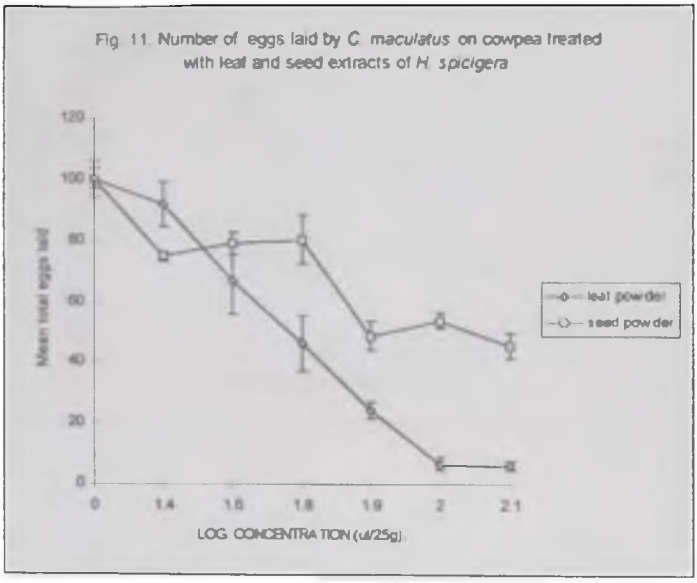
**Table 11. Percentage germination of *H. spicigera* crude essential oil treated seeds**

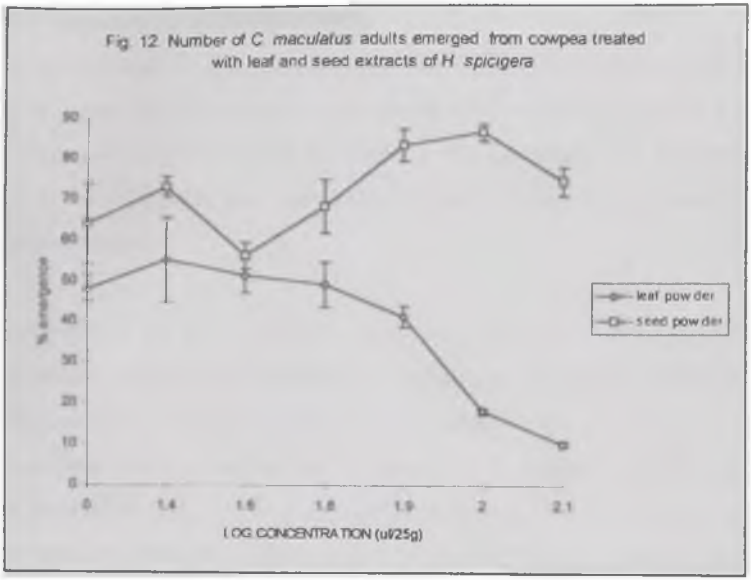
| Concentration (µl/25g) | Mean percent germination± S.E | |
|---|---|---|
| | Maize | Cowpea |
| 0 | 100 ± 0.0a | 100 ± 0.0a |
| 10 | 100 ± 0.0a | 100 ± 0.0a |
| 20 | 100 ± 0.0a | 100 ± 0.0a |
| 30 | 100 ± 0.0a | 100 ± 0.0a |
| 40 | 100 ± 0.0a | 100 ± 0.0a |
| 50 | 100 ± 0.0a | 100 ± 0.0a |

Mean of five replicates of 10 seeds each. Values followed by the same letter(s) are not significantly different at 0.05 level. LSD test

### 4.8 Effect of leaf and seed extracts of *H. spicigera* on oviposition of *C. maculatus*

Increasing concentration of seed or leaf extract of *H. spicigera* decreased oviposition of *C. maculatus* ( Fig. 11 ). More eggs were laid on cowpea treated with seed extract than cowpea treated with leaf extract. For instance, at concentration of 130 µl/25g, 6 and 45 eggs were laid on leaf and seed extract treated beans, respectively. Also emergence of adults declined with increase in concentration, noticeably from 50 µl/25g of the leaf extract. On the other hand, the trend in adult emergence in seed extract did not show a clear pattern (Fig. 12).





# CHAPTER 5

## 5.0 DISCUSSION

### 5.1 Toxicity of plant products.

In the present study, isoeugenol and limonene were not toxic to *S. zeamais* but killed over 30% of the *C. maculatus* and *T. castaneum* exposed within 24 hours. The essential oil extract of *H. spicigera* was non-toxic to any of the three beetle species tested.

Obeng-Ofori *et al.*, (1997a), demonstrated that 1,8 cineole, a major component of essential oil extract of *Ocimum kenyense* (Ayobangira) was highly toxic to *S. zeamais, S. granarius, T. castaneum* and *P. truncatus.* Isoeugenol is a minor component of essential oil of *O. kenyense* comprising 8% of the total collection (Bekele, 1994). The inability of isoeugenol to cause any appreciable mortalities even at higher concentrations to these insects suggests that the toxic effect of the crude essential oil of *O. kenyense* demonstrated by Bekele (1994) was mainly due to the major component 1,8 cineole which was 37% of the total collection. The same reason can be attributed to limonene which accounted for 6% of the total collection of essential oil extract of *O.*

*kilimandscharicum*. The major component, camphor comprised about 70% of the total collection and evoked 100% mortalities of *S. granarius, S. zeamais* and *P. truncatus* at even lower levels (Obeng-Ofori *et al.*, 1997b). This could also mean that the toxic effect of essential oil extract of *O. kilimandscharicum* demonstrated in the laboratory by Bekele (1994) might be attributed mostly to the major component, camphor.

**5.2 Effect on developmental stages**

Isoeugenol and limonene caused complete inhibition of the development of eggs and larvae of *C. maculatus* but had no effect on the pupae and all stages of *S. zeamais* and *T. castaneum* tested (Tables 4, 5 & 6). It could be that like the adults, the compounds were not toxic to the various developmental stages of *S. zeamais* or did not penetrate the grains and therefore had no contact with the eggs, larvae and pupae of *S. zeamais*.

**5.3 Insect damage to treated grains**

Maize seeds treated with isoeugenol and limonene were damaged due to feeding by *S. zeamais* but the damage was not as extensive as that for the controls (Fig. 4). Similar results

were obtained for *C. maculatus* on treated cowpea grain (Fig. 6). However, the number of $F_1$ progenies did not correlate with the extent of damage(Table 8). This is in contrast with Bekele,(1989) and Cotton (1963),( cited by Bekele, 1994) that under conditions favourable for storage insects, the extent of damaged is related to the number of insects present. The rather low persistence of limonene or isoeugenol might explain the discrepancy obtained in the present study.

Weight loss caused by *S. zeamais* and *C. maculatus* was significantly lower in isoeugenol and limonene treated maize and cowpea seeds, compared to the untreated seeds. Since the two compounds were not toxic to all stages of *S. zeamais* when applied topically or mixed with maize grains, they might have acted as antifeedants by reducing insect feeding and subsequent weight loss and quantity of dust produced. According to Nawrott *et al.*, (1989), some secondary plant metabolites may act as both insecticides and antifeedants as observed for rotenone against *T. castaneum* and other rotenoids against lepidopterous pests such as *Spodoptera exempta* Wlk., *Eldana saccharina* Wlk.and *Maruca testulalis*(Geyer) (Hassanali and Lwande, 1989).

**5.4 Repellency effects on the insects**

The esential oil of *H. spicigera* evoked very strong repellent action against *S. zeamais* and moderate repellency against *C. maculatus* and *T. castaneum* (Fig.10). According to Abbiw (1990), small-scale West African farmers mix leaves of *H. spicigera* with grains as protectants. Also the leaves are burned to repel mosquitoes. The results confirm the scientific basis behind the use of the plant as a repellent. Identification of the constituents of the oil would throw more light on the biologically active compounds responsible for its repellent effect.

Similarlly, isoeugenol and limonene showed repellent activity against *T. castaneum* and *C. maculatus* but the repellency was low against *S. zeamais*. All the three beetles are highly specialized to stored products and are mainly confined to stores, and their sensitivity to volatile secondary compounds from exotic sources may be a reflection of this specialized mode of life. Bekele (1994), showed out that the essential oil extract of *O. kenyense* and *O. kilimandscharicum* gave repellency of 73% and 79% at 750 mg/ 250g seeds, respectively. The major components of essential oil extract of *O. kenyense* and *O. kilimandscharicum* which are

1,8 cineole and camphor, respectively also showed moderate repellent action against *S. zeamais, S. granarius, T. castaneum* and *P. truncatus* (Obeng-Ofori *et al.*, 1997a). From the results of this study, it can be concluded that isoeugenol and limonene which are minor components of the essential oils of these plants contributed enormously to the results obtained by Bekele (1994). Thus the repellency could be additive considering the various components in the essential oil extracts. This additive effect may confirm why crude essential oil extract of *H. spicigera* had higher repellent activity to the three beetles, especially against *S. zeamais*. This repellent action increases the protectant potential of these products for protection of foodstuffs against insect pest infestation in storage.

**5.5 Effect on germination**

All the oils tested had no effect on the germination of maize and cowpea seeds. Earlier, Bekele (1994) reported that essential oils of *O. kenyense* and *O. kilimandscharicum* did not have significant effect on the germination of seeds. Thus these products may be used to protect seed stock for subsequent propagation.

**5.6 Efeect of crude extracts of *H. spicigera* on oviposition and adult emergence of *C. maculatus***

Leaf and seed extracts of *H. spicigera* caused a highly significant reduction in the number of eggs laid by *C. maculatus*. This result confirms that of Ellis (1990) who observed a decline in the oviposition of the beetle with increasing concentration of acetone extract of *H. spicigera*.

Similarlly, adult emergence also declined with increasing concentration of the leaf and seed extracts of *H. spicigera*. It is possible the oils might have affected the development of the eggs by depriving them of oxygen. Isolation, identification and testing of the various constituents would help to explain the results better.

# CONCLUSIONS

From the study, the following conclusions can be made.

1. Isoeugenol and limonene killed at least 30% of *C. maculatus* and *T. castaneum*.
2. Isoeugenol and limonene did not inhibit the development of the different stages of *S. zeamais* and *T. castaneum* but inhibited the development of eggs and larvae of *C. maculatus*.
3. Adults that survived the toxicity of the two essential oils could live to reproduce their $F_1$ progenies.
4. Maize and cowpea seeds treated with isoeugenol and limonene were less damaged by the beetles compared to the controls.
5. The essential oil extract of *H. spicigera* evoked the strongest repellent activity against *S. zeamais* .
6. All the oils did not have effect on the viability of maize and cowpea seeds and hence did not affect their germination.
7. Acetone extracts of leaf and seed powder of *H. spicigera* caused a decline in oviposition of *C. maculatus*.

# RECOMMENDATIONS

1. Identification and evaluation of the biologically active components of the essential oil of *H. spicigera* needs to be carried out.
2. Determination of the specific mode of action of *H. spicigera* oils and their effect on mammals fed on treated grain also needs to be evaluated.
3. Preparation of plant-based insecticides and repellents for use by resource-poor farmers needs to encouraged.

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# APPENDICES

**Analysis of variance tables**

Appendix 1(Table3)

Toxicity of isoeugenol and limonene by topical application

*T. [illegible]*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 16.367 | 1.488 | 1.743 | 0.123 |
| Residual | 24 | 20.490 | 0.854 | | |

*C. maculatus*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 27.318 | 2.483 | 198.108 | 0.0001 |
| Residual | 24 | 0.301 | 0.013 | | |

Appendix 2 (Table 4)

Toxicity of isoeugenol and limonene on treated grains

*T. castaneum*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 57.816 | 5.256 | 38.722 | 0.0001 |
| Residual | 24 | 3.258 | 0.136 | | |

*C. maculatus*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 75.810 | 6.892 | 603.998 | 0.0001 |
| Residual | 24 | 0.274 | 0.011 | | |

Appendix 3

Toxicity of isoeugenol and limonene on different stages of life cycle

*C. maculatus* (Table 5)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 47 | 2317.638 | 49.311 | 234.691 | 0.0001 |
| Residual | 96 | 20.171 | 0.210 | | |

*S. zeamais* (Table 6)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 47 | 3987.367 | 84.838 | 23.748 | 0.0001 |
| Residual | 96 | 342.955 | 3.572 | | |

*T. castaneum* (Table 7)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 47 | 36.835 | 0.784 | 2.539 | 0.0001 |
| Residual | 96 | 29.631 | 0.309 | | |

**Appendix 4 (Table 8)**

Progeny production of adults emerged in grains treated with isoeugenol and limonene

*S. zeamais*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 167.257 | 15.205 | 10.184 | 0.0001 |
| Residual | 24 | 35.833 | 1.493 | | |

*T. castaneum*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 29.575 | 2.689 | 8.192 | 0.0001 |
| Residual | 24 | 7.877 | 0.328 | | |

**Appendix 5**

Percent weight loss of isoeugenol and limonene treated seeds as a results of feeding

*S. zeamais* (Fig. 5)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 1541.386 | 140.126 | 1.596 | 0.046 |
| Residual | 24 | 2019.755 | 87.815 | | |

Table 8. Mean percentage weight loss of isoeugenol and limonene treated maize grain infested with *S. zeamais*

| Concentration (µl/100g) | Mean percent weight loss ±S.E |
|---|---|
| Isoeugenol | |
| 0 | 7.3 ± 2.6b |
| 12 | 5.5 ± 1.3b |
| 24 | 2.3 ± 0.4a |
| 36 | 2.7 ± 0.3a |
| 48 | 1.9 ± 0.2a |
| 60 | 1.2 ± 0.6a |
| Limonene | |
| 0 | 24.1 ± 9.1d |
| 12 | 12.6 ± 7.8c |
| 24 | 12.2 ± 8.0c |
| 36 | 7.5 ± 3.3b |
| 48 | 6.4 ± 8.5b |
| 60 | 1.3 ± 0.6a |

Mean of three replicates of 40 insects each. Values followed by the same letter(s) are not significantly different at the 0.05 level. LSD test.

*C. maculatus* (Fig. 6)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 152.264 | 13.842 | 2.682 | 0.021 |
| Residual | 24 | 123.859 | 5.161 | | |

Mean percentage weight loss of isoeugenol and limonene treated cowpea infested with *C. maculatus*

| Concentration (µl/100g) | Mean percent weight loss ±S.E |
|---|---|
| Isoeugenol | |
| 0 | 1.6 ± 0.5b |
| 12 | 0.4 ± 0.1a |
| 24 | 0.4 ± 0.1a |
| 36 | 0.3 ± 0.1a |
| 48 | 0.3 ± 0.1a |
| 60 | 0.3 ± 0.1a |
| Limonene | |
| 0 | 2.3 ± 0.1c |
| 12 | 1.7 ± 0.3b |
| 24 | 1.1 ± 0.4b |
| 36 | 0.8 ± 0.1b |
| 48 | 0.2 ± 0.1a |
| 60 | 0.1 ± 0.0a |

Mean of three replicates of 40 insects each. Values followed by the same letter(s) are not significantly different at the 0.05 level. LSD test.

Appendix 6 (Table 9)
Mean weight of dust produced as a result of feeding by *S. zeamais* on isoeugenol and limonene treated maize grain

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 11 | 31.047 | 2.822 | 2.558 | 0.026 |
| Residual | 24 | 26.483 | 1.103 | | |

**Appendix 7**

**Mean percent repellency of isoeugenol and limonene against the three beetle species**

*S. zeamais* (Figs 8 & 9)

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 9 | 2301.321 | 255.703 | 22.755 | 0.0001 |
| Residual | 20 | 224.743 | 11.237 | | |

*C. maculatus*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 9 | 1107.861 | 123.096 | 14.65 | 0.0001 |
| Residual | 20 | 168.021 | 8.401 | | |

*T. castaneum*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 9 | 2187.668 | 243.074 | 26.346 | 0.0001 |
| Residual | 20 | 184.527 | 9.226 | | |

Mean percent repellency (PR) values for different concentrations of isoeugenol and limonene against each of the three beetle species

| Concentration (µl/50g) | Mean percent repellency ± S.E | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| Isoeugenol | | | |
| 6 | 15 ± 2.6b | 37 ± 3.6b | 33 ± 0.6bc |
| 12 | 30 ± 3.2d | 41 ± 4.2bc | 40 ± 1.1c |
| 18 | 30 ± 1.3d | 49 ± 3.5cd | 53 ± 0.3d |
| 24 | 44 ± 4.3ef | 51 ± 1.3d | 53 ± 0.5d |
| 30 | 52 ± 1.5f | 53 ± 2.6d | 58 ± 0.8d |
| Limonene | | | |
| 6 | 7 ± 2.3a | 21 ± 1.3a | 15 ± 0.3a |
| 12 | 17 ± 1.0bc | 35 ± 2.6b | 25 ± 0.8b |
| 18 | 25 ± 2.8cd | 48 ± 3.6cd | 30 ± 0.8b |
| 24 | 28 ± 2.6d | 53 ± 2.5d | 51 ± 0 8d |
| 30 | 39 ± 4.6e | 53 ± 0.6d | 54 ± 1.2d |

Mean of three replicates of 50 insects each. Values followed by the same letter(s) in each column are not significantly different at 0.05 level. LSD test.

Appendix 8 (Fig. 10)
Mean percent repellency of crude essential oil of *H. spicigera* against the three beetle species

*S. zeamais*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 4 | 5128.544 | 0.1280 | 16.545 | 0.0001 |
| Residual | 20 | 1549.864 | 77.493 | | |

*C. maculatus*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 4 | 2516.888 | 129.222 | 2.637 | 0.0001 |
| Residual | 20 | 979.977 | 48.999 | | |

*T. castaneum*

| Source of variation | DF | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| Treatment | 4 | 2187.668 | 243.074 | 26.346 | 0.0001 |
| Residual | 20 | 184.527 | 9.226 | | |

Mean percent repellency (PR) values for different concentrations crude essential oil of *H. spicigera* against each of the three beetle species

| Concentration (µl/50g) | Mean percent repellency±S.E | | |
|---|---|---|---|
| | *S. zeamais* | *C. maculatus* | *T. castaneum* |
| 20 | 17 ± 4.6a | 39 ± 4.5a | 22 ± 2.7a |
| 40 | 37 ±3 4b | 39 ± 3.9a | 29 ± 2.7b |
| 60 | 50 ± 10.0c | 40 ± 11.0a | 47 ± 2.6c |
| 80 | 64 ± 3.4d | 53 ± 4.0b | 50 ± 2.0c |
| 100 | 82 ± 4.5e | 58 ± 4.2c | 52 ± 3.1c |

Mean of five replicates of 50 insects each. Values followed by the same letter(s) are not significantly different at 0.05 level. LSD test.

Appendix 9(Fig. 12)

Oviposition of *C. maculatus* on cowpea treated with acetone extracts of *H. spicigera*.

| Concentration (µl/25g) | No. of eggs laid ±S.E | |
|---|---|---|
| | Leaf | Seed |
| 0 | 169 ± 5.8e | 95 ±3.4d |
| 30 | 155 ± 7.3e | 71 ± 1.7c |
| 50 | 113 ± 11.1d | 75 ± 3.7c |
| 70 | 78 ± 9.1c | 76 ± 8.1c |
| 90 | 41 ± 2.7b | 46 ± 4.8b |
| 110 | 11 ± 2.0a | 51 ± 2.6b |
| 130 | 10 ± 1.4a | 43 ± 4.0b |

Mean of three replicates of 15 pairs of insects each. Values followed by the same letter(s) are not significantly different at the 0 05 level. LSD test.