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EFFECT OF SMOKE FROM *SENNA SIAMEA* (LAMBK) ON *SITOPHILUS ZEAMAIS* (MOTSCHULSKY) IN STORED MAIZE

BY

ANSAH, KWAME DUODU

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EFFECT OF SMOKE FROM *SENNA SIAMEA* (LAMBK) (FABACEAE) ON

SITOPHILUS ZEAMAIS (MOTSCHULSKY) (COLEOPTERA: CURCULIONIDAE) IN

STORED MAIZE

A Thesis submitted to the School of Graduate Studies, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, in partial fulfillment of the requirement for the award of MPhil. Crop Protection (Entomology) degree.

ANSAH, KWAME DUODU

BSc. Agriculture

JUNE 2009

DECLARATION

I hereby declare that this research work presented in this thesis is my own work and that, to the best of my knowledge, it contains no material previously published by another person for the award of a degree in any other University, except where acknowledgement has been made in the text

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Ansah, Kwame Duodu

(Student)

02-10-09

Date

.....

Dr. J.V.K. Afun

(Supervisor)

05/10/2009

Date

.....

Dr. J.V.K Afun

(Head of Department)

5th Oct. 2009

Date

ABSTRACT

The use of botanical protectants in traditional management of storage pests in the tropics cannot be overemphasized. Earlier studies on the use of smoke from twigs of *Senna siamea* (Lambk) (Syn. *Cassia Siamea*) revealed that the smoke minimized insect pest damage and prevented the growth of some storage fungi. The objective of the study was to evaluate the insecticidal, repellence, persistence and fungicidal properties of the smoke on maize in smoke fumigation chambers. In a completely randomized design, clean and whole-grained shelled and dehusked Obatanpa maize variety was infested and fumigated against the various developmental stages of *Sitophilus zeamais* (Motshulsky) (Coleoptera:Curculionidae). The differences in numbers of the weevil that emerged when maize containing the different developmental stages of the weevil was fumigated, were not significant. The smoke did not exhibit any significant insecticidal effect on the insects on dehusked (intact) cobs. Increasing the duration of fumigation from 1 hour to 4 hours, had no significant effect on the insects on shelled maize. Fumigating undehusked cobs, for 1, 2, 3 and 4 hours and sampled at 1, 4, and 8 weeks after fumigation, showed significant reduction in infestation and damage when compared with unfumigated maize for all the durations of fumigation. To assess the repellency of the smoke, a modified Mohan and Field (2002) technique was used. Plastic bottles; measuring 20 cm x 5 cm (height and base diameter respectively) with holes created at an interval of 1 cm were

used. The set - up was replicated three times with 300 g of maize fumigated for 4 h. The greatest number of the weevil was repelled during the 1st hour of exposure with decreasing numbers at 24h and 48 h. Three hundred grams of shelled maize was also fumigated for 4 h and assessed for contact toxicity. Mortality was recorded at 1, 24 and 48 hours after fumigation. The fumigated maize grains did not exhibit any contact toxicity on the pest. Fumigating the adult *S. zeamais* for 1 or 4 hours did not kill the weevils. Obatanpa maize variety, fumigated for 1, 2, 3 and 4 hours, were evaluated for their health status using the Blotter method. There were no significant differences in the incidence of *Aspergillus flavus, Fusarium verticelloides* and *Penicillium* sp after fumigation. Although the fumigated maize had no contact toxicity and the smoke did not kill the fumigated weevils, the fumigation exhibited a repellent activity on *S. zeamais*. The twigs of *S. siamea* can therefore be used in traditional post harvest systems where fire is set under maize barns and platforms. Burning the twigs under such structures will result in the pest been repelled by the smoke.

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DEDICATION

To God as first fruit offering

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CHAPTER ONE

INTRODUCTION

Maize (*Zea mays* L.) is the most domesticated and evolutioned plant of the vegetal kingdom (FAO, 2007). It is one of the most important staple crops in Ghana and in terms of production, it ranks only after the roots and tubers and plantain (MoFA, 2001). About 793 000 ha of arable land was put to maize cultivation in the year 2006 with a yield of 1 188 840 Mt in Ghana (MoFA, 2007). The crop is cultivated in all the 10 regions of the country with the Eastern region being the largest producer (MoFA, 2001).

Every part of the maize crop has economic value; the grain, leaves, stalk, tassel and cob can be used to produce a wide variety of food and non food products. The crop is used as food for human consumption, animal feed and for many industrial purposes (Morris, 2001, FAO, 2007). In Ghana, the bulk of maize produced is directly used for human consumption. Out of the 1158 000 Mt produced in 2004, 810 000 Mt were for human consumption. It thus has the greatest (42.5 kg / annum) per capita consumption among the cereals (MoFA, 2004).

Larger and Hill (1991), reported that the maize crop is a valuable source of carbohydrates (70 - 72%), protein (9.5-11%) and oils (4-4.5%). In Ghana, it is an important source of protein ranking only after meat, fish and legumes in terms of annual protein consumption (Twumasi-Afriyie *et al.*, 1992). Despite its importance, the crop is subject to severe losses both on the field and in storage. The losses during the short period of storage from

harvest to sale at the farm gate have been estimated to range between 10-20% (FAO, 1991).

Organisms that attack maize include rodents (Gwinner *et al.,* 1996), fungi and insects (Beti *et al.,* 1995), with fungi and insects being the most important (Pitt and Hocking, 1996).

The problem of fungal infection of post-harvest grain is mainly due to high moisture content or inadequate drying before storage (FAO, 1980) but the interaction of temperature with moisture is critical for rate of fungal spoilage (Burrell *et al.,* 1980).
Several insect pests attack stored maize. Vowotor *et al* (2005), reported that the dominant insect pests of maize stored in West Africa are the Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). Earlier on, Ofosu (1980) had reported the maize weevil, *S. zeamais* as the most important primary pest of stored maize in Ghana. According to Youdeowei and Service (1986), about 15% of maize grains harvested in Ghana are lost annually to *S. zeamais*.

Damage caused consists mainly of reduction in dry matter, contamination with insect faeces, dead or live insects and depreciation of nutritional and commercial values of end products as well as loss of viability through their feeding and metabolic activities (Caneppele *et al.,* 2003). In addition, the weevil's attack predisposes the stored grains to fungal colonization (Beti *et al.*, 1995; Osei-Akrasi, 1999) and infestation by secondary

insect pest such as *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae)

Some of the storage fungi produce Mycotoxins (secondary metabolites produced by moulds), which cause mycotoxicosis in both animals and humans (D'mello *et al.*, 1997).

Management of these insect pests is achieved mainly through the use of synthetic chemicals, physical and biological control, resistant varieties and botanicals. The use of synthetic chemical to control storage pest, even though continues to play an important role in reducing storage losses due to insect pest activities (Niber, 1994), raises issues such as development of insect resistance (Perez-Mendoza, 1999), toxic residues in food and the environment, killing of beneficial and non-target insects, worker's safety and the high cost of the chemicals (Niber, 1994; Obeng-Ofori and Reichmuth, 1997; Asawalam and Hassanali 2006). Physical means, though environmentally friendly, do not provide maize with residual protection and requires specific environmental conditions over an extended time (Kumar, 1984). In biological control methods, the technicalities involved, such as survey for hyperparasitism, superparasitism and the general impact on natural enemies serves as a disincentive to farmers.

There are maize varieties that have been bred with traits that are known to contribute to maize weevil resistance but resistance to the maize weevil is not available in improved varieties (Derera *et al.*, 2001; Thanda and Pixley 2001; Kim and Kossou, 2003).

The use of botanical protectants have played important role in traditional method of storage pest control in the tropics (Hassanali *et al.*, 1990; Niber, 1994; Bekele and Hassanali, 2001). It is patronized and suitable for small scale farmers as it is eco-friendly, non toxic to consumers and readily available (Hassanali *et al.*, 1990; Niber, 1994; Asawalan *et al.*, 2006).

Smoke has been reported to contain phenolic compounds with insecticidal, fungistaltic and fungitoxic properties (Zagory and Parmeter, 1984). Smoke fumigation is not new to peasant farmers who produce the bulk of maize in the country. They set fire under maize cribs to generate smoke intended to prevent and or protect the maize from pest attack. According to Hell *et al.* (2000), lower aflatoxin contamination is associated with smoke fumigation of maize in Benin. Awuah (2005) also reported that smoke from twigs of *S. siamea* (Lambk) have fungitoxic and insecticidal properties. *S. siamea* also known as the Cassod tree or Siamese cassia is an evergreen tree which grows fast and under optimum conditions can reach 30 m in height. It has a spreading, multibranched crown with dense foliage. It has medicinal properties as the leaves, flowers and heartwood are used to cure a variety of ailments and as laxatives (Padumanonda and Gritsanapan, 2006).

This study evaluated the effectiveness of smoke from *S. siamea* in controlling *S. zeamais* and maize storage fungi.

The specific objectives of the study were

- To determine the effect of the smoke on the developmental stages of *S. zeamais* in maize in a fumigation chamber.
- To determine the effect of the smoke on *S. zeamais* damage and weight loss of maize.
- To evaluate the insecticidal effect of the smoke on adult *S .zeamais*
- To assess the repellence of the smoke to *S. zeamais*.
- To determine the optimum duration of fumigation to achieve effective control of *S. zeamais* and maize storage fungi.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conditions for Maize cultivation

Maize is cultivated between latitudes 50° north and south of the equator and from sea level to 3600 m elevation, in cool and hot weathers, with variable growing cycles (Morris, 2001). Its tremendous genetic variability enables it to thrive well under lowland tropical, subtropical, and temperate climates. It is grown in more countries than any other cereal (Morris, 2001; FAO, 2007). In Ghana, cultivation is done over a wide range of soil and climatic conditions but production is concentrated in the Forest-Savanna transition zone comprising the Ashanti, Brong-Ahafo and Eastern Regions, but mainly in the Ejura-Sekyedumase-Techiman-Nkoransa-Wenchi- area considered as the maize belt of Ghana (GGDP, 1991; Onumah and Coulter, 2000).

2.2 Maize production trends

According to Pingali and Pandey (2001), maize plays a vital role in the economies of many countries. This is because its demand has been on the increase and the challenge of meeting this demand is daunting. Worldwide production was over 600 million metric tonnes in 2003 — slightly more than rice and wheat (http: //www.wikipedia.htm). Between the periods of 2000-2002, about six hundred millions tonnes of maize was produced in the world on 139 million hectares of land, of which 70 percent of this area was in developing countries. However, only 50 percent of the global maize production was harvested from these countries due to several factors. Africa produced 7% of the world's maize with South Africa and Egypt being the main producers (FAO, 2007).

In Ghana, production has increased from an average of 296,700 tons in 1977 to over one million tons in 1996 (http: //www.cropsresearch.org). During the period 1985-89, maize production improved by over 20% per annum and output growth averaged 7.4% per annum in the 1990s with total production stabilizing around 1 million tonnes (Onumah and Coulter, 2000). In 2006, about 793, 000 ha of land was put under cultivation in Ghana with a yield of 1,188,840 tons representing 1.5% increase over the previous year's yield (MoFA, 2007).

In sub-Sahara Africa, the bulk of maize is produced by peasant farmers who cultivate small-scale farms individually or in groups (Odogola and Henriksson, 1991; Dowswell *et al*, 1996). In Ghana, farm holdings are small, between 1-2 hectares with only about 15% of maize farmers cultivating more than 2 hectares in the major maize producing areas (Onumah and Coulter, 2000).

2.3 Nutritional value and uses of maize

Maize is nutritionally superior to other cereals in several ways, except in protein value. However, with the development of the quality protein maize (QPM), this deficiency has been corrected thus, making maize, nutritionally, the most superior cereal grain (IITA, 2007; FAO, 2007). It is also an important source of carbohydrate, protein, oils, vitamin B, and minerals (IITA, 2007).

In developed countries, the bulk of maize produced is largely used as livestock feed and as a raw material for industrial products, while in developing countries it is mainly used for human consumption. In sub-Sahara Africa, maize is a staple for an estimated 50% of

the population (IITA, 2007). Of the total maize produced in Ghana between 1995 and 1997, 75% and 6% were used for human and livestock consumption, respectively (Aquino *et al.*, 2001). Human consumption of maize is concentrated particularly, in the coastal areas, where it is a traditional staple (Onumah and Coulter, 2000).

Maize has a number of industrial uses including the production of ethanol (used as bio fuel and industrial reagent), cosmetic or skin care products, beverages, crayons, soaps, absorbent material for diapers, food additives, biodegradable plastics and food supplements (http: //www.ienica.net.pdf.).

2.4 Storage of maize

Storage and management of stored maize play a vital role in the determination of quality grains (Olakojo and Akinlosotu, 2004). The choice of storage system, structures and options will thus affect grain quality. At small rural farm level, in Ghana, there is no clear cut distinction between storage and drying as the two occur simultaneously. However, storage occurs after the crop has been dried. Storage of cobs with sheath, which does not significantly impair the rate of grain drying in well aerated structures, reduces the status of the maize weevil as a pest and will be beneficial where the weevil is the main insect threat (FAO, 2007). Even without the sheath, grains on the cob are considerably less susceptible to maize weevil attack than the shelled grains (Anon, 1989). According to Vowotor *et al.* (1995), storing unshelled maize reduces the rate of infestation of the maize weevil, thus reducing the rate of population build up of the insect. This practice results in no extra cost and thus readily employed by resource-poor farmers.

There are three main traditional storage systems based on type and location, classified as; indoor, outdoor and underground systems (Osei-Akrasi, 1999). The indoor and outdoor structures are usually used to store both shelled and unshelled maize but the underground storage is for shelled maize and it is used in drier regions. Maize storage structures tend to be specific to a climatic zone and are constructed to meet the requirements of that particular area (Nicol *et al.*, 1997). Small quantities of seed maize are usually stored indoors using calabashes, gourds and earthenware clay-pots at the rural household level. Large-scale storage is undertaken in jute sacks or bins after shelling and treating with the appropriate pesticides. The quantity of grain produced in a season, influences the nature of storage and the extent of the storage period (Owusu, 1981). Maize storage in Ghana is predominantly in traditional cribs packed with the undehusked cobs that dry out gradually through natural ventilation. There is also the improved narrow crib which enhances faster drying and storage (Nicol *et al.*, 1997).

2.5 Fungi associated with stored maize

The predominant grain storage fungi genera are *Aspergillus*, *Fusarium*, *Penicillium* and *Cladosporium* (Simpanaya *et al.*, 2001). Two fungi that are prevalent on maize in Africa are *Aspergillus flavus* Link:Fr., and *Fusarium verticillioides* Sacc. (Nirenberg) (Synonymous *F. moniliforme* Sheld) (Cardwell *et al.*, 2000). Borgemeister *et al.* (1998) identified *Fusarium*, *Aspergillus* and *Penicillium* as the dominant storage fungi on stored maize in Central Benin.

In a survey on seed maize in Ghana, Dadey (1997) reported that *Fusarium verticillioides* and *Acremonium strictum* (Corda) W. Gams were found in seed produced by registered and traditional seed growers in all the four Agro-ecological zones. *Phoma* spp and *Botryodiplodia* spp. were recorded in all zones except the semi-deciduos forest whilst *Fusarium semitectum* (Berk & Ravenel) was found in all zones except the Guinea Savannah zones. Other fungi identified were *Aspergillus, Penicillium, Rhizopus* and *Curvularia* species.

2.5.1 Mode of invasion of maize storage fungi

Maize storage fungi invade the grains during storage and in some cases, growth starts in the field. In the later case, they are usually not problematic before harvest. For example, *A. flavus* spores may land on the silk of a maize cob, germinate, and enter the cob just before pollination and subsist on senescent silks within the husks for a long time (Marsh and Payne, 1984). Invasion of the grain by fungi may be directly through damaged spots in the pericarp, such as silk scars and stress cracks, through the pedicel, or through damage due to insect feeding sometimes before harvest (Wicklow, 1994). Pre-harvest infestation, poor handling at harvest and storage equipment or structures are the sources of inoculum. Under poor storage conditions such inoculum can increase rapidly leading to significant problems (http: //www.agebb.missouri.edu/storage/diseases/fungi.htm).

2.5.2 Factors affecting fungi colonization

The development of storage fungi in stored grain is influenced by the moisture content of the stored grain, temperature within the stored grain, condition of the grain going into

storage, duration of storage period and the amount of insect and mite activity in the grain (http:// www.hgca.com/publication/cropresearch/fungi.pdf.).

2.5.3 Effects of fungi colonization on maize

The two main problems caused by storage fungi are; maize spoilage from fungal growth and production of mycotoxins. Maize spoilage results in loss of grain nutrient, discoloration, reduction in germination ability, hardening or caking of the maize and mouldy smell and taste (FAO, 2007.) Mycotoxins are secondary metabolites of certain strains of fungi. Having relatively small molecular weight and being non infectious, mycotoxins do not accumulate in fat tissues of the body (Amoako-Atta, 2007, citing Wilson and Romer, 1990). Once produced, mycotoxins are highly stable and are not destroyed by boiling or processing. If feed contaminated by some mycotoxins are consumed by livestock, the toxins appears in animal's milk and can be passed on to humans through products prepared from the milk (FAO, 2007) This means that contaminated produce has to be destroyed (FAO, 2007). Some of the effects of some mycotoxins include human oesophageal carcinoma, human hepatocellular carcinoma and immunotoxicity to both livestock and man (Bankole and Adebanjo, 2003).

2.5.4 Management of fungal contamination and mycotoxin production

There is no effective way of eliminating fungi but there are effective measures that control their growth on grains. The prevention of fungal contamination of maize grains depends mainly on the successful drying to optimum moisture content and the control of insect infestation. Rapid drying of harvested maize to 15.5% moisture content or lower

within 24 to 48 hours will reduce the risk of fungal growth and possible mycotoxin production (Bankole and Adebanjo, 2003). This recommendation is not achievable at most resource poor farmers' level because they dry their harvested maize grain under the sun which takes longer period to achieve such moisture content. Solar and mechanical dryers which can achieve such rapid drying are not used by farmers due to the huge capital investment involved.

The feeding activities of insects often create conditions conducive for fungal growth. The destruction of the protective covering of grains exposes their moist interiors to contamination. Prevention of insect attack will ultimately reduce fungal growth. The physical separation of damaged and infected grains from healthy ones is a simple approach to preventing mycotoxin contamination.

Synthetic chemicals can be used on contaminated grains. Propionate acid and gentian violet can be used (Chow, 1984). The use of synthetic chemicals to control mycotoxin contamination has generated a lot of concern due to possible risks of abuse with attendant health hazard.

Research has confirmed fungistatic effects of some plant parts or products used traditionally by farmers in Africa to protect stored grain against fungi (http://www.asabe.org/resource/kk). An extract of dried fruits of *Xylopia aethiopica* (Dunal) and dry seeds of *Piper guineense* (Schum & Thonn) were able to completely prevent development of *Aspergillus flavus* Link:Fr. on maize in laboratory studies. For

practical large scale fungus control purposes botanicals do not seem reliable enough (http://www.asabe.org/resource/kk). According to Bankole (1997), the essential oil of *Azadirachta indica* (Juss) and *Morinda lucida* (Benth) inhibited the growth of *Aspergillus flavus* and significantly reduced aflatoxin synthesis in inoculated maize grains. Using crude acid protein extracts from matured sorghum seeds, Modhumitha and Ulaganathan (1996) reported the inhibition of spore germination and growth of aflatoxin producing *Aspergillus flavus* strains.

In Ghana, Awuah and Ellis (2001) confirmed the fungicidal properties of *Ocimum gratissimum* (L) and *Syzigium aromaticum* (L) Merr & Perry. The botanicals in combination with various packaging methods against *Aspergillus parasiticus* (Speare) were effective. *S. aromaticum* powder proved most effective and in combination with jute bag packaging, provided absolute protection of intact whole groundnut kernels (Awuah and Ellis, 2001).

According to Bankole and Adebanjo (2003) smoking is also an efficient method of protecting maize against infestation by fungi.

2.6 Insects pests associated with stored maize

Mould (1973), reported that there are about 20 different insect pests that attack stored maize in Ghana, of which the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is the most important primary pest (Ofosu, 1980). However, the larger grain borer, *Prostephanus truncatus* is assuming primary pest status (Vowotor *et al.*, 2005).

In a survey conducted in the Ashanti Region to identify the type of storage insect pest associated with maize damage, Owusu (1981) reported that *S. zeamais* occurred in greater numbers in all the stores sampled. On the average, 15% of maize harvested in Ghana is lost annually to *S. zeamais* (Youdeowei and Service, 1986) with localized heavy losses in parts of the country.

2.6.1 Description and biology of *Sitophilus zeamais*

The maize weevil, *S. zeamais*, is a cosmopolitan pest that primarily attacks stored maize (Markham *et al.*, 1994; Thanda and Pixley, 2001) but can survive on other cereals such as rice and wheat (Haines, 1991).

The adults of *S. zeamais* have a cylindrical body and a pronounced snout or rostrum that functions as a boring tool. It measures 3.5-4.0 mm from the snout to the tip of the abdomen and usually reddish-brown to black in colouration with four reddish-orange circular markings on the wings (Haines, 1991, Bosque-Perez, 1992). The thorax is densely pitted with irregularly shaped punctures except, for a smooth narrow strip extending down the middle of the dorsal side.

Adult weevils live up to a year (Bosque-Perez, 1992) and each female has the potential to lay 200-300 eggs throughout most of its adult life (Ileleji *et al.*, 2004) with about 50% being laid in the first four to five weeks (Haines, 1991). The eggs are white and oval in shape and each female may deposit about five eggs per day (Mattah, 2001). Before oviposition, the female bores a small hole into the maize grain and the eggs are laid

individually into these holes. The hole is then sealed with a gelatinous material (egg plug) secreted by the female (Haines 1991). The eggs hatch into tiny grubs in four to nine days. Larval development is normally 25 days under favorable conditions of 30^0C and 70% relative humidity but under unfavourable environmental conditions, the larval stage may last for up to 98 days (Mattah, 2001). The larvae feed inside the grain and are therefore responsible for most of the grain damage. Pupation occurs within the grain and lasts three to six days (Haines 1991). A hole is left behind as the newly developed adult chews its way out of the maize. Under optimum conditions the total developmental period lasts for 35 days whereas under unfavourable condition it may extend to 110 days (Haines, 1991).

2.6.2 Infestation of maize by *S. zeamais*

Being an active flier, infestation usually starts in the field some weeks before the crop is harvested and later continues in the store (Sallam, 2007; Mejia, 2007). Importation of maize and cereal products also plays an important role in infestation.

Grain moisture content considerably affects pest status. *S. zeamais* appears to infest the maize grain in the field only after moisture in the grain has dropped to around 30%. Moisture levels of 10% or less are considered safe where *S. zeamais* is the dominant pest (Bosque-Perez, 1992).

Neglected and unkempt maize stores usually serve as breeding grounds where the weevil population builds up before moving to maize fields. Structures with poor ventilation also cause in moisture and temperature build up hence enhanced *S. zeamais* damage.

The weevil population increase is greater in maize with greater levels of initial field infestation than in maize with low level of infestation. Adult maize weevils seek kernels that have been contaminated by semiochemicals coming from the same species. As a result, high populations may build up quickly around small sources of infestation (Trematerra *et al.*, 2007). However, the difference evens out with prolonged storage periods (Boateng *et al.*, 2001). Other factors such as tightness of the tip of cob sheath and the time of harvest of matured maize from the field also affect the infestation level (Borgemeister *et al.*, 1998).

2.6.3 Damage caused to maize by *S. zeamais*

According to Chow (1984) damage caused by *S. zeamais* include weight loss, quality loss, health risk and economic loss. These losses arise from the feeding activities of insects and fungal growth and these are interrelated (Chow, 1984). Bored holes in the grain, disappearance of a large portion of the endosperm, injury to the germ reducing the nutritive value and loss of viability of the grain, caking and fungal growth on the grain all constitute quality loss. Contamination with insect's excrement, foul odour, micro-organisms and production of mycotoxins also give cause for concern (Mejía, 2007).

Damaged grain is undesirable in the market, causing great economic loss to the producer and quality loss to the consumer. The cost incurred in inspection of grains to maintain quality standards, especially, in developed countries cannot be discounted (Chow, 1984; FAO, 2007).

2.7 Current protective measures against *S. zeamais*

The approaches for protecting maize against weevil attack are numerous. They include the following: cultural, physical, chemical, use of resistant varieties and biological control.

2.7.1 Cultural control

Cultural methods involve early harvesting of maize as soon as it attains physiological maturity as leaving matured maize in the field for extended period results in severe field infestation (Borgemeister *et al.*, 1998) and ensuring general cleanliness in and around the place of storage.

2.7.2 Physical control

This involves the elimination of the pest or alteration of the environment to make it inimical for pest survival and development. Use of dry inert material such as sand, crushed limestone and wood ash to fill up the inter-grain spaces hinders insect's movement through the stored maize. Friction (abrasive effect) between these particles and the insect's cuticle lead to desiccation and hamper the survival and development of the pest (Mejia, 2007). Drying or exposure of maize to sunlight to reduce the moisture content to about 10 °C, prolongs the development of the insect. This practice is not new to farmers as they air-dry their maize during the dry season.

In Japan, Nakakita and Ikenaga (1997) demonstrated that *S. zeamais* could be successfully managed at temperatures between 5°C and 15°C on rice. The authors reported that over four million tonnes of brown rice was commercially stored in low temperature warehouses during the summer season. An ambient temperature of 15°C in

warehouses was maintained by ventilating with refrigerated air to prevent insect pest development and subsequent destruction. In the USA, cooling stored grain in low-volume aeration to limit insect development forms an important component in insect pest management in the Midwestern and North central states (Ileleji *et al.*, 2004). Using simulations, Arthur *et al.* (2001) predicted that aeration alone without the use of insecticides could provide adequate management of maize weevil in maize stored in the northern United States. The above-named and other physical methods although environmentally friendly, do not leave residual protection and also require specific environmental conditions during the period of storage. According to Kumar (1984), these methods are expensive and unreliable.

2.7.3 Chemical control

The use of chemicals to prevent or control the insect infestation has been the simplest and most cost effective way of dealing with the maize weevil (Hidalgo *et al.*, 1998; Quiniones, 2007). Chemical control is generally viewed as a therapeutic measure and involves the use of synthetic insecticides and botanicals.

2.7.3.1 Use of synthetic chemicals

Synthetic chemicals commonly used in the control of *S. zeamais* are of two types; contact insecticides and respiratory poisons or fumigants. Contact insecticides are applied in the form of dust by small-scale farmers although, emulsifiable solutions occur. Pirimiphos-methyl (Actellic), Malathion and Actellic Super (Pirimiphos-methyl + Cypermethrin) are examples of such synthetic insecticides.

Fumigation is widely used all over the world on small and large scale but requires air-tight granaries or silos. Examples of fumigants used are phosphine (PH_3) and Methyl bromide (CH_3Br).

Despite its important role in reducing storage losses due to insect pest activities, the use of synthetic insecticides is fraught with problems; the over-reliance on synthetic insecticides for the control of the maize weevil and other stored grain pests in tropical areas has made insecticide resistance a frequent problem (Ribeiro *et al.*, 2003).

Perez-Mendoza (1999), bioassayed DDT, Lindane, Malathion, Pirimiphos-methyl, Deltamethrin, and Permethrin on eleven field strains of *S. zeamais* from nine states in Mexico and reported that populations of the maize weevil from the different states of the country were resistant to DDT, Lindane, and Malathion . Resistance to Pirimiphos-methyl, Deltamethrin, and Permethrin was however, in its initial stages. Fragoso *et al.* (2007) citing Guedes *et al.*, (1995) and Ribeiro *et al.*, (2003) reported that resistance to DDT and Pyrethroids occured in Brazilian populations of *S. zeamais* in the early 1990s and suggested the possibility of cross and multiple resistances to DDT, organophosphates and pyrethroids.

Toxic residues of synthetic insecticides in food and humans are rampant. Lalah and Wandiga (2002), assessing the effect of storage and processing on Malathion degradation and persistence reported that Malathion and its degradation products Malaoxon, Malathion a- and Malathion b-monocarboxylic acid were present in stored beans and

maize grains after 12 months storage. Although Malathion and its polar metabolites, Malathion a- and Malathion b-monocarboxylic acids were completely eliminated by boiling, Malaoxon was still detected in quite high quantities in the solvent extracts of cooked beans and maize.

2.7.3.2 Hindrances to the use and effectiveness of synthetic Pesticide

The high temperatures and humidity associated with the tropics interfere with shelf-life and insecticidal activity of most insecticides (Hamacher *et al.*, 2002). In developing countries, the high cost of exporting synthetic insecticides may result in a drain on the limited reserves of foreign exchange (Obeng-Ofori and Reichmuth, 1997), the cost of recommended synthetic insecticides do not allow the resource poor farmers to patronize them (Muyinza and Agona, 2005). The problem is compounded by the difficulty of packaging and distributing the chemicals in the forms suitable at the farm level. This has led to the adulteration of pesticides by unscrupulous vendors. Other problems associated with synthetic insecticide use are the killing of beneficial non-target organisms and user abuse as farmers are rarely sufficiently trained to handle them.

2.7.3.3 Use of botanicals to control *S. zeamais* in maize

With the increasing concern about the safety of synthetic insecticides, the need to find alternatives that are readily available, affordable, less poisonous and less detrimental to the environment was apparent (Niber, 1994). Plant products and their secondary metabolites are receiving increasing attention in stored product management (Arthur,

1996; Haque *et al.*, 2000). The technology is not new as peasant farmers have used it to protect their grains in the small scale and rural settings.

Several workers have evaluated the insecticidal, repellent or antifeedant and development inhibiting effects of various plants parts and plant products on *S. zeamais* with varying degrees of success (Obeng-Ofori and Reichmuth, 1997; Bekele, 2002; Udo, 2005; Asawalam *et al.*, 2006; Arannilewa *et al.*, 2006; Asawalam and Hassanali, 2006).

In Ghana, Obeng-Ofori and Armiteye (2005) used coconut, groundnut and soybean oil applied at 2, 5 and 10 ml/kg and Pirimiphos methyl at 1/8 and 1/16 of the recommended dosage and reported significant mortality of *S. zeamais* within 24 h of exposure compared with untreated controls. Other workers including Cobbinah and Appiah-Kwarteng (1989) and Owusu-Akyaw (1991) have reported the insecticidal, antifeedant and development inhibiting activity of some local plants and plant parts against *S. zeamais*.

2.7.4 The role of resistant varieties in the management of *S. zeamais*

The use of resistant varieties promises the most cost effective control measure against the pest (Muyinza and Agona, 2005). Resistance in stored maize to *S. zeamais* attack has been attributed to a number of factors (Thanda and Pixley, 2001). Arnason *et al.* (1994) reported that some Mexican landraces of maize were resistant to *S. zeamais* and attributed the resistance to the phenolic acid content of the maize. Bergvinson (2001) reported that there were strong correlations between insect resistance, kernel hardness and elevated levels of diphenolic acids located within the pericarp of the kernel. Kernel hardness as a

resistance mechanism was only limited by moisture content. Moisture content above 16% renders resistant maize genotypes susceptible thus the importance of grain conditioning before storage (Bergvinson, 2001).

Maize genotypes with elevated levels of cell wall cross-linking components in the pericarp are known to be more resistant to the maize weevil. The principal cell wall components associated with this resistance are phenolic acids, diferulates and structural proteins, which have strong negative correlations with susceptibility parameters and a positive correlation with grain hardness (Garcia-Lara *et al.*, 2004). Tripsacorn, hybrid maize developed from a perennial teosinte, *Zea diploperennis*, and eastern gamagrass, *Tripsacum dactyloides*, may have resistance to storage insect pests that could be incorporated into commercial maize hybrids. Whole Tripsacorn grains are not attacked by *S. zeamais*. The grains are difficult to grind because of the hardness of the fruitcase, and the inability of the weevil to lay eggs is also attributed to this same factor. There is also the possibility that the fruit case contains a repellent that deters oviposition (Throne and Eubanks, 2002).

Chicken avidin has been known to possess insecticidal property causing mortality in many species of stored-product insects by preventing the absorption of dietary biotin (Flinn *et al.*, 2006). The avidin gene has been incorporated into maize plants and avidin maize grains are resistant to insects, especially when the grains are ground into a meal or powder. When avidin content level in transgenic maize grains are about 100 ppm or higher, it inhibited the development of almost all insect pests that damage grain during storage, including the maize weevil, *S. zeamais* (Flinn *et al.*, 2006).

According to Thanda and Pixley (2001) superior maize cultivars can reduce losses due to weevil infestation but no maize grain will be immune to attack by the weevil. The use of resistant varieties alone may not provide a permanent solution to the problems of maize storage but rather may contribute to integrated pest management (Gudrups *et al.*, 2001; Credland *et al.*, 2005).

2.7.5 Biological control of *S. zeamais* on stored maize

The use of biological control as an alternative to chemical treatment in stored products has received increased attention over the past few decades (Wakefield *et al.*, 2005). According to Scholler *et al.* (1997), biological control agents in stored products function effectively in the early stages of pest infestation. Predators, parasitoids and entomopathogens are the principal agents employed (Haines, 1991).

Anipsoteromalus calandrae Howard (Hymenoptera: Pteromalidae), a larval parasitoid, has been reported to provide sufficient control of *S. zeamais* if introduced early in the storage season (Arbogast and Mullen, 1990). Flinn *et al.* (2006) used *Theocolax elegans* Westwood (Hymenoptera: Pteromalidae), a wasp that attacks primary grain pests, whose immature stages develop inside the grain kernels, to achieve a 70% reduction in the population of *S. zeamais* developing on maize.

Hidalgo *et al.* (1998) using various formulations of *Beauveria bassiana* reported that 24 hours of direct contact of *S. zeamais* with a fat pellet formulation containing 10^{10} conidia/g gave 100% mortality after seven days, the dustable powder (DP) formulation

achieved up to 90% control after 15 days with an application of 20g of DP per kg of maize ($2x10^{10}$ conidia/kg maize). Oduor *et al.* (2000) stated that maize mixed with conidia of *Beauveria brongniartii* (Sacc.) Petch and infested with *S. zeamais*, was protected from damage after a period of six months. Ethiopian isolates of highly virulent entomopathogenic fungi, *B. bassiana* and *M. anisopliae* strains controlled *S. zeamais* and *Prostephanus truncatus* (Kassa *et al.*, 2002). *M. anisopliae* and *B. bassiana* were virulent to *S. zeamais* causing 92-100 % mortality, and with median survival time ranging from 3.58 to 6.28 days (Kassa *et al.*, 2002).

Entomopathogenic fungi are generally considered safe, in terms of low risks compared to chemical pesticides (Steenberg, 2005) but, have health issues such as allergic reaction to dustable powder formulation of conidia (Hidalgo *et al.*, 1998; Scholler *et al.*, 1997). Other challenges are short persistence, slow action, high specificity, need for highly skilled management techniques and consumer acceptability (Steenberg, 2005).

Some of the factors limiting the use of biological control for stored products protection are the low economic injury level and the long time required for such control measures to be effective (Scholler *et al.*,1997).

2.7.6 Other methods

Stored product insects can be killed by exposing them to irradiation. Plarre *et al.* (1997) reported that microwave with a power source of 200 kW, 28 GHz with a gyrotron that delivered energy to an applicator flowing at an instantaneous rate of 0. 50 tonnes/ h

effectively controlled all the developmental stages of *S. zeamais*. The advantages of irradiation include residue-free grains; inability to develop resistance and quick result after treatment. Since there is no residual protection of the grains, the irradiated grain must be protected from re-infestation by other methods. However, due to the technical skills required for application, the cost involved and the poor acceptance by consumers, it does not seem probable that irradiation will gain much importance for the treatment of grains any sooner.

2.8 Use of smoke to control fungi and insects

The use of smoke as grain protectant is ancient. Archaeological remains of Paquimé/Anasazi cultures of Mesoamerica (AD 1250-1425) revealed long-term maize storage strategies including pest control and smoke/tannin preservation in the design of their granaries (Fisher, 2004). Smoke and tannins from the oak wood fire were used as a preservative, protecting the stored grain from mold, mildew, insects, and rodents, while adding flavor as well. The granary smoker/storage technology helped Oasis America cultures survive periodic severe, prolonged droughts (Fisher, 2004).

The nature and constituent of smoke is not constant but mainly influenced by factors such as temperature of combustion, condition in the combustion environment and oxidative changes in the compound formed. Smoke contains a broad range of chemicals but phenols, organic acids, carbonyl compounds and hydrocarbons are generally considered most important in killing or repelling storage pests (Kramlich *et al.*, 1973).

Smoking preserves food through dehydration and also the various tars, phenols, and other chemicals in the smoke are toxic to both microorganisms and insects (Pimentel and Pimentel, 2007). According to Hill (1987), the active ingredient of a smoke formulation is usually dispersed as smoke circulates the enclosed chamber. Its usefulness, therefore, lies in the ability to repel insects, interfere with olfaction and thus, delay pest development in inaccessible sites such as thatched roofs and therefore provide quick and labour-saving method for the general de-infestation of houses, warehouses and similar structures.

Smoke fumigation forms an integral part of traditional systems of managing maize in storage (Odogola and Henriksson, 1991). Farmers in small-scale production preserve and protect stored maize on barns, platforms and traditional granaries with smoke. In a survey conducted in four districts of the Volta region of Ghana on the extent of adoption of recommended maize storage practices against the larger grain borer, it was reported that smoke fumigation was practised by farmers but smoking alone was not a sufficient response to insect threats (Addo *et al.*, 2002).

2.8.1 Fungicidal properties of smoke

Hell *et al.* (2000) reported that in Benin, farmers lit fire under storage structures to reduce humidity and control insects and fungi inside the store. Udoh (1997) also reported that between 3.6 and 12% of the farmers in the different agro-ecological zones of Nigeria used smoke to protect their maize against insects and fungi. Osei-Akrasi (1999 citing Parmeter and Uhrendholdt, 1975) stated that smoke was fungitoxic as it prevented the growth of mycelia and the germination of fungal spores.

2.8.2 Insecticidal properties of smoke

In Ghana, the use of smoke as insecticide even though wide spread, its efficiency has not been fully quantified. Osei-Akrasi (1999) reported that smoke from *Ocimum gratissimum* reduced kernel damage by *S. zeamais* in smoked maize cobs. In a survey conducted in 12 out of 15 districts in the forest areas of the Ashanti Region on plants used as domestic insecticides, 26 different plant species were found to be used as grain storage protectants. However, smoking maize stores was the most common method of control in most districts with the exception of the two major maize producing areas (Ejura and Mampong) where conventional synthetic insecticides were preferred (Cobbinah, *et al.*, 1999).

2.9 Uses of *S. siamea.*

Senna siamea has multiple uses. In its native habitat, it is used to establish windbreaks and to provide shade to coffee plantations. It is planted to recover degraded soils since the foliage is rich in organic matter and serves as green manure (http: //www.winrock.org/forestry/factnet). It has medicinal properties as the leaves, flowers and heartwood are used to cure a variety of ailments and as laxatives (Padumanonda and Gritsanapan, 2006). In an ethnobotanical survey on five species of *Senna* including *S. siamea* within and around Oyo State, Nigeria, Ogunkunle and Ladejobi (2006), reported their relevance in the local herbal medicine. Phytochemical screening of their leaves revealed some major groups of pharmacological importance including alkaloids, flavonoids, tannins, phlobatannins, saponins and anthraquinones. Ogunkunle and

Ladejobi (2006, citing Sofowora, 1982) reported that *S. taro* contains an antifungal substance called chrysophanic acid-9-anthrone.

2.9.1. Use of smoke from *S. siamea* to control fungi and insects

The insecticidal properties of the genus *Cassia* is known (Cobbinah *et al.*, 1999; Ogunkunle and Ladejobi, 2006; Kestenholz *et al.*, 2007) but the use and effectiveness of its smoke on *S. zeamais* has not been reported. Awuah (2005) reported that smoke from *S. siamea* (Lambk) has both fungicidal and insecticidal properties. The smoke from the plant in laboratory maize storage studies minimised the damage by *S. zeamais* and mouldiness of stored maize

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The experiments were conducted at the Pathology and Entomology laboratories of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology.

3.2 Maize variety

Fifty kilograms of shelled untreated Obatanpa maize variety was obtained from the Crops Research Institute, Fumesua, Kumasi. The maize was sorted and clean whole grains were used for the experiments. Basal insect infestations in the maize were removed by deep freezing for two weeks (Kossou *et al.*, 1992). The maize was then air-dried under a screen to prevent possible re-infestation. The moisture content of the maize samples was determined before use.

3.3 Collection and preparation of test botanical

Dry twigs of *Senna siamea* were harvested from the Department of Horticulture, KNUST. The twigs were further air-dried for one week and milled into powder, using Christy & Norris Junior® laboratory mill.

3.4 Smoke fumigation boxes

Fumigation boxes measuring 340 mm x 330 mm x 320 mm, with two equal compartments seperated by a wire mesh, were constructed (Fig.3.1). A 10 mm diameter hole was provided on top of the upper chamber through which a thermometer was inserted to measure the temperature within the treatment chamber during fumigation. Another hole of bigger size (40 mm squared) was made at the bottom to serve as inlet for the smoke. The treatment chamber was then mounted on a smoke generation chamber to

form one unit. To prevent escape of the smoke through the joints of the boxes, adhesive tapes were used to seal all possible leaks.

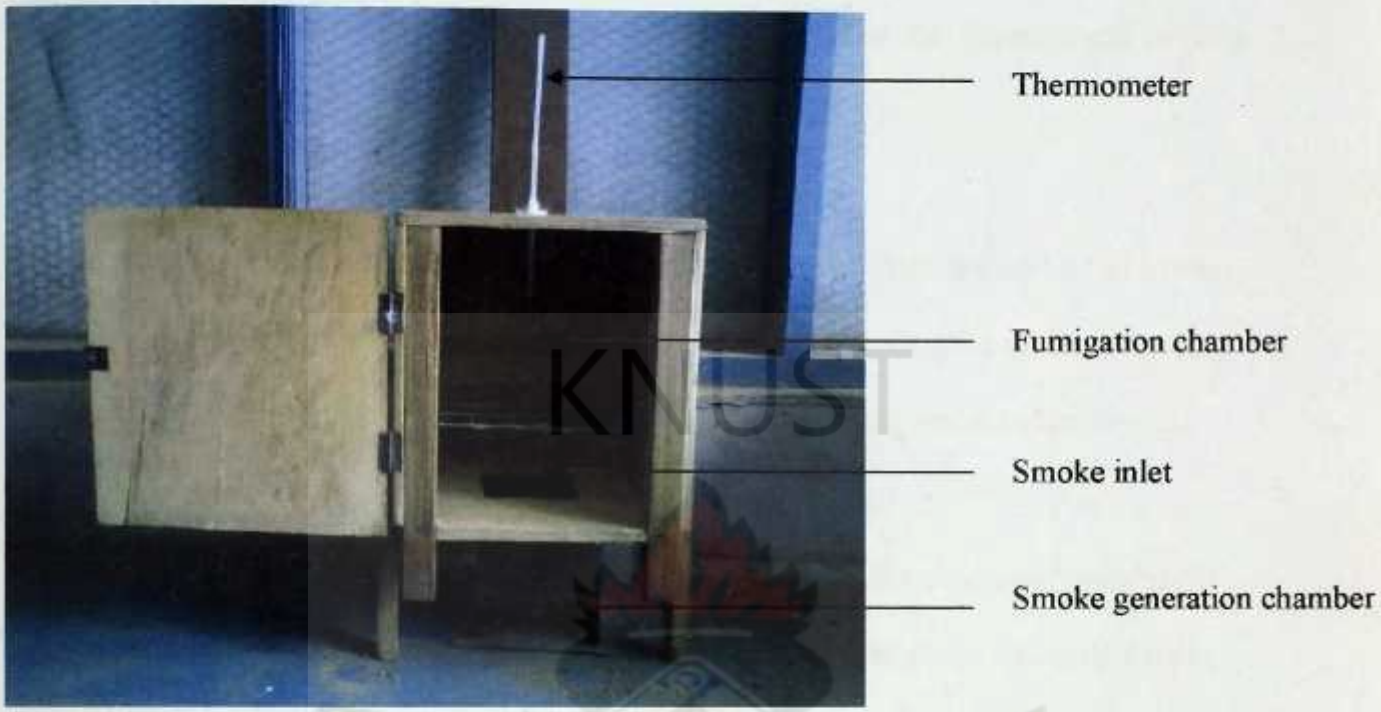

Figure 3 1: Fumigation box with a thermometer.

3.5 Fumigation method

S. siamea powder were placed on a metal plate at the base of the incinerating chamber. The powder was lit. The smoke produced then moved up into and filled the fumigation box. Fifteen grams of the powder burned for a fumigation duration of one hour.

3.6 Laboratory breeding of experimental maize weevils

Three hundred unsexed adult maize weevils taken from a laboratory stock of *S. zeamais* in the Entomology Section of the Department of Crops & Soil Sciences, KNUST, were introduced onto 1000 g maize contained in a plastic jar. The insects were allowed to oviposit for seven days after which they were sieved out. The plastic jar was covered with metal gauze and muslin net firmly secured with rubber bands to prevent possible escape or reinfestation. The F_1 adults that emerged were introduced onto a sample of the test

maize and the resulting F_2 adult weevils were used for the various experiments. Four days prior to infestation, all the adult weevils were sieved out. The fresh adults that emerged during the subsequent 96 hours were sieved and used to infest the experimental maize stock

3.7 Determination of median developmental period

The median developmental period was estimated as the time from the mid-point of the duration allowed for oviposition to the time of 50 per cent emergence of the offspring (Kossou *et al.*, 1992). The ovipostion period was four days for the various experiments.

3.8 Determination of weight loss due to insect feeding

Samples of 100 grains were randomly taken from each jar and the number of undamaged and damaged grains counted and weighed. Per cent weight loss was calculated using FAO (1985) method as follows:

% Weight loss = [UaN-(U+D)] / UaN × 100

Where:

N= total number of grains in the sample

U= weight of undamaged fraction in the sample

Ua= average weight of one undamaged grain

D= weight of damaged fraction in the sample

3.9 Determination of moisture content

The moisture content of the maize was determined gravimetrically by oven drying 20 g samples in the oven at 65°C to constant weights. The change in weight was expressed as per cent moisture content.

3.10 Determination of type and frequency of fungi on experimental maize samples

The deep freezer-blotter method of seed health testing (Mathur and Kongsdal, 2003) was used to determine the type of fungi present. The fungal frequency was determined by critically identifying and scoring for the individual fungi per grain on Petri - dishes. The Petri dishes containing the experimental maize were placed in a deep freezer for seven hours. The samples were removed from the deep freezer and incubated in a dark room under alternating cycles of 12 hours near ultra violet light (Philips TLD 36w/08) and 12 hours darkness for seven days at a temperature of 22 ± 2°C. On the eighth day, the fungi on the maize grains were identified using a simple stereo microscope and in doubtful cases, slides of fungi were confirmed under a compound microscope.

3.11 Maize shoot emergence test

Per cent shoot emergence was determined by sowing 50 seeds in seed boxes containing sterilized sandy-loam soil. The number of emerged seedlings were counted seven days after sowing and expressed as a percentage of the total number sown.

3.12 Effect of smoke on oviposition deterrence of *S. zeamais* on shelled maize

Two Kilner jars each of one kilogram capacity with muslin net lids were filled with maize fumigated one hour earlier. One hundred unsexed 0-4 days old adult *S. zeamais* were introduced into each Kilner jar for a period of four days to oviposit on the grains after which they were sieved out. Three lots of 500 g samples of the fumigated maize were then weighed into different Kilner jars. The three jars containing the fumigated maize, was set up to determine the effect of the smoke on oviposition. A control

experiment was set up in which there was no fumigation. The total number of adult insects emerged were counted and used as a measure of oviposition deterrence.

3.13 Effect of smoke on the developmental stages of *S. zeamais*

Experiment 1: Effect of smoke on the developmental stages of *S. zeamais* within shelled grain

Six one-kilogramme capacity Kilner jars with muslin net lids were filled with clean, uninfested and unfumigated maize. One hundred unsexed 0-4 days old adult *S. zeamais* were introduced into each Kilner jar for a period of four days to oviposit on the grains after which they were sieved out. Three lots of 500 g samples of the unfumigated maize were then weighed into 12 different Kilner jars. Three jars were randomly selected and the maize fumigated in the fumigation box at 4, 28 and 34 days after the initial infestation (DAI) (these days coincide with the egg, larva, and pupa stages of development respectively) The fumigated maize samples were transferred back into Kilner jars and observed for adult emergence. The following data were collected:

a. Total number of adult insects emerged

b. Median developmental period

c. Per cent damaged grains

d. Per cent weight loss

Experiment 2: Effect of smoke on the developmental stages of *S. zeamais* in dehusked cobs

Untreated undehusked maize was obtained from the Crops Research Institute, Fumesua, Kumasi. The maize was dehusked and sorted and clean whole cobs were selected for the experiment. The cobs were placed in a deep freeze for two weeks to clear them of any

basal insect infestation after which they were air-dried under screen to prevent fresh infestation.

Ten cobs were placed in each of eight plastic containers measuring 22 cm x10 cm x 24 cm (top internal diameter, base diameter and height respectively). The ten cobs in each of the plastic containers were infested with 100 unsexed 0-4 days old adult *S. zeamais*. After four days the insects were brushed off the cobs.

To determine the effect of the smoke on the developmental stages of the weevil, 12 lots of five cobs were randomly picked and transferred into twelve plastic containers. At 4, 28 and 34 days after infestation (DAI) three of the 12 plastic containers with the five cobs were randomly selected and fumigated for one hour in the fumigation box.

The fumigated cobs were transferred back into the plastic containers and firmly secured with muslin net lids and observed for adult emergence. The following data were collected:

a. Total number of adult insects emerged
b. Median developmental period
c. Per cent damaged grains
d. Per cent weight loss

3.14 Determination of minimum effective duration of fumigation for the control of *S. zeamais*

Experiment 1: Determination of minimum effective duration of fumigation for control of in shelled grains

Five Kilner jars with a capacity of 1000 g were filled with the experimental maize. One hundred unsexed 0-4 days old adult *S. zeamais* were introduced into each Kilner jar for a period of four days to oviposit on the grains after which they were sieved out. Three hundred grams of the infested maize were then weighed into 15 different Kilner jars. Three of these Kilner jars were randomly selected at a time, as one replication, and fumigated in the fumigation box for periods of one, two, three and four hours. The fumigated maize were transferred back into the Kilner jars and observed for adult emergence. The following data were collected:

a Total number of adult insects emerged

b Per cent damaged grains

c Per cent weight loss

d Per cent emergence

Experiment 2: Determination of minimum effective fumigation time for optimum control of on undehusked maize cobs

Undehusked Obatanpa maize was obtained from the Ghana Seed Company Offices at Asuoyeboa, near Kumasi. Fifteen undehusked cobs were then randomly picked and stacked into each fumigation box. The cobs were then fumigated weekly for 8 weeks for one, two, three and four hours. A control experiment was set up in which there was no fumigation.

At the end of one, four and eight weeks after fumigation, three cobs were selected from each fumigation box, dehusked and observed for:

a. Total number of adult insects on the cobs

b. Per cent damaged grains

c. Per cent weight loss.

3.15 Persistence of smoke from *S. siamea* on dehusked cobs

Dehusked cobs (110) were fumigated in the fumigation chamber for 1 hour. The fumigated dehusked cobs were kept in an airtight container and at 1, 7, 14, 21, 28, and 35, days after fumigation, 15 cobs (3 replications of 5 cobs each) were randomly selected and infested with 150, 0-4 days old adult S. *zeamais* for a period of 4 days to oviposit on the grains after which they brushed off.

They were placed in plastic containers measuring 22 cm x10 cm x 24 cm with muslin net firmly secured with a rubber band and observed for adult emergence. The following data were collected:

a. Total number of adult insects emerged

b. Per cent damaged grains

3.16 Repellency test

A modified Mohan and Field (2002) technique for assessing repellents and attractants in stored products was used. Plastic bottles measuring 20 cm x 5 cm (height and base diameter respectively) with 2 mm holes created all round at an interval of 1 cm were used. Three hundred grams of shelled maize fumigated for 4 hours were put into the plastic bottles. This was placed in a plastic cup with holes at the base measuring 5 cm x 6

cm (height and base diameter respectively). The set up was placed in a Petri dish filled with water (Fig.3.2).

Twenty 0-7 days old adult *S. zeamais*, starved for 24 hours were introduced into the fumigated maize samples through a long stemmed funnel. A measuring cylinder (50 cm tall and 10 cm wide) was inverted over the set up (Fig.3).

Repellence was tested at 1, 24, 48 and 72 hours after the fumigation. Each set up had a three reps with a control in which unfumigated maize was used. The numbers of *S. zeamais* that moved out of the plastic bottles were counted at 1, 2 and 12 hours after infestation. Random departure was eliminated using Abbot's (1925) correction formular.

Figure 3.2: A modified Mohan and Field apparatus for assessing repellence of smoke on fumigated maize.

Figure 3.3: A modified Mohan and Field apparatus for assessing repellence of smoke on fumigated maize.

3.17 Contact toxicity of smoke fumigated maize on adult *S. zeamais*

Maize samples weighing 300 g were fumigated for 4 hours in a fumigation chamber and each transferred into a Kilner jar with Muslin net as lids. Twenty 0-7 days old adult *S. zeamais* were introduced into the fumigated maize samples and observed for mortality at 1, 24 and 48 hours after the introduction. Adult weevils that did not respond to needle probe were considered dead. The set up was replicated three times and a control

experiment was also set up using unfumigated maize. Abbott's (1925) correction formular (Appendix 30) was used in statistical computing to account for mortalities due to factors other than the smoke.

3.18 Effect of smoke from *S. siamea* on adult *S. zeamais*

Twenty 0-7 days old adult *S. zeamais* were placed in inverted Kilner jars with wire gauze as lids. The insects were fumigated in a fumigation chamber for 1 or 4 hours after which they were brought out and mortality recorded. The set up was replicated three times and a parallel experiment was also set up using unfumigated adult *S. zeamais*.

3.19 Effect of smoke from *S. siamea* on fungi associated with stored shelled maize

Shelled maize was obtained from the Ghana Seed Company Offices at Asuoyeboa. The Health status of the maize was determined by the Blotter method (sees section 3.10). A working sample of 200 grains (four replications of 50 maize grains per replication) was used for the each of the maize fumigated for 1, 2, 3 and 4 hours. Forty eight hours after fumigation, 10 grains from each lot of maize were plated on a moist mat of three blotter papers in plastic Petri dishes. A control experiment was set up with unfumigated grains.

3.20 Data Analysis

All percentage data with more than 40% range were arcsine transformed ($Sine^{-1}((x+0.5/100)$. While count data were square root transformed $\sqrt{(x+0.5)}$ (Clewer and Scarisbrick, 2001) and median developmental period was $\log_{10}$ transformed. GenStat Release 7.2 Discovery Edition (2007) Computer package was used to analyze variances and least significant differences (LSD) were used to separate means that showed significant differences at a Probability level of 5% ($P \leq 0.05$).

CHAPTER FOUR

RESULTS

4.1. Effect of fumigated maize grains on oviposition deterrence of *S. zeamais*

Adult emergence was used as a measure of oviposition on the maize grains. Significantly smaller numbers of the weevils emerged from samples fumigated prior to infestation than from the unfumigated maize samples (Figure 4.1). This implies that the fumigation significantly deterred *S. zeamais* from ovipositing on the treated maize.

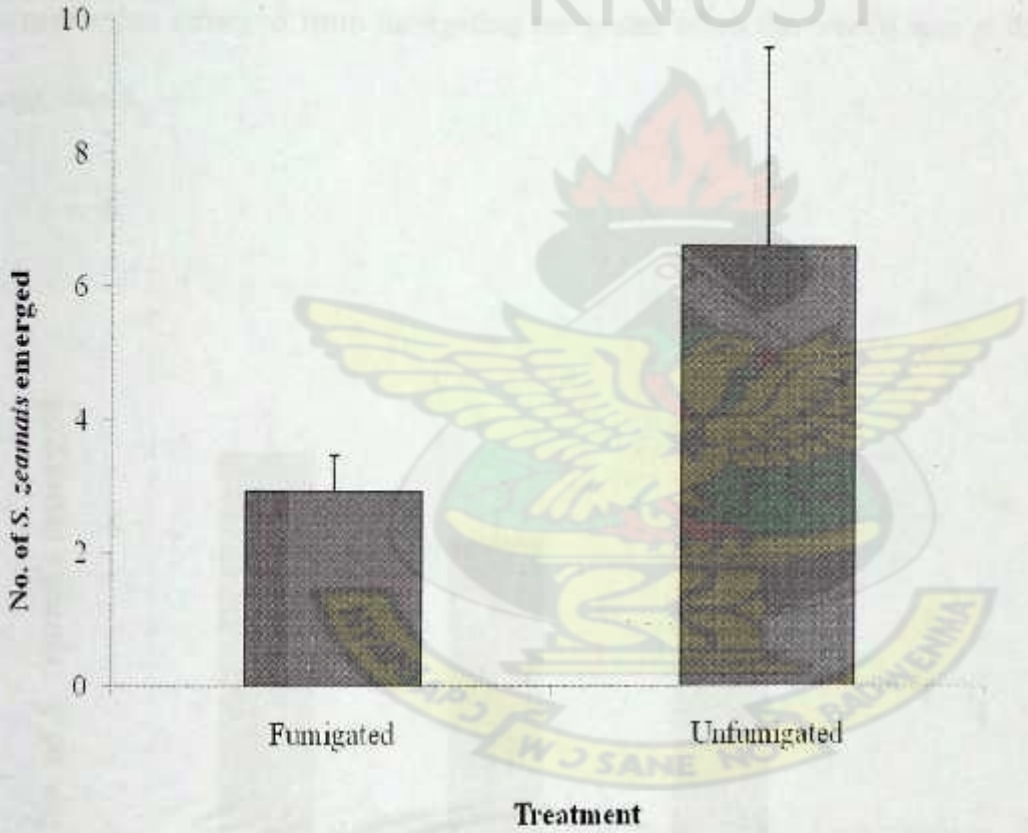

Figure 4.1: Mean number of *S. zeamais* emerged from fumigated and unfumigated maize grains.

The error bars represent standard error of means (SEM).

4.2. Fumigating maize grains containing the various developmental stages of *S. zeamais*

4.2.1 Effect of fumigation on number of *S. zeamais* emerged from shelled maize

Figure 4.2 illustrates the effect of fumigating shelled maize with smoke from *S. siamea* on the number of adult *Sitophilus zeamais* that emerged.

Smaller numbers of the weevils emerged from samples fumigated when the pest was at the larval stage. However, this was not significantly different from the numbers of the weevils that emerged from fumigating the grains when the weevil was at the pupal and egg stages.

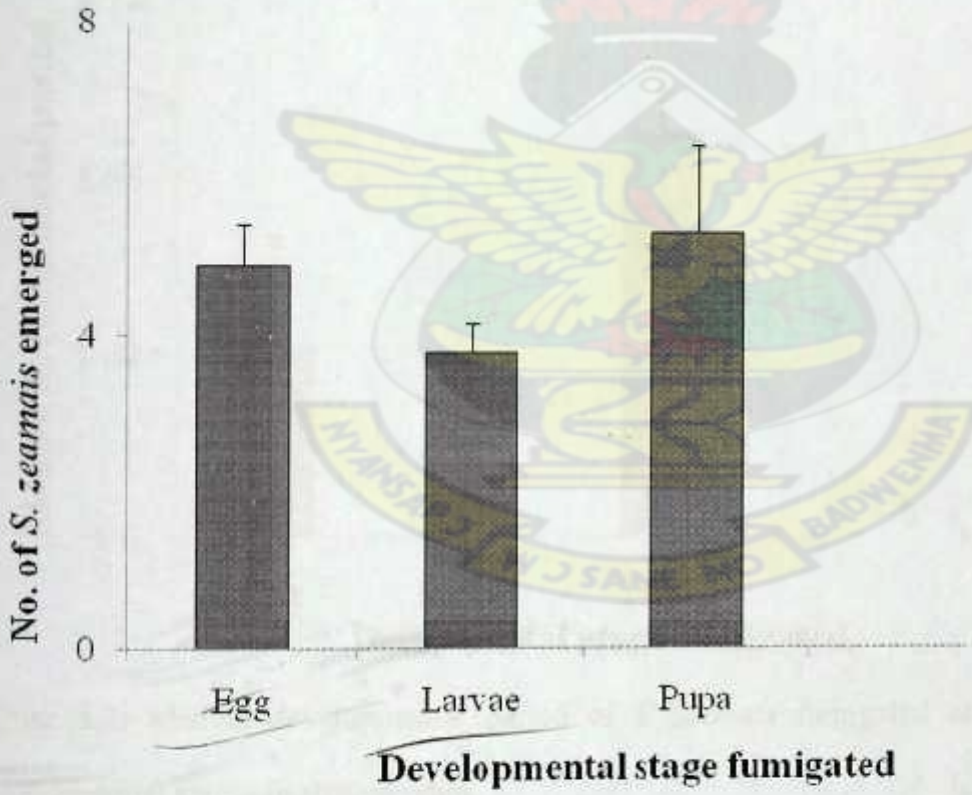

Figure 4.2: Mean emergence of *S. zeamais* from maize grains fumigated at the different developmental stages.

The error bars represent standard error of means (SEM).

4.2.2 Effect of fumigation on median development period of *S. zeamais* in shelled maize

Figure 4.3 shows the effect of fumigating the grain containing the different stages of the pest on the median developmental period of *S zeamais*.

Fumigating at the pupa stage resulted in a significantly longer median developmental period of the weevil than at the larval and egg stages. The median developmental periods of samples fumigated at the egg and larvae stages were however, not different.

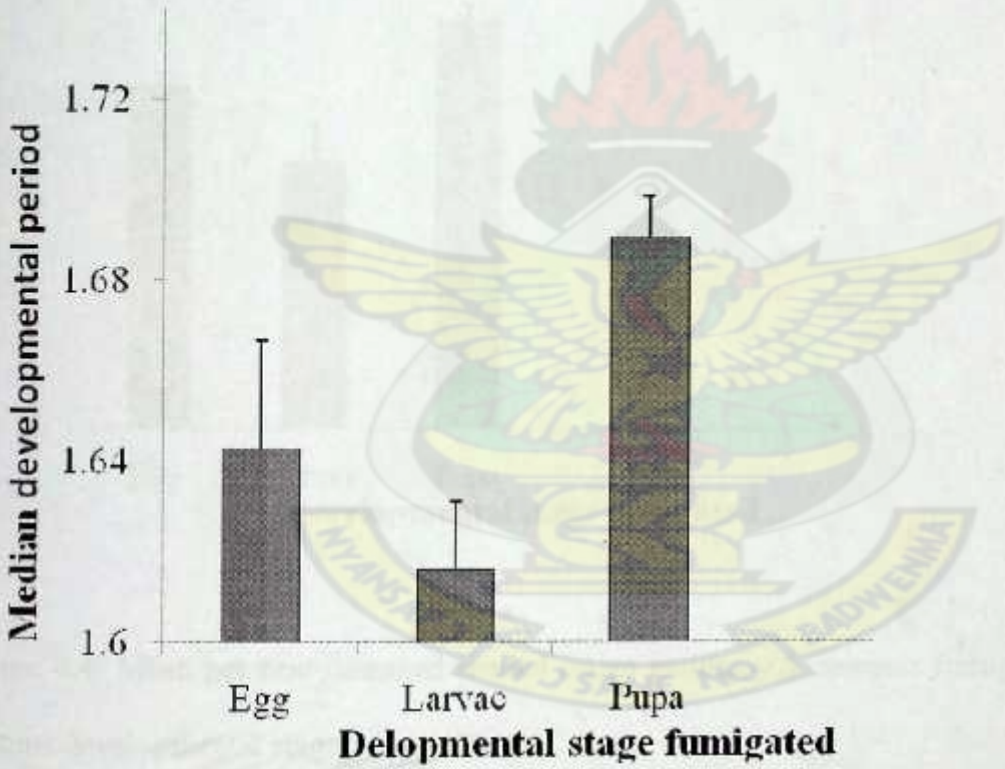

Figure 4.3: Median developmental period of *S zeamais* fumigated at the different developmental stages in shelled grain

The error bars represent standard error of means (SEM).

4.2.3 Effect of fumigation on per cent damaged shelled maize grains.

Delaying fumigation until the pest reached the pupal stage within the grain resulted in the greatest damage, which was significantly greater than fumigating earlier (Figure 4.4). There were however no significant differences between fumigating at the egg and larval stages.

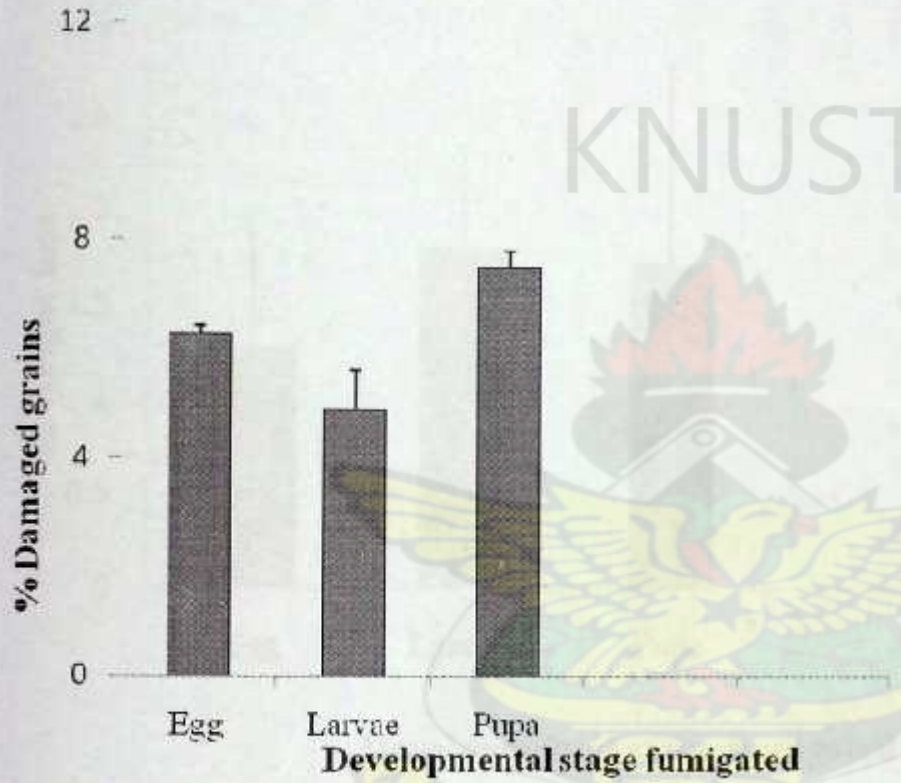

Figure 4.4: Mean per cent damaged shelled maize grains by *S .zeamais* fumigated at the various developmental stages.

The error bars represent standard error of means (SEM).

4.2.4 Effect of fumigation on per cent weight loss of shelled maize grains

Fumigating at any of the developmental stages of the pest had no significant effect on the per cent grain weight loss (Fig.4.5), even though fumigating at the egg stage resulted in the least weight loss.

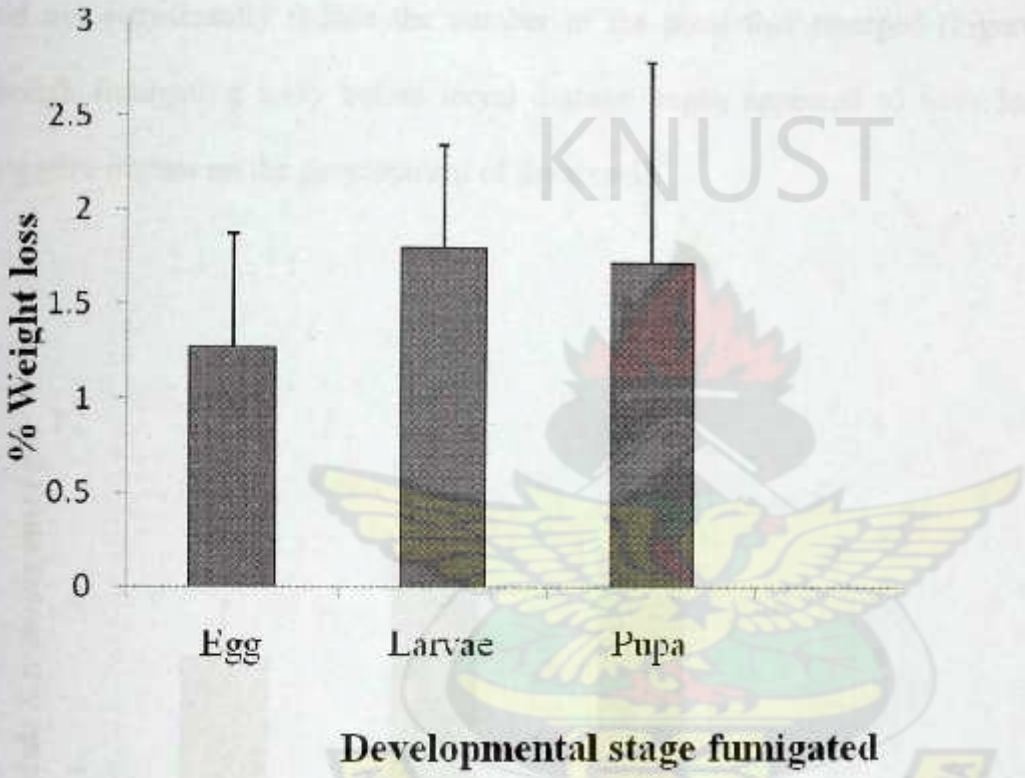

Figure 4.5: Mean per cent weight loss of shelled maize fumigated at the various developmental stages of *S. zeamais*.
The error bars represent standard error of means (SEM).

4.3 Fumigating intact maize cobs (dehusked maize) containing the different developmental stages of *S zeamais* with smoke from *S. siamea*

4.3.1 Effect of fumigation on number of *S zeamais* emerged

Fumigating the cobs containing the different developmental stages of the pest for 1 hour did not significantly reduce the number of the pests that emerged (Figure 4.6) even though fumigating early before larval damage began appeared to have had a greater negative impact on the development of the weevil.

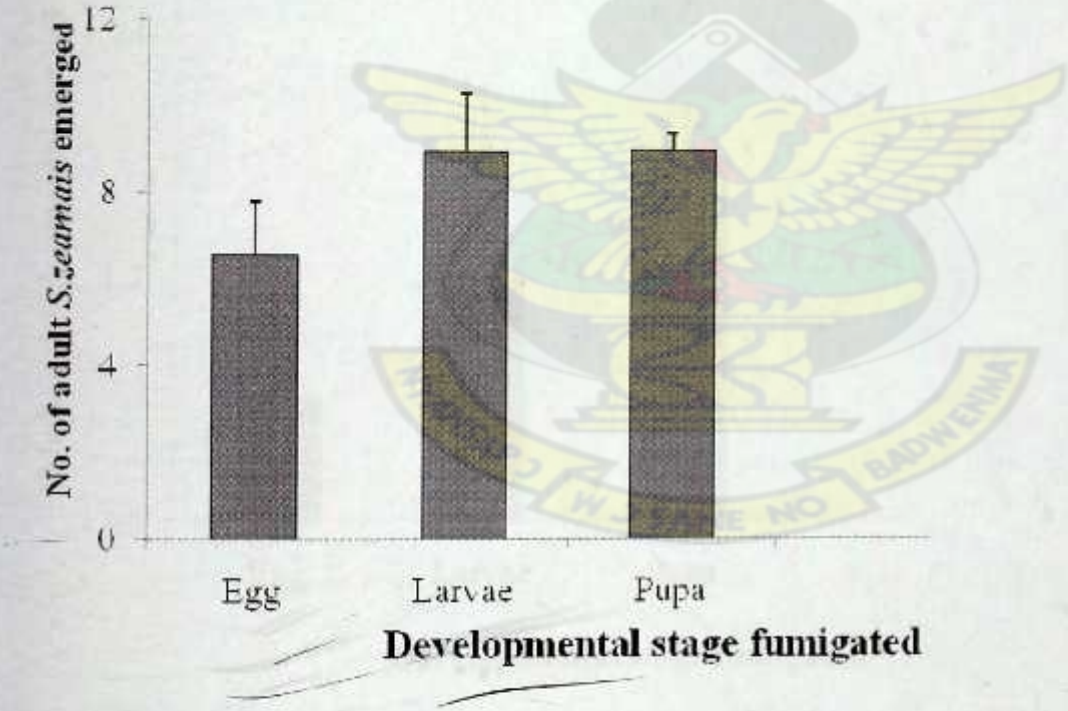

Figure 4.6: Mean number of *S zeamais* emerged from dehusked maize fumigated at the various developmental stages.

The error bars represent standard error of means (SEM).

4.3.2 Effect of fumigation on median developmental period of *S zeamais* in dehusked cobs

The median developmental periods of *S. zeamais* on the dehusked maize cobs fumigated at the different developmental stages of the pest are presented in Fig. 4.7.

Although fumigating the cobs at the pupal stage resulted in the longest time taken to reach the adult stage, this was not significantly different from fumigating at any of the other stages of the pest. However, there was a gradual increase in the median developmental period with instar age.

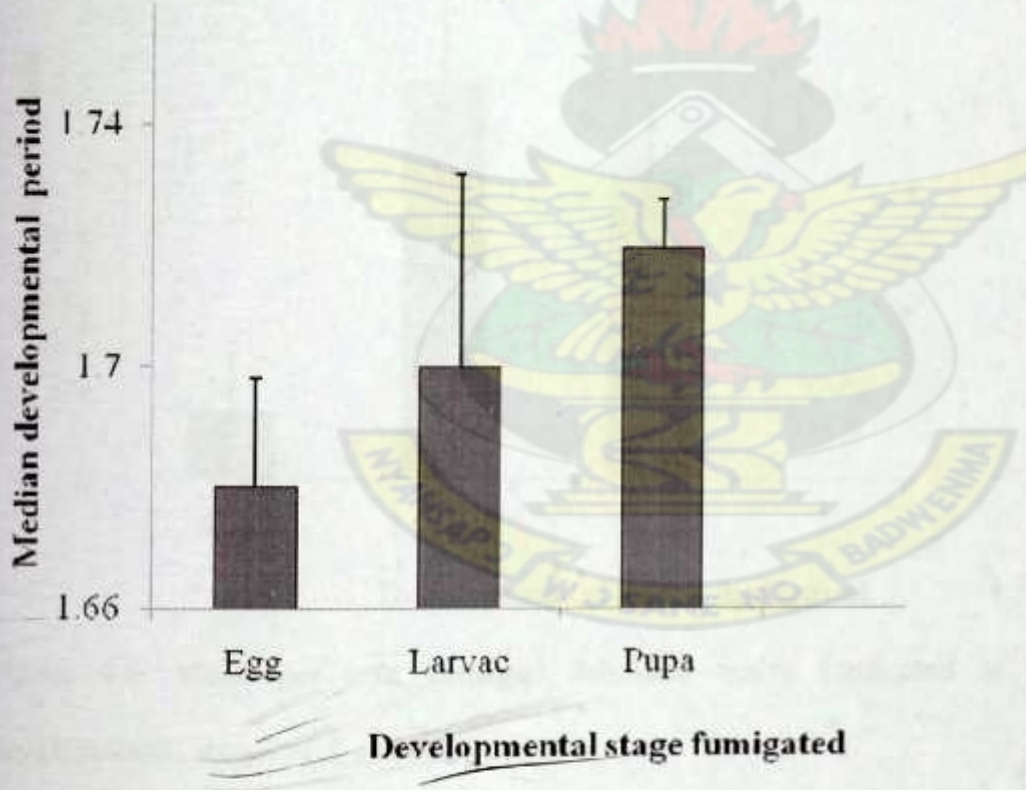

Figure 4.7: Median developmental period of *S. zeamais* on dehusked maize fumigated at the different developmental stages.

The error bars represent standard error of means (SEM).

4.3.3 Effect of fumigation on per cent damaged grains of *S zeamais* in dehusked cobs

Figure 4.8 shows the mean per cent damaged grains on the cobs fumigated at the various developmental stages of *S. zeamais*. Fumigating the cobs when the pest was at the egg stage resulted in the least damage and was significantly smaller than at the other stages of development. Damage increased more than two fold when fumigation of the cobs started after the egg stage.

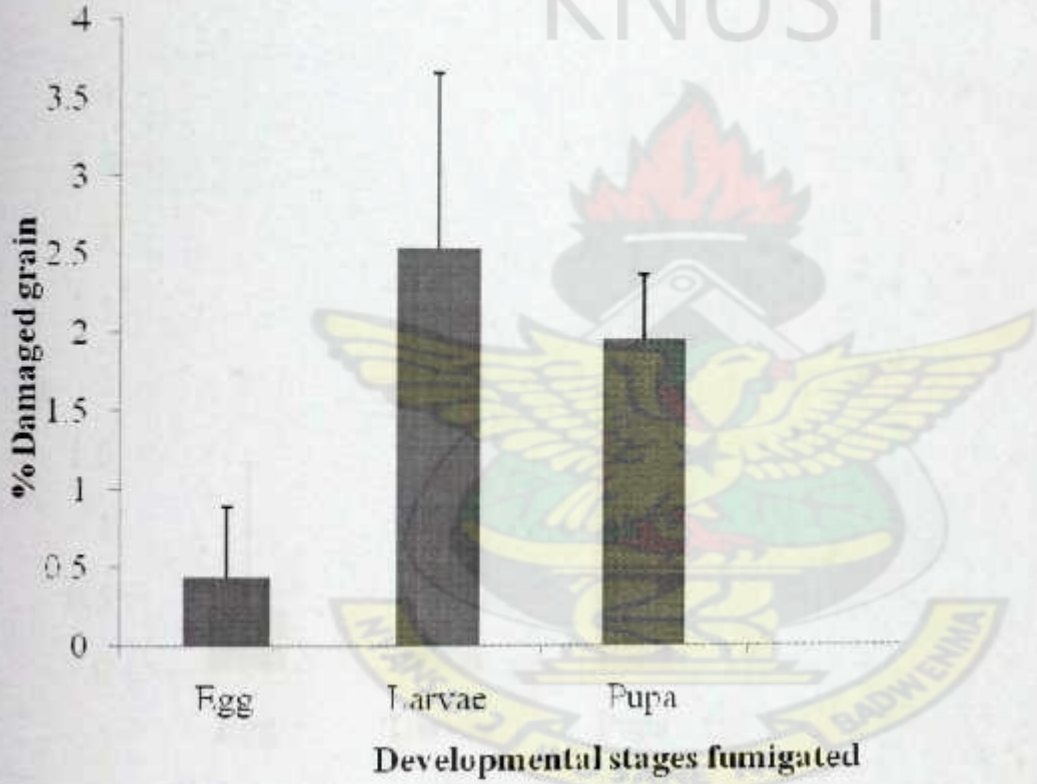

Figure 4.8: Mean per cent damaged dehusked maize fumigated at the various developmental stages of *S. zeamais*

The error bars represent standard error of means (SEM).

4.3.4 Effect of fumigation on per cent weight loss of *S zeamais* in dehusked cobs

Figure 4.9 indicates the mean per cent weight loss of dehusked cobs fumigated at the various developmental stages of *S. zeamais* with smoke from *S. siamea*.

The greatest per cent grain loss occurred when the pests were at the larval stage but this was however not significantly different from fumigating at the pupal, but greater than at the egg stage.

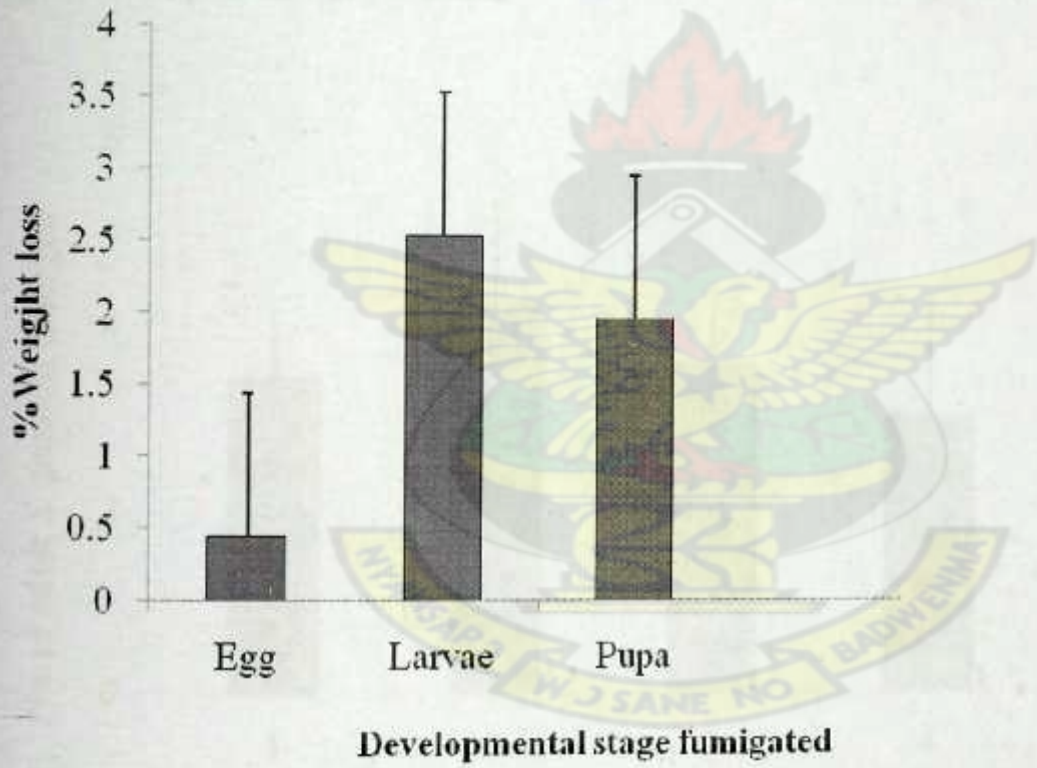

Figure 4.9: Mean per cent weight loss of dehusked maize fumigated at the different developmental stages of *S. zeamais*.

The error bars represent standard error of means (SEM).

4.4 Effect of different durations of fumigation on the different stages of *S. zeamais*

4.4.1 Fumigation of *S. zeamais* in maize grain

4.4.1.1 Mean weevil emergence

As results of fumigating for 1h duration were not consistent, fumigation periods to determine an optimum duration was varied.

Fumigating the grain at the different durations did not significantly affect the number of the pest that emerged, even though fumigating for four hours resulted in the smallest number of the pest emerging as adults.

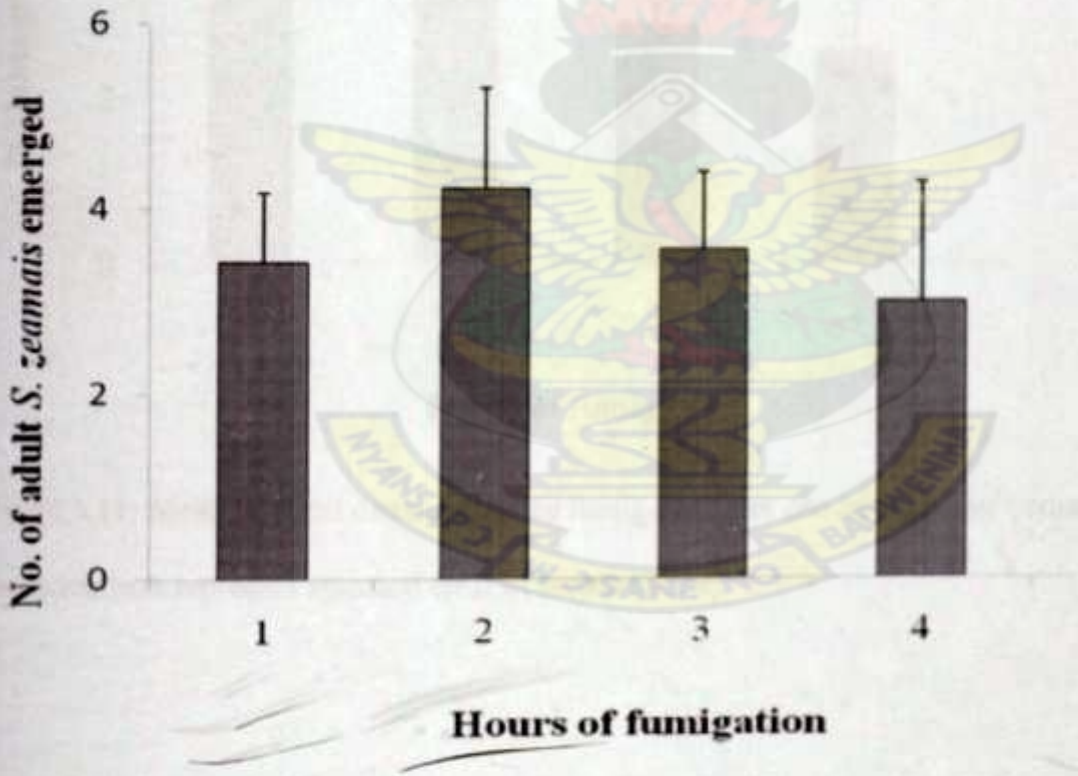

Figure 4.10: Mean number of *S. zeamais* emerged from shelled maize fumigated at different duration.

The error bars represent standard error of means (SEM).

4.4.1.2 Per cent damage by weevil

Figure 4.11 shows the per cent damaged maize, fumigated from 1 h to 4 h . The lowest damage occurred when the maize was fumigated for 4 hours but this was however not significantly different from the other durations of fumigation.

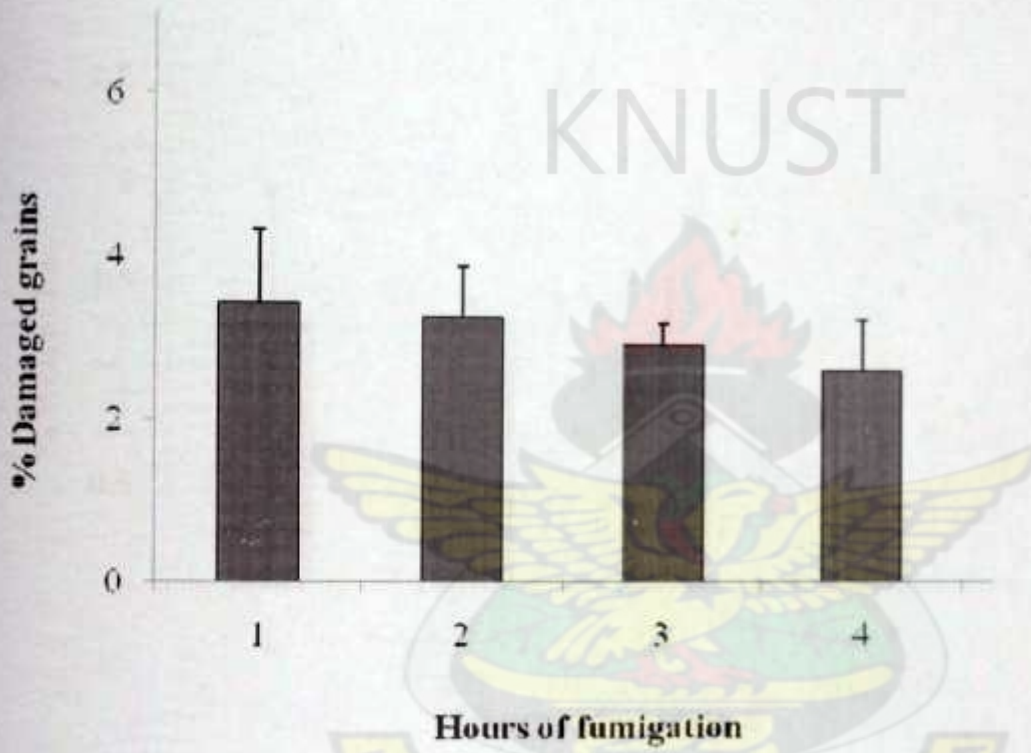

Figure 4.11: Mean per cent damaged maize fumigated from one hour to four hours. The error bars represent standard error of means (SEM).

4.4.1.3 Mean per cent maize weight loss

From Figure 4.12, the smallest loss occurred when the maize was fumigated for four hours but this was however not significantly smaller than the other durations of fumigation, a pattern similar to the per cent damaged grains (Figure 4.11).

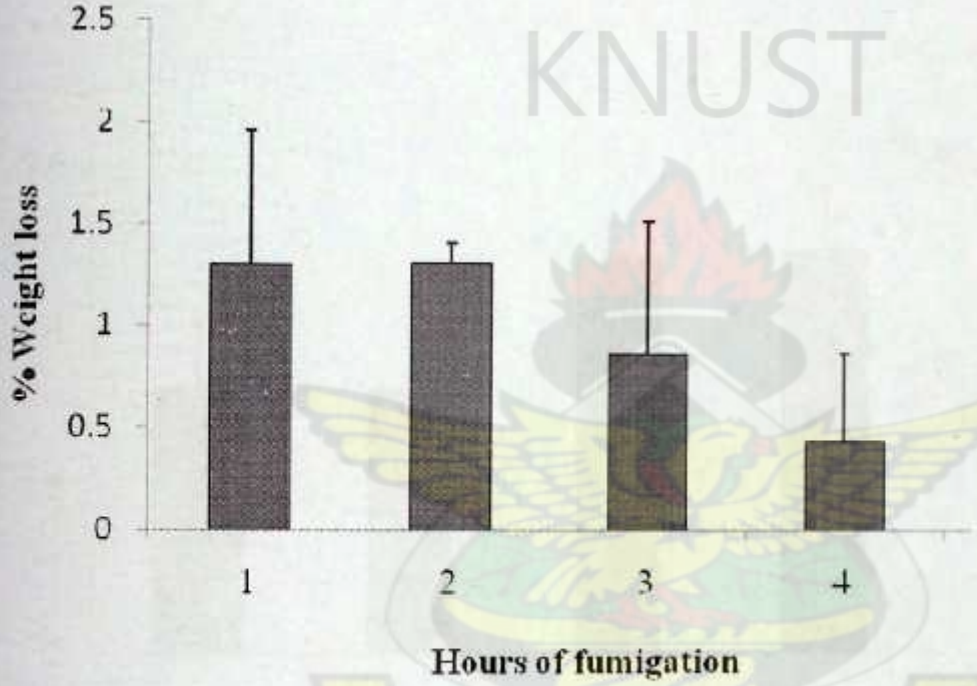

Figure 4.12: Mean per cent weight loss of shelled maize fumigated from one hour to four hours.

The error bars represent standard error of means (SEM).

4.4.1.4 Mean per cent maize shoot emergence

It was neccesary to determine the effect of the smoke on the germ of the maize seed. The greatest per cent shoot emergence was observed in the grains fumigated for four hours but this was however not significantly different from the other durations of fumigation fumigated grains.

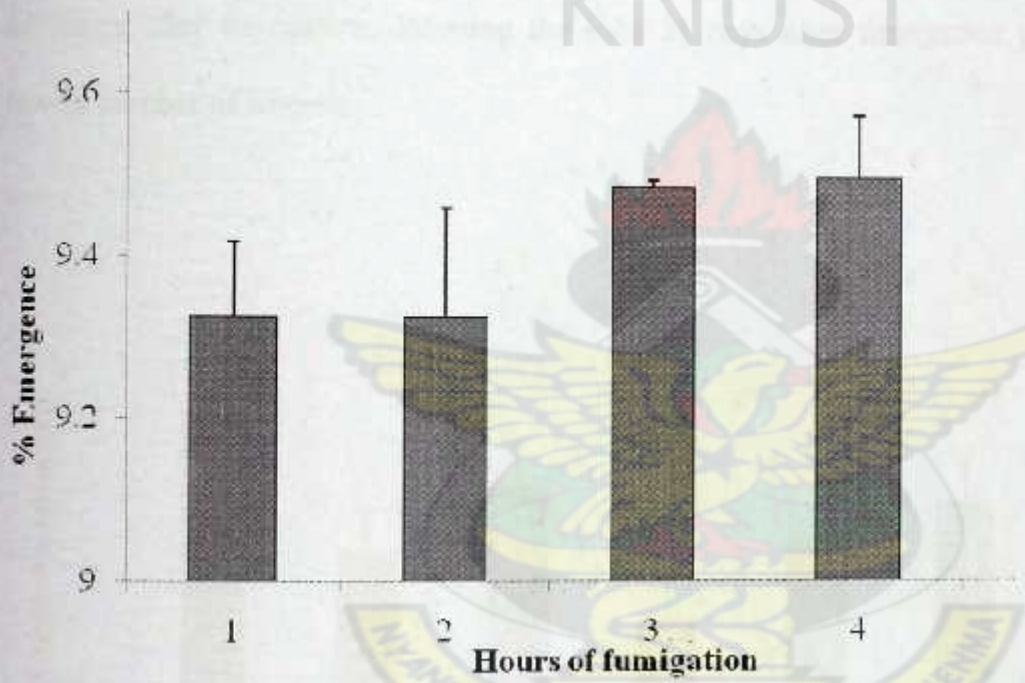

Figure 4.13: Mean per cent shoot emergence of shelled maize fumigated from one hour to four hours and sown in sterilized sandy loam soil.

The error bars represent standard error of means (SEM).

4.5 Persistence of the smoke on fumigated dehusked maize cobs

4.5.1. Mean number of *S. zeamais* emerged

Figure 4.14 shows the effect of infesting maize at different intervals after fumigation. The numbers of weevils emerging from the unfumigated cobs and those fumigated 24 hours earlier were not different ($p<0.001$). But infesting maize 7-35 days after fumigation resulted in significantly fewer weevils than from the unfumigated cobs and those infested 24 hours after fumigation. Infesting the cobs 21 days after fumigation produced the fewest number of weevils.

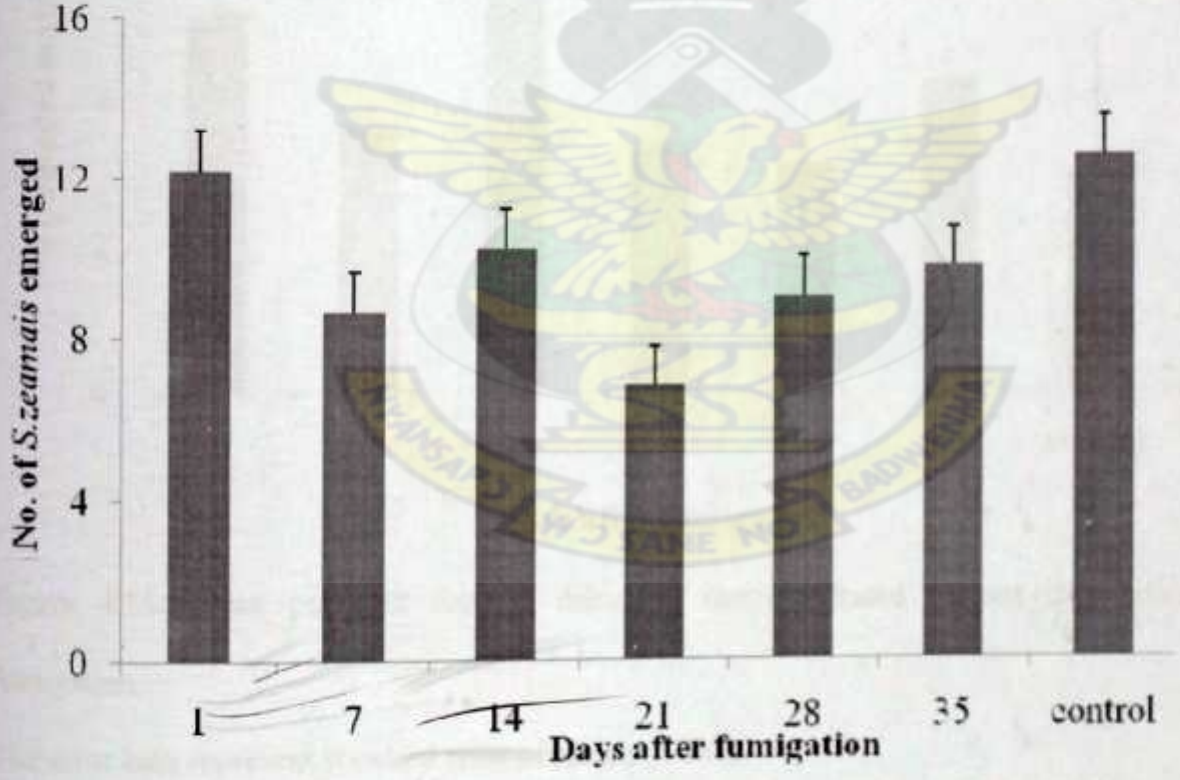

Figure 4.14: Mean number of *S. zeamais* emerged from dehusked maize infested various days after fumigation.

The error bars represent standard error of means (SEM).

4.5.2 Mean per cent damaged grains

Contrary to expectation, the greastest damage occurred when the cobs were infested a day after fumigation but this was not different from infesting 14 days after fumigation. Even though not better than the control, infesting cobs 7, 21 and 28 days after fumigation suffered less damage than infesting 1 day after fumigation (Fig.4.15).

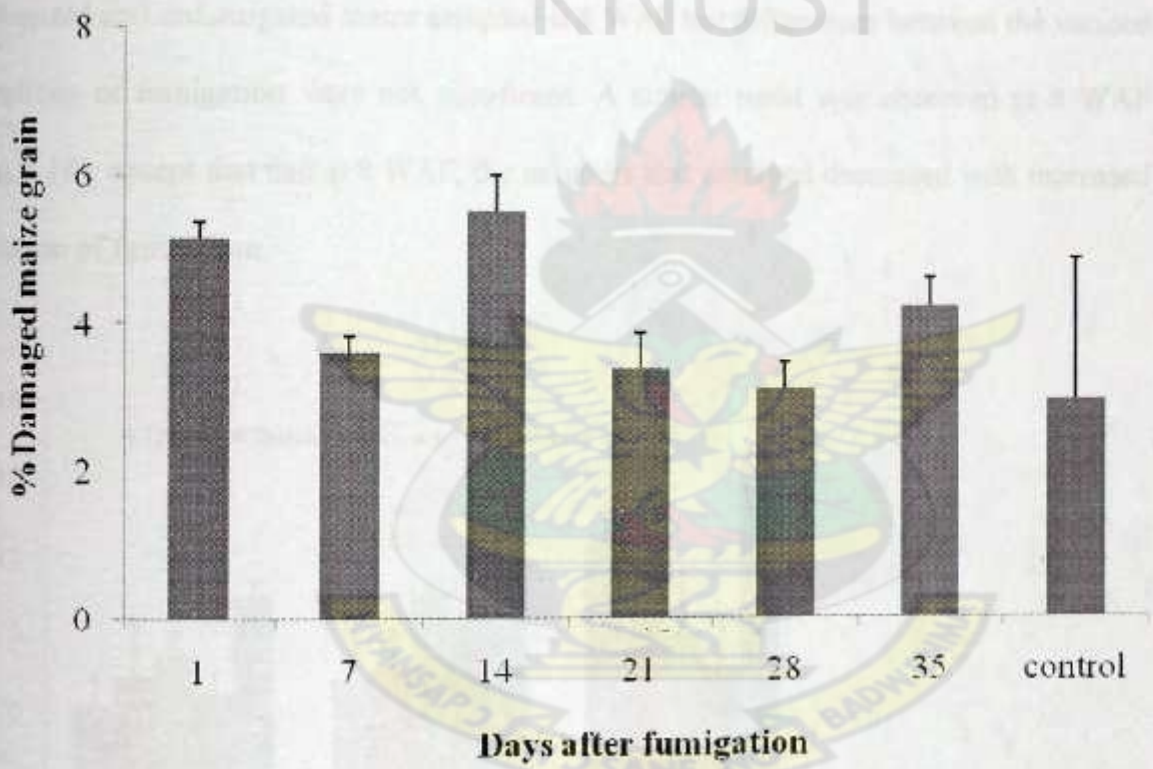

Figure 4.15: Mean per cent damage dehusked maize infested various days after fumigation.

The error bars represent standard error of means (SEM).

4.6 Effect of different durations of fumigation on persistence of the smoke on undehusked cobs

4.6.1 Mean number of *S. zeamais* emerged

The differences in numbers of the pest that emerged from the fumigated and unfumigated maize sampled at 1 week after fumigation (WAF) were not significant. The differences in the mean numbers of weevils that emerged were however significant between the fumigated and unfumigated maize sampled at 4 WAF but differences between the various durations of fumigation were not significant. A similar trend was observed at 8 WAF (Fig.4.16), except that that at 8 WAF, the numbers that emerged decreased with increased duration of fumigation.

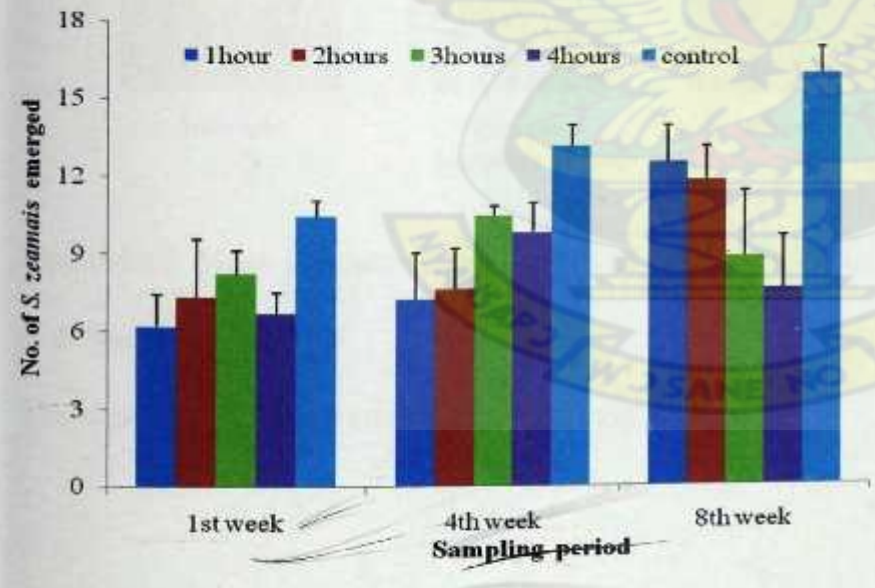

Figure 4.16: Mean number of *S. zeamais* emerged from undehusked maize fumigated for different durations and sampled at different time intervals

The error bars represent standard error of means (SEM).

4.6.2 Mean per cent damaged grains

With the exception of the 3 h of fumigation, the differences in damage incurred between the fumigated and unfumigated maize, sampled at 1 WAF were significant ($p=0.04$) but the differences between the other durations of fumigation were not significant. There was however no significant differences in damage on the grains sampled at the 4 and 8 WAF.

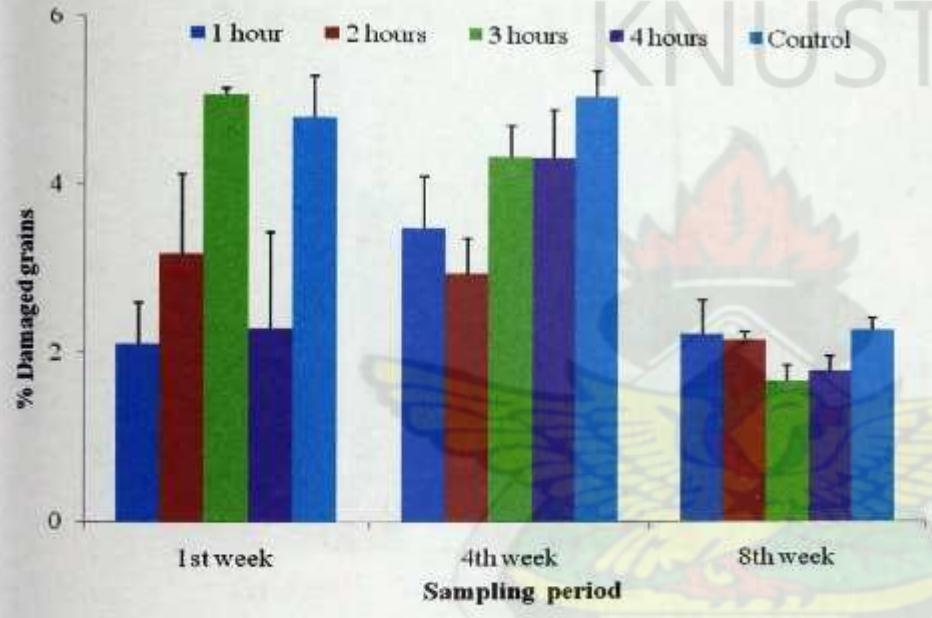

Figure 4.17: Mean per cent damage grains of undehusked maize fumigated and sampled at different time interval

The error bars represent standard error of means (SEM).

4.6.3 Effect of smoke fumigation on per cent maize weight loss

The unfumigated maize recorded the greatest weight loss at 1 WAF. Between the fumigated maize, a significantly ($p=0.02$) greater weight loss was recorded for the 3-h fumigation. However weight losses recorded on the fumigated and unfumigated maize sampled at the 4 and 8 WAF were not significantly different.

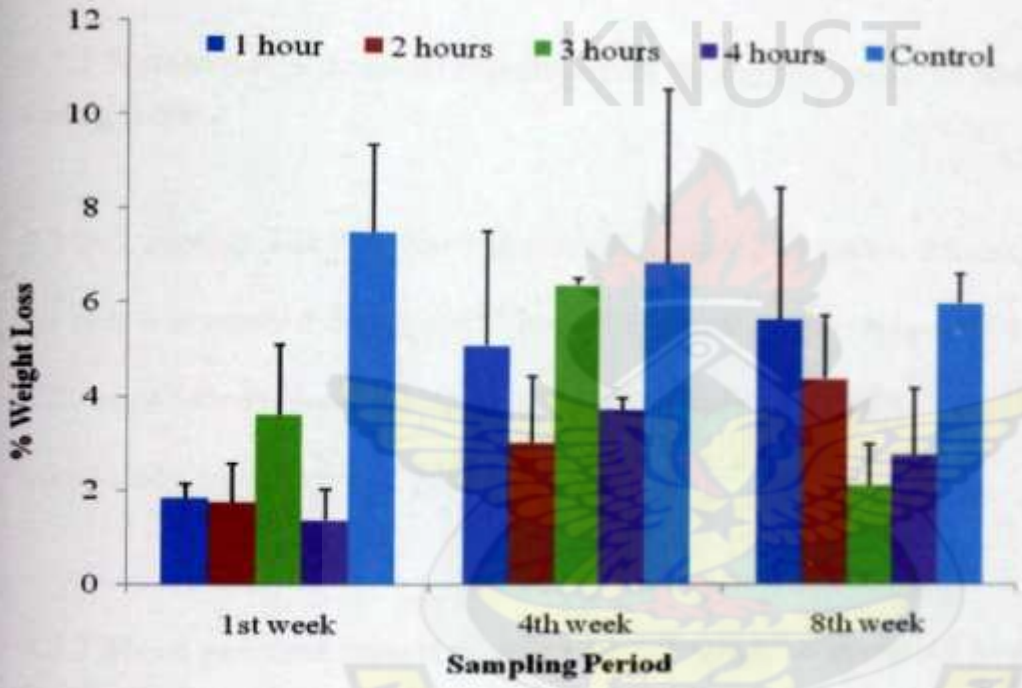

Figure 4.18: Mean per cent weight loss of undehusked maize fumigated and sampled at different time interval

The error bars represent standard error of means (SEM).

4.7 Repellence of *S. zeamais* from maize fumigated for four hours with smoke from *S. siamea*

4.7.1 Mean number repelled 1 hour after fumigation

The greatest number of the pest was repelled during the first one hour of introduction onto the maize grains fumigated 1 h earlier. The numbers of the pest repelled at 2 h and 12 h after introduction onto the grains were not significantly different. (Table 4.1).

4.7.2 Number of *S. zeamais* repelled from fumigated maize 24 hours after fumigation

When *S. zeamais* was introduced on maize fumigated 24 h earlier, the greatest number of the pest was repelled during the 1st hour of exposure. There was however no significant difference between the numbers of the weevils repelled during the 2nd and 12th hours after introduction onto the grain (Table 4.1).

4.7.3 Mean per cent insects repelled from fumigated maize 48 hours after fumigation.

The pattern of repellence 48 h after fumigation was similar to what was observed at 1 h and 24 h after fumigation (Table 4.1).

4.7.4 Number of *S. zeamais* repelled from fumigated maize 72 hours after fumigation

The differences in the mean numbers of the weevils repelled after 1 h, 2 h and 12 h of exposure to the grains fumigated 72 h earlier were not significant (Table. 4.1).

Table 4.1: Per cent *S. zeamais* repelled from shelled maize fumigated for 4 hours and exposed to the weevil at different durations after fumigation

Duration of exposure of weevil to fumigated maize (h)	Cumulative mean % *S. zeamais* repelled from fumigated maize exposed to the weevil at different durations after fumigation ± SE			
	1h	24h	48h	72h
1	46.8 ±5.2	29.5 ± 5.1	39.3 ± 0.9	12.2 ± 7.5
2	60.4 ± 0.0	39.9 ± 3.1	49.7 ± 3.2	26.2± 4.9
12	64.5± 0.0	44.3 ± 0.0	53.7 ± 0.0	30.6 ± 0.0
P (0.05)	0.001	0.03	<.001	0.12
LSD	11.7	13.4	7.6	10.9

4.8 Contact toxicity of maize fumigated with smoke from *Senna siamea* to adult *S. zeamais*

Smoke is known to contain chemicals that can kill insects but after exposing *S. zeamais* for 1 h, 24 h and 48 h to maize fumigated for 4 hours, no significant mortality occurred (Table 4.2).

Table 4.2: Mean per cent mortality of the weevil killed on maize fumigated for 4 hours and exposed for different hours after fumigation.

Duration after fumigation (Hours)	% Mean weevil mortality ± SE
1	7.22 ±3.2
24	4.05 ± 0.0
48	4.05 ± 0.0
P (0.05)	0.44
LSD	7.19

4.9 Effect of direct fumigation on adult *S. zeamais*

Fumigating adult *S. zeamais* for 1 or 4 hours did not kill the weevils but kept them moving erratically in the kilner jar and remained alive and active 24 hours after the fumigation. This observation was contrary to the knowledge that smoke blocks the tracheal system of insects and kills them through asphyxiation.

4.10. Fumigating maize grains for different durations against maize storage fungi

The dominant storage fungi identified on the maize fumigated for 1, 2, 3 and 4 h were: *Aspergillus flavus*, *Fusarium verticelloides* and *Penicillium* species.

4.10.1 Effect of fumigation on mean per cent occurrence of *Aspergillus flavus*

The differences in the various durations of fumigation on the incidence of *A. flavus* on shelled maize were not significant. Four hours fumigation did not significantly reduce the occurrence of the fungus on the maize compared to one hour fumigation (Figure 4.19).

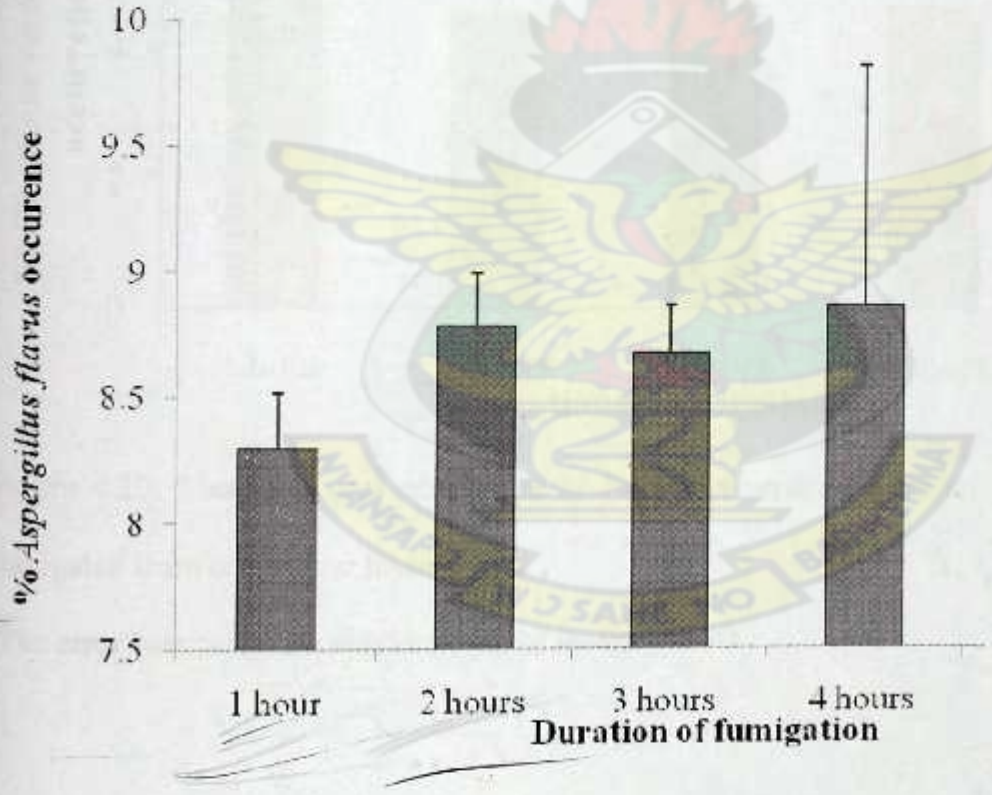

Figure 4.19: Mean per cent occurrence of *Aspergillus flavus* on shelled maize fumigated at different durations.

The error bars represent standard error of means (SEM).

4.10.2 Effect of fumigation on mean per cent occurrence of *Fusarium verticellioides*

The lowest frequency of *F. verticellioides* was recorded when the grains were fumigated for two and four hours but these were not significantly different from the one and three hours of fumigation (Fig.4.20).

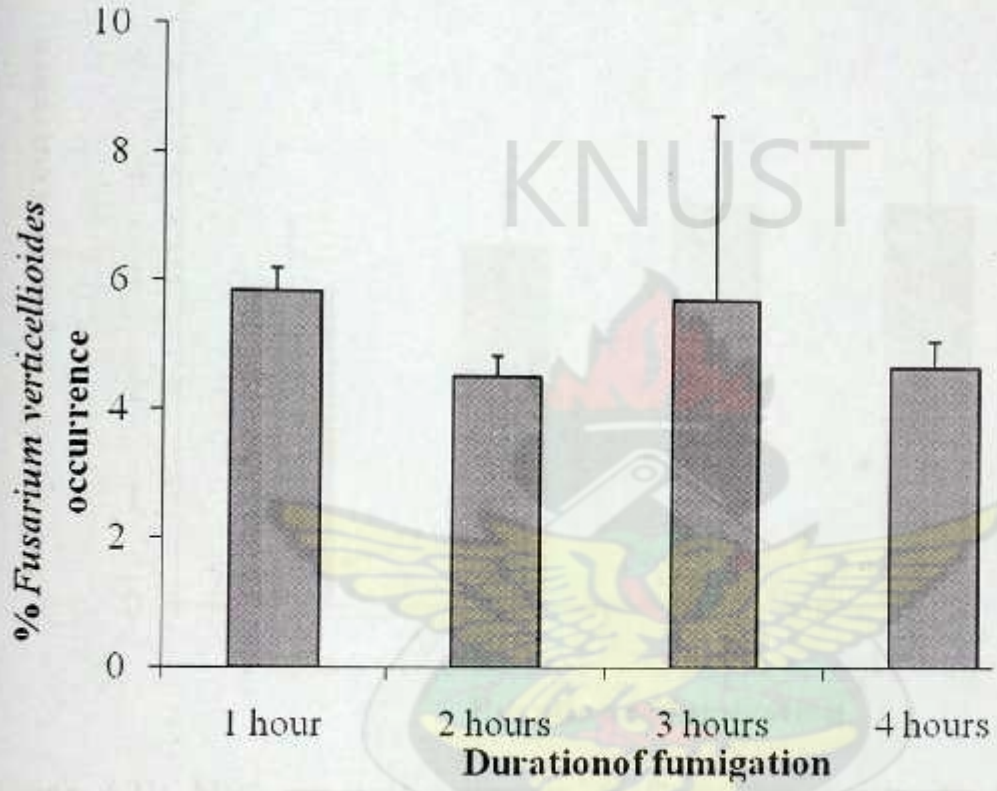

Figure 4.20: Mean per cent occurrence of *Fusarium verticellioides* on shelled maize fumigated from one to four hours

The error bars represent standard error of means (SEM).

4.10.3 Effect of fumigation on mean per cent occurrence of *Penicillium* species

Penicillium species did not respond any differently to the fumigation from *Aspergillus* and *Fusarium* and the differences in their incidence on the differentially fumigated maize samples were not significant (Fig. 4.21).

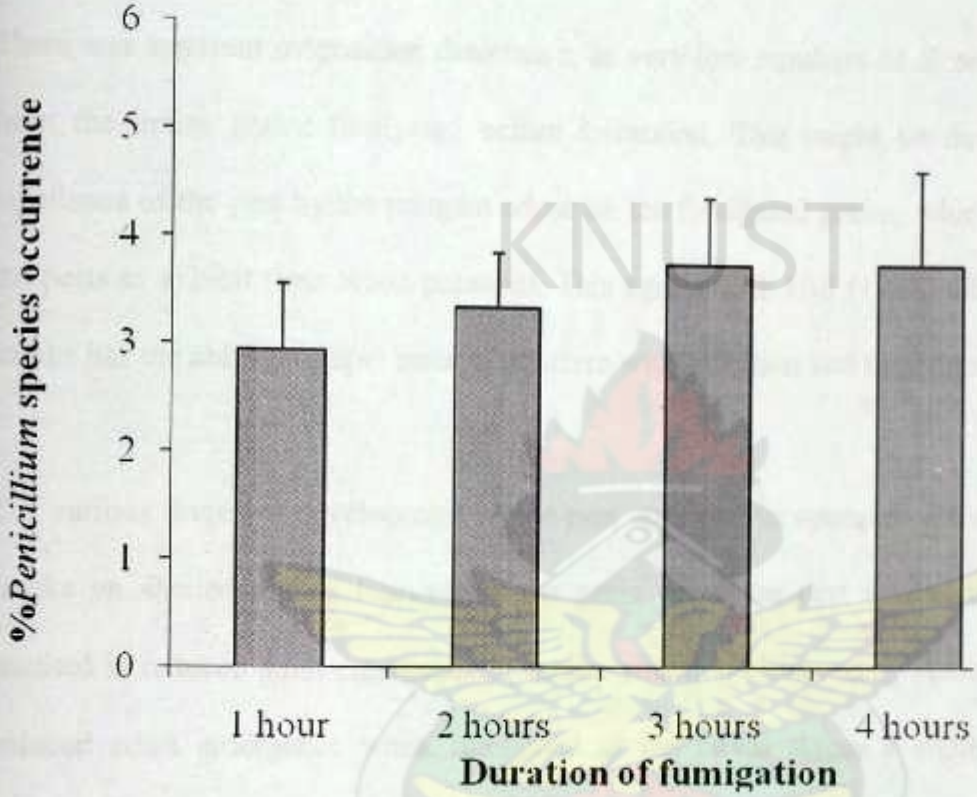

Figure 4.21: Mean per cent occurrence of *Penicillium* species. on shelled maize fumigated from one to four hours

The error bars represent standard error of means (SEM).

CHAPTER FIVE

DISCUSSION

5.1 Effect of smoke fumigation on oviposition deterrence and on the development of the various developmental stages of *S. zeamais.*

There was apparent oviposition deterrence, as very low numbers of *S. zeamais* emerged from the maize grains fumigated before infestaion. This might be due to the initial repellence of the pest by the pungent odour on the fumigated grains, which did not allow the pests to exhibit their biotic potential. This agrees with Hill (1987) who reported that smoke has the ability to repel insects, interfere with olfaction and thus delay proliferation.

The various stages of development of the pest appeared to succumb differentially to the smoke on shelled maize. Fumigating the grain when the pest was at the larval stage resulted in reduced adult emergence, a similar situation observed by Ansah (2003). With reduced adult emergence when fumigated at the larval stage, a significantly longer median developmental period with a corresponding significantly lower weight loss was expected, but was not the case. This was because the larval stage is the longest and any significant interference would prolong the median developmental period. Also, at during the larval stage, that much feeding occurs inside the grain with corresponding weight loss, since weight loss is related to the number of insects and feeding duration (Asawalam and Hassanali, 2006). A very obvious reason was that the smoke did not have any appreciable insecticidal properties on the various developmental stages of the pest on shelled maize. It is also probable that the smoke provided a limiting environment

therefore a faster developmental rate thus the shorter median developmental period at the larval stage.

The results obtained when the maize on cobs (dehusked maize) was fumigated were similar to the observations for the fumigated maize grains. The number of the pest that emerged, per cent damaged grains and median developmental period, fumigating at the various developmental stages, were not significantly different.

Increasing the duration of fumigation from 1 h to 4 h on shelled maize also did not show significant differences. Should the smoke have any significant killing or antifeedant properties, increasing the duration of fumigation (dosage) would have improved grain protection but this was generally not realized. This observation is in contrast to several authors (Talukder and Howse, 1995; Obeng-Ofori and Reichmuth, 1997; Udo, 2005; Asawalam and Hassanali, 2006) who had reported decreased insect emergence with better grain quality, as insecticide dosage increased. As stated earlier, it is probable that the smoke may not have a killing or antifeedant effect.

5.2 Persistence of smoke on stored maize

It was expected that the effect of the fumigation would decrease with time of storage and with it, increasing emergence of the pests. But this was not observed, as differences between the increasing storage periods before insect infestation were not significant. This results contrast those of Obeng-Ofori and Reichmuth (1997) who reported a decrease in the persistence of eugenol with the increase of length of storage after application.

Since the nature and constituents of smoke are influenced by the temperature of combustion (29-34^{0}C in the fumigation chamber), and volatile organic compounds (VOCs) are released from smoke at ambient temperatures of combustion (Todd, 2003), it is probable that the smoke evaporated quickly from the surface of the treated materials thus its failure to persist. As the weevil was able to oviposit and develop well on the treated maize, the inconsistent result obtained for the persistence studies, could not be attributed to the smoke.

5.3 Effect of fumigation on undehusked maize

The mean numbers of *S. zeamais* that emerged when the maize was fumigated for the various duration of fumigation and sampled at 4 and 8 weeks later were not significantly different from that of the samples taken 1 week after fumigation. This is in contrast to the report of several authors (Asawalam and Hassanali, 2006; Udo, 2005; Obeng-Ofori and Reichmuth, 1997; Talukder and Howse, 1995) who reported decreased insects emergence and better quality grain with increasing insecticide dosage. The differences could be because these authors used essential oils and plant powders that were directly applied to the surface of the grain.

In this study, the sheaths may have acted as a barrier to the smoke and thus provided the normal atmosphere for the pest's development. Even if the fumes penetrated the sheath, as stated earlier, the smoke was not persistent and also did not demonstrate insecticidal properties, thus the non apparent change in the insect numbers with increasing dosage and time.

5.4 Repellence of smoke

A significantly large proportion of the *S. zeamais* was repelled from the treated maize for up to 48 h after treatment, however greatest within the first 1 h of exposure. This suggests a repellent ability of the smoke from *S. siamea* twigs, but not in a persistent manner. This finding agrees with Isman (2006) who reported that there are several plant oil repellants against some insects, but are effective for less than 1 h. The failure of the smoke to persist might be due to volatile organic compounds (VOCs) released from smoke at ambient temperatures of combustion (Todd, 2003). It is also possible that there is a behavioural plasticity in which the pest adapts to the odour rendering it ineffective in a matter of hours (Isman, 2006). The decreased activity might be due to air movement. The situation might be different if air tight containers were used (Udo, 2005).

5.5 Contact toxicity and effect of smoke on adult *S. zeamais*

Most insecticides used against *S. zeamais* are contact or respiratory poisons (Hill, 1987), and it was expected the smoke will at least choke the tracheal system of the adult weevils. The inability of the smoke to immobilize, let alone kill the adult weevil, after 4 h of continuous fumigation suggests that the smoke has no killing effect on the adult weevil, and if at all not at this duration. This finding confirms the report of Ansah (2003) who reported that smoke from five botanicals including *S. siamea* had no killing effect on *S. zeamais*. Addo *et al.* (2002) also reported that in the Jasikan district of Ghana, smoking was particularly useful in drying maize and that when used alone, was not sufficient in preventing the threats of insects.

Since most insecticides have two main ways (namely acute and chronic effect) of eliciting their toxic effect, it is possible that a comparatively longer exposure to the smoke may evoke a chronic toxic effect should they be persistent and not readily hydrolysed. This is because the constituents of smoke have been reported to contain phenols, organic acids, carbonyl compounds and hydrocarbons, which are generally considered important in killing storage pest (Kramlich *et al.*, 1973).

5.5 Effect of the fumigation on Fungi on shelled maize

Increasing the duration of the fumigation up to 4 hours did not have any significant effect on *Aspergillus flavus, Fusarium verticelloides* and *Penicillium* sp. even though the fungitoxicity of the smoke *in vitro* has been reported (Awuah, 2005). This apparent difference in results might be due to differences in the testing procedure. In the *in vitro* set up of Awuah (2005), the fungi were directly fumigated on the growth media Potato dextrose agar. Dharmaputra *et al.* (1990) also reported that increasing the concentrations of carbon dioxide had no significant effect on the total population of fungi including *A. flavus* and *Penicillium* spp on storage maize. This, however, contrasts Essien *et al.* (2008) who reported a dose dependent effect in the control of several fungi including *Penicillium* spp. with essential oil of *Citrus medica* on groundnut.

CHAPTER 6

Conclusion and Recommendation

6.1 Conclusions

The study has shown that;

1 smoke from *Senna siamea* interfered with the developmental stages of *Sitophilus zeamais,*

2 the smoke was not persistent on the stored grain,

3 increasing the duration of fumigation of undehusked maize did not significantly affect the number of the weevil that emerged, per cent damaged grains and grain weight loss maize,

4 the smoke from *S. siamea* exhibited a repellent effect on the weevil,

5 the greatest number of the weevil was repelled during the first hour of exposure to the shelled maize,

6 maize fumigated for four hours did not exhibit any contact toxicity to the weevil,

7 direct fumigation of the adult *S. zeamais* for 4 hours did not kill the adult pest,

8 smoke from *S. siamea* twigs cannot be used in the management of *A. flavus, F. verticilliodes* and *Penicillium* species on maize grains

9 results of this study showed that the smoke might be of practical use only if farmers harvested early, stacked the harvested maize quickly in narrow cribs and then burned twigs from S. siamea to repel colonizing weevils.

6.2 Recommendations

Only dry twigs of the *S. siamea* were used as source of smoke. This might have caused the loss of vital ingredients from the twig. It is necessary therefore, to test fresh stems, cut and air dried.

Even though the adult pest did not die after four hours of continuous fumigation, one needs to study the effect of the smoke on the fecundity of females and their offspring. A negative effect of the smoke on the biotic potential of the weevil will have a long term effect on the weevil.

The repellency of the smoke should be tested in the field.

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APPENDICES

Appendix 1: Analysis of variance on the effect of fumigating shelled maize containing different developmental stages of *S. zeamais* on its emergence.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	8.065	4.033	2.26	0.186
Residual	6	10.722	1.787		
Total	8	18.787			

Appendix 2: Analysis of variance on the effect of fumigating shelled maize containing different developmental stages of *S. zeamais* on median developmental period.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	0.0084292	0.0042146	12.19	0.008
Residual	6	0.0020746	0.0003458		
Total	8	0.0105038			

Appendix 3: Analysis of variance on the effect of fumigating shelled maize containing different developmental stages of *S. zeamais* on per cent damage grains.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	10.2507	5.1253	5.49	0.044
Residual	6	5.6065	0.9344		
Total	8	15.8572			

Appendix 4: Analysis of variance on the effect of fumigating shelled maize containing different developmental stages of *S. zeamais* on weight loss.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	0.507	0.253	0.09	0.920
Residual	6	17.865	2.977		
Total	8	18.371			

Appendix 5: Analysis of variance on the effect of fumigating maize cobs (dehusked maize) containing different developmental stages of *S. zeamais* on emergence.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	11.561	5.781	2.75	0.142
Residual	6	12.615	2.102		
Total	6	24.176			

Appendix 6: Analysis of variance on fumigating dehusked maize containing different developmental stages of *S. zeamais* on median developmental period.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	0.002721	0.001361	1.28	0.343
Residual	6	0.006354	0.001059		
Total	8	0.009075			

Appendix 7: Analysis of variance on the effect of fumigating dehusked maize containing different developmental stages of *S. zeamais* on per cent damage grains.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	6.893	3.446	2.28	0.184
Residual	6	9.079	1.513		
Total	8	15.972			

Appendix 8: Analysis of variance on the effect of fumigating dehusked maize containing different developmental stages of *S. zeamais* on weight loss.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	7.0631	3.5315	6.91	0.028
Residual	6	3.0652	0.5109		
Total	8	10.1283			

Appendix 9: Analysis of variance on the effect of different duration of fumigation on number of *S. zeamais* emerged from shelled maize.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	3	2.260	0.753	0.24	0.863
Residual	8	24.675	3.084		
Total	11	26.936			

Appendix 10: Analysis of variance on the effect of different duration of fumigation on per cent damaged shelled maize grains.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	3	5.243	1.748	0.56	0.659
Residual	8	25.164	3.145		
Total	11	30.407			

Appendix 11: Analysis of variance on the effect of different duration of fumigation on per cent weight loss of shelled maize grains.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	3	2.2911	0.7637	0.94	0.464
Residual	8	6.4811	0.8101		
Total	11	8.7722			

Appendix 12: Analysis of variance on the effect of different duration of fumigation on per cent shoot emergence of shelled maize.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	3	0.09014	0.03005	0.78	0.537
Residual	8	0.30804	0.03851		
Total	11	0.39818			

Appendix 13: Analysis of variance on the effect of persistence of smoke on dehusked maize infested various days after fumigation on the emergence of *S. zeamais*

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	6	74.687	12.448	8.30	<.001
Residual	14	20.986	1.499		
Total	20	95.673			

Appendix 14: Analysis of variance on the effect of persistence of smoke on dehusked maize infested various days after fumigation on damaged grains

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	6	14.2798	2.380	7.88	<0.001
Residual	14	4.2296	0.3021		
Total	20	18.5094			

Appendix 15: Analysis of variance on the effect of different duration of fumigation on number of *S. zeamais* emerged from undehusked cobs sampled one week after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	33.606	8.401	1.70	0.227
Residual	10	49.535	4.954		
Total	14	83.141			

Appendix 16: Analysis of variance on the effect of different duration of fumigation on number of *S. zeamais* emerged from undehusked cobs sampled four weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	68.377	17.094	3.69	0.043
Residual	10	46.352	4.635		
Total	14	114.729			

Appendix 17: Analysis of variance on the effect of different duration of fumigation on number of *S. zeamais* emerged from undehusked cobs sampled eight weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	129.759	32.440	3.50	0.049
Residual	10	92.586	9.259		
Total	14	222.344			

Appendix 18: Analysis of variance on the effect of different duration of fumigation on per cent damage of undehusked cobs sampled one week after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	23.995	5.999	3.76	0.041
Residual	10	15.943	1.594		
Total	14	39.937			

Appendix 19: Analysis of variance on the effect of different duration of fumigation on per cent damage of undehusked cobs sampled four weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	8.1589	2.0397	3.08	0.068
Residual	10	6.6243	0.6624		
Total	14	14.7832			

Appendix 20: Analysis of variance on the effect of different duration of fumigation on per cent damage of undehusked cobs sampled eight weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	0.92798	0.23200	2.64	0.098
Residual	10	0.88037	0.08804		
Total	14	1.80835			

Appendix 21: Analysis of variance on the effect of different duration of fumigation on per cent weight loss of undehusked cobs sampled one week after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	77.913	19.478	4.74	0.021
Residual	10	41.088	4.109		
Total	14	119.000			

Appendix 22: Analysis of variance on the effect of different duration of fumigation on per cent weight loss of undehusked cobs sampled four weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	33.10	8.27	0.63	0.651
Residual	10	130.95	13.09		
Total	14	164.05			

Appendix 23: Analysis of variance on the effect of different duration of fumigation on per cent weight loss of undehusked cobs sampled eight weeks after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	4	36.484	9.121	1.16	0.383
Residual	10	78.340	7.843		
Total	14	114.824			

Appendix 24: Analysis of variance on number of *S. zeamais* repelled one hour after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	53.96	26.98	1.00	0.001
Residual	2	3016.32	26.98		
Total	4	107.91			

Appendix 25: Analysis of variance on the number of *S. zeamais* repelled 24 hours after fumigation.

Source of variation	DF	SS	MS	VR	F.pr
Treatment level	2	79.54	604.42	1.14	<.001
Residual	2	1052.20	19.63		
Total	4	139.67			

Appendix 26: Analysis of variance on the number of *S. zeamais* repelled 48 hours after fumigation

Source of variation	DF	SS	MS	VR
Treatment level	2	20.39	10.19	0.91
Residual	2	2120.26	11.18	
Total	4	44.71		

Appendix 27: Analysis of variance on the effect of different duration of ımigation on mean per cent occurrence of *Aspergillus flavus.*

Source of variation	DF	SS	MS	VR
Treatment level	3	0.7899	0.2633	0.77
Residual	12	4.1026	0.3419	
Total	15	4.8925		

Appendix 28: Analysis of variance on the effect of different duration of fumigation on mean per cent occurrence of *Fusarium verticilliodes.*

Source of variation	DF	SS	MS	VR
Treatment level	3	5.4968	1.8323	2.97
Residual	12	7.3954	0.6163	
Total	15	12.8922		

Appendix 29: Analysis of variance on the effect of different duration of fumigation on mean per cent occurrence of *Penicillium* species.

Source of variation	DF	SS	MS	VR
Treatment level	3	1.9499	0.6500	0.88
Residual	12	8.8135	0.7345	
Total	15			
		10.7634		

Appendix 30: Abbotts correction formula:

Corrected % = [1-(N in T after treatment /N in Co after treatment)] x 100

Where N = Insect population

T = Treated

Co = Control