EFFECTIVENESS OF DIATOMACEOUS EARTH, SELECTED BOTANICALS AND PESTICIDES AGAINST *SITOPHILUS ZEAMAIS* (MOTSCH) AND *ACANTHOSCELIDES OBTECTUS* (SAY)

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REG NO 2002/HD02/992U

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTERS OF SCIENCE IN AGRICULTURE (CROP SCIENCE) OF MAKERERE UNIVERSITY.

DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE

2009

Declaration

I hereby declare that the work presented in this thesis is original and has not been submitted to any other university or college.

Signed...Date........10/11/2009.....

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

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Dedication

To parents, brothers, sisters and Christopher my son

Acknowledgement

Am very grateful to my supervisors, Professor Samuel Kyamanywa and Dr Ambrose Agona for their advice, guidance, encouragement and constructive criticism throughout the course of the study.

I would like to acknowledge the tireless support of the Head of Post-harvest Programme for his effort in accessing funds which enabled me to conduct this study. Special thanks go to the European Union Support to National Agricultural Research Organization for funding this study.

My heart felt thanks go to all Post-harvest staff especially the technicians Mr. Godfrey Musitala, Ms Jennifer Adomu and Ms Gloria Aguti who assisted me in data collection.

Finally, my sincere thanks to my family and friends for their encouragement, prayers and love.

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Abstract

Among the storage problems of maize and beans, insect pests are a major constraint. In order to minimize storage losses, farmers usually apply different methods which include physical or inert materials, cultural means, botanical and chemicals to control storage pests. However, none of the individual control method has been successful.

Diatomaceous earth (DE), a natural mineral product from fossilized silica shell or remains of unicellular organisms is reported to be effective on storage pests. Of recent, DE has been found in Nebbi district in Uganda. The efficacy of DE of Uganda origin is not well known. A study was therefore conducted to evaluate the effectiveness of DE of Uganda origin against *S. zeamais* and *A. obtectus,* the major storage pests of maize and beans respectively.

Three experiments were conducted; first was to determine the DE optimum dosage rate, the second one was to study the effect of DE on hatchability and survival of *A. obtectus* and third was to compare efficacy of DE, selected botanicals and pesticides against the two storage insect pests. Optimum DE application rate was determined by investigating six different dosage rates of 0.5,1,2,3,4 and 5% weight per weight basis. The control was untreated grain. The treated and non treated grains were artificially infested with 5 males and 10 females of each of the species obtained from routine stock culture, each treatment replicated four times. 2-3 days old *A. obtectus* and 14 days old *S. zeamais* were used. The insects were allowed to mate and oviposit for seven days and then removed and discarded. To count the eggs of *S. zeamais,* the grains were treated with acid fuschin to stain the eggs. For *Acanthoscelides obtectus,* eggs were separated from DE-bean mixture and counted under a binocular microscope. After counting, the eggs together with the pre-determined DE dosages and grains were put back to their respective containers and incubated.

The infested cultures were placed in shelves in laboratory under prevailing ambient conditions until the F_1 progeny started to emerge. The adults that emerged were counted daily, recorded and then discarded.

Results showed that there were significant differences ($P < 0.05$) in number of eggs laid and adult emergents in grains treated with DE compared to the controls. The untreated grain registered the highest number of eggs laid and adult insect emergence while grains treated with DE at 5% dosage rate recorded the least number of eggs and insect emergence. However, there were no significant differences ($P > 0.05$) in the mean number of emergent adults in grains treated with DE at different dosage rates.

The highest hatchability percentage and survival of *A.obtectus* was in the controls and lowest in grains treated with DE at 5% dosage level.

The effect of botanicals, DE (0.5, 1, and 2%) and insecticides (actellic super1%, malathion 2% dust) were evaluated. Botanicals tested included; tobacco leaf and seed powder, neem leaf and seed powder. The same protocol used in experiment 1 was adopted.

Neem leaf and tobacco seed powder were not toxic to *S. zeamais* and *A. obtectus.* Tobacco leaf and neem seed extracts were effective against the two candidate storage insect pests. The mean number of *S. zeamais* and *A. obtectus* emergence in maize and beans treated with DE at 2% and 1% respectively were not statistically different from those treated with synthetic pesticides. There were no significant differences ($P < 0.05$) in mean number of adult that emerged and seed damage in grains treated with DE, tobacco leaf and neem seed powder.

The incorporation of DE in integrated pest management of *S.zeamais* and *A.obtectus* and their application on-farm is recommended. On-farm trials, should however, precede recommendation for wider use.

CHAPTER I

GENERAL INTRODUCTION

1.1 Storage constraints

In the tropics, it is estimated that 25-40% of stored agricultural products are lost due to inadequate farm level storage (Proctor, 1994). In Uganda, post-harvest losses of agricultural crops are estimated between 30-40% (Silim *et al.*, 1990). It is reported that within three to six months of storage, between 5-15% loss in weight occurs to legumes and cereals in the post-harvest chain, from the farm through trader storage and marketing chains (Silim *et al.*, 1990).

Among the most commonly stored produce are maize and beans. In Uganda, maize (*Zea mays* L) is the major cereal crop, while beans (*Phaseolus vulgaris* L.) is the main grain legume, and both are widely grown in almost all parts of the country (NARO Annual Report, 2003). Maize production in Uganda was estimated at 900,000 metric tonnes from 600,000 hectares (NAARI Annual Report, 1999/2000). The increased production was attributed to high adoption of improved varieties. Maize is widely consumed as a staple food and is an important raw material in brewery industry and animal feed.

According to the National Bean Programme Annual Report, (1997/1998) acreage planted to bean in Uganda has been consistently increasing over the years from 574,000 ha in 1994 to 685,000ha in1996. The increment to production area is attributed to farmers' response to increasing demand, for beans in the export market. On average, Uganda produces about 500,000 metric tons of beans annually which provide 25% and 45% of the caloric and protein intake in human nutrition, respectively (NARO Annual Report, 2003). Beans are valuable source of vitamin B-complex, iron, zinc, folic acid, complex carbohydrates and many other essential minerals. Both maize and beans are cash generators for small-scale farmers and important in rural food security.

In storage, maize and beans are prone to insects, rodents, microbes, birds and adverse changes in environmental conditions which lead to high losses. According to Hall (1980), insect pests are the major cause of deterioration of stored produce in the tropics and sub-tropics. Losses caused by storage insects are manifested in several ways. These include, weight loss resulting from direct insect damage, reduce seed quality and viability. Insect infestation also results into severe losses in nutritive value of food (Hall, 1980).

Hall (1980) reported that losses of thiamine in maize stored for eight months were 10-15% greater in infested than uninfected samples. Kabeere *et al.*, (2002) reported that more than 96% of farmers in Masindi district lost their stored beans to weevil infestation and this resulted into poor quality seed with low germination capacity (60%) compared to the 80% standard set by the National Seed Certification Service. In East Africa, weight loss of 30% has been reported for stored legumes in Kenya (De Lima, 1979), and 21% due to bruchids have been reported in grain legumes in Uganda after 6 months of storage (Silim *et al.,* 1990). In Zimbabwe, 8% of stored maize is lost mainly due to storage insect pests (Mvumi *et al.*, 1995), while in Uganda, weight loss of 1-3% on maize grain stored for six months caused by storage insect pests have been reported (Silim *et al.*, 1990)

Major insect pests of stored maize include; *Sitophilus zeamais* (Motsch), *Rhyzopertha dominica* (Fabricius), *Sitotroga cerealella* (Olivier), *Prostephanus truncatus* (Horn), *Lasioderma serricorne* (Golob and Hanks, 1990), while on beans *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh) are common (Silim *et al.*,1990). Among the storage insect pests, the maize weevil, *S. zeamais* and the bean bruchids, *A. obtectus,* are the most important pests on stored maize and beans respectively, (Silim *et al.*, 1990).

1.2 Problem Statement

In order to minimise or eliminate the damage caused by *S.zeamais* and *A.obtectus*, a number of control measures have been used which include; chemical, cultural, host plant resistance, biological and physical methods (Van Huis, 1991). Conventional insecticides have been used for long, as grain protectants and most of them were reported to be effective against storage insect pests. However, the increased availability and use of insecticides has led to insects developing resistance which render most of them ineffective (Taylor and Webley, 1979; Sriharen *et al.*, 1990; Golob and Hanks, 1990). In addition, chemicals are not usually available and/or affordable by many subsistence farmers and are user and environmental unfriendly.

Although biological control agents are possible candidates for pest management, they are usually very specific, can easily be out competed by pests and require special features of storage environment which limit their use (Gwinner et al., 1996).

Cultural pest control methods have been used since time immemorial. However, the effectiveness of cultural practices in management of storage pests is variable. Cultural control practices are less costly but their effect is limited, which excludes the possibility of any general application of such measures (Van Huis, 1991). On the other hand, use of host resistance may take long to develop resistant varieties of desirable traits. Host plant resistance has been mostly restricted to control of field pests with little effort being directed towards developing resistance against storage pests.

Some of the physical methods which have been tested include; solarisation, (Agona and Silim, 1998), air tight bins (Kyamanywa *et al*., 1999), use of inert materials such as ash (Kyamanywa, 1991), diatomaceous earth (Stathers *et al*., 2000). However, solarisation may hamper seed germination and there is re-infestation of grains (Agona and Silim, 1998) while sealed storage may not be practical and /or affordable by most resource poor farmers.

Some of the botanicals which have been found effective against storage pests include; *Cassia nigricans* (Lambert *et al*.,1985), *Azadirachta indica, Melia azedarach*, (Van Huis, 1991), *Tephrosia vogelli, Chenopodium sp* ,*Tagetes minuta* and *Nicotiana tabacum* (Kyamanywa *et al*., 1999; Silim, 1999). However, very few of the plant materials have been studied under traditional storage conditions (Van Huis, 1991). Consequently their practibility and effectiveness under field usage have been questioned (Taylor and Webley, 1979). In addition, botanicals may taint the grain with either undesired smell or colour (Van Huis, 1991).

In view of the above storage problems, studies elsewhere have been going on to explore other means of protecting grains against storage insect pests. Among the alternative methods is the use of diatomaceous earth (DE) which is a natural and non toxic inert material.

1.3 Justification

Diatomaceous earth is natural and non toxic. It has been tested and found to be effective on storage pests and gives long grain protection unlike chemicals (MacDonald, 1986; Stathers *et al.*, 2000; Garrett, 2004). DE had been approved by the United States Food and Drug Administration for use. According to NARO Annual Report (2004), DE is available in Uganda along the River Nile in Nebbi district. Its exploitation and utilisation is currently at a very low scale mainly for plastering walls. The efficacy of DE of Uganda origin in controlling insect pests is not well known and optimal dosage against major storage pests of maize and beans has not been established, hence the need to conduct this study. It is believed that once its effectiveness is proven, there are great opportunities for its commercialization and use by farmers which can lead to optimization of their incomes by extending the shelf life of stored products until a good market price is attained.

1.4 Objectives

The overall objective of the study was to evaluate the efficacy of diatomaceous earth on *S. zeamais* and *A. obtectus* and compare it with other management methods.

The specific objectives of the study were to:

1. Determine the optimum dosage rate of diatomaceous earth for control of S. *zeamais* on stored maize grain.
2. Determine the optimum dosage rate of diatomaceous earth for control of A. *obtectus* on stored bean.
3. Determine the effect of DE on hatchability and survival of first larval instar of *A. obtectus*
4. Compare the efficacy of DE, botanicals and pesticides in the management of *S. zeamais* and *A. obtectus* in stored maize and beans, respectively.

1.5 Hypotheses

- DE is effective against *S. zeamais* and *A. obtectus* irrespective of dosage rate.
- DE is as good as botanicals and pesticides in controlling *S. zeamais* and *A. obtectus*.

CHAPTER II

LITERATURE REVIEW

2.1 Biology and economic importance of maize weevil (*Sitophilus zeamais*) (Motsh) (Coleoptera: Curculionidae)

An adult maize weevil is about 2-4 mm in length with a well defined snout or rostrum and reduced mouthparts (Haines, 1991). The female has a smooth and slender rostrum with regular spaced pits while the male has a rough surface and shorter rostrum with irregular pits (Halstead, 1963).

According to Longstaff (1981), the adult weevil is long-lived, attaining an average of about six months to one year. Eggs are laid throughout most of the adult life, although 50% may be laid in the first two to five weeks. Up to 150 eggs or more may be laid per female (Haines, 1991). Eggs are laid individually in small cavities chewed into the cereal grain by the female, after which the egg cavity is sealed by mucilaginous material produced by the female (Haines, 1991).

Longstaff (1981) reported that when the female bores a hole in the grain, they never damage the embryo, hence the eggs are mostly oviposited on the endosperm. Under optimal conditions eggs take six to seven days to hatch and there are four larval instars (Kenneth, 1988). After hatching, the larva feeds on the endosperm and moults four times (Haines, 1991). The total lengths of larval and pupal stages are 18-22 and 6-7 days, respectively. Pupation takes place within the grain (Haines, 1991). The pupal stage is inactive which later metamorphoses into an adult. The young weevil remains within the grain for sometime; two to three days from where it eats through to the outer seed coat and emerges (Haines, 1991). Under warm favourable conditions, the life cycle takes some 26-35 days, depending on the maize variety. However, the development period may be prolonged to two or to three months in cooler weather (Zakladnoi and Ratanova, 1987). According to Dobie (1974) and Muyinza (1998) developmental period of *S. zeamais* greatly varied among different maize varieties. Dobie (1974) reported that maize varieties with floury and or soft endosperm were highly susceptible, had shorter developmental periods and were greatly infested by *S. zeamais*. Optimum conditions for development are 25-27^{0}C and 70% relative humidity (Kenneth, 1988). Both the larvae and adults feed on the grain and are primary pests of sound, dry cereal grains (Hall, 1980).

S. zeamais is one of the most important storage insect pest of maize (Kenneth, 1988; Dowswell *et al.*, 1996; Silim *et al.*, 1990). It is a primary pest of cereal grains but has been reported to attack other commodities like split peas (Haines, 1991) and dried cassava and sweet potato chips (Agona, 1998).

S. zeamais has been widely reported to infest maize cobs in the field as the crop approaches maturity (Giles and Ashman, 1971; Silim *et al.*, 1992).

The adults can fly and eggs may be laid on the grain when still in the field or in store (Giles and Ashman, 1971; Markham, 1981; Kenneth, 1988; Silim *et al.*, 1992).

The ability of *S. zeamais* and other post-harvest pests to infest growing maize plants appear to be influenced by factors related to the maturity of the grains. The most important factor being the moisture content of the grain. Moisture content of the grain has great influence on the development of storage insect pests. In Kenya, Giles and Ashman (1971) reported that *S. zeamais* survival depends on moisture content of the grain. Markham (1981) found no evidence of *S. zeamais* infestation of the grains which had a mean moisture content of 57%, but three weeks later, 45% of the harvested cobs were infested by *S. zeamais*, when moisture content of the grain had dropped to 30%. Dix and All (1985) suggested that immature maize at a high moisture content lacks certain chemical compounds attractive to weevils, which are produced by maturing grains.

In storage infestation builds up rapidly with corresponding high levels of damage (Giles and Ashman, 1971; Silim *et al.*, 1992). Silim *et al.*, 1992) reported that an apparently undamaged grain at harvest becomes heavily infested only after four to eight weeks of storage. The degree of damage varies according to the grain type, adequacy of husk cover, store condition, duration of storage and pest combination in storage (Kenneth, 1988; Silim *et al.*, 1992). Maize with soft and starchy kernels are more readily infested than hard kernels (Dobie, 1974; Muyinza, 1998). Similarly, in the field, maize ears that are not completely covered by the husk, which is a frequent shortcoming of improved maize varieties are more preferred than the well covered local varieties (Giles and Ashman, 1971; Markham, 1981; Silim *et al.*, 1992). Studies conducted in Zambia demonstrated losses due to storage insect pests at smallholder level varied with maize varieties. Local varieties had the lowest yield losses (1-3%), improved varieties (5-6%) and highest in the hybrids (8-9%) (Adams and Harman, 1977). This suggested that improved varieties were more susceptible to insect damage than the local varieties.

According to studies conducted in Kenya on maize cobs taken from farmers' stores after nine months of storage had on average 1.6% and 1.3% weight loss as a result of infestation by *S. zeamais* and *S. cerealella*, respectively (De Lima, 1979). In Uganda, weight loss of 1-3% on maize grain stored for six months have been reported (Silim *et al.*, 1990). Zakladnoi and Ratanova, (1987) reported that 75% of maize grain damage were attributed to *S. zeamais* infestation. The major effect of infestation by *S. zeamais* is the damage of the grain by feeding activities of adults and the developing immature stages within the grain which affect the quantity and quality of the grain (De Lima, 1979). This does not only reduce the quality of the grain but also produces considerable amount of grain dust, and excreta which make the grain completely unpalatable. In addition to this, as a result of their respiratory activities, heat is produced in the grain causing rise in local temperature in the packed grain. The rising temperatures usually speed up insect development so that weevil populations rise sharply (Hall, 1980). The rising temperatures lead to the movement of moisture to the surface of grain bulk where conditions become optimal for development of various fungal species some of which are very toxic to man (Hall, 1980).

Golob (1984) reported that insect infestation of grain often results in decreased levels of essential amino acids and seed viability. As a result of the weevil feeding activities, the quality of the remaining grain is lowered, germination is reduced or abnormalities occur during germination (Hall, 1980). In Uganda, where small farmers form the basis for maize production, *S. zeamais* has come to be one of the biggest constraints on economic returns from maize production. Due to the weevil infestation, farmers are forced to sell off their surplus maize produce immediately after harvest and this coincides with the time when prices are usually very low (Silim *et al.*, 1990).

2.2. Biology and economic importance of bean bruchid (*Acanthoscelides obtectus*) (Say) (Coleoptera: Bruchidae)

The adult bruchid is a small, hard-shelled beetle, about 5 mm long, with broad rounded end, and a dull, grayish-brown colour. In the course of its development, *A.obtectus* passes through four developmental stages; eggs, larvae, pupae and adults (Howe and Currie, 1964). *A. obtectus* female lays its eggs loosely among the beans (Schoonhoven, 1976). It may infest the grain in the field where eggs are laid in cracks or cuts on the growing pods and egg laying usually begins within two or three days after the weevil emerges and continues throughout the female's life (Haines, 1991).

Haines (1991) reported that larval development is very rapid at room temperature and in three to four days the head and body segments of the developing larva can be seen by microscope through the eggshell. The newly hatched larva promptly bores into the bean seed, where it tunnels and feeds until full grown (Haines, 1991). The growing larva within the bean molts four times and then becomes a pupa (Haines, 1991). The pupa develops from the full grown larva within the bean and the adult weevil transforms from the pupa, and immediately bores out of the bean leaving a round clear hole (Zakladnoi and Ratanova, 1987). The male and female bruchids mate immediately after emergence and the female lay the eggs within 2-3 days for the next generation.

On average, a female may lay 63 eggs, and the largest number of eggs per female are deposited at temperatures between 21.0^{0}C and 27.1^{0}C and at this temperature, 95% of eggs are deposited during the first six days (Schoonhoven, 1976). Total developmental period of the bean bruchid was observed to range from 28 days at 32^{0}C to 61 days at 24.6^{0}C (Howe and Currie, 1964).

According to Haines (1991), the optimum conditions for rapid development were 70% relative humidity and 30^{0}C, when the insects spent 22-23 days inside the bean. Oviposition starts rapidly after emergence as adults are short-lived, adults live 12 days at 30^{0}C and 70% relative humidity and the adult bruchids do not feed (Schoonhoven, 1976). The larva is the major destructive stage of the bean weevil.

The principal stored insect pest of beans is the bruchid, *A.obtectus* (Say) (Schoonhoven, 1976; Taylor, 1981; Silim and Agona, 1993). *A. obtectus* cause losses similiar to *S. zeamais* such as qualitative, quantitative, and reduced seed viability. Unlike *S. zeamais* where both adults and larvae damage the grains, for *A. obtectus,* it is only the larvae that cause damage as the adults do not feed (Haines, 1991). Bean infestation by *A. obtectus* starts in the field (Schoonhoven, 1976; Silim *et al.*, 1990).

In East Africa, losses attributed to bruchid damage have been estimated between 30 - 73% (Karel and Khamala, 1978). In Uganda, loss in weight levels of 3 and 8% on beans stored for three and six months respectively have been attributed to *A. obtectus* (Silim *et al.*, 1990).

2.3 Control measures against storage pests of maize and beans

A number of methods are available for management of storage insect pests. These include, cultural, botanicals/biorationals, host resistance, biological, physical and chemical control methods (Silim *et al;* 1990). The choice of control measure applied depends on a number of factors like type and biology of the pest, storage conditions, extent of damage, environmental conditions e.t.c. Most of these control techniques are inherently related to certain forms of storage and none of them is perfectly complete and without disadvantages. Therefore an integrated approach of pest management is deemed necessary.

.3.1 Chemical pest control method

Chemical means of controlling pests include both bio-pesticides, organic and norganic insecticides. Chemical control is the most commonly used method nd it involves use of mainly contact insecticides and respiratory poisons or umigants (Hall, 1980). Most of the contact insecticides used against storage nsect pests are dry formulations and in dust form. These are ready to use nd contain low percentage of active ingredient plus a very fine inert carrier nade from talc, chalk, clay e.t.c. The recommended storage insecticides nclude; Pirimiphos-methyl and Permethrin (actellic super 1%), malathion 2% ust and other grain dusts.

umigants provide no residual effect but unlike contact insecticides, have the ower to penetrate stocks of bulks and be absorbed into individual grains illing all stages of insects within. The method if properly used can ensure otal elimination of pests within the shortest time. Aluminium phosphide, that elds phosphine, the active ingredient, is the common fumigant used against orage pests. Fumigants are highly toxic and need to be applied under rtight conditions and by experts to ensure safety. Fumigation gives grain mporary protection and there is a problem of grain re-infestation if no other ontrol measures are undertaken.

ontact insecticides tend to be specific in their effect upon insect species d, pests tend to develop resistance to them more than respiratory poisons arkin *et al.,* 1962; Hall,1980; Shriharen *et al.,* 1990). Georghiou and Taylor, 86; Haubruge and Arnaud, (2001) reported pesticide resistance in stored oduct insects in different countries and in particular against malathion.

part from insects developing resistance, chemical control methods have itations and are unsuitable under small scale subsistence agriculture as most of the pesticides are unavailable, prone to user abuse, expensive, have low shelf-life, are highly toxic (phosphine) and farmers generally lack the technical expertise in the handling and application of chemicals (Taylor and Webley, 1979; Hall, 1980; Agona, 1998).

In Uganda, a number of plant materials are used by farmers to control storage pests. Van Huis, (1991); Kyamanywa *et al.*, (1999) and Silim, (1999), reported that *Azadirachta indica, Dennettia tripetala*, *Cassia nigricans Nicotiana tabacum, Mexican marigold, Melia,* and *Chenopodium* are the most effective in controlling the storage pests of beans.

Elsewhere, plant materials in form of oil extracts, plant parts or plant products have been used to control storage pests (Nazan, 1983; Ajayi *et al.*, 1987; Dunkel *et al.*, 1990; Jembere *et al.*, 1995). Most of these plants are known to have either antifeedant, repellent or insecticidal effects on storage pests (Van Huis, 1991). *Azadirachta indica* (neem kernel extract), *Nicotiana tabacum, Melia* and *Cassia nigricans* have been reported to have insecticidal effect and repulsive, inhibition of insect development (Van Huis, 1991).

However, very few of these plants have been studied under traditional storage conditions and in many cases some may offer partial protection (Van Huis, 1991). As a result of this, their practicability and effectiveness under field conditions is inconclusive (Taylor and Webley, 1979; Van Huis, 1991).

There is paucity of information on the toxicological levels of botanicals on stored grains which may restrict their usage. In addition, botanicals may taint the grain with either undesired smell or colour, even though they are cheap, safe and easily available to the farmers.

2.3.2 Biological pest control of storage pests

Biological control involves use or manipulation of natural enemies/living organisms to reduce or control pest population and damage. These natural enemies are characterised as predators, parasitoids and micro-organisms/microbial. The use of biological control agents can keep storage insects at a low level although they cannot be eradicated. As storage pests are tolerated up to certain level in small farm storage, there are excellent possibilities for use of such control methods (Gwinner *et al.*, 1996).

Natural enemies can reduce insect population build-up before crop harvest for example deployment of *Teretrius nigrescens* in control of *P. truncatus* (Golob and Hanks, 1990).

Predators are free-living insects, which rapidly feed on the whole prey and attack all stages of development of a pest. The predators of storage insect pests according to Hodges, (1986); Golob and Hanks, (1990) include *Teretrius nigrescens*. Although very few predators have been tested, Golob and Hanks, (1990) demonstrated that *Teretrius nigrescens* was able to reduce *Prostephanus truncatus* (Larger Grain Borer), a serious pest of stored maize to minimum levels. Nangayo and Hill (1996) reported 85% reduction in Larger Grain Borer with release of *T. nigrescens* in two sites in Kenya in three years.

Parasitoids are the insects that feed on one living individual of a different species until it completes one of its growth stages. Parasitoids are parasitic in their immature stages only, while the adults are free living. Parasitic wasps are examples that occur in stores. They lay their eggs on immature stored products pests especially on the larvae of moths and beetles. Wasps then complete their development which usually results in the death of the pest (Haines, 1991).

Pathogenic micro-organisms like fungi, bacteria, viruses, nematodes and protozoa have been reported as disease causing agents of insects. According to Agona *et al.*, (2003) use of *Beaurveria bassiana* prior to harvest reduced bean bruchid; *A.obtectus* emergence and seed damage.

However, practical application of biological control methods against stored product insects, however, is still very limited because of some special features of the storage environment. For example, most biological control agents are naturally very susceptible to commonly used broad-spectrum insecticides, they also don't find very attractive living conditions in large storage facilities which make it difficult to establish themselves (Gwinner *et al.*, 1996).

According to Van Huis (1991), use of microbial agents for control of insect pests of stored products is still in its infancy and a lot of research work on these products is still to be undertaken. In addition to this, the market potential for these agents of durable foodstuff is still limited and costs, risks and time for their development are very high, thus making this method of control not very feasible (Gwinner *et al.*, 1996). The use of bio-control agents is suitable for pest in the wild, otherwise in storage their presence is unwanted since they are treated as any other insects. There is still limited information on use of natural enemies in the field to control pre-harvest infestation of *S. zeamais* and *A. obtectus.*

2.3.3 Host plant resistance

It has been ascertained that some varieties of crop grains are less susceptible than others to insect damage. Such varieties are described as being resistant or less susceptible to insect attack (Schoonhoven *et al.*, 1983; Dobie, 1984). Varietal differences in field infestation of common beans, *P. vulgaris* L; by bean weevil, *A obtectus* (Say), and the Mexican bean weevil *Zabrotes subfasciatus* (Boh), have been reported (Schoonhoven *et al.*, 1983). For example, according to studies by (Schoonhoven *et al.*, 1983), the small white varieties and wild types were less infested by *A. obtectus* than were the improved varieties.

Dobie (1984) investigated inherent resistance to attack by *S. zeamais* and *S. cerealella* of a large number of genotype at CIMMYT's maize bank in Mexico and found out that there was a good linear relationship in the susceptibility of maize varieties to the two insect species. Mechanisms of resistance in grains are attributed to the physical and chemical characteristics of the grain (Schoonhoven *et al.*, 1983, Arnason *et al.*, 1992). However, breeding for resistance is usually a long-term process and expensive in terms of human resources.

2.3.4 Physical pest management practices

Physical pest management methods have been in use since time immemorial under subsistence peasantry agriculture. Some of these methods include; solarisation, sealed bins, and sealed polyethylene bags (Agona and Silim, 1998; Kyamanywa, *et al.,* 1999) and use of inert materials like ash (Kyamanywa, 1991). The efficacies of the different methods in reducing damage levels are varied depending on the type of crop, species of pest, prevailing weather conditions and duration of storage.

According to (Kyamanywa *et al.,* 1999; Silim *et al.,* 1992; Agona and Silim, 1998), use of physical methods like sealed bins, polyethylene bags, inert materials, natural cover and solarisation were found to be as effective as the chemical control in the management of storage pests such as bean bruchids and maize weevil. Storage of grain in air tight containers depletes oxgyen and increases accumulation of carbondioxide which leads to suffocation and death of insects. Use of physical methods in controlling storage insect pests, have many benefits, they are cheap, user and environmental friendly. However, solarisation may hamper seed germination and there is re-infestation of grains (Agona and Silim, 1998).

Use of various ashes and inert dusts such as mixing wood ash, diatomaceous earth (DE) with the grains is reported to act as a physical barrier and/ or cause desiccation which leads to dehydration of the insect and can give grain some protection against storage pests (Kyamanywa, 1991; MacDonald, 1986). Inert dusts have been used as an insecticide for thousand of years by aboriginal peoples in North America and Africa (Golob, 1997). The main advantage of inert dusts is that they have low-mammalian toxicity, they are effective for long durations and do not affect end use quality (Desmarchelier and Dines,1987; Korunic *et al.,* 1996). Mode of action of inert dusts is generally accepted as desiccation due to abrasion of cuticular lining and are more effective when the grain moisture content and relative humidity is low. Abrasion of the cuticle is thought to be more important as a mode of action for the silica based inert dusts and is the main mechanism for the most inert dusts.

Another physical insect control mechanism is the grain cover. Several grain types have some form of natural cover when they are harvested. Maize is covered by a husk or sheath, beans are covered by pods which provide intact cover that act as an effective barrier to pests but this will vary with variety type, prevailing weather conditions and time of harvest (Markham, 1981; Silim *et al.*, 1992). However, Schwartz and Pastor-Carroles, (1989) reported that storing beans un-threshed or delaying harvest greatly enhanced *A. obtectus* attack. Many studies revealed that maize with good tight husk cover were less damaged by pests both in field and storage (Giles and Ashman, 1971; Markham, 1981; Silim *et al.,* 1992).

2.3.4.1 Diatomaceous Earth (DE)

Diatomaceous earth is a natural mineral substance made from the fossils of fresh water unicellular organisms (diatoms) and marine life which lived in oceans, rivers and lakes for million years (http:w.w.w.ighawii.com/naturally/naturalanimal/de.html). Diatoms which live in the surface layers of the ocean and fresh water lakes, remove silica from the water to make their exoskeletons, which is an amorphous form of silica

(MacDonald, 1986). When diatoms die, they sink to the bottom and accumulate into a sedimentary layer, which over centuries build up and becomes compressed and fossilised into a soft chalky rock called diatomaceous earth (Garrett, 2004). These can then be mined, dried and ground to render a soft whitish powder which may be put to different uses. So diatomaceous earth is a fine white powder made up of fossilized shells of diatoms. It is light weight, gritty and porous (Garrett, 2004).

According to MacDonald, (1986) much of the DE being used today originated more than 20 million years ago in the lakes and seas. DE is formulated into two grades: swimming pool grade and food grade. Pool grade is chemically treated and partially melted and toxic while food grade DE, is in natural form and non toxic.

Chemical/physical composition of DE

The major composition of DE is approximately Silicon (86%), calcium (19%), Sodium (5%), Magnesium (3%), Iron (2%) and many other trace minerals such as titanium, boron, manganese, copper, vanadium, strontium and zirconium (Garrett, 2004). When observed under a microscope, the crushed particles resemble bits of broken glass and these particles are deadly to insects but harmless to animals, fish, fowl or food http://w.w.w.hydromall.com/happy.grower).

Uses of DE

Diatomaceous earth has countless uses in today products and processes. It is used for grain protection and is reported to be effective against field pests like corn worm, ants, aphids, bollworm, leaf hoppers, mites and many other insects (http:// w.w.w.hydromall.com/happy-grower). It is sometimes mixed with other natural or synthetic insecticides to increase efficiency. MacDonald (1986) reported that in several tests, DE gave better grain protection than malathion, especially over the long term storage. Whereas with contact pesticide the insect dies quite quickly, DE control may take several days. However, the effect of protection provided by the chemical is short-lived, whereas DE controls the pests as long as the powder remains (MacDonald, 1986).

Apart from controlling insect pests on crops, diatomaceous earth can be added to animal feed as it controls internal parasites, increases digestion and provides valuable trace minerals (MacDonald, 1986). According to Korunic (1998) DE promotes good animal growth as it controls endo and ecto-parasites, reduces fly incidences, provides better feed conversion, reduce manure odour and acts as an anti-caking agent. There is no residual danger of contamination, and it is beneficial to the soil as it contains trace minerals (Garrett, 2004). It is also used in processing of toothpaste, in cosmetics, drugs, paints, plastics, as filter media in swimming pools, in beer, juice and water filtering, fish tanks and many others (http.// w.w.w.hydromall.com/happy-grower.html; MacDonald, 1986; Garrett, 2004).

DE mode of action

Insect control methods using DE are similar to traditional methods applied in most parts of Africa where sand or ash is used to limit insect development. As an insecticide, DE functions by mechanical action by absorbing the cuticular wax and or abrasion of the cuticle that results into desiccation and death of an insect (MacDonald, 1986; Garrett, 2004). DE is considered natural and inert in its mode of activity. According to Garrett, (2004), DE affects all insect developmental stages which take place outside the grain but does not affect those inside the grain. According to Ebeling (1971) insects die when they have lost approximately 60% of their water or about 30% of their body weight. Insects are small and therefore have high surface to volume ratio, and this even makes it more difficult for them to maintain water balance.

MacDonald (1986) reported that insects have several mechanisms to deal with desiccation, but the cuticle is one of the more important ones and it is sensitive to inert dusts. The role of the cuticle is evident by how much DE is picked by the cuticle and a sensitive species such as *Cryptolestes furrugineus* (S) picks up much more than *Tribolium castaneum* (H) (Golob, 1997). It is also believed that behaviour may play a role; insects that move extensively through grain, such as *C. furrugineus* may be more damaged than sedentary insects such as *Ryzopertha dominica* (Golob, 1997).

According to Fields and Korunic (2000), insect species differ in their sensitivity to DE and that the most susceptible tend to be insects with large surface to volume ratios, body hair and thin cuticle as opposed to those with hardened waxy cuticle. Further grain protection is provided by the powder's property of repelling many insects (Korunic, 1997).

Factors that influence DE efficacy

According to MacDonald, (1986), the efficacy of DE depends on its composition, the higher the silicon dioxide (SOi_{2}) content, the more efficacy it confers. However, not all diatomaceous earths are of the same quality, many variations are found. The wide disparity of the quality that exists among deposits of DE stem primarily from the following causes; the species of diatoms comprising the sample, the age of the deposit, the presence of various impurities and the extent of skeletal deterioration (http://w.w.w.safeuse-products/diatomaceous/diatomaceous-earth.htm).

The toxicity of DE is reported to vary by more than twenty times depending on the geological origin and physical characteristics (MacDonald, 1986; Golob, 1997; Korunic, 1998). According to NARO Annual Report (2004/2005) there were variations in the performance of DE from different origin or location and other inert materials evaluated in Uganda. The differences in performance were attributed to DE quality or composition and DE of Alwi origin was more effective than that from Boro (Table 1). Fields and Korunic (2000) reported that factors such as climatic conditions and adherence of the DE to the commodity also affect its efficacy.

Stathers *et al.*, (2000) reported that Protect-it and Dryacide the two commercially available diatomaceous earths were extremely effective and persistent as grain protectants against the major insect storage pests.
Stathers *et al.*, (2000) further reported that the efficacy of DE was closely linked to the dosage rates and could differ between stored grains and they recommended Dryacide 0.1% w/w for maize and cowpea, 0.2% w/w for sorghum while Protect-it dosage rate of 0.1% w/w was required for maize, cowpea and sorghum.

Advantages and disadvantages of DE

According to Korunic, (1997), diatomaceous earth of food grade which is amorphous silicon dioxide has extremely low toxicity to mammals and therefore very safe to mix with food. DE used for grain protection is amorphous and not treated unlike the one used for swimming pool filters which is ineffective for insect control because it is heated, chemically treated and crystalline in nature and is very dangerous to breath (Garrett, 2004). As an inert dust, DE has some other advantages which include; long residual activity, mechanical by action and thus no resistance development and does not affect end-use quality (Korunic *et al.*, 1996).

However, DE is very dusty and can cause lung problems if breathed heavily, so it is recommended when applying it to wear good dust mask (Garrett, 2004). DE sold for swimming pool filters is ineffective for insect control because it is heated and chemically treated, therefore does not kill insects (MacDonald, 1986). Another disadvantage is that DE will kill beneficial insects too, so it is advised to use it sparingly and should target only harmful pests (Garrett, 2004)

Diatomaceous earth is already registered and commercially being used as a stored grain protectant and for structural treatment in other countries like Australia, Canada, USA, Croatia, Germany, China and some Asian countries (MacDonald, 1986; Korunic, 1998; Stathers *et al.*, 2000; Garrett, 2004; http:// w.w.w.hydromall.com/happy-grower).

However, in Uganda, DE exploitation and utilization is currently at a very low scale mainly used by the local people in Nebbi district for plastering of their houses (NARO Annual Report (2004/2005). Although, DE is known to be effective against storage insect pests, information on efficacy and applications of DE of Uganda origin is lacking, hence the need to conduct this study.

Table 1 Composition of diatomaceous earth of Uganda origin

Mineral	DE-Alwi (DE-Ug 1) (%)	DE-Boro (DE-ug 2) (%)
Cao	16.01	22.21
Fe_20_3	0.17	0.93
Mg0	0.995	0.663
$Si0_2$	78.84	72.4
Mn	1.00 ppm	2.8 ppm

Source (NARO Annual Report, 2004/2005)

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Materials

The study was conducted in the Post-Harvest Entomology Laboratory based at National Agricultural Research Laboratories (NARL- Kawanda) which is situated at 13 Km on Kampala-Gulu highway. The experiments were conducted under ambient laboratory conditions of temperature and relative humidity regimes of $25^0C \pm 2^0C$ and $70\% \pm 5\%$ respectively. Temperature and R.H were determined using a thermo-hygrograph. Maize variety Longe 5 and beans variety K132 used in the study were obtained from Post-Harvest Programme. The maize and bean grains were sun dried to 13% moisture content (MC). The moisture content was determined by drying 5g of ground sample in an oven at 130^0C for one hour (ISTA, 1996). The grain lots, each weighing 20kg were fumigated with Aluminium phosphide tablets at the dosage of $3gm^{-3}$ for 96 hours in order to get rid of any prior insect infestation, after which the grain was removed and aerated for one day to remove any un decomposed phosphide residues. The maize and bean seed lots were temporally stored in airtight containers to avoid re-infestation.

The treatments studied included application of DE as grain admixture at different rates and efficacy evaluation of DE against selected botanicals and pesticides.

3.1.1 *Sitophilus zeamais* and *Acanthoscelides obtectus* stock cultures

The adult insects used for inoculation were obtained from routine stock cultures raised under ambient conditions in the Post-harvest Entomology Laboratory, NARL- Kawanda. The food media were whole maize grains for *S. zeamais* and bean seed for *A. obtectus*.

S. zeamais adults of two weeks old were sexed and used. *S. zeamais* sexing was based on dimorphic sexual characteristics of the rostrum (Halstead, 1963). The female has a smooth and slender rostrum with regular spaced pits while the male has a rough surface and shorter rostrum with irregular pits. According to Kenneth (1988), *S .zeamais* females lay most eggs in the first 2-5 weeks of adult life. *A. obtectus* of 2-3 days old were used and males were identified from females by use of hues and patterns of the pygidium coloration (Haines, 1991; Silim and Agona, 1993).

3.1.2 Diatomaceous Earth sample

The diatomaceous earth sample of Uganda origin obtained from Nebbi district was used. In order to retain its natural state and effectiveness, DE material was sun dried and then grounded into powder using a pestle and mortar. The powder was sieved using 353 micron aperture sieve and this was used in the study. The powder was kept in sealed plastic bags to avoid absorption of moisture.

3.1.3 Plant materials (Botanicals)

Plant materials: leaves and seeds of *Azadirachta indica,* and *Nicotiana tabacum* were collected from Kawanda and other surrounding areas. The leaves were dried in the shade to full dryness. The dried materials were grinded into powder using a mortar and pestle and sieved using the same sieve size (353 micron). *A. indica* seed powder was obtained by grinding seeds using a laboratory mill and the powder sieved using the same sieve size mentioned above. *N. tabacum* seed was used whole since they are very tiny.

3.2 Experimental design and Treatments

The experimental design was Completely Randomised Design (CRD).

Three studies were conducted and these included;

(i) Effect of varying DE levels as grain admixture on oviposition and adult emergence of *S. zeamais* and *A. obtectus*.

(ii) Effect of varying DE levels as grain admixture on hatchability and survival of *A. obtectus*

(iii) Effectiveness of DE in comparison to selected botanicals and pesticides against *S. zeamais* and *A. obtectus*.

3.2.1 Effect of DE dosage levels on oviposition and adult emergence of *S.zeamais* and *A. obtectus*

The effect of DE on oviposition and adult emergence of *S. zeamais* and *A. obtectus* was studied by varying the DE dosage levels. DE was applied on 100-g samples of maize and beans at the following dosage rates: 0, 0.5, 1, 2, 3, 4 and 5% w/w. 0% rate was the control/ untreated grains. 100 g of maize and bean grain were weighed and put separately into 400-ml plastic containers each replicated four times. Different dosages of DE (0, 0.5, 1, 2, 3, 4, 5% w/w) were separately added to the grain in each of the plastic containers. The parent adults used were 2- week old adults of *S. zeamais* and 2-3 day old *A. obtectus*. For each species 15 adults composed of 10 females and 5 males were used to infest the grain samples in each container depending on the crop species. The adult ratio of 2:1 (female: male) was to avoid egg-laying interference by the males and the age of inoculation of each species was chosen deliberately to coincide with periods when egg-laying is optimum. The insects were allowed seven days to oviposit. After seven days of oviposition, the insects were removed and discarded. It was assumed that the insects would have oviposited enough eggs in seven days.

To determine the number of eggs oviposited on maize grain, maize grain in each sample were stained using acid fuschin (Frankenfield, 1950). The eggs appear as cherry red spots and were counted under binocular microscope. After counting the eggs, the treated grains were dried with blotting paper and incubated until the adult insects start to emerge. Emerging insects were removed and counted on daily basis after which were discarded.

For A. obtectus, eggs together with DE were separated from the grain by placing on a plain sheet of paper and a thin layer of DE and eggs were gently placed in a Petri dish. Adult insects both live and dead were removed from the grain and discarded. The eggs were observed under a binocular microscope, counted and recorded. To avoid egg counting repetition, grid paper was placed under the Petri dish. *A. obtectus* eggs are more white than DE with one end broadly rounded (Haines,1991). After counting, the eggs and DE were returned to the grains in their respective containers. The containers were tightly covered with their perforated top covers to allow maximum aeration. The containers were placed on shelves in the laboratory under room temperature until adult insects started to emerge. Emerging adults were removed, counted and recorded every day and then discarded. Insect counting was continued until there were no more emergent adults.

The data collected included;

- Total number of eggs laid in 7 days
- Total number of adult insects which emerged

3.2.2 Effect of DE dosage rates on hatchability and emergence of *A. obtectus*

The same treatments (0, 0.5, 1, 2, 3, 4, 5% w/w DE), replicates and procedure as in sub-section 3.2.1 above were followed. Three days old *A. obtectus* were placed in vials and allowed to oviposit for 24 hours after which the insects were removed. Twenty eggs were randomly picked and introduced into containers with beans at different dosage levels of DE. Controls/non treated grains were also prepared. On the ninth day after egg introduction, the DE-egg mixture was carefully separated from the bean seed. The DE-egg mixture from each treatment was thinly spread in a Petri dish and the eggs observed under a microscope to determine the hatched and un hatched ones. The number of larvae that hatched was determined by counting the empty egg shells and those of the un-hatched were counted and recorded. DE together with the larvae and egg shells were then put back to the bean samples in their respective containers. The samples were left on shelves under ambient condition of temperature and relative humidity regimes of $25^0C \pm 2^0C$ and $70\% \pm 5\%$, respectively until adult insects emerged. The number of insects that emerged were counted after every two days and recorded. Hatchability was expressed as the number of eggs out of 20 eggs inoculated which successfully hatched while percentage adult survival is the number of larvae that successfully developed into adults.

Data collected included;

- Number of un hatched eggs
- Number of hatched eggs
- Number of emerging adults

3.2.3 Comparison of DE, selected botanicals and pesticides in management of *S. zeamais* and *A .obtectus.*

100-g maize and bean grain samples were separately weighed and inoculated with *S. zeamais* and *A. obtectus* respectively, following the same procedure and replicates as in sub-section 3.2.1 above. Two different plant materials (botanicals); *A. indica*, and *N. tabacum*, (dried ground leaves and seed) and two standard synthetic chemicals (Actellic super 1%, Malathion 2% dust) and DE dosage levels of 0.5, 1 and 2% were evaluated against *S. zeamais* and *A. obtectus*.

The recommended dosage rate of 2 kg tobacco leaf powder per 100-kg of grain, corresponding to 2% w/w (NARO Annual Report 2002/2004).was used for the botanicals (2 g/100-g of grain) while the manufacture's recommendation rate of 50 g/100-kg grain ie 0.05 g/100 g grain of the actellic and malathion was applied. The weighed botanicals, pesticides and DE were then added to 100-g of maize and bean samples in the containers and the same insect ratio, age and replicates as in sub-section 3.2.1 were used. After seven days, both live and dead adults were removed and then discarded.

The experiment was set under ambient temperature and relative humidity regimes of $25^0C \pm 2^0C$ and 70% ± 5% respectively. Number of emerging adult insects, F1 progeny were counted and recorded. Insect counting was on daily basis until there were no more emergences. Seed damage assessment was carried out on both treated and untreated grains. The initial damage of maize and beans used in the study was 1%. At the end of the study, 100 seeds were randomly taken from each container and the number of damaged grains (with characteristic round holes) and undamaged seeds were counted and recorded. The final percentage seed damage was then determined. Square root data transformation $\sqrt{(X+1)}$ was used for seed damage only to normalize the data. Seed germination was conducted following ISTA 1996 standards. The initial germination percentage of maize used in the study was 79.5% while for bean was 85%.

Data collected included;

- Total number of adult insects that emerged
- Number of damaged and undamaged grains
- Seed germination capacity

3.3 Data Analysis

The data were analysed as completely randomised design using MSTAT-C statistical package ANOVA. The standard error of difference (SED) was applied to measure the differences between the means. Two means were considered significantly different when the observed differences between them were more than twice SED.

CHAPTER IV

RESULTS

4.1 Effect of Diatomaceous Earth dosage rates on *S. zeamais*

(i) Effect of DE dosage on oviposition of *S. zeamais*

The results on effect of DE dosage rates on oviposition of *S. zeamais* are presented in Table 2. There were significant differences ($P < 0.05$) in the number of eggs laid by *S. zeamais.* The number of eggs oviposited decreased with DE increased application rates. The highest number of eggs laid were in the non treated grains while the lowest was observed in grains treated with DE at 5% dosage level. The mean number of eggs laid in maize seeds treated with DE at 0.5, 1, 2 and 3% concentrations were not significantly different.

(ii) Effect of DE dosage rate on adult emergence of *S. zeamais*

The effect of DE dosage levels on *S. zeamais* are shown in Table 2. There were significant variations ($P< 0.05$) in the mean number of *S.zeamais* adult emergence between treatments. The number of weevil emergence decreased with increase in DE dosage levels. The highest weevil emergence was observed in grains that never received any treatment and the lowest in grains treated with DE at 5% dosage level (Table 2).

Table 2 **Effect of DE dosage rates on oviposition and adult emergence of *S. zeamais* on maize.**

DE dosage % w/w	Number of eggs laid/100g (Mean ± se)	Number adult emergence/100g (Mean ± se)
0	20.8 ± 5.8	19.3 ± 5.3
0.5	12.8 ± 2.9	8.3 ± 3.0
1.0	11.3 ± 0.6	8.5 ± 1.0
2.0	7.8 ± 0.9	5.50 ± 0.7
3.0	7.0 ± 2.5	6.3 ± 2.6
4.0	4.3 ± 1.9	2.3 ± 1.6
5.0	2.8 ± 0.5	1.3 ± 0.3
CV (%)	**15.6**	**30.4**
SED (21 d.f.)	**3.9**	**3.7**

There was a negative and significant relationship between DE dosage rates and number of eggs laid and *S. zeamais* emergence, indicating that oviposition and insect emergence decreased with an increase in DE dosage rate as shown in Fig 1 and 2 respectively. A negative slope indicated the amount of reduction in number of eggs laid and adult emergence for every change in DE dosage rate. Thus, 84% and 70% of total variation in oviposition and adult emergence respectively, could be attributed to change in DE dosage rates as indicated by coefficient of determination (R^2) (Fig 1 and 2).

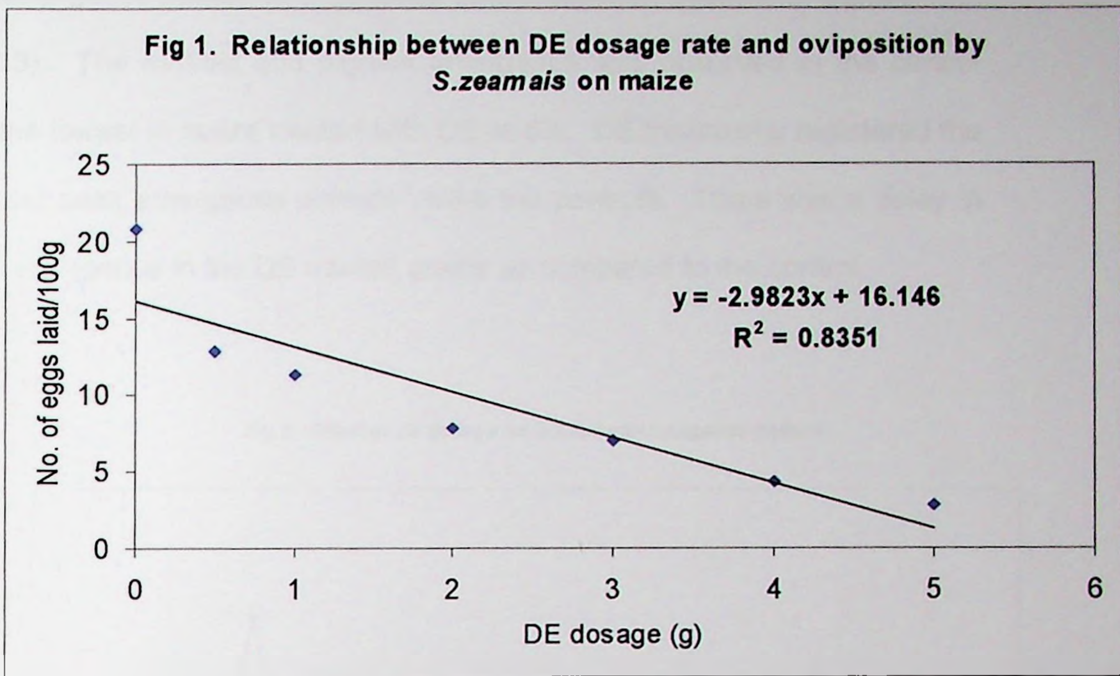


Fig 1. Relationship between DE dosage rate and oviposition by *S.zeamais* on maize
25
20
15
10
5
0
No. of eggs laid/100g
y = -2.9823x + 16.146
R^2 = 0.8351
0
1
2
3
4
5
6
DE dosage (g)

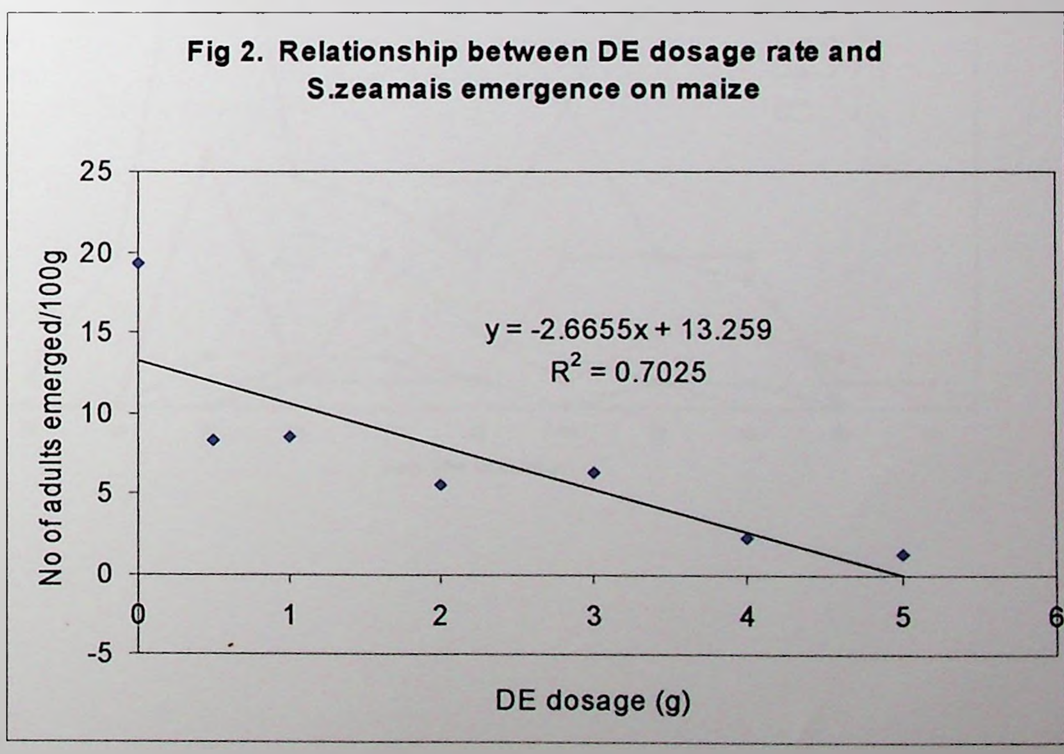
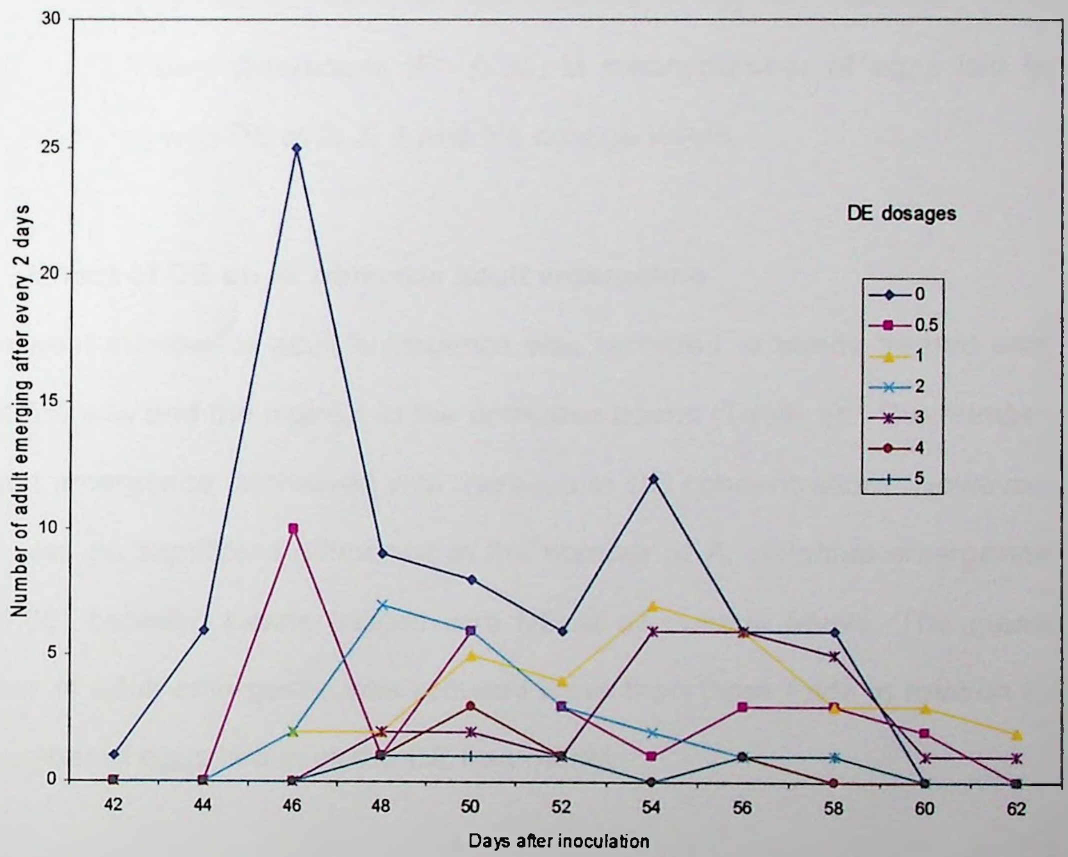


Fig 2. Relationship between DE dosage rate and S.zeamais emergence on maize
25
20
15
10
5
0
-5
No of adults emerged/100g
y = -2.6655x + 13.259
R^2 = 0.7025
0
1
2
3
4
5
6
DE dosage (g)

(iii) Effect of DE dosage levels on emergence pattern of *S.zeamais*

The emergence pattern of *S .zeamais* varied between treatments applied (Fig 3). The earliest and highest emergence was observed in the control and the lowest in maize treated with DE at 5%. DE treatments registered the shortest peak emergence periods unlike the controls. There was a delay in adult emergence in the DE treated grains as compared to the control.

Fig 3. Effect of DE dosage on *S.zeamais* emergence pattern



4.2 Effect of Diatomaceous Earth dosage rates on *A. obtectus*

(i) Effect of DE on oviposition of *A. obtectus*

The results of DE dosage rates on *A .obtectus* oviposition are shown in Table 3. The DE concentrations had variable effects on oviposition. The number of eggs laid decreased with increase in DE dosage levels. There was significant difference in oviposition ($P < 0.05$) between the DE treatments. The control or untreated beans registered the highest mean number of eggs laid compared to the grains treated with DE at different dosage levels. Beans treated with DE at 5% dosage rate had the least mean number of egg laid. However, there were no significant differences ($P > 0.05$) in mean number of eggs laid in beans admixed with DE at 2, 3, 4 and 5% dosage levels.

(ii) Effect of DE on *A. obtectus* adult emergence

The lowest number of adult emergence was recorded in seeds treated with DE at 5% w/w and the highest in the untreated beans (Table 3). The number of adult emergence decreased with increase in DE concentration. However, there was no significant difference in the number of *A. obtectus* emergence ($P > 0.05$) between beans treated with DE at all dosage levels. The mean number of adult emergence was reduced more than three folds in relation to the number of eggs laid in all the DE treatments.

Table 3 Effect of Diatomaceous Earth dosage rates on oviposition and emergence of *A. obtectus* on beans.

DE dosage % w/w	Number of eggs laid/100g (Mean ± se)	Number of adult emergence/100g (Mean ± se)
0	118.4 ± 14.0	66.6 ± 12.3
0.5	99.8 ± 13.0	22.3 ± 3.9
1.0	49.0 ± 12.6	11.0 ± 4.0
2.0	10.8 ± 1.7	3.3 ± 1.3
3.0	10.3 ± 3.5	2.3 ± 1.3
4.0	10.0 ± 4.3	2.0 ± 0.9
5.0	6.0 ± 2.0	1.0 ± 0.4
CV (%)	30.82	34.7
SED (21 d.f.)	16.4	11.2

The estimated linear regression relationships between oviposition, *A. obtectus* emergence and DE dosage rates are shown in Fig 4 and 5 respectively. There was a negative and significant association between DE dosage rates and number of eggs laid and adult emergence. The number of eggs laid and adult emergence decreased with an increase in DE dosage levels as indicated by the negative slope. 72% and 52% of total variation in oviposition and adult emergence respectively, could be attributed to change in DE dosage rate.

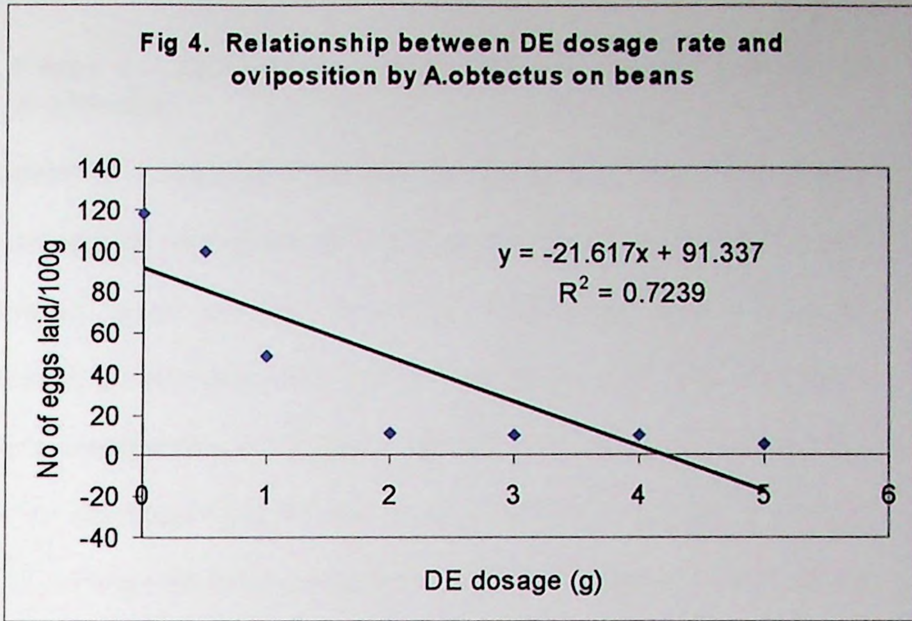


Fig 4. Relationship between DE dosage rate and oviposition by A.obtectus on beans
y = -21.617x + 91.337
R^2 = 0.7239
No of eggs laid/100g
DE dosage (g)
140
120
100
80
60
40
20
0
-20
-40
0
1
2
3
4
5
6

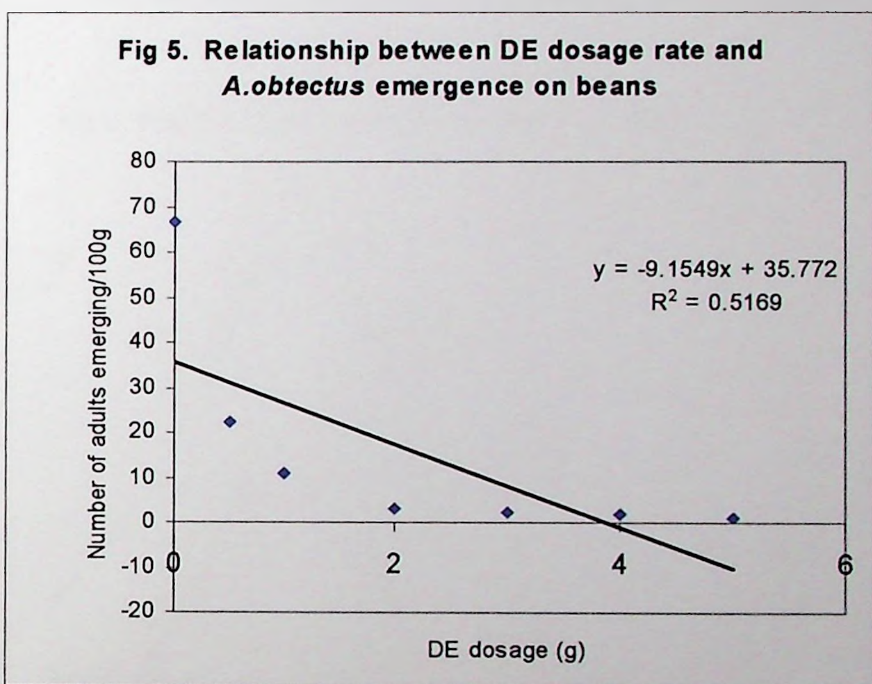
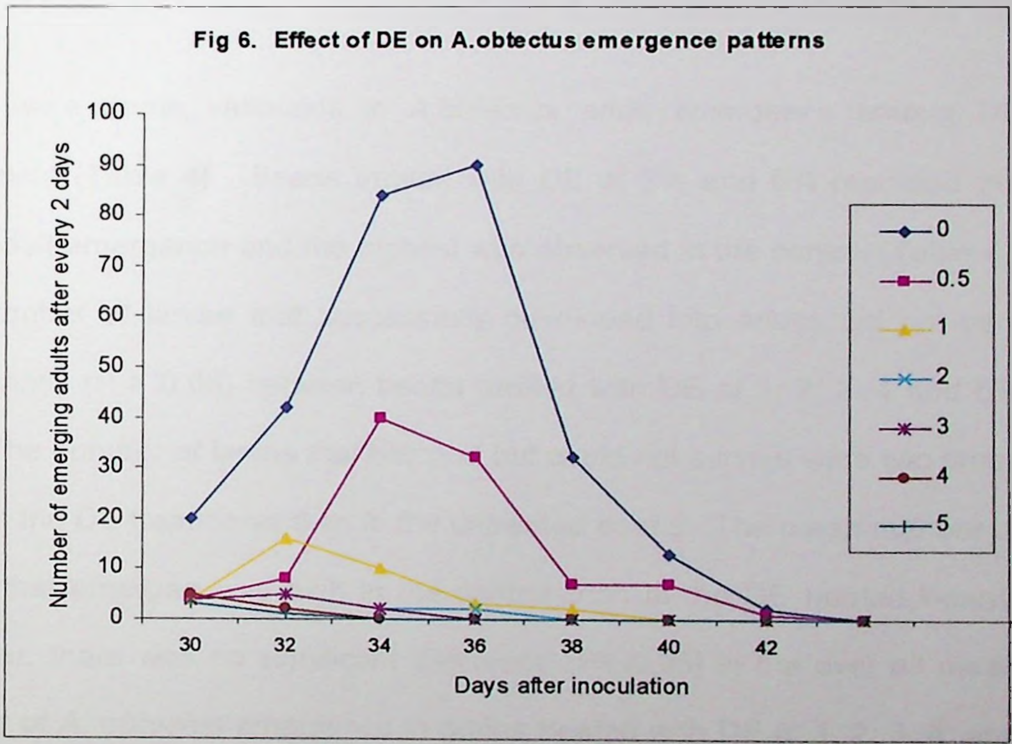


Fig 5. Relationship between DE dosage rate and *A.obtectus* emergence on beans
y = -9.1549x + 35.772
R^2 = 0.5169
Number of adults emerging/100g
DE dosage (g)
80
70
60
50
40
30
20
10
0
-10
-20
0
2
4
6

(iii) Effect of DE dosage levels on emergence pattern of *A.obtectus*

Emergence pattern of *A. obtectus* from different treatments is shown in Fig. 6. The highest emergence was observed in the control and lowest in DE treated grains. However, there was no difference in developmental period as emergence started 30 days from the time of inoculation in all the treatments. Generally, peak emergence period was attained on the thirty sixth day after inoculation in all the treatments for the 0% and 0.5% DE w/w concentration while for the remaining higher concentration, peak adult emergence occurred around 32 days after inoculation.



4.3. Effect of Diatomaceous Earth dosage rates on hatchability and adult emergence of *A. obtectus*

The effect of DE dosage rates on percentage hatchability, adult survival and overall emergence of *A. obtectus* are presented in Table 4. There were significant differences ($P < 0.05$) in hatchability between treatments applied against *A. obtectus*. The highest percentage egg hatchability was observed in the untreated bean seeds and lowest in beans admixed with DE at 5% dosage level. Hatchability decreased with increase in DE dosage levels. However, there were no significant differences ($P > 0.05$) in egg hatchability of *A. obtectus* in the untreated beans and beans treated with DE at 0.5, and 1% DE dosage levels.

There were some variations in *A.obtectus* adult emergence among DE treatments (Table 4). Beans treated with DE at 2% and 5% recorded the least adult emergence and the highest was observed in the control (Table 4). The number of larvae that successfully developed into adults did not vary significantly ($P > 0.05$) between beans treated with DE at 1, 2, 3, 4 and 5% w/w. The number of larvae that hatched but could not survive were two times more in the DE treatments than in the untreated beans. The mean number of adults that emerged was high in the control than in the DE treated beans. However, there was no significant difference ($P < 0.05$) in the over all mean number of *A. obtectus* emergence in grains treated with DE at 1, 2, 3, 4, and 5% w/w dosage rates. There were remarkable reductions in mean percentage hatchability and *A. obtectus* adult emergence in DE treated beans.

Table 4 **Effect of DE dosage rates on hatchability and over all *A. obtectus* emergence on beans.**

Treatments (% w/w)	Hatchability (%) (Mean ± se)	Adult emergence/100g (Mean ± se)
0	92.5 ± 3.2	14.5 ± 1.8
0.5	87.5 ± 3.2	7.8 ± 1.3
1.0	82.5± 3.2	2.0 ± 0.6
2.0	53.8 ± 6.2	1.5 ± 0.5
3.0	52.5 ± 3.2	1.8 ± 1.0
4.0	48.8 ± 3.2	1.8 ± 0.8
5.0	40.0 ± 2.0	1.5 ± 0.5
CV (%)	**11.26**	**22.3**
SED (21 d.f.)	**5.2**	**1.4**

4.4. Comparison of DE, selected botanicals and pesticides in the management of *S. zeamais*

The results of DE, botanicals and pesticides evaluation on emergence of *S. zeamais* are presented in Table 5. There were significant differences ($P< 0.05$) in *S. zeamais* emergence between treatments applied. Grains treated with conventional insecticides (malathion and actellic) recorded no adult emergence while the highest weevil emergence was observed in the untreated grains followed by tobacco seed and neem leaf powder. However, there were no significant differences ($P> 0.05$) in mean number of adult emergence in the maize treated with DE at 0.5, 1 and 2%, tobacco leaf and neem seed powder concentration. The performance of tobacco seed and neem leaf powder did not vary significantly from the control in terms of number of *S. zeamais* emergence. Among the botanicals evaluated, neem leaf and tobacco seed powder performed poorly while tobacco leaf and neem seed powder were more effective in reducing F1 progeny emergence.

The mean number of *S. zeamais* which emerged from the maize treated with conventional pesticides (actellic super and malathion), neem seed powder and DE at 2% dosage level was not significantly different (P< 0.05).

Of the treatments evaluated, synthetic insecticides/pesticides were the most effective in controlling the weevils and this was demonstrated by zero *S. zeamais* emergence. However, DE performance was rated high and compared well with the neem kernel extract and tobacco leaf powder.

The difference in adult emergence between DE, tobacco leaf and neem seed powder were not significant suggesting that DE at all the tested dosages was effective as tobacco leaf and neem seed powder in controlling *S. zeamais*.

Table 5. **Effect of DE, botanicals and pesticides on emergence of *S. zeamais***

Treatment	Number adult emergence (Mean ± se)
Control	85.0 ± 5.3
DE 0.5	28.8 ± 4.5
DE 1.0	25.8 ± 5.3
DE 2.0	10.5 ± 3.2
Neem seed powder	16.8 ± 5.2
Neem leaf powder	72.0 ± 9.1
Tobacco seed	84.0 ± 14.4
Tobacco leaf powder	29.5 ± 9.0
Malathion dust 2%	0.0
Actellic super dust 1%	0.0
CV (%)	**19.50**
SED (30 d.f.)	**9.8**

4.4.1 Treatment effect on maize seed damage and germination by *S. zeamais.*

Table 6 shows treatment effect on maize seed damage and germination. The control registered the highest mean seed damage and the lowest percentage germination while maize treated with actellic super recorded the least seed damage and highest seed germination (Table 6). There were no significant differences ($P > 0.05$) in the percentage seed damage in maize seeds treated with DE, neem seed and tobacco leaf powder. The differences in percentage seed damage between untreated grains and those treated with tobacco seed and neem leaf powder were not statistically significant.

There were variations in maize seed germination percentage in all the treatments applied. Maize seeds treated with actellic recorded the highest germination percentage while the control registered the lowest Table 6. Generally, initial germination capacity of the maize seed was below the ISTA (1996) recommended standard of $\geq$ 80% and this could be attributed to other factors like processing methods used on the grains. However, the untreated maize registered the lowest mean percentage seed germination and this in addition could be attributed to high insect damage inflicted on the seeds.

Table 6. **Effect of DE, botanicals and pesticides on maize seed damage and germination infested by *S. zeamais*.**

Treatment	Final seed damage % (Mean ± se)	Final germination % (Mean ± se)
Control	5.4 ± 0.4	46.5 ± 4.4
DE 0.5	3.7 ± 0.4	64.0 ± 1.7
DE 1.0	2.8 ± 0.6	66.5 ± 1.4
DE 2.0	2.9 ± 0.7	72.0 ± 2.5
Neem seed powder	2.5 ± 0.5	64.0 ± 0.8
Neem leaf powder	5.1 ± 0.2	54.0 ± 1.4
Tobacco seed	5.3 ± 0.4	58.5 ± 4.3
Tobacco leaf powder	3.3 ± 0.5	63.0 ± 3.7
Malathion dust 2%	1.5 ± 0.2	73.5 ± 2.2
Actellic super 1%	1.0	79.0 ± 1.5
C.V (%)	25.53	8.5
SED (30 d.f.)	0.60	3.9

4.5. Comparison of DE, botanicals and pesticides in management of *A. obtectus*

The effect of DE, selected botanicals and pesticides on *A. obtectus* adult emergence are shown in Table 7. Different treatments had variable effects on adult emergence of *A. obtectus.* There were significant differences ($P<0.05$) in adult emergence among different treatments applied against *A. obtectus.* Unlike in maize where malathion achieved 100% control of *S.zeamais*, some *A. obtectus* emergence were observed in the malathion treated beans Table 7.

The highest adult emergence was observed in beans treated with tobacco seed followed by the control and neem leaf powder. Beans treated with actellic super recorded no adult emergence while malathion treated seeds had some insect emergence. There were no significant differences ($P > 0.05$) in mean number of adults which emerged in beans treated with actellic super, malathion, tobacco leaf, neem seed powder, and DE at 1 and 2% dosage rates. DE at 1% dosage level showed high potential in controlling *A. obtectus* as it reduced the number of emerging adults compared to DE at 0.5 and 2% w/w.

Like for *S. zeamais*, tobacco seed and neem leaf powder were not effective against *A. obtectus*. The untreated beans and those treated with tobacco seed and neem leaf powder were highly infested with *A. obtectus*.

Table 7. **Effect of DE, botanicals and pesticides on adult emergence of *A. obtectus.***

Treatment	Number of emerged adults (Mean ± se)
Control	81.0± 9.3
DE 0.5	24.5 ± 3.8
DE 1.0	2.5 ± 0.3
DE 2.0	3.3 ± 0.6
Tobacco seed	99.0 ± 13.7
Neem leaf powder	64.8 ± 16.8
Neem seed powder	1.5 ± 1.0
Tobacco leaf powder	2.8 ± 0.9
Malathion dust	1.5 ± 1.2
Actellic super	0.0
C.V (%)	23.94
SED (30 d.f)	10.7

4.5.1. Effect of DE, botanicals and pesticides on bean seed damage and germination infested by *A. obtectus*.

Results on effect of different treatments on bean seed damage and germination are presented in Table 8. Results showed significant differences among various treatments applied. The tobacco seed treated beans registered the highest percentage mean seed damage, followed by the controls and neem leaf powder (Table 8). Beans treated with actellic super and tobacco leaf powder recorded the least percentage seed damage. However, there were no significant differences (P> 0.05) in the seed damage levels in beans treated with synthetic pesticides (actellic, malathion), DE 1%, tobacco leaf and neem seed powder (Table 8).

The highest mean percentage seed germination was observed in beans treated with actellic super and lowest in seeds treated with DE concentration at 0.5%. However, there was no significant difference in mean seed germination in all the treatments applied.

Table 8. **Effect of DE, botanicals and pesticides on bean seed damage and germination infested by *A. obtectus***

Treatment	Final seed damage% (Mean ± se)	Final germination % (Mean ± se)
Control	4.1 ± 0.4	79.0 ± 2.4
DE 0.5	2.6 ± 0.4	78.0 ± 2.5
DE 1.0	1.2 ± 0.2	81.0 ± 0.6
DE 2.0	1.8 ± 0.3	79.5 ± 1.7
Tobacco leaf powder	1.00	81.0 ± 3.1
Tobacco seed	4.7 ± 0.2	79.5 ± 1.0
Neem seed powder	1.3 ± 0.2	82.0 ± 2.5
Neem leaf powder	3.8 ± 0.3	79.5 ± 3.4
Actellic super 1%	1.00	81.5 ± 4.2
Malathion dust 2%	1.4 ± 0.3	84.0 ± 2.8
C.V (%)	**22.08**	**6.38**
SED 30 (d.f.)	**0.36**	**3.71**

CHAPTER V

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Effect of DE dosage rates on oviposition and adult emergence of *S.zeamais* and *A.obtectus*

The results of effect of Diatomaceous Earth dosage rates on *S. zeamais* and *A. obtectus* have shown that DE is effective on the two storage insect pests. This was manifested in significant reductions in the number of eggs laid and subsequent low adult emergences in DE treatments as compared to the controls which registered high number of eggs and adult emergence. DE dosage rates, however, affected *S. zeamais* and *A. obtectus* oviposition and adult emergence differently.

Although there were more eggs laid by *A. obtectus*, fewer adults emerged in the DE treatments as compared to the control. This could be attributed to the effect of DE on first larval instar which takes place outside the grain. This is in agreement with the findings by (Garrett, 2004) who reported that DE affects all insect developmental stages which take place outside the grain but does not affect those inside the grain. For *S. zeamais*, the mean number of eggs laid and adult emergence showed smaller differences of less than five in all the treatments. This further confirms that in insects whose developmental stages take place inside the grain are less affected by DE.

Although there were variations in the number of eggs laid among treatments applied against *S. zeamais* and *A. obtectus*, there were no significant differences (P> 0.05) in mean number of adult emergence between DE treatments. DE at varying dosage levels greatly reduced number of eggs laid and adult emergence of *S .zeamais* and *A. obtectus* unlike in the untreated grains. The results showed that DE dosage rate at 5% w/w reduced the mean number of eggs laid and adult emergence of *S. zeamais* and *A. obtectus* to very low levels. However, there was no significant difference in mean number of adult emergence between DE treatments. The use of DE at high dosage level may be uneconomic as it increases costs and too much dust in grain is a health hazard to the applicant. Therefore a lower but effective DE dosage rate is more justifiable.

In Zimbabwe, the two commercial diatomaceous earths: Protect-it and Dryacide are extremely effective and application rate of 0.1% w/w was recommended for grain protection (Stathers *et al.*, 2000). MacDonald (1986) reported that the use of improved DE formulations that contains baits and attractants as little as 0.5kg/tonne could provide full grain protection.

However, DE of Uganda origin used in this study had slightly lower silicon dioxide content (NARO Annual Report, 2004/2005) and nothing was added to it which means it may need to be applied at a slightly high dosage level unlike those with some additions which are already recommended elsewhere in order to achieve full grain protection.

The emergence patterns for both *S. zeamais* and *A. obtectus* in DE treatments were lower as compared to the controls. This strongly suggest that DE was effective on *S. zeamais* and *A .obtectus*.

5.2. Effect of DE dosage rates on hatchability and adult emergence of *A. obtectus*

According to the results of the study, it was observed that there were significant variations in the hatchability and adult emergence of *A. obtectus* due to admixing of beans with DE at different dosage levels. The number of larvae that hatched but failed to develop into adults or survive were more than two folds in the DE treatments than in the untreated beans. This possibly suggests that DE caused high mortality and or interfered with mobility of the hatched larvae to penetrate the bean seeds which was reflected by the low number of adult insects that emerged.

A. obtectus oviposit its eggs loosely among the grain and the hatched larva has to move and penetrate the grain unlike the *S. zeamais* where eggs are laid inside the grain. Garrett (2004) reported that DE affects all insect stages that take place outside the grain but does not affect the developmental stages inside the grain. DE could have also affected the egg hatchability since it decreased with increase in DE dosages.

5.3. Effect of DE, botanicals and pesticides on adult emergence, seed damage and germination infested by *S. zeamais* and *A. obtectus*

The study results on comparison of DE, botanicals and pesticides showed that DE at 1%w/w dosage rate was as effective as conventional insecticides: Actellic 1% ai and Malathion 2% ai, tobacco leaf powder and neem seed powder in controlling *A. obtectus*. This was demonstrated by the insignificant differences in the number of test insects that emerged and seed damage levels exhibited among those treatments. DE at 2%w/w performed well and was as effective as actellic and malathion, and neem seed powder in controlling *S. zeamais* adult emergence. The mean number of *S. zeamais* and *A. obtectus* that emerged were greatly reduced in the DE treated grains as compared to untreated and those treated with tobacco seed and neem leaf powder.

DE has no insecticidal properties and its mode of action like any inert material, is mechanical, through abrasion of insect cuticle which leads to dehydration and death of the insect (MacDonald, 1986; Golob, 1997; Garrett, 2004). The reduction in number of emergent adults observed in DE treated maize could therefore be attributed to a number of factors. For instance, DE could have affected mobility and mating ability of *S. zeamais* which reduced oviposition level and hence low subsequent number of adult emergence and seed damage. High reduction in the number of *A. obtectus* emergence observed could have been due to low oviposition and DE effect on the first larval instar development.

Resistance development against malathion 2% ai by *A. obtectus* was shown in this study due to *A. obtectus* emergence observed. This is in support of (Dyte and Rowlands, 1968; Beeman and Nanis, 1986; Georghiou and Taylor, 1986; Sriharen *et al.*, 1990; Haubruge and Arnaud, 2001) who reported pesticide resistance in stored product insects in different countries and in particular against malathion. Parkin *et al* (1962) reported the first case of malathion-resistance in *Tribolium castaneum* (Herbst) and attributed resistance development to the intensive use of malathion in grain storage. Sriharen *et al.*(1990), reported actellic resistance development in three stored product insects infesting sorghum and beans in Rwanda. This further confirms that exclusive use of pesticides could not provide a lasting solution to storage pest problem. Apart from resistance development, pesticides have other limitations as they are not always available to the farmers, unaffordable, require technical skills in application, have high residual effect, unsafe to users and environment. Therefore use of chemicals alone for protection of stored products is not reliable and alternative means have to be explored.

In this study, DE performance was rated good and compared well with neem kernel extract and tobacco leaf powder in reducing adult emergence and minimising seed damage levels. Among the botanicals which have been tested and reported to be effective include; neem seed or kernel extract (Van Huis,1991), *Cassia nigricans* and *Hyptis spicigera* (Lambert *et al.*, 1985), tobacco leaf powder(Silim 1999; Kyamanywa *et at.*, 1999). Tobacco seed and neem leaf powder proved to be ineffective against *S. zeamais* and

A. *obtectus* and seemed to provide favourable conditions for insect development. This was shown by high number of adult emergence and seed damage levels from maize and bean samples treated with neem leaf and tobacco seed powder. Although some of these plant extracts are effective and recommended for protection of stored grains, their toxicological levels is not well known which may limit their usage.

5.4 General Discussion

Although there have been reports on use and effectiveness of diatomaceous earth as grain protectant against storage pests (MacDonald, 1986; Golob, 1997; Stathers *et al.*, 2000; Garrett, 2004), information on efficacy of DE of Uganda origin has been lacking.

The main objectives of this study were to determine the optimal DE dosage rate, effect of DE on hatchability and survival of *A. obtectus* and to compare DE efficacy with some selected botanicals and pesticides in controlling *S. zeamais* and *A. obtectus* the major storage pests of maize and beans respectively.

The study results confirmed that DE of Uganda origin is effective on *S. zeamais* and *A. obtectus* in stored maize and beans respectively. The study on effect of DE dosage rates on *S. zeamais* and *A. obtectus* showed there were significant variations in oviposition and adult emergence in DE treatments. DE efficacy increased with the increase in dosage rates and at the highest rate of 5%, the lowest oviposition and adult emergence were achieved. However, there was no significant difference in the number of adult insects that emerged in all the DE treatments. This means that DE at lower dosage level of less than 5% could still be effective in the management of the two insect pests. The results showed that *A. obtectus* was more affected by DE than *S. zeamais* and this was indicated by low adult emergence in relationship to the number of eggs laid observed on *A. obtectus* as compared to *S. zeamais* subjected at the same DE dosage rate. This could be attributed to the difference in biology of the two insect species.

S. zeamais oviposit its eggs inside the grain and all the developmental stages take place in the grain unlike *A. obtectus* which lays eggs outside the grain. This may require different DE application regimes for effective control of the two insect species. For effective control, *S. zeamais* may require a higher DE dosage rate than *A. obtectus*. DE treatment affected egg hatchability and development of *A. obtectus* and this was shown by low hatchability and adult emergence in DE treated beans than the controls.

The results on comparison of DE, botanicals and pesticides effectiveness showed that DE at 2% and 1% dosage levels had similar performance like actellic 1% ai and malathion 2% ai in minimizing emergence and damage caused by *S. zeamais* and *A. obtectus* respectively. This was demonstrated by non significant difference in the number of adult emergence in those treatments. Use of large quantities of DE to control storage pests may be unnecessary since it increase storage costs, bulkiness of the grain and pose health hazard to the applicants which may result from inhalation of dust.

DE performance compared well with tobacco leaf and neem seed powder in controlling the test pests and this was indicated by insignificant number of adult emergence and seed damage levels registered in same treatments.

The use of DE may be limited due to the presence of crystalline silica, but DE of Uganda origin was confirmed to mainly contain silicon dioxide (NARO Annual Report, 2004/2005) which is considered safe by the Food and Drug Administration of United States Department of Agriculture.

5.5 Conclusion

According to the results from this study, DE is a viable alternative means of protecting stored grains in Uganda. It affected the number of emergent adult of both *S. zeamais* and *A. obtectus* depending on dosage rates applied. DE at 1%w/w and 2%w/w showed high potential in controlling *A .obtectus* and *S. zeamais* respectively when compared with actellic 1% ai and malathion 2% ai dust admixtures.

Basing on the results obtained, it is concluded that DE of Uganda origin is effective on the candidate storage pests evaluated and its efficacy compared well with the synthetic pesticides and botanicals like neem seed and tobacco leaf powder extracts. The use of DE as an alternative means of protecting stored grain has many advantages as it is non toxic regarding health and environment, have extremely low toxicity to mammals, is effective and most of it 98% is removed during processing and can be used for pest control in stored organic products.

5.6 Recommendations

For effective control, DE in dosage range of 1-2% is recommended for the management of *A. obtectus* and *S. zeamais*. More studies on efficacy of DE on other storage pests of economic importance should be conducted and application regimes determined. DE efficacy under farmer storage field conditions should be validated since DE is affected by other factors like moisture content and relative humidity, considering storage conditions and systems in the rural areas in Uganda.

Studies on DE efficacy against storage insect pests under prolonged storage durations should be investigated and Synergetic effects/admixture of DE with other promising botanicals or other inert materials and or pesticides should be studied. Further studies on DE of different dosage levels should be conducted and compared with the recommended rate. Finally, it is recommended that cost effective analysis, consumer acceptability studies in use of DE as grain protectant and environmental impact assessment in case of DE exploration be conducted.

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