PATHOGENICITY OF ENTOMOGENEOUS FUNGI ISOLATED FROM *ELDANA SACCHARINA*

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other Degree of the University, except where due acknowledgement has been made in the text.

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Abstract

Maize is one of the most important cereal crops, grown in Africa by commercial and resource-poor small-scale farmers, serving as a staple food for millions of people in Africa. Maize production is however constrained by many biotic factors including lepidopteran stemborers, which feed inside plant stems, and the parasitic weed belonging to the genus *Striga*, which rob the maize plant of water and nutrients. Many of the currently existing methods of controlling stemborers are either too expensive or inefficient. The objective of this study was to isolate and identify entomogenous fungi on dead *Eldana saccharina* larvae collected from selected sites in Kumasi in the Ashanti Region of Ghana and to test the pathogenicity of the isolated fungi as potential biological control agents. Percentage larval mortality was determined for all isolated fungi using equal spore concentrations. Median Lethal Time and Median Lethal concentrations were also determined for each of the isolated fungi. Fungal species isolated and identified were *Aspergillus flavus, Verticillium albo-atrum, Trichothecium sp., Fusariunm oxysporum and Alternaria brassicicola.* Of the five fungi isolated and identified, *Aspergillus flavus* caused the largest percentage mortality of 80 % while *Alternaria brassicicola* caused the least percentage mortality of 22.5 % at a concentration of $5x10^7$c.f.u./ml. *Aspergilllus flavus,* and *Verticillium albo-atrum*., were pathogenic to *E. saccharina. Trichothecium sp.* was moderately pathogenic while *Fusarium oxysporum* and *Alternaria brassicicola* were not pathogenic *to E. saccharina*.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cereals, especially maize and sorghum, are the most important field crops grown in Africa by commercial and resource-poor small-scale farmers (Kfir, 1998; Seshu Reddy, 1998). Maize is one of the most important cereal crops, which serves as a staple food for millions of people in Africa. Several African countries have focused attention on increasing maize production in the small holder agricultural sector, but such efforts have been ineffective particularly because of extremely heavy pre- and post-harvest losses due to pests and weeds (Khan *et al*., 1997b).

Among the most important biotic constraints to maize production are lepidopteran stemborers, which feed inside plant stems, and the parasitic weed belonging to genus *Striga*, which rob the maize plant of water and nutrients. Among the insect pests attacking maize in Africa, the lepidopteran stemborers are by far the most injurious (Youdeowi, 1989). Because of their economic importance, stemborers have been the subject of many studies, which resulted in an enormous amount of literature. Stemborers seriously limit potentially attainable maize yields by infesting the crop throughout its growth, from seedling stage to maturity (Youdeowi, 1989).

Seventeen stemborer species in three families, Pyralidae, Noctuidae and Crambidae have been found to attack maize in various parts of Africa. However, *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* Strand, *Busseola fusca* Fuller, *Sesamia calamistis*

Hampson, and *Eldana saccharina* Walker are of greatest importance. *E. saccharina* is found throughout sub-saharan Africa and is considered a pest of maize in West Africa (Moyal, 1988).

The pest complex of maize in Ghana consists of stemborers, shootflies, grasshoppers, termites, beetles and rodents. According to Tams and Bowden (1953), the major maize stemborers in Ghana are *Sesamia nonagrioides botanephaga* and *Eldana saccharina*.Stemborers belong to the Order Lepidoptera. Adults are harmless but the larvae bore into the stem and cause the damage while feeding inside the stem. Moths are nocturnal, attracted to light and are strong fliers. Maes (1998) listed 21 economically important lepidopteran stemborer species of cultivated grasses in Africa, including 7 noctuids, 2 pyralids, and 12 crambids. Out of these 21 species, 7 are primarily pests of rice, and one mainly attacks pearl millet in the Sahelian region.

Among the noctuids, *Busseola fusca* and six *Sesamia spp*. are considered economically important. Two pyralids are serious pests: the rice borer, *Maliarpha separatella*, and *Eldana saccharina*, a pest of sugarcane and maize (Maes, 1998).The largest group (12 species) of injurious stem borers are the crambids, with the majority (7 species) belonging to the genus *Chilo* Zincken. Within specific crops and geographic regions, fewer species are considered to be important pests. In South Africa, *Busseola fusca* and *Chilo partellus* are the only important stem borers of maize and sorghum (Kfir 1998), while in sugarcane in the same region, only *Eldana saccharina* is considered to be a serious pest (Conlong De, 1997). In East Africa, *C. partellus*, *Chilo orichalcociliellus*, *E. saccharina*, *B. fusca*, and *Sesamia calamistis* are important and widely distributed stem

borers of maize and sorghum (Seshu Reddy 1998). Major stem borers of maize and sorghum in West Africa include *B. fusca*, *Sesamia calamistis*, and *E. saccharina* (Bosque-P´erez and Schulthess, 1998).

Two of the economically important cereal stem borers in Africa are introduced species: *C. partellus* and *C. sacchariphagus. C. partellus* is an Asian species (Bleszynsky, 1970). Using Geographic Information System (GIS) tools, Overholt *et al.* (2000) predicted the eventual distribution of *C. partellus* in Africa based on the climate at locations where it was known to occur and then comparing to other locations with similar climates. The prediction included several countries in Southwestern and West Africa where *C. partellus* is not yet known to occur. The other invasive species, *C. sacchariphagus*, is a serious pest of sugarcane in the Indian Ocean Islands. *C. sacchariphagus* may have been accidentally introduced into the islands either from Sri Lanka or Java with the introduction of sugarcane around 1850 (Williams, 1983).

Some of the more important native species have restricted distributions, whereas others are found throughout sub-Saharan Africa. *C. orichalcociliellus*, a pest of maize and sorghum, occurs in coastal East Africa, Malawi, and Madagascar at altitudes below 600 feet (Delobel, 1975). *C. aleniellus* has been reported as a pest of rice in West and Central Africa and more recently as an important pest of maize in Ivory Coast (Moyal and Tran 1992). *C. ignefusalis* is the dominant stem borer of millet in the Sahelian zone of West Africa. In southern Africa, *E. saccharina* is a major pest of sugarcane but rarely causes damage in maize. In contrast, *E. saccharina* is considered to be a pest of maize, sugarcane, and rice in West Africa (Bosque-P´erez and Mareck 1990). Of the *Sesamia*

spp., *S. calamistis* is the most widely distributed and economically important species, but several others, including *S. cretica*, which occurs in Somalia, Sudan, and Ethiopia, and *S. nonagrioides botanephaga*, which is found in both East and West Africa, are also important. As with *E. saccharina*, the pest status of *B. fusca* varies by region. In East and Southern Africa it is a pest at higher altitudes (>600 m), but in West Africa, *B. fusca* occurs from sea level to over 2000m, but is primarily a pest in the dry savanna zone (Sithole, 1989).

1.2 Statement of the problem

Lepidopteran stemborers are one of the major constraints to maize production in Africa, and losses due to this pest are estimated to be 20-40% of the potential grain yield (Seshu Reddy and Walker, 1990). The lepidopteran pests, particularly the stemborer complex, are a major constraint to increased productivity, and are of economic importance in most maize-growing countries throughout the world. Nearly half (46%) of the maize area in the 25 key maize-growing countries have medium (40% area infested in temperate areas) to high levels (60% area infested in tropics/subtropics) of infestation with lepidopteran pests. The high yield losses of maize, results in increased poverty and subsequent starvation and mortality. This is because maize is the staple food for many people in developing countries especially Africa. In addition, large sums of money are lost by already poor famers. Some of these famers go for loans to cultivate the crop and are unable to pay back. This leads to an overall national economic loss since agriculture plays a major role in a nation's development.

1.3 Justification

Many of the currently existing methods of controlling stemborers are either too expensive or inefficient. The most widely used method is the use of chemical insecticides but this has many disadvantages which include their toxicity which can result in the death of man and non target species which include some of the natural enemies of the insect pests. Most of the chemicals have a broad spectrum activity and therefore tend to kill more species than necessary. Since most of these chemicals, especially the organochlorines are persistent in the environment; they remain in the soil as well as in the plants for a long period of time and enter the food chain eventually.

Most of the chemicals are also expensive and for large farms, a lot of money is required. The use of cultural practices can only be effective before or after planting and therefore during the growing season they may not be useful. Biological control agents such as entomogenous fungi are specific in their attack of pests and also, they are already part of the natural ecosystem and therefore would not kill other beneficial organisms.

The success of the study would provide a solution to most of the above problems since the natural enemies are more specific and therefore attack only target pests. They are also less harmful to man and other organisms.

1.4 Objectives of Research

The main objective of this study was to isolate and identify pathogenic fungi from *Eldana saccharina* for biological control.

The specific objectives were:

- To investigate the presence of entomogenous fungi on *Eldana saccharina* through isolation and culturing.
- To test the pathogenicity of the fungal isolates against *Eldana saccharina* by measuring the mortality rate due to the fungi.
- To compare the pathogenicity of the fungal isolates against *Eldana saccharina* by estimating their median lethal times (LT_{50}) and median lethal concentrations (LC_{50}).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Life cycle of *Eldana saccharina*

The females begin laying eggs around the flowering time of the maize plants. Eggs are yellow and oval and are laid on the plants or on debris on the soil. Adult females oviposit in batches of 50-100 eggs between leaf sheaths and stalks or on dry leaves at the base of the plant and may lay up to 500 eggs over a period of one week (Cohen, 2002).

The eggs hatch in five or six days and larvae penetrate the stems or cobs. Larvae are grey or black and more active in habit than other stem borers. Larval development takes 21-35 days. Pupation occurs inside the stem and the pupa is covered by a cocoon made of silk and plant debris. Adults emerge in 7-14 days, mate, and start the cycle again (Atkinson, 1980).

2.2 Characteristics and general life cycle of Fungi

Fungi are characterized by non-motile bodies (thalli) constructed of apically elongating walled filaments (hyphae), a life cycle with sexual and asexual reproduction stages, usually from a common thallus, haploid thalli resulting from zygotic meiosis, and heterotrophic nutrition. The characteristic wall components are chitin (beta-1,4-linked homopolymers of N-acetylglucosamine in microcrystalline state) and glucans primarily alpha-glucans (alpha-1,3- and alpha-1,6- linkages) (Griffin, 1994).

Organisms of fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, moulds, and yeasts, as well as many less well-known organisms (Alexopoulos *et al*., 1996). More than 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth, 1991; Hawksworth *et al*., 1995). The life cycle of fungi can follow many different patterns. For most of the moulds indoors, fungi are considered to go through a four-stage life cycle: spore, germ, hypha, mature mycelium. Other life cycle patterns differ from this four-stage cycle in that different reproduction mechanism and physiology characteristics are present, especially for non-mouldy fungi (such as wood rots, sapstains etc.)

Fungi reproduce by releasing spores, which have different shapes and dimensions. Through spore liberation (the process of detachment of spore from the spore-bearing structure) and spore dispersal (the subsequent movement of the spore before settling on a material surface), spores travel through air, water and even on insects from fungal infesting areas into homes. Concentrations of spores in outdoor and indoor air have been the target for much research (Ingold, 1971).

2.3 Control of stemborers

Stem borers can be managed using cultural control measures, biological control agents, use of resistant varieties, chemical control and the use of natural enemies.

2.3.1 Cultural control methods

Cultural control is among the oldest traditional practices and normally cannot be used as a tactical means of control (Dent, 1991). Cultural control is considered the first line of

defense against pests and includes techniques such as destruction of crop residues, intercropping, crop rotation, manipulation of planting dates, and tillage methods (Van den Berg *et al.,* 1998). The latter three agronomic practices can directly affect crop yield.

However, an understanding of stem borers' behaviour and the relationships with their respective crops are important for the development of efficient management strategies. The differences in the behaviour of *E. saccharina* in South and Eastern Africa provide an example of the importance of understanding pest behaviour in making control decisions. In South Africa, larvae of *E. saccharina* mainly infest the lower parts of sugarcane stalks. It is recommended that farmers cut stalks low and leave the tops, which are then cut off and left in the field. In East Africa, on the other hand, recommendations are to not leave the upper parts of plants in fields because *E. saccharina* larvae largely occur in upper-plant parts, and these residues serve as a store for the carryover population (Van den Berg *et al.,* 1998).

2.3.2 Chemical control

Chemicals used to control stemborers include organic compounds like Dichloro-diphenyl- trichloroethane (DDT), fumigants such as mythyl bromide, ethylene oxide, phosephine, and carbon disulphide, carbamates like carbaryl and botanicals like nicotine, rotenone and pyrethrum (Meyer, 2003).

2.3.3 Biological control methods

Biological control is the action of natural enemies (parasites, predators and microbial agents) including naturally occurring agents and agents which are introduced and managed by humans for pest control (also referred to as "classical biological control").

Biological control agents include braconid, eulophid, mymarid, scelionid, chalcid, pteromalid and trichogrammatid wasps that parasitize the eggs of the yellow stem borer. Ants, ladybird beetles, staphylinid beetles, gryllid, green meadow grasshopper, and mirid bug also eat eggs (Dent, 1991). The larvae are parasitized by phorid and platystomatid flies, bethylid, braconid, elasmid, eulophid, eurytomid and ichneumonid wasps. They are attacked by carabid and lady bird beetles, chloropid fly, gerrid and pentatomid bugs, ants, and mites. Bacteria and fungi also infect the larvae. A mermithid nematode also attacks the larvae (Dent, 1991). Chalcid, elasmid and eulophid wasps parasitize the pupae. Ants and earwigs also eat the pupa. Bird, asilid fly, vespid wasp, dragonflies, damselflies, and spiders prey upon the adults (Dent, 1991).

2.3.4 Habitat management

Napier grass (*Pennisetum purpureum*), a commercial fodder grass, can provide natural control to stem borers by acting as trap plant, and as reservoir for their natural enemies. Although the stemborers oviposit heavily on the attractive Napier grass, only very few larvae are able to complete their life cycles (Hutter, 1996). Napier grass has its own defense mechanism against crop borers. When the larvae enter the stem, the plant produces a gumlike substance, which causes the death of the pest. Sudan grass (*Sorghum*

sudanense), also a commercial fodder grass, can provide natural control to stemborers by acting as trap plants for stemborers, and as reservoirs for their natural enemies. Planting Sudan grass around maize field reduced stemborer infestation on maize and also increased efficiency of natural enemies (Khan *et al.*, 1997b). Molasses grass, (*Melinis minutiflora*), when intercropped with maize, not only reduced infestation of crops by stemborers, but also increased parasitism particularly by the native larval parasitoid, *Cotesia sesamae* (Khan *et al.*, 1997a). The plant releases volatiles that repel stemborers. Such plants with an inherent ability to release such stimuli could be used in ecologically-sound crop protection strategies.

2.4 Ecological considerations for the use of entomopathogens in pest management

Pathogens have their own ecological niches, which must be thoroughly understood for the manipulation of epizootics. Epizootics are characterized by rapid change in prevalence of the disease. This change depends on massive reproduction of the pathogen interacting with or reinforced by host and environmental factors. Something usually triggers epizootics, such as stress of host insect or an increase in the proportion of susceptible hosts in the population. Initially, the host population must be largely susceptible to the microorganism, but later the proportion of susceptible hosts decline and the increased number of pathogen units become less important (Fuxa, 1987).

2.5 Fungi as insect pathogens

Deuteromycetes (Moniliales and Sphaeropsidales) and Phycomycetes (Blastocladiales, Chytridiales, and especially Enthomophthorales) are the two most important groups. The genus *Beauveria* (Moniliales), certainly the best known genus of the group, has been

reviewed by De Hoog (1972) and now contains two species, *B. bassiana* and *B. brongniarti* (*B. tenella*). Also, the agent of green muscardine, *Metarhizium*, has two species, *M. flavor-viride* (Gams and Rozsypal, 1973) and *M. anisopliae*, with var. *anisopliae* and var. *major* separated by size of the conidia (Tulloch, 1976). The genus *Paecilomyces* was reviewed successfully by Brown and Smith (1957) with description of the three entomogenous species, *P. farinosus, P. fumoso roseus* (Bajan, 1973) and *P. amoeno roseus*. The genus *Akanthomyces* (Samson, and Evans, 1974) develops synemata similar to those of *Paecilomyces.* According to Samson and Evans (1974), *Akanthomyces gracilis* pathogenic on lepidopterans and Cercopidae in Ghana, can be considered a transitory form between the two genera. The same authors gave new descriptions of the genera *Gibellula* and *Pseudogibellula*, both of which are pathogens of Arachnidae, Hymenoptera Formicidae, Homoptera, Cereopidae, and the genus *Hymenostilbe* (Weiser, 1966).

Among the Stilbaceae, the genus *Hirsutella* shows morphological analogies with the genus *Akanthomyces* in that the formation of synnema has lateral phialides. These characteristically produce a terminal sterigmata that bears a single conidium surrounded by mucus. This genus is pathogenic to all systematic groups of insects and comprises a large number of species, including *Hirsutella gigantea* (Swiezynska and Balazy, 1970) and *Hirsutella thompsonii* (McCoy and Selhime, 1974; Villalon and Dean, 1974). The genus *Massospora*, characterized by its development that is limited to the terminal portion of the insect abdomen, can be readily separated from other entomogenous genera of the Enthomophthoraceae by (a) the production of conidia in chambers within a mycelia mass, whereas in other genera the conidia are ejected from conidiophores, and

(b) the production of reticulated resting spores. Other genera of the family produce either smooth-walled resting spores, or if they are ornamented they are not reticulate. Finally, species of *Massospora* appear to grow only in cicadas with the type species *M. cicadina* described from *Magicicada septemdecim* (Soper, 1974, Soper, *et al.,* 1976).

Among the Phycomycetes, in addition to the Entomophthorales, entomogenous species belong to the Blastocladiales (*Coelomomycetaceae, Coelomomyces*) and to the Chytridiales (Chytridiaceae, *Coelomycidium and Myophagus*). Species of *Coelomomyces* (Dubitskij *et al*., 1972) characterized by their specificity for mosquitoes and chironomids, as well as by their multiplication under the form of sporangia in the body cavity of the insect, have been studied by entomologists and public health scientists before being able to promote a new method of controlling these vectors of human diseases (Soper *et al.*, 1976). The potentials of new genera *Coelomycidium* (Weiser and Zizka, 1974) and *Lagenidium* (Umphlett and Haung, 1972) have been examined.

2.6 Modes of insect infection

Unlike bacteria and viruses, fungi can infect insects not only through the gut, but also through spiracles and particularly through the surface of the integument. This property leads directly to the possibility of infesting insects independently of their feeding activity. Attempts to define the mode of infection by a fungus have often resulted in contradictory positions because of the fact that all possible ways of infection are not necessarily exploited by a fungus for a given insect: for instance, artificial contamination of the intestinal tract of larvae of *Melolontha melolontha* by *Beauvaria brongniaetii* does not necessarily bring about infection of the host (Ferron, 1967), whereas

contamination of the integument or of the buccal appendages causes the development of the white muscardine (Delmas, 1973).

If the mycelia penetration through the integument can be favoured by the spores (David, 1967), then according to Zacharuk and Tinline (1968), it is probable that Robinson's theory, which involves both physical and enzymatic mechanisms with or without the development of appresorial structures, must be considered. This penetration is frequently followed by a melanic reaction of the integument at the sites of infection, probably because of changes in phenoloxidase activity caused by the fungus (Aoki and Yanase, 1970b). It is important to remember that the insect integument is essentially composed of proteins and chitin associated with lipids and phenolic compounds. The very thin outer layer or epicuticle contains lipids (fatty acids and paraffin) whose antifungal activities have been demonstrated, but in higher concentrations than those present in insect integument. Furthermore, it was demonstrated that entomogenous fungi such *as Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Aspergillus flavus* and *Entomophthora muscae* have lipolytic enzymes (Gabriel, 1968; Leopold, *et al*., 1973).

The ability of the enzymatic properties of entomogenous fungi to function is due to systems capable of attacking the proteo-chitin complex. It is estimated that only 10% of the total proteins of the integument are not linked. Therefore it is necessary that hydrolysis of the proteins takes place before the action of chitinases begins (Samsinakova *et al.,* 1976). It is known that in addition to the lipases, proteases and chitinases are effectively produced by these fungi and that lipolytic and proteolytic

activity precede chitin breakdown (Kalvish, 1974). The production of proteases was also reported in 10 species of Entomophthorales (Jonsson, 1968).

Electron microscope studies on the histopathology of fungal infections of wireworms by *Metarhizium anisopliae* (Zacharuk, 1970, 1971, 1973 and 1974) show that the fungus develops through at least six identifiable stages: external infective spores, which produce the germ tube; appressorial cells formed from the germ tube against the surface of the cuticle, which produce the penetrant peg through the epicuticle; subepicuticular penetrant plate, which produces hyphae that give rise to the hyphal bodies; irregular-walled and smooth-walled penetrant hyphal bodies that give rise to hyphae that penetrate the procuticle to the coelom; detached coelomic hyphal bodies produced by the penetrant hyphae, which circulate in the haemolymph, germinate to produce new hyphae, and thus spread the fungus in the body cavity; and chlamydospores produced after death of the host, which can maintain the fungus in a viable state within the host cadaver. These spores subsequently germinate to form emergent hyphae that sporulate on the surface of the host to produce new external infective spores (Kish and Allen, 1976; Kish *et al*., 1976).

It is important to emphasize that because of arthropod moulting the infection can be considered successful only when the fungus has reached the hypodermis. This phenomenon is even more important if the insect under consideration undergoes frequent moults. In fact, these infectious spores may vary as a result of the coincidence between fungal penetration and the moulting stage. Therefore, *B. bassiana* can cross through the integument of third instar larvae of *Leptinotarsa decemlineata* just prior to

moulting. In this instance, the hyphae and blastophores invade the exuvial fluid and infect the newly formed integument, which cause localized lysis of the hypodermis by the usual process of primary infection of larvae between moults. In other times, the fungal action is more advanced when ecdysis occurs, and the cuticle is apparently wounded during ecdysis because of the adhesion of the two cuticles caused by the mycelium. These wounds are an open door not only to mycelial elements of the exuvial fluid, but also to bacteria that can cause lethal septicemia (Fargues and Vey, 1974; Zacharuk, 1974). However, if the penetration into the old integument is only superficial during the moult, the host insect can escape infection by rejecting the infectious inoculum.

2.7 Development of mycosis

After crossing the cuticular barrier the fungus comes up against the cellular defence reactions of the host (Seryczynska and Bajan, 1975). Plasmatocytes, normally dispersed in the haemolymph accumulate around the fungus and give rise to melanization. This haemocytic pseudotissue becomes lamellae. By using invertebrate tissue culture techniques, it was noted that the haemocytes are attracted from a distance by the mycelium (Vey and Quiot, 1975). After a few days, these hemocytic formations may become large and visible to the naked eye. Either the infection is blocked and the insect continuous its normal development, or the mycelium may also overcome this haemocytic barrier and invade the rest of the organism. The degree of cyst formation is related to the virulence of the fungus. With less virulent species such as *Apergillus niger*, the infection seems to be easily overcome, but with wound pathogens, such as *Mucor hiemalis*, cyst formation is not very effective because of the rapid development of

the hyphae and the secretion of toxins. Finally, in true pathogens such as *Beauveria*, alteration of cyst formation is probably affected by the production of toxins, in which case the mycelium may rapidly resume its normal growth and invade the haemocoel by means of blastospores (Kawakami, 1965; Aoki and Yanase, 1970a).

The death of the insect marks the end of the parasitic phase of the fungus development, but the lethal result of the disease is only one aspect of an infection. Until recently, entomologists and pathologists gave only limited attention to the consequences for the host insect of an infection that does not kill it. In fact, knowing that the lethal development of a mycosis is closely connected with the number of infected spores, it is eventually important to examine what happens to insects infected by an inoculum at a level lower than the optimum, as occurs frequently in the case of field application as well as in epidemiological development of the in nature. Several workers, mostly from the Soviet Union have made new observations on the phenomena relating to disturbance in the fecundity and diapause of surviving imagos, and they also have confirmed some older observations along these lines (Bajan and Kmitowa, 1972; Diehtiarova, 1967; Gorskhova, 1966). Similar observations were made in Western Europe (N'Doye, 1976). It also reported that insects surviving an infection can have a reduced resistance to cold, which results in a high hibernal mortality (Litvinenko, 1974). Despite the practical and theoretical interest of these observations, no fundamental work, either physiological or pathological, seems to have been undertaken to explain the mechanisms of the secondary effects.

2.8 Favourable conditions for mycosis development

All stages of insect development, eggs, larvae, pupae, and imagos, are generally susceptible to mycosis. Contradictory data were found, however with regard to susceptibility of the egg (Lappa and Goral, 1974) but it frequently concerns a stage of short duration that only later manifests the disease. On the other hand, all insect species are not susceptible to the same fungus. It is well known that the families of fungi are characterized by certain specificity. In the presence of virulent strain and a susceptible host insect, the development of the infection is closely connected with the dose of infecting spores, the physiological state of the host, and abiotic conditions. Until recently, physical phenomena of the environment, especially hygrometry, were considered as prominent factors to the extent that, for certain workers, prospective for the practical utilization of entomogenous fungi seemed to be dependent on specific, if not exceptional, ecological conditions (Ferron, 1978).

2.8.1 Influence of humidity

Water, either liquid or vapour, has long been recognized as essential for the germination of spores of most fungi, and furthermore, high atmospheric humidity is known to favour the development of epizootic mycosis. At present, various information permits us to distinguish between the role of atmospheric humidity during the process of infection and on various epizootic phenomena. It is known, that the development of the fungus on the cadaver, and therefore the sporulation, is associated with humidity close to the saturation point. Under these conditions the auto-multiplication of the inoculums results in a heavier contamination of healthy insects, favouring an epizootic development of mycosis. On the other hand Ferron (1977) demonstrated that, the infection of insects can

be obtained independently of the environmental humidity. This would lead to the supposition that physical phenomenon of the boundary layer of the insect integument facilitates spore germination even if the atmosphere is practically dehydrated.

It is evident that spore longevity depends upon the relative humidity (Clerk and Madelin, 1965): conidia of *Beauveria bassiana* and *Paecilomyces farinosus* lived longer at a RH of 0 % or 34% than at75%; on the other hand, conidia of *Metarhizium anisopliae* survived the longest at higher and lower humidities and the least at humidities near 45%. In the case of *Entomophthora,* the conidia could not survive exposure to humidities below 75% (Newman and Carner, 1975).

2.8.2 Influence of temperature

The rapidity of mycelia development and therefore the rapidity of the evolution of infection depends on temperature (Kalvish, 1974). In general, optimum temperature values fall between 20^{o}C and 30^{o}C (for example 23^{o}C for *Beauveria brongniartii*, 24^{o}C for *Entomophthore obscura*, 25^{o}C for *Beaurveria bassiana* and 30^{o}C for *Entomophthora*) with the limits between 5^{o}C and 35^{o}C . Temperatures lower than the optimum distinctly retards the development of mycosis without necessarily affecting the total mortality (Ferron, 1967). Furthermore, the enzymatic activity of the fungus, whose importance in the penetration through the cuticle, does not always appear to follow strictly the development of mycelia as a function of temperature. The optimum temperature for the development of the fungus is not necessarily the same for the development of the disease; however, the influence of temperature on the host insect must be taken into consideration, since very short periods between moults resulting from

high temperature may reduce, for example, the duration of the instar to an extent that penetration of the fungus through the integument is impeded.

2.8.3 Influence of Health of the host.

Lappa (1964) showed that it is necessary to consider the state of health of populations to permit an estimation of their biotic potential. Telenga *et al.* (1967, 1976), recommended the use of combinations of entomogenous preparations with low doses of chemical insecticides. This relies upon a detailed knowledge of the haemolymph, especially the haemocytes, to diagnose different diseases before the appearance of the classic external symptoms, which often appear too late to be of great interest in epidemiology. Thus it was possible to characterize physiological alterations resulting not only from bacteria, viruses, fungi, and protozoa, but also from a series of diseases and unfavourable conditions. Examples of a synergistic effect were pointed out by Ferron (1970, 1971) with *Melolontha melolontha* larvae attacked by the muscardine, caused by *Beaurveria bongniartii* in the presence of low doses of organochlorines or organophosphates. Under these conditions, there was an increase in the incidence of muscardine and an acceleration of the infectious process and the sensitivity of the insect to mycosis, which appears at a concentration of spores that does not cause any mortality without the addition of an insecticide.

2.8.4 Influence of the quantity of spores.

It has already been established by many authors that there was a positive correlation between the number of infective spores and the mortality by mycosis, but the influence of low doses, which do not lead to lethal development of disease, has not yet been

demonstrated (Berrios and Hidalgo-Salvatierra, 1971; Broome *et al.*, 1976; Ferron, 1967). The estimation of the LD_{50} varies according to the fungal strain and species of a given insect and to the modes of contamination: by topical application of the inoculums; by spraying a spore suspension directly on the insect or by treating a plant or inert substance upon which the insects are placed; by free or force ingestion; by contamination of the rearing substrate, particularly for subterranean insects; by immersion of insects in a titrated suspension of spores (Ferron, 1978).

2.9 Characterization and selection of strains of entomogenous fungi

Virulence and specificity of strains, the capacity of mass production of resistant forms for satisfactory storage of the biological preparations, and their innocuousness for vertebrates are the main properties that must be considered to develop techniques of microbiological control of crop pests (Ferron, 1978).

2.9.1 Characterization of strains

In all cases, the classification of entomogenous fungi is still based on morphological criteria. Further knowledge of virulence and specificity has revealed, however, the necessity of more precise characterization. Fargues *et al*. (1975) have used electrophoretic and immunoelectrophoretic methods associated with characteristic reactions of enzymatic activity in order to compare two strains of *Beauveria bassiana* and *Beauveria tenella*. They then applied the same techniques to four stains of two varieties of *Metarhizium anisopliae,* whose specificity of action on three different insects had been established previously.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was conducted in the Kumasi Metropolis. Five communities (KNUST campus, Atonsu, Fumesua, Ahinsan and Kentinkron) were selected with each community representing a sampling site. The communities were selected because of the large numbers of maize farms present in those areas. Maize farms were selected at random and 10 maize farms, both abandoned (after harvest) and those yet to be harvested were visited within each community for larvae collection.

3.2 Sample collection

Dead larvae of *Eldana saccharina* were collected from both new and abandoned maize farms. The collection was done at the end of the minor maize growing season in February and March. On each field, the plants were inspected carefully for signs of stemborer infections and damage. Maize stems were carefully split into two to expose larvae present in them. Dead larvae were collected and kept in sterile glass bottles with tight covers.

3.3 Sterilization of media and equipments.

All media were sterilized in an autoclave at 121 ^{o}C and a pressure of 15 psi for 15 minutes.

Petri dishes and glass bottles were sterilized in hot air oven at 160 ^{o}C for 2 hours.

Other materials such as metals and plastics were sterilized using 70% ethanol (alcohol). Inoculating loops were sterilized by holding them in a flame until red hot.

The laboratory benches were disinfected by swabbing the working surface with 70% ethanol before and after each day's work.

3.4 Preliminary screening

All dead larvae were screened through a series of simple laboratory steps which included direct observation and observation under the microscope to detect the presence of microbes. For those samples on which fungal sporulations were visible on cadavers, or where the cadaver was stiff and subsequent incubation under humid conditions (moist tissue paper in petri dish) led to sporulation, spores were examined in lactophenol mounts, and cultured by plating onto Potato Dextrose Agar (PDA) containing the antibiotic chloramphenicol at 0.05 g/l. After 3 days at 28 ^{o}C, single colonies were re-isolated onto ordinary PDA slants, incubated until sporulation, and then refrigerated at a temperature of 5^{o}C. Those larvae that showed no sign of fungal growth were discarded.

3.5 Identification of fungi

Isolated fungal species were identified based on colony morphology as well as hyphal and spore shapes. Morphological features used included: colour, shape and diameter of colonies (Lomer and Lomer, 1996). Lactophenol mounts of the isolated fungi were also observed under the microscope at X400 magnification.

3.6 Spore production and bioassay of isolated fungal species

Five fungal species that were isolated from the dead larvae of *Eldana saccharina* were used in a pathogenicity test on live larvae of *Eldana saccharina* which were reared in the insectary.

3.6.1 Insect breeding

Live larvae of *Eldana saccharina* were collected from maize fields and fed with maize stems (cut into pieces) and were observed daily until pupation occurred. The sexes of the pupae were determined using the size of pupae and as well their mating organs. Male and female pupae were kept in different wooden cages of size 60 cm x 60 cm x 50 cm and observed daily until the adults emerged. Male and female adults that emerged on the same day were transferred into new cages in pairs (male and female) and fed daily with sugar solution. Fresh maize stems with leaves were placed in each cage to serve as oviposition sites. Eggs layed by the female were collected and incubated in Petri dishes at a temperature of 28 ^{o}C. Larvae that emerged were fed with soft young maize leaves. Second instar larvae of about ten to twelve days old were used for the pathogenicity test. The average weight of the larvae was determined using an electronic balance.

3.6.2 Spore production

Five isolated fungal species were used for the pathogenecity test. The fungi were grown in 25 ml universal bottles on PDA until sporulation.

3.6.3 Bioassay

3.6.3.1 Preparation of spore suspensions

Groundnut oil without added antioxidants was used for the spore suspensions. The oil was added to the fungal spores in the 25ml universal bottles and a spatula was used to scrape the spore off the media into the oil. The suspension was then transferred into another bottle. An electronic shaker was used to shake the suspension for four minutes to separate spores from one another. The procedure was carried out for all the fungal species.

3.6.3.2 Spore counting

Spore counting was done using an Improved Neubea Haemacytometer and a light microscope. The bottle containing the spore suspension was well shaken to ensure that the spores are evenly distributed in the suspension. 1 ml of the spore suspension was then taken and added to 9 ml of groundnut oil in order to dilute the suspension.

Ethanol was used to clean the haemacytometer to remove grease. A clean finger (washed with a detergent) was used to smear saliva over two opposite edges of the cover slip and placed on the haemacytometer. A small amount of the diluted solution was dropped at the edge of the cover slip using a Pasteur pipette. After 40 minutes, the haemacytometer was placed under a light microscope and the spores counted under the X400 magnification.

3.6.3.3 LT_{50} determination (Median Lethal Time)

For each of the five fungal species, 20 second instar *E. saccharina* larvae were used for each single set-up during the pathogenicity test. Larvae were sprayed with 50µl of groundnut oil containing $1x10^7$c.f.u ml^{-1} of fungal spores. Larvae were kept in separate bottles and fed with maize (cut into pieces) at room temperature. They were observed every day and dead larvae were counted. The number of larvae that survived infection and thus pupated was noted, and their sexes determined. The number of pupae that emerged into adults was also recorded. Each set-up was replicated 3 times. Dead larvae were incubated in humid conditions to facilitate sporulation.

A control experiment was also set up and each of the 20 larvae sprayed with 50µl of groundnut oil only (ie. groundnut oil without spores).

3.6.3.4 Determination of LC_{50} (Median Lethal Concentration)

A series of fungal spore suspensions, obtained through serial dilutions, of concentrations ranging from $1x10^2$ to $1x10^8$c.f.u./ml were used for this test. For each of the five fungal species, 50µl each of four different spore concentrations ($5x10^0$, $5x10^2$, $5x10^4$ and $5x10^6$ c.f.u/ml) were used to spray 15 second instar *E. saccharina* larvae. A control was set up with 15 larvae each of which was sprayed with 50µl of groundnut oil. Each set-up was replicated 3 times for each fungal species. Each larva was kept in a separate bottle and fed with maize stems. The set-up was observed every day and the number of dead larvae was recorded. Larvae were observed till adults emerged. The LC_{50} of each fungal species was determined using probit analysis. Bioassays in this study were carried out at room temperature.

3.7 Statistical analysis

Statistical packages used to analyze the results were SPSS (version 14) and Excel. One way ANOVA at a 95% confidence interval was used to compare the larval mortality produced by the fungal isolates. It was also used to compare mortalities produced by different concentrations of each fungal isolate. Regression analysis was used to determine the median lethal concentrations of the fungal isolates using probits analysis. Correlation analysis also performed on increasing spore concentration and resulting mortality produced by each fungus.

CHAPTER FOUR

4.0 RESULTS

4.1 Fungal isolates

A total of 60 dead *Eldana saccharina* larvae collected from the field were taken through the preliminary screening procedure. Out of that number, 38 fungal isolates were obtained from all the sites and from these, 5 different fungal species were identified. The collection sites and the fungal species are shown in Table 1.

Table 1: Fungal species isolated from five different sites.

Sampling site	Number of larvae collected and screened	Fungi identified	No. of fungi isolated
KNUST Campus	12	*Aspergillus flavus*	6
Atonsu	14	*Verticillium albo-atrum* *A. flavus*	5 4
Kentinkrono	10	*Trichothecium sp.* *A. flavus*	6 3
Fumesua	14	*Fusarium oxysporum*	5
Ahinsan	10	*Alternaria brassicicola* *F. oxysporum*	6 3

At least one fungal species was isolated on larvae from each sampling site. *Aspergillus flavus* was isolated on larvae collected from three of the five sampling sites (KNUST campus, Atonsu and Kentinkrono); *Fusarium oxysporum* was isolated from larvae

collected from two sampling sites (Fumesua and Ahinsan); *Verticillium albo-atrum* was isolated on larvae collected from Atonsu; *Trichothecium sp.* was isolated on larvae collected from Kentinkrono; *Alternaria brassicicola* was isolated on larvae collected from Ahinsan (Table 1).

4.1.1 Aspergillus flavus

Growth of vegetative body was observed 24 hours after incubation and colonies grew rapidly and covered the entire agar surface in two weeks. On Potato Dextrose Agar (PDA), the isolates were initially yellow to green in colour (Plate 1a), but changed to dark red brown in one month old cultures (Plate 1b). Colonies were also granular to woolly in texture. Under the microscope, hyphae were branched and conidia were globose in shape (Lomer and Lomer, 1996).

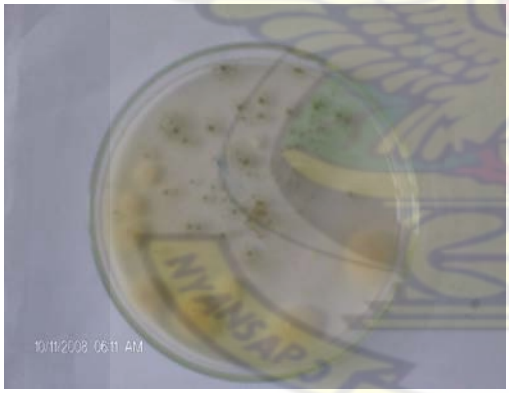

Plate 1a. Six-day-old *A. flavus* on PDA

Plate 1b. One-month-old *A. flavus* on PDA

4.1.2 Verticillium albo-atrum

Growth of this fungus was observed after 24 hours of incubation. Colonies were velvety to wooly and grew rapidly on PDA in Petri dish to cover the entire agar surface in two

weeks. Colonies were initially white to cream but later became deep yellow about eight days after incubation (Plate 2a). Colonies became dark brown to black after about one month (Plate 2b). When observed under the microscope, conidiophores were simple and conidia were oval in shape and were usually aggregated (Larone, 1995).

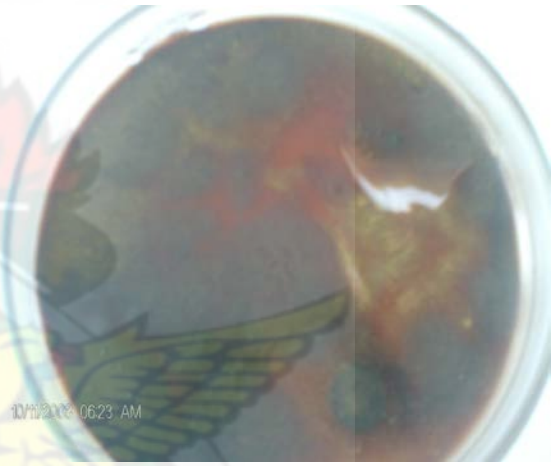

Plate 2a. 8-day-old *V. albo-atrum* on PDA Plate 2b. One-month-old *V. albo-atrun* on PDA

4.1.3 Trichothecium sp.

Growth was observed after 48 hours of incubation. The fungus grew rapidly on PDA and colonies were flat and powdery. Colonies on agar were initially white but became pink and the reverse side of the plate was pale. On agar slants in 25ml universal bottles, colonies grew and covered the entire agar surface in one month (Plate 3).When observed under the microscope, conidiophores were unbranched and conidia were pear shaped (St-Germain and Summerbell, 1996).

Plate 3: One-month-old-*Trichothecium sp*. on PDA slant

4.1.4 Fusarium oxysporum

Growth was observed 48 hours after incubation. Colonies on PDA were initially white but became purple nine days after incubation (Plate 4a and 4b). Growth of the fungus on PDA was slow and colonies covered the entire agar surface in one and half months. Under the microscope, conidia were oval to cylindrical in shape (Lomer and Lomer, 1996).

Plate 4a. Ten-Day-old *F. oxysporum on* PDA Plate 4b Plate 4a enlarged**.**

4.1.5 Alternaria brassicicola

Growth was observed after 48 hours of incubation and colonies were wooly and covered by grayish aerial hyphae after ten days of incubation (Plate 5). Colonies on PDA were grayish becoming black with the appearance of conidia. The hyphae easily spread over the entire agar surface within two weeks and growth was rapid. Under the microscope, conidia were oval in shape and darkly pigmented (Lomer and Lomer, 1996).

Plate 5: Ten-day-old *A. brassicicola* on PDA Plate 5b Plate 5a enlarged

4.2 Effect of fungal isolates on mortality and adult emergence

All the five different fungal isolates were used for the pathogenicity test. Mean percentage mortality ranged from 22.5 % (corrected to 0 % using Abbot's (1925) formula) by *Alternaria brassicicola* to 80% (corrected to 73.3 % to using Abbot's (1925) formula) by *Aspergillus flavus* (Table 2). The control gave a 25.0 % (corrected to 0 % using Abbot's (1925) formula) larval mortality (Table 2).

When the mean mortality values of the isolates were compared using the one-way-ANOVA at 95% confidence interval and at a 0.05 significance level, there was a significant difference between the percentage mortality of the fungal species (p= 0.008)

(Appendix 1). LSD was performed to separate the pair of means. When mortality by *A. flavus* was compared with that of the other isolates using the LSD, mortality by *A. flavus* was found to be significantly different from those caused by *Trichothecium sp.*,(p= 0.016) *F. oxysporum* (p= 0.009) *A. brassicicola* (p= 0.002) and the control (p=0.002). There was however no significant difference between mortalities produced by *A. flavus* and *V. albo-atrum* (p= 0.283) (Appendix 1).

When the mortality value of *V. albo-atrum* was compared with that of the other isolates using the LSD, mortality by *V. albo-atrum* was found to be significantly different from those produced by *F. oxysporum* (p= 0.041*), A. brassicicola* (p= 0.005) and the control (p= 0.007). There was however no significant difference between mortalities produced by *V. albo-atrum* and *Trichothecium sp.* (p= 0.078) (Appendix 1).

Comparing the mortality value of *Trichothecium sp*. with that of the other isolates using the LSD, mortality by *Trichothecium sp.* was not significantly different from those produced by *A. brassicicola* (p= 0.078) *F .oxysporum* (p= 0.654*)* and the control (p= 0.108).

When the mortality value of *F. oxysporum* was compared with that of the other isolates using the LSD, mortality by *F. oxysporum* was not significantly different from those by *A. brassicicola* (p= 0.150) and the control (p= 0.207) (Appendix 1).

In comparing the mortality value of *A. brassicicola* with that of the other isolates using the LSD, mortality by *A. brassicicola* was not significantly different from that by the control (P= 0.822) (Appendix 1).

Table 2: Effect of fungal isolates on mortality and adult emergence of *E. saccharina*

Isolate	Mean number of dead larvae	Mean number of larvae that pupated	Mean number of adults emerging	% larval mortality	Corrected % larval mortality
A. flavus	16.0	4.0	3.5	80.0	73.3
V. albo-atrum	13.5	6.5	6.5	67.5	56.7
Trichothecium sp.	11.5	8.5	7.5	57.5	43.3
F. oxysporum	8.0	12.0	12.0	40.0	20.0
A. brassicicola	4.5	14.5	14.5	22.5	0.0
Control	5.0	15.0	15.0	25.0	0.0

4.3 Median Lethal Time (LT_{50}) of isolated fungi.

4.3.1 Aspergillus flavus

A mean of sixteen (16) dead larvae were recorded representing an 80% larval mortality and this was corrected to 73.3% using Abbott's (1925) formula (Table 2). A mean of 3.5 adults emerged from an average number of four (4) pupae (Table 2). Using cumulative daily mortality, the LT_{50} value obtained was 6 days (Fig 1).

4.3.2 Verticillium albo-atrum

A mean of 13.5 dead larvae were recorded representing 67.5% larval mortality which was corrected to 56.6% using Abbott's (1925) formula. A mean of 6.5 pupae were produced all of which emerged into adults (Table 2). Using cumulative daily mortality, the LT_{50} value obtained was 7.20 days (Fig. 1).

4.3.3 Trichothecium spp.

A mean of 11.5 dead larvae were recorded representing 57.5% larval mortality which was corrected to 43.33% using Abbott's (1925) formula. A mean of 8.5 pupae were produced of which an average of 7.5 emerged as adults (Table 2). Using cumulative daily mortality, the LT_{50} obtained was 9.80 days (Fig. 1).

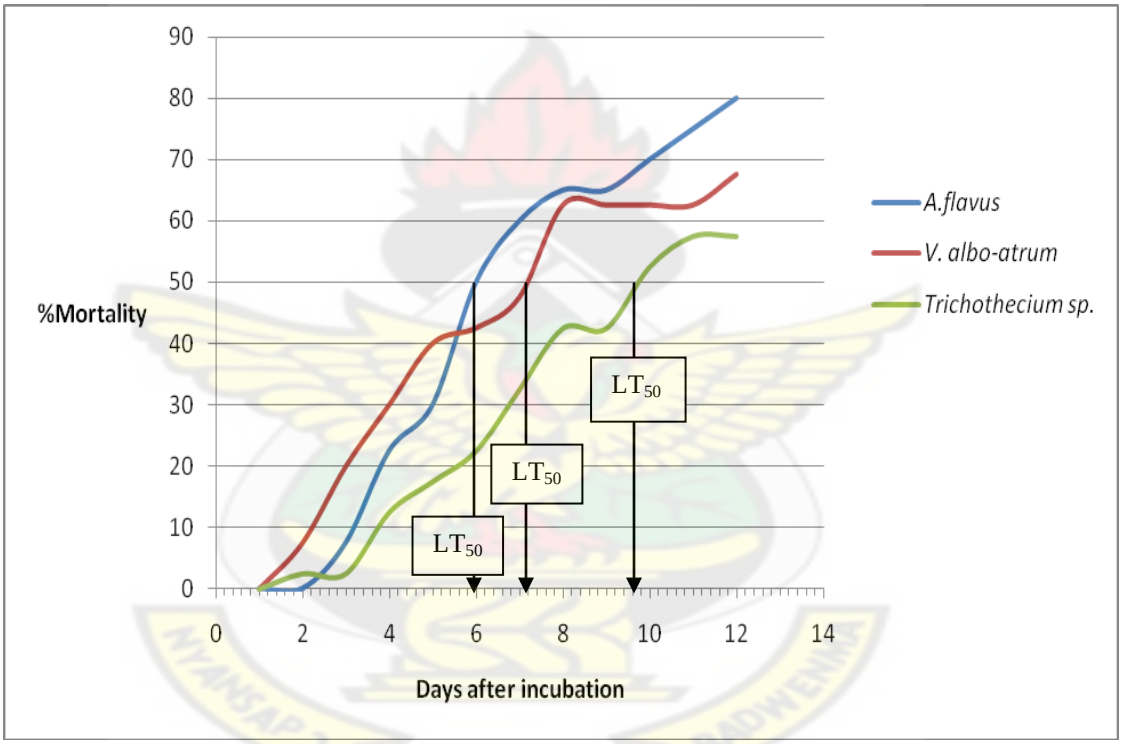

Figure 1: Cumulative mortality of *Eldana saccharina* larvae by three fungal species showing the LT_{50} for each fungus.

4.3.4 Fusarium oxysporum

An average of eight (8) dead larvae was recorded representing 40% mortality which was corrected to 20% using Abbott's (1925) formula. An average of 12 pupae was produced

all of which emerged as adults (Table 2). LT_{50} was however not determined because mortality did not exceed or equal to 50%.

4.3.5 Alternaria brassicicola

An average of 4.5 dead larvae was recorded representing 22.5% mortality which was corrected to 0% using Abbott's (1925) formula. An average of 14.5 pupae was recorded and all of them emerged as adults. LT_{50} was not determined because mortality was not up to 50% of the larvae.

4.4 Mean Daily Mortality of fungal isolates

4.4.1 Aspergillus flavus

There was no larval mortality two days after incubation. Larval mortality started on the 3rd day after incubation and ended on the 12th day. The highest mortality occurred on day 6. Thereafter, mortality decreased to day eight. There was no mortality on the 9th day. Mortality resumed on day ten and remained constant till day 12th. (Fig 2) (Appendix 2).

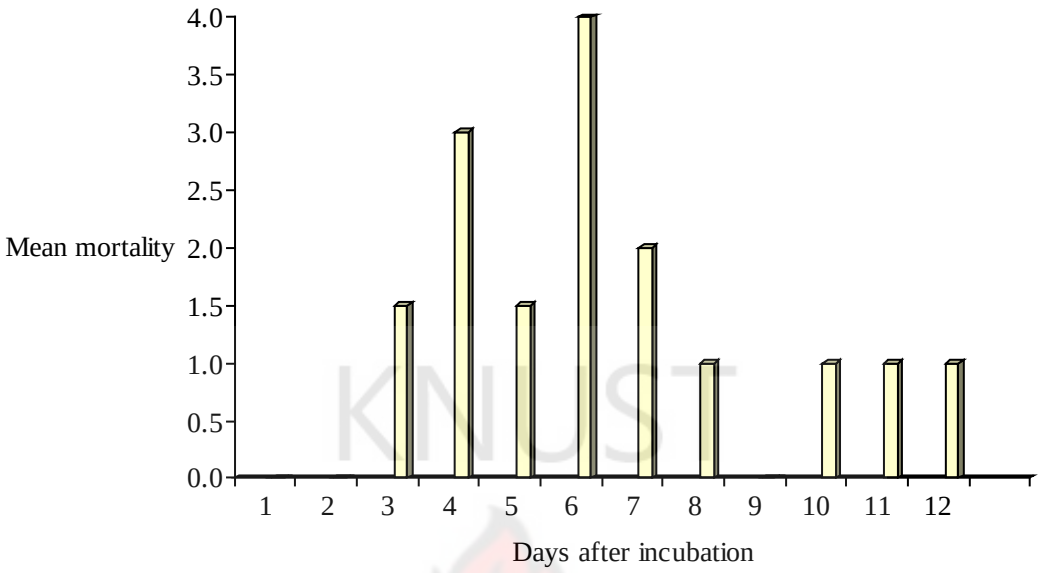

Figure 2: Daily larval mortality caused by *A. flavus*.

4.4.2 Verticillium albo-atrum

A mean larval mortality of 1.5 was recorded on the 2nd day after incubation and mortality reached its peak on the 8th day after incubation (Fig.5). There was no mortality from the 9th to the 11th day. However, mortality was recorded on day 12th (Fig.3) (Appendix 2).

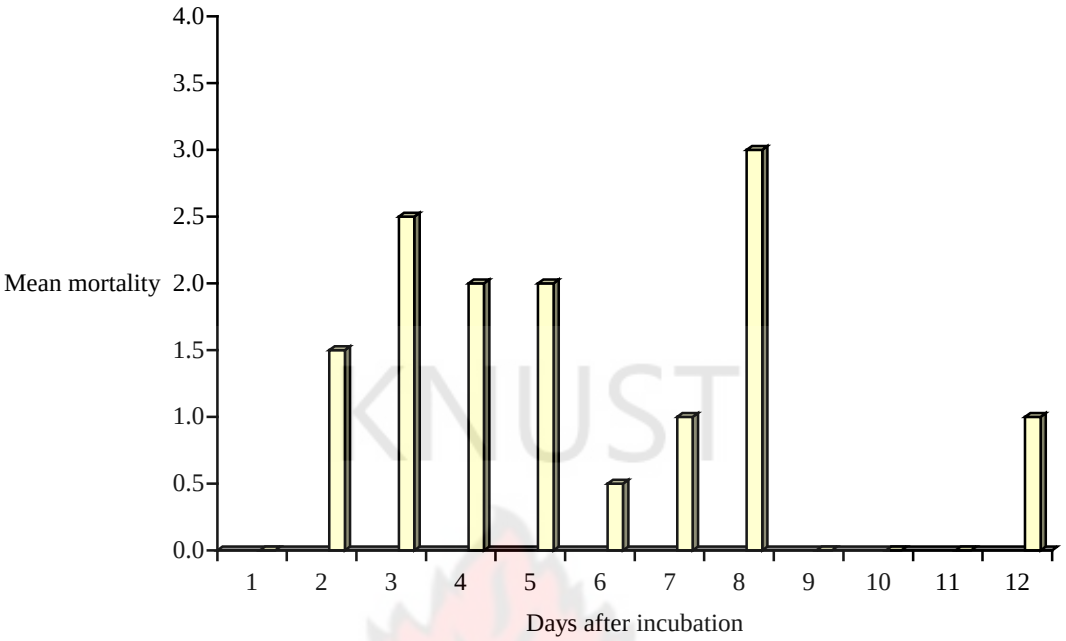

Figure 3: Daily larval mortality caused by *V. albo-atrum*

4.4.3 Trichothecium sp.

Larval mortality was recorded on the 2nd day after incubation. The highest larval mortality of 2 was recorded on three separate days. The first was on day 4 after which mortality dropped. Mortality went up again on day 7 and remained constant until day eight. There was no mortality on day nine. Mortality was then recorded on days ten and eleven (Fig. 4) (Appendix 2).

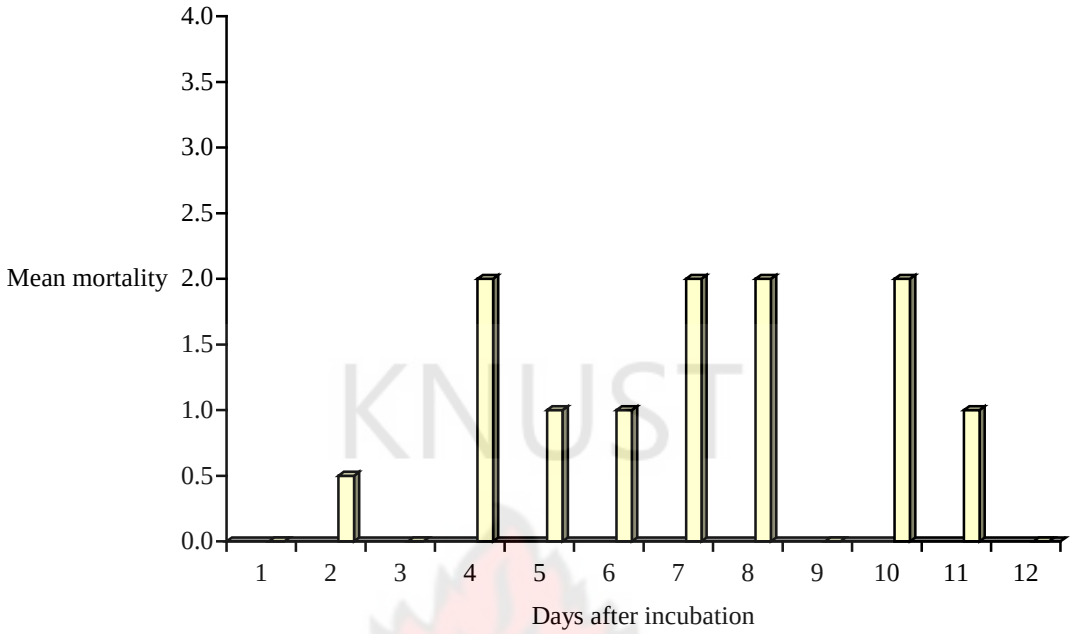

Figure 4: Daily larval mortality caused by *Trichothecium sp*.

4.4.4 Fusarium oxysporum

There was no mortality for the first three days after incubation. Larval mortality started on the 4th day after incubation and ended on the 9th day. The highest mortality of 2 was recorded on days five and eight after incubation. (Fig. 5) (Appendix 2).

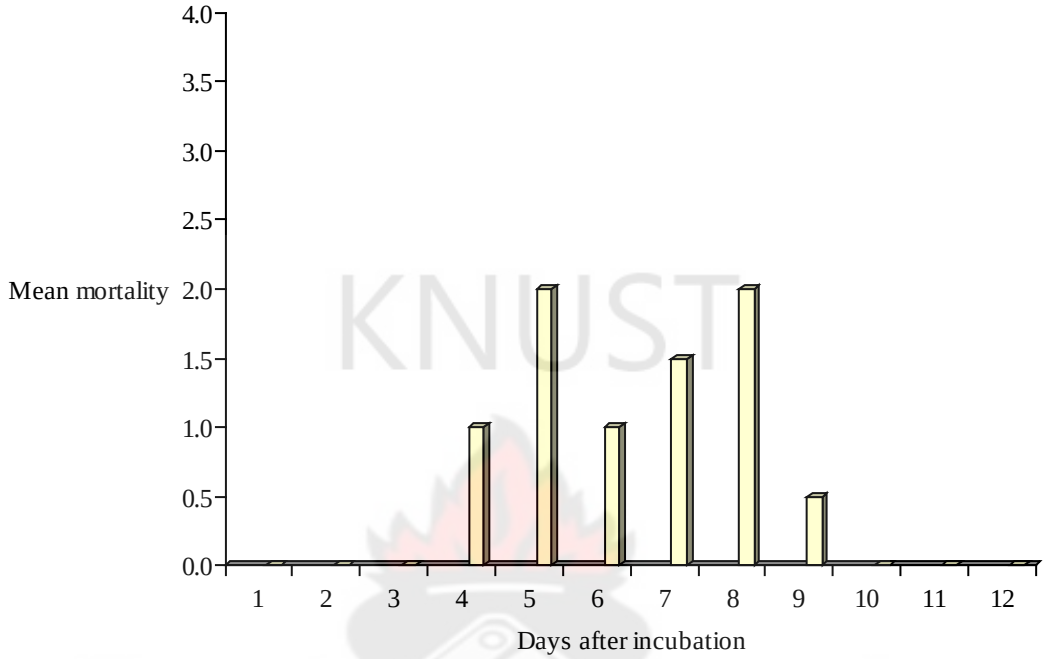

Figure 5: Daily larval mortality caused by *F. oxysporum*

4.4.5 Alternaria brassicicola

There was no larval mortality until the 5th day after incubation. Mortality occurred from day 5 to day 7 with highest mean mortality occurring on the 7th day after incubation, which was also the last day on which mortality was observed (Fig. 6) (Appendix 2).

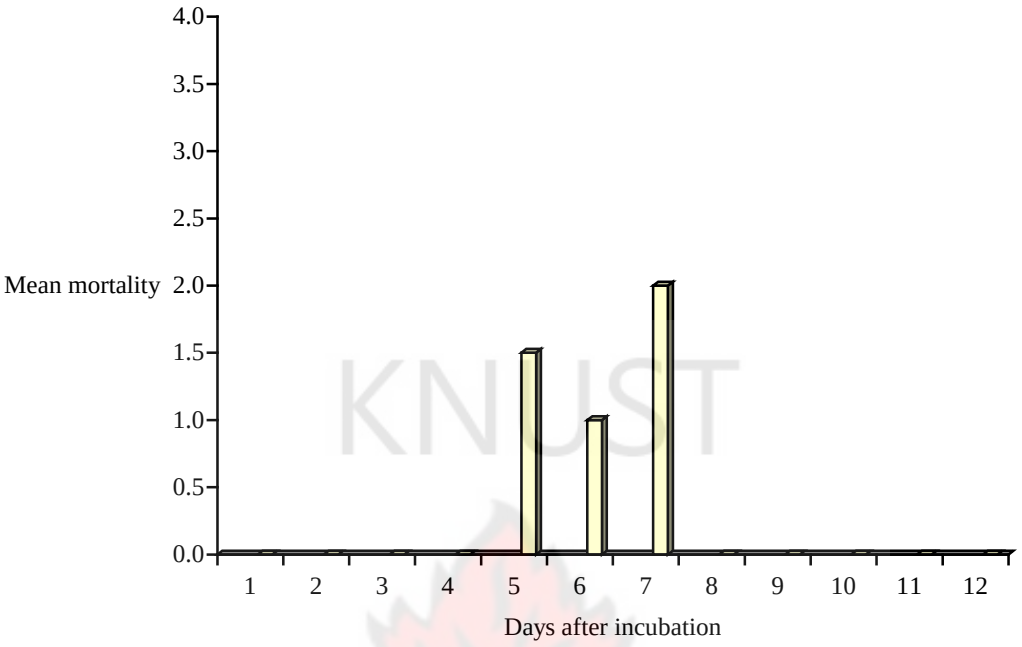

Figure 6: Daily larval mortality caused by *A. brassicicola*

4.5 Median lethal concentration (LC_{50}) of fungal isolates

Percentage mortality calculated for the different concentrations of all fungal species showed increasing mortality with increasing spore concentration.

4.5.1 Aspergillus flavus

Aspergillus flavus had the least percentage mortality of 16.7% at the concentration of $5x10^0$c.f.u/ml and the highest mortality of 86.6% at the concentration of $5x10^6$c.f.u/ml (Table 3). The LC_{50} determined from the probit transformed dose- response graph was log inverse of 3.00 ($1x10^3$ c.f.u/ml) (Fig. 7).

Table 3: Larval mortality caused by different spore concentrations of *A. flavus*

Spore concentration (Conidia/ml)	Mean number of dead larvae	Mean number of pupae	Mean Number of adults emerging	% larval mortality	% adult emergence
$5x10^0$	2.5	12.5	12.5	16.7	100
$5x10^2$	7.0	8.0	8.0	46.6	100
$5x10^4$	12.5	2.5	2.0	83.3	80
$5x10^6$	13.0	2.0	2.0	86.6	100

Data was statistically analyzed by one-way ANOVA at 95% confidence interval and at a 0.05 significance level.

Mortality produced by the four different concentrations of *A. flavus* showed a significant difference (p= 0.004) (Appendix 1). There was significant difference between mortalities produced by the following pairs of spore concentrations; $5x10^0$ c.f.u. /ml and $5x10^2$ c.f.u /ml (p= 0.027), $5x10^0$ c.f.u. /ml and $5x10^4$ c.f.u /ml (p= 0.002), $5x10^0$ c.f.u /ml and $5x10^6$ c.f.u. /ml (p= 0.001), $5x10^2$ c.f.u. /ml and $5x10^4$ c.f.u./ml (p= 0.014) and between $5x10^2$ c.f.u. /ml and $5x10^6$ c.f.u. /ml (p= 0.011) (Appendix 1). There was no significant difference between mortalities at concentrations $5x10^4$ c.f.u. /ml and $5x10^6$ c.f.u. /ml (p= 0.725) (Appendix 1).

Correlation analysis was used to compare the different spore concentrations and mortality caused by *A. flavus*. This gave a correlation coefficient of 0.576 and a p-value of 0.424. This suggests that increase in concentration of *A. flavus* spores is positively correlated to the mortality caused since the coefficient (0.576) is relatively close to

positive 1. A p-value of 0.424 is not significant and therefore concentration and mortality are not linearly related (Appendix 1).

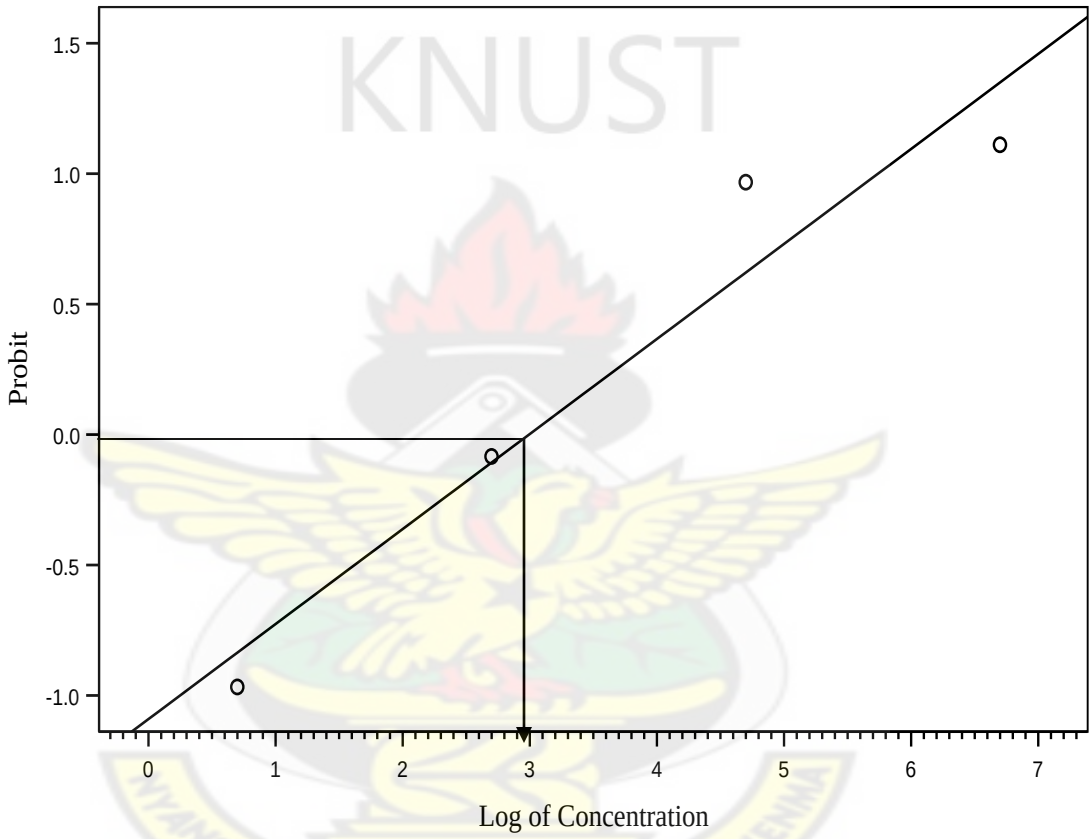

Figure 7: Probit transformed dose-response graph indicating the LC_{50} of *A. flavus* against *E. saccharina*

4.5.2 Verticillium albo-atrum

Verticillium albo-atrum produced the least mortality of 9.9%, at the concentration of $5x10^0$c.f.u./ml and the highest mortality of 73.6% was recorded for the concentration of

$5x10^6$c.f.u/ml (Table 4). LC_{50} determined from probit transformed dose-response graph was log inverse of 4.31 ($20.417x10^3$ c.f.u./ml) (Fig.8).

Table 4: Larval mortality caused by different spore concentrations of *V. albo-atrum*

Spore concentration (Conidia/ml)	Mean number of dead larvae	Mean number of pupae	Mean number of adults emerging	% larval mortality	% adult emergence
$5x10^0$	1.5	13.5	13.5	9.9	100.0
$5x10^2$	5.5	9.5	9.0	36.6	94.7
$5x10^4$	8.0	7.0	6.0	53.3	85.7
$5x10^6$	11.5	3.5	2.0	76.6	57.1

One-way ANOVA at 95% confidence interval and a 0.05 significance level showed a significant difference in mortalities produced by the different concentrations of *Verticillium albo-atrum* (p= 0.008) (Appendix 1). Mortalities produced by the following pairs of concentrations showed significant differences when LSD was calculated : $5x10^0$ c.f.u./ml and $5x10^2$ c.f.u./ml (p= 0.043), $5x10^0$ c.f.u./ml and $5x10^4$ c.f.u./ml (p= 0.009), $5x10^0$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.002), and $5x10^2$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.012). There was no significant difference between mortalities produced by the pairs of concentrations of $5x10^2$ c.f.u./ml and $5x10^4$ c.f.u./ml (p= 0.142) and also $5x10^4$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.063) (Appendix 1) .

When the different spore concentrations and mortality caused by *Verticillium albo-atrum* were compared using correlation, a correlation coefficient of 0.777 and a p-value of 0.223 were obtained. This suggests that the increase in spore concentration of *V. albo-atrum* is positively correlated to mortality caused since the coefficient (0.777) is

relatively close to positive 1. A p-value of 0.223 is not significant and therefore concentration and mortality are not linearly related (Appendix 1).

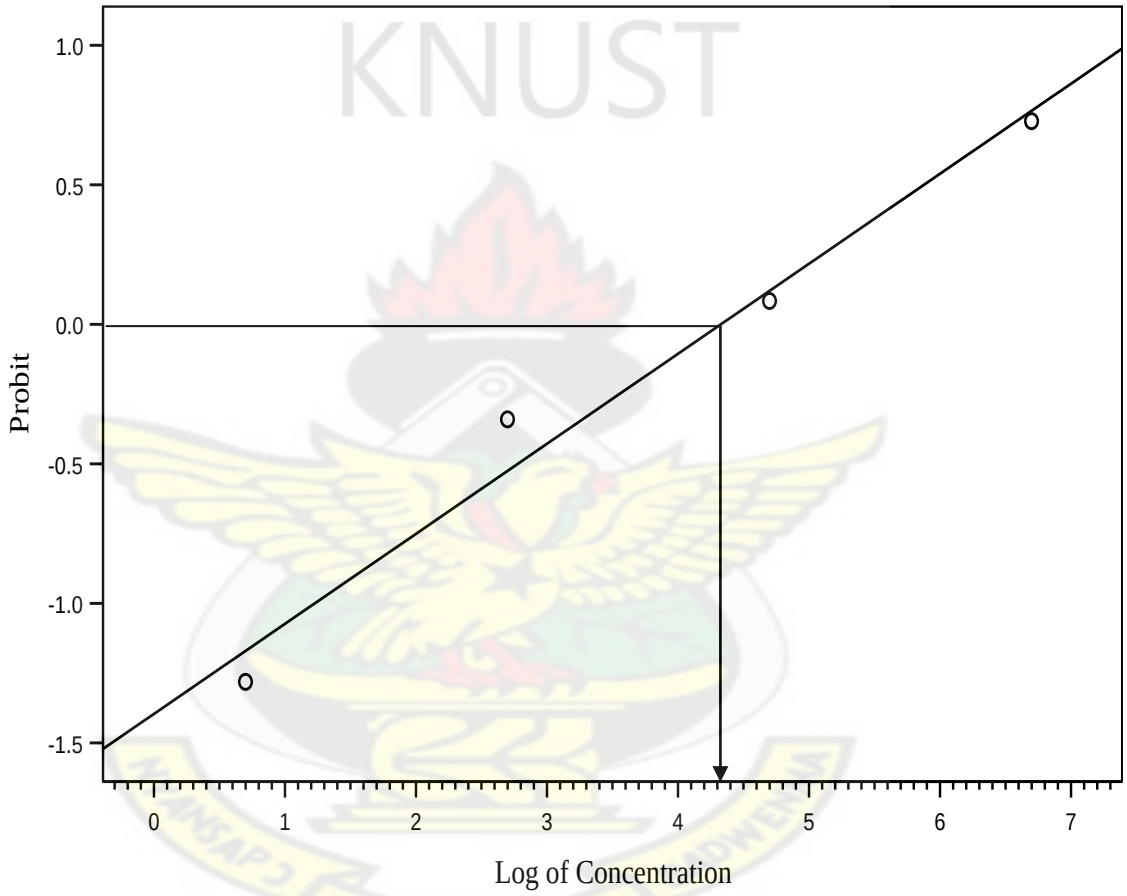

Figure 8: Probit transformed dose-response graph indicating the LC_{50} of *V. albo-atrum* against *E. saccharina*

4.5.3 Trichothecium sp.

The least mortality of 13.3% was produced at the concentration of $5x10^0$ c.f.u. /ml, whilst the highest mortality of 59.5% was produced at the concentration of $5x10^6$c.f.u/ml, (Table 5). It can be seen that increase in the spore concentration of *Trichothecium sp* produced corresponding increase in mortality of *E. saccharina* larvae. LC_{50} determined from probit transformed dose-response graph was log inverse of 5.71 ($512.8x10^3$c.f.u/ml) (Fig 9).

Table 5: Larval mortality caused by different spore concentrations of *Trichothecium sp.*

Spore concentration (Conidia/ml)	Mean number of dead larvae	Mean number of pupae	Mean number of adults emerging	% larval mortality	% adult emergence
$5x10^0$	2.0	13.0	13.0	13.3	100.0
$5x10^2$	3.5	11.5	11.5	23.3	100.0
$5x10^4$	6.0	9.0	9.0	40.0	100.0
$5x10^6$	9.0	6.0	4.0	59.9	66.7

One-way ANOVA at 95% confidence interval showed no significant difference between larval mortalities produced by the different spore concentrations of *Trichothecium sp.* (p= 0.105) (Appendix 1). However when mortality produced by the individual concentrations were compared using the LSD, there was a significant difference between mortalities produced by the concentrations of $5x10^0$ c.f.u./ml and $5x10^6$ c.f.u./ml (p = 0.031) (Appendix 1) .

When the different spore concentrations of *Trichothecium sp.* were compared to mortalities produce, a correlation coefficient of 0.847 and a p-value of 0.153 were obtained. This suggests that the concentration increase in *Trichothecium sp*. is positively correlated to mortality since the coefficient (0.847) is relatively close to positive 1. A p-value of 0.153 is not significant and therefore concentration and mortality are not linearly related (Appendix 1).

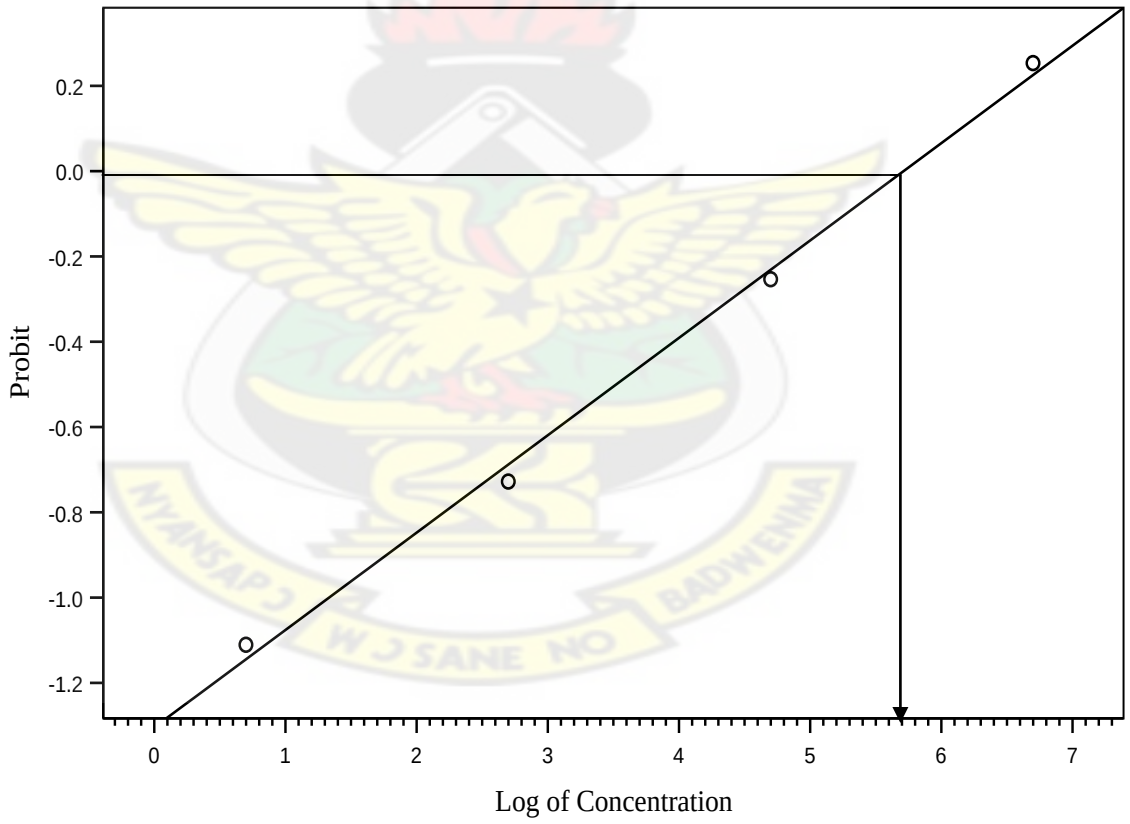

Figure 9: Probit transformed dose-response graph indicating the LC_{50} of *Trichothecium sp.* against *E. saccharina***.**

4.5.4 Fusarium oxysporum

There was no mortality (0.0%) at the concentration of $5x10^0$ c.f.u./ml. The least mortality of 3.5 was produced at the concentration of $5x10^2$, whilst the highest mortality of 53.3% was produced at the concentration of $5x10^6$ c.f.u./ml (Table 6). LC_{50} determined from probit transformed dose-response graph was log inverse of 6.03 ($1071.5x10^3$ c.f.u/ml) (Fig. 10).

Table 6: Larval mortality caused by different concentrations of *F. oxysporum*

Spore concentration (Conidia/ml)	Mean number of dead larvae	Mean number of pupae	Mean number of adults emerging	% larval mortality	% adult emergence
$5x10^0$	0.0	15.0	15.0	0.0	100.0
$5x10^2$	3.5	11.5	11.5	23.3	100.0
$5x10^4$	6.5	8.5	8.0	43.3	94.1
$5x10^6$	8.0	7.0	5.0	53.3	71.4

Comparing the mortalities of the different spore concentrations of *F. oxysporum* using One-way ANOVA, there was no significant difference between the groups (concentrations) (p= 0.095) (Appendix 1). However, there was a significant difference between mortalities at the concentrations of $5x10^0$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.029) when an LSD was calculated for the pairs of concentrations(Appendix 1) .

A correlation coefficient of 0.666 and a p-value of 0.334 were obtained when the different spore concentrations of *F. oxysporum* were compared with mortality using correlation analysis. This suggests that the concentration increase in *F. oxysporum* is

positively correlated to mortality since the coefficient (0.666) is relatively close to 1. A p-value of 0.334 is not significant and therefore concentration and mortality are not linearly related (Appendix 1).

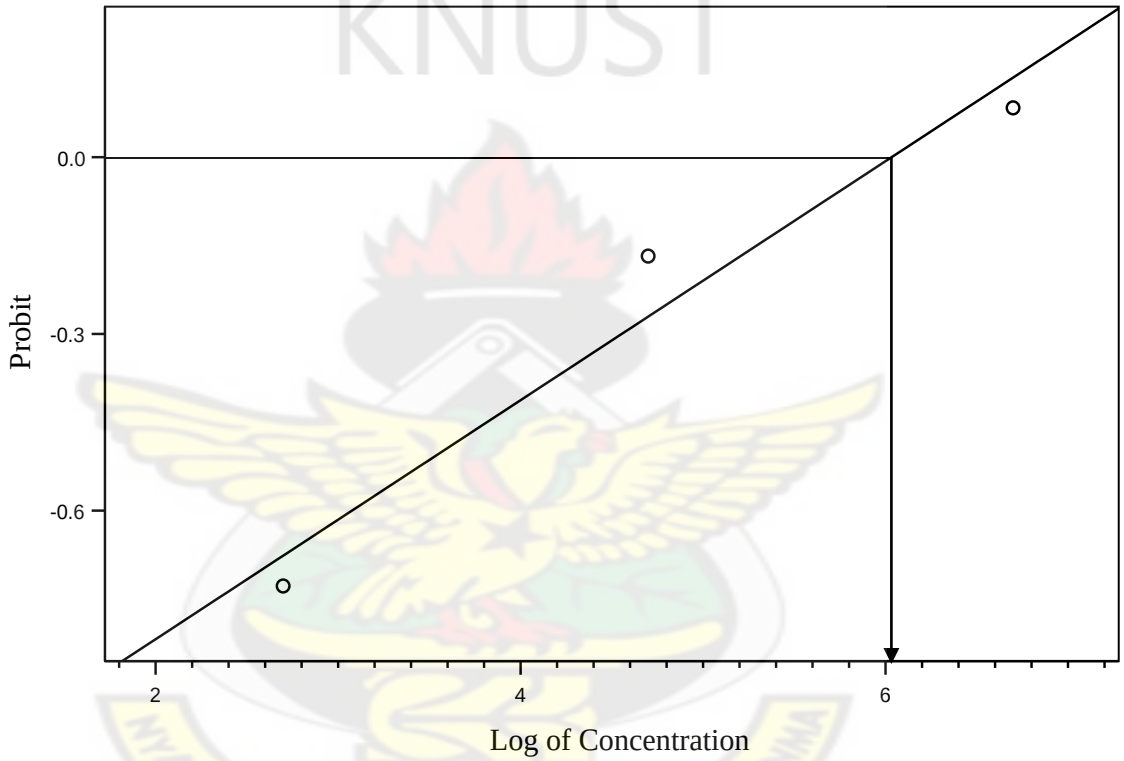

Figure 10: Probit transformed dose-response graph indicating the LC_{50} of *F. oxysporum* against *E. saccharina*.

4.5.5 Alternaria brassicicola

The concentration of $5x10^0$c.f.u /ml caused the least mortality of 3.3%, whilst the highest larval mortality of 46.6% was caused by the concentration of $5x10^6$c.f.u /ml

(Table 7). LC_{50} was not determined since none of the concentrations produced mortality equal to or above 50%.

The control produced a 20% larval mortality (Table 7).

Table 7: Larval mortality caused by different concentrations of *A. brassicicola* and the control.

Spore concentration (Conidia/ml)	Mean number of dead larvae	Mean number of pupae	Mean number of adults emerging	% larval mortality	%adult emergence
$5x10^0$	0.5	14.5	14.5	3.3	100.0
$5x10^2$	1.0	14.0	14.0	6.6	100.0
$5x10^4$	3.5	11.5	11.5	23.3	100.0
$5x10^6$	7.0	8.0	7.0	46.6	87.5
Control	3.0	12.0	11.0	20.0	91.6

One-way ANOVA at 95% confidence interval and a 0.05 significance level showed a significant difference between the groups (concentrations) (p= 0.014) (Appendix 1). There was significant difference between mortalities produced by the following pairs of concentrations: $5x10^0$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.004), $5x10^2$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.006) and also $5x10^4$ c.f.u./ml and $5x10^6$ c.f.u./ml (p= 0.035). There was no significant difference between the following pairs: $5x10^0$ c.f.u./ml and $5x10^2$ c.f.u./ml (p= 0.678), $5x10^0$ c.f.u./ml and $5x10^4$ c.f.u./ml (p= 0.055) and also $5x10^2$ c.f.u./ml and $5x10^4$ c.f.u./ml (p= 0.089) (Appendix 1).

Correlation analysis was used to compare increased concentration with mortality. When the different spore concentrations were compared to mortality in *A. brassicicola,* a

correlation coefficient of 0.906 and a p-value of 0.099 were obtained. This suggests that the concentration increase in *A.* is positively correlated to mortality since the coefficient (0.906) is relatively close to positive1. A p-value of 0.099 is not significant even though it is close to 0.05 and therefore concentration and mortality are not linearly related (Appendix 1).

CHAPTER FIVE

5.0 DISCUSSION

Stemborers are the most damaging pests of maize in Africa (Youdeowi, 1989). The stemborers seriously limit potentially attainable maize yields by infesting the crop throughout its growth from seedling stage to maturity (Youdeowi, 1989).

Control of maize stemborers in Africa presents a unique set of biological, technical and socio-economical challenges (Cherry *et al.*, 1999). Firstly, in ecozones such as the forest-savanna transition zone, with rapidly increasing human population pressure, deforestation, and overlapping maize plantings, pest attacks can be highly variable between seasons and fields. Secondly, all species are cryptic and spend the majority of their life cycle hidden within the maize stalk or cob, protected from most mortality factors. Thirdly the same plant may be attacked by several species preferring different plant growth stages for oviposition, and thereby varying in time of attack. Such multi-species infestations, which are common in West Africa, require several treatments over a cropping cycle and may complicate use of entomopathogens which tend to be species specific (Cherry *et al.*, 1999).

Various methods are employed in the control of stemborers which include Chemical control, Cultural control and Biological control. Chemicals used in the control of stemborers include organic compounds like DDT; fumigants such as methyl bromide, ethylene dibromide ;carbamates such as carbaryl and botanicals such as nicotine and pyrethrum (Meyer, 2003). However, control of stemborers with chemical insecticides is

neither affordable nor sustainable for the mostly resource poor African maize farmers (Cherry *et al.*, 1999).

Cultural control is among the oldest traditional practices but normally cannot be used as a tactical means of control (Dent, 1991). Cultural control is considered the first line of defence against pests and includes techniques such as destruction of crop residues, intercropping, crop rotation, manipulation of planting dates, and tillage methods (Van den Berg *et al.*, 1998).

Biological control is the action of natural enemies (parasites, predators, and microbial agents) including naturally occurring agent and agents which are introduced by humans for pest control (also referred to as "classical biological control").

Entomopathogenic fungi are considered excellent candidates for biopesticides due to their safety, relatively limited host range, ease of production and suitability of large scale production (Ferron, 1981). The ability of a fungus to grow on an insect host depends on both biological and physical factors which include biological-virulence of fungal spores and host health and physical- temperature and humidity.

5.1 Fungal isolates

The different fungal species isolated from *Eldana saccharina* showed that *Aspergillus flavus* was very common on *E. saccharina* than the other four species since it had the largest number of isolates (13). It was also isolated from three of the five sampling sites. The colour of fungal colonies and arrangement of the heads of conidiophores of indicates the fungus belongs to the *Aspergillus* group (Raper and Fennell, 1965). The *Aspergillus* group contains species of economic importance throughout the world.

Several species cause food spoilage, others are used in oriental food fermentations; some strains can be insect pathogens and many produce potent mycotoxins (Klich and Pitt, 1988).

Fusarium oxysporum was isolated from two fields and the colour of the colonies the shape of conidia as well as the arrangement of conidiophores agree with the work by Lomer and Lomer (1996) that Colonies on PDA were initially white but became purple and under the microscope, conidia were oval to cylindrical in shape. Work done by Pristavko *et al.* (1975) showed that the codling moth, *Cydia pomonella* is known to be attacked by different entomogenous fungi which include *A. flavus, Verticillium lecanii* and *F. oxysporum.*

Verticillium albo-atrum was also identified using the colony colour, morphology and the arrangement of conidiophores as well as the shape of the conidia Lomer and Lomer (1996). According to Baudu *et al.* (2002), some commonly encountered fungal pathogens of insects include *Beauveria bassiana, Metarrhizium anisopliae* and *Verticillium sp*. Sikorowski and Lawrence (2002) also mentioned *Verticillium sp*. as an entomogenous fungus.

Trichothecium sp. identified had features similar to those described by Larone, (1995), and St-Germain and Summerbell (1996).

Alternaria brassicicola was also identified based on colony colour, spore shape and arrangement of conidia Larone (1995). Cherry *et al.* (1999), in a similar work done in Benin isolated *Beauveria spp*. and *Metarrhizium spp.* on *E. saccharina* as entomogenous fungi. In their work, isolates of *M. anisopliae, B. bassiana* and *Hirsutella spp.* were

found infecting *E. saccharina* and preliminary bioassays with *M. anisopliae* and *B. bassiana* indicated a high degree of virulence towards *E. saccharina.* Maniania (1993) reported a reduction in damage on maize in Kenya due to *Chillo partellus* following application of *B. bassiana* isolate ICIPE 35.

5.2 Mean daily mortality of fungal isolates

From the results, each of the fungal species produced mortalities starting and ending on different days. This was due to the fact that different fungal species have different growth rates at different temperatures and humidity. In addition, different fungi produce different mycotoxins that have different potencies.

Daily mortality in *E. saccharina* caused by *A. flavus* showed that there was no well laid pattern for larval death. An irregular pattern of mortality occurred during the study period. This could be due to the fact that each larva has its own defence mechanism against which the fungus has to compete to be able to overcome it, and so it takes different times for larvae to die. The same pattern was observed for the other four isolates, *Verticillium albo-atrum, Trichothecium sp., Fusarium oxysporum,* and *Alternaria brassicicola*.

5.3 Median Lethal Time (LT_{50}) of isolated fungi

The LT_{50} also known as the median lethal time is an estimation of the time that a chemical would take to cause mortality in 50% of a test population during a bioassay. This is used to compare two or more chemicals by administering the same quantity of each chemical to the same number of test animals. Using daily mortalities, the time

taken for 50% of the test population to die is determined by plotting a graph of daily mortality frequencies against time. This therefore shows which of two or more chemical is able to produce mortality faster and also to determine the survival times of the test population.

Entomopathogens have been successfully tested against stemboring species in some parts of the world, and these have been developed into commercial products, for example Ostrinil (a granular formulation of *Beauveria bassiana* from NPP, Pau, France), and DiPel G (a granular *Bacillus thuringiensis* formulation from Abbott, Chicago, USA) (Bing and Lewis, 1991).

Of the five fungal species used for the bioassay, *A. flavus* produced the highest larval mortality of 73.3% and also the fastest Median Lethal Time of 6 days at a concentration of $1x10^7$c.f.u/ml. Mortality was significant when compared to the control. When dead larvae were incubated, there was growth of *A. flavus* which confirms that this fungus was responsible for the death of the insects. This observation agreed with the work of Robert and Yendol (1971) in which, *Entomophora*, *Beauvera*, *Metarhizium* and *Aspergillus* were found to be the most commonly encountered entomogenous fungi in nature which are pathogenic to almost all groups of insects.

This makes *A. flavus* a potential pathogen on *E. saccharina*. The high mortality produced by *A. flavus* and the early time of 50% mortality may be due to its mode of infection which involves the production of lypolytic enzymes (Leopold *et al*.,1973). The lipolytic enzymes are able to attack and hydrolyse the protein-chitin complex of the

integument of insects. This action precedes chitin breakdown and gives the fungus easy access to the haemolymph of the insect.

Mortality produced by *Verticillium albo-atrum* was 56.7% and this was significant when compared to the control and this demonstrates that the fungus is pathogenic to *E. saccharina*. Larvae just after death were stiff but without external growth of fungus. However when the dead larvae were incubated under humid conditions, there was growth of the fungus on the larvae. According to Cloyd (1999) some entomogenous fungi can be used as biological control agents of stemborers as being done in the use of Veralec^{R} (commercially produced *Verticillium lecanii* for control of stemborers in Europe.

Mortality by *Trichothecium sp*. was 43.3% and this was not significantly different from that produced by the control. It could be said to be moderately pathogenic to *E. saccharina* but not enough to bring about significant death. This may be due to the inability of the fungus to overcome the cellular defence reactions of the *E. saccharina* larvae. Before mycosis can develop, the fungus after crossing the cuticular barrier (Kawakami and Mikuni, 1965), comes up against the cellular defence reaction of the host (Seryczynska and Bajan, 1975). In this case, either the infection is blocked and the larvae continue their normal development, or the mycelium may also overcome the hemocytic barrier and invade the rest of the bodies of the larvae.

Fusarium oxysporum produced a 20% larval mortality. The fungus can be said to be non pathogenic to the host since mortality was not significantly different from those produced by the control ($p= 0.207$). This shows that death may be as result of natural

causes rather than by fungal infection and the fungus might be saprophytic rather than pathogenic. Pristavko *et al*. (1975) however showed that, the codling moth, *Cydia pomonella* is known to be attacked by different entomogenous fungi which include *A. flavus, Verticillium lecanii a*nd *F. oxysporum*.

Inability of *F.oxysporum* to cause death could also be due to unfavorable growth conditions such as temperature or humidity or both. *F. oxysporum* is a common laboratory contaminant and also naturally occurring and could be a saprophyte (Lomer and Lomer, 1996).

Alternaria brassicicola also produced a larval mortality of 22.5% which is not significantly different from the control and was therefore non pathogenic to *E .saccharina*. This shows that, death was caused by natural means. This could be because the fungus was not able to infest the larvae and subsequently was not able to produce significant death. The inability of the fungus to infest the larvae may be due to the ability of the haemoctic barrier of the larvae to overcome the fungus. In addition since *Alternaria* species are cosmopolitan and ubiquitous in nature and are also common laboratory contaminants (Pritchard and Muir, 1987).

5.4 Median Lethal concentration (LC_{50}) of isolated fungi

The characteristics of exposure of a chemical and the spectrum of effects produced come together in a correlative relationship customarily referred to as the dose–response relationship. This relationship can be presented graphically to produce what is called the median Lethal dose or the Median Lethal concentration of a chemical and it is the quantity of that chemical which is able to cause death in 50% of a test population during

a bioassay. This is done by administering a series of different doses of a single chemical to equal numbers of the test animals.

The estimation of the LC_{50} varies according to the fungal strain and species of a given insect and to the modes of contamination: by topical application of the innoculum; by spraying a spore suspension directly on the insect or by treating a plant or an inert substance upon which the insects are placed; by free or forced ingestion; by contamination of rearing substrate or by immersion of insects in a titrated suspension of spores.

Small scale field tests indicated that *B. bassiana, B. brongniartii*, and *M. anisopliae* have good potential for control of soil inhabiting insects such as wireworms and cockchafers, and of others such as lepidoptereous larvae (NAS, 1979).

In Russia, *B. bassiana* is being used for the control of the Colorado potato beetle, *Leptinotarsa decemlineata* (NAS, 1979).

LC_{50} values were determined for four of the five isolates. Figures obtained from this study showed that *A. flavus* requires a lesser dose ($1x10^3$ c.f.u./ml) than the other four isolates to produce death in 50% of the test population and therefore can be said to be more potent than the other isolates (Fig 7). Mortalities produced by individual concentrations, when compared statistically showed no significant difference between the concentrations $5x10^4$ and $5x10^6$ ($p= 0.725$) suggesting that both concentrations when selected for biological control may produce similar effects. Also, the LC_{50} value of *A. flavus* falls within the range of concentrations used for the experiment which ranged from $5x10^0$ to $5x10^6$.

Correlation was used to compare the increase in concentration of spores of *A. flavus* and mortality. The correlation coefficient 0.576 and p-value of 0.424 suggested that concentration increase and mortality by *A. flavus* were not strongly correlated and were also therefore not linearly related. This means that increase in concentration did not have a direct effect on increased mortality.

Verticillium albo-atrum also produced 50% mortality at a concentration of $20.417x10^3$ c.f.u/ml and this falls within the range of concentrations used for this research (Fig. 10). This concentration was however higher than that of *A. flavus* making it less potent and therefore less pathogenic to *E. saccharina* compared to *A. flavus*. Comparing individual concentrations statistically, there was no significant difference between the concentrations $5x10^2$ and $5x10^4$ and also between $5x10^4$ and $5x10^6$. This suggests that the concentration of $5x10^4$ can produce effects similar to that producible by $5x10^2$ and $5x10^6$. The correlation coefficient 0.777 suggested that concentration increase and mortality by *Verticillium albo-atrum* were positively correlated but the p-value of 0.223 however suggested that the two were not linearly related. This means that increase in concentration had a direct effect on mortality and therefore increased mortality. However the two are not linearly related (Appendix 1).

LC_{50} determined for *Trichothecium sp*. was higher than that of both *A. flavus* and *V. albo-atrum* suggesting that a very high dose is required to obtain the desired effects. Statistical analysis showed that the only significant difference in mortalities is between those produced by the concentrations $5x10^0$ and $5x10^6$ (p= 0.029). This suggests that, any other pair of concentrations selected for a test would produce similar effects. A correlation coefficient of 0.847 suggested that concentration increase and mortality by

Trichothecium sp. were positively correlated but the p-value of 0.153 however suggested that the two were not linearly related. This means that increase in concentration had a direct effect on mortality and therefore increased mortality. However the two are not linearly related (Appendix1).

Fusarium oxysporum had an LC_{50} value larger than *A. flavus*, *V. albo-atrum* and *Trichothecium sp.* This makes *F. oxysporum* the less potent of all the isolates since a much higher concentration is required to produce an effect similar to those produced by the three isolates discussed above. Statistical comparison of individual concentrations showed only $5x10^0$ and $5x10^6$ to be significantly different. This suggests that any other concentration pair selected for a test would produce similar effects. The correlation coefficient 0.666 suggested that concentration increase and mortality by *F. oxysporum* were positively correlated but the p-value of 0.334 however suggested that the two were not linearly related. This means that increase in concentration had a direct effect on mortality and therefore increased mortality. However the two are not linearly related.

Alternaria brassicicola was unable to produce mortality in 50% or more of the test population and therefore LC_{50} was not determined. This confirms the earlier suggestion that the *Alternaria brassicicola* isolated could be a contamination or a saprophyte (Pritchard and Muir, 1987). When individual concentrations were compared using ANOVA, the mortality produced by the concentration of $5x10^0$ was significantly different from the other three concentrations which suggest that each concentration would produce an effect different from $5x10^0$. The concentrations of $5x10^2$ and $5x10^4$ as well as $5x10^4$ and $5x10^6$ showed no significant difference. This suggests that the

concentration of $5x10^4$ can be selected to produce an effect similar to both $5x10^2$ and $5x10^6$.

A correlation coefficient 0.906 suggested that concentration increase and mortality by *Alternaria brassicicola* were positively correlated but the p-value of 0.099 however suggested that the two were not linearly related. This means that increase in concentration had a direct effect on mortality and therefore increased mortality. However the two are not linearly related but however close to linearity since the significance of 0.099 is close to 0.05.

CHAPTER SIX

6.0 CONCLUSION

All five fungal isolates are entomogenous to *E. saccharina*. However, only *A. flavus and Verticillium albo-atrum* produced mortalities that were significant enough for them to be considered pathogenic to *E. saccharina* and can therefore by employed for biological control of stemborers. The other species, *Trichothecium sp., F. oxysporum* and *A. brassicicola* are not pathogenic to *E. saccharina*.

It can also be concluded that, *A. flavus* is more pathogenic to *E. saccharina* than *V. albo-atrum* and it also took a shorter time for *A. flavus* to produce mortality in 50% of the population. *A. flavus* also requires a lesser dose to produce 50% mortality.

All the fungal species also demonstrated that pathogenicity is influenced by dose and therefore higher doses might produce higher mortalities.

Finally, fungi can be present on insects and interact with the insect in biological relationships other than being pathogenic and therefore not all entomogenous fungi are pathogenic to their insect hosts.

6.1 RECOMMENDATIONS

The outcome of this study suggests that *A. flavus* and *V. albo-atrum* are potential biopesticides against *E. saccharina*. However, further studies should be carried out to test for their potential to control *E. saccharina* on the field since field conditions may be different from laboratory conditions. Specific growth conditions should be provided in further research especially conditions similar to those encountered in the field taking

into consideration the maize growing seasons. More work should also be done using other modes of administration of the fungal spores other than liquid formulations.

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APPENDIX 1

Statistical analysis

ANOVA of the % mortality of the five fungal species (LT_{50})

ANOVA

No. of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	211.667	5	42.333	9.407	.008
Within Groups	27.000	6	4.500		
Total	238.667	11			

Multiple Comparisons (LSD)

Dependent Variable: No. of dead larvae
LSD

(I) Isolates	(J) Isolates	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Asp	Ver	2.50000	2.12132	.283	-2.6907	7.6907
	Tri	7.00000(*)	2.12132	.016	1.8093	12.1907
	Fus	8.00000(*)	2.12132	.009	2.8093	13.1907
	Alt	11.50000(*)	2.12132	.002	6.3093	16.6907
	Con	11.00000(*)	2.12132	.002	5.8093	16.1907
Ver	Asp	-2.50000	2.12132	.283	-7.6907	2.6907
	Tri	4.50000	2.12132	.078	-.6907	9.6907
	Fus	5.50000(*)	2.12132	.041	.3093	10.6907
	Alt	9.00000(*)	2.12132	.005	3.8093	14.1907
	Con	8.50000(*)	2.12132	.007	3.3093	13.6907
Tri	Asp	-7.00000(*)	2.12132	.016	-12.1907	-1.8093
	Ver	-4.50000	2.12132	.078	-9.6907	.6907
	Fus	1.00000	2.12132	.654	-4.1907	6.1907
	Alt	4.50000	2.12132	.078	-.6907	9.6907
	Con	4.00000	2.12132	.108	-1.1907	9.1907
Fus	Asp	-8.00000(*)	2.12132	.009	-13.1907	-2.8093
	Ver	-5.50000(*)	2.12132	.041	-10.6907	-.3093
	Tri	-1.00000	2.12132	.654	-6.1907	4.1907
	Alt	3.50000	2.12132	.150	-1.6907	8.6907
	Con	3.00000	2.12132	.207	-2.1907	8.1907
Alt	Asp	-11.50000(*)	2.12132	.002	-16.6907	-6.3093
	Ver	-9.00000(*)	2.12132	.005	-14.1907	-3.8093
	Tri	-4.50000	2.12132	.078	-9.6907	.6907
	Fus	-3.50000	2.12132	.150	-8.6907	1.6907
	Con	-.50000	2.12132	.822	-5.6907	4.6907
Con	Asp	-11.00000(*)	2.12132	.002	-16.1907	-5.8093
	Ver	-8.50000(*)	2.12132	.007	-13.6907	-3.3093
	Tri	-4.00000	2.12132	.108	-9.1907	1.1907
	Fus	-3.00000	2.12132	.207	-8.1907	2.1907
	Alt	.50000	2.12132	.822	-4.6907	5.6907

* The mean difference is significant at the .05 level.

LC_{50}

ANOVA of mortality produced by different concentrations of *A. flavus*

ANOVA

no. of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	148.500	3	49.500	28.286	.004
Within Groups	7.000	4	1.750		
Total	155.500	7			

Multiple Comparisons

Dependent Variable: no. of dead larvae

LSD

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5	500	-4.50000(*)	1.32288	.027	-8.1729	-.8271
	50000	-10.00000(*)	1.32288	.002	-13.6729	-6.3271
	5000000	-10.50000(*)	1.32288	.001	-14.1729	-6.8271
500	5	4.50000(*)	1.32288	.027	.8271	8.1729
	50000	-5.50000(*)	1.32288	.014	-9.1729	-1.8271
	5000000	-6.00000(*)	1.32288	.011	-9.6729	-2.3271
50000	5	10.00000(*)	1.32288	.002	6.3271	13.6729
	500	5.50000(*)	1.32288	.014	1.8271	9.1729
	5000000	-.50000	1.32288	.725	-4.1729	3.1729
5000000	5	10.50000(*)	1.32288	.001	6.8271	14.1729
	500	6.00000(*)	1.32288	.011	2.3271	9.6729
	50000	.50000	1.32288	.725	-3.1729	4.1729

* The mean difference is significant at the .05 level.

ANOVA of mortality produced by different concentrations of *Verticillium spp.*

ANOVA

No of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106.375	3	35.458	18.911	.008
Within Groups	7.500	4	1.875		
Total	113.875	7			

Multiple Comparisons

Dependent Variable: No of dead larvae
LSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5	500	-4.00000(*)	1.36931	.043	-7.8018	-.1982
	50000	-6.50000(*)	1.36931	.009	-10.3018	-2.6982
	5000000	-10.00000(*)	1.36931	.002	-13.8018	-6.1982
500	5	4.00000(*)	1.36931	.043	.1982	7.8018
	50000	-2.50000	1.36931	.142	-6.3018	1.3018
	5000000	-6.00000(*)	1.36931	.012	-9.8018	-2.1982
50000	5	6.50000(*)	1.36931	.009	2.6982	10.3018
	500	2.50000	1.36931	.142	-1.3018	6.3018
	5000000	-3.50000	1.36931	.063	-7.3018	.3018
5000000	5	10.00000(*)	1.36931	.002	6.1982	13.8018
	500	6.00000(*)	1.36931	.012	2.1982	9.8018
	50000	3.50000	1.36931	.063	-.3018	7.3018

* The mean difference is significant at the .05 level.

ANOVA of mortality produced by different concentrations of *Trichothecium spp.*

ANOVA

No of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.375	3	18.792	4.063	.105
Within Groups	18.500	4	4.625		
Total	74.875	7			

Multiple Comparisons

Dependent Variable: No of dead larvae

LSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5	500	-1.50000	2.15058	.524	-7.4710	4.4710
	50000	-4.00000	2.15058	.136	-9.9710	1.9710
	5000000	-7.00000(*)	2.15058	.031	-12.9710	-1.0290
500	5	1.50000	2.15058	.524	-4.4710	7.4710
	50000	-2.50000	2.15058	.310	-8.4710	3.4710
	5000000	-5.50000	2.15058	.063	-11.4710	.4710
50000	5	4.00000	2.15058	.136	-1.9710	9.9710
	500	2.50000	2.15058	.310	-3.4710	8.4710
	5000000	-3.00000	2.15058	.235	-8.9710	2.9710
5000000	5	7.00000(*)	2.15058	.031	1.0290	12.9710
	500	5.50000	2.15058	.063	-.4710	11.4710
	50000	3.00000	2.15058	.235	-2.9710	8.9710

* The mean difference is significant at the .05 level.

ANOVA of mortality produced by different concentrations of *F. oxysporum*

ANOVA

No of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.000	3	25.000	4.348	.095
Within Groups	23.000	4	5.750		
Total	98.000	7			

Multiple Comparisons

Dependent Variable: No of dead larvae
LSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5	500	-3.50000	2.39792	.218	-10.1577	3.1577
	50000	-6.50000	2.39792	.053	-13.1577	.1577
	5000000	-8.00000(*)	2.39792	.029	-14.6577	-1.3423
500	5	3.50000	2.39792	.218	-3.1577	10.1577
	50000	-3.00000	2.39792	.279	-9.6577	3.6577
	5000000	-4.50000	2.39792	.134	-11.1577	2.1577
50000	5	6.50000	2.39792	.053	-.1577	13.1577
	500	3.00000	2.39792	.279	-3.6577	9.6577
	5000000	-1.50000	2.39792	.566	-8.1577	5.1577
5000000	5	8.00000(*)	2.39792	.029	1.3423	14.6577
	500	4.50000	2.39792	.134	-2.1577	11.1577
	50000	1.50000	2.39792	.566	-5.1577	8.1577

* The mean difference is significant at the .05 level.

ANOVA of mortality produced by different concentrations of *A. brassicicola*

ANOVA

No of dead larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53.000	3	17.667	14.133	.014
Within Groups	5.000	4	1.250		
Total	58.000	7			

Multiple Comparisons

Dependent Variable: No of dead larvae
LSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5	500	-.50000	1.11803	.678	-3.6042	2.6042
	50000	-3.00000	1.11803	.055	-6.1042	.1042
	5000000	-6.50000(*)	1.11803	.004	-9.6042	-3.3958
500	5	.50000	1.11803	.678	-2.6042	3.6042
	50000	-2.50000	1.11803	.089	-5.6042	.6042
	5000000	-6.00000(*)	1.11803	.006	-9.1042	-2.8958
50000	5	3.00000	1.11803	.055	-.1042	6.1042
	500	2.50000	1.11803	.089	-.6042	5.6042
	5000000	-3.50000(*)	1.11803	.035	-6.6042	-.3958
5000000	5	6.50000(*)	1.11803	.004	3.3958	9.6042
	500	6.00000(*)	1.11803	.006	2.8958	9.1042
	50000	3.50000(*)	1.11803	.035	.3958	6.6042

* The mean difference is significant at the .05 level.

Correlations

A. flavus

Correlations(a)

		concentration	number of dead larave
concentration	Pearson Correlation	1	.576
	Sig. (2-tailed)		.424
number of dead larave	Pearson Correlation	.576	1
	Sig. (2-tailed)	.424	

a Listwise N=4

Verticillium spp.

Correlations

		no of dead larvae	concentration
no of dead larvae	Pearson Correlation	1	.777
	Sig. (2-tailed)		.223
	Sum of Squares and Cross-products	53.188	24443161.875
	Covariance	17.729	8147720.625
	N	4	4
concentration	Pearson Correlation	.777	1
	Sig. (2-tailed)	.223	
	Sum of Squares and Cross-products	24443161.875	18625600061268.750
	Covariance	8147720.625	6208533353756.250
	N	4	4

Trichothecium spp

Correlations

		no of dead larvae	concentration
no of dead larvae	Pearson Correlation	1	.847
	Sig. (2-tailed)		.153
	Sum of Squares and Cross-products	28.188	19417921.875
	Covariance	9.396	6472640.625
	N	4	4
concentration	Pearson Correlation	.847	1
	Sig. (2-tailed)	.153	
	Sum of Squares and Cross-products	19417921.875	18625600061268.750
	Covariance	6472640.625	6208533353756.250
	N	4	4

F. oxysporum

Correlations

		no of dead larvae	concentration
no of dead larvae	Pearson Correlation	1	.666
	Sig. (2-tailed)		.334
	Sum of Squares and Cross-products	37.500	17599477.500
	Covariance	12.500	5866492.500
	N	4	4
concentration	Pearson Correlation	.666	1
	Sig. (2-tailed)	.334	
	Sum of Squares and Cross-products	17599477.500	18625600061268.750
	Covariance	5866492.500	6208533353756.250
	N	4	4

A. brassicicola

Correlations

		no of dead larvae	concentration
no of dead larvae	Pearson Correlation	1	.901
	Sig. (2-tailed)		.099
	Sum of Squares and Cross-products	26.500	20023987.500
	Covariance	8.833	6674662.500
	N	4	4
concentration	Pearson Correlation	.901	1
	Sig. (2-tailed)	.099	
	Sum of Squares and Cross-products	20023987.500	18625600061268.750
	Covariance	6674662.500	6208533353756.250
	N	4	4

APPENDIX 2

Table 8: Mean daily larval mortality.

	Mean number of dead larvae					
Day	*A. flavus*	*V.albo-atrum*	*Trichothecium sp.*	*F. oxysporum*	*A. brassicicola*	Control
1	0	0	0	0	0	0
2	0	1.5	0.5	0	0	0
3	1.5	2.5	0	0	0	0
4	3	2	2	1	0	0
5	1.5	2	1	2	1.5	2
6	4	0.5	1	1	1	3
7	2	1	2	1.5	2	0
8	1	3	2	2	0	0
9	0	0	0	0.5	0	0
10	1	0	2	0	0	0
11	1	0	1	0	0	0
12	1	1	0	0	0	0