EVALUATION OF THE POTENTIAL OF NATIVE FUNGAL ISOLATES FOR GREATER (*GALLERIA MELLONELLA*) AND LESSER (*ACHROIA GRISELLA*) WAX MOTHS

BY

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Dedication

I wish to dedicate this work to my beloved parents, Mr. and Mrs. Kifuko, my Uncle John Divo Wancha and to all my brothers and sister. Their encouragement and support has made this work a success.

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TABLE OF CONTENTS

List of Figures

List of Tables

List of Annexes and Appendices

ABSTRACT

The effects of six fungal isolates; DLCO-AA-5, DLCO-AA-14, IITA 18, IMI 330189, DLCO-AA-83 and DLCO-AA-109 were evaluated in the laboratory for their pathogenicity to adults, 5th and 6th larval instars of both the greater wax moth (*Galleria mellonella*) and lesser wax moth (*Achroia grisella*). Conidial dilutions were prepared in 0.5% Tween 80. The adults of both species were treated by spraying while dipping the larvae in varied fungal concentrations ranging from $2x10^4$ to $2x10^7$ conidia/ml. Assessments of larval infection of both the greater and lesser wax moth was made daily for 14 days before entering the pupal stage after treatment. Mortality assessment for the lesser wax moth adults was made daily for 8 days post treatment. Adults of both the greater and lesser wax moths were found to be susceptible to all fungal isolates and concentrations, ranging from $2x10^4$ to $2x10^7$ conidia/ml. Comparison of mortality results in adults of the greater wax moth at $2x10^4$ conidia/ml revealed that infection of over 90% could be achieved in 13 days after inoculation. Similar comparison with the adults of the lesser wax moth showed that over 78% mortality could be achieved by day 8 after treatment except isolate IMI33089 that caused low mortality of 67.05%. Comparison of mortality results of the 5th larval instars of both species of wax moth at $2x10^7$ conidia/ml showed varying degrees of virulence among the fungal isolates. With the 5th larval instars of the greater wax moth, isolate IITA 18 caused percentage mortality of 68.52% while with isolates DLCO-AA-5 and DLCO-AA-14 a cumulative percentage mortality of 61.11% was recorded in the lesser wax moth by day 14 of post treatment. Low mortality in the 5th larval instars of both species was recorded with isolate IMI 330189. With the greater wax moths 5th larval instars, mortality in the control was less than 2 % while in the lesser it was less than 4%. Results on percentage emergence of the adults from pupae of the greater wax moth adults showed no significant difference ($P > 0.05$) between the different fungal isolates and controls. With the lesser wax moth a slight difference in emergence of adults from pupae was observed between $2x10^7$ conidia/ml and the controls. With all fungal isolates and concentrations used, percentage emergence of over 86.1% was observed in the greater and lesser adults. This showed that, the isolates at the different fungal concentrations had no effect on adult emergence when applied on the 6th instars. Laboratory experiments were also conducted to investigate host specificity of two isolates of *Metarhizium* spp (DLCO-AA-83 and IMI 330189) and 1 *Beauveria* spp (IITA 18) all deuteromycete

entomopathogenic fungi that were tested as entomopathogenic agents against the honeybees (*Apis mellifera* (Ethiopian race). Each isolate was applied topically by spraying 10ml of $2x10^7$ conidia/ml into the beehives. Isolate IITA 18 caused 0.5% mortality of the treated surface sterilized honeybees, isolate DLCO-AA-83 resulted in 1% mortality of the treated surface sterilized honeybees and isolate IMI 330189 had no effect on the honeybees. In cases were infection with the applied fungal isolates occurred, fungal growth on the cadavers was observed after surface sterilization. Therefore, the present study confirm the pathogenicity of all the tested isolates against the adults of the greater and lesser wax moths and 4 of these isolates in i.e. isolates in degree of virulence. Isolates IITA 18, DLCO-AA-14, DDLCO-AA-5 and DLCO-AA-83 for the greater wax moth while isolates DLCO-AA-5, DLCO-AA-14 & DLCO-AA-83 and isolate IITA 18 for the lesser 5^{th} larval instars as potential microbial control agents for the management of the greater and lesser wax moths.

INTRODUCTION

The order Lepidoptera contains some of the most damaging pests known to man among which are one or both of the wax moths; the greater, *Galleria mellonella* L. and the lesser, *Achroia grisella* (Fabr.). These two species of wax moth occur naturally or have been introduced by man in almost all regions of the world where apiculture is practiced. Sheltered environment of a hive or a cavity occupied by colonies of bees may be utilized by a variety of insects and other organisms, of which some are parasitic and harmful, while others are harmless. Perhaps, among a wide variety of pests and predators, which may attack, the apiaries or individual hives resulting in a significant degree of damage is the wax moth (FAO, 1987; Desalegn Begna, 2001).

The larvae mainly eat and destroy beeswax combs where the bees can store pollen, honey and lay eggs for their normal activities. The larvae of these moths form a silken feeding tunnel, which enlarge along the mid-rib of the comb or make borings through the thin wax caps of honey cells causing honey to leak out. When infestation is severe, silken tunnels with caterpillars wriggling in them are noticed and eventually the whole comb is a mass of webbing's in which excreta of caterpillars are enmeshed. This leads to cessation of further brood rearing, fieldwork is virtually suspended and the colony deserts leaving it to intruders. Frequently, the larvae cement the cocoons inside a cavity in the wood. Chewed frames are weakened and easily broken. Adults of the wax moth can also transfer pathogens of serious diseases like foulbrood as they move from hive to hive (Tompkins and Griffith, 1977; Singh, 1982; Crane, 1990; Charriere and Imdorf, 1999; MAAREC, 2000).

In Ethiopia, both the greater and lesser wax moths are widely distributed throughout the country and are believed to be the most troublesome of the pests of honeybees though their economic injury level has not yet been estimated (Ayalew Kasaye, 1983). Reports of these wax moths being the most serious and threatening pests are taken to Holleta Bee Research Centre by beekeepers and different institutions engaged in the field (Desalegn Begna, 2001). Distribution of these two species of wax moths varies i.e. the lesser is predominant in mid-highland areas like Holleta (Suba) and Gedo, the greater at lowland areas like Nazareth, Bahir-Dar, Awasa, and Bako. In some places both species are found (Desalegn Begna, 2001).

A survey conducted in south and southwest parts of Ethiopia in areas of Asossa, Begi, Anffilo, Gambella, Tepi, Bebeka, Negele, Moyale, Mega, Yabello, Tetele, DoloAdo, Segen, Konso, Keyi Afer, and Jinka, shows existence of these two species of wax moths (Desalegn Begna and Amsalu Bezabeh, 2001).

Williams (1978) estimated the annual economic loss to beekeepers who have capital invested in framed combs built on comb foundation in the USA as US $500,000 and anticipated the damage to be more in hotter parts of the world. The combs are vulnerable to wax moths when in store as well as parts of the hives not occupied by bees. Nevertheless, in temperate zones, storage of framed combs from one active season to the next, away from the hive, is usually cost effective. In the tropics such stored combs are likely to be destroyed by wax moths (Crane, 1990).

Entomopathogenic fungi have the potential as viable control agents for many insect pests (Reinecke, 1990). The entomopathogenic fungi, particularly *Metarhizium* and *Beauveria* in the class *Deuteromycetes* are attractive as biopesticides for use in Integrated Pest Management (IPM) systems because they combine host specificity with proven safety (Bateman *et al.*, 1993).

Current information on the effect of fungal pathogens on insects indicates that these occur via a series of integrated systematic events progressing from spore attachment to germination, penetration, growth and proliferation within the body of the host, interaction with the insects defense mechanisms and finally re-emergence on the cadaver. The interaction that occurs between the fungus and insect are exceedingly complex and are dependent upon specific host-pathogen interactions (Hajek and Leger, 1994; Hegedus and Khachatourians, 1996).

The increasing problem of resistance to conventional pesticides, plus environmental and public safety concerns emphasize the need to continue isolating and testing microbial organisms to develop a long term management for the wax moths. Thus, this study tested the pathogenicity of the standard isolates of *Metarhizium anisopliae* var. *acridium* (IMI 330189) and *Beauveria* (IITA 18). Two of each of the local isolates of the same types of fungi against the adults, fifth and sixth larval instars of both the greater and lesser wax moths were also used for comparison.

Objectives of the present study included:

- To compare the efficacy of native isolates of *Metarhizium* and *Beauveria* against adults, fifth larval and sixth larval instars and lesser of the greater wax moths with known entomopathogenic strains.
- To test the local isolates for the pathogenicity against adults, fifth larval and sixth larval instars and lesser of the greater wax moths.
- To compare the susceptibility levels of the adults, fifth and sixth larval instars of the greater and lesser wax moths to the fungal isolates.
- To test possible effect of three fungal isolates tested on the wax moth on the honeybee colony.

2. LITERATURE REVIEW

2.1 Distribution and biology of the wax moths

There are a number of wax moths that attack honey combs i.e. Greater wax moth (*Galleria mellonella*); Lesser wax moth (*Achroia grisella*); the Mediterranean flour moth (*Ephestia kuehniella*); the fig moth (*E. cautella*); Fruit (pollen) moth (*Vitula edmansii*) and (*Vitula scrratilineclla*); the Indian meal moth (*Plodia interpunctella*); death's head or hawk moths (*Acherontia atropos*) and moth from bumble-bee nests (*Aphomia sociella*). All these moths may present an especially serious problem in the tropics and subtropics where the warm climate favour their rapid development, but the greater wax moth, in particular, is a major pest in all regions (Singh, 1982; Crane, 1990).

2.1.1 Greater wax moth

2.1.1.1 Distribution

Wax moths are thought to originate from Asia and now inhabit much of the world today. They are found in most of the honeybee colonies worldwide. Their geographical distribution corresponds with that of the honeybee. Distribution of the greater wax moth is limited by its inability to withstand prolonged period of cold. This explains why the greater wax moth problems are less acute in higher altitude locations or do not occur at all (Charriere and Imdorf, 1999).

2.1.1.2 Biology

The greater wax moth (*Galleria mellonella*) (Lepidoptera: Pyralidae) undergoes complete metamorphosis. The adult is pale brown, quite large and about 3/4 inches long. It has a wingspread of $1^{1}/_{4}$ inches. The males are slightly smaller than the females and may be

differentiated from them by the shape of the outer margin of the forewings, which has a semi lunar notch in males, but smooth in females. The wings fold roof like over the body; wing scales and body are non- descript grayish brown. Adults emerging from pupae in the hive leave it and expand their wings, and then soon after dark, they fly to trees and mate. Mated females enter the hives when the bees are no longer active and each lay about 500 eggs in batches. The eggs are small, white, and slightly oblong with the greatest diameter of less than $1/50^{th}$ of an inch. They are inserted in crevices or cracks between hive parts out of the way places like between supers, between hive body and bottom of board, or between the top super and cover and occasionally on open surfaces (Burges, 1978; Singh, 1982; MAAREC, 2000).

The eggs hatch into larval stage within 5-8 days. When fully grown, the larvae (caterpillars) are $3/4^{th}$ of an inch. They have a reddish brown hard head capsule with chewing mouthparts and four tinny simple eyes (stemmata) one each side; three pairs of small segmented legs occur on the thorax and 5 pairs of fleshy prolegs on abdominal segments. The upper surface is gray in color while the lower surface is nearly white. There are no conspicuous hairs on the body (MAAREC, 2000).

Development from egg laying to emergence of adult from pupae takes at least one month at the most favorable temperature of $35\ ^0$C but the period is protracted in temperate climate, often limiting the pest to one generation a year (Burges, 1978). Overwintering can take place as egg, larva or pupa (Charriere and Imdorf, 1999). The incubation period of the eggs varies from a week to 18 days depending on temperature. The eggs cannot withstand temperatures below 15.6^0C and above 35^0C. Males of the greater wax moths are known to live for about three to four weeks. The female starts depositing eggs 4-10 days after she emerges from the cocoon; she continues depositing as long as her vitality lasts and die within a week after

laying eggs (Tompkins and Griffith, 1977; Singh, 1982).

When fully grown, the wax moth larva spins a rough silken cocoon, within which the larva changes to pupa. Frequently, the larva cements the cocoon inside a boat-shaped cavity chewed into the wood. When making their cocoons, they seem to relish woody fibre for they often eat in the wood of the frames or of the hives in which they are allowed to pupate. They usually pile their cocoon one on the other, or join them firmly together in long rows. Within the cocoon, the larva changes to pupa and overwinters in the pupal stage. Under warm conditions, adults may emerge at almost any time of the year (Tompkins and Griffith, 1977; MAAREC, 2000).

2.1.2 The lesser wax moth

2.1.2.1 Distribution

On the contrary, the lesser wax moth has a temperate geographical distribution i.e. it is noticed at a comparatively higher altitude than where the greater wax moth is found (Charriere and Imdorf, 1999).

2.1.2.2 Biology

The lesser wax moth (*Achroia grisella*) (Lepidoptera: Pyralidae) also undergoes complete metamorphosis. The adults, larvae and pupae of this moth are smaller than those of the greater wax moth. The female moth lays from 300 to 500 eggs in its eight-day long life. The larvae are about 16-20mm much smaller than that of the greater wax moths. They usually have all the three pairs of jointed thoracic legs, and a series of five pairs of abdominal prolegs bearing series of small hooks (crochets) on their walking surfaces. The prolegs are located on abdominal segments 3-6 and 10, though these may be variously modified or lost particularly

the most anterior ones: a very few groups have prolegs on other abdominal segments. The egg stage lasts from two to four days, larval from 34 to 48 days, pupal from 5 to 12 days, and the adults live for about a week (Crane, 1990)

2.2 Damage and losses caused by the wax moths

The newly hatched larvae of the wax moths run aimlessly for some hours and become distributed around the colony. They eat away combs or damage them by making silken feeding tunnels (galleries) with their side tunnels near the midribs. After several days, the tunnels enlarge and lengthen as the larva grows (Tompkins and Griffith, 1977; Burges, 1978; Singh, 1982; Crane, 1990). The first indication of entry of the larva in the comb is the presence of small masses of minute particles of wax outside the comb holes. Later, faint webbings are observable over some cells of the comb. When infestations have progressed far enough, silken tunnels with caterpillars wriggling in them are noticed and eventually the whole comb is a mass of webbings in which the excreta of the caterpillars are enmeshed (Tompkins and Griffith, 1977).

Caterpillars of the lesser wax moth are more like scavengers as they are usually found in debris. They also attack stored combs and usually feed on the outer surface. Larvae of the lesser inflict damage similar to that of the greater wax moth larvae, but the tunnels are smaller, the webs finer and webbings are more confined to the outer surface of the comb. The webbings are interspersed with dark fecal pellets (Singh, 1982).

In capped honey, young larvae make small rather inconspicuous tunnels and borings through the wax caps on the honey cells just below the cappings. Eggs are probably laid on the comb or section boxes before the comb honey supers are removed from the hives, but the damage

does not become evident until some time after the honey has been placed in storage. This cause harvested honey to leak from the packages and makes the affected sections less attractive and unmarketable (Tompkins and Griffith, 1977; Morse, 1994; MAAREC, 2000).

Adults of wax moths cause no damage because their mouthparts are suctorial. However, adult wax moths can transfer pathogens of serious diseases (e.g. foulbrood), since they move from hive to hive in colonies infested with foulbrood, the feces of wax moths contain large amounts of Paenibacillus spores (Charriere and Imdorf, 1999; MAAREC, 2000).

Wax moths fly mainly at night, during daylight they rest in dark places. Adults often walk fast before they take flight when disturbed. Female adults have acute sensory capability to find and exploit wax. Males and females mate within a day of their emergence and the female enters a hive usually at night but occasionally during the day in the case of weak colonies (Singh, 1982). The female easily finds and gains entry even to the strongest bee colony beehives to lay eggs in clusters, one or two days after mating. This is because a component of the female sex pheromone Nonanal is also found in beeswax and may help explain how wax moths find beeswax for oviposition (MAAREC, 2000).

For both the greater and lesser wax moths, in severe cases of infestation, further brood rearing by honeybee is stopped, fieldwork is virtually suspended and the colony deserts its home, to seek fortune elsewhere, leaving it to intruders. It is in the stored honey combs or weakened or died out colonies that their number explodes. This is evidenced by presence of all the four stages; tunnels of silk through out the combs, especially near the mid ribs; deposits of dark fecal matter since the larva of wax moth feeds on combs and reduce them into a pile of debris that falls to the hive floor; cocoons stuck to the frames every where disintegrating comb structure (Tompkins and Griffith, 1977; MAAREC, 2000).

2.3 Control measures of the wax moths

Many attempts have been made to control the wax moths but these are available for combs and other equipment not occupied by bees (Ali, 1974; IBRA, 1978; Seegeren *et al.*, 1996; Holleta Bee Research Centre, 1999; Desalegn Bagne, 2001).

2.3.1 Management practices

These prevent infestation in the hive, apiary, and in comb storage. The practices include: ensuring that all colonies are strong, healthy, replacing floorboard yearly, and sterilizing those removed, reducing hive entrance to widths that can be guarded by bees, removing and destroying all debris from the bottom board of the hive that is assumed to attract the moths; paying special attention to preventing infestation of any hive containing colonies that are weak or have just died, replacing supers and placing them in well aerated area the dearth period and control the factors (disease, pests, predators and pesticides) that might substantially weaken the colony (Singh, 1982; Crane, 1990; MAAREC, 2000).

2.3.2 Physical methods

Involves exposure to heat or cold, irradiation and exposure to light but are used in the absence of bees. Temperature extremes can be used to control this pest because the growth and development of the wax moths depend on temperature. During cooling, infested combs are put in the refrigerator at 0^0C for 4 hours. At 15^0C, all large larvae survived 8 weeks with virtually no increase in size (Burges, 1978). Killing by freezing when there is a large mass of greater wax moth protected deep inside hive bodies and insulated with webbing may be difficult (Heather, 2001). Heating is used for empty hives and kills all stages of *Galleria* in 24

hours at 50% RH and 60^0C (Cantwell and Matthenius, 1973).

Irradiation is a more advanced physical method using gamma rays. All stages of the wax moths are killed by moderate exposure, or possibly to sterilize the males so that mated females produce no offspring or induce genetic defect, which would for instance, reduce egg viability (Bee World, 1984). Exposure to light is effective in some conditions i.e. combs are stored in full light in an out building, enclosed so that there is ample ventilation (Crane, 1990).

2.3.3 Chemical methods

Many chemicals have been applied to vacant combs or apiary structures and to stored combs. Fumigant gasses are satisfactory as they can penetrate into crevices in structures and through out the combs, and be completely removed afterwards by airing (Burges, 1978). A number of fumigants like Phostoxin pellets can be used: newly hatched eggs are more resistant than any other stages of the life cycle, Ethylene dibromide: kills all stages of the greater wax moth in 24 hours. Sulphur dioxide is ineffective against eggs, Paradichlorobenzene (PDCB) kills adults and immature stages, but not the eggs. Carbondioxide can be used to destroy all stages of the wax moth on comb honey or stored combs (Tompkins and Griffith, 1977; Charriere and Imdorf, 1999; MAAREC, 1999). Some contact insecticides like cidial 0.008%, endosuifan 0.002%, endosulfan with fenitrothion 0.001%, toxaphene 0.004% trichlorphon 0.008%; can also be used (Ali, 1974).

2.3.4 Biological control

Galleria mellonella is attacked by a number of parasitic hymenoptera for instance;

Trichogramma, which is a tiny stingless parasitic wasp that lays its eggs in a greater wax moth egg and as a result a wasp emerges instead of a moth. This wasp can only control the egg stage of the moth (Burges, 1978; Bollhalder, 1999). *Didrachys cavus* (Pteromalidae) belonging to a group of wasps called the Chalcid wasps parasitizes the cocoons of the wax moths. After the eggs hatch, the wasp larvae feed on the immature wax moth, leaving it when the larval stage is complete. These wasps pupate within the wax moth cocoons, not on the out side. The wasps as adults, chew through the wax moth cocoon (Gauld and Bolton, 1988).

Other wasp species, *Bracon hebtor*, *Venturia canescens* and *Apanteles galleriae* target the larval stages of wax moths .The wasps inject their eggs into the caterpillars through the ovipositor and the wasp eggs hatch inside the caterpillar. Larvae of the wasps upon emergence feed on inside parts of the caterpillar, eating it alive. They do not cause death too soon to the caterpillars because the larval wasps might also die before completing their developmental stages. They then ooze through the skin in different places. Eventually, each larva spins a cocoon, which is attached to the caterpillar. Once wasps finish pupating; they emerge from their cocoons and search for other caterpillars (Bambara and Ambrose, 1980).

2.4 Microbial insect control

Entomopathogens (i.e. viruses, bacteria, rickettsia, fungi, protozoans and nematodes) are commonly isolated from their insect hosts. Their natural occurrence in insect populations contributes to regulation of injurious pests of humans and their crops, households and domestic animals. Entomopathogens have potential for microbial control programs and many of them have been exploited for pest control through inoculative, inudative and augmentative releases (Lacey and Goettel, 1995). Spectacular success has been reported from some entomopathogens as classical biological control agents (Zelazny *et al.*, 1992;

Bedding, 1993; Hajek *et al.*, 1996) but their inudative use for suppression of pest insect is much more common.

In the past few decades, there has been growing interest in the use of pathogens as biological control agents for insect pests (Anderson, 1982; Hall and Papierok, 1982). This trend has in part been stimulated by realization that pesticides are not the panacea that were once said to be (Anderson, 1982). Although chemical control has been efficacious, the drawbacks: - pesticide resistance; resurgence of target organism and emergence of secondary pests to pest status because of destruction of parasitoids and predators; impact on non target organisms, including humans; environmental pollution through the accumulation of pesticides in the soil, water and air; and the residues on agricultural products and animals, have necessitated the development of more selective control measures compatible with the environment. Moreover, legislation now and in future will limit or eliminate many chemical pesticides because of the aforementioned drawbacks.

Insect pathogens overcome many of the problems of chemical pesticides, but they are not extensively used despite of their positive features (Falcon, 1985). The key reason for their lack of use varies, but one key for greater acceptance of these biological control agents is education. People have been conditioned to the "quick kill" of chemical pesticides and expect similar results with pathogens.

Microbial pathogens play a vital role in the natural regulation of many arthropod populations in agricultural systems. Their importance has been under emphasized compared to parasitoids and predators because of less specialist work on pathogens and they are less noticeable than arthropod natural enemies are. Because of the disturbed nature of the

agroecosystem and the need for rapid control of the arthropod pests in crops, many of the entomologists are of the opinion that naturally occurring pathogens have a limited value in Integrated Pest Management (IPM). This assumption is inaccurate. Ecologists have become increasingly aware of the role microorganisms play in population regulation. May (1988) argues that the significance of pathogens has been under estimated in ecology.

A dozen of microbial control agents have been identified in the last few years of which the principal groups include viruses, bacteria, fungus and protozoa (Burges, 1981; Federici 1990; Charnley, 1991). To date about 100 insect pathogenic species of bacteria (Luthy, 1986a cited in Payne, 1988), more than 1600 disease-causing virus isolates (Mortignoni and Iwai, 1986) and about 400 species of entomopathogenic fungi (Hall and Papierok, 1982) have been identified and documented.

Despite the success of the past, there is a continuing need to discover and develop new entomopathogens if we are to meet the future needs of increased food and fiber production with concomitant reduction in use of chemical pesticides. Sustainable agriculture in the 21st century will relay increasingly on microbial control and other interventions for pest management that are environmentally friendly (Lacey and Goettel, 1995). There is plenty of evidence that microbial pesticides can effectively control insect pests as long as the biology of the pathogen is taken into account (Payne, 1988).

2.4.1 Bacteria and viruses as biological control agents of wax moths

When the wax moth ingests minute quantities of *Bacillus thuringiensis* spores, the conidia germinate and multiply to cause lethal infection. *B. thuringiensis* is effective against the

greater wax moth, but its use in controlling the wax moths diminishes after one year and this makes it uneconomical. Moth larvae are most susceptible when small. The relatively less important pest of comb, the lesser wax moth *Achroia grisella* is resistant to *B. thuringiensis* and not adequately controlled (Burges, 1978).

The Nucleopolyhedrosis Virus (NPV) of *Galleria mellonella* is protected in the proteinaceous inclusion bodies of the same order of size as bacterial spores. It is also incorporated into the foundation as *B. thuringiensis*. NPV is lethal to wax moth larvae (Martignon and Iwai, 1986).

2.4.2 Entomopathogenic fungi as microbial control agents

Fungal diseases of insects have been known since 1934 when the Italian, Agostino Bassi showed the fungal nature of white muscardine disease of silk worms (Hall and Papierok1982). At present about 700 species of fungi in 90 genera are recognized to cause infection among insects (Goettel, 1992). These fungi vary widely in ease of mass production, host range, epizootic potential, temperature and moisture optima etc. and include a wide spectrum of organisms with potential for development as pest control agents. Nevertheless, only a few fungi are currently being developed for pest control.

According to Ferron (1978), entomopathogenic fungi do not occupy definite systematic places, but are distributed among all groups from Phycomycetes to Fungi Imperfecti, Ascomycetes and Basidiomycetes. Majority of entomopathogenic fungi belong to the group Deuteromycetes and Entomophthorales (Samson, 1981) and according to Ferron (1978), there are two most important groups when compared with the rest of entomopathogenic fungi. Class Deuteromycetes alone contains a bout 150 species of insect pathogens (Samson, 1981).

Metarhizium and *Beauveria* species are best known genera in class Deuteromycetes because of their wide geographical distribution and host range including many insects of economic importance (Hall and Papierok, 1982). According to De Hoog (1972), the genus *Beauveria* contains two insect pathogenic species i.e. *B. bassiana* and *B. brongniartii* (*B. tenella*). *Beauveria* species have been identified from a bout 500 host species, principally Lepidoptera and Coleoptera (Hall and Papierok, 1982). Collection of *Beauveria* infested insects from tropical ecosystem, have revealed a considerable variation between isolates that have made species identification difficult (Mugnai *et al.*, 1989). According to Mugnai *et al.* (1989), the following species of *Beauveria* are recognized: *B. alba, B. armoph, B. bassiana, B. bronnaiartii, B. velata, B. vermiconia* and undescribed taxon close to *B. armoph* but morphologically and biochemically distinct.

The genus *Metarhizium* also contains two species, *M. anisopliae* and *M. flavoviride* (Tulloch, 1976). *Metarhizium anisopliae* var. *acridium* (previously referred to as *M. flavoviride* Gams and Rozsypal), is a pathogen specific to locusts and grasshoppers (Lomer *et al.*, 1997). *M. anisopliae* is by far the most important species, known to infect more than 200 insects and it has a wide host range than *Beauveria* species. The major insect orders infected by *Metarhizium* and *Beauveria* spp are Coleoptera, Lepidoptera, Orthoptera, and Hemiptera (Hall and Papierok, 1982). *Metarhizium* species consist of two forms characterized by the size of their conidia i.e. *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium anisopliae* var. *major* (Tulloch, 1976; Samson, 1981). According to Payne (1988) individual strains of the same fungal species often have different host ranges or pathogenicities.

Other well studied entomopathogenic Deuteromycetes include *Nomuraea rileyi*, mainly

parasitizing lepidopteran pests (Ignoffo, 1981). The genus *Paecilomycetes*, is a common parasite of many insect orders while *Hirsutella*, and *Verticillium lecanii* are pathogens of aphids, scales and whitefly (Hall, 1982). Entomopathogenic fungi have a strong potential as viable control agents for insect pests (Reinecke, 1990), and have been shown not to infect the African honeybee (Price and Müller, 1994).

2.4.3 Mechanism of fungal infection

The most common route of host invasion is through the external integument, although infection through the digestive tract is possible. Conidia attach to the cuticle, germinate and penetrate the cuticle Infection and disease development by fungus occurs when there is contact between a virulent infectious inoculum and a susceptible insect cuticle in the presence of favorable conditions (Microclimate). Development of mycosis can be divided into three phases: adhesion and germination of the spore on insect cuticle, penetration into the haemocoel and development of the fungus, which generally results in death of the insect (Boucias *et al.,* 1988; Charnley, 1992).

2.4.3.1 Spore adhesion and germination

The adhesion of the spore on to the insect's cuticle may be a passive mechanism and involves mucilagenous material and spore surface structures (McCoy *et al.*, 1988; Samson *et al.*, 1988). The conidia of Entomophthorales for example, *Entomophthora muscae*, are covered with amorphous mucus, and those of certain Deuteromycetes (e.g. *Verticillium lecanii* and *Hirsutella thompsonii*) are slimy and these attach the conidia to the substrate or cuticle. Dry conidia of deuteromycetous fungi have well-organized fascicles of rodlets

(Boucias *et al.,* 1988). In these conidia, the attachment is non-specific and is due to hydrophobicity of the rodlets and insect cuticle. In some other fungi, adhesion is specific but little is known regarding the specificity and adhesion. Electrostatic forces and molecular interaction maybe involved in adhesion (Samson *et al.*, 1988).

Following spore attachment to the host, germination begins forming a germ tube that serves as a penetrant hypha (Hegedus and Khachatourians, 1996). Interaction between the fungus and the insect results in appressorium formation in addition to the germ tube and extrusion of an adhesive mucus layer (Penaland and Boucias, 1984; Brey *et al.*, 1986 Bidochka *et al.*, 1997). Mucilagenous material may coat the germ tube and appressorium.

Spore germination is largely dependent on environmental humidity and temperature, to a lesser extent on light conditions and nutritional environment. There is still little information on the availability of nutrients on the surface of the cuticle (Charnley, 1982). He further indicated that successful germination not only requires the assimilation of utilizable nutrients but also tolerance of any toxic compound present at the cuticular level.

2.4.3.2 Penetration through the integument

Mode of penetration depends on the property of the cuticle, its thickness, sclerotization and the presence of anti fungal and nutritional substances (Charnley, 1984). The epicuticle of the living insect is not inanimate structure and several factors such as microbial antagonists may influence the susceptibility of the host to the fungus species or strains (Hall and Papierok, 1982). For instance, the newly molted larvae and the newly formed pupae are more susceptible to infection than those in which the cuticle is fully hardened (Kurisu and Manabe, 1978).

Studies conducted on *M. anisopliae* conidia showed that low levels of complex nitrogen compounds, surface topography and some catabolites stimulate differentiation into appressorium (Clarkson and Charnley, 1996), then the appressorium penetrates through the host's cuticle and eventually emerges through the insect's haemocoel. Deviation has also been noted in some Entophthorales and *Beauveria bassiana* that can penetrate the host cuticle without appressorium formation (Hall and Papierok, 1982).

Penetration of insect cuticle by germinating spore results from a combination of mechanical pressure by the germ tube and enzymatic degradation of the cuticle (Ferron, 1978 & 1981; Hall and Papierok, 1982; Gillespie *et al.*, 1989). For infection to occur, the fungus must reach the hypodermis without being affected by the moulting process of the insect (Ferron, 1978). *In vitro* studies indicate that the digestion of the integument follows a sequential lipase-protease-chitinase process of digestion (Smith *et al.*, 1981; St. Leger *et al.*, 1986).

Penetration of the fungus may be restricted to certain areas of the host e.g. thorax and abdomen of a pea aphid, *Acyrthosiphon pisum*, by the entomophthorus fungus, *Conidiobolus obscurus* (Brey *et al.*, 1986). In thin integuments as found in homopteran and lepidopteran insects, the germinating hypha (tube) penetrates directly without altering its form (Tomiyama and Aoki, 1982). Many writers report that membranes at joints and between segments are often favored site of penetration by the entomopathogenic fungi (Schable, 1978). At the penetration phase, the thickness and composition of cuticle may influence pathogenicity of the fungus, which prevents penetration of the germ tube (Charnley, 1982).

Fungus can infect insects through the bucal cavity, spiracle or other external openings of an insect. In the alimentary canal, the spores may germinate readily since moisture is not a problem but on the other hand, the digestive juice may destroy the spores or germinating

hypha. In some cases the digestion of fungal structures may cause death by toxicosis rather than by mycosis (Charnley, 1984). Pre-oral transmission has been reported; particularly at the bucal cavity and esophagus. *Metarhizium anisopliae* occasionally infects wireworm larvae through the spiracle and pores of sense organs (McCauley *et al.*, 1986).

The insects' humoral and cellular defense reaction plays an important part in preventing penetration of hyphae through the insect tissue (Hall and Papierok, 1982). However, these can be overcome by fungal toxins, which weaken the multicellular defense mechanism of the host. Many reports indicate that entomopathogenic fungi are known to produce extracellular proteolytic, chitinolytic and lipolytic enzymes *in Vitro* during penetration (Roberts, 1981; Charnley, 1982; Hall and Papierok, 1982).

Generally, penetration of host exoskeleton appears to involve both mechanical and enzymatic components. In soft cuticles e.g. caterpillars growth across the cuticle is more or less direct, in hard cuticles e.g. wireworms the fungus proceeds in a step-wise fashion. The production of cuticle-degrading endoproteases with similar modes of action by all *Deuteromycetes* studied suggests that it is unlikely that they contribute to host specific or virulence, though the common occurrence implies that, an indispensable fungus differ only in charge. However, this does have practical significance, as binding to cuticle with different charge may be favorable or unfavorable to binding by individual enzymes, with consequences for the parts of the body, which can be invaded by enzymatic action. This can influence the speed of penetration and thus virulence (Samson *et al.*, 1988; Goettel *et al.*, 1989 and St. Leger *et al.*, 1991).

2.4.3.3 Invasion of haemocoel and death of the insect

Initial growth of the fungus may be localized around the epidermis at the point of entry but finally the fungus invades the rest of the insect bodies. On entering the haemocoel, penetrant

species of certain entomopathogenic fungi continue filamentous development. Most of the entomopathogenic fungi soon or later produce a variety of structures called hyphal bodies, essentially blastospores that multiply by budding (Bidochka *et al.*, 1997). These circulate in the haemolymh before germinating into mycelium (Charnley, 1982). Inside the haemocoel, the fungus proliferates rapidly and spreads throughout the body by means of the open circulatory system of the insect (Hegedus and Khachatourians, 1996).

Many studies suggest that invasion of the insect cannot be considered successful until the fungus reaches the epidermis, because inoculum may be lost with the exuviate of ecdysis, if the insect moults too soon (Zacharuk, 1973; Vey and Fargues, 1977). After crossing the integument, muscardine disease develops in the haemocoel in presence of cellular defense reaction of the host (Ferron, 1981; Charnley, 1982). At this stage, the fungus produces toxins, which erode the host defense reaction (Hall, 1982). Several toxic cyclodepsipeptidase, desmethyl destruxin from *Metarhizium anisopliae* and beauverin from

Beauveria bassiana have been isolated and recently beauvellide has been identified from *B. brongniartii* (Ferron, 1981). It has been found that lepidopteran larvae injected with destruxins exhibited sustained muscle contraction followed by flaccid paralysis (Charnley, 1992).

Histopathological studies of insect tissues attacked by an entomopathogenic fungus showed that toxins kill the host by inciting progressive dehydration of the host tissues, due to loss of structural integrity of membranes and the dehydration of cells by fluid loss (Ferron, 1981, Charnley, 1982). Dehydration of host tissue may range from limited (Brobyn and Wilding 1977) to extensive (Mohamed *et al.*, 1978). Roberts (1981) suggested that the lower entomopathogenic fungi like *Coleomomyces*, *Legenidium* and *Entomophthora* overcome the

host insect primarily when starved or using up soluble host reserves rather than via toxins. The fungus may avoid the immune defense of an insect by developing protoplasts that are not recognized by the insects' hemocyte population (Latgé *et al.*, 1986), by forming yeast like hyphal bodies that multiply and disperse rapidly (Samson *et al.*, 1988) and by producing mycotoxins.

2.4.3.4 Death of the insect and saprophytic development of the fungus

Death of the insect ends the parasitic development of the fungus (Ferron 1981). The fungus then grows saprophytically in the haemocoel to form a mycelial mass that turns into a hard sclerotium. Reproductive spores are produced within the sclerotium or on the sporophores (sporangiosphore or conidiospore). The sporophores and sterile hyphae emerge from the cadavers under favourable conditions especially of humidity and temperature to form a characteristic mycelial growth on the insects' integument (Hall, 1982) forming a white mass

of fungi. Hyphae that emerge from the cadavers produce aerial spores capable of initiating the cycle once again (Ferron, 1981; Hegedus and Khachatourians, 1996). Under unfavorable conditions, the fungus produces a resistant or resting propagule.

2.4.3.5. Signs and symptoms of mycosis

At an early stage of infection, the insect shows little or no sign except for few necrotic spots, which may develop at the invasion sites. At a later stage of infection, the insect generally becomes restless, less active, their appetites are reduced, and they lose coordination (Ferron, 1981). Infected insects normally move to higher places, or if subterranean, rise to the soil surfaces (McCoy *et al.*, 1988).

Tissue disintegration differs with fungal species, mode of invasion, and host species. The cells and tissues of an infected insect may begin to disintegrate prior to the insects' death (Zacharuk, 1973; Mohamed *et al.*, 1978) or may break down after death .The hyphae continue to grow usually resulting in mummification, and the dead insects retain their form and shape. However, in lepidopteran larvae infeeted with certain Entomopthorales become flaccid with watery contents and fragile integuments.

In many lepidopteran insects infected with *Beauveria bassiana,* the hemolymph protein increases up to the last stage of infection then decreases when the insect ceases feeding prior to death (Gardner *et al.*, 1979). In the silkworm, larva infected with *Beauveria bassiana*, the pH of the hemolymph drops within one day after infection and rises slightly in 2 to 4 days but never higher than that of the uninfected hemolymph (Kusunoki and Watanabe, 1982).

2.5 Host specificity

Host specificity varies considerably; some fungi infect a broad range of hosts (Ferron, 1981), and other are restricted to a few or single insect species. Those with a broad range may consist of a variety of pathotypes (McCoy *et al.*, 1988). *Beauveria bassiana* and *Metarhizium anisopliae* infect over 100 different insect species, but their isolates have a high degree of host specificity (Ferron, 1981). Studies have indicated that host specificity may first be manifested at the level of the host cuticle. Brobyn and Wilding (1977) showed that *Nomuraea fresenii* conidia germinated on the cuticle of a susceptible and resistant aphid host but infected only the former.

Host specificity may be associated with the physiological stage of the host cuticle like insect maturation and host plant (McCoy *et al.*, 1988), properties of the insect's integument and/or

with the nutritional requirement of the fungus (Kerwin and Washino; 1986), and cellular defense (Ferron, 1978).

Similarly, Goettel *et al.* (1990) suggested that the cuticle is of major importance in host specificity, because differences in host specificity are not usually present when the fungus is injected in the host hemocoel. St Leger *et al.* (1991) have shown that enzymes located at the surface of the spore are involved in penetration events. Rath *et al.* (1995) suggested that antigens are the primary factors determining of host specificity.

In general, mechanisms of pathogenicity and specificity appear to be complex and successful penetration can be said to be dependant upon three major factors: the ability of the spore to adhere to the cuticle, to germinate and to penetrate enzymatically (Hall and Papierok, 1982).

2.6 Host defense

Successful virulent entomopathogenic fungi must be able to adhere, penetrate the cuticle and overcome the resistance mechanism of the host inside the haemocoel (Bidochka *et al.,* 1997). The primary barrier to penetration of the fungus is the integument or cuticle (Hall and Papierok, 1982). Phenoloxidase, an enzyme that is localized in the cuticle and haemocoel, has been implicated in wound healing and defense reaction against the pathogen (Bidochka *et al.*, 1989). It has also been found in melanic patches on wounds, cuticle or fungal infection (Bidochka *et al.*, 1989). Melanic granules in the insect cuticle also appear to impede the penetration of *M. anisopliae* (Goettel *et al.*, 1989).

Fungi that successfully cross the integument are challenged by cellular and humoral immune responses (Hall and Papierok, 1982). Cellular defense mechanisms involve cellular

encapsulation and phagocytosis of pathogens. It has been found that the *B*-1, 3-glucan component of the fungal wall is recognized as non-self by the haemocytes and triggers the insect's immune response such as cellular encapsulation (Bidochka *et al.,* 1997).

Despite the insect's cellular defense, the fungi have also evolved evading mechanisms. One is assuming of different morphology in form of blastospores, hyphal bodies or protoplasts that enable the disease to be disseminated to other tissues (Hall and Papierok, 1982). The growth rate of the fungus inside the haemocoel also helps to out compete the insects' immune response (Bidochka *et al.,* 1997). Toxins, destruxins produced by *M. anisopliae* may assist in establishing and extending infection by interfering with the immune response (Clarkson and Charnley, 1996).

Humoral defense reaction does not involve the participation of free cells. It includes haemagglutinins, which play an important role in insect defense reaction by opsonizing the invading fungus (Wheeler *et al.*, 1993). Agglutinins purified from *Melanlophus differentialis* hemolymph have been shown to have the *in vitro* association of *B. bassiana* blastospores with haemocytes. It appears that agglutinins are involved in the recognition of invading fungi (Bidochka *et al.,* 1997).

Three types of defense reactions have been observed in the insects' haemocoel: phagocytosis, cellular encapsulation (capsule, nodule, "giant" cell), and humoral encapsulation (Charnley, 1984). Although humoral encapsulation plays an important role in prevention of mycoses due to several fungi, Gotz and Vey (1974) have shown that *B. bassiana* could break out of the encapsulation.

Deposition of oxidized phenols (melanin) in cuticle by host phenoloxidase is the first overt response to infection. Anti-microbial effects of phenols are well established, but in insect

cuticles melanization appears to be primarily an effective defense against more virulent pathogens. Protease inhibitors within the cuticle may serve to restrict pathogen enzyme activity (Charnley, 1989). Within the haemocoel, the main cellular response of the insect is a multihaemocytic encapsulation of the fungal element following initial recognition of the fungus by the haemocytes. The yeast like blastospores, produced by Deuteromycetes in the insect haemolymph, reduce the effectiveness of the cellular defences by sheer weigh of numbers and not being as antigenic as the mycelium (Charnley, 1989). Finally the cyclodepsipeptide toxins, destruxin produced by *M. anisopliae* appear to interfere with haemocyte function, especially by suppressing prophenoloxidase activation (Huxham *et al.*, 1989).

However, recent evidence is consistent with destruxins being a determinant of virulence for *M. anisopliae.* Destruxins are active in causing symptoms, principally by paralyzing muscles of caterpillars, while in other insect hosts of *M. anisopliae* such as Orthoptera, whose muscles are not susceptible to destruxins, and in susceptible insects infected with low destruxins producing strains, the toxin may act indirectly to assist the pathogen to overcome host defence; perhaps as stated earlier by interfering with haemocyte activity (Samuels *et al.*, 1988).

2.7 Mass production of insect pathogenic fungi

Microbial control agents, many of which are facultative pathogens, can be produced on artificial media in large quantities. Fungi such as *Beauveria bassiana, Metarhizium anisopliae,* and *Hirsutella thompsonii* are produced by fermentation procedures (Weiser, 1982). Previous reviews on mass production of entomopathogenic fungi include Bartlette

and Jaronskin, (1988); Goettel and Roberts, (1992); Feng *et al.*, (1994). There are several methods available for mass production of entomopathogenic fungi. These are *in vivo*, submerged and surface culture. The method used depends largely on the growth requirements of the fungus in question and the end product desired (Goettel and Roberts, 1992).

2.7.1 Production *in vivo*

In vivo systems most commonly involve the production of pathogens within the natural host. Occasionally, alternative susceptible organisms that can be more easily mass-reared can be used (Higby *et al.*, 1979). Entomopathogenic fungi such as *Entomophaga grylli* (Fresenius) have complex nutritional requirements and are difficult to be grown in artificial cultures. The best alternative is to grow them *in vivo* (Ignoffo and Hink, 1971). *In vivo* production is relatively expensive and questionable if it can be used for mass production of fungus for use as a microbial pesticide.

2.7.2 Submerged culture or liquid fermentation method

According to Auld (1992), industrial fermentation provides the cheapest and most economical way to produce large numbers of fungal spores for the existing equipment can be used without modification. It can also have limitation in that most hyphomycete fungi grow as mycelium and produce blastospores in submerged liquid culture while conidia are the desired end products (Jaronski and Goettel, 1997) or resting spores of entomophthoralean fungi (Carruthers *et al.*, 1997). Blastospores are short lived and, consequently, have not been adopted for field application (Ferron, 1981). An exception is *Verticillium lecanii* (Zimmermann) Viégas that has been used successfully as dried blastospore formulation

against green white flies and aphids (Lisansky and Hall, 1982). In this case, much of the mortality actually occurred from infection by conidia, which developed after the formulation had been applied to the plant surface.

Blastospores are unpigmented, hydrophobic and compared to conidia, are less resistant towards environmental stress (Van Winklehof and McCoy, 1984). Nevertheless, blastospores may have potential use in biological control, as they are infective and can be produced easily in deep fermentors and could potentially be produced economically in deep tank fermentors (Kleespies and Zimmerman, 1992).

Few entomopathogenic fungi produce conidia in submerged cultures, for example *B. bassiana*, which produce conidia as opposed to blastospores, in submerged cultures (Thomas *et al.*, 1987). Hegedus *et al.* (1992) carried out a comparative study on the difference between virulence, stability and cell wall structure of aerial conidia, submerged conidia and blastospores of *B. bassiana*. Virulent studies on the three types indicated that, although blastospores were slightly more virulent in comparison to aerial and submerged conidia, both conidia types retained greater viability during storage than blastospores.

Production of submerged conidia was also possible by *Metarhizium flavoviride* (now identified as *Metarhizium anisopliae* var. *acridium*) in liquid medium containing sucrose and brewers yeast with limited nitrogen and excess carbon (Jenkins and Prior, 1993). Bioassay and semi field trials against *S. gregaria* also indicated that both aerial and submerged conidia have comparable virulence (Jenkins and Thomas, 1996). However, submerged conidia are hydrophobic as blastospores, which affect formulation in oil for ultra low volume (ULV) application (Moore and Caudwell, 1997).

Some fungi that produce mycelium only in submerged culture can be dried and applied as

fragments or pellets in the field. A recent break through has been the development of a method for producing dry, viable mycelium, which can be stored under refrigeration (Andersch *et al.*, 1990). When introduced in the field, particles of dry mycelium rapidly produce infectious conidia following rehydration from dew, rain or soil moisture. This method has been used for many entomopathogenic fungi including *M. anisopliae* and *B. bassiana* (Pereira and Roberts, 1990). It has been field-tested successfully (Rombach *et al.*, 1988). A commercial product, Bayer 1020 has been produced (Pereira and Roberts, 1990).

Media for growth of biocontrol organisms should be as simple as possible, utilizing a standard set of inorganic salts and source of carbon and nitrogen (Auld, 1992). Animal manure and waste products could be suitable as nitrogen sources and are effective nutrients for mass production of fungi. Waste products such as molasses and Naturpur™ may be used to produce blastospores of *M. anisopliae* var. *acridium* in high concentrations. The mycelial production is reduced. Therefore, a separation of blastospores from the mycelium is not necessary and the processing of blastospores is simplified (Auld, 1992).

Submerged fermentation is most often used in diphasic method of production; blastospores and mycelium are produced in submerged culture and then transferred to surface culture for production of conidia.

2.7.3. Surface culture

Many entomopathogenic fungi grow profusely on damp solid media/substrate and produce conidia aerially (Goettel and Roberts, 1992). Substrates, which provide a large surface area to volume ratio such as rice grains, are often used for conidial production (Mendonca, 1992). Rice has been shown to be superior to other substrates such as sweet potato, tapioca, papaya,

or coconut (Ibrahim and Low, 1993). Bartlett and Jaronski (1988) reported production of *M. anisopliae* on beer mash.In general methods for the production of fungal conidia on solid substrates are numerous but most systems have evolved from the above techniques.

2.7.4 Diphasic liquid-solid Fermentation/Two phase system.

Diphasic fermentation system combines the benefit of high biomass production in liquid fermentation, and production of stable, hydrophobic (lipophilic) aerial conidia on solid substrate (Goettel and Roberts, 1992). Currently, this method is being used to produce *M. flovoviride* by LUBILOSA Programme (Lomer *et al.*, 1997)

2.8 Formulation of insect pathogenic fungi

Large-scale field application of fungal isolates requires a suitable formulation. Formulation refers to the addition of substances to infective units to enhance their activity during application (Daoust *et al.*, 1982). Successful use of fungal pathogens in agricultural pest control requires appropriate formulation that is suitable during storage and compatible with existing technology (Prior *et al.*, 1992).

The type of formulation that is chosen is determined by the pathogen biology and the physical properties, and location and habit of the target pest (Daoust *et al.*, 1983). Good formulation can improve the shelf life, persistence and efficacy (Goettel, 1992). The formulation may incorporate additives such as humectants, UV protectants, and thickening agent to adjust the viscosity of the pesticide (Goettel and Roberts, 1992; Auld, 1992).

The ingredients added to infective units of microbial control agents contribute to the

viability, virulence and the acceptance of the product by the user (Couch and Ignoffo, 1981). The user must find the product easy to use and apply. Daoust *et al.* (1982) have noted 10 different *M. anisopliae* formulations that showed low virulence than unformulated conidia. Oil formulations that reduced conidial viability of *M. anisopliae* have been recorded (Daoust *et al.*, 1983). They include mineral oils, cod-liver oil and 12 vegetable oils. Stability of formulations under high temperatures and UV levels of the tropics is very important. Studies on the survival of *M. flavoviride* conidia suspended in Soya bean oil and mineral kerosene have shown that 5% viability loss occurs after 2-4 weeks at 25^0C, and less than a week at 35^0C (Prior *et al.*, 1992). The lethal effect of radiation in the UVB range can be overcome by adding UVB absorbing compounds during formulation (Prior *et al.*, 1992).

2.9. Application of insect pathogenic fungi

Since fungal spores/propagules are microscopic and most can be applied as conventional chemical pesticides in the form of suspension, application methods have to take into consideration the multitude of factors affecting host-pathogen relationship like sunlight, temperature, humidity, host age, inoculum, targeting and other factors (Auld, 1992, Goettel, 1992).

Basically, the role of a biopesticide is to kill the maximum number of target pests by direct contact, as is the case with non-persistent chemicals, rather than to create epizootics (Bateman *et al.*, 1993). It has been found that topical application of conidia to some insects may proceed independently of ambient relative humidity (Ferron, 1981). This indicates that epizootics in the field are limited by the inhibiting effect of low relative humidity on sporulation rather than infection. It also implies that if conidia are delivered to the target pest, they may infect under ambient conditions too dry for their *in situ* production. Therefore, the

concern is how to deliver the inoculum to the target and ensure the infectivity of this inoculum under conditions of low ambient humidity (Bateman *et al.*, 1993).

Entomopathogenic fungi may be applied in dry state as dust or granules or as liquid in presence of liquid. This may be as wettable powder, in oil as aqueous concentration or emulisifiable concentrate. The range of techniques available for application is as broad as those available for conventional pesticides and herbicides. These include the high volume (about 1000 l/ha), medium (350 l/ha), low to very low volumes (3-150 l/ha), ultra low volumes (0.5-3 l/ha), controlled droplet application and electrostatic spraying (Auld, 1992). One of the advantages of microbial control agents is that they can be applied using the existing technology. Thus the use of controlled droplet application (Bateman *et al.*, 1993) and electrostatic spraying has been reported to be promising for application of entomopathogenic fungi formulated in oils.

The method of application depends on the type of formulation available (Goettel and Roberts, 1992). For example *Beauveria bassiana* produced using Mycotech's surface culture method has been formulated as bran bait or oil formulation and was applied using a hand held low volume spinning disc sprayer (Johnson *et al.*, 1992). Bran baits have been a successful method of application of the protozoan *Nosema locustae* for control of rangeland grasshoppers. Bait formulations have been suggested to provide environmental advantages such as efficacy at reduced dosages, elimination of spray drift, reduced microbial degradation and UV radiations (Ewen and Mukerji, 1987). In China, the use of mortar shells, firecrackers and landmines have been used for application of *Beauveria bassiana* (Auld, 1992).

3. MATERIALS AND METHODS.

3.1 Collection and rearing of the wax moths

The initial stage of this experiment was the establishment of both *Galleria mellonella* and *Achroia grisella* cultures in order to get uniform generation and age group insects. The initial stock culture of both species of wax moths and the wax combs for rearing the insects were obtained from Holleta Bee Research Center. The insects were found in infested honeycombs. The colony of both species were reared at the Biology Department Insectary Addis Ababa and at the Desert Locust Organization for Eastern Africa (D.L.C.O-E.A) on natural (wax combs) and artificial diets using method described by previous workers (Haydak, 1936; Smith, 1937; Good *et al.*, 1953). Wax combs were put into the old culture for females to oviposit since it contains a component of the female sex hormone Nonanal (MAAREC, 2000). The initial stock was maintained in an empty rectangular water bath (62x32 cm) and the open end covered with wire mesh (1.5x2 mm) to prevent emerging adults from escaping.

A total of 10 newly emerged females and 5 newly emerged males were introduced into new rearing containers with artificial diet and a piece of wax comb. The containers were then placed in a warm room with temperature maintained at 30 ± 2^{0}C. Humidity in the rearing room was maintained by spraying 500ml of water using a screw nozzle sprayer daily. This was an improvisation since no hygrometer was available.

In the rearing containers, a folded wire mesh was placed, to act as a support on to which the adults rested. Soft cardboard was also put in the rearing containers for pupation of fully-grown larvae. In some, the fully-grown larvae pupated on the folded wire mesh. As soon as the moths emerged, they were transferred to new rearing containers. The food remained soft as honey

absorbs moisture from the air and glycerine prevented the developing of moulds. This made the production of many insects easy.

For rearing and feeding of inoculated 5th and 6th larval instars of both the wax moths, the recipe used to prepare the artificial diet consisted of two parts (Haydak, 1936; Smith, 1937; Good, *et al.*, 1953) i.e. the liquid and solid component.

Mixture 1	**Mixture 2**
300 ml clear honey	200 g Milk powder (with low fat content).
400 ml glycerol (=glycerine)	200 g whole-wheat flour
150 ml Water	I00 g yeast powder (dried brewer's yeast)
	100 g wheat germ
	400 g corn flour
	400 g bran

Equal parts of mixture 1 and mixture 2 were mixed by weight. The mixture was allowed to stand for 24 hours in order for the liquid to penetrate the dry food. The mass of the mixture was placed in locally improvised rearing containers (62x32 cm) and covered with wire mesh (1.5x2 mm).

3.2 Preparation of media for culturing of fungi

The media was prepared using Sabouraud Dextrose Agar (SDA) and constituted of:

Mycological peptone	10.0 g
Glucose	40.0 g
Agar	15.0 g

65 grams of the ready mixed powdered SDA was suspended in 1 litre of distilled water, boiled and autoclaved at 121^{0}C and 15psi for 15 minutes (according to procedures on the agar bottle provided by Decton Dickinson & company, Microbiology Systems - USA). After preparation

of the media, 0.05 g of chloramphenicol powder (an antibiotic) was weighed in a clean, dry universal bottle and 10ml of 90% alcohol added to it and agitated. The antibiotic solution was then added to the sterilized agar at a rate of 1ml in 100 ml agar medium (i.e. 10 ml in 1 litre). The bottle containing the mixture of agar and antibiotic was then gently inverted several times to distribute the antibiotic solution evenly throughout the medium. The medium was autoclaved again for 10 minutes at 10psi and 115^{0}C.

3.3 Source of entomopathogenic fungi and preparation of inoculum used for infection.

3.3.1 Source of entomopathogenic fungi

For this study, *Beauveria*: DLCO-AA-5, DLCO-AA-14 and IITA 18 and *Metarhizium*: DLCO-AA-109 and DLCO-AA-83 were obtained from Desert Locust Organization for Eastern Africa (D.L.C.O-E.A), Addis Ababa Ethiopia. The standard isolate, *Metarhizium anisopliae* Var. *acridium* (IMI 330189) was provided by Dr. Emiru Seyoum, Addis Ababa University. Each isolate had a different host and origin.

3.3.2 Preparation of inoculum

Production of each isolate in sufficient amount was imperative. Thus all isolates of *Metarhizium anisopliae* and *Beauveria bassiana* were cultured on Sabouraud Dextrose Agar (SDA) at a pH of 6.8 and Malt Extract Agar plates at 25^{0}C for 10-14 days to achieve maximum growth and sporulation before harvesting (Kaaya, 1989; Khada *et al.,* 1990; Ibrahim and Low, 1993). Conidia were harvested by flooding 10 ml of sterile distilled water containing 0.5% Tween 80 on the plates. The plates were then scraped carefully using a spatula to recover the spores. Subsequent to filtration through loosely plugged cotton wool to remove hyphal debris and diluted (1:9) in sterile distilled water containing 0.5% Tween 80 into a test tube.

The suspension was agitated for approximately 5-8 minutes on a vibrating shaker to dissociate the conidial chains and produce a homogenous conidial suspension. The conidial suspension was adjusted from $2x10^4$ to $2x10^7$ ml^{-1} with sterile distilled water containing Tween 80. Numbers of conidia were estimated using a phase contrast the light microscope and a stage haemocytometer (Prior *et al.*, 1992; Raid and Cherry, 1992).

3.4 Effect of entomopathogenic fungi on the honeybees (*Apis mellifera*) Ethiopian race

Four viable active *Apis mellifera* colonies were purchased from farmers at Debra zeit. They were confined in locally made wooden honeybee cages and maintained under a confined room at Desert Locust Control for Eastern Africa before being treated with three of fungal isolates i.e. IMI 330189, IITA 18 and DLCO-AA-83 used to treat the wax moths. The cages were placed at 3-meter interval. The bee colonies were maintained on pure honey and 30% sugar solutions.

3.5 Spore viability tests

Viability tests of the spores were performed 24 hours before each bioassay and spore batches with >85 % viability were used. This was performed by pipetting 200µl of spore formulation onto a 9 cm glass petri dish containing SDA and spread evenly using a spreader. Twenty-four hours later, the number of spores with an extrusion as big as or greater than the width of the spore in a sample of at least 300 spores were counted, and expressed as a percentage of germinating spores (Hall, 1976).

3.6 Bioassay

3.6.1 Treatment of larvae

Although the dose can't be measured precisely, dipping of the insects into suspensions of propagule has been used successfully in bioassay of entomopathogenic hyphomycetes.

Conidial concentrations ranging from $2x10^4$ to $2x10^7$ conidia/ml were prepared as described in section 3.3.2. Six experimental insects were each inoculated by dipping singly in 1.00ml of conidial concentrations for 3 seconds (Sewify and Sharaf, 1993). Six larvae in the control were treated with sterile distilled water containing 0.5% Tween 80. In order to ensure that the dose received by each insect was as constant as possible, a separate conidial suspension was used for each insect dipped. Since the treatment of the larvae was done under a laminar flow hood, the suspension was quickly drained off by suction.

After inoculation, the treated insects (larvae) were transferred into plastic petri dishes (9-cm diameter) for 24 hours without food. This was done to prevent removal of fungal spore from the insects' body onto the food (artificial diet). After 24 hours, grated artificial diet was introduced.

While in the petri dishes, the larvae formed silken feeding tunnels, these were removed daily to probably increase susceptibility to the fungus and easy observation. For each treatment, six larvae were used in three replicates and the experiments repeated three times adopted from similar experiments conducted on larvae of *Galleria mellonella* (Sewify and Sharaf, 1993).
To investigate the effect of fungal isolates and concentrations used on the emergence of the adults from pupae, six 6^{th} larval instars in three replicates were inoculated prior to pupation and the experiment repeated three times

3.6.2 Treatment of adults

Six adults were introduced into a lunch box (15x11x9 cm) lined with wire mesh (1.5x2 cm). The top of the lunch box was covered with nylon mesh and held in position by a rubber band. They were then inoculated by spraying 2.00 ml of each conidial suspension in three replicates and experiment repeated three times. This method of treatment was adopted from similar experiment conducted on the bean and maize weevils (Adane *et al.* 1998). The treated adult

insects were maintained on sugar solution (10%) soaked in cotton wool balls, which were changed after every 3 days. All the treated insects were then kept in a room maintained at 28 ± 2^{0}C. Temperature was maintained using a room heater.

3.7 Mortality assessment

Insect mortality was recorded daily at 24 hours interval recorded for 14 days with the adults of the greater wax moths, 8 days with adults of the lesser wax moths since it has a short life span and 14 days for the larvae of both moth species beginning from the day 2 following treatment. All cadavers in each replicate of both species were removed immediately before the fungus sporulated to prevent horizontal transmission.

3.8 Comparison of the different fungal isolates used to treat the adults and larvae

Comparisons between the different fungal isolates were made to select the most virulent of the six fugal isolates used. This comparison was done with at all fungal concentrations ranging from $2x10^{4}$ to $2x10^{7}$ conidia/ml used to treat the adults, 5^{th} and 6^{th} larval instars of both the greater and lesser wax moths.

3.9. Examination of sporulating fungi on cadavers

To determine whether the insect died of mycosis due to the inoculated fungi the cadavers were incubated under high humidity in petri dishes containing moistened sterilized filter paper (Prior *et al.*, 1992; Bateman *et al.*, 1993; Odino, 1994; Inglis *et al.*, 1996). Dead insects following inoculation were surface sterilized by dipping in 70% ethanol for 3 seconds and 5% sodium hypochrolite for 2 seconds. The insects were then rinsed three times in distilled sterile water and transferred to petri dishes with moistened sterilized filter paper lined. The petri dishes were then tightly sealed with parafilm to increase moisture content and prevent contamination.

Following incubation at 25^0C in the dark for 7 days, cadavers were checked for external sporulation.

3.10. Treatment of the honeybee colonies

Two isolates, IMI 3 30189 and DLCO-AA-83 from the genus *Metarhizium* and one isolate, IITA 18 from genus *Beauveria* were used for this study. Estimate of wax moths that could be in the bee colony (30 in number) was made before inoculation (*per comm.*) The spores were inoculated by spraying 10 ml of fungal formulation into the beehives with the highest concentration used on the wax moths of $2x10^7$conidia/ml. One hive served as a control and was treated with the same a mount of distilled water containing 0.5% Tween 80. The bee colonies were observed before treatment and daily after treatment. Dead bees were removed daily, surface sterilized to check for external growth of the conidia as described in section 3.9.

3.11 Statistical analysis.

Data from the three experiments were pooled and to correct for control mortality, mortality, all data was corrected using Abbots formula (1925) cited in Lawrence Lacey (1997).

Corrected % mortality =100 x (T %-C %)/(100 %-C %)

Where T % = the percentage of the dead test organisms and C % = the percentage of dead control organisms.

Analysis of cumulative percentage mortality data was then subjected to one-way analysis of variance using SPSS version 10.0 (SPSS, 1999). Two-way analysis of variance was used to find out if there is interaction between the concentrations and fungal isolates. Waller-Duncan Multiple Range Test was used to separate the means.

4. RESULTS

All fungal isolates used in the present study were able to infect and cause death in adults of both the greater and lesser wax moths at all concentrations ranging from $2x10^4$ to $2x10^7$ conidia/ml (Tables: 1-12). The 5th larval instars were susceptible at higher concentrations of $2x10^7$ conidia/ml with some fungal isolates while the 6th larval instars of both the greater and lesser wax moths were not susceptible to all fungal isolates at the different fungal concentrations used during the pupal stage. However, the degree of infection varied greatly between the isolates and concentrations based on speed and magnitude of mortality especially in the 5th larval instars (Tables: 13-24). Cumulative percentage mortality data presented in the tables and figures and days not shown in the tables are incorporated in the day that follows.

4.1 Bioassays on adults, 5th and 6th larval instars of the greater and lesser wax moths

4.1.1 Effect of isolate DLCO-AA-83 on adult greater and lesser wax moths with a range of conidial concentrations over time.

Mortality of the adult greater wax moth due to mycosis by isolate DLCO-AA-83 increased gradually from the second day after treatment to 100% on day 13 post treatment with conidial concentrations of $2x10^4$ and $2x10^7$conidia/ml. By day 3, there was no significant difference ($P > 0.05$) in mortality between the different fungal concentrations and the control (Table: 1).

As the number of post treatment days progressed, i.e. from days 7 to 13, there were no significant differences in mortality of the treated adults with the different fungal concentrations. All fungal concentrations used in this study had similar efficacy (Table: 1 and Appendix: 1a)

Mortality of the lesser wax moth adults due to mycosis by isolate DLCO-AA-83 also ranged from approximately 2% to 94.44% on day 8 post treatment. No mortality was observed on day 1 with all the concentrations used and the control. Mortality began on day 2 and there was a significant difference ($P < 0.05$) between the different fungal concentrations and the control. There was no significant difference ($P > 0.05$) in mortality due to mycosis between $2x10^6$ and $2x10^7$ conidia/ml. Higher mortality of the lesser wax moth adults on day 2 was recorded with the concentration of $2x10^4$ conidial/ml. From day 3 to day 8, no significant difference in mortality of the treated lesser adults was observed between the different fungal concentrations. However, a significant difference in mortality was observed between the different concentrations and the control (Table: 2 and Appendix: 1b).

Table 1: Mean percent mortality of *Galleria mellonella* adults treated with isolate DLCO-AA-83 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3(NS)	5	7	9	11	13
$2x10^4$	23.84±2.7	56.94±11.81b	74.03±6.41a	82.62±4.84a	88.04±1.52a	100.00±0.00a
$2x10^5$	25.69±4.22	61.97±6.03ab	70.83±4.17a	74.68±5.60a	83.12±10.57a	91.67±8.33a
$2x10^6$	25.23±9.72	60.10±5.46ab	76.65±4.83a	84.26±4.63a	88.53±3.40a	100.00±0.00a
$2x10^7$	28.01±5.41	82.84±6.45a	86.31±8.27a	89.56±6.45a	90.91±5.25a	95.24±4.76a
Control	7.41±3.70	18.52±7.41c	35.19±3.70b	46.30±3.70b	59.26±1.85b	61.11±3.21b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test) (Pooled N=18)

Table 2: Mean percent mortality of *Achroia grisella* adults treated with isolate DLCO-AA-83 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2	3	4	5	6	7	8
$2x10^4$	7.41±1.85a	27.04±7.04ab	46.16±7.69ab	62.18±3.53a	77.68±5.61ab	86.11±7.35ab	91.67±3.70a
$2x10^5$	1.85±1.85b	21.44±2.70ab	43.60±5.13ab	57.61±6.04a	79.68±12.89ab	88.89±11.11a	91.67±3.70a
$2x10^6$	5.56±0.00ab	29.54±4.80a	46.72±4.44ab	68.63±1.00a	83.74±6.18a	94.44±5.56a	94.44±1.85a
$2x10^7$	5.56±0.00ab	25.62±1.05ab	48.72±6.68a	62.52±3.89a	87.44±8.43a	94.44±5.56a	94.44±1.85a
Control	0.00±0.00c	12.62±3.70b	27.78±0.00b	35.19±4.90b	50.00±6.41b	62.96±3.70b	62.96±3.70b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test) (Pooled N=18)

4.1.2 Effect of IITA 18 on adults greater and lesser wax moths with different conidial concentrations over time

Mortality of the greater wax moth adults treated with isolate IITA 18 increased gradually from the second day of treatment to 88.89% with concentration $2x10^7$ Conidia/ml (Table: 3). On day 7, a higher mortality was observed with $2x10^5$ and $2x10^7$ conidia/ml and a significant difference ($P < 0.05$) in mortality following treatment by the different fungal concentrations was observed with $2x10^4$ conidia/ml and $2x10^7$ conidia/ml. On day 11, no significant differences ($P > 0.05$) in mortality were observed between the different treatments and the control. On day 13, there was no significant difference ($P > 0.05$) death following treatment by the different fungal concentrations and the control (Appendix: 2a).

In contrast to the greater wax moth, death due to infection in adult lesser wax moths started as early as day 2 but no statistically significant differences ($P > 0.05$) in mortality were observed between the different conidial concentrations and the control (Table: 4). By days 4, 5 and 8, no significant differences in mortality due to mycosis were also observed between different conidial concentrations but a significant difference ($P < 0.05$) was observed between the concentrations and in the control (Appendix: 2b).

Table 3: Mean percent mortality of *Galleria mellonella* adults treated with isolate IITA 18 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e))					
	3	5	7	9	11(NS)	13
$2x10^4$	6.00±0.12abc	11.76+3.40b	16.42±3.75c	36.01±3.37b	60.00±10	78.44±5.33ab
$2x10^5$	12.03±3.17a	42.95+9.94ab	43.39±4.16a	49.75±6.78b	60.00±10	79.36±6.35ab
$2x10^6$	4.99±2.60c	28.25+6.77a	30.96±8.18ab	38.20±5.60b	51.25±6.96	65.07±4.20b
$2x10^7$	11.76±0.00ab	22.28±1.85ab	41.67±8.74a	66.62±4.81a	82.78±12.56	88.89±11.11a
Control	5.56±0.00bc	16.67±5.56b	31.48±4.90ab	38.89±3.21b	50.00±3.21	57.41±3.70b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 4: Mean percent mortality of *Achroia grisella* adults treated with isolate IITA 18 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2 (NS)	3	4	5	6	7	8
$2x10^4$	7.73±5.21	29.90±5.01ab	52.22±4.00a	66.67±2.38a	80.23±5.49a	91.84±0.47a	88.33±2.55a
$2x10^5$	3.81±1.91	35.27±2.95a	42..56±3.82a	53.02±4.97a	56.86±7.75b	75.89±5.01b	85.00±3.47a
$2x10^6$	3.81+1.91	27.18±6.22ab	40.00±4.97a	51.11±7.26a	71.51±3.76ab	78.92±4.97ab	88.08±2.39a
$2x10^7$	1.96+1.96	15.48±5.02bc	31.81±5.99ab	46.35±3.49a	61.41±7.75ab	72.86±6.63b	88.33±7.26a
Control	1.85±1.85	5.56±3.21c	12.96±1.85b	20.37±1.85b	22.22±3.21c	29.63±4.90c	37.04±3.70b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test) NS: not significant. (Pooled N=18)

4.1.3 Effect of DLCO-AA-5 on greater and lesser wax moth adults with different conidial concentrations over time

On day 3, there was a significant difference ($P < 0.05$) in mortality between the different conidial concentrations and the control of the greater wax moth adults. By day 5, there was a significant difference in target mortality between the concentrations and the control (Table: 5). However, no significant effects ($P > 0.05$) in mortality were observed between the concentrations of $2x10^4$, $2x10^5$ and $2x10^6$ conidia/ml, respectively. Higher mortalities were observed with $2x10^7$ and $2x10^6$ conidia/ml. On days 7, 9, and 11, significant differences in mortality were observed between the different fungal concentrations and the control. However by day 13, 100% mortality was achieved with the lower concentration of $2x10^4$ conidia/ml and the higher concentration of $2x10^7$ conidia/ml and significant difference ($P < 0.05$) in mortality was observed between results of the fungal concentrations and the control (Appendix: 3a).

Similar to that of the greater wax moth adults, no mortality was recorded in the lesser wax moth adults on day 2. No significant differences in mortality were observed between the different fungal concentrations from days 3 to 8 (Table: 6). Significant effects in mortality following treatment were observed between different fungal concentrations and the control (Appendix: 3b).

Table 5: Mean percent mortality of *Galleria mellonella* adults treated with isolate DLCO-AA-5 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	22.92±2.63ab	34.03±1.84a	49.23±6.41ab	69.04±8.31a	85.60±5.30a	100.0±0.00a
$2x10^5$	19.91±116b	44.97±8.84a	47.52±11.02ab	56.88±10.75ab	774.49±10.23ab	77.26±10.10ab
$2x10^6$	26.85±6.48ab	43.39±4.16a	68.38±4.52a	75.35±3.64a	76.51±2.73a	92.59±3.70a
$2x10^7$	34.49±5.08a	49.54±6.81a	72.49±13.16a	86.11±13.89a	93.93±6.06a	100.00+0.00a
Control	3.70±3.70c	11.11±6.42b	24.07±6.42d	33.33±3.21c	44.44±5.56b	50.00±6.42c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 6: Mean Percent Mortality of *Achroia grisella* adults treated with isolate DLCO-AA-5 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2	3	4	5	6	7	8
$2x10^4$	12.96±1.85ab	47.28±7.01a	62.05±4.46a	72.94±6.69a	84.72±9.72a	94.84±2.60a	97.43±2.56a
$2x10^5$	14.82±7.41ab	43.57±5.68a	52.35±3.92a	71.24±2.85a	83.28±2.21a	87.06±6.00a	90.91±9.09a
$2x10^6$	16.67±5.56a	43.63±8.97a	54.05±2.57a	73.46±1.70a	87.47±0.45a	92.62±0.496a	94.59±2.76a
$2x10^7$	18.52±1.85a	43.36±1.09a	61.68±4.99a	79.35±4.54a	93.61±3.61a	94.84±2.60a	94.59±2.83a
Control	0.00±0.00	1.85±3.21b	7.41±4.90b	9.26±3.70b	11.11±3.21b	24.07±4.90b	29.63±4.90b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

4.1.4 Effect of isolate DLCO-AA-14 on adult greater and lesser wax moths at different conidial concentrations over time

Mortality of the greater wax moth adult due to mycosis by isolate DLCO-AA-14 increased gradually to 100% by day 13 after inoculation. On day 5, 7 and 9, significant differences ($P < 0.05$) in mortality were observed between the concentrations and the control (Table: 7). On days 11 to 13, although no significant differences ($P > 0.05$) in mortality were observed between the different conidial concentrations, there was a significant difference in mortality between the concentrations and the control (Appendix: 4a).

Similar to that of the greater wax moth adults, no mortality was observed on day 1 in the lesser wax moth adults but death commenced on day 2. A significant difference ($P < 0.05$) in mortality was seen between the different concentrations and the control. Although there was a significant difference in mortality between the different concentrations and the control, no significant difference ($P > 0.05$) in mortality was observed between the different spore concentrations of $2x10^4$, $2x10^5$ and $2x10^6$ conidia/ml (Table: 8). By days 4 and 6, no significant difference in mortality was also observed between the different conidial concentrations. A significant difference in mortality was however observed between the different conidial concentrations and the control. On days 7 and 8 post treatment, there was a significant difference in mortality between the different conidial concentrations and the control (Appendix: 4b).

Table 7: Mean percent mortality of *Galleria mellonella* adults treated with isolate DLCO-AA-14 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3(NS)	5	7	9	11	13
$2x10^4$	12.96±7.41	46.90±4.85a	65.56±8.68b	84.75±3.23b	91.67±4.81a	95.83±4.17a
$2x10^5$	22.22±8.47	51.25±4.73a	68.41±6.43ab	81.44±3.61b	90.00±5.77a	97.22±2.78a
$2x10^6$	16.67±9.62	61.94±9.19a	82.50±2.50a	93.64±3.19a	97.22±2.78a	97.46±2.56a
$2x10^7$	24.07±7.40	52.22±7.78a	74.76±6.50ab	94.64±2.91a	100.0±0.00a	100.0±0.00a
Control	0.00±0.00	9.26±3.70b	18.51±1.85c	35.18±1.85c	40.74±3.21b	50.00±3.21b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
(Pooled N=18)

Table 8: Mean percent mortality of *Achroia grisella* adults treated with isolates DLCO-AA-14 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2	3	4	5	6	7	8
$2x10^4$	14.81±1.85b	26.85±3.34ab	52.59±4.36a	53.05±4.82b	67.63±6.73a	79.29±3.31b	78.52±0.00b
$2x10^5$	24.07±3.70a	44.21±9.62a	62.96±3.70a	73.87±9.82a	82.42±11.22	88.64±4.90ab	96.30±1.85a
$2x10^6$	12.96±3.70b	32.41±10.94ab	60.00±6.67a	68.81±4.52ab	86.90±5.024a	96.97±3.03a	96.30±1.85a
$2x10^7$	12.96±1.85b	34.26±5.63b	54.44±4.20a	71.03±2.41a	86.90±5.024a	94.20±2.91a	96.30±1.85a
Control	0.00±0.00c	3.70±3.70b	5.56±5.56b	16.67±3.21c	29.63±4.90b	37.03±1.85c	48.15±1.85c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test).
(Pooled N=18)

4.1.5 Effect of different conidial concentrations of isolate DLCO-AA-109 on adults greater and lesser wax moth over time

Mortality of adult greater wax moths due to infection with isolate DLCO- AA-14 increased gradually with time after inoculation with the different fungal concentration. On day 3, no significant difference ($P < 0.05$) in mortality between the fungal concentrations of $2x10^4$ conidia/ml and the control was observed and the control on. On day 5, significant difference ($P < 0.05$) in mortality due to the applied fungus was also observed between the different conidial concentrations and the control (Table: 9). No significant differences in mortality were observed among comparisons between the different fungal concentrations. A significant difference in mortality between the different concentrations and the control (Appendix: 5a) was however seen by day 13 after inoculation.

Mortality of the lesser wax moth adults due to infection with isolate DLCO-AA-109 began on day 2. But no significant difference ($P > 0.05$) in mortality was observed between the different conidial concentrations at day 2. From days 3 to 8, significant difference ($P < 0.05$) in mortality was observed between the different fungal concentrations and the control (Table: 10). A total mortality of 100% was achieved with higher concentrations of $2x10^6$ and $2x10^7$conidia/ml post treatment, respectively (Appendix: 5b).

Table 9: Mean percent mortality of *Galleria mellonella* adults treated with isolate DLCO-AA-109 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	5.88±3.39b	35.91±2.88b	50.79±8.94b	79.49±5.13b	92.59±7.41a	96.31±3.70a
$2x10^5$	15.69±1.96ab	34.07±2.14b	52.05±4.12b	66.67±2.55c	78.45±7.00ab	86.74±7.88a
$2x10^6$	23.53±5.88a	45.96+3.57a	64.92±4.68ab	84.98±4.46b	93.26±3.42a	93.52±3.33a
$2x10^7$	25.49±3.92a	50.00±1.70a	69.68±2.75a	98.44±2.56a	100.0±0.00a	100.0±0.00a
Control	5.56±0.00b	7.41±1.85c	20.37±1.85c	27.78±0.00d	37.04±1.85c	53.00±3.70b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 10: Mean percent mortality of *Achroia grisella* adults treated with isolates DLCO-AA-109 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2(NS)	3	4	5	6	7	8
$2x10^4$	3.7±1.85	30.17±1.65ab	41.92±6.52abc	45.98±6.04b	65.12±2.08b	85.00±4.90a	88.89±3.21a
$2x10^5$	5.56±3.21	22.87±6.50bc	29.15+6.78bc	55.46±10.62ab	76.13±6.95b	85.99±1.85a	89.56±0.00a
$2x10^6$	3.70±3.70	44.57±2.29a	49.27±5.52ab	86.53±6.83a	96.97±3.03a	100.00±0.00a	100.00±0.00a
$2x10^7$	6.14±3.41	33.37±4.90ab	52.26±6.92a	78.95±7.67a	93.27±3.42a	100.00±0.00a	100.00±0.00a
Control	1.85±1.85	12.96±3.70c	25.93±4.90c	37.04±3.21b	40.74±3.21c	46.30±3.21b	55.56±5.56b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

4.1.6 Effect of isolate IMI 330189 on adults of greater and lesser wax moth at different fungal concentrations over time

Mortality of the greater wax moth adults due to isolate IMI 330189 ranged from 3.7% on day 3 to 100% on day 13 post treatment. From days 3 to 7, a significant difference ($P < 0.05$) in mortality was observed between the different fungal concentrations and the control (Table: 11). However on day 9, significant difference in mortality was observed between $2x10^5$ and $2x10^7$ conidia/ml. By day 13, no significant differences in mortality were observed among the different fungal concentrations although a significant difference in mortality was observed between the different fungal concentrations and the control (Appendix: 6a).

Mortality due to infection by isolate IMI 330189 in the lesser wax moth adults began on day 2. No significant difference in target mortality was however observed in comparison of results between the different concentrations and the control. From days 3 to 8, there was a significant difference ($P < 0.05$) in mortality due to mycosis between the different fungal concentrations and the control (Table: 12). On day 8, higher mortality was observed with $2x10^7$conidia/ml post treatment (Appendix: 6b). This may witness that the concentration of $2x10^7$conidia/ml was the most virulent in causing mycosis.

Table 11: Mean percent mortality of *Galleria mellonella* adults treated with isolate IMI 330189 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	32.57±4.46ab	50.14±3.19ab	68.32±2.11b	82.75±0.93b	91.84±4.46ab	100.0±0.00a
$2x10^5$	36.71±5.61a	54.90±4.90ab	64.16+1.48b	70.30±2.46c	81.44±3.21b	100.0±0.00a
$2x10^6$	13.40±3.21cd	40.95±6.42b	68.75±4.90b	83.51±3.21b	88.14±4.90ab	93.94±3.70a
$2x10^7$	20.92±4.90bc	57.50±1.25a	82.97+2.22a	97.44±1.85a	100.0±0.00a	100.00±0.00a
Control	3.70±3.70d	12.96±1.85c	24.07±1.85c	31.48±3.70d	42.59±3.70c	55.56±0.00b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 12: Mean percent mortality of *Achroia grisella* adults treated with isolate IMI 330189 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)						
	2 (NS)	3	4(NS)	5	6	7	8
$2x10^4$	3.70±1.85	23.28±2.60a	32.64±3.91	44.29±2.97ab	51.85±1.85ab	59.88±1.55bc	67.05±3.99b
$2x10^5$	1.85±1.85	12.92±3.49b	21.93±3.81	52.38±2.38ab	54.81±2.89ab	64.85±7.74bc	85.00±0.00a
$2x10^6$	3.70±3.70	32.64±3.70a	36.87±6.79	58.97±11.14a	67.89±6.79a	80.58±10.02ab	91.66±8.33a
$2x10^7$	5.56±5.56	12.22±4.90b	21.14±3.71	50.00±4.12b	61.48±4.55ab	88.33±7.26a	95.83±4.17a
Control	0.00±0.00	14.81±1.83c	31.48±4.90	33.33±3.21c	42.59±0.85b	50.00±3.21c	55.56±0.00b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test) NS: not significant. (Pooled N=18)

4.1.7 Effect of isolate DLCO-AA-83 on 5th larval instars of the greater and lesser wax moth at a range of different fungal concentrations over time.

Mortality of the greater wax moth 5th larval instars increased gradually with all the fungal concentrations. On days 3 to 5, mortality of the 5th larval instars of the greater wax moth due to the applied fungus was no significantly different ($P > 0.05$) between the different fungal concentrations and the control. From days 7 to13, high mortalities were recorded at $2x10^7$ and $2x10^6$ conidia/ml and there was a significant difference ($P < 0.05$) in mortality between concentrations of $2x10^7$ conidia/ml and the control (Table: 13). $2x10^7$ conidia/ml caused the highest larval mortality of the greater wax moth (50%) by day 13 post treatment followed by $2x10^6$ conidia/ml (31%), then $2x10^4$and $2x10^5$ conidia/ml (16.67%). Mortality in the control was very low (5.56 %) throughout the experimental period (Table: 13 and Appendix: 7a).

On day 5 no significant difference ($P > 0.05$) in mortality due to the applied fungus was observed in the 5th larval instars of the lesser wax moth between concentrations of $2x10^4$, $2x10^5$, and $2x10^6$ conidia/ml and the control (Table: 14). Comparison of percentage mortality at $2x10^6$ and $2x10^7$ conidia/ml was not shoed no significant difference. Higher mortality (42.59%) was recorded with $2x10^7$ conidia/ml on day 7 post treatments. By day 13, there was a significant difference ($P < 0.05$) in mortality between results of the concentrations applied but no significant difference larval mortality was found when results of $2x10^4$ conidia/ml and the controls were compared (Appendix: 7b).

Table 13: Mean percent mortality of *Galleria mellonella* 5th larval instars treated with isolate DLCO-AA-83 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3 (NS)	5 (NS)	7	9	11	13
$2x10^4$	0.00±0.00	3.70±1.85	3.70±1.85b	7.41±1.85bc	14.81±1.85cd	16.67±0.00c
$2x10^5$	9.23±4.85	9.23±4.90	9.23±4.90b	12.96±1.85bc	16.67±3.21c	16.67±3.21c
$2x10^6$	7.41±3.70	12.96±6.68	16.67±8.47ab	22.22±8.49ab	29.63±3.70b	31.48±4.90b
$2x10^7$	7.41±1.85	16.67±3.21	31.48±1.85a	35.19±4.90a	42.59±6.68a	50.00±8.49a
Control	3.70±0.70	3.70±3.70	3.70±3.70b	3.70±3.70c	3.70±3.70d	5.56±3.21c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
NS: not significant. (Pooled N=18)

Table 14: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate DLCO-AA-83 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	3.70±3.70b	5.56±3.21ab	5.56±3.21b	7.41±4.90c	9.26±3.70cd	9.26±3.70cd
$2x10^5$	3.70±3.70b	7.41±4.90ab	7.41±4.90b	9.26±3.21c	14.81±4.90c	14.81±4.90c
$2x10^6$	1.85±1.85b	16.67±4.90ab	29.63±6.68a	31.48±4.90b	31.48±4.90b	31.48±4.90b
$2x10^7$	14.81±1.85a	25.93±3.21a	42.59±6.68a	48.15±6.68a	55.56±3.21a	55.56±3.21b
Control	1.85±1.85b	1.85±1.85b	1.85±1.95b	1.85±1.85c	1.85±1.85d	1.85±1.85d

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
(Pooled N=18)

4.1.8 The kill effects of isolate IITA on 5th larval instars of the greater and lesser wax moths with different fungal concentrations over time

On day 3, no significant difference in target mortality was observed between the different fungal concentrations and the control of the greater wax moth 5th larval instars. From days 5, to 9, there were no significant differences ($P > 0.05$) in mortality of the larvae between applied concentrations of $2x10^4$, $2x10^5$ and $2x10^6$ conidia/ml. A significant difference ($P < 0.05$) in mortality due to mycosis was however recorded between the above concentrations, $2x10^7$ conidia/ml and the control. On day 11, there was a significant difference in mortality of the larvae between concentrations of $2x10^4$, $2x10^5$ and $2x10^6$ conidia/ml and $2x10^7$ conidia/ml but no mortality was recorded in the control. On day 13, there was a significant difference in mortality due to mycosis between the different concentrations and the control. A higher significant difference in the death of treated larvae of 64.81% was observed with $2x10^7$ conidia/ml on day 13 after treatment (Appendix: 8a).

On day 3, there was a significant difference in mortality of the lesser wax moth larvae between the different concentrations of $2x10^6$, $2x10^7$ and $2x10^5$ conidia/ml and the control, respectively. No significant difference ($P > 0.05$) in the mortality of the treated larvae was observed between the concentrations of $2x10^4$, $2x10^5$ conidia/ml and the control by day 7 post treatments (Table: 16). On days 9 to 13, significant differences ($P < 0.05$) in mortality due to the applied fungus were observed between the different concentrations but no significant effects ($P > 0.05$) in mortality of the larvae were observed between the lower concentrations of $2x10^4$ and $2x10^5$ conidia/ml and the control. Generally, increased mortalities due to the applied fungal isolate by day 13 post treatment were recorded with $2x10^7$ conidia/ml (40.74%), and $2x10^6$ conidia/ml (27.78%), respectively (Table: 16 and Appendix: 8b).

Table 15: Mean percent mortality of *Galleria mellonella* 5th larval instars conidia/ml treated with isolate IITA 18 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3(NS)	5	7	9	11	13
$2x10^4$	5.56±0.00	5.56±0.00ab	11.11±3.21bc	11.11±3.21bc	11.11±3.21bc	16.67±3.21bc
$2x10^5$	1.85±1.85	5.56±3.21ab	12.96±4.90bc	12.96±4.90bc	18.52±6.68b	25.93±11.26c
$2x10^6$	5.56±3.21	11.11±3.21a	16.67±3.21ab	22.22±6.42ab	24.07±8.07b	31.90±4.90b
$2x10^7$	9.26±4.90	24.07±4.9a	44.44±9.26a	51.85±6.68a	61.11±6.42a	68.52±3.21a
Control	0.00±0.00	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	1.85±1.85c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 16: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate IITA 18 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00b	1.85±1.85c	5.56±3.21b	7.41±1.85c	7.41±1.85c	7.41±1.85c
$2x10^5$	1.85±1.85ab	3.70±1.85bc	5.56±0.00b	7.41±1.85c	7.41±1.85c	7.41±1.85c
$2x10^6$	9.26±3.70a	14.81±1.85a	25.93±1.85a	27.78±3.21b	27.78±3.21b	27.78±3.21b
$2x10^7$	3.70±3.70ab	7.41±1.85b	27.78±3.21a	40.74±1.85a	40.74±1.85a	40.74±1.85a
Control	0.00±0.00b	0.00±0.00c	0.00±0.00b	0.00±0.00d	1.85±1.85c	1.85±1.85c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test) NS: not significant. (Pooled N=18)

4.1.9 The toxic effects of isolate DLCO-AA-5 on 5th larval instars of greater and lesser wax moths with different spore concentrations over time

A significant difference ($P < 0.05$) in mortality following treatment by varied spore concentrations and the control was observed on days 3 and 5 of the 5th larval instars of the greater wax moths. No significant differences ($P > 0.05$) in mortality were however observed between $2x10^4$, $2x10^5$, $2x10^6$ conidia/ml and the control (Table: 17). A significant difference in mortality of the treated larvae was observed between $2x10^7$ conidia/ml, the above conidial concentrations and control on day 7 post treatments. On days 9, 11, and 13, there were significant differences in mortality due to the applied fungus between the different fungal concentrations although no significant difference in mortality was observed between $2x10^4$, $2x10^5$ conidia/ml and the control (Appendix: 9a).

With the 5th larval instars of the lesser wax moth i.e. from days 5 to 13, significant differences ($P < 0.05$) in mortality following treatment by the different fungal concentrations and the control were observed (Table: 18). An increased mortality of 61.11% was observed with $2x10^7$ conidia/ml on day 13 post treatment (Appendix: 9b).

Table 17: Mean percent mortality of *Galleria mellonella* 5th larval instars treated with isolate DLCO-AA-5 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00b	0.00±0.00b	1.85±1.85c	1.85±1.85c	1.85 ±1.85c	3.70±1.85c
$2x10^5$	0.00±0.00b	1.85±1.85b	1.85±1.95c	1.85±0.85c	3.70±1.85c	5.56±3.21c
$2x10^6$	0.00±0.00b	3.70±1.85ab	14.81±4.9b	18.52±4.90b	24.07±1.85b	27.78±3.21b
$2x10^7$	5.56±5.56a	14.81±6.67a	38.89±3.21a	46.29±4.9a	53.70±4.90a	55.56±5.56a
Control	1.85±1.85b	1.85±1.85b	3.7±1.85c	3.70±1.85c	3.70±1.85c	3.70±3.21c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
NS: not significant. (Pooled N=18)

Table 18: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate DLCO-AA-5 at different concentrations over time

Concentration (Conidia/ml)	Percent mortality by day after treatment (Mean±s.e)					
	3 (NS)	5	7	9	11	13
$2x10^4$	1.85±1.85	7.41±1.85cd	14.81±1.85c	18.52±1.85c	22.22±3.21b	22.22±3.21b
$2x10^5$	1.85±1.85	14.81±3.70bc	20.37±1.85bc	22.22±0.00bc	22.22±0.00b	22.22±0.00b
$2x10^6$	7.41±4.90	22.22±0.00b	33.33±5.56b	37.04±6.68b	37.04±6.68b	37.04±6.68b
$2x10^7$	16.67±4.90	42.59±7.80a	51.85±8.07a	61.11±8.49a	61.11±8.49a	61.11±8.49a
Control	0.00±0.00	0.00±0.00d	0.00±0.00d	1.85±1.85d	1.85±1.85c	1.85±1.85c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
NS: not significant. (Pooled N=18)

4.1.10 Effect of isolate DLCO-AA-14 on 5th larval instars of the greater and lesser wax moths with different conidial concentrations over time

No mortality due to mycosis with isolate DLCO-AA-14 was observed in the 5th larval instars of the greater wax moth on day 3 after inoculation with the different spore concentrations and the control. However, from days 5 to 11, no significant difference ($P > 0.05$) in mortality of the treated larvae was observed between concentrations $2x10^4$, $2x10^5$ conidia/ml, and the controls. Significant differences ($P < 0.05$) in mortality due to the applied fungus were observed between concentrations of $2x10^6$ $2x10^7$ conidia/ml and the control on day 13 after treatment (Table: 19 and Appendix: 10a).

Mortality of the lesser 5th larval instars due to isolate DLCO-AA-14 increased gradually with time after inoculation. A significant difference in mortality was observed between fungal concentrations of $2x10^4$ and $2x10^7$ conidia/ml throughout the experimental period (Table: 20). No mortality was observed in the control on day 13 after inoculation (Appendix: 10b)

Table 19: Mean percent mortality of *Galleria mellonella* 5th larval instars treated with isolate DLCO-AA-14 at different concentrations over time

Concentration (Conidia/ml)	Percent mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00	1.85±1.85b	1.85±1.85c	3.70±1.85b	5.56±0.00b	5.56±0.00b
$2x10^5$	0.00±0.00	1.85±1.85b	3.70+3.70c	9.26±3.70b	16.67±5.56b	16.67±5.56b
$2x10^6$	0.00±0.00	7.41±4.90ab	20.37±1.85b	40.74±4.90a	53.70±8.07a	55.56±6.42a
$2x10^7$	0.00±0.00	12.96±7.41a	37.04±3.70a	50.00±6.42a	55.56±5.56a	61.11±8.49a
Control	0.00±0.00	1.85±1.85b	1.85±1.85c	5.56±3.21b	9.26±4.90b	9.26±4.90bc

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

Table 20: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate DLCO-AA-14 at different concentrations over time

Concentration (Conidia/ml)	Percent mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	1.85±1.85	1.85±1.85c	1.85±1.85c	1.85±1.85cd	1.85±1.85c	1.85±1.85c
$2x10^5$	0.00±0.00	9.26±1.85bc	12.96±1.85bc	14.81±3.70bc	14.81±3.7bc	14.81±3.7bc
$2x10^6$	1.85±1.85	12.96±6.15b	16.67±5.56b	27.78±6.42b	29.63±8.07b	29.63±8.07b
$2x10^7$	0.00±0.00	33.33+3.2a	51.85±3.2a	53.70±3.2a	55.56±3.2a	55.56±3.2a
Control	0.00±0.00	0.00±0.00c	0.00±0.00c	0.00±0.00d	0.00±0.00c	0.00±0.00c

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test). (Pooled N=18)

4.1.11 Effect of isolate DLCO-AA-109 on 5th larval instars of greater and lesser wax moths at different fungal concentrations over time

Mortality due to isolate DLCO-AA-109 in the 5th larval instars of the greater wax moth also ranged from approximately 2% to 24.07% on day 13 post treatment. On day 3, mortality due to mycosis was observed only with $2x10^6$ conidia/ml (1.83%). From days 5 to 13 post treatment, significant differences ($P < 0.05$) in larval mortality following treatment by different fungal concentrations were observed (Table: 21). No significant difference ($P > 0.05$) in mortality was observed between $2x10^4$, $2x10^5$, $2x10^6$ conidia/ml and the control. However, a significant difference in mortality was observed between concentrations of $2x10^4$, $2x10^5$, $2x10^6$ conidia/ml and $2x10^7$ conidia/ml. Mortality increased gradually over time and on day 13, the highest mortality (Table: 21 and Appendix: 11a) was recorded in insects treated with $2x10^7$ conidia/ml.

On day 3, a significant difference ($P < 0.05$) in target mortality was observed between the different fungal concentrations and controls in the 5th larval instars of the lesser wax moths. From days 5 to 13, higher significant differences ($P < 0.05$) in larval mortality following treatment with different fungal concentrations and control were observed (Table: 22). No mortality was recorded during the observation period following treatment with $2x10^4$ conidia/ml while mortality of 14.81% was attained with $2x10^7$ conidia/ml by day 13 after treatment (Appendix: 11b).

Table 21: Mean percent mortality of *Galleria mellonella* 5th larval instars treated with isolate DLCO-AA-109 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3 (NS)	5	7	9	11	13
$2x10^4$	0.00±0.00	0.00±0.00b	0.00±0.00b	1.85±1.85b	1.85±1.85b	1.85±1.85b
$2x10^5$	0.00±0.00	0.00±0.00b	0.00±0.00b	0.00±0.00b	1.85±18.5b	5.56±3.21b
$2x10^6$	1.85±1.85	1.85±1.85b	1.85±1.85b	5.56±0.00b	5.56±0.00b	7.40±1.85b
$2x10^7$	0.00±0.00	5.56±3.21a	9.26±3.70a	9.26±3.70a	18.52±4.90a	24.07±4.98a
Control	0.00±0.00	0.00±0.00b	0.00±0.00b	0.00±0.00b	1.85±1.85b	1.85±1.85b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
NS: not significant. (Pooled N=18)

Table 22: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate DLCO-AA-109 at different concentrations over time

Concentration (Conidia/ml)	Percentage mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00b	0.00±0.00a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
$2x10^5$	3.70±3.70a	5.56±3.21b	5.56±3.21ab	5.56±3.21ab	5.56±5.56ab	5.56±3.21ab
$2x10^6$	0.00±0.00b	1.85±1.85b	1.85±1.85b	1.85±1.85b	3.70±1.85b	5.56±5.56ab
$2x10^7$	0.00±0.00b	7.41±1.85a	9.26±1.85a	9.26±1.85a	14.81±4.90a	14.81±4.90a
Control	0.00±0.00b	1.85±1.85b	1.85±1.85b	1.85±1.85b	1.85±1.85b	1.85±1.85b

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test).
(Pooled N=18)

4.1.12 Effect of isolate IMI 330189 on 5th larval instars of the greater and lesser wax moth at different fungal concentrations over time

Mortality of the 5th larval instars of the greater wax moth due to isolate IMI 330189 ranged from approximately 2 % on day 3 to 9.3% by day 13 post treatment. No significant difference ($P > 0.05$) in larval mortality was observed between the different fungal concentrations and the controls on day 1 to 13 (Table: 23). However, mortality of 9.3% was observed with $2x10^6$ and $2x10^7$conidia/ml, respectively. No mortality was observed in the control throughout the experimental period (Appendix: 12a).

With the 5th larval instars of the lesser wax moth, no mortality due to isolate IMI 330189 was observed on day 3 post treatments. Further more, throughout the experimental period, no significant difference ($P > 0.05$) in mortality was observed between the different fungal concentrations and the control. Low mortality due to isolate IMI 330189 was observed through out the experimental period in the 5th larval instars of both the greater and lesser wax moth (Table: 24 and Appendix: 12b). This may imply that the fungus was not effective in causing mycosis to the larvae within that period of time.

Table 23: Mean percent mortality of *Galleria mellonella* 5th larval instars treated with isolate IMI 330189 at different concentrations over time

Concentration (Conidia/ml)	Percent mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85
$2x10^5$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.85±1.85
$2x10^6$	0.00±0.00	0.00±0.00	0.00±0.00	5.56±1.85	5.56±1.85	9.30±3.70
$2x10^7$	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85	5.56±1.85	9.30±3.70
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
All were not significant. (Pooled N=18)

Table 24: Mean percent mortality of *Achroia grisella* 5th larval instars treated with isolate IMI 330189 at different concentrations over time

Concentration (Conidia/ml)	Percent mortality by day after treatment (Mean±s.e)					
	3	5	7	9	11	13
$2x10^4$	0.00±0.00	0.00±0.00	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85
$2x10^5$	0.00±0.00	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85	1.85±1.85
$2x10^6$	0.00±0.00	1.65±1.85	1.85±1.85	1.85±1.85	1.85±1.85	3.70±1.85
$2x10^7$	0.00±0.00	0.00±0.00	3.70±1.85	3.70±1.85	3.70±1.85	3.70±1.85
Control	0.00±0.00	3.70±3.70	3.70±1.85	3.70±1.85	3.70±1.85	3.70±1.85

Means within a column followed by the same letter are not significantly different at $P > 0.05$ (Waller-Duncan Multiple Range Test)
All were not significant. (Pooled N=18)

4.1.13 Comparison of different fungal isolates on adult *Galleria mellonella* and *Achroia grisella* at $2x10^4$ conidia/ml

Adults of the moths were found to be susceptible to all fungal concentrations ranging from $2x10^4$ to $2x10^7$conidia/ml. Comparison of mortality results among the different fungal isolates applied was therefore made at lower concentration of $2x10^4$conidia/ml to select the most promising (virulent) isolate. Data from tables (1-12) at a concentration of $2x10^4$ conidia/ml is presented in the figures (1& 2). Percentage mortality of the greater wax moth adults due to the applied fungal isolates ranged from approximately 2 to 100% on day 14 post treatment with some fungal isolates. On day 8, there was no significant difference ($P > 0.05$) in mortality of the treated adults between isolates DLCO-AA-5 (61.26%), IMI 330189 (68.32%), DLCO-AA-83 (76.77%), DLCO-AA-14 (73.85%) and DLCO-AA-109 (62.27%), respectively. But a significant difference in mortality was observed between these isolates and isolate IITA 18 (Fig. 1).

As the number of days progressed, isolates DLCO-AA-5, IMI 330189 and DLCO-AA-83 caused high mortality (100%) in 13 days after inoculation of the adults. Isolates DLCO-AA-109, DLCO-AA-14, and IITA 18 and showed a relatively high mortality of 96.30%, 95.83% and 78.44%, respectively. In all cases, mortality of the greater wax moth adults treated with the different fungal isolates increased gradually post treatment until 100% was recorded with isolates DLCO-AA-5, IMI 330189, DLCO-AA-83 and DLCO-AA-109, respectively. On day 14, isolates DLCO-AA-14 and IITA 18 had cumulative percent mortalities of 96.30% and 84.92%, respectively (Fig.1). Analysis of variance (ANOVA) results showed that on day 2, no significant difference ($P > 0.05$) in mortality existed between the different fungal isolates (Appendix: 13).

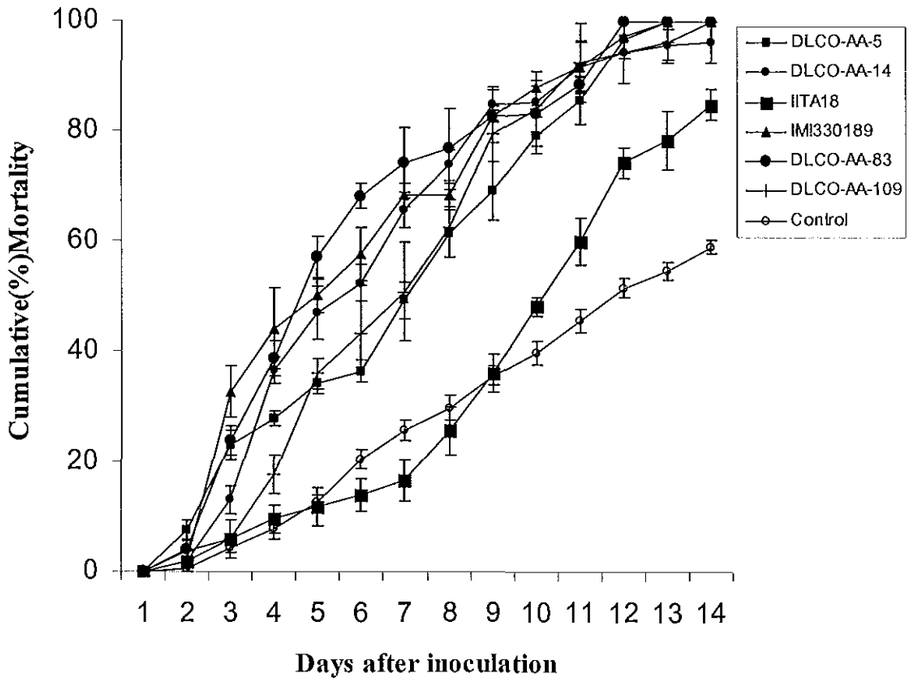

Figure 1. Cumulative mortality of *Galleria mellonella* adults treated with six fungal isolates at a concentration of $2x10^4$ conidia/ml.

Cumulative percentage mortality of the lesser wax moth adults following treatment with different fungal isolates ranged from 3.71% with fungal isolate IMI 330189 on day 2 to 97.43% with fungal isolate DLCO-AA-5 on day 8 post treatment (Fig. 2). On day 7, significant differences in mortality due to the applied fungi were observed with isolate IMI 330189, which caused low mortality through out the experimental period.

Similar to the adult greater wax moths, inoculated lesser wax moth adults started dying of mycosis on day 2 after treatment. Comparison of the fungal isolates revealed that isolates DLCO-AA-5, DLCO-AA-83, DLCO-AA-109 and IITA 18 caused high percentage mortalities of 97.43%, 91.67%, 88.89% and 88.33%, respectively on day 8 post treatment while isolates DLCO-AA-14 and IMI 330189 registered cumulative percentage mortalities of 78.52% and 67.05%. Mortality in the control increased gradually with time after treatment (Appendix: 14).

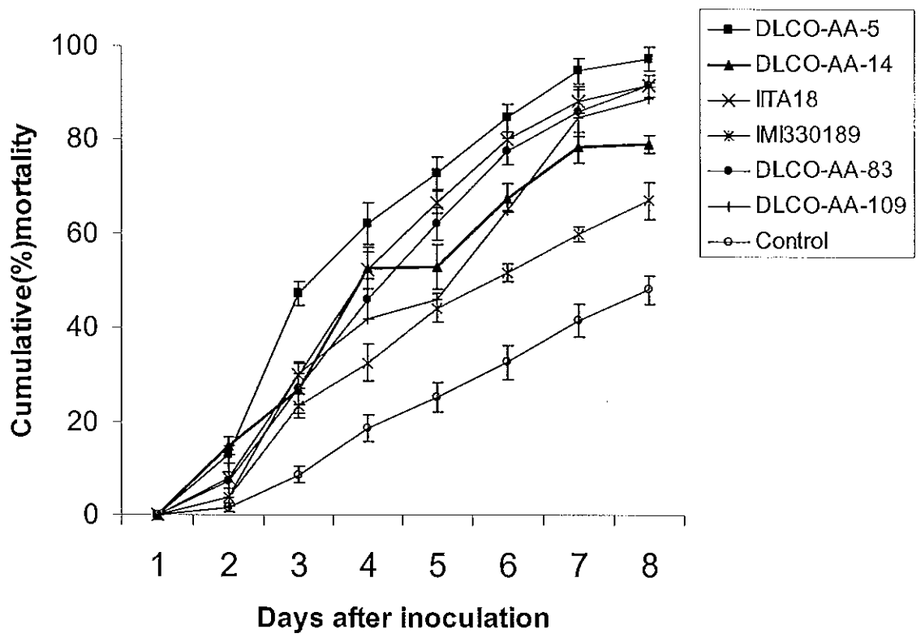

Figure 2. Cumulative mortality of *Achroia grisella* adults treated with six fungal isolates at a concentration of $2x10^4$ conidia/ml

4.1.14 Comparison of different fungal isolates on 5th larval instars of *Galleria mellonella* and *Achroia grisella* treated with $2x10^7$conidia/ml

Comparisons to select the most virulent isolates were carried out at a higher concentration of $2x10^7$ conidia/ml with the 5th larval instars of both the greater and lesser wax moths. This was done with an assumption that the higher conidial concentrations would result in a higher percentage mortality of the experimental insects. Data from tables (13-24) at a concentration of $2x10^7$ conidia/ml is presented in the figures (3&4). Mortality of the 5th larval instars of the greater wax moth due to infection by different fungal isolates began on day 1 with isolate DLCO-AA-5. There was no significant difference ($P > 0.05$) in mortality with all the fungal isolates on day 2. No mortality was recorded on day 2 with isolates DLCO-AA-14, IMI 330189, DLCO-AA-83 and DLCO-AA-109, respectively (Fig. 3). On day 3, all the different fungal isolates had registered some mortality except isolate DLCO-AA-14 with which mortality began on day 4 post treatment. Mortality of 5th larval instars of the greater wax moth ranged from approximately 2% to 64.81% by day 13 post treatment with fungal isolate IITA 18 (Fig. 3).

However, as the number of days progressed after treatment, isolates IITA 18, DLCO-AA-14, DLCO-AA-5, and DLCO-AA-83 showed relatively higher mortality by day 14 of 68.52%, 61.11%, 55.56% and 50%, respectively. Isolates DLCO-AA-109 and IMI 330189 showed a lower percentage mortality of 24.07% and 9.26%, respectively on day 14 post treatment (Fig. 3 and Appendix: 15). Mortality in the control was low by the end of the experimental period.

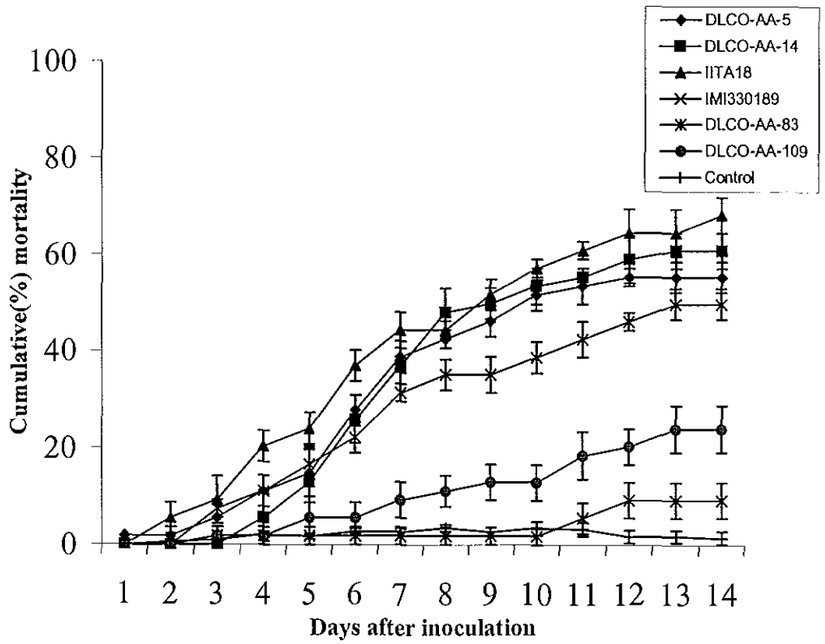

Figure 3. Cumulative mortality of *Galleria mellonella* 5th larval instars treated with six fungal isolates at a concentration of 2x10^{7}conidia/ml

Mortality due to infection with different fungal isolates in the 5th larval instars of the lesser wax moth began on day 2 with isolates DLCO-AA-5 (5.56%) and DLCO-AA-83 (5.56%) while no mortality was observed with the rest of the fungal isolates. On day 3, no significant differences ($P > 0.05$) in mortality due to mycosis were observed between the different fungal isolates (Fig. 4.) However, as the number of days post treatment progressed, isolates DLCO-AA-5 and DLCO-AA-14 caused high mortality of 61.11% followed by isolates DLCO-AA-83 (55.56%), IITA 18(40.74%), DLCO-AA-109 (14.81%) and IMI 330189 (3.7%), respectively (Appendix: 16) by day 14 after inoculation. Cumulative mortality in the control was 3.40% by the end of the experimental period.

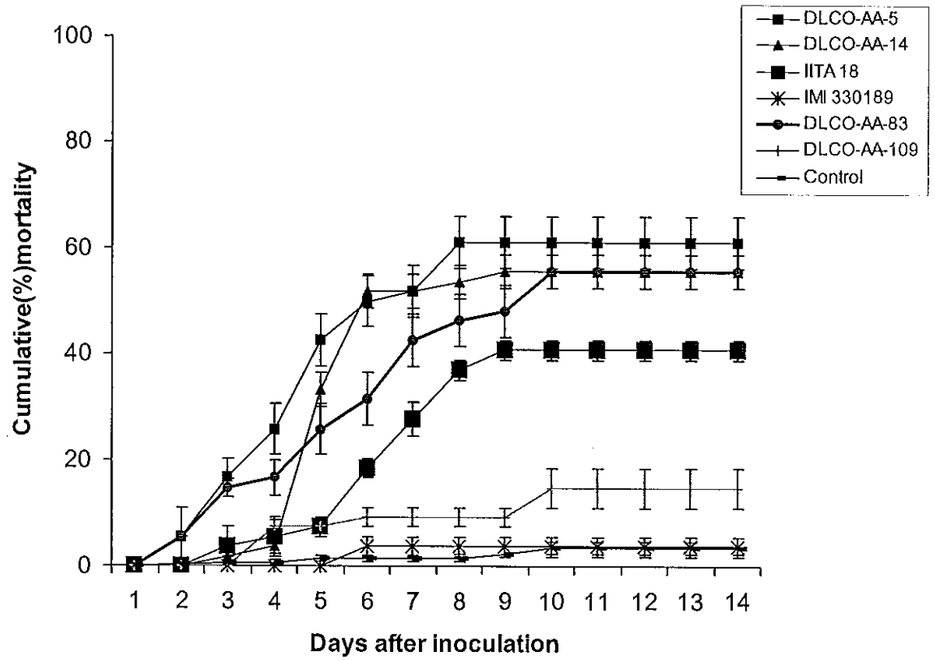

Figure 4. Cumulative mortality of 5th larval instars of *Achroia grisella* treated with six fungal isolates at a concentration of 2x10^{7}conidia/ml

4.1.15 Effect of different fungal isolates and concentrations on emergence of adult of *Galleria mellonella* and *Achroia grisella* from pupae.

Comparison of results on percentage emergence of both the greater and lesser adults showed that there was no significant difference ($P > 0.05$) in emergence of the adults after inoculation with each fungal isolate (Table: 25). Comparison of mortality results with the different conidial concentration of each fungal isolate showed that there was no significant difference in percentage emergence of the greater wax moth from pupae. All fungal isolates and concentration achieved percentage emergence of over 88.9 %. This shows that both the concentrations and fungal isolates were not effective in causing infection to the 6^{th} larval instars during the pupal stage (Appendix: 17)

The emerged insects mated in the petri dishes and started ovipositing masses of eggs on the food and in between the upper and lower lids of the petri dish. After 6-7 days, newly emerged larvae were observed. This evidenced that the eggs remained viable and probably horizontal fungal infection occurred after eclosion.

Table 25: Percentage emergence of *Galleria mellonella* and *Achroia grisella* adults after treatment with six different fungal isolates at different concentrations

		Greater Wax moth	Lesser Wax moth
Isolate	Concentration (Conidia/ml)	Percentage emergence	Percentage emergence
DLCO-AA-5	$2x10^4$	97.2±2.8	97.2±2.8
	$2x10^5$	94.4±2.8	94.4±2.8
	$2x10^6$	91.7±0.0	94.4±2.8
	2x107	86.1±7.4	100.0±0.0
	Control	97.2±2.8	97.2±2.8
DLCO-AA-14	$2x10^4$	97.2±0.0	94.4±2.8
	$2x10^5$	91.7±0.0	94.4±2.8
	$2x10^6$	91.7±0.0	94.4±2.8
	$2x10^7$	88.9±5.6	91.7±4.8
	Control	97.2±2.8	97.2±2.8
IITA I8	$2x10^4$	97.2±2.8	94.4±2.8
	$2x10^5$	97.2±2.8	91.7±4.8
	$2x10^6$	97.2±2.8	97.2±2.8
	$2x10^7$	94.4±0.0	88.9±2.8
	Control	100±0.0	100.0±0.0
IMI 330189	$2x10^4$	100±0.0	100.0±0.0
	$2x10^5$	100±0.0	97.2±2.8
	$2x10^6$	91.7±4.8	97.2±2.8
	18 $2x1810^7$	97.2±2.8	94.4±2.8
	Control	97.2±2.8	100.0±0.0

Table 25 cont'd

DLCO-AA-83	$2x10^4$	97.2±2.8	94.4±2.8
	$2x10^5$	100.0±0.0	97.2±2.8
	$2x10^6$	97.2±2.8	94.4±2.8
	$2x10^7$	91.7±4.8	94.4±2.8
	Control	97.2±2.8	88.9±7.4
DLCO-AA-109	$2x10^4$	97.2±2.8	100.0±0.0
	$2x10^5$	97.2±2.8	100.0±0.0
	$2x10^6$	91.7±4.8	94.4±2.8
	$2x10^7$	94.4±2.8	94.4±2.8
	Control	100.0±0.0	97.2±2.8

Means within a column followed by the same letter are not statistically significant at $P > 0.05$ (Waller -Duncan Multiple Range Test). (Pooled N=18).

4.2 Examination of sporulating fungi on cadavers

All the infected insects gave characteristic external sporulation of the respective fungi when surface sterilized as described in section 3.9. The first symptoms observed with the larvae were the appearance of brown spots (necrotic spots) at the invasion sites at the cuticle, which covered other parts of the body. They then lost their appetite, as they failed to make silken feeding tunnels, become apathetic, made no spontaneous movement and their irritability decreased more and more their bodies becoming stiff and mummified. The adults after surface sterilization, the fungus first emerged from the parts like the antennae, legs in weak membranes at the joints for all the surface sterilized stages of the test insects.

Summary of the effect of all tested fungal isolates on 5th larval instars of both the greater and lesser wax moths are presented (Annex: 2). The dead pupae were observed at the end of the experimental period by opening the pupal case. Those pupae, which did not show any movement of the tip of the abdomen when touched with a sharp needle, at least four times, were regarded as dead. Pupal mortality due to mycosis was generally found to be low.

4.3 Bioassay on honeybees, *Apis mellifera* (Ethiopian race)

4.3.1 Effect of three fungal isolates DLCO-AA-83, IITA 18 and IMI 330189 on honeybee colonies

With each fungal isolate, a total of 200 dead honeybees collected from the inoculated colonies in the different cages were surface sterilized. Of all the three fungal isolates tested against the honeybees, as non-target organisms, isolate IMI330189 (*Metarhizium* spp) had no effect; with isolate IITA 18 (*Beauveria* spp) 1 honeybee (0.5%) was infected while with isolate DLCO-AA-83 (*Metarhizium* spp) 2 honeybees (1%) were infected. The *Beauveria* infected honeybees produced a characteristics white sporulation while the *Metarhizium* infected honeybees produced a green characteristic sporulation when the spores were streaked on malt extract agar and incubated at 25^0C in the dark for 7 days.

5. DISCUSIONS

Fungal diseases of insects have been known since 1934 and at present, about 700 species of fungi in 700 genera are recognized to cause infection in insects (Goettel, 1992). Nevertheless of a few that have been developed for pest control, the potential of entomopathogens against the wax moth has not been established. In the present work, laboratory studies have shown that fungal isolates belonging to the genera *Beauveria* and *Metarhizium* have the potential towards the management of wax moths. Two standard isolates IMI 330189 (*Metarhizium anisopliae* var. *acridium*) and IITA 18 plus local isolates of DLCO-AA-5, DLCO-AA-14, DLCO-83, DLCO-AA-109, respectively were tested for their efficacy against adults, 5^{th} and 6^{th} larval instars of both the greater and lesser wax moths.

Since previous works on the wax moths using entomopathogens are not available, comparisons of results herein are based on previous similar works but on other groups of insects although it is however difficult to compare and reach conclusions based on previous and the present results for the target insects, the isolates, fungal concentrations, and conditions under which the experiments were conducted are all different.

Adults of both the greater and lesser wax moths were found to be susceptible to all fungal isolates and different fungal concentrations ranging from $2x10^4$ to $2x10^7$ conidia/ml used in the present work. Comparison of mortality results due to the applied fungal isolates was carried out at a lower concentration of $2x10^4$ conidia/ml on adults of the greater wax moth. The results revealed that infection of over 90% mortality could be achieved by day 13 post treatment with all fugal isolates except isolate IITA 18 (Fig. 1). Similar comparisons with the lesser wax moth at the same concentration of $2x10^4$ conidia/ml showed that, over 67%

mortality could be achieved in 8 days after inoculation. The present findings differ from similar work on adults of silverleaf whitely where mortality obtained using *Beauveria bassiana* (isolates MLC3 and L3009), resulted in 12.0% and 32.5%, respectively on 14 day post treatment (Adhanom Negasi *et al*., 1998).

However, findings with the adults of the greater and lesser wax moths in the present laboratory investigations; are in agreement with previous work using *Metarhizium flavoviride* (F1985). In which *Metarhizium flavoviride* (F1985) was topically applied on *Valanga irregularis* (Walker) an occasional pest of horticultural crops in northern Australia.100% mortality of *Valanga irregularis* (Walker) adults was observed with 85% of the dead insects producing conidia (Milner and Prior 1994), following post death incubation of target insects. Observations of *Valanga irregularis* (Walker) were also made at 13 day of post treatment. Similar results in the present work were obtained with the adults of the greater wax moths where 100% mortality was recorded with isolates DLCO-AA-5, IMI 330189 and DLCO-AA-109. Over 85% produced conidia when cadavers of infected insects were surface sterilized and incubated.

The present findings with the adult wax moths of both species in the laboratory investigations are also in agreement with similar findings on adults of *Glossina morsitans morsitans*. Tested isolates of *Beauveria bassiana* and *Metarhizium anisopliae* were found to be pathogenic for adult tsetse with high mortalities ranging between 60 and 95% on days 15-18 post infection and 100% mortalities by week 4 post treatments using an aqueous spore suspension of $2x10^7$ conidia/ml (Kaaya, 1989)

Findings with the adults of both the greater and lesser wax moths in the present work showed that even low fungal concentrations of $2x10^4$conidia/ml could cause mortality. This is because once the conidia penetrate the insect cuticle; the fungus multiplies inside the host. Mortality in

the control also increased gradually with time, but was low. The cause of death in the controls might have been due to aging, loss of appetite and stress. Females were observed to die after oviposition. Comparison of results of the fungal treated insects, by day 14 for the greater wax moths and by day 8 for the lesser wax moths showed that, no significant difference in mortality due to the applied fungi existed among the different concentration and isolates (Figures: 1 & 2). This may therefore witness that all the isolates had more or less similar virulence despite the difference in host origins (Annex: 1).

Interesting results were obtained with the 5th larval instars of both species. The different fungal isolates used in the present work were found to have varying degrees of virulence at the same concentration of $2x10^7$ conidia/ml. With the 5th larval instars of the greater wax moth, highest percentage mortality of 67.93% was recorded with isolate IITA 18 using $2x10^7$ conidia/ml while no mortality was obtained with isolate IMI 330189 using the same concentration (Fig. 3) However, with the lesser 5th larval instars, isolate DLCO-AA-5 registered the highest percentage kill of 60.38% on day 14 post treatment while low mortality was recorded with isolate IMI 330189 (Fig. 4)

Findings with the 5th larval instars of both the greater and lesser wax moths of isolates that caused over 60% mortality are in agreement with isolate *Beauveria bassiana* used on the first and third instars of the silverleaf whitefly. A significant difference in mortality based on variations in concentrations and isolates used was observed. The higher concentrations of $1x10^7$ conidia/ml caused more mortality due to mycosis as opposed to the lower concentrations of $1x10^4$ conidia/ml (Adhanom Negasi *et al.*, 1998). The present findings with the 5th larval instars are also in agreement with results obtained on *Scolytus scolytus* larvae and *Cylas formicarius* adult weevils where *Beauveria bassiana* required higher spore concentration and shorter post treatment periods to kill 50% of the worker population (Burdeos and Villacarlos, 1989). Unfortunately, the concentration used to treat *Scolytus*

scolytus larvae was not specified in the same literature cited. In the present work, other isolates including DLCO-AA-14 and DLCO-AA-83 also caused cumulative percentage mortality of above 50% of the treated larvae on day 14 of post treatment (Figs. 3 & 4).

The present findings further indicated that, the standard isolate IMI 330189 that gained much attention for locusts and grasshoppers programme biological control in Africa caused low mortality in the 5th larval instars of both the greater and lesser wax moths using $2x10^7$ conidia/ml by day 14 after treatment. This is possibly due to the host specificity of this isolate to acridid insect groups (Figs. 3 & 4)

Results obtained with 5th larval instars are also in agreement with findings of previous workers (Aguda and Rombach, 1986), who stated that fungal infection could best be evaluated after 3 to 7 days and up to 2 months or longer post application. Pachamuthu *et al.* (1999) also stated that, despite being effective biopesticide agents, the different strains of *Metarhizium anisopliae* required 21-42 days to achieve 90% mortality in the german cockroach. In the present work, some of the 5th larval instars of both the lesser and greater wax moths, that didn't die at the larval stage following inoculation, pupated and in most cases emerged as adults.

Lack of infection in 5th larval instars of both the greater and lesser wax moths treated with isolate IMI 330189 using $2x10^7$ conidia/ml was observed in the present work and is in agreement with the findings of low virulence to termites and failure to infect several species of beetles, coreid bugs, crickets and cockroaches (Kooyman, 1999). Low mortality of less than 50% and failure to infect the 5th larval instars of both species of wax moths may be due to a number of reasons including; failure to shed old cuticle and replace the new developing cuticle during the moulting process; or due to enzymatic defence mechanism of the target insects. These findings with the 5th larval instars of both the greater and lesser wax moths are

in agreement with the work on the elaterid larvae where failure to infect was attributed to the moulting process (Zacharuk, 1973; Vey and Fargues, 1977). Results of the present work (Tables: 15-20) are also in agreement with the work on *Beauveria bassiana*. This fungus was applied to tree trunks for the control of over wintering larvae of the codling moths resulting in negligible mortality in some cases and with those studied with highest control, less than 50% was observed (Falcon and Huber, 1991). The fungal concentration used was not however specified.

In general, observations in the present mortality data due to mycosis of the 5^{th} larval instars of both the greater and lesser wax moths showed that death increased more rapidly with more virulent isolates while was slower for less virulent ones. A similar trend using different fungal concentrations was observed where more mortality was obtained using higher concentration of $2x10^6$ and $2x10^7$ conidia/ml as opposed to low concentrations, which resulted in low or no mortality of the target insects. These findings in the present work with fungal isolates are in agreement with previous workers (Adane *et al.*, 1998) who found some isolates of *Beauveria* to be more virulent than others against the control of maize weevils and bean beetles.

Mortality results due to the toxic effect of the different isolates and concentrations on the emergence of adults from the pupae of both the greater and lesser wax moths showed that, the isolates had no effect on adult emergence when applied on the 6^{th} larval instars. No significant difference was thus observed between the treated and control insects suggesting lack of infection or inability of the fungus to penetrate the cuticle. Although still the experimental insects are of different groups, these findings are in agreement with work on *Glossina morsitans morsitans* (Kaaya, 1989). Newly larviposited larvae of *Glossina morsitans morsitans* were allowed to crawl and pupate on petri dishes containing well sporulated cultures of *Metarhizium anisopliae* and *Beauveria bassiana*. Normal adults emerged with no

increase in pupal mortality between exposed and non-exposed pupae. This might have been attributed to the molting process when the larvae change to pupa or the conditions in the pupal case might not be favorable for fungal growth or the fungus might require longer latency period than in the present study.

Findings on lack of infection due to the applied fungal isolates on the 6th larval instars before pupation in the present work are also in agreement with the previous work on *Metarhizium anisopliae* where an extended time period was required to cause sufficient mortality (McCoy, 1990). The 6th larval instars of both the greater and lesser wax moths entered pupation after treatment, adults emerged and oviposited viable eggs that hatched to larvae. These findings are also in agreement with previous work on the silverleaf whitefly (Aguda and Rombach, 1986).

Findings on emergence adults from pupae of the greater and lesser wax moths in the present study were also in disagreement with similar work on the larvae of *Glossina morsitans morsitans*. The larvae were allowed to pupate in the sand and high mortality due to treatment with *Beauveria bassiana* and *Metarhizium anisopliae* at 1.0g/litre spore concentration was observed in the newly emerged adults between 2 and 10 days post emergence (Kaaya and Munyinyi, 1995). This was not however the case with the wax moths as the newly emerged continued living, mated and the females laid viable eggs.

Immature insects are in general believed to be the most susceptible developmental stages of infection by hyphomycetous fungi (Osborne and Landa, 1992). However, the present findings under laboratory conditions are in disagreement with the greater and lesser wax moths for some fungal isolates. The 6th larval instars were not susceptible in the pupal stage as opposed to the adults that were found to be susceptible at all fungal isolates and concentrations used in the present study (Table: 25). This might be due to the molting

process, which may remove the penetrating fungus prior to the colonization of the insect or long incubation period required by some fungal isolates or inability of the fungus to penetrate the cuticle. The adults were found to be more susceptible than the larvae. This was probably due to stress resulting from confinement or loss of appetite following infection.

From laboratory investigations of the present work, it was observed that not all stages of the greater and lesser wax moths are equally susceptible to infection by applied entomopathogenic fungi. Pupal stages were the least affected while adults were more susceptible. The 5^{th} larval instars of both the greater and lesser wax moths were less or not susceptible at a concentration of $2x10^7$ conidial/ml to infection by the fungal isolates applied. The present laboratory based findings are in agreement with work on the larvae of the thrips, *Frankliniella occidentalis* that were found to be less susceptible to *Verticillium lecanni* and *Metarhizium anisopliae* than adults while later instars were less susceptible than earlier instars (Vestergaard *et al.*, 1995). Larvae of *Ostrina nubilalis* were found to be the most susceptible to infection by *Beauveria bassiana* when exposed at first instar while the fourth instars were most tolerant (Feng *et al.*, 1994).

Zimmermann (1986) used *Galleria mellonella* larvae to bait from moist soil. This insect was found to be very susceptible to pathogens, probably because of its unusually sheltered in beehives as normal habitats where fungal pathogens such as *Metarhizium anisopliae* may not reach and/or develop. Therefore, observations with adults and 5^{th} larval instars of both the greater and lesser wax moths in the present work have several implications for the use of myco-insecticides towards wax moth control. For instance, there was a reasonable correlation between mortality and fungal concentration with the 5^{th} larval instars of the greater wax moth with isolates IITA 18 and DLCO-AA-14 (Fig. 3) while the lesser wax moth with isolates DLCO-AA-5 and DLCO-AA-14 (Fig. 4) on day 14 of post treatment.

Other factors that might have attributed to the low mortality of 5^{th} and 6^{th} larval instars of both the greater and lesser wax moths may include:

- The insect behavior that may affect the site of penetration. In the present work, the larvae were observed to burrow into the artificial diet to make silken feeding tunnels; there is a possibility of scraping off the infectious inoculum before it penetrates the cuticle.
- Successful penetration also depends on the host diet. For example some insects maintained on artificial diet can be more susceptible to infection than insects fed on natural diet (Boucias *et al*, 1984, Goettel *et al*, 1993). Likewise, laboratory reared insects can be more susceptible than field fed collected ones (Bell and Hamalle, 1971). Both species of moths were reared on artificial diet. It might be one of the reasons why the adults were more susceptible.
- Low virulence with the 5^{th} and 6^{th} larval instars by some isolates may have been limited by humidity. The only humidity available was from the artificial diet in the petri dishes. Papierok and Hajek, (1997) stated that; for some fungi, humidities approaching saturation must be maintained in order to obtain infection.

For the control of the 5^{th} larval instars increasing conidial concentration from $2x10^7$ conidia/ml tested in the present work to $2x10^8$ conidia/ml would be recommendable since less than 50% mortality was observed with most isolates used. With the increased concentration, the number of conidia would be more and possibly increase chances of infection. With the adults, a lower conidial concentration of $1x10^4$ conidia/ml needs to be further investigated because they were found to be susceptible even at the lower of $2x10^4$ conidia/ml that was used in the present work.

Beneficial insects such as the honeybees are a group of non-target organisms meriting special attention when applying entomopathogens as myco-insecticides. Both *Beauveria* and *M.*

anisopliae have wide host ranges and numerous records of infection of other hymenoptera have been reported (Goettel *et al.*, 1990). Ideally, isolates of pathogens used as a myco-insecticide should have a narrow host range, not infecting important groups of beneficial arthropods and should not pose a large risk of creating epizootics in non-target species after release. Data obtained from the present laboratory investigations showed that with isolate DLCO-AA-83 (*Metarhizium* spp) 2 honeybees were infected; with isolate IITA 18 (*Beauveria* spp) 1 honeybee was infected while isolate IMI 330189 had no effect. Results obtained using isolate IMI 330189 in the present work are in agreement with the research findings in South Africa, where four viable and reproductive active *Apis mellifera scutellata* colonies were dusted with dry spores (approximately $5x10^{10}$ conidia/ml). The hives were situated near *Acacia galpinii* trees but all remained healthy with no trend, such as decline in food or brood reserves 7 months post-application (Price and Müller, 1994).

Ball *et al.* (1994) found less than 1% infection in honeybees (*Apis mellifera* Linnaeus) after exposure in the laboratory to the *Metarhizium* isolate IMI 330189. This is in disagreement with the observations made on the honeybee colonies (Ethiopian race) in the present work using isolate IMI 330189 under laboratory conditions. No spore sporulation on the surfacesterilized and incubated honeybees cadavers occurred. This isolate was therefore probably infective or required a long infection period or the spores were still in the resting phase in the hemocoel.

Limited mortality found in the honeybee as the result of infection by isolate IMI 330189 (Ball *et al.*, 1994) as indicated above, is in agreement with the mortality data recorded using isolate DLCO-AA-83, in which conidia sporulated when 2 cadavers obtained from a colony treated at a spore concentration of $2x10^7$ conidia/ml. Results of this study confirm the conclusion by Goettel *et al.* (1990) that fungal pathogens pose minimum risks to non-targets invertebrates.

Testing of these isolates under field conditions seems to be less pronounced than in laboratory assays as conditions differ significantly. Further investigations are needed to assess the performance of these isolates that caused some mortality in the beehive situation.

Isolate IITA 18 (*Beauveria* spp) showed external growth (sporulation) from only 1 dead honeybee after surface sterilizing cadavers from the treated colony. These findings are in agreement with work by Vandenberg (1990) who demonstrated that strains of *Beauveria bassiana* caused mycosis among honeybees treated with high doses of conidia, although the results were not pronounced for the fact that mortality was not significantly different from the control.

The present study confirms the pathogenicity of all the tested fungal isolates against adults of the greater and lesser wax moths and four of these isolates i.e. DLCO-AA-5, DLCO-AA-14, IITA 18 and DLCO-AA-83 for 5th larval instars could be used as potential microbial control agents. However, infection of the wax moths with these isolates was obtained under controlled laboratory conditions. These conditions might have enhanced the performance of the pathogen more than what could be expected in nature where sub-optimal conditions for growth of the pathogen, many different antagonists and adverse weather conditions prevail.

Therefore, beneficial insects such as the honeybee should be included in impact monitoring when groups of myco-insecticides are used under operational circumstances.

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Many genera and species of entomopathogenic fungi e.g. *Beauveria, Metarhizium* and *Paecilomycetes* have been extensively studied for their use as biological control agents for crop pests and some are already in commercial production. No information is available in literature regarding pathogenicity and biocontrol potentials of entomopathogenic fungi for the control of honeybee pests and predators that inhabit beehives especially the greater and lesser wax moths.

Entomopathogenic fungi constitute a unique group of insect pathogens in that ingestion by the host is not a prerequisite since infection results from direct penetration of the cuticle. Although used widely for the control of agricultural and forest pests, nothing has been made to evaluate entomopathogenic fungi for pests of apiculture such as the wax moths.

The effectiveness of a fungal pathogen is measured in terms of pathogenicity and speed within which it kills the target host. Taking this variable into account, for controlling adults of both species, all the fungal isolates are equally important at both early and late dates after treatment. The adults were found to be susceptible to all fungal concentrations ranging from $2x10^4$to $2x10^7$conidia/ml. With the 5^{th} larval instars however, a concentration of $2x10^7$ conidia/ml showed a degree of variation in percentage mortality with different isolates by day 14 post treatments. Isolate IITA 18 resulted in the highest percentage kill of the target insects by 68.52%, followed by isolate DLCO-AA-14 (61.11%), DLCO-AA-5 (55.56%), and DLCO-AA-83 (50%) while for the lesser wax moths, high mortality was observed with DLCO-AA-5 (61.11%), followed by, DLCO-AA-14 and DLCO-AA-83 (55.56%) and IITA 18 (40.74%).

The time taken to kill the larvae of the wax moths is a disadvantage, but reduced feeding behavior is an additional symptom of myco-insecticides. This could probably reduce the degree of damage caused by the wax moth larvae.

From findings of the present work, isolates of *Beauveria* performed better in terms of causing mortality in the 5th larval instars of both the greater and lesser wax moths than the *Metarhizium* isolates (IMI 330189 and DLCO 109) except isolate DLCO-AA-83. From the these laboratory findings, a myco-insecticide like fungus that suppresses the pest population by day 3 would be a better option but unfortunately fungal infection are slow and can not arrest damage by the pest if its population builds up. Fungus infection can be best evaluated after 3 to 7 days and up to two months or longer after application. Results in the present work obtained with the adults, 5th and 6th larval instars of both species of wax moths showed an increased rate of infection due to the applied fungus over time post treatment.

The adults were equally susceptible to all fungal isolates and at the different fungal concentrations while with the 5th larval instars mortality depended on the fungal isolate and concentration. The higher fungal concentrations caused more mortality than the lower ones that caused low mortality. Similarly, the more virulent isolates caused higher mortality than the less virulent ones. Therefore the most significant result is that the younger instars can be readily infected and killed, providing the most susceptible stage to target in a control strategy.

The standard Isolate IMI 330189 that gained much attention for locusts and grasshopper programme caused low mortality in the 5th larval instars of both the greater and lesser wax moths using $2x10^7$ conidia/ml by day 14 of post treatment. This isolate may therefore not be recommendable for use in their control.

The tested fungal isolates were not able to cause infection to 6th larval instars of both the greater and lesser wax moths larvae during the pupal stage. This might be because of the molting process that takes place immediately when the insect turns to the next stage, the conditions in the pupal case might not be favorable for fungal growth, or the fungus might require a long latency period to cause mortality.

The present study confirms pathogenicity of all the tested fungal isolates against the adults of the greater and lesser wax moths and five of the isolates i.e. DLCO-AA-5, DLCO-AA-14, IITA 18, DLCO-AA-83 and DLCO-AA-109 against the 5th larval instars. Further screening to determine the effective dose, exposure period, persistence, and application methods in the beehives are necessary. The potential of the fungal pathogens for bio-control of the greater wax moth and lesser wax moth need to be confirmed in large-scale field trials.

Beneficial insects like honeybees are a group of non-target insects meriting special attention when applying entomopathogens as myco-insecticides. Since both *Beauveria bassiana* and *Metarhizium anisopliae* have wide host ranges and numerous records of infection of other Hymenoptera have been reported (Goettel *et al.*, 1990). Therefore, the actual risk of using myco-insecticides needs to be determined under conditions that are close to the ones occurring in the field (Beehive situation). Unfortunately the interpretation of field experiments assessing side effects on non-target arthropods tend to be difficult, especially since these pathogens act relatively slowly and effects might theoretically even occur the year after treatment.

Despite the success of the past with microbial control of insect pests, there is a continuing need to discover and develop new entomopathogens if we are to meet the future needs of increased food and fibre production with concomitant reduction in use of chemical pesticides. Sustainable agriculture in the 21st century will relay increasingly on microbial

control and other interventions for pest management that are environmentally friendly (Lacey and Goettel, 1995). There is plenty of evidence that microbial pesticides can effectively control insect pests as long as the biology of the pathogen is taken into account (Payne, 1988).

The increasing problem of resistance to convectional pesticides, plus environmental and public safety concerns emphasizes the need to continue isolating and testing microbial organisms to develop a long term management of the wax moth but integrated with other control methods available.

6.2 RECOMMENDATIONS

- It is difficult to reduce pest population without affecting another component of the ecosystem e.g. pest's predators and parasites that inhabit the beehives. Therefore fungal control could be integrated with other available control methods used in the control of wax moths like management practices, physical control, biological and use of pheromone traps.
- The host-pathogen interaction with the desired out come of fungal infection with subsequent death of the host is a very intricate biological system. In order to successfully utilize the pathogen, a number of facts relating to the fungal and insect lifestyle needs to be addressed. For example, the mature larvae of the wax moths feed in silken galleries and female adults enter the hives at night when the bees are not active to lay eggs.
- There is need to fully understand the behavior of the honeybees before and after application of microbial control agents as it may affect their foraging and other activities.

- There is need to search for acceptable and cheap alternative to control the wax moth, which may be integrated into the existing control strategies for example specific entomopathogenic fungi and testing of these fungi in the beehive conditions.
- Continuous monitoring during field use after preliminary laboratory and field safety testing is recommended to ensure safety to non-target invertebrate especially the honeybees, which have a role of pollinating plants in the ecosystem.
- There is need to identify the local isolates. This would be a better option as compared to exotic ones, which have a potential to persist in the environment spreading over other areas and may pose risk to non-target organisms. Once exotic agents become established, it will likely be impossible to eradicate.
- Information gathering and dissemination on the on-going activities of bee pests and predators management need to be addressed and assist in the retrieval of appropriate literature. For example both species of the wax moth are widely distributed in Ethiopia, but its economic injury level has not been estimated.

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Annex 1: Fungal isolates used in the present work against the greater wax moth (*Galleria mellonella*) and the lesser wax moth (*Achroia grisella*) and their country of origin

Code	Isolate	Source	Country of origin
DLCO-AA-5	*Beauveria*	Grasshopper	Ethiopia
DLCO-AA-14	*Beauveria*	Grasshopper	Ethiopia
IITA 18	*Beauveria*	Coleoptera	Ethiopia
IMI 330189	*Metarhizium anisopliae* var. *acridium*	Grasshopper	Niger
DLCO-AA-83	*Metarhizium*	Grasshopper	Ethiopia
DLCO-AA-109	*Metarhizium*	Soil	Ethiopia

DLCO: Desert Locust Organization for Eastern Africa
IITA: International Institute of Tropical Agriculture
IMI: International Mycological institute

Annex 2: Summary of the effect of all tested fungal isolates on 5th larval instars of both *Galleria mellonella* and *Achroia grisella*

Fungal isolate	Concentration		Nº died at larval Stage		Nº died at in pupation	
		N	Greater Wax moth	Lesser Wax moth	Greater Wax moth	Lesser Wax moth
DLCO-AA- 5	$2x10^4$	18	2	12	4	1
	$2x10^5$	18	3	12	8	3
	$2x10^6$	18	17	20	6	2
	$2x10^7$	18	32	33	7	1
	Control	18	2	1	0	1
DLCO-AA-14	$2x10^4$	18	3	1	1	1
	$2x10^5$	18	10	11	6	1
	$2x10^6$	18	31	11	3	2
	$2x10^7$	18	31	31	7	4
	Control	18	5	0	0	0
IITA 18	$2x10^4$	18	7	5	3	4
	$2x10^5$	18	16	4	6	4
	$2x10^6$	18	26	15	6	1
	$2x10^7$	18	32	13	9	7
	Control	18	1	1	2	0
IMI 330189	$2x10^4$	18	1	1	4	0
	$2x10^5$	18	3	1	2	3
	$2x10^6$	18	6	3	3	1
	$2x10^7$	18	5	2	4	1
	Control	18	0	3	0	0
DLCO-AA-83	$2x10^4$	18	7	6	2	2
	$2x10^5$	18	10	8	0	0
	$2x10^6$	18	16	17	3	1
	$2x10^7$	18	28	28	4	2
	Control	18	3	1	0	0
DLCO-AA- 109	$2x10^4$	18	2	0	2	1
	$2x10^5$	18	6	3	5	2
	$2x10^6$	18	13	5	7	1
	$2x10^7$	18	17	5	5	2
	Control	18	1	1	2	1

Appendix 1: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* treated with DLCO-AA-83 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentrations	829.604	4	207.401	2.114	.154
	Within Groups	981.224	10	98.122		
DAY5	Concentrations	6535.012	4	1633.753	9.017	.002
	Within Groups	1811.897	10	181.190		
DAY7	Concentrations	4588.146	4	1147.037	11.669	.001
	Within Groups	982.936	10	98.294		
DAY9	Concentrations	3536.152	4	884.038	11.200	.001
	Within Groups	789.344	10	78.934		
DAY11	Concentrations	2030.605	4	507.651	5.398	.014
	Within Groups	940.507	10	94.051		
DAY13	Concentrations	3191.988	4	797.997	12.987	.001
	Within Groups	614.449	10	61.445		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	111.111	4	27.778	6.750	.007
	Within Groups	41.152	10	4.115		
DAY3	Concentration	505.940	4	126.485	2.225	.139
	Within Groups	568.499	10	56.850		
DAY4	Concentration	849.806	4	212.452	2.343	.125
	Within Groups	906.856	10	90.686		
DAY5	Concentration	2005.691	4	501.423	9.778	.002
	Within Groups	512.809	10	51.281		
DAY6	Concentration	2634.686	4	658.672	3.153	.064
	Within Groups	2088.780	10	208.878		
DAY7	Concentration	2038.117	4	509.529	3.357	.055
	Within Groups	1517.992	10	151.799		
DAY8	Concentration	2195.572	4	548.893	4.266	.029
	Within Groups	1286.585	10	128.658		

Appendix 2: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* adults treated with IITA 18 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
D1	Concentration	.000	4	.000	.	.
	Within Groups	.000	10	.000		
D3	Concentration	148.106	4	37.027	3.595	.046
	Within Groups	102.991	10	10.299		
D5	Concentration	1749.131	4	437.283	3.826	.039
	Within Groups	1142.999	10	114.300		
D7	Concentration	1391.931	4	347.983	2.925	.077
	Within Groups	1189.813	10	118.981		
D9	Concentration	1907.599	4	476.900	6.513	.008
	Within Groups	732.263	10	73.226		
D11	Concentration	2076.008	4	519.002	1.980	.174
	Within Groups	2620.923	10	262.092		
D13	Concentration	1874.780	4	468.695	3.495	.049
	Within Groups	1340.913	10	134.091		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	67.951	4	16.988	.679	.622
	Within Groups	250.189	10	25.019		
DAY3	Concentration	1727.645	4	431.911	4.467	.025
	Within Groups	966.808	10	96.681		
DAY4	Concentration	2608.971	4	652.243	4.896	.019
	Within Groups	1332.266	10	133.227		
DAY5	Concentration	3444.502	4	861.125	4.852	.020
	Within Groups	1774.771	10	177.477		
DAY6	Concentration	5905.096	4	1476.274	10.332	.001
	Within Groups	1428.837	10	142.884		
DAY7	Concentration	6687.420	4	1671.855	23.610	.000
	Within Groups	708.124	10	70.812		
DAY8	Concentration	6120.222	4	1530.056	28.110	.000
	Within Groups	544.303	10	54.430		

Appendix 3: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* adults treated with DLCO-AA-5 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	1555.877	4	388.969	7.223	.005
	Within Groups	538.516	10	53.852		
DAY5	Concentration	2819.507	4	704.877	6.299	.008
	Within Groups	1119.004	10	111.900		
DAY7	Concentration	4483.707	4	1120.927	5.045	.017
	Within Groups	2221.949	10	222.195		
DAY9	Concentration	4902.515	4	1225.629	5.093	.017
	Within Groups	2406.727	10	240.673		
DAY11	Concentration	4222.146	4	1055.536	4.396	.026
	Within Groups	2400.903	10	240.090		
DAY13	Concentration	5360.587	4	1340.147	6.285	.009
	Within Groups	2132.304	10	213.230		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	646.091	4	161.523	2.907	.078
	Within Groups	555.556	10	55.556		
DAY3	Concentration	4391.117	4	1097.779	10.990	.001
	Within Groups	998.904	10	99.890		
DAY4	Concentration	6259.634	4	1564.909	28.745	.000
	Within Groups	544.406	10	54.441		
DAY5	Concentration	10248.661	4	2562.165	47.423	.000
	Within Groups	540.282	10	54.028		
DAY6	Concentration	14108.754	4	3527.188	47.810	.000
	Within Groups	737.757	10	73.776		
DAY7	Concentration	11306.280	4	2826.570	63.495	.000
	Within Groups	445.167	10	44.517		
DAY8	Concentration	10112.497	4	2528.124	32.701	.000
	Within Groups	773.093	10	77.309		

Appendix 4: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* a dults t reated w ith D LCO-AA-14 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentrations	1098.765	4	274.691	1.669	.233
	Within Groups	1646.091	10	164.609		
DAY5	Concentrations	4970.980	4	1242.745	9.637	.002
	Within Groups	1289.493	10	128.949		
DAY7	Concentrations	7582.522	4	1895.631	20.156	.000
	Within Groups	940.465	10	94.046		
DAY9	Concentrations	7191.037	4	1797.759	65.740	.000
	Within Groups	273.465	10	27.346		
DAY11	Concentrations	7190.823	4	1797.706	33.969	.000
	Within Groups	529.218	10	52.922		
DAY13	Concentrations	5470.195	4	1367.549	54.346	.000
	Within Groups	251.639	10	25.164		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	884.774	4	221.193	10.750	.001
	Within Groups	205.761	10	20.576		
DAY3	Concentration	2738.040	4	684.510	3.073	.068
	Within Groups	2227.688	10	222.769		
DAY4	Concentration	6684.280	4	1671.070	22.141	.000
	Within Groups	754.733	10	75.473		
DAY5	Concentration	6788.294	4	1697.073	18.094	.000
	Within Groups	937.931	10	93.793		
DAY6	Concentration	7074.409	4	1768.602	11.997	.001
	Within Groups	1474.175	10	147.417		
DAY7	Concentration	7221.857	4	1805.464	35.220	.000
	Within Groups	512.618	10	51.262		
DAY8	Concentration	5295.144	4	1323.786	48.888	.000
	Within Groups	270.782	10	27.078		

Appendix 5: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* adults treated with DLCO-AA-109 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	1066.000	4	266.500	6.796	.007
	Within Groups	392.157	10	39.216		
DAY5	Concentration	3322.561	4	830.640	41.704	.000
	Within Groups	199.173	10	19.917		
DAY7	Concentration	4441.664	4	1110.416	14.238	.000
	Within Groups	779.889	10	77.989		
DAY9	Concentration	8558.852	4	2139.713	60.132	.000
	Within Groups	355.835	10	35.583		
DAY11	Concentration	7747.237	4	1936.809	27.112	.000
	Within Groups	714.383	10	71.438		
DAY13	Concentration	4206.391	4	1051.598	17.404	.000
	Within Groups	604.243	10	60.424		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	31.138	4	7.784	.206	.929
	Within Groups	378.327	10	37.833		
DAY3	Concentration	1682.679	4	420.670	6.308	.008
	Within Groups	666.903	10	66.690		
DAY4	Concentration	1666.796	4	416.699	3.640	.044
	Within Groups	1144.723	10	114.472		
DAY5	Concentration	5412.871	4	1353.218	4.663	.022
	Within Groups	2901.901	10	290.190		
DAY6	Concentration	6263.868	4	1565.967	18.819	.000
	Within Groups	832.103	10	83.210		
DAY7	Concentration	5811.000	4	1452.750	19.204	.000
	Within Groups	756.484	10	75.648		
DAY8	Concentration	4010.067	4	1002.517	23.056	.000
	Within Groups	434.820	10	43.482		

Appendix 6: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* adults treated with IMI 330189 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	concentrations	2209.763	4	552.441	8.216	.003
	Within Groups	672.391	10	67.239		
DAY5	concentrations	3926.588	4	981.647	14.554	.000
	Within Groups	674.476	10	67.448		
DAY7	concentrations	5902.550	4	1475.638	42.163	.000
	Within Groups	349.987	10	34.999		
DAY9	concentrations	7600.935	4	1900.234	65.123	.000
	Within Groups	291.791	10	29.179		
DAY11	concentrations	6013.980	4	1503.495	27.477	.000
	Within Groups	547.181	10	54.718		
DAY13	concentrations	4505.663	4	1126.416	51.111	.000
	Within Groups	220.386	10	22.039		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Concentration	53.498	4	13.374	.433	.782
	Within Groups	308.642	10	30.864		
DAY3	Concentration	914.009	4	228.502	5.015	.018
	Within Groups	455.671	10	45.567		
DAY4	Concentration	578.561	4	144.640	1.810	.204
	Within Groups	799.336	10	79.934		
DAY5	Concentration	1116.568	4	279.142	2.365	.123
	Within Groups	1180.512	10	118.051		
DAY6	Concentration	1107.424	4	276.856	2.655	.096
	Within Groups	1042.636	10	104.264		
DAY7	Concentration	2904.957	4	726.239	5.364	.014
	Within Groups	1353.846	10	135.385		
DAY8	Concentration	3517.070	4	879.267	9.098	.002
	Within Groups	966.391	10	96.639		

Appendix 7: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* 5th larval instars treated with DLCO-AA-83 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	Sig.
DAY3	Concentration	164.609	4	41.152	1.250	.351
	Within Groups	329.218	10	32.922		
DAY5	Concentration	390.947	4	97.737	1.696	.227
	Within Groups	576.132	10	57.613		
DAY7	Concentration	1625.514	4	406.379	5.809	.011
	Within Groups	699.588	10	69.959		
DAY9	Concentration	1921.811	4	480.453	6.868	.006
	Within Groups	699.588	10	69.959		
DAY11	Concentration	2687.243	4	671.811	13.060	.001
	Within Groups	514.403	10	51.440		
DAY13	Concentration	3539.095	4	884.774	12.647	.001
	Within Groups	699.588	10	69.959		

(b)

		Sum of Squares	df	Mean Square	F	p-value
D3	Concentration	358.025	4	89.506	8.700	.003
	Within Groups	102.881	10	10.288		
D5	Concentration	1139.918	4	284.979	2.716	.091
	Within Groups	1049.383	10	104.938		
D7	Concentration	3798.354	4	949.588	12.473	.001
	Within Groups	761.317	10	76.132		
D9	Concentration	4580.247	4	1145.062	13.573	.000
	Within Groups	843.621	10	84.362		
D11	Concentration	5502.058	4	1375.514	30.386	.000
	Within Groups	452.675	10	45.267		
D13	Concentration	5502.058	4	1375.514	30.386	.000
	Within Groups	452.675	10	45.267		

Appendix 8: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* 5th larval instars treated with IITA 18 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	156.379	4	39.095	1.727	.220
	Within Groups	226.337	10	22.634		
DAY5	Concentration	1008.230	4	252.058	3.311	.057
	Within Groups	761.317	10	76.132		
DAY7	Concentration	3279.835	4	819.959	4.239	.029
	Within Groups	1934.156	10	193.416		
DAY9	Concentration	4641.975	4	1160.494	5.937	.010
	Within Groups	1954.733	10	195.473		
DAY11	Concentration	6432.099	4	1608.025	10.561	.001
	Within Groups	1522.634	10	152.263		
DAY13	Concentration	6551.440	4	1637.860	10.904	.001
	Within Groups	1502.058	10	150.206		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	176.955	4	44.239	2.389	.120
	Within Groups	185.185	10	18.519		
DAY5	Concentration	411.523	4	102.881	12.500	.001
	Within Groups	82.305	10	8.230		
DAY6	Concentration	1995.885	4	498.971	34.643	.000
	Within Groups	144.033	10	14.403		
DAY7	Concentration	3456.790	4	864.198	70.000	.000
	Within Groups	123.457	10	12.346		
DAY11	Concentration	3279.835	4	819.959	56.929	.000
	Within Groups	144.033	10	14.403		
DAY13	Concentration	3279.835	4	819.959	56.929	.000
	Within Groups	144.033	10	14.403		

Appendix 9: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b *Achroia grisella* 5th larval instars treated with DLCO-AA-5 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	Sig.
DAY1	Concentration	8.230	4	2.058	1.000	.452
	Within Groups	20.576	10	2.058		
DAY3	Concentration	69.959	4	17.490	8.500	.003
	Within Groups	20.576	10	2.058		
DAY5	Concentration	423.868	4	105.967	3.219	.061
	Within Groups	329.218	10	32.922		
DAY7	Concentration	3016.461	4	754.115	28.192	.000
	Within Groups	267.490	10	26.749		
DAY9	Concentration	4390.947	4	1097.737	31.382	.000
	Within Groups	349.794	10	34.979		
DAY11	Concentration	5938.272	4	1484.568	65.591	.000
	Within Groups	226.337	10	22.634		
DAY13	Concentration	6185.185	4	1546.296	44.206	.000
	Within Groups	349.794	10	34.979		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentrations	555.556	4	138.889	1.023	.441
	Within Groups	1358.025	10	135.802		
DAY5	Concentrations	3201.646	4	800.412	11.788	.001
	Within Groups	679.012	10	67.901		
DAY7	Concentrations	4609.053	4	1152.263	18.667	.000
	Within Groups	617.284	10	61.728		
DAY9	Concentrations	5954.733	4	1488.683	20.097	.000
	Within Groups	740.741	10	74.074		
DAY11	Concentrations	5773.663	4	1443.416	18.461	.000
	Within Groups	781.893	10	78.189		
DAY13	Concentrations	5773.663	4	1443.416	18.461	.000
	Within Groups	781.893	10	78.189		

Appendix 10: Summary table for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* 5th larval instars treated with DLCO-AA-14 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY5	Concentration	296.296	4	74.074	1.385	.307
	Within Groups	534.979	10	53.498		
DAY7	Concentration	2901.235	4	725.309	32.045	.000
	Within Groups	226.337	10	22.634		
DAY9	Concentration	5707.819	4	1426.955	19.264	.000
	Within Groups	740.741	10	74.074		
DAY11	Concentration	7209.877	4	1802.469	19.909	.000
	Within Groups	905.350	10	90.535		
DAY13	Concentration	8477.366	4	2119.342	21.020	.000
	Within Groups	1008.230	10	100.823		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentraion	12.346	4	3.086	.750	.580
	Within Groups	41.152	10	4.115		
DAY5	Concentraion	2127.572	4	531.893	14.361	.000
	Within Groups	370.370	10	37.037		
DAY7	Concentraion	5246.914	4	1311.728	21.250	.000
	Within Groups	617.284	10	61.728		
DAY9	Concentraion	5855.967	4	1463.992	23.717	.000
	Within Groups	617.284	10	61.728		
DAY11	Concentraion	6337.449	4	1584.362	21.389	.000
	Within Groups	740.741	10	74.074		
DAY13	Concentraion	6337.449	4	1584.362	21.389	.000
	Within Groups	740.741	10	74.074		

Appendix 11: Summary tables for analysis of variance (ANOVA) of (a) *Galleria mellonella* and *Achroia grisella* 5th larval instars treated with DLCO-AA-109 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	8.230	4	2.058	1.000	.452
	Within Groups	20.576	10	2.058		
DAY5	Concentration	74.074	4	18.519	3.000	.072
	Within Groups	61.728	10	6.173		
DAY7	Concentration	193.416	4	48.354	4.700	.022
	Within Groups	102.881	10	10.288		
DAY9	Concentration	320.988	4	80.247	6.500	.008
	Within Groups	123.457	10	12.346		
DAY11	Concentration	625.514	4	156.379	7.600	.004
	Within Groups	205.761	10	20.576		
DAY13	Concentration	1020.576	4	255.144	9.538	.002
	Within Groups	267.490	10	26.749		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	32.922	4	8.230	1.000	.452
	Within Groups	82.305	10	8.230		
DAY5	Concentration	111.111	4	27.778	2.250	.136
	Within Groups	123.457	10	12.346		
DAY7	Concentration	164.609	4	41.152	3.333	.056
	Within Groups	123.457	10	12.346		
DAY9	Concentration	164.609	4	41.152	3.333	.056
	Within Groups	123.457	10	12.346		
DAY11	Concentration	399.177	4	99.794	4.042	.033
	Within Groups	246.914	10	24.691		
DAY13	Concentration	390.947	4	97.737	3.393	.053
	Within Groups	288.066	10	28.807		

Appendix 12: Summary table for analysis of variance (ANOVA) of (a) *Galleria mellonella* and (b) *Achroia grisella* 5th larval instars treated with IMI 330189 at different concentrations over time

(a)

		Sum of Squares	df	Mean Square	F	P-Value
DAY3	Concentration	12.346	4	3.086	.750	.580
	Within Groups	41.152	10	4.115		
DAY5	Concentration	12.346	4	3.086	.750	.580
	Within Groups	41.152	10	4.115		
DAY7	Concentration	12.346	4	3.086	.750	.580
	Within Groups	41.152	10	4.115		
DAY9	Concentration	61.728	4	15.432	1.500	.274
	Within Groups	102.881	10	10.288		
DAY11	Concentration	94.650	4	23.663	1.643	.239
	Within Groups	144.033	10	14.403		
DAY13	Concentration	238.683	4	59.671	2.231	.138
	Within Groups	267.490	10	26.749		

(b)

		Sum of Squares	df	Mean Square	F	p-value
DAY3	Concentration	8.230	4	2.058	1.000	.452
	Within Groups	20.576	10	2.058		
DAY5	Concentration	28.807	4	7.202	1.167	.382
	Within Groups	61.728	10	6.173		
DAY7	Concentration	12.346	4	3.086	.300	.871
	Within Groups	102.881	10	10.288		
DAY9	Concentration	12.346	4	3.086	.300	.871
	Within Groups	102.881	10	10.288		
DAY11	Concentration	12.346	4	3.086	.300	.871
	Within Groups	102.881	10	10.288		
DAY13	Concentration	12.346	4	3.086	.300	.871
	Within Groups	102.881	10	10.288		

Appendix 13: Summary table for analysis of variance (ANOVA) of *Galleria mellonella* adults treated with different fungal isolates at $2x10^4$ conidia/ml over time

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Fungal isolates	70.893	5	14.179	1.345	.311
	Within Groups	126.455	12	10.538		
DAY3	Fungal isolates	1752.960	5	350.592	6.877	.003
	Within Groups	611.730	12	50.978		
DAY4	Fungal isolates	2668.316	5	533.663	8.395	.001
	Within Groups	762.840	12	63.570		
DAY5	Fungal isolates	3852.054	5	770.411	7.848	.002
	Within Groups	1178.051	12	98.171		
DAY6	Fungal isolates	5365.719	5	1073.144	12.683	.000
	Within Groups	1015.343	12	84.612		
DAY7	Fungal isolates	6553.837	5	1310.767	10.245	.001
	Within Groups	1535.343	12	127.945		
DAY8	Fungal isolates	5184.957	5	1036.991	11.921	.000
	Within Groups	1043.820	12	86.985		
DAY9	Fungal isolates	5249.848	5	1049.970	14.843	.000
	Within Groups	848.878	12	70.740		
DAY10	Fungal isolates	3324.415	5	664.883	15.500	.000
	Within Groups	514.755	12	42.896		
DAY11	Fungal isolates	2350.601	5	470.120	4.117	.021
	Within Groups	1370.385	12	114.199		
DAY12	Fungal isolates	1303.470	5	260.694	5.876	.006
	Within Groups	532.408	12	44.367		
DAY13	Fungal isolates	1054.666	5	210.933	7.098	.003
	Within Groups	356.631	12	29.719		
DAY14	Fungal isolates	546.956	5	109.391	2.656	.077
	Within Groups	494.203	12	41.184		

Appendix 14: Summary table for analysis of variance (ANOVA) of *Achroia grisella* adults treated at $2x10^4$ conidia/ml with different fungal isolates over time

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Fungal isolates	322.509	5	64.502	2.912	.060
	Within Groups	265.767	12	22.147		
DAY3	Fungal isolates	1076.745	5	215.349	2.181	.125
	Within Groups	1184.680	12	98.723		
DAY4	Fungal isolates	1537.761	5	307.552	3.578	.033
	Within Groups	1031.375	12	85.948		
DAY5	Fungal isolates	2014.272	5	402.854	6.127	.005
	Within Groups	788.999	12	65.750		
DAY6	Fungal isolates	2190.546	5	438.109	4.187	.020
	Within Groups	1255.604	12	104.634		
DAY7	Fungal isolates	2340.418	5	468.084	7.055	.003
	Within Groups	796.174	12	66.348		
DAY8	Fungal isolates	1767.353	5	353.471	5.045	.010
	Within Groups	840.687	12	70.057		

Appendix 15: Summary table for analysis of variance (ANOVA) of *Galleria mellonella* 5th larval instars treated at 2x10^{7}.conidia/ml with different fungal isolates over time

		Sum of Squares	df	Mean Square	F	p-value
DAY1	Fungal isolate	8.573	5	1.715	1.000	.458
	Within Groups	20.576	12	1.715		
DAY2	Fungal isolate	75.446	5	15.089	2.200	.122
	Within Groups	82.305	12	6.859		
DAY3	Fungal isolate	198.903	5	39.781	2.320	.108
	Within Groups	205.761	12	17.147		
DAY4	Fungal isolate	754.458	5	150.892	1.956	.158
	Within Groups	925.926	12	77.160		
DAY5	Fungal isolate	955.075	5	191.015	1.663	.218
	Within Groups	1378.601	12	114.883		
DAY6	Fungal isolate	2786.351	5	557.270	3.869	.025
	Within Groups	1728.395	12	144.033		
DAY7	Fungal isolate	4540.466	5	908.093	5.634	.007
	Within Groups	1934.156	12	161.180		
DAY8	Fungal isolate	5612.140	5	1122.428	6.175	.005
	Within Groups	2181.070	12	181.756		
DAY9	Fungal isolate	6592.936	5	1318.587	7.394	.002
	Within Groups	2139.918	12	178.326		
DAY10	Fungal isolate	8184.156	5	1636.831	11.932	.000
	Within Groups	1646.091	12	137.174		
DAY11	Fungal isolate	7585.734	5	1517.147	11.344	.000
	Within Groups	1604.938	12	133.745		
DAY12	Fungal isolate	7674.897	5	1534.979	12.097	.000
	Within Groups	1522.634	12	126.886		
DAY13	Fungal isolate	7498.285	5	1499.657	9.718	.001
	Within Groups	1851.852	12	154.321		
DAY14	Fungal isolate	7992.112	5	1598.422	11.098	.000
	Within Groups	1728.395	12	144.033		

Appendix 16: Summary table for analysis of variance (ANOVA) of *Achroia grisella* 5th larval instars treated at 2 x10^7 conidia/ml with different fungal isolates over time

		Sum of Squares	df	Mean Square	F	p-value
DAY2	Fungal isolate	123.457	5	24.691	.800	.571
	Within Groups	370.370	12	30.864		
DAY3	Fungal isolate	913.923	5	182.785	1.719	.205
	Within Groups	1275.720	12	106.310		
DAY4	Fungal isolate	1332.305	5	266.461	3.790	.027
	Within Groups	843.621	12	70.302		
DAY5	Fungal isolate	3655.693	5	731.139	7.227	.002
	Within Groups	1213.992	12	101.166		
DAY6	Fungal isolate	4171.811	5	834.362	8.689	.001
	Within Groups	1152.263	12	96.022		
DAY7	Fungal isolate	5406.379	5	1081.276	15.380	.000
	Within Groups	843.621	12	70.302		
DAY8	Fungal isolate	7875.514	5	1575.103	17.332	.000
	Within Groups	1090.535	12	90.878		
DAY9	Fungal isolate	8259.602	5	1651.920	19.661	.000
	Within Groups	1008.230	12	84.019		
DAY10	Fungal isolate	8429.355	5	1685.871	26.573	.000
	Within Groups	761.317	12	63.443		
DAY11	Fungal isolate	8609.396	5	1721.879	23.910	.000
	Within Groups	864.198	12	72.016		
DAY12	Fungal isolate	9020.919	5	1804.184	22.874	.000
	Within Groups	946.502	12	78.875		
DAY13	Fungal isolate	9252.401	5	1850.480	19.985	.000
	Within Groups	1111.111	12	92.593		
DAY14	Fungal isolate	9252.401	5	1850.480	19.985	.000
	Within Groups	1111.111	12	92.593		

Appendix 17: Summary table for analysis of variance (ANOVA) of *Galleria mellonella* and *Achroia grisella Achroia grisella* after treatment with six different fungal isolates at different concentrations

Species	Fungal Isolate	Variation	Sum of Squares	Mean Square	df	F	p-value
Greater Wax moth	DLCO-AA-5	Concentration	64.815	16.204	4	.875	.52
		With groups	185.185	18.519	10		
	DLCO-AA-14	Concentration	46.296	11.574	4	.250	.903
		With groups	462.963	46.296	10		
	IITA 18	Concentration	231.481	57.87	4	2.08	.158
		With groups	277.78	27.78	10		
	IMI 330198	Concentration	64.81	16.204	4	1.17	.38
		With groups	138.89	13.889	10		
	DLCO-AA-83	Concentration	203.704	50.926	4	1.10	.408
		With groups	462.963	46.296	10		
	DLCO-AA-109	Concentration	92.593	23.143	4	.83	.534
		With groups	277.778	27.778	10		
Lesser Wax moth	DLCO-AA-5	Concentration	259.259	64.815	4	1.40	.303
		With groups	462.963	46.296	10		
	DLCO-AA-14	Concentration	166.667	41.667	4	1.50	.274
		With groups	277.778	27.778	10		
	IITA 18	Concentration	46.296	11.574	4	.625	.655
		With groups	185.185	18.519	10		
	IMI 330198	Concentration	138.889	34.722	4	1.50	.274
		With groups	231.481	23.143	10		
	DLCO-AA-83	Concentration	111.111	27.778	4	1.0	.452
		With groups	277.778	27.778	10		
	DLCO-AA-109	Concentration	120.370	30.093	4	1.083	.452
		With groups	277.778	27.778	10		