

SUSCEPTIBILITY OF THE RICE WEEVIL, *SITOPHILUS ORYZAE*
(COLEOPTERA: CURCULIONIDAE)
TO NATIVE ENTOMOPATHOGENIC FUNGAL ISOLATES

ASHENAFI KASSAYE



A THESIS SUBMITTED TO
THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE MASTER OF SCIENCE IN BIOLOGY (INSECT SCIENCE)

ADDIS ABABA UNIVERSITY
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List of abbreviations

DE	Diatomaceous earth
DLCOEA	Desert locust control Organization for East Africa
IPM	Integrated Pest Management
LT ₅₀	50 % lethal time
MEA	Malt extract agar
PDA	Potato dextrose agar
SDA	Sabouraud dextrose agar

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ABSTRACT

The experiments on susceptibility of the rice weevil, Sitophilus oryzae (coleoptera: curculionidae) to native entomopathogenic fungal isolates were conducted at Addis Ababa University Insect Science Research Laboratory. The initial cultures of Sitophilus oryzae were obtained from Holeta Agricultural Research centre. Five different local fungal isolates were used in the study. The isolates obtained from Addis Ababa University Insect Science Research laboratory and Desert Locust Control Organization for East Africa (DLCO-EA) Addis Ababa. The target concentrations of 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia mL^{-1} were adjusted for liquid formulations. All experiments were replicated three times. The fungal isolates were evaluated by direct spraying of spore suspensions over the target insects. All fungal isolates used in the present study were able to infect and cause death in adults of Sitophilus oryzae at all concentrations ranging from 1×10^4 to 1×10^8 conidia mL^{-1} . The present study showed that Sitophilus oryzae treated with 1×10^8 conidia mL^{-1} , AAU D is observed to be virulent with the least LT_{50} value 3.52 days and 96.6% mortality in day 10, DLCO 131 killed 83.33% of the target insect in day 10 with LT_{50} value 4.45 days, DLCO 141 with LT_{50} value 5.32 days and 76.67% mortality, DLCO 26 with LT_{50} value 6.53 days and 70.00% mortality 7 and DLCO 91 with LT_{50} value 6.21 days and 60.00 % mortality in day 10. The present study suggests that biological control of Sitophilus oryzae with local entomopathogens make in Ethiopia.

Key words: Sitophilus oryzae, Entomopathogen, pathogenecity, Metarhizium, Beauveria

1- INTRODUCTION

Cereals are an important part of both human and livestock diets. After harvesting unprocessed cereal product, it will be stored for various lengths of time. Subsequent to the initial storage period it may also be stored at processing premises. Whilst in storage, cereals are at risk to infestation by a wide range of stored product insects and mites (Wakefield, 2006).

For the past five decades, humans have almost been wholly dependent upon synthetic/organic insecticides. Agriculture has been revolutionized by the use of chemicals for crop protection. Consequently, increasing attention has been directed toward natural enemies such as predators, parasitoids, and pathogens. It has become evident that there is an urgent need for a biological agent, possessing the desirable properties of a chemical pesticide making it highly toxic to the target organism, which can be mass produced on an industrial scale, has a long shelf life and can be safely transported (Moazami, 2008).

The use of biological control agents as biopesticides has wide scope in Integrated Pest Management (IPM). Their use has resulted in environmentally sound pest control methods with less danger towards the development of resistance, and no lethal effect on non-target organisms (Dhuyo and Selman, 2007).

Insect pathogens have a long history of recognition despite the relatively recent understanding of microbial infections. Descriptions of entomopathogenic fungi can be found in drawings of several centuries ago (Samson *et al.*, 1988). By 1762 the first successful importation of an organism from one country to another for biological control took place with the introduction of the Mynah bird from India to the island of Mauritius, for locust control. In the last 50 years, microbial control of pests and plant diseases showed an amazing development associated with

pronounced good results under optimized laboratory conditions, followed many times by disappointing results in the field applications (Samson *et al.*, 1988). Thus, we need to understand the important concepts required to produce reliable, effective, and safe entomopathogens for microbial control (El-Husseini, 2006). Under natural conditions, fungi are a frequent and often important natural mortality factor in insect populations. All groups of insects may be affected and over 700 species of fungi have been recorded as pathogens. Some species are facultative generalist pathogens, such as *Aspergillus* and *Fusarium*. However, most species are obligate pathogens, often quite specific and rarely found (Milner, 2000). The potential of entomopathogenic fungi as biocontrol agents against several weevil species has been also evaluated (Adane *et al.*, 2003).

Entomopathogenic fungi have been shown to be effective biological control agents against several insect pests. Recently, there is also a growing interest in the use of mycopathogens for the development of biological control strategies for stored product pests. Stored-product insects are serious pests of dried, stored, durable agricultural commodities, and of many value-added food products and non food derivatives of agricultural products worldwide (Phillips and Throne, 2009). Stored-product insects can cause serious postharvest losses, estimated from up to 9% in developed countries to 20% or more in developing countries (Pimentel, 1991).

There is an increasing need to examine alternatives to the use of chemical pesticides in grain storage and biological control methods have received renewed interest over the past decade. The various biological control agents that could be used include macroorganisms such as parasitoids and predators and microorganisms such as bacteria, viruses and fungi. There is an increasing evidence that entomopathogenic fungi have the potential for control of arthropod pest species in the storage environment (Wakefield, 2006).

Until present, limited number of published articles on biocontrol of stored grain insects using entomopathogenic fungi are available. *Beauveria bassiana*, for example, has proven to be highly effective against the major stored grain insects: *Sitophilus oryzae*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis* (Smith *et al.*, 1999).

The rice weevil *Sitophilus oryzae* is a cosmopolite insect theoretically originated in India and spread all around the world through infested and ship-transported grains (Metcalf and Flint, 1962). Its morphological and biological characteristics are similar to the *S. zeamais* species (Gallo *et al.*, 2002).

Stored grain is vulnerable to both external and internal damage by insects; internal infestations are generally considered the most damaging. granary weevil develop through larval stages and into adults while inside the wheat kernels and leaves an exit hole in the seed once the insect reaches the adult stage and emerges. Internal insect infestation of wheat kernels degrades quality and value of wheat and is one of the most difficult defects to detect. Insect infestation causes grain loss by consumption, contaminating the grain with excrement and fragments, causes nutritional losses, and degrades end-use quality of flour (Pearson *et al.*, 2003).

Objective

General objective

The general objective of this study is to develop an IPM control for Rice weevil, *Sitophilus oryzae* using native fungal isolates

Specific objective

- To evaluate the potential of local Entomopathogenic fungal isolates against adults *S. oryzae* under laboratory conditions
- To optimize lethal dose of fungal isolates under laboratory condition
- To compare the media preference of native isolates of *Metarhizium* and *Beauveria* against adult Granary weevils.

2. LITERATURE REVIEW

The Class Insecta contains nearly one million described species which comprise approximately 67% of the world's described fauna and flora. Insects are central to the performance of many ecosystem processes (Shah and Pell, 2003).

For the past 15 years, research with entomogenous fungi has benefited from a renewed interest, which arose from a better understanding of the role of natural phenomena in the regulation of insect populations, among which epizootics caused by fungi have played a determinant part in certain cases. Furthermore, there was a need to develop new techniques of biological control for the expanding application of the concepts of integrated pest control (Draganova *et al.*, 2006).

A number of insect pathogens have been tested for control of stored-product insects, but none is in common use because of lack of sufficient, broad-spectrum efficacy. Many tests have been conducted to synergize pathogens with other control technologies, particularly those that might be expected to increase efficacy of pathogens. Laboratory evaluations of the commercially available fungi *Beauveria bassiana* and *Metarhizium anisopliae* and the bacterium *Bacillus thuringiensis* (Bt), alone or in conjunction with another insecticidal material such as DE, generally result in complete control of only some stages of some species, while other stages or species are poorly controlled (Kavallieratos *et al.*, 2006).

Fungal diseases in insects are common and widespread and often decimate insect populations in spectacular epizootics. Virtually all insect orders are susceptible to fungal diseases. Fungi infect insects by breaching the host cuticle; they are the principal pathogens among sucking insects because these hosts cannot ingest other pathogens that infect through the gut wall. Fungi are also

particularly important for control of Coleoptera, because viral and bacterial diseases are rare among beetles. Entomopathogenic fungi are associated with insects living in diverse habitats, including fresh water, soil, soil surfaces, and aerial locations (Hajek and St. Leger, 1994).

There are 100 genera and 1000 fungal species that are entomopathogenic (Jenkins and Goettel, 1997; Bidochka *et al.*, 2000). Entomopathogenic fungi are found in the divisions Zygomycota, Ascomycota and Deuteromycota (Samson *et al.*, 1988).

The most studied for the control of insects and mites belong to the genera *Beauveria* and *Metarhizium* (Dolinski and Lacy, 2007). Entomopathogenic fungi such as *M. anisopliae* (Metschnikoff) Sorokin and *Beauveria* spp. are among the biological control agents used against insect pests (Emiru and Merid, 2007).

2.1 Storage insect Pests

The coleopteran and Lepidoptera are of primary importance as pest of stored grain. Larvae and adults of the former group and only larvae of the latter are doing the damage (El-Husseini, 2006). Over 60 species of insects infest stored grains. Lesser grain borer, rice weevils, maize weevils, Cadelle beetles, Flat grain beetles, Rusty grain beetles, Sawtoothed grain beetles, Foreign grain beetles, Mealworm beetles, Red flour beetles, Confused flour beetles, Indian meal moths, book lice, and grain mites are considered to be the 14 main pests (Herbert, 2009).

The stored-grain environment is unique among most agroecosystems in that it is entirely Human-made and not subject to rapid and extreme changes in environmental conditions (Phillips and Throne, 2009). There are complexes of insect pests that infest grain, and the particular species present depend upon the type of grain. All cereal grains and many stored legumes are infested by pests whose larvae either feed and develop inside the kernel or develop outside intact kernels.

The internal-feeding insects have been referred to historically as primary pests, while those feeding outside the kernels on broken and fine material have been referred to as secondary pests (Phillips and Throne, 2009).

2.2 Biology of Rice weevil

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most widespread pests and causes heavy losses of stored grain both quantitatively and qualitatively throughout the world (Madrid *et al.*, 1990; Lucas and Riudavets, 2000; Park *et al.*, 2003).



Figure 1: Rice Weevil (Canadian Grain Commission, 2009)

The rice weevil is small, 1/10 inch (2 to 3 mm) and stout in appearance. It is very similar in appearance to the granary weevil. However, the rice weevil is reddish-brown to black in color with four light yellow or reddish spots on the corners of the elytra (the hard protective forewings). The snout is long (1 mm), almost 1/3 of the total length. The head with snout is as long as the prothorax or the elytra. The prothorax (the body region behind the head) is strongly pitted and the elytra have rows of pits within longitudinal grooves. The larva is legless and stays

inside the hollowed grain kernel. It is fat with a cream colored body and dark head capsule (Koehler, 2008).

The adult is small; it is 2.5 to 4 mm long. It is dark brown. The adult has a long, distinctive snout and elbowed antennae. It has 4 distinct reddish patches on the elytra. The adult can fly. Breeding conditions are temperatures between 15°C and 34°C and a relative humidity at 40%. The female lays most eggs within the first 4 weeks after it emerges. It chews a small hole in the grain kernel to lay one egg and covers the hole with a waxy secretion. The female lays approximately 150 eggs in its lifetime (Canadian Grain Commission, 2009).

The larva develops in the kernel. The larva is white, grub-like and legless. Under optimal temperature conditions between 27°C and 31°C, the rice weevil's life cycle is complete in 5 to 8 weeks. The shortest development time is 25 days at 30°C and 70% relative humidity. Development stops if the temperature falls below 17°C. The larva pupates in the kernel. (Koehler, 2008). Once the adult emerges from the kernel, it mates and lays eggs immediately. Adults live from 4 to 12 months. The rice weevil is distributed in tropical environments. However, it is becoming established in temperate environments due to changes in the transportation and storage of rice (Canadian Grain Commission, 2009).

2.3 Economic importance

Rice weevils mainly attack whole grains such as wheat, corn, barley and rice, however they have been found actively breeding in such foods as macaroni and spaghetti when those materials are old and have become caked. They also have been reported to feed on beans, nuts, cereals, and have been observed sucking the juice from apples and pears (Park et al., 2003)

The adults feed basically on the same foods as the larvae but not as restricted in their diets because the larvae need to develop inside whole grains. Flight also helps rice weevils infest grains stored at far ends of warehouses. Most infestation, however originated from nearby sources on infested grain (White and Leesch, 1995)

In good production years farmers in Africa harvest and store cereals and pulse crops for several months mostly in simple storage structures at farm or village levels. However, as in field crops, all stored products are attacked by a wide range of insect pests (Subramanyam and Hagstrum, 1995). Despite the fact that the stored grain insect pests are categorized as one of the most important pests, they have not received much attention for research priority in Ethiopia (Emana *et al.*, 2003). Consequently, little information is available on the identity of the species involved in the infestation, their relative importance and their control options. In the store, in Ethiopia insect pests causes loses ranging from 20 to 30 % (Abraham, 1996). Ethiopia's annual pesticide purchase amount more than 3000metric tones, valued at nearly US\$ 20 million and insecticides constitute about 71% of the total purchase (Tsedeke, 1997). It is therefore crucial to reduce these loses by applying feasible and environmentally friendly pest management strategies (Alemnesh , 2007).

Recent explorations for entomopathogens conducted in Ethiopia, have resulted in the collection of hundreds of isolates of entomopathogenic fungi with potential for control of a number of major insect pests of stored grain (Adane *et al.*, 2003)

Wheat is one of the major cereal crops in the Ethiopian highlands which range between 6 and 16°N, 35 and 42°E, and from 1500 to 2800 m. Ethiopian wheat production is increased each year

from the year 1960 up to date, (U.S. Army Centre for Health Promotion and Preventive Medicine, Entomological Sciences Program, 2007).

2.4 Control measures

Control of these insect populations around the world is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticides and the fumigants methyl bromide and phosphine (Subramanyam and Hagstrum, 1995). These are still the most effective treatments for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance. It has had undesirable effects on non-target organisms, and fostered environmental and human health concerns (White and Leesch, 1995). The use of methyl bromide will be prohibited in the near future because of its ozone depletion potential and high toxicity (Subramanyam and Hagstrum, 1995). These problems have highlighted the need for the development of selective insect-control alternatives.

2.5 Microbial control of insects

Recent years have seen rapid development of the field of insect pathology, and our knowledge of microbial diseases of insects is fast accumulating. Moreover, the use of microorganisms in the control of insect pests appears to be entering a renaissance. A pathogen is a microorganism, including viruses, that causes disease (Brewer, 1994).

Experimentation during the past decade has proved that microbial control, or the use of pathogenic microorganisms, is an integral part of applied insect control. Microbial control is of special value when other methods have been found inadequate, for example, when insecticidal

residues are toxic to man, insect parasites, and predators. Although some insect pathogens may replace certain insecticides, parasites, and predators, most of the successful applications of pathogens in the future will probably be made in conjunction with these and other agents of control (Tanada, 1959).

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often considerable scope as plant protection agents against several insects (Noris *et al.*, 2002). These pathogens when conserved and augmented would keep some of the pests under check within the economic injury levels. In other cases, the pathogens cultured in vitro are employed either for short-term control equivalent to chemical control or long-term biological control (Jayaraj, 1986).

As to Harper (1987) Cited in Lacy *et al* (2001), strategies for the use of entomopathogenic organisms for insect control are basically the same as that for other biological control agents. They may be used to augment naturally occurring pathogens (augmentation), conserved or activated in nature (conservation), introduced into pest populations as classical biological control agents to become established and exert long-term regulation of the pest (inoculative release), or used inundatively for rapid short-term control (inundative release). In this presentation some of the successes on entomopathogenic fungi microbial control have been high lighted speculate on future developments (Lacy *et al.*, 2001).

2.6 Entomopathogenic fungus as a microbial control

Entomogenous fungi in nature cause a regular and tremendous mortality of many pests in many parts of the world and do, in fact, constitute an efficient and extremely important natural control factor (Evans, 1999). Fungal pathogens can be exploited as biological agents for the management of agricultural pests, including the most problematic groups: weeds, insects (and invertebrates in

general) and diseases (Evans, 1999). Entomopathogenic fungus *B. bassiana* and some parasitoids have been tested for control of *S. granarius* and some other storage pests (Trdan, *et al.*, 2005).

Most of entomopathogenic fungi belong to Deuteromycetes. Some 700 species of entomopathogenic fungi have been reported, but only 10 of these have been or are currently being developed for insect control (Hajek and St. Leger, 1994). Most of them are included in 11 genera, i.e., *Lagnidium*, *Entomophthora*, *Neozygites*, *Erynia*, *Ashersonia*, *Verticillium*, *Nomuraea*, *Hirsutella*, *Metarhizium*, *Beauveria*, and *Paecilomyces* (Roberts and Wraight, 1986).

The early successful application with an entomopathogenic fungus dated back to 1878 by the Russian scientist Metchnikoff using *M. anisopliae* against the wheat leaf beetle, *Anisoplia austriaca* and the beet weevil *Cleonus punctiventris* in sugar beet fields (Narayanan, 2004).

2.6.1 Mechanism of fungal infection

Pathogenicity is the qualitative ability of a pathogen to cause disease and it is determined by a variety of factors including the physiology of the host (e.g. defence mechanisms), the physiology of the fungus (e.g. pathogenicity factor such as production of enzymes and toxins) and environment (Butt *et al.*, 2001).

Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their hosts but invade directly through the cuticle and so can, potentially, be used for control of all insects including sucking insects (Milner, 2000). Differing from bacteria and viruses fungi can infect insects not only through the gut, but also through spiracles and particularly through the surface of the integument. This property leads directly to the theoretical possibility of infecting insects independently of their feeding activity (Ferron, 1978).

Asexually produced fungal spores or conidia are generally responsible for infections and are dispersed throughout the environment in which the insect hosts are present. When conidia land on the cuticle of the suitable host, they attach and germinate initiating cascades of recognition enzyme activation reactions both by the host and the fungal parasite (Samson *et al.*, 1988).

Unlike other insect pathogens, fungi infect the host by contact, penetrating the insect cuticle. The host can be infected both by direct treatment and by transmission of inoculums from treated insects or cadavers to untreated insects or to subsequent developmental stages via the new generation of spores. These unique characters make entomopathogenic fungi especially important for the control of concealed insects. (Lacey *et al.*, 1999; Quesada-Moraga, *et al.*, 2004)

The fungus multiplies within the insect and kills it; death is due to toxin production by the fungus or multiplication to inhabit the entire insect (Kannan *et al.*, 2008). The conidia germinate on contact with the cuticle of the insect, produce a penetrating germ tube and establish a systemic infection which kills the host in 7 to 21 days depending on conditions, especially temperature and dose (Milner, 2000). Fungi usually cause insect mortality by one or more of the following: nutritional deficiency, invasion and destruction of tissues, and release of toxins (Tanada and Kaya, 1993).

Following death, the fungus often grows saprophytically within the host. Soon after host death, and under favourable conditions, hyphae emerges from the cadaver; they produce conidiogenous cells, sporulation occurs on the host surface and the conidia are liberated. Conidial dispersal is passive, relying principally on wind and other factors, such as rain, can play a role in dissemination (Butt *et al.*, 2001).

Insect mycopathogens may overcome the internal defence response by utilizing one or more of the following strategies. Either the fungal cells developing within the insect may possess an outer coat, which is neutral to circulating haemocytes or they are effectively masked by host proteins or by producing immuno- modulating substances which suppress the cellular defence system; thereby the fungal cells may be tolerant to the humoral and cellular defence system of the insects (Hung *et al.*, 1993). Insect mycopathogens such as *B. bassiana* and *M. anisopliae* undergo *in vivo* development cycle which includes the following stages in Chart 1 (Narayanan, 2004).

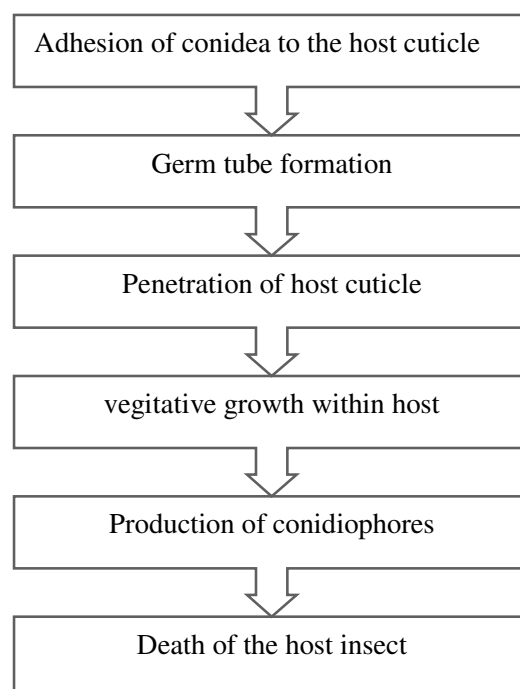


Chart 1 Insect mycopathogens developmental stages *in the insect* (Narayanan, 2004).

2.6.2 Insect resistance to fungus

Insect immunity more closely resembles the innate immune system of vertebrates. This system entails rapid, relatively nonspecific responses, both cellular and humoral, to invading organisms

and may have appeared quite early in the evolution of animals. Host specificity observed with many insect pathogens demonstrates that insect species are naturally resistant to these microorganisms (Narayanan, 2004).

Insects are continuously exposed to potentially pathogenic microorganisms and eukaryotic parasites, but only a few encounters result in infection. Insects possess a complex and efficient system of biological defense against pathogens and parasites (Gillespie *et al.*, 1997).

A pathogen or parasite that succeeds in invading the body is recognized as foreign. This chemical/physical recognition may involve soluble proteins present in the hemolymph, as well as proteins (receptors) localized at the surface of hemocytes or other cells. The initial recognition event may bring about communication to other populations of cells through molecules that act as signals to stimulate a response. The recognition stimulus and the secondary signals trigger signal transduction pathways that produce an action by a cell: cell adhesion followed by phagocytosis or encapsulation by hemocytes, release of signalling factors, or induced transcription of genes for production of antimicrobial proteins in fat body and other tissues as shown in Chart 2 (Janeway, 1994).

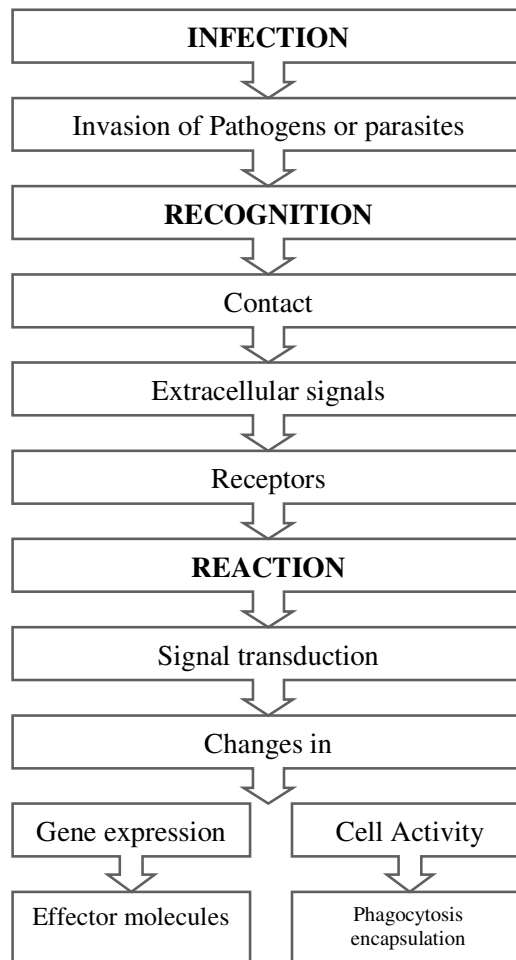


Chart 2 A model for insect immune responses (Janeway, 1994).

2.6.3 Abiotic factors affecting mass production and pathogenicity isolates

Both biotic and abiotic factors are responsible for the losses in activity of various microbial control agents (Alves *et al.*, 1996). The stored grain ecosystem is dynamic. The interactions of abiotic and biotic factors influence storage conditions and the deterioration of stored products. The best way to maintain the quality of stored products is to ensure that storage conditions do not favour the development of insects and fungi (Niewiada *et al.*, 2005).

Fungal species, and sometimes even their strains or isolates, behave in a very different way depending both on biotic (insect host range, infection level, germination rate) and abiotic (humidity and especially temperature) factors (Slantas *et al.*, 2008).

Two of the most important environmental factors affecting the ability of an entomopathogenic fungus to infect and overcome host are humidity and temperature (Erron *et al.*, 1991). Temperature is an important factor in selecting an isolate for development as a mycoinsecticide. Most isolates grow well between 15° and 30°C, although some develop at temperatures as low as 5-10°C and others grow even at 35-40°C (Milner, 2000).

The knowledge on growth requirements of entomopathogenic fungi is more essential for their mass production and pathogenicity (Sreeramakumar *et al.*, 2002). The nutritional requirements of entomopathogenic fungi vary with the fungal species and even the fungal strains under consideration.

Generally fungi require oxygen, water, source of carbon, inorganic or organic nitrogen besides minerals that play a major role in growth and their pathogenicity including novel metabolite production (Samson, 1988). Optimization of glycerol and erythritol content has increased the germination rate of these fungi, enhanced germination at low water availability, and improved pathogenicity at low relative humidity (Hallsworth and Magan, 1995).

2.6.4 The use of entomopathogenic fungus to control granary weevil

Biological control for stored products has been used as early as 1887 in London, England, when an infestation of the Mediterranean flour moth in a warehouse was controlled by a parasitoid

(Brower *et al.*, (1996). A few other incidents have been reported over the intervening years but biological control has never become an accepted practice because there have been few successful trials with biological control agents in stored grain, no possible procedure or agent can be recommended at this time. A general knowledge of biological control methods is necessary to consider future developments (Muir, 2000).

3. MATERIALS AND METHODS

The experiments were conducted at Addis Ababa University Insect Science Research Laboratory. Infested wheat seed with *Sitophilus oryzae* was obtained from Holeta Agricultural Research Centre and the adults were removed by sieving in every five days and the wheat grains were kept at the same conditions until the emergence of the next generation.

Fungal isolates obtained from Addis Ababa University Insect Science Research Laboratory and Desert Locust Control Organization for East Africa (DLCO-EA) was used. None of the isolates were obtained from the pest insects tested here but had been isolated originally from other coleopteran in different agro-ecological zones of Ethiopia (Table 1). In this study, one *Beauveria* isolate, DLCO 141, and four *Metarhizium* isolates, DLCO 131, AAU, DLCO 26, and DLCO 91 were used.

Table 1: Fungal isolates used in the study

No	Code	Isolated from	Collected by	Collected from	Isolated by	Genera
1	DLCO-131	Grasshopper	Esayas & Yilma			<i>Metarhizium</i>
2	DLCO-141	Grasshopper	Esayas & Yilma			<i>Beauveria</i>
3	DLCO-91	Grasshopper	Esayas & Yilma	Debre-Zeit	Esayas	<i>Metarhizium</i>
4	DLCO-26	Grasshopper	Emiru, Esayas & Ketena	Harmokale	Emiru & Esayas	<i>Metarhizium</i>
5	AAU- D	<i>pachnoda interrupta</i>	Merid Negash		Merid Negash	<i>Metarhizium</i>

3.1 Fungal suspension preparation

Spores from preserved fungal isolates were grown on Sabouraud Dextrose Agar (SDA) media with ingredients: Mycological pepton 10g/l, Dextroes 40g/l, Agar 15g/l under a laminar flow hood using sterilized loop. The media then prepared by using distilled water and the amount of the agar to be taken was determined according to the instruction on the label. Freshly incubated fungal isolates were kept at 18-20⁰C .This process was repeated several times until a pure culture was obtained.

Suspension of fungal isolate were prepared by scraping the surface of plates with a sterile scalpel and suspended in a sterile, aqueous solution of 0.5% Tween 80. The resulting suspension was homogenized by thoroughly blending the mixture with a vortexer for two minutes. The suspension was then filtered through sterile muslin to remove mycelia and debris. The spore concentration was counted with haemocytometer under a compound microscope (400 x magnifications).

According to Lacey (1997), to obtain an estimation of the number of propagules per ml in the original suspension, the average number of propagules per cell was then multiplied by the volume conversion factor 2.5×10^5 . The original suspension was then diluted to obtain the desired target concentration. This is obtained by dividing the target concentration to the actual concentration. For example if the actual concentration of the fungal solution is 1.25×10^7 , and the target concentration is 1×10^6 , then the required amount is obtained by dividing the target concentration to the actual and obtained 0.08ml . Therefore 0.08 ml of original suspension is

added to the 0.92ml of an appropriate carrier would result in 1 ml of a suspension of 1×10^6 propagules per ml (target concentration). In view of that the target concentrations of 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia ml⁻¹ are adjusted for liquid formulations.

3.2 Spore viability test

Viability of conidia was assessed by germination on SDA, PDA and MEA. Potato Dextros Agar (PDA) with ingredients: Potatoes infusion from 20g/l, Dextroes 20g/l, Agar 15g/l with pH 5.6 ± 0.2 (at 25°C), Malt extract agar (MEA) ingredients : Malt extract 300g/l, Mycological pepton 5g/l, Agar 15 g/l, with pH 5.4 ± 0.2 (at 25°C), and Sabourd Dextros Agar (SDA) with ingredients: Mycological pepton 10g/l, Dextroes 40g/l, Agar 15g/l was used as in the instruction on the label.

The media was prepared as in the instruction labelled on the bottles. Accordingly, PDA was prepared by suspending in the proportion of 39g in 1000ml of distilled water and heated to boil to dissolve the medium completely by mixing well while boiling and sterilized by autoclaving at 15lbs pressure (121°C) for 15 min. MEA was prepared by suspending in the proportion of 50g in 1000ml of distilled water and soaked for 15min and sterilized for 10min at 10lbs (115°C). By suspending in the proportion of 65 g/l in 1000ml distilled water, SDA was prepared and heated to boil to dissolve the medium completely by mixing well while boiling and sterilized by autoclaving at 15lbs pressure (121°C) for 15 min.

A conidial suspension of 100µl of 1×10^6 conidia per ml was spread on a 9cm Petridish evenly. Each culture medium was replicated three times for each isolate. The petridishes were sealed with parafilm and incubated at 23°C. 300 spores were examined after 8hr, 16hr and 24hr. Spores

were considered germinated if the germ tube was at least twice longer than the width of each conidium .

3.3 Bioassays

Wheat grains obtained from Holeta Agricultural research centre were obtained. The grain was weighted to 20g and placed in sterilized paper cup. Ten insects per one experimental unit were placed for treatment. Experiments were carried out for 5 fungal isolates of the genera *Metarhizium* and *Beauveria*.

Conidia of 1 month old culture were harvested using similar methods as described previously. Inoculations were made by spraying 2ml of the suspension of 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 conidia ml⁻¹ to the test insects while they are in the wheat grain inside a paper cups with a mesh cloth lid and kept at 25°C and 60% RH for 11 days. The control insects were treated with Tween 80 solution (0.5% v/v). Ten insects per one experimental unit were placed for treatment and each test will be replicated 3 times this also done for the 5 fungal isolates. Mortality was checked on daily basis for 10 days. The *Metarhizium* isolate AAU D was used as a standard for comparison.

3.4 Statistical analysis

Mortality between doses of the given Isolates was subjected to two way ANOVA analysis of variance using the general linear model procedure (GLM). Means were separated by using Tukey's post hock test, and similarly mortality between days of the given isolates were compared using ANOVA and means separated using Student-Newman-Keuls. pathogenicity of the different isolates with a given dose, also analysed in the same way. Probit analysis was used to estimate the LT₅₀ of the isolates with 95 % confidence. SPSS Version 17 was used to analyse the data

4. RESULTS

4.1 Study on pathogenicity

4.1.1 Effect of isolate DLCO 131 on *S. oryzae* with a range of conidial concentration over time

Cumulative mean percent mortality of *S. oryzae* treated with DLCO 131 with different concentrations was observed to increase with dose (Table 2). Cumulative mean percent mortality due to DLCO 131 showed significant difference on day 5, (F=14, P<0.0042), on day 6 (F=7.84, P<0.039), on day 7 (F=5.8, P<0.011), on day 8 (F=5.9, P<0.010), and on day 10 (F=3.6, P<0.043).

A significant difference in cumulative mean percent mortality were observed for concentrations 1×10^8 conidia ml⁻¹ (F=21, P<0.010), 1×10^7 conidia ml⁻¹ (F=22.4, P<0.010), 1×10^6 conidia ml⁻¹ (F=8.8, P<0.010), 1×10^5 conidia ml⁻¹ (F=13.3, P<0.010), 1×10^4 conidia ml⁻¹ (F=9.05, P<0.010).

Table 2 Mean mortality (%) of *S. oryzae* treated with DLCO 131 with different concentrations over time

	1×10^8 conidia ml⁻¹	1×10^7 conidia ml⁻¹	1×10^6 conidia ml⁻¹	1×10^5 conidia ml⁻¹	1×10^4 conidia ml⁻¹
	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error
Day 1	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 2	3.33 $\pm$ 3.33aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	3.33 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA
Day 3	16.67 $\pm$ 6.67aA	10.00 $\pm$ 5.77abA	6.67 $\pm$ 3.33abA	6.67 $\pm$ 3.33aA	6.67 $\pm$ 3.33abA
Day 4	26.67 $\pm$ 3.33aA	13.33 $\pm$ 6.67abA	10.00 $\pm$ 5.77abA	10.00 $\pm$ 0.00aA	6.67 $\pm$ 3.33abA
Day 5	53.33 $\pm$ 6.67bA	20.00 $\pm$ 0.00abB	13.33 $\pm$ 3.33abB	20.00 $\pm$ 5.77abB	13.33 $\pm$ 3.33abB
Day 6	63.33 $\pm$ 8.82bA	30.00 $\pm$ 5.77cB	26.67 $\pm$ 3.33abcB	23.33 $\pm$ 8.82abB	16.67 $\pm$ 3.33abB
Day 7	70.00 $\pm$ 10.00bA	46.67 $\pm$ 8.82dAB	33.33 $\pm$ 8.82abcB	36.67 $\pm$ 3.33cdB	20.00 $\pm$ 5.77abB
Day 8	73.33 $\pm$ 6.67bA	50.00 $\pm$ 5.77dB	40.00 $\pm$ 11.55bcdB	40.00 $\pm$ 5.77cdB	26.67 $\pm$ 3.33bcB
Day 9	73.33 $\pm$ 6.67bA	53.33 $\pm$ 3.33dA	53.33 $\pm$ 12.02cdA	50.00 $\pm$ 5.77dA	36.67 $\pm$ 8.82cA
Day 10	83.33 $\pm$ 8.82bA	70.00 $\pm$ 5.77eAB	63.33 $\pm$ 12.02dAB	53.33 $\pm$ 8.82dAB	40.00 $\pm$ 5.77cB

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p>0.05$) Student Newman Keuls (SNK) test.

4.1.2 Effect of isolate AAU D on *S. oryzae* with a range of conidial concentration over time

Cumulative mean percent mortality of *S. oryzae* treated with AAU D with different concentrations was observed to increase with dose (Table 3). Cumulative mean percent mortality due to AAU D showed significant difference for all concentrations on day 2, (F=4.5, P<0.025), on day 4 (F=4.8, P<0.020), on day 5 (F=10.9, P<0.01), on day 6 (F=11.29, P<0.01), and on day 7 (F=6.3, P<0.008) on day 8, (F=6.65, P<0.008), on day 9, (F=8.63, P<0.03), on day 10, (F=10, P<0.002).

A significant difference in cumulative mean percent mortality were observed over time for concentrations 1×10^8 conidia ml⁻¹ (F=27.6, P<0.010), 1×10^7 conidia ml⁻¹ (F=35.8, P<0.010), 1×10^6 conidia ml⁻¹ (F=42.4, P<0.010), 1×10^5 conidia ml⁻¹ (F=9.7, P<0.010), 1×10^4 conidia ml⁻¹ (F=5.1, P<0.010).

Table 3 Mean percent mortality of *S. oryzae* treated with AAU D at different concentrations over time

	1×10^8 conidia ml⁻¹	1×10^7 conidia ml⁻¹	1×10^6 conidia ml⁻¹	1×10^5 conidia ml⁻¹	1×10^4 conidia ml⁻¹
	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
Day 1	0.00±0.00aA	0.00±0.00aA	0.00±0.00aA	0.00±0.00aA	0.00±0.00aA
Day 2	10.00±0.00aA	6.67±3.33aAB	3.33±3.33aAB	0.00±0.00aB	0.00±0.00aB
Day 3	20.00±5.77abA	13.33±6.67abA	6.67±3.33aA	3.33±3.33aA	0.00±0.00aA
Day 4	33.33±8.82bcA	23.33±6.67bcAB	13.33±3.33abAB	10.00±0.00abB	3.33±3.33aB
Day 5	46.67±8.82cdA	33.33±3.33cdAB	20.00±0.00bBC	16.67±3.33abcBC	6.67±3.33aC
Day 6	53.33±8.82cdA	43.33±3.33deAB	23.33±3.33bB	30.00±5.77bcBC	6.67±3.33aC
Day 7	63.33±6.67deA	50.00±5.77efA	40.00±5.77cAB	36.67±12.02bcAB	13.33±3.33aB
Day 8	70.00±0.00deA	60.00±5.77fgA	50.00±5.77cdAB	40.00±10.00bcAB	23.33±8.82abB
Day 9	83.33±3.33efA	66.67±3.33ghAB	56.67±3.33deABC	43.33±8.82bcBC	30.00±11.55abC
Day 10	96.67±6.67fA	83.33±3.33hAB	66.67±3.33eBC	56.67±6.67cBC	40.00±11.55bC

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p>0.05$) Student Newman Keuls (SNK) test.

4.1.3 The Virulence of isolate DLCO 141 on *S. oryzae* with a range of conidial

concentration over time

A significant difference in cumulative mean percent mortality were observed over time for concentrations 1×10^8 conidia ml^{-1} ($F=7.8$, $P<0.010$), 1×10^7 conidia ml^{-1} ($F=10.4$, $P<0.010$), 1×10^6 conidia ml^{-1} ($F=11.6$, $P<0.010$), 1×10^5 conidia ml^{-1} ($F=22.8$, $P<0.010$), 1×10^4 conidia ml^{-1} ($F=34.8$, $P<0.010$).

Cumulative mean percent mortality of *S. oryzae* treated with DLCO 141 with different concentrations was observed to increase with dose (Table 4). Cumulative mean percent mortality due to DLCO 141 showed significant difference for all concentrations on day 4, ($F=3.76$, $P<0.04$), on day 5 ($F=4.2$, $P<0.029$), on day 6, ($F=4.23$, $P<0.02$) and on day 7 ($F=4.33$, $P<0.027$).

Table 4 Mean percent mortality of *S. oryzae* treated with DLCO 141 at different concentrations over time

	1×10^8 conidia ml⁻¹	1×10^7 conidia ml⁻¹	1×10^6 conidia ml⁻¹	1×10^5 conidia ml⁻¹	1×10^4 conidia ml⁻¹
	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error
Day 1	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 2	3.33 $\pm$ 3.33aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 3	13.33 $\pm$ 3.33abA	6.67 $\pm$ 3.33aA	6.67 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA	3.33 $\pm$ 3.33abA
Day 4	26.67 $\pm$ 3.33abcA	16.67 $\pm$ 6.67abAB	13.33 $\pm$ 3.33abAB	10.00 $\pm$ 0.00abAB	6.67 $\pm$ 3.33abB
Day 5	36.67 $\pm$ 8.82abcdA	33.33 $\pm$ 8.82abcA	20.00 $\pm$ 0.00abcA	13.33 $\pm$ 3.33abA	10.00 $\pm$ 0.00abA
Day 6	53.33 $\pm$ 8.82bcdA	46.67 $\pm$ 14.53bcAB	30.00 $\pm$ 0.00abcdAB	20.00 $\pm$ 0.00abAB	13.33 $\pm$ 3.33bB
Day 7	60.00 $\pm$ 11.55cdA	60.00 $\pm$ 11.55cA	40.00 $\pm$ 5.77bcdeA	26.67 $\pm$ 3.33bA	26.67 $\pm$ 3.33cA
Day 8	66.67 $\pm$ 14.53cdA	63.33 $\pm$ 8.82cA	46.67 $\pm$ 12.02cdeA	46.67 $\pm$ 8.82cA	33.33 $\pm$ 3.33cdA
Day 9	70.00 $\pm$ 17.32cdA	70.00 $\pm$ 11.55cA	56.67 $\pm$ 12.02deA	53.33 $\pm$ 8.82cA	33.33 $\pm$ 3.33cdA
Day 10	76.67 $\pm$ 14.53dA	73.33 $\pm$ 12.02cA	66.67 $\pm$ 12.02eA	60.00 $\pm$ 5.77cA	40.00 $\pm$ 0.00dA

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p>0.05$) Student Newman Keuls (SNK) test

4.1.4 The pathogenicity of isolate DLCO 26 on *S. oryzae* with a range of conidial concentration over time

A significant difference in cumulative mean percent mortality were observed over time for concentrations 1×10^8 conidia ml^{-1} ($F=9.1$, $P<0.010$), 1×10^7 conidia ml^{-1} ($F=4.6$, $P<0.02$), 1×10^6 conidia ml^{-1} ($F=5.3$, $P<0.01$), 1×10^5 conidia ml^{-1} ($F=10.8$, $P<0.01$), 1×10^4 conidia ml^{-1} ($F=15.25$, $P<0.01$).

Cumulative mean percent mortality of *S. oryzae* treated with DLCO 26 with different concentrations was observed to increase with dose (Table 5). Cumulative mean percent mortality due to DLCO 26 showed significant difference for all concentrations on day 4, ($F=3.76$, $P<0.040$), on day 5 ($F=4.1$, $P<0.031$), and on day 6, ($F=5.1$, $P<0.016$) .

Table 5 Mean percent mortality of *S. oryzae* treated with DLCO 26 at different concentrations over time

	1×10^8 conidia ml⁻¹	1×10^7 conidia ml⁻¹	1×10^6 conidia ml⁻¹	1×10^5 conidia ml⁻¹	1×10^4 conidia ml⁻¹
	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error
Day 1	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 2	3.33 $\pm$ 3.33abA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 3	13.33 $\pm$ 3.33abcA	6.67 $\pm$ 3.33abA	6.67 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA
Day 4	26.67 $\pm$ 3.33abcdA	16.67 $\pm$ 6.67abcAB	13.33 $\pm$ 3.33abAB	10.00 $\pm$ 0.00aAB	6.67 $\pm$ 3.33aB
Day 5	36.67 $\pm$ 8.82bcdeA	33.33 $\pm$ 8.82abcA	16.67 $\pm$ 3.33abA	13.33 $\pm$ 3.33aA	10.00 $\pm$ 0.00aA
Day 6	46.67 $\pm$ 6.67cdeA	46.67 $\pm$ 14.53abcAB	23.33 $\pm$ 3.33abcAB	20.00 $\pm$ 0.00abAB	10.00 $\pm$ 0.00aB
Day 7	53.33 $\pm$ 8.82deA	53.33 $\pm$ 17.64abcA	33.33 $\pm$ 8.82abcA	26.67 $\pm$ 3.33abA	23.33 $\pm$ 3.33bA
Day 8	60.00 $\pm$ 11.55deA	56.67 $\pm$ 14.53bcA	40.00 $\pm$ 15.28abcA	40.00 $\pm$ 10.00bcA	26.67 $\pm$ 3.33bA
Day 9	63.33 $\pm$ 14.53deA	63.33 $\pm$ 17.64bcA	50.00 $\pm$ 15.28bcA	46.67 $\pm$ 12.02bcA	26.67 $\pm$ 3.33bA
Day 10	70.00 $\pm$ 11.55eA	66.67 $\pm$ 18.56cA	60.00 $\pm$ 15.28cA	53.33 $\pm$ 8.82cA	33.33 $\pm$ 6.67bA

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p>0.05$) Student Newman Keuls (SNK) test.

4.1.5 The Virulence of isolate DLCO 91 on *S. oryzae* with a range of conidial concentration over time

Cumulative mean percent mortality of *S. oryzae* treated with DLCO 91 with different concentrations was observed to increase with dose (Table 6). Cumulative mean percent mortality due to DLCO 91 showed significant difference for all concentrations on day 4, (F=3.7, P<0.04), on day 5 (F=4.2, P<0.03), and on day 6 (F=5.1, P<0.01).

A significant difference in cumulative mean percent mortality were observed over time for concentrations 1×10^8 conidia ml⁻¹ (F=20.6, P<0.010), 1×10^7 conidia ml⁻¹ (F=7.0, P<0.010), 1×10^6 conidia ml⁻¹ (F=6.0, P<0.035), 1×10^5 conidia ml⁻¹ (F=16.4, P<0.010), 1×10^4 conidia ml⁻¹ (F=15.1, P<0.010).

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Table 6 Mean percent mortality of *S. oryzae* treated with DLCO 91 at different concentrations over time

	1×10^8 conidia ml⁻¹	1×10^7 conidia ml⁻¹	1×10^6 conidia ml⁻¹	1×10^5 conidia ml⁻¹	1×10^4 conidia ml⁻¹
	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error	Mean $\pm$ Std. Error
Day 1	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 2	3.33 $\pm$ 3.33aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA	0.00 $\pm$ 0.00aA
Day 3	13.33 $\pm$ 3.33abA	6.67 $\pm$ 3.33abA	6.67 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA	3.33 $\pm$ 3.33aA
Day 4	26.67 $\pm$ 3.33bcA	10.00 $\pm$ 0.00abAB	13.33 $\pm$ 3.33aAB	10.00 $\pm$ 0.00abAB	6.67 $\pm$ 3.33aB
Day 5	30.00 $\pm$ 5.77bcdB	26.67 $\pm$ 3.33abcB	13.33 $\pm$ 3.33aB	13.33 $\pm$ 3.33abB	10.00 $\pm$ 0.00aB
Day 6	30.00 $\pm$ 5.77bcdA	40.00 $\pm$ 10.00bcAB	16.67 $\pm$ 6.67aAB	16.67 $\pm$ 3.33abAB	10.00 $\pm$ 0.00aB
Day 7	36.67 $\pm$ 3.33cdeA	46.67 $\pm$ 13.33cA	26.67 $\pm$ 12.02aA	23.33 $\pm$ 3.33bA	23.33 $\pm$ 3.33bA
Day 8	46.67 $\pm$ 3.33defA	50.00 $\pm$ 10.00cA	33.33 $\pm$ 18.56aA	36.67 $\pm$ 6.67cA	26.67 $\pm$ 3.33bA
Day 9	50.00 $\pm$ 5.77efA	53.33 $\pm$ 12.02cA	43.33 $\pm$ 18.56aA	43.33 $\pm$ 8.82cA	26.67 $\pm$ 3.33bA
Day 10	60.00 $\pm$ 5.77fA	56.67 $\pm$ 14.53cA	53.33 $\pm$ 18.56aA	50.00 $\pm$ 5.77cA	33.33 $\pm$ 6.67bA

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p>0.05$) Student Newman Keuls (SNK) test.

4.2 Comparison of LT₅₀ of all fungal isolates used

The median lethal time of *S. oryzae* treated with 1×10^8 conidia / ml of isolate DLCO 26 was took the longest 6.53, as compared with others, to kill the target insect which is 6.53 day. AAU D was observed to kill the target insect with this concentration from day 3 onwards.

DLCO 91's median lethal time as compared with the rest with 1×10^7 conidia per ml was the least performing and took 7 days.

In all doses AAU D has shown to have the shortest median lethal time DLCO 26 and DLCO 91 on the other hand has observed to have the longest median LT₅₀ value (Table 7).

Table 7 median lethal time of *S. oryzae* for different concentrations

Median Lethal time (LT ₅₀) ±SE					
	1×10^8 conidia ml ⁻¹	1×10^7 conidia ml ⁻¹	1×10^6 conidia ml ⁻¹	1×10^5 conidia ml ⁻¹	1×10^4 conidia ml ⁻¹
DLCO 131	4.45±0.23	5.29±0.03	6.74±0.03	7.91±0.02	9.56±0.03
AAU D	3.52±0.06	4.52±0.04	6.17±0.02	7.23±0.02	7.87±0.02
DLCO 141	5.32±0.03	6.32±0.02	7.30±0.02	8.05±0.02	9.32±0.02
DLCO 26	6.53±0.04	6.99±0.03	8.39±0.02	9.10±0.02	9.47±0.02
DLCO 91	6.21±0.03	7.30±0.03	7.73±0.03	9.67±0.03	10.18±0.02

5. DISCUSSION

This study demonstrated a different degree of susceptibility of *S. oryzae* to various entomopathogenic fungi. All fungal isolates used in the present study were able to infect and cause death in adult of *S. oryzae* at all concentrations ranging from 1×10^4 to 1×10^8 conidia / ml but no mortality were observed in day 1 post treatment for all isolates and concentrations used. However, the degree of infection varied greatly between the isolates and the concentration used. Mortality caused by the isolates was confirmed by external sporulating of mycelial insects bodies and 89% of all dead insects showed mycosis of the respective isolates.

The results obtained in the present study demonstrated the susceptibility of *S. oryzae* to non specific local entomopathogenic fungi. The result also showed that there is a difference in susceptibility of the insect for all isolates. Fungal species of the genera *Beauveria* and *Metarhizium* are recognized to cause disease of insects (Dhuyo and Soomro, 2008).

In the present study, adults *S. oryzae* were found to be susceptible to all fungal isolates and different fungal concentrations ranging from 1×10^4 to 1×10^8 conidia per ml with water formulation and spray application. The fungus used in this work was isolated from different hosts and geographical location. As to Maniania (1992) entomopathogenic fungi generally are more pathogenic to the species of insects from which they are obtained or closely related species.

In the present finding, local isolates including DLCO 131 and AAU D which are members of the genus *Metarhizium* were found to be more virulent than DLCO 141 which is member of the genera *Beauveria*. Similar to the present study, Adane *et al.*, (2003) showed that when either *B. bassiana* or *M. anisopliae* were applied to *S. zeamais* at a lower concentration ($1 \times$

10⁶ conidia ml⁻¹), the latter caused a significantly higher mortality. *Metarhizium* has a number of advantages as a mycoinsecticide: it is relatively easy to mass produce, strains can be found with appropriate levels of virulence and specificity, and the conidia can be dried to ensure good persistence in the field and storage for over one year in cool conditions (Milner, 2000).

Less target mortality in the first two –three days were observed in all isolates. This condition is similar to Draganova *et al*, (2006) findings where Differences between cumulative mortality due to mycosis in treated populations began to be visible after the 3rd day. It is important to remember that the insect integument is essentially composed of proteins and chitin associated with lipids and phenolitic compounds. The very thin outer layer or epicuticle contains lipids (fatty acids and paraffin) whose antifungal activities have been demonstrated, but in higher concentrations than those present in the insect integument (Newman, and Cherry, 2005).

The isolate AAU D is more effective than others. The fungus was isolated from the coleopteran *Pachnoda intrupta* and preserved at Addis Ababa University Insect Science Research Laboratory. It is summed that he medium in which the spores were cultured might have a very strong bearing on the virulence of the fungual isolates. Differences in mortalities between the media used can be attributed to differential level of conidia adhesion to the insect cuticle. Adhesion is facilitated by hydrophobic interactions between the mucus produced by the conidia and the lipids and waxes on the cuticle. Lipids and waxes make the epicuticle highly lipophilic and thus highly hydrophobic. Oil and water may thus contribute in different ways to this adhesion process, oil being a better facilitator than water, allowing the conidia to stick with ease on the insect body wall because of the presence of lipids and waxes on the

cuticle surface, unlike those with a water film around them where a “water-off-the-duck’sback” problem has to be overcome (Prior *et al.*, 1988).

As time progresses, cumulative mortality for all isolates increased but at differing rates. Adane *et al* result in (2003) showed that cumulative mortality of *S. zeamais* due to different local *Metarhizium* and *Beauveria* isolates exceeded 38-75 % mortality on day 4 and exceeded 95% by day 6 and in contrast to this, the present study showed that in which by day 5, cumulative mean percent mortality for the isolates DLCO 131, and AAU D were 53 % and 46.6% respectively. This could be due to the insects are of different species.

In contrast with Adane *et al.*, (2003) mortality due to infection by Mortality caused by a *Beauveria* isolate, PPRC-CC exceeded 60 % by day 5. In present study 53% cumulative mortality was recorded in day 6 for the *Beauveria* isolate DLCO 141.

Adane *et al* (2003) further reported that, 6 days after treatment mortality for all isolates exceeded 97 % with out apparent difference between isolates. In contrast, the present study showed that DLCO 131 and AAU D, showed more than 45% mortality. This could be due to the difference in the fungal isolates used both in the present study and Adane’s study.

Earliest mortalities and 50% target mortalities occurred with higher concentrations i.e. speed and magnitude of infection were proportional to dose. This could be attributed to (among others) adhesion of spores to the lipophilic insect cuticle as fungal disease development is the attachment of the infective unit (conidia) to the insect’s body which in turn is dependent on whether the insect comes into contact with the unit or not (Roberts, 1981). Various laboratory application rates have thus been recommended, concentrations ranging from 10^4 to 10^8

conidia/ml (Ferron, 1971). It is also possible that the fewer the conidia the longer it will take for them to achieve adequate growth to cause mycoses. Low conidia concentrations may also not be able to overcome the healthy insects' resistance mechanisms and thus the insects could delay or survive the infection, with only the older and unhealthy ones succumbing to the infection. Muller-Kogler (1967) noted that there is a minimum threshold required to cause a lethal infection below which there is no disease (Makaka, 2008).

In bioassay tests of pathogens, LT_{50} is the indicative of their pathogenicity intensity. Nevertheless the pathogenicity and its intensity are influenced by various factors. The difference in virulence may be due to the fungal genetic material and other internal physiological factors within the host (Miranpuri & Khachatourians, 1995).

Miranpuri and Khachatourians (1995) showed that LT_{50} of different isolates on *Sitobion avenae* was 3 - 5.4 days. Cherry *et al.* (2005) investigated the effects of *B. bassiana* on *Callosobruchus maculatus* and using 1×10^8 spore/insect obtained 3.11 - 6.13 days for LT_{50} . In this study, LT_{50} value were in the above-mentioned range and the difference observed about LT_{50} values is probably is due to difference in physiological combinations. Also according to Todorove *et al.* (2002) LT_{50} depends on the spore suspension and with increase in spore suspension; there is a decrease in its quantity proportionately (Alizadeh et al , 2007). The lowest LT_{50} time recorded for *S. oryzae* in this study is 3.52 day Adane *et al.*, (1996) demonstrated that the lowest time recorded was 2.74 day and for Khashaveh *et al* 2008, the lowest time were 5.54. This difference could be due to the difference in application of spore suspension and the amount used and the age of the insects used plus the condition the experiments were conducted.

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

- Its observed in many research works that insect are susceptible to different pathogens including fungi. In view of that, *S. oryzae* is found to be susceptible for all isolates used in this work at all fungal concentrations ranging from 1×10^4 to 1×10^8 conidia/ml
- The local isolate AAU D, which was used as a standard as compared with the rest of the isolates was found to be effective highly pathogenic in that it kills faster and was observed to be effective even at a lowest concentration, 1×10^4 . This is probably due to it is the isolate from beetle, insects with the toughest chitinous exoskeleton, where as other isolates are obtained from grasshopper.
- Dose is one of the major factors for the determination of the amount to be used in the application. For that reason, the target insect showed different degree of susceptibility for different doses of the different isolates used. The most virulent isolates do have the lowest median survival time and are observed to be effective in minimum dose. In this study it was observed that lowest LT_{50} values were obtained for higher doses in all isolates. Virulent strains always observed to have lowest LT_{50} values. Therefore from the present results it could be concluded that most of the isolates used were virulent enough against to *S. oryzae*.

7.2 Recommendations

- The present study focuses on the effect of entomopathogens on the pathogenicity of the storage insect *S. oryzae*. In view of that Entomopathogens of the genus *Metarhizium* and *Beauveria* are found to be virulent which in turn are promising in the control of the insect. Therefore, further studies should be carried out for the control of the target insect for a better outcome.
- The results of this study magnificently demonstrate that it is possible to achieve a successful level of control for *S. oryzae* on stored and infested cereals using liquid formulations of conidia of *Beauveria* and *Metarhizium* isolates. Further studies should be carried out on different formulations like oil formulation.
- *Beauveria* and *Metarhizium* isolates used in the present study have the potential use as biological control agents against *S. oryzae*. Other local fungal isolates should be tested for virulence against this insect.
- It can be inferred that developments being made now on the scope would most probably make biological control of insect pests attractive from the practical point of view and also from an economic perspective in Ethiopia. Therefore, countries like Ethiopia should focus on biological control of pests than chemical control for the latter costs much.

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9. APPENDIX

Appendix 1 Fungal isolates used in the study

No	Code	Isolated from	Collected by	Collected from	Isolated by	Genera
1	DLCO-131	Grasshopper	Esayas & Yilma			Metarhizium
2	DLCO-141	Grasshopper	Esayas & Yilma			Beauveria
3	DLCO-91	Grasshopper	Esayas & Yilma	Debre-Zeit	Esayas	Metarhizium
4	DLCO-26	Grasshopper	Emiru, Esayas & Ketena	Harmokale	Emiru & Esayas	Metarhizium
5	AAU- D	<i>pachnoda interrupta</i>	Merid Negash		Merid Negash	Metarhizium

Appendix 2 Percent germination of all fungal isolates grown on MEA, PDA and SDA on different time

Isolate	MEA			PDA			SDA		
	8hr	16hr	24hr	8hr	16hr	24hr	8hr	16hr	24hr
DLCO 131	39.67%	74.11%	96.33%	48.67%	90.33%	97.11%	47.89%	94.11%	98.22%
AAU D	41.11%	82.78%	95.33%	52.00%	85.56%	96.44%	48.00%	92.00%	99.22%
DLCO 141	44.00%	81.78%	93.00%	51.44%	83.89%	96.11%	51.67%	92.11%	98.33%
DLCO 26	46.11%	83.67%	96.78%	50.33%	92.33%	98.44%	57.22%	87.56%	98.89%
DLCO 91	50.89%	86.33%	94.33%	49.78%	91.00%	97.00%	56.00%	88.67%	93.11%



A- *S. oryzae* treated with DLCO 131



B- *S. oryzae* treated with AAU D



C- *S. oryzae* treated with DLCO 141

Appendix 3 mycosis with external growth of mycelia on died experimental insects



Laminar air flow



Compound microscope

Micropipette



Balance



Vortexer



Incubator



Autoclave sterilizer



Appendix 4 Laboratory equipments used