

SHORT COMMUNICATION

EVALUATION ON THE POTENTIAL OF ENTOMOPATHOGENIC FUNGAL ISOLATES FOR THE CONTROL OF MEXICAN BEAN BRUCHID (*ZABROTES SUBFASCIATUS*) (COLEOPTERA: BRUCHIDAE)

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ABSTRACT: The efficacy of five isolates of entomopathogenic fungi belonging to the genus *Metarhizium* was assessed against Mexican bean bruchid, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) in a laboratory experiment. Four local isolates namely META-B, META-D, PPRC-6, PPRC-29 and one standard isolate ICIPE-30 were evaluated. Pathogenicity of the isolates was conducted with three different conidia concentrations including 10^6 , 10^7 and 10^8 conidia/ml. The adults of Mexican bean bruchid were treated by spraying one ml of fungal concentrations ranging from 1×10^6 to 1×10^8 . All the isolates tested were virulent at all concentrations ranging from 1×10^6 to 1×10^8 conidia/ml. However, pathogenicity (based on speed and magnitude of mortality) varied among isolates and concentration. Among the *M. anisopliae* isolates, ICIPE-30 and META-D were most pathogenic. A significant difference ($p < 0.05$) in mortality was recorded between treatments of fungal concentrations and the control. None of the control treatment units gave more than 15% mortality. All tested isolates were able to infect and cause mycosis in adult Mexican bean bruchid, out of which isolate META-B showed the least virulence compared to others with less than 67.5% mortality with confirmed symptom of mycosis. The study suggests that entomopathogenic fungi may be used as a component of IPM in stored beans under Ethiopian conditions.

Key words/phrases: Entomopathogenic fungi, *Metarhizium*, Mexican bean bruchid, Mycosis, Pathogenicity, *Zabrotes subfasciatus*.

INTRODUCTION

Bruchids are major pests of stored haricot bean, *Phaseolus vulgaris* L., in Ethiopia. Little information is available on the identity, importance and control of these pests in this country. Efforts at improving agricultural production have always been concentrated on increasing production through breeding of high-yielding varieties with little emphasis on conservation of what is produced (Boxall, 1998). In the past, haricot bean in Ethiopia was

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cultivated on small subsistence farms. In recent years, however, large hectares of haricot bean is under production. Among the major biotic factors contributing to low yields are pre and post-harvest insect pests. Available information shows that about 10% of post-harvest food grains were lost in developing countries due to different factors out of which insects alone were responsible for about 2.5% of the loss (Jiliani and Saxena, 1988). The most economically important insects of stored product belong to orders Coleoptera and Lepidoptera (Olkowski *et al.*, 1991).

Among the various insect pests of beans, the oriental cowpea bruchid, *Callosobruchus chinensis* (L.), the spotted cowpea bruchid, *C. maculatus* (F.), the bean beetle, *Acanthoscelides obtectus* (say) and the Mexican bean bruchid, *Zabrotes subfasciatus* (Boheman) are the most important insect pests of stored-grain legumes. These insects belong to the Family Bruchidae, which are prolific and rapid in their breeding and can quickly cause a serious reduction in the nutritive value of stored seeds (NRI, 2003). Their biology was studied by Hill (1990) and Deny and Credland (1991). Storage insect control has been based on the use of synthetic pesticides for many years. It has been reported by many workers that synthetic insecticides like fumigants, dusts for admixture of seeds, and sprays are effective for the control of bruchids (El-Nahal *et al.*, 1984; Tsedeke Abate, 1986; Hill, 1990). The widespread use of insecticides against insect pests is of global concern with respect to environmental hazards, insecticide resistance, chemical residues in food and feed stuffs, side effects on non-target organisms and the associated high protection costs (Beeman and Wright, 1990). To this effect, the increased public awareness and concern for environmental safety has directed research to the development of alternative control strategies such as the use of microbial control agents against insect pests on stored-products (Brower *et al.*, 1995). Entomopathogenic fungi have been shown to be effective in controlling several insect pests of stored beans (Burges, 1981; Inglis *et al.*, 2001).

The objectives of the study were to evaluate pathogenicity of different fungal isolates against Mexican bean bruchid, compare the efficacy of local fungal isolates with a standard isolate (ICIPE-30), and optimize lethal dose of local fungal isolates under laboratory conditions.

MATERIALS AND METHODS

Mass rearing of test insects

Establishment of efficient mass rearing system is an essential step to sufficiently supply test insects for bioassay. Accordingly, adult Mexican bean bruchid were reared in one litre volume glass jars at the Department of Zoological Sciences, Addis Ababa University. The colony of *Z. subfasciatus* was obtained from Melkassa Agricultural Research Centre (MARC). The insectary where rearing was carried out had a temperature of $27\pm 3^{\circ}\text{C}$ and 55-70% RH. Seeds of haricot bean (variety Awash-1) to be used for mass rearing were obtained from local farmers at Melkassa. It was cleaned and kept in an oven at 40°C for 4 hours to disinfect the seeds from any prior infestation (Bekele Jembere, 2002). Fifty pairs of adult *Z. subfasciatus* were placed in one litre volume glass jars containing 250 g non-infested seed. Individual insects were picked using a fine paint brush and released into each jar. The mouth of the jar was covered with a nylon mesh and held in place with rubber bands to prevent the insects from escaping. The parent bruchid were sieved out after 13 days of oviposition and seeds were kept under laboratory condition until F1 progeny emergence. When the F1 progeny started to emerge (often after 30 days), they were sieved out and used for experiment.

Media for culturing fungal isolates

Sabouraud Dextrose Agar (SDA) nutrient medium was used for fungal growth. The concentration needed for the bioassays was suspended in sterile distilled water in the proportion of 65 g of the ready mixed powdered media to 1 litre of distilled water, boiled and autoclaved at 121°C and 15 psi for 20 minutes. After preparation of the media, 0.05 g of chloramphenicol powder (an antibiotic) was weighed in 10 ml of 90% alcohol added to it and agitated. The antibiotic solution was then added to the sterilized agar at a rate of 1 ml 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 5 psi and 121°C . Similarly, the petridish was autoclaved to protect from contamination, then the sterilized media was poured into sterilized petridish under a laminar flow hood (Seneshaw Aysheshim *et al.*, 2003; Tesfaye Haile *et al.*, 2012).

Source of fungal isolates and preparation of conidial suspension

The fungal isolates used in this investigation were obtained from Insect Science Research Laboratory, Department of Zoological Sciences, Addis Ababa University and Plant Protection Research Centre (PPRC), Ambo, Ethiopia (Table 1). Conidia were obtained from seven day-old sporulating cultures in 9 cm diameter petridishes at 25°C, on Sabouraud Dextrose Agar (SDA). The conidia of each isolate were harvested by scrapping the surface of the culture with a sterile loop into a 500 ml glass beaker containing 50 ml sterile distilled water with tween-80 (0.01% v/v solution). The conidia suspension was prepared via mixing the solution with magnetic stirrer, vortexed and ultrasonicated for approximately 3 minutes to break up the conidial chains (confirmed by microscopic examination) then filtered through a 75 µm sieve to remove debris. It was then adjusted to the desired concentration using an improved haemocytometer. Numbers of conidia were estimated and serial dilutions were made and adjusted to 1×10^6 , 1×10^7 , and 1×10^8 conidia/ml (Dhaliwal and Koul, 2007).

Table 1. List of fungal isolates tested against Mexican bean bruchid, their species, origin, and host insects.

No.	Isolate code	Species	Location collected	Host insect
1	AAU Insect-B	<i>Metarhizium anisopliae</i>	Rasa (N.Shoa), Ethiopia	<i>Pachnoda interrupta</i> (Coleoptera: Scarabaeidae)
2	AAU Insect-D	<i>Metarhizium anisopliae</i>	Melka Worer, Ethiopia	<i>Pachnoda interrupta</i> (Coleoptera: Scarabaeidae)
3	PPRC-6	<i>Metarhizium anisopliae</i> var <i>anisopliae</i>	North Shoa, (Kewot, 1950) Ethiopia	<i>Pachnoda interrupta</i> (Coleoptera: Scarabaeidae)
4	PPRC-29	<i>Metarhizium anisopliae</i>	Gobenayetu (N.Shoa), Ethiopia	<i>Pachnoda interrupta</i> (Coleoptera: Scarabaeidae)
5	ICPIE-30	<i>Metarhizium anisopliae</i> var <i>anisopliae</i>	Kenya	Tsetse fly

Spore viability tests

Viability tests of the spores were performed 24 hours before application of each experiment. This was carried out by pipetting 200 µl of spore suspension onto a 9 cm glass petridish containing SDA and spread evenly using a glass-rod spreader. Twenty four hours later, 300 spores were examined and spores with an extrusion of germ tube as big as or greater than the width was recorded as germinated spore in a sample. This value was expressed as a percentage of spore germinating for each experimental unit

(Hall, 1976) and spore batches with more than 85% viability were used for bioassay.

Pathogenicity tests

Treatment of adults

Serial dilutions of spores were made to obtain three different conidial concentrations that included 1×10^6 , 1×10^7 , and 1×10^8 conidia/ml. Four replicates of each conidial concentrations plus a control treatment that included 0.01% of tween-80 aqueous solution were used for the series of laboratory experiments. Laboratory-reared adult Mexican bean bruchid (1-8 days old) were used for the study. The bruchids were mixed and ten adult bruchids were randomly picked and introduced to each of sixty four petridishes containing filter paper. One ml of the appropriate conidial suspension was sprayed with a small deodorant sprayer to each batch of ten bruchids. After spraying, the treated insects were transferred into plastic petridishes (9 cm diameter) and kept without food for 24 hours. This was done in order to prevent removal of fungal spores from the insects during contact with the grain. After 24 hours, bruchids were transferred into bottles (1000 ml capacity) containing undamaged bean and each bottle was covered with a nylon mesh. All tests were replicated four times and maintained at 27°C and 70% RH (Tesfaye Haile *et al.*, 2012).

Mortality follow ups and examination of sporulating isolates on cadavers

Mortality of bruchids was recorded daily at 24 hours interval for about six days. All cadavers in each replicate were removed immediately before the fungus was sporulated to prevent and minimize contamination and possible horizontal fungal transmission. To determine whether the insect died of mycosis, cadavers were incubated under high humidity in petridishes containing moistened sterilized filter paper (Prior *et al.*, 1992). The insects in this set were rinsed three times in distilled sterile water and transferred into petridishes with moistened sterilized filter paper. The petridishes were then tightly sealed with parafilms to increase moisture and prevent contamination. Following incubation at 25°C for seven days, cadavers were checked for fungal infection and sporulation. From mycosed bruchids, spores were inoculated into media and the fungus was compared under microscope by preparing on slide culture. The comparison was made based on morphology of mycelia, spore shape and size with the aid of phase contrast microscope.

Statistical analysis

Analysis was performed on mean cumulative percentage mortality data, following correction for control mortality using Abbot's formula (Abbotts, 1925). All the experiments were laid in a completely randomized design (CRD). Cumulative percentage mortality data was subjected to one-way ANOVA using SPSS version 13.0. In cases where significant results were obtained, mean separations were conducted using Tukey's Multiple Range Test at 5% level of significance. Homogeneity of variance was tested using Levene's test.

RESULTS

Efficacy of local isolates

In the present study, adults of Mexican bean bruchid were found to be susceptible to all fungal isolates tested and levels of conidial concentrations applied (Table 2).

Mexican bean bruchids were found to be susceptible to all fungal concentrations ranging from 1×10^6 to 1×10^8 conidia/ml (Table 2). No mortality was observed on day-1 in all the concentrations used and the control. It was apparent that results on target mortality showed significant differences particularly fungal-treated and those in the control group (Table 2). Mortality of adults due to mycosis caused by isolates PPRC-6, PPRC-29, META-D, and META-B ranged from approximately 2.5% on day-2 to 87.5%, 85%, 89.5% and 67.5%, respectively by day-6 post treatment observation with conidial concentrations of 1×10^6 and 1×10^8 conidial/ml (Table 2). However, as the number of post-treatment days progressed, i.e., from day-3 to day-6 there were significant differences in mortality due to variation in doses. This may indicate that even the lowest concentration (1×10^6 conidial/ml) had caused infection sufficient to cause as much target death as the highest conidial concentration used (1×10^8 conidial/ml) (Table 2). Higher mortality of the bruchid on day-6 was recorded with the concentration of 1×10^8 .

Mortality of adult bruchid due to infection with isolate ICIPE-30 increased gradually with time after inoculation with the different fungal concentration. Similar to that of the local isolates, no mortality was recorded on day-1. In all days except day-2, there was a significant difference ($p < 0.05$) in mortality between the different conidial concentrations (Table 2). In addition, no significant difference in mortality was observed between the different fungal concentrations except in day-6 at 1×10^6 (Table 2). Higher

mortality was observed at day-6 in the highest concentration of 1×10^8 conidia/ml which was not significant ($p > 0.05$). In general, local isolates were equality effective against bruchid in common bean as standard isolates.

Table 2. Mean percent mortality of bruchid adults treated with PPRC-6, PPRC-29, META-B, META-D and ICIPE-30 isolates at different concentrations over days after treatment.

Fungal isolates	Fungal concentration (Conidia/ml)	Mean percent mortality in days after treatment (Mean \pm SE)				
		2	3	4	5	6
PPRC-6	1×10^6	2.50 \pm 2.50 ^a	12.50 \pm 2.50 ^a	25.0 \pm 2.89 ^b	40.0 \pm 0.0 ^b	55.0 \pm 2.89 ^b
	1×10^7	15.0 \pm 2.89 ^b	15.0 \pm 2.50 ^b	40.0 \pm 4.08 ^{bc}	57.50 \pm 4.80 ^{bc}	77.50 \pm 6.29 ^c
	1×10^8	20.0 \pm 0.0 ^b	35.0 \pm 2.89 ^b	55.0 \pm 6.45 ^c	70.0 \pm 7.07 ^c	87.50 \pm 6.29 ^c
	Control	0.0 \pm 0.0 ^a	2.50 \pm 2.50 ^a	12.50 \pm 1.80 ^a	20.50 \pm 3.20 ^a	30.0 \pm 4.0 ^a
PPRC-29	1×10^6	2.50 \pm 2.50 ^a	15.0 \pm 2.89 ^b	42.50 \pm 7.50 ^b	57.50 \pm 4.79 ^b	65.0 \pm 6.45 ^b
	1×10^7	17.50 \pm 2.50	35.0 \pm 2.89 ^c	50.0 \pm 7.07 ^b	67.50 \pm 4.79 ^c	82.50 \pm 4.79 ^c
	1×10^8	7.50 \pm 4.79 ^a	35.0 \pm 2.89 ^c	57.50 \pm 8.54 ^b	72.50 \pm 2.50 ^c	85.0 \pm 5.0 ^c
	Control	0.0 \pm 0.0 ^a	2.50 \pm 2.50 ^a	5.50 \pm 5.50 ^a	10.50 \pm 4.50 ^a	20.53 \pm 0.0 ^a
META-B	1×10^6	0.0 \pm 0.0 ^a	10.0 \pm 4.08 ^{ab}	25.0 \pm 5.0 ^b	42.50 \pm 4.79 ^b	50.0 \pm 4.08 ^b
	1×10^7	5.0 \pm 2.89 ^a	22.50 \pm 2.50 ^c	32.50 \pm 2.50 ^{bc}	47.50 \pm 4.79 ^b	65.0 \pm 2.89 ^b
	1×10^8	7.50 \pm 2.50 ^a	20.0 \pm 0.0 ^{bc}	42.50 \pm 2.50 ^c	57.50 \pm 2.50 ^b	67.50 \pm 2.50 ^b
	Control	0.0 \pm 0.0 ^a	1.80 \pm 0.0 ^a	2.0 \pm 2.50 ^a	7.40 \pm 4.00 ^a	10.0 \pm 3.0 ^a
META-D	1×10^6	2.50 \pm 2.50 ^{ab}	17.50 \pm 2.50 ^b	40.0 \pm 0.0 ^b	57.50 \pm 4.79 ^b	60.0 \pm 4.08 ^b
	1×10^7	15.0 \pm 2.89 ^c	30.0 \pm 4.08 ^{bc}	52.50 \pm 4.79 ^b	70.0 \pm 4.08 ^{bc}	80.0 \pm 4.08 ^c
	1×10^8	10.0 \pm 0.0 ^{bc}	35.0 \pm 2.89 ^c	70.0 \pm 4.08 ^c	82.50 \pm 2.50 ^c	89.50 \pm 2.50 ^c
	Control	0.0 \pm 0.0 ^a	2.50 \pm 2.50 ^a	12.50 \pm 1.80 ^a	20.50 \pm 3.20 ^a	30.0 \pm 4.0 ^a
ICIPE-30	1×10^6	5.0 \pm 2.89 ^a	20.0 \pm 0.0 ^b	42.50 \pm 2.50 ^b	60.00 \pm 4.08 ^b	67.50 \pm 4.79 ^b
	1×10^7	5.0 \pm 2.89 ^a	17.50 \pm 2.50 ^b	32.50 \pm 2.50 ^b	52.50 \pm 4.79 ^b	87.50 \pm 2.50 ^c
	1×10^8	5.0 \pm 2.89 ^a	17.50 \pm 2.50 ^b	32.50 \pm 4.79 ^b	50.0 \pm 7.07 ^b	92.50 \pm 4.79 ^c
	Control	0.0 \pm 0.0 ^a	3.50 \pm 0.70 ^a	5.50 \pm 5.56 ^a	15.0 \pm 3.00 ^a	30.0 \pm 1.80 ^a

Means within a column followed by the same letter are not significantly different at $p < 0.05$

Comparison of different fungal isolates on Mexican bean bruchids at 1×10^6 to 1×10^8 conidia/ml

Mexican bean bruchids were found to be susceptible to all fungal concentrations ranging from 1×10^6 to 1×10^8 conidia/ml (Table 3). At a concentration of 1×10^6 , percentage mortality of the bruchid ranged from approximately 0% with fungal isolate META-B on day-2 to 67.5% with fungal isolate ICIPE-30 on day-6. On day-2, -3, and -5, there was no significant difference ($p > 0.05$) in mortality between isolates META-B, META-D, PPRC-6, PPRC-29, and ICIPE-30 (Table 3). From day-3 to day-6, there was no significant difference in mortality between isolates. Cumulative percentage mortality of the Mexican bean bruchid following treatment with different fungal isolates ranged from 5% with fungal isolate

META-B on day-2 to 87.5% with fungal isolate ICIPE-30 on day-6 at 1×10^7 conidia/ml (Table 3). In all days, except day-2, significant differences in mortality due to the applied fungi and control were observed with different concentration (Table 3), but from day-2 to day-6 no significant differences ($p > 0.05$) was seen between META-D, PPRC-6, and PPRC-29. Furthermore, on days-2, -4, -5 significant differences ($p < 0.05$) was recorded between isolates META-B and META-D (Table 3). At a concentration of 1×10^8 conidia/ml, bruchids started dying of mycosis on day-2 after treatment. Comparison of the fungal isolates revealed that isolates META-D, PPRC-6, PPRC-29 and ICIPE-30 caused high percentage mortalities on day-6 even though no significant difference ($p > 0.05$) was observed among them. META-B showed lowest mortality on day-6.

Table 3. Percentage mycosis of adult bruchids after treatment with five fungal isolates.

Fungal isolate	Concentration (conidia/ml)	Percentage mycosis
META-B	1×10^6	57.50 ± 6.29^b
	1×10^7	75.00 ± 5.00^c
	1×10^8	77.50 ± 9.46^c
	Control	70.00 ± 5.00^a
META-D	1×10^6	65.00 ± 2.9^b
	1×10^7	77.50 ± 2.50^{bc}
	1×10^8	90.00 ± 4.10^c
	Control	60.00 ± 5.00^a
PPRC-6	1×10^6	60.00 ± 4.08^b
	1×10^7	75.00 ± 2.89^c
	1×10^8	87.50 ± 2.5^c
	Control	55.00 ± 3.50^a
PPRC-29	1×10^6	65.00 ± 6.045^b
	1×10^7	80.00 ± 4.08^c
	1×10^8	85.00 ± 5.00^c
	Control	70.00 ± 5.00^a
ICIPE-30	1×10^6	65.00 ± 6.045^b
	1×10^7	80.00 ± 4.08^c
	1×10^8	95.00 ± 2.89^c
	Control	70.00 ± 4.50^a

Means within a column for each fungal isolate followed by the same letter are not significantly different at $p < 0.05$

Effect of different fungal isolates on mycosis of adult Mexican bean bruchids

All the fungal treated insects gave characteristic feature of external sporulation of the respective fungi isolates when incubated in moistened filter paper. External white mycelial growth from all cadavers was evident within 24-48 hours of incubation. Mycelium started to appear after 24 hours. Comparison of results on percentage mycosis of adult Mexican bean

bruchids showed that there was no significant difference ($p>0.05$) in mycosis after inoculation with each fungal isolate at concentration 1×10^7 conidia/ml and 1×10^8 conidia/ml (Table 3). However, a significant difference ($p<0.05$) in each fungal isolate at 1×10^6 concentration was recorded. The mortality in the treated bruchids was due to fungal infection as the external growth was apparent for 57-95% of the dead bruchids depending on the dose and isolate. However, no mycosis was observed in bruchids from the control group.

DISCUSSION

In the present work, laboratory studies have shown that fungal isolates belonging to the genus *Metarhizium* have the potential towards the management of Mexican bean bruchids. One standard isolate ICIPE-30 and four local isolates META-B, META-D, PPRC-6, and PPRC-29, were tested for their efficacy against Mexican bean bruchids. Since previous works on entomopathogens against Mexican bean bruchids in Ethiopia were not noted by the authors, comparison of results of the present study are based on previous similar works on different groups of insects. However, it was difficult to directly compare and reach conclusions based on results of unrelated target insects, isolate types, fungal concentrations (doses), and conditions under which the experiments were conducted.

The viability of all of the isolates tested in this bioassay exceeded 80%. Similar results have been obtained in laboratory bioassays and field experiments with conidia viability of more than 80% (Lomer *et al.*, 1997). The results of this experiment, therefore, measure true differences in pathogenicity among the fungal isolates tested. All the isolates of *M. anisopliae* were pathogenic to adult Mexican bean bruchids. According to Humber and Soper (1986), both species of *M. anisopliae* and *B. bassiana* fungi are known to be ubiquitous insect pathogens, and have been isolated from different species of insects, including beetles. The pathogen of the different isolates varied considerably between species and within species and this was more pronounced within the *M. anisopliae* isolates than within the *B. bassiana* isolates. The results gained in our research using Ethiopian fungal strains corroborated with those of similar studies carried out on various stored product insect pests (Adane Kasa *et al.* 1996). The results obtained in the present work showed that dose-mortality relationship studies further indicate that differences in pathogenicity were apparent among the five potent isolates tested. There were also differences in pathogenicity within the isolates of *M. anisopliae*. Isolate META-B was the least

pathogenic compared to other isolates (Table 3). The entomopathogenic fungi used in this study were isolated from different hosts and geographic origins. The results indicated that the Mexican bean bruchid was susceptible differently to all of the fungal isolates at different concentrations. Among the *M. anisopliae* isolates, the virulence and speed of action of isolate ICIPE-30 and META-D were superior to those of all other isolates. Many authors agree that isolates of entomopathogenic fungi are generally more pathogenic to the species of insect from which they are obtained or to closely related species (Zimmermann, 1982). However, there are also many investigators in agreement with the present study who reported that the pathogenicity is not necessarily related to the origin of insects or geographic origins (Moore and Prior, 1993).

Studies by Moore *et al.* (1992), Emiru Seyoum *et al.* (1994) have all shown significant reductions in feeding as early as 1-4 days after treatment with *Metarhizium flavoviride* (Gams & Rozypal), in the desert locust *Schistocerca gregaria* and the variegated grasshopper, *Zonocerus variegatus* (L.), respectively. *Beauveria bassiana* infection in the Colorado potato beetle has been observed to cause a decrease in food consumption three days after treatment (Fargues *et al.*, 1994). Infection by *Nomuraea rileyi* (Farlow) Samson and *M. anisopliae* can cause reduced feeding two days after treatment in *Heliothis zea* and *Megalurothrips sjostedti* (Trybom) (Mohamed *et al.*, 1982; Ekesi, 1999). Tesfaye Demis and Emiru Seyoum (2010) showed that META-B killed 41.67% of sorghum chafer (*Pachnoda interrupta*) in an experiment aimed at attracting and infecting under field condition in 30 days, however, META-D killed above 75% in fifteen days under field conditions. The findings in the present work are in agreement with previous ones in that META-D at higher dose of 1×10^7 and 1×10^8 shows more pathogenicity than META-B.

CONCLUSION

The use of entomopathogenic fungi for the control of insect pests offers environmentally acceptable strategy. Major obstacles to their application include variability in effectiveness due to uncontrolled environmental conditions (mainly temperature and humidity) and development of suitable formulation techniques. Strains of fungi differ considerably in their ability to colonize and establish within the test bruchid. This pathogenicity is the result of complex processes including the interaction of individual traits determining germination and penetration, followed by successful growth within the target host (bruchid), which is prerequisite for induction of

mortality. From the present investigation, it was made clear that mortality in Mexican bean bruchid was found to be dose-dependent regardless of the isolate types and the results have shown the potential of local entomopathogens, which can be used for management of Mexican bean bruchid. Moreover, higher mortality was achieved as the days after treatment proceeded. High germination rate of the biocontrol agent, percent mortality of the target pest and external mycelial growth of the biocontrol agent on the tested insect may authenticate the potential of fungi as a candidate of biological control agent.

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