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EVALUATION OF NATIVE FUNGAL ISOLATES OF *METRAHIZIUM ANISOPLIAE* VAR. *ACRIDUM* AND *BEAUVERIA BASSIANA* AGAINST THE GREATER WAX MOTH, *GALLERIA MELLONELLA* (L) (PYRALIDAE: LEPIDOPTERA) IN ETHIOPIA

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ABSTRACT: The greater wax moth (GWM) is a widely distributed and devastating insect pest to honey production sector in Ethiopia. The present study investigated the potential of native fungal isolates against GWM and assessed the non-target effect of one isolate of Beauveria (IITA 18) and five isolates of Metarhizium (IMI 330189, DLCO-AA83, DLCO-AA 109, DLCO-AA5, DLCO-AA14) by inoculating Ethiopian honeybee race, *Apis mellifera bandasii*. The effects of these six fungal isolates were evaluated in the laboratory for their pathogenicity to adult greater wax moths. Adult GWMs were found to be susceptible to all isolates and concentrations used. Greater than 90% adult greater wax moth (GWM) mortality was achieved 13 days post-inoculation. The on host specificity test of Metarhizium and the Beauveria isolates on Apis *melifera bandasii* also showed no significant effects on honey bees. No significant effects ($P>0.05$) of mycosis on adult emergence from last larval instar GWMs inoculated by different spore concentrations ($2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ conidia /ml) before entering into pupation.

Key words/phrases: ***Beauveria bassiana*,** greater wax moth**, *Metarhizium anisopliae*,** pathogenicity

INTRODUCTION

Wax moths are found in most of the honey bee colonies world wide. Their geographical distribution corresponds with that of honey bee (Charriere and Imdorf, 1999). The greater wax moth (GWM) (Pyralidae: Lepidoptera**)** is a widely distributed and devastating insect pest to honey production in Ethiopia through unabated damage to honey bees (Desalegn Begna, 2001; Desalegn Begna and Amsalu Bezabeh, 2001). Damage to honey bees is the result of the direct impacts of GWM larvae and the indirect effects inflicted by adult wax moths. The damage by larvae is manifested in such a way that they eat and destroy beeswax combs, form silken feeding tunnels, bore through honey wax caps and cement the cocoon in cavities of beehive frames. Severe infestation by larvae often leads to comb collapse (Desalegn Begna, 2001).

On the other hand, adult greater wax moths affect honey bees indirectly as vectors of honey bee diseases, the worst of which is the foulbrood, an invasive mycosis produced by the fungus *Ascosphaera apis*, affecting stretched larvae (Floyd and Paul, 1976). Various management measures have been used against the greater wax moth but use of pathogens in general and fungi in particular. It has been well documented that different isolates of the entomopathogenic fungi, Metarhizium and Beauveria have proved to be potential candidates for many insect pests (Burge, 1988). However, an effective control method of this pest has not been developed. Physical, chemical, and biological methods are imperfect (Cantwell and Smith, 1970; Ali *et al*., 1973; Burges, 1978), and further studies are needed to find more effective control methods. Therefore, this study is intended to evaluate the potential effects of different fungal isolates as possible GWM control options.

The study hence investigated the potential of local isolates in comparison with (one isolate taken) the standard isolate, *Metarhizium anisopliae* var. *acridium* (IMI 330189) and in comparison with recently explored five local isolates against adult wax moths (Emiru Seyoum and Merid Negash, 2007; Emiru Seyoum and Hellen Namusana, 2010) as environmentally friendly management options. The possible effect of the fungal isolates on the honeybee colonies (as non-target organisms) was also investigated. The results in

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this part of the work proved that the applied fungal isolates had no significant effects on the non-target honey bees inoculated.

MATERIALS AND METHODS

Sources of experimental insects (GWM, honey bees) and fungal isolates

The initial stock culture of the target greater wax moth and wax combs for rearing of honey bees were obtained from Holleta Bee Research Centre, and reared at the insect rearing facilities of the Faculty of Life Sciences, Addis Ababa University and that of the Desert Locust Control Organization for Eastern Africa (D.L.C.O-E.A), Addis Ababa, Ethiopia. The greater wax moths were fed with natural (wax combs) and artificial diet prepared using methods described by previous workers (Haydak, 1936; Good *et al*., 1953). Wax combs were put into the old culture for females to lay eggs for they contain a component of the female sex hormone Nonanal (MAAREC, 2000).

A total of 10 newly-emerged female and 5 male GWM were introduced into new rearing containers with artificial diet and pieces of wax comb. The containers were then placed in a warm room with temperature set at 30 ± 2°C. Humidity in the rearing room was maintained by spraying 500 ml of water using a screw nozzle sprayer daily. A soft cardboard was placed in the rearing containers for pupation of fully-grown larvae. Soon after emergence, the immature adult GWM were transferred to a new rearing container so that same age GWM could be used for subsequent experiments. On the other hand, four viable active *Apis mellifera bandasii* (Ethioipian race) were purchased from farmers in Bishoftu about 45 Km from Addis Ababa and were confined in locally-made wooden cages placed at three meters interval. The colonies were fed with pure honey and 30% sugar solutions.

The entomopthogenic fungal isolates of Beauveria (DLCO-AA5 , DLCO-AA14 and IITA 18) and of Metarhizium (DLCO-AA 109 and DLCO-AA83) were obtained from the stocks of DLCO collections whereas the standard isolate, IMI 330189 was obtained from BCP Ltd.(a South African company that produces the pathogen at commercial scale) (Table 1).

The pathogens were cultured on Sabouraud Dextrose (C6H12C6) Agar (SDA) plates. Antibiotic solution of chloramphenicol was added to the sterilized agar medium. Isolates were cultured at a pH of 6.8 and temperature 25°C for 10–14 days to achieve maximum growth and sporulation (Kaaya, 1989; Khada *et al.,* 1990; Seneshaw Aysheshim *et al.,* 2003; Ibrahim and Low, 1993). Conidia were harvested by flooding 10 ml of sterile distilled water containing 0.5% Tween 80 on agar plates. Conidial suspensions were adjusted to $2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ conidia /ml. A phase contrast light microscope and a stage haemocytometer were deployed to determine conidial concentrations following that used by Prior *et al.* (1992). Spore viability tests were carried out routinely throughout the experiments by pipetting 200 µl of spore formulation onto a 9 cm glass petri dish; SDA and viability were determined based on percentage germination (Hall, 1976; Emiru Seyoum, 2001). Spore batches with >85% germination were considered to be viable and were used.

Treatment of greater wax moths

Six adult GWMs were introduced into a transparent white plastic box (19 x 15 x 10 cm in length, width and height, respectively) lined with wire mesh (1.5 x 2 cm). The plastic box was covered with nylon mesh and held in position by a rubber band. Adults were inoculated by spraying 2 ml of each conidial suspension including, $2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ conidia/ml in three replicates; each experiment was repeated three times (Adane Kasa *et al*., 1998). Treated insects were provided with sugar solution (10%) soaked in cotton wool balls, which were changed at 24 hr interval and kept in a room, temperature set at 25 ± 2°C. Similarly, sixth larval instars were inoculated prior to entering into pupation and the effects of the pathogens (isolates) on emergence to adults following treatments were examined. Six last stage larvae (6^{th} larval instars) were used for each isolate and concentration. The experiments had three replicates and were repeated three times.

Treatment of honeybees

The honeybee (*Apis mellifera bandasii*) colonies (as non-target insects) were treated by six fungal isolates including, IMI 330189, DLCO-AA83, DLCO-AA 109, DLCO-AA5, DLCO-AA14) and IITA 18 in order to investigate the possible impact of the pathogens (isolates) on the non-target beneficial honeybee colonies. Post-treatment mortality of adult locusts was, for instance, recorded at 24 hrs interval for a period of 14 days (Emiru Seyoum and Merid Negash, 2007).

Table 1. Fungal isolates used, source substrates and country of origin.

Code	Isolate	Sources	Country of origin
DLCO-AA5	Beauveria	Grasshopper	Ethiopia
DLCO-AA14	Beauveria	Grasshopper	Ethiopia
IITA 18	Beauveria	Coleoptera	Ethiopia
IMI 330189	*M. anisopilae var. acridum*	Grasshopper	Niger
DLCO-AA83	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.

Confirmation of mycosis

In order to check whether or not mortality was due to mycosis, each cadaver was removed immediately, surface sterilized with 70% ethanol for 3 sec (Odindo, 1994) and kept in Petri dishes with moisten tissue papers laid in and incubated under high relative humidity (>90%) at temperature set at 25 ± 2°C for 7 days. Mortality was considered to be due to mycosis only when external growth of mycelia (external sporulation) following incubation of dead insects was apparent (Emiru Seyoum *et al.,* 1994; Emiru Seyoum, 2001). Comparison of pathogencity of isolates was based on speed of kill (Prior *et al.,* 1992; Seneshaw Aysheshim *et al.,* 2003).

All mortality data were corrected using Abbot's formula (1925) and cumulative percentage mortality data were subjected to one-way ANOVA using SPSS Computer Program. Student Newman Keul's Test (SNK) at 5% level of significance was used to separate the means.

RESULTS

Effects of the standard isolate, IMI 330189 and the local isolate IITA 18

Percentage mortality of GWM due to mycosis in targets infected with isolate IITA 18 increased, reaching 88.89% with the highest conidial concentration (2 x 10^7 conidia/ml) (Table 2). By day 3 post-applications, a significant difference in percent target mortality was due to differences in conidial concentration. This variation in percent mortality based on dose was not, however, observed by days 3 and 5 after treatment (Table 2). This is contrary to the expectation.

Mycosis in experimental GWM treated with IMI 330189 grew steadily and constantly from day 3 post-treatment and finally mortality reached 100% by day 13 post-application (Table 3).

Effects of local isolates (DLCO-AA-5, DLCO-AA-14, DLCO-AA-83 and, DLCO-AA-109)

Target mortality due to mycosis by the local isolates was time dependent and increased from 23.84% by day 3 (Table 4) to 100% (Tables 4, 5, 6 and 7) adult mortality by day 13 after treatment. All results showed significant differences in target mortality between the fungal treated and insects in the control group (Tables 4, 5, 6 and 7). No significant difference in mortality due to variation in doses (conidial concentration) was however recorded. This may indicate that even the lowest concentration (2 x 10^4 conidia/ml) caused infection sufficient to cause target death, similar to the highest conidial concentration used (2 x 10^7 conidia/ml) (Tables 4, 5, 6 and 7).

Table 2. Mean percent mortality of greater wax moth adults treated with isolate IITA 18 at different concentrations over time.

Conidia/ml	Percent 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±12.6	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 5% significance level [Student Newman Keul's Test (SNK)]. NS= not significant, s.e.= standard error, *= significant.

Table 3. Mean percent mortality of greater wax moth adults treated with isolate IMI 330189 at different concentrations over time.

Conidia/ml	Percent 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

Notations as in Table 2.

Table 4. Mean percent mortality of greater wax moth adults treated with isolate DLCO-AA-83 at different concentrations over time.

Conidia/ml	Percent mortality by day after treatment (Mean ±s.e)					
	3(NS)	5	7	9	11	13
$2x10^4$	23.84±2.67	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

Notations as in Table 2.

Table 5. Mean percent mortality of greater wax moth adults treated with isolate DLCO-AA-5 at different concentrations over time.

Conidia/ml	Percent 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

Notations as in Table 2.

Table 6. Mean percent mortality of greater wax moth adults treated with isolate DLCO-AA-14 at different concentrations over time.

Conidia/ml	Percent 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	00±0.00	9.26±3.70b	18.51±1.85c	35.18±1.85c	40.74±3.21b	50.00±3.21b

Notations as in Table 2.

Table 7. Mean percent mortality of greater wax moth adults treated with isolate DLCO-AA-109 at different concentrations over time.

Conidia/ml	Percent 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

Notations as in Table 2.

Adult mortality of >90% was achieved by day 13 post-treatment with all fungal isolates, except the isolate IITA 18 which did not show significant effects compared with the controls until day 9 after treatment. Results revealed that even the lowest conidial concentration ($2x10^4$ conidia/ml) of almost all isolates tested resulted in significant level of target greater wax moth post-treatment mortality (Table 2–7). In the course of post-inoculation examination, only one honey bee was found infected with the IITA 18 (*Beauveria* spp) and 2 honeybees with DLCO-AA-83 (*Metarhizium* spp). No honey bee was found infected with IMI 330189 and with the remaining isolates used (DLCO-AA-109, DLCO-AA-5, DLCO-AA-14).

In the present series of investigations, adult moths were found to be susceptible to all fungal isolate concentrations including, $2x10^4$, $2x10^5$, $2x10^6$ and $2x10^7$ conidia/ml. Percentage mortality of the GWM adults due to the applied fungal isolates reached up to 100% on day 13 post-treatment with some fungal isolates.

As the number of days progressed, isolates DLCO-AA-5, IMI 330189 and DLCO-AA-83 caused high mortality (100%) by day 13 after inoculation of the adults with the lowest concentration ($2x10^4$ conidia/ml). Isolates DLCO-AA-109, DLCO-AA-14, and IITA 18 also showed target mortalities of 96.31%, 95.83% and 78.44%, respectively by day 13 with the lowest concentration. In all cases, mortality of 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 (Tables 2–7) by day 13 after treatment with the lowest concentration of each isolate. This may show the virulence of the fungal isolates even when used at low concentration.

On the other hand, bioassays on the possible effect of fungal isolates applied one day prior to GWM larvae entered into pupation had no apparent (significant) effects of mycosis (Table 8) although this was not as expected. This result was interesting because it was contrary to what happened when larvae were treated at early stage i.e. larvae treated 3 or more days before entering into pupation had shown apparent (significant) impact of mycosis. The effect was found to be significant when compared with the controls and was also dose-dependent (Emiru Seyoum and Namusana, 2010). Although previous similar works are not available currently, we assume that pupation prior to infection seems to have impact on the progress/development of infection.

Confirmation of mycosis

Treated insects showed the characteristics of external sporulation of the respective fungi when surface sterilized as means of confirmation of mycosis (Emiru Seyoum *et al.*, 1994; Emiru Seyoum, 2001; Prior *et al.*, 1992). Similarly, post-mortality examination were carried out to check whether death of honeybees, *Apis mellifera bandasii* (Ethiopian race) were due to fungal infection. A total of 200 dead honeybees were collected from the inoculated colonies with the different isolates and maintained in the different cages and were surface sterilized. Of all the three fungal isolates tested against the honeybees (as non-target organisms), the post-death examination showed that isolate IMI 330189 (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 found infected. The Beauveria-infected honeybees produced a green characteristic sporulation when the spores were streaked on malt extract agar and incubated at 25°C in the dark for 7 days while the other cadavers showed no external growth of mycelia.

Table 8. Percentage emergence of Galleria mellonella adults following treatment of 6th instar larvae with six fungal isolates one day before entering pupation at different concentrations.

Fungal isolate	Concentration (conidia/ml)	Percent mortality
	$2x10^4$	97.2±2.8
	$2x10^5$	94.4±2.8
DLCO-AA-5	$2x10^6$	91.7 ±0.0
	$2x10^7$	86.1±7.4
	Control	97.2±2.8
	$2x10^4$	97.2±0.0
	$2x10^5$	91.7 ±0.0
DLCO-AA-14	$2x10^6$	91.7 ±0.0
	$2x10^7$	88.9±5.6
	Control	97.2±2.8
	$2x10^4$	97.2±2.8
	$2x10^5$	97.2±2.8
IITA-18	$2x10^6$	97.2±2.8
	$2x10^7$	84.4±0.0
	Control	100.0±0.0
	$2x10^4$	100.0±0.0
	$2x10^5$	91.7±4.8
IMI 330189	$2x10^6$	97.2±2.8
	$2x10^7$	94.4±2.8
	Control	97.2±2.8
	$2x10^4$	94.4±2.8
	$2x10^5$	100.0±0.0
DLCO-AA-83	$2x10^6$	97.2±2.8
	$2x10^7$	91.7±4.8
	Control	97.2±2.8
	$2x10^4$	97.2±2.8
	$2x10^5$	97.2±2.8
DLCO-AA-109	$2x10^6$	91.7±4.8
	$2x10^7$	94.4±2.8
	Control	100.0±0.0

Means within a column followed by the same letter are not significantly different at 5% levels of (SNK).

DISCUSSION

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, relatively few fungal diseases have thus far been developed for pest control. The potential of entomopathogens against the wax moth has not been established yet. In the present work, laboratory studies have shown that fungal isolates belonging to genera Beauveria and Metarhiziuzm have the potential towards the management of the GWM adults.

Owing to the fact that no previous published works on GWM using entomopathogens are currently available, comparisons of results herein are made based on previous similar works but on different groups of insects. It is, however, apparent that factors such as difference in target insects, isolates, fungal concentrations and conditions under which the experiments were conducted would contribute towards variations between the results of the present work and previous works under different situations.

As elucidated earlier (Tables 2–7) adult GWM were found to be susceptible to all fungal isolates at different concentrations, ranging from $2x10^4$ to $2x10^7$ conidia/ml used. This implies that the subject insects, GWM adults were susceptible to even the lowest dose (concentration). This is probably related to the fact that once the conidia penetrated the insect cuticle the post-penetration defence mechanism of the insects was weak, which might have led to the fast multiplication and invasion by conidia, as reported by Charnley *et al.* (1989). Hence, comparison of target mortality results due to mycosis was carried out at the lowest concentration ($2x10^4$ conidia/ml) of each isolate used. The findings revealed that infection of over 90% mortality was recorded by day 13 post-treatment with all fungal isolates except isolate IITA 18 (Tables 2–7). This finding is in agreement with results of 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 as a means for confirmation of mycosis. Over 85% of dead insects produced conidia when cadavers of infected insects were surface sterilized and incubated. This finding is also in accord with that recorded on adults *of Glossina morsitans morsitans* in which tested isolates *of Beauveria bassiana* and *Metarhizium anisopilae* were found to be pathogenic against adult tsetse with high mortalities ranging between 60 and 95% on days 15–18 post-infection and 100% mortalities by week 4 post-treatment using an aqueous spore suspension of $2x10^7$ conidia/ml (Kaaya, 1989).

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. anisopilae* 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 (Prior, 1997). Results obtained on non-target effects using isolate IMI 330189 in the present work are in agreement with the research findings in South Africa (Price and Muller, 1994), 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 Muller, 1994).

Ball *et al.* (1994) also found less than 1% infection in honeybees (*Apis mellifera,* Linnaeus) after exposure in the laboratory to the Metarhizium isolate IMI 330189. This is again in disagreement with the observations made on the honeybee colonies (Ethiopian race) in the present work using isolate IMI 330189 under laboratory conditions. This isolate was, therefore, probably infective or required a long infection period or the spores were still in the resting phase in the hemocoel. These findings are also 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.

Tested isolates have shown promising results in the present laboratory-based experiments although the conditions in the laboratory might have enhanced the performance of the pathogen more than what could be expected in nature where sub-optimal conditions for the growth and viability, many different antagonists and adverse weather conditions may prevail. Therefore, beneficial insects such as the honeybee should be included in impact monitoring when myco-insecticides are evaluated under large scale operations.

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