VIRULENCE OF *METARHIZIUM* ISOLATES AGAINST THE POLYPHAGOUS DEFOLIATOR PEST, *SPILARCTIA OBLIQUA* (LEPIDOPTERA: ARCTIIDAE)

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SAPNA BAI N, SASIDHARAN TO, REMADEVI OK, RAJAN PD & BALACHANDER M. 2010. Virulence of *Metarhizium* isolates against the polyphagous defoliator pest, *Spilarctia obliqua* (Lepidoptera: Arctiidae). Species of the entomopathogenic fungus *Metarhizium* are some of the most promising biocontrol agents against Lepidopteran pests. Laboratory bioassays were conducted to study the virulence of five isolates of *Metarhizium* sp. against larvae of the polyphagous pest, *Spilarctia obliqua* (Lepidoptera: Arctiidae). The *Metarhizium* isolates used in the study were recovered from soil and various insect hosts belonging to Lepidoptera, Coleoptera and Isoptera. Initially, a preliminary assay was carried out involving 18 *Metarhizium* isolates at a spore concentration 10^7 conidia ml^{-1} and five isolates, MIS 1, MIS 2, MIS 3, MIS 8 and MIS 9, which caused more than 50% mortality were chosen for the detailed bioassay. The assay was carried out with four different spore concentrations of each isolate, ranging from 10^4–10^7 conidia ml^{-1}. The lethal concentration LC_{50} of these five isolates ranged from 2.1×10^5 to 38.9×10^5 conidia ml^{-1}. The lethal time LT_{50} of the isolates ranged from 7.0–8.1, 6.0–7.7, 5.1–6.7, 4.6–5.4 days at concentrations 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia ml^{-1} respectively. The most pathogenic among the five isolates was the MIS 2 isolate with the lowest LC_{50} of 2.11×10^5 conidia ml^{-1} and LT_{50} of 4.6, 5.1, 6.0 and 7.0 days respectively at concentrations 1×10^7, 1×10^6, 1×10^5, 1×10^4 conidia ml^{-1}. Isolate MIS 3 was second in effectiveness. These two isolates showed promise for use as biocontrol agents against *S. obliqua*.

Keywords: Lepidopteran pests, entomopathogen, pathogenicity, LC_{50}, LT_{50}, biocontrol

SAPNA BAI N, SASIDHARAN TO, REMADEVI OK, RAJAN PD & BALACHANDER M. 2010. Virulens pencilan *Metarhizium* terhadap perosak peluruh daun polifagus, *Spilarctia obliqua* (Lepidoptera: Arctiidae). Spesies kulat entomopatogen *Metarhizium* merupakan agen pengawal biologi yang paling berkesan terhadap perosak Lepidoptera. Biocerakinan makmal dijalankan untuk mengkaji kevirulenan lima pencilan *Metarhizium* sp. dalam menentang larva *Spilarctia obliqua* (Lepidoptera: Arctiidae) yang merupakan sejenis perosak polifagus. Pencilan *Metarhizium* yang diguna dalam kajian ini diperoleh daripada tanah dan pelbagai serangga perumah daripada order Lepidoptera, Coleoptera dan Isoptera. Pada mulanya, cerakinan dijalankan untuk 18 pencilan *Metarhizium* pada kepekatan spora 10^7 konidium ml^{-1} dan kemudiannya, lima pencilan yang menyebabkan lebih daripada 50% kematian, iaitu MIS 1, MIS 2, MIS 3, MIS 8 dan MIS 9, dipilih untuk biocerakinan terperinci. Cerakinan setiap pencilan dijalankan pada empat kepekatan spora yang berbeza iaitu 10^4–10^7 konidium ml^{-1}. Kepekatan maut LC_{50} bagi kelima-lima pencilan ini berjulat antara 2.1×10^5 konidium ml^{-1} hingga 38.9×10^5 konidium ml^{-1}. Tempoh maut LT_{50} pencilan ini pula berjulat antara 7.0–8.1 hari, 6.0–7.7 hari, 5.1–6.7 hari dan 4.6–5.4 hari masing-masing pada kepekatan 1×10^4 konidium ml^{-1}, 1×10^5 konidium ml^{-1}, 1×10^6 konidium ml^{-1} dan 1×10^7 konidium ml^{-1}. Pencilan yang paling patogenik ialah pencilan MIS 2 yang mempunyai LC_{50} paling rendah iaitu 2.11×10^5 konidium ml^{-1} dan LT_{50} sebanyak 4.6 hari, 5.1 hari, 6.0 hari dan 7.0 hari masing-masing pada kepekatan 1×10^7 konidium ml^{-1}, 1×10^6 konidium ml^{-1}, 1×10^5 konidium ml^{-1} dan 1×10^4 konidium ml^{-1}. Pencilan MIS 3 berada di tempat kedua dari segi keberkesanan menentang perosak. Kedua-dua pencilan ini menunjukkan potensi untuk diguna sebagai agen kawalan biologi terhadap *S. obliqua*.

INTRODUCTION

The Bihar hairy caterpillar, *Spilarctia obliqua* (Lepidoptera: Arctiidae) is a polyphagous pest having a very wide range of host plants. It has been recorded feeding on as many as 33 host plants including many agricultural crops and garden plants and also on numerous species of forest shrubs and trees including *Butea frontosa, Cedrela toona, Colebrookia oppositifolia, Lantana camara, Morus alba, Morus indica, Tectona grandis* and *Vitex negundo* (Yadav *et al.* 2001). This pest

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has been recorded in India, Pakistan, Sri Lanka, eastern Asia, Borneo, China and Japan (Biswas 2006). It occurs almost throughout the year and infestation is severe from August till December and also sometimes in January. There are six generations of this pest in a year, indicating the potential of this pest to cause severe defoliation of host plants. Young caterpillars are gregarious and they feed on green soft tissues of tender leaves, mostly on the under surface of the leaves leaving behind only veins. Grown-up caterpillars are solitary and feed voraciously on entire leaves causing defoliation of the plants leading to significant reduction in yield. Destroying one field, they move in swarms to another field (Yadav *et al.* 2001).

Use of various pesticides is widespread among farmers and plantation growers for the control of this pest. Though application of pesticides have yielded considerable success, continued use of these chemicals has resulted in adverse impact on humans, animals and the environment due to toxic effects in food chain, development of resistance in target pests and destruction of natural enemies (Paray & Rajabalee 1997, Joshi *et al.* 2000). Due to these ill effects of pesticides, alternative eco-friendly strategies have been evolved and increasingly practised for pest management (Padmaja *et al.* 2004). Pathogens differ from chemical insecticides in that they can replicate in the host insect and spread through the population leading to sustained suppression of pest population. This spread of infection is an important aspect of the successful use of entomopathogens as insecticides (Ahmed & Leather 1994, Hauxwell *et al.* 2001).

Among the entomopathogens, fungi are of significant importance because, unlike bacteria and viruses which need to be ingested for causing diseases, fungus enters the insect by penetrating its cuticle, although infection through the digestive tract occurs with some species. Spores attach to the cuticle, germinate and penetrate the integument by means of a combination of physical pressure and enzymatic degradation of the cuticle. Host death is usually due to a combination of nutrient depletion, invasion of organs and the action of fungal toxin (Butt & Goettel 2000). Of the about 750 species of entomopathogenic fungi, the Hyphomycetes group is reported to have a broad spectrum of activity (Ihara *et al.* 2003). Most entomopathogenic Hyphomycetes are facultative pathogens and are relatively easily grown on defined or semi-defined media (Goettel & Inglis 1997). Although many major fungal entomopathogens have basic diagnostic characters which make them quickly identifiable, species such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* are complex and their resolutions will depend on correlating molecular, morphological, patho-biological and other characters (Humber 1997).

Various studies have revealed the efficiency of different Hyphomycetes fungi such as *B. bassiana, M. anisopliae, Nomuraea releyii, Debaromyces hansenii* and *V. lecanii* as potential mycoinsecticides. *Metarhizium* spp. are capable of infecting insects living in diverse habitats including fresh water, soil and aerial location (Hajek & Leger 1994) and are infective to more than 200 insect species of various orders. *Metarhizium anisopliae* has gained significant attention as a biocontrol agent due to its broad spectrum of pathogenicity and infectivity to a wide range of insect pests (Freire *et al.* 2001, Wright *et al.* 2005, Ypsilos & Magan 2005).

In the present study, initially, 18 *Metarhizium* isolates were evaluated for their efficacy using a single concentration of spores against the larvae of *S. obliqua*. From these, the most promising five isolates were selected for which detailed laboratory bioassays were carried out. The lethal concentration LC_{50} and lethal time LT_{50} for these isolates were determined and presented in this paper.

MATERIALS AND METHODS

Insect culture

Spilarctia obliqua larvae were collected from the field and reared on teak leaves in a Biological Oxygen Demand (BOD) incubator at 26 ± 1 °C and 70 ± 5% relative humidy (RH) under 14 hours L/10 hours D photoperiod. The rearing was conducted as follows. A fresh piece of tender teak leaf along with a small piece of moistened cotton wool were placed in a polystyrene vial (15 mm diameter × 45 mm high) covered with double-layered muslin cloth. Individual larva was transferred to each vial which was then placed in the BOD incubator. The vials were cleaned periodically and fresh leaves were provided. After pupation and emergence, 10 pairs of adults were transferred to a glass container (200 × 190 mm) covered with muslin cloth in the BOD incubator for mating and oviposition. Eggs were mostly laid

on the muslin cloth cover. The cloth with eggs were removed, covered with tender teak leaves, placed in glass bottles and kept in the BOD incubator. After hatching the larvae were reared till the third instar as described above and used for bioassay.

Metarhizium culture

The *Metarhizium* isolates (Table 1) used in this study were recovered from infected natural hosts and soil. The fungus was isolated on potato dextrose agar medium (Himedia), fortified with 1% yeast extract. The medium was sterilised at 121 °C and 15 psi for 30 min, poured into sterile Petri plates, cooled and inoculated with infected part of the cadavers or serially diluted soil sample under aseptic conditions. The Petri plates were then incubated at room temperature (28 ± 2 °C). After seven days of incubation, the fungus was subcultured and purified. Slants were prepared from purified culture and stored at 4 °C.

Inoculum preparation

The conidia of *Metarhizium* isolates were produced by growing the fungus on potato dextrose agar yeast extract (PDAY) medium in Petri plates and incubated at 28 ± 2 °C for 14 days. Conidia were harvested by flooding the plates with sterile distilled water containing 0.05% Tween 80. Conidial concentration was counted under phase contrast optics at a magnification of 600 × using an improved Neubauer haemocytometer and adjusted to four concentrations of 10^4, 10^5, 10^6 and 10^7conidia ml^{-1} for bioassay.

Bioassay

Initial virulence test for all the 18 isolates was carried out with a single concentration (10^7conidia ml^{-1}) of conidial suspension. Four replicates of 10 third instar *S. obliqua* larvae each, were maintained for each isolate. The test larvae were dipped in the conidial suspension

Table 1 *Metarhizium* isolates used in the study

Isolate	Strain	Host insect/source
MIS 1	*Metarhizium* sp.	Mummified larvae (Lepidoptera)
MIS 2	*Metarhizium* sp.	Mummified larvae (Lepidoptera)
MIS 3	*Metarhizium* sp.	Mummified larvae (Lepidoptera)
MIS 4	*Metarhizium* sp.	*Eutectona machaeralis* (Lepidoptera)
MIS 5	*Metarhizium* sp.	*Myllocerous discolor* (Coleoptera)
MIS 6	*Metarhizium* sp.	Orthoptera
MIS 8	*Metarhizium* sp.	Soil
MIS 9	*Metarhizium* sp.	Soil
MIS 11	*M. anisopliae*	*Agrianome spinicollis* (Coleoptera)
MIS 12	*M. anisopliae* var. *anisopliae*	*Oryctes rhinocerous* (Coleoptera)
MIS 13	*M. anisopliae* var. *anisopliae*	*Diabrotica speciosa* (Coleoptera)
MIS 14	*M. anisopliae* var. *anisopliae*	*Spodoptera* sp.(Lepidoptera)
MIS 15	*M. anisopliae* var. *anisopliae*	Coleoptera
MIS 16	*M. frigidium*	*Coptotermes lacteus* (Isoptera)
MIS 18	*Metarhizium* sp.	*Oryctes rhinocerous* (Coleoptera)
MIS 19	*Metarhizium* sp.	Soil
MIS 20	*Metarhizium* sp.	Soil
MIS 21	*Metarhizium* sp.	Soil

(1×10^7 conidia ml^{-1}) for 10 s and transferred to separate Petri plates with tender teak leaves. Control larvae were similarly dipped in a solution of Tween 80 (0.05%) in sterile distilled water. Mortality of larvae was recorded at 24 hour intervals for six days. Mycelial growth and conidial formation on the dead larvae were confirmed by further incubation of the cadavers at 28 ± 2 °C and 90 ± 5% RH.

From results obtained, the most promising five isolates which caused > 50% mortality were selected and a detailed bioassay was performed as described above using inoculum concentrations ranging from 10^4–10^7 conidia ml^{-1}.

Statistical analysis

For the single concentration assay, cumulative mortality after six days of treatment of each isolate was compared. Mortality data were normalised using arcsine transformation and subjected to analysis of variance (ANOVA). Means were separated using the Duncan's multiple range test (DMRT) at $p \leq 0.05$. For multiple-concentration assay, Probit analysis (Finney 1971) was used to estimate LC_{50} and LT_{50} values of the five selected promising isolates.

RESULTS AND DISCUSSION

All the 18 isolates were pathogenic to *S. obliqua* at 10^7 conidia ml^{-1} and the mean mortality ranged from 36.0 (MIS 11 and 13 isolates) to 60.2% (MIS 2 isolate) (Table 2). Isolates MIS 1, 2, 3, 8 and 9 (mortality > 50%) were considered as more potent against *S. obliqua*. In a detailed bioassay with these promising five isolates, larval mortality of *S. obliqua* ranged from 16.4–32.8, 26.2–42.1, 32.8–51.0 and 50.7–60.2% for concentrations of 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia ml^{-1} respectively (results not shown). LC_{50} values of the five isolates ranged from 2.1×10^5 to 39.9×10^5 conidia ml^{-1} but isolate MIS 2 was the most virulent (Table 3). LT_{50} values for these isolates ranged from 4.6–5.4 days in the highest concentration tested (Table 4). MIS 2 needed the lowest concentration (LC_{50} value of 2.1×10^5 conidia ml^{-1}) and shortest time (LT_{50} values of 7.0, 6.0, 5.1 and 4.6 days at concentrations 1×10^4, 1×10^5, 1×10^6 and 1×10^7 respectively) to cause the highest mortality (Table 4). Thus, among the isolates tested, MIS 2 exhibited the highest pathogenicity against *S. obliqua*.

Table 2 Mean per cent mortality of *Spilarctia obliqua* treated with *Metarhizium* sp. at 10^7 conidia ml^{-1}

Isolates	% Mortality (mean ± SE)
MIS 1	51.0 ± 4.9 abc (45.57)
MIS 2	60.2 ± 3.1 a (50.89)
MIS 3	57.1 ± 3.6 ab (49.08)
MIS 4	48.1 ± 5.7 bcd (43.91)
MIS 5	42.1 ± 2.8 cde (40.46)
MIS 6	47.8 ± 2.8 bcde (43.74)
MIS 8	50.7 ± 5.9 abcd (45.40)
MIS 9	51.0 ± 4.9 abc (45.57)
MIS 11	36.0 ± 3.1 e (36.87)
MIS 12	42.1 ± 2.8 cde (40.46)
MIS 13	36.0 ± 3.1 e (36.87)
MIS 14	39.2 ± 5.9 cde (38.76)
MIS 15	42.1 ± 2.8 cde (40.46)
MIS 16	47.8 ± 2.8 bcde (43.74)
MIS 18	45.0 ± 3.3 cde (42.13)
MIS 19	45.0 ± 3.3 cde (42.13)
MIS 20	47.8 ± 2.8 bcde (43.74)
MIS 21	38.9 ± 4.9 de (38.59)

Values in parentheses are angular transformed. Means followed by different alphabets differ significantly at $p \leq 0.05$.

Larval mortality occurred 3–6 days after treatment. During early stages of the disease, no symptom was noticed but later, larvae were sluggish and lagged behind in development. The diseased larvae were also found less responding to physical stimuli. The infected larvae remained undersized and shrunken even after the control larvae completed pupation and emergence. In the cadavers, fungal mycelium emerged through the inter-segmental membranes and eventually covered most parts of the body within 2–5 days (Figure 1).

This study indicated that although all 18 *Metarhizium* isolates were pathogenic to *S. obliqua*, the five selected isolates were most promising. Isolate MIS 2 was exceptionally effective and can be regarded as the best for further investigation. Studies which elucidate mechanisms affecting host–pathogen interactions will be of great value in the selection of hyper virulent strains for the future. A toxicity study of *B. bassiana* and

Table 3 Concentration–mortality response of *S. obliqua* larvae exposed to *Metarhizium* isolates

Isolates	No. of larvae[a]	Slope ± SE	LC_{50} ($\times 10^5$)	$\chi^{2\,b}$
MIS 1	320	2.78 ± 0.80	39.9	0.329
MIS 2	320	2.11 ± 0.74	2.1	0.004
MIS 3	320	3.41 ± 0.82	15.2	0.664
MIS 8	320	2.45 ± 0.77	32.6	0.332
MIS 9	320	2.81 ± 0.80	31.1	0.058

[a]Total number of larvae used for each isolate
[b]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$)

Table 4 Time–mortality response of *S. obliqua* larvae exposed to *Metarhizium* isolates

Isolates	Concentrations	Slope ± SEM	LT_{50} (days)	$\chi^{2\,a}$
MIS 1	1×10^4	7.2 ± 3.5	8.1	0.15
	1×10^5	5.3 ± 1.7	7.5	0.22
	1×10^6	5.0 ± 1.4	6.7	0.62
	1×10^7	6.1 ± 1.4	5.4	0.54
MIS 2	1×10^4	4.3 ± 1.2	7.0	1.55
	1×10^5	3.7 ± 0.8	6.0	0.91
	1×10^6	3.9 ± 0.8	5.1	0.54
	1×10^7	4.0 ± 0.8	4.6	0.45
MIS 3	1×10^4	7.4 ± 3.3	7.6	0.23
	1×10^5	4.7 ± 1.3	7.0	0.95
	1×10^6	4.9 ± 1.2	6.1	0.76
	1×10^7	5.7 ± 1.2	5.1	1.08
MIS 8	1×10^4	7.4 ± 3.3	7.6	0.23
	1×10^5	5.5 ± 1.8	7.7	0.26
	1×10^6	5.3 ± 1.4	6.5	0.43
	1×10^7	4.7 ± 1.0	5.4	0.06
MIS 9	1×10^4	7.2 ± 3.5	8.1	0.15
	1×10^5	5.5 ± 1.8	7.7	0.26
	1×10^6	5.2 ± 1.4	6.4	0.40
	1×10^7	4.7 ± 1.0	5.4	0.06

[a]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$)

M. anisopliae carried out against the tobacco caterpillar *Spodoptera litura* and *S.obliqua* indicated that the activity of the fungi decreased with the increase in age of larvae (Purwar & Sachan 2005). It was also reported that mortality of *S. obliqua* increased with time after treatment with *B. bassiana* (Bhattacharya *et al.* 2003). Fortifying the growth medium with various supplements may increase the virulence of fungal isolates. Increased virulence of fungal isolates against *S. obliqua* larvae was observed when they were cultured on medium fortified with host insect larval extract and chitin powder or *S. obliqua* integument (Mondal & Bhattacharya

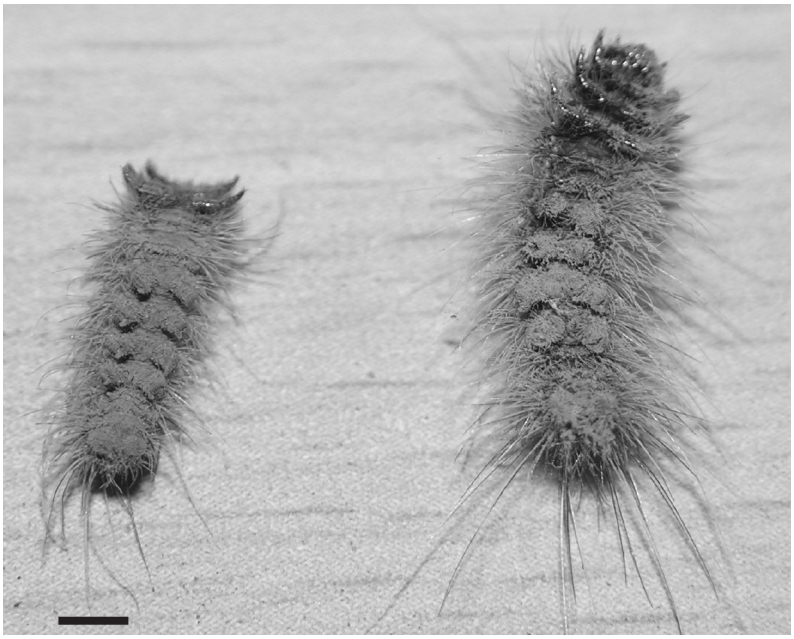

Figure 1 *Metarhizium*-infected *S. obliqua* larvae showing profuse fungus growth on the bodies; scale bar = 2.5 mm

2003, Pandey & Kanaujia 2004). Similarly, combination treatments of certain insecticides and entomogenous fungi also showed improved toxicity over sole treatments for the control of *S. obliqua* (Purwar & Sachan 2006).

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