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Virulence of Native Isolates of *Metarhizium anisopliae* and *Beauveria bassiana* Against *Helicoverpa armigera* in Nepal

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ABSTRACT

The native isolates of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana,* were evaluated against the third instar larvae of the chickpea pod borer, *Helicoverpa armigera,* for mortality, infection rate and LT_{50} under laboratory conditions in Nepal. Out of four isolates of *M. anisopliae* and two isolates of *B. bassiana* evaluated, *M. anisopliae* M1 and *B. bassiana* B3, were the most virulent ones of all isolates evaluated, with a concentration at 10^7 conidia/ml. The M1 and B3 isolates had the highest mortality rates and required the shortest time to kill the larvae of *H. armigera*. After treatment with M1 and B1 isolates for 10 days, the larval mortality exceeded 85%. These data lay the ground work for further studies in optimizing the effectiveness of indigenous virulent isolates, *M. anisopliae* M1 and *B. bassiana* B3 against *H. armigera* in Nepal.

Key words: *Beauveria bassiana, Metarhizium anisopliae,* LT_{50}, *Helicoverpa armigera*

Introduction

The chickpea pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), is reported as a cosmopolitan, polyphagous pest and considered as one of the major biotic constraints of chickpea production in the world (Mehto *et al.*, 1985; Manjunath, 1997; Pawar, 1998; Pande *et al.*, 2000). In Nepal, *H. armigera* is considered a major legume pest across the country (Manandhar, 1997). This pest has gained a 12 to 103 fold resistance to the common pyrethroids in Nepal (Armes and Pandey, 1995). The alternative to the chemical insecticides, the mycopesticides have low or no resistance problem, are host specific, cheaper and ecologically sound (Ferron *et al.,* 1991;

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Mendoca, 1992).

Metarhizium anisopliae has been employed against a variety of insect pests including *H. armigera*. Likewise, *Beauveria bassiana* has also been reported as an effective fungus against *H. armigera* under both laboratory and field conditions (Sandhu *et al.,* 2001). Several studies and developmental activities have indicated many opportunities of using indigenous isolates of entomopathogenic fungi, *M. anisopliae* and *B. bassiana* which were reported being very effective against caterpillar pests in Nepal (GC. and Keller, 2000; GC. *et al.,* 2004). However, there have been no reports on the evaluation of indigenous pathogenic fungi (*M. anisopliae* and *B. bassiana*) isolates against the *H. armigera* in Nepal. Therefore, this study aimed to evaluate the native isolates of *M. anisopliae* and *B. bassiana* for use against the third instar of *H. armigera* under laboratory conditions in Nepal.

Materials and Methods

Helicoverpa armigera larvae

The third instar larvae of *H. armigera* were collected from a chickpea field from the farm fields of the Chitwan district in Nepal near our research laboratory. The larvae collected were reared under laboratory conditions at 25 ± 2°C, 70 ± 10% RH and natural light and dark conditions. All larvae were kept in quarantine for two days with sterilized water soaked chickpea grains. Two days after the quarantine, only healthy and normal third instar larvae were used for further studies.

Fungal isolates

The fungal isolates were obtained from stock maintained at the Insect Pathology Laboratory, Department of Entomology, IAAS, Rampur, on Saboraud Dextrose Agar supplemented with antibiotics (0.6 g streptomycin, 0.05 g tetracycline, and 0.05 g cyclohexamide and 0.1 mL dodine (AS: 460g/l) as reported by Strasser *et al.* (1996). The isolates were obtained from insects that had succumbed (*M. anisopliae* and *B. bassiana*) and soil from different parts of Nepal in 2003/04 (GC *et al.*, 2008).

Fungal preparation

Four isolates of *M. anisopliae* (M1, M48, M65 and M70) and two isolates of *B. bassiana* (B1 and B3) were grown on the selective medium (SM) adapted from Strasser *et al.* (1996). The 10 g peptone from meat pancreatically digested, 20 g glucose and 18 g agar were all dissolved in 1 l. of distilled water and autoclaved for 20 min at 120°C. After the medium was cooled down to 60°C, 0.6 g streptomycin, 0.05 g tetracycline, and 0.05 g cyclohexamide (dissolved in 20 ml sterilized distilled water) and 0.1 ml dodine (AS: 460g/l) were mixed in. To induce growth and sporulation, the fungi were incubated at 25°C and 75 % RH for 15 days. The conidia were collected by scraping off the contents of each Petri dish.

From the original solution, 1 ml was dropped onto a Thoma haemocytometer, observed under a microscope (TIEFA, Germany) and adjusted to 10^7 conidia/ml. The original solution was diluted for ease of counting concentrations. The fungal concentration was calculated by using a haemocytometer. The hydrophobic conidia were dispersed in water using two drops of Tween 80 (0.1%). The enumeration of conidia was done separately for respective bioassay experiments.

Experimental design

The Completely Randomized Design with three replications and seven treatments was used for this study. Each experimental unit included 25 third instar larvae, collected from the chickpea field and reared in the laboratory for 2 days. The four isolates of *M. anisopliae* (M1, M48, M65 and M70) and two isolates of *B. bassiana* (B1 and B3) were assayed for their virulence against *H. armigera*. The treatment with water was used as a

Table 1. Mortality of *H. armigera* larvae caused by different isolates of *M. anisopliae* and *B. bassiana* (1×10^7 conidia/ml) in the laboratory

Isolates	Larval mortality (%) (Mean ± SE)*					
	3 DAT	7 DAT	10 DAT	14 DAT	21 DAT	LT_{50} (day)
M1	6.67 ± 1.33a	65.33 ± 7.42a	86.67 ± 4.81ab	92.00 ± 2.31b	94.67 ± 1.33ab	5.33 ± 0.33bc
M48	0.00 ± 0.00c	60.00 ± 4.00ab	84.00 ± 0.00b	84.00 ± 0.00bc	88.00 ± 0.00b	6.00 ± 0.00bc
M65	0.00 ± 0.00c	54.67 ± 8.74ab	84.00 ± 2.31b	85.33 ± 3.53bc	89.33 ± 1.33b	6.66 ± 0.33ab
M70	5.33 ± 1.33ab	56.00 ± 10.58ab	81.33 ± 5.53b	86.67 ± 2.67bc	93.33 ± 1.33b	6.00 ± 0.58bc
B1	2.67[b] ± 1.33b	37.33 ± 10.67b	66.67 ± 7.06b	80.00 ± 4.62c	85.33 ± 5.33b	7.66 ± 0.67a
B3	4.00 ± 0.00b	64.00 ± 6.11a	94.67 ± 5.33a	98.67 ± 1.33a	98.67 ± 1.33a	5.00 ± 0.58c
Control	0.00 ± 0.00c	0.00 ± 0.00c	2.67 ± 2.67c	8.00 ± 2.31d	10.67 ± 3.52c	-
CV (%)	19.54	19.33	12.96	7.42	7.45	14.43
CD ($P \leq 0.05$)	2.643	14.16	13.28	8.20	8.68	1.323
SEM (±)	0.87	4.67	4.38	2.71	2.86	0.4363

* Numbers with the same letters in the same column are not significantly different at 5 % by DMRT.

SEM (±): Standard error of mean difference.

CV (%): Coefficient of variation.

CD ($P = 0.05$): Critical difference at probability value 0.05.

control.

Bioassay

The conidial suspension (10^7 conidia/ml) was prepared in a glass Petri dish. That concentration was found to be most effective against the common white grubs (GC *et al.*, 2008), *Lygus lineolaris* (Li and Holdom, 1994) and *Bombyx mori* (Acharya, 2005). Each larva was allowed to crawl over the prepared conidial suspension on the glass Petri dish for 30 seconds. After treatment, the larvae were kept individually and were provided with soaked chickpea grains as a source of food overnight.

The numbers of dead and mycosed larvae were recorded daily. The dead larvae were kept individually in a plastic vial (60 ml) containing moist cotton to facilitate fungal growth. The dead larvae with fungal growth in the plastic vial were observed under the microscope and confirmed as death due to the fungal infection. Larvae with fungal growth were recorded as mycosed larvae (Mazodze and Zvoutete, 1999).The medial lethal time (LT_{50}) and mortality assessment were calculated based on the daily observations of the number of dead larvae. To determine the percentage of mycosed larvae from all of the dead larvae, the number of larvae infected with fungus and those dead without fungal growth were recorded. Then the following equation was used to determine the mortality, infection and LD_{50}.

$$\text{Mortality / Infection (\%)} = \frac{\text{No. of dead / Infected larvae}}{\text{Total no. of larvae}} \times 100$$

All of the means were compared using Duncan's multiple range test (DMRT) (MSTAT-C, 2002).

Results

Mortality of larvae and LT_{50}

At 3 days after treatment (DAT), a significantly higher percentage of *H. armigera* larvae were killed by the M1 isolate than by the B1 and B3 isolates. However, the percentages of *H. armigera* larvae killed by M1 and M70 isolates or M70, B1 and B3 isolates were significantly different among each other. No larva died

Table 2. Infection and mycosis time by fungal isolates (1×10^7 conidia/ml) on *Helicoverpa* larvae in the laboratory

Isolates	Infection after death (days)* (Mean ± SE)	Infection (%) (Mean ± SE)
M1	7.49 ± 0.23b	45.33 ± 1.33c
M48	8.15 ± 0.25a	37.33 ± 3.52de
M65	8.48 ± 0.05a	36.00 ± 2.31e
M70	7.39 ± 0.16b	44.00 ± 2.31cd
B1	7.36 ± 0.03b	66.67 ± 3.52b
B3	7.22 ± 0.20b	80.00 ± 0.00a
Control	0.00 ± 0.00c	0.00 ± 0.00 f
CV (%)	4.24	5.99
CD ($P \leq 0.05$)	0.4891	4.161
SEM (±)	0.1612	1.372

* Numbers with same letters in the same column are not significantly different at 5 % by DMRT.

SEM (±): Standard error of mean difference.

CV (%): Coefficient of variation.

CD ($P = 0.05$): Critical difference at probability value 0.05.

as a result of the treatment with isolates M48, M65 and the control. At 7 DAT, a significantly higher percentage of *H. armigera* larvae were killed by M1 and B3 isolates than by the rest of the isolates. However, the percentages of *H. armigera* larvae killed by M1, M48, M65, M70 and B3 isolates or M48, M65, M70 and B1 isolates were significantly different among each other. Similarly, at 10 DAT, a significantly higher percentage of *H. armigera* larvae were killed by B3 isolate than by the rest of the isolates. However, the percentages of *H. armigera* larvae killed by M1 and B3 isolates or M1, M48, M65, M70 and B1 isolates were significantly different among each other (Table 1).

Furthermore, at 14 DAT, a significantly higher percentage of *H. armigera* larvae were killed by B3 isolate than by the rest of the isolates. However, the percentages of *H. armigera* larvae killed by M1, M48, M65 and M70 isolates or B1, M1, M48, M65 and M70 isolates were significantly different among each other. At 21 DAT, a significantly higher percentage of *H. armigera* larvae were killed by B3 isolate than by the rest of the isolates. However, percentages of *H. armigera* larvae killed by B3 and M1 isolates or M1, M48, M65, M70 and B1 isolates were significantly different among each other (Table 1).

Based on the daily observation of mortality of *H. armigera* larvae with 10^7 conidia/ml, isolate B3 had a significantly lower LT_{50} than the B1 and M65 isolates. The LT_{50} of isolates M1, M48, M65, M70, and B3 were not significantly different among each other (Table 1).

Infectivity and mycosis of larvae

The infection of the larvae differed with the fungal isolates. The number of days required to the dead larvae to be infected was significantly less after treatment by the B3 than by the M48 and M65 isolates. However, the number of days required to the dead larvae to be infected for the M1, M70, B1 isolates and the B3 or M48 and M65 isolates were not significantly different. The highest percentages of larvae were infected by isolate B3 followed by B1 and M1, and a significantly lower percentage of larvae were infected by the M65 isolate. However, the percentages of infection by M1 and M70, M48 and M70

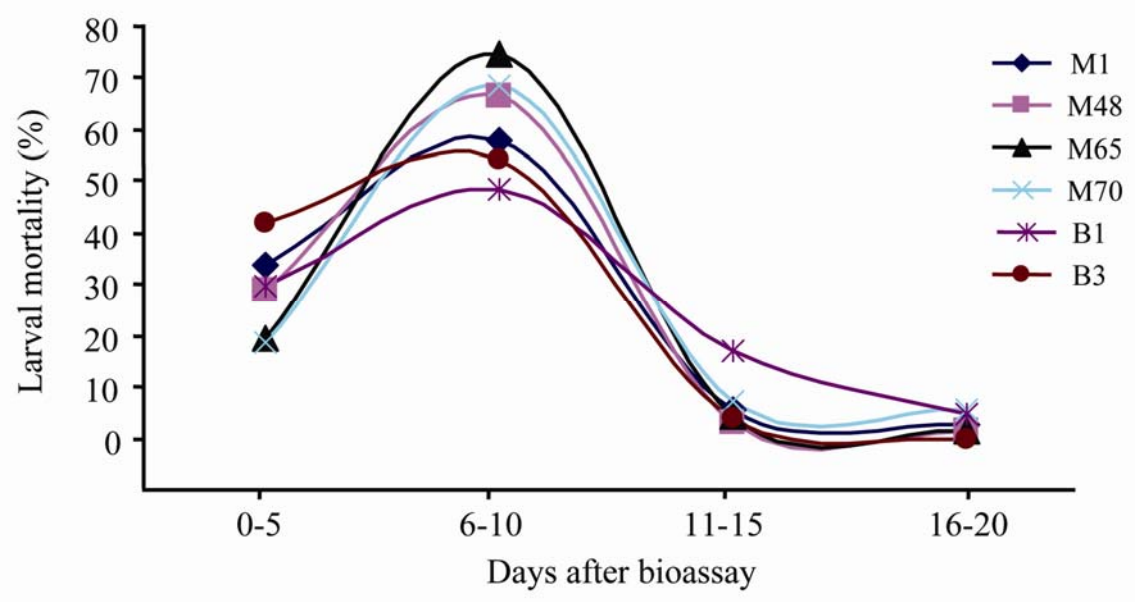

Fig. 1. Mortality of *H. armigera* caused by different isolates of *M. anisopliae* (M1, M18, M65 and M70) and *B. bassiana* (B1 and B3) in the laboratory.

isolates or M48 and M65 isolates were not significantly different (Table 2).

Effective mortality time

As shown in Figure 1, the mortality of larvae for all isolates at four time intervals (0-5, 6-10, 11-15, 16-20 days of bioassay) followed the same trend, withthe mortality increasing up to the 10th day and then decreased. During the 0-5 days of the bioassay, the larval mortality was highest with B3 (41.89%) followed by M1 (33.80%) and then B1 (29.69%). Both isolates of *B. bassiana* showed higher percent mortalities during 0-5 days, followed by all *M. anisopliae* isolates. Similarly, during the 6-10 days of the bioassay, the larval mortality was highest with M65 (74.63%) followed by M70 (68.57%), M48 (66.67%), M1 (57.75%), B3 (54.05%) and then B1 (48.44%). In addition, during the 11-15 days of the bioassay, the larval mortality declined drastically compared to the 6-10 days of the bioassay. The larval mortality was highest with B1 (17.2%) followed by M70 (7.16%), M1 (5.63%), M65 (4.48 %), B3 (4.06%) and then M48 (3.05%). Finally, during the 16-20 days of the bioassay, the larval mortality was highest with M70 (5.71%) followed B1 (4.69%), M1 (2.82%), M48 (1.52%), M65 (1.49%) and then B3 (0.00%).

Discussion

All four isolates of *M. anisopliae* were pathogenic to *H. armigera* larvae tested, but with great variability among the isolates. Patil *et al.* (2005) also reported that the isolates of different fungi could result in different rates of pathogenicity under laboratory and field conditions.

Several indigenous isolates of *B. bassiana* have been reported to be pathogenic to *H. armigera* (Sandhu *et al.*, 2001). The findings of this study also confirmed that Nepalese isolates were pathogenic to *H. armigera* larvae. In this study, the overall mortality of *H. armigera* ranged from 85.3% to 98.7%. It has been reported that

the average mortality of *Lygus lineolaris*, *Plutella xylostella* and *Sylepta deroga* was 86.7%, 86.2% and 89.4%, respectively (Li and Holdom, 1994; Sood *et al.*, 2001; Ramesh *et al.*, 1999). The overall mortality of *H. armigera* in our study was slightly higher than the mortality of those insects, but that might be due to the different species of insects used for the test.

The LT_{50} of both fungi, *Beauveria* isolates and *Metarhizium,* was almost similar with the finding of Doberski (1981) regarding the Elm beetle larvae. The percent mycosis was highest with B3 isolate of *Beauveria* and M1 of *Metarhizium*. Both isolates of *Beauveria* resulted in a higher mycosis percentage than that of *M. anisopliae*. This result agrees with Ramesh *et al.* (1999) who reported 58 to 63% of mycosis in *Sylepta derogates* by *B. bassiana*. The time for developing mycosis after treatment was least with B3 followed by B1 and was the longest for the *M. anisopliae* isolates. Zimmermann (1982) reported that *M. anisopliae* required 5 days for mycosis under optimal temperature and humidity. The differences might be due to the fact that in this study different fungi isolates were used (Zimmermann, 1982).

The native isolates of *Metarhizium anisopliae* and *Beauveria bassiana* were recently isolated and evaluated against several insect pests and proved to be especially effective against white grubs (Rodríguez-del-Bosque *et al.*, 2005; GC *et al*., 2008), sweet potato weevils (Ondiaka *et al*., 2008) and cherry slugworm (Aslantaş *et al*., 2008). In addition, in Nepal we also evaluated the native isolates and observed them to be also effective against *H. armigera* larvae. Based on the facts observed in this study, the indigenous insect pathogenic fungal isolates have shown to have the potential for *H. armigera* management and could be included in the integrated pest management strategy. However, more advanced studies need to be done both in the field and in the laboratory to understand the efficacy of the native fungi isolates under diverse field conditions in Nepal.

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黑殭菌（*Metarhizium anisopliae*）與本土白殭菌（*Beauveria bassiana*）之本土分離株對蕃茄夜蛾（*Helicoverpa armigera*）之毒性於尼泊爾之研究

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摘　要

在尼泊爾，於實驗室條件下，將蟲生真菌——黑殭菌（*Metarhizium anisopliae*）與本土白殭菌（*Beauveria bassiana*）的本土分離株對於蕃茄夜蛾（*Helicoverpa armigera*）三齡幼蟲的致死率、感染率及 LT50 進行評估。加上 4 個黑殭菌分離株與 2 個本土白殭菌分離株，黑殭菌 M1 與本土白殭菌 B3 是所有分離株在濃度 10^7 conidia/ml 下毒性最強者。M1 與 B3 分離株具有最高的致死率，且可用最短時間殺死蕃茄夜蛾幼蟲。於施用 M1 與 B3 分離株十天後，幼蟲致死率超過 85%。在尼泊爾欲善加利用本土毒性分離株——黑殭菌 M1 與本土白殭菌 B3，以達最佳功效，這些數據可作為未來研究的基礎。

關鍵詞：本土白殭菌、黑殭菌、LT_{50}、番茄夜蛾。

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