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Efficacy of *Metarhizium anisopliae* and *Beauveria bassiana* against *Helicoverpa armigera* in Chickpea, under Field Conditions in Nepal

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ABSTRACT

A field study was conducted to evaluate the efficacy of two most virulent native isolates of insect pathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) and compared with four commercial biopesticides against Chickpea pod borer (*Helicoverpa armigera* Hubner) at Chitwan, Nepal. The number of *H. armigera* lavae observed in plots treated with *M. anisopliae* and *B. bassiana* were significantly lesser than the control plots during vegetative, flowering and pod setting stage of chickpea. Similarly, the chickpea yield was significantly higher in the plots treated with *M. anisopliae* and *B. bassiana* than control, however lesser than NPV and Bt treated plots. Based on this study, the native isolates have potential to be a biocontrol agent against the *H. armigera* in Nepal.

Key words: *Helicoverpa armigera*, *Beauveria bassiana, Metarhizium anisopliae,* HaNPV, Bt, Margosom

Introduction

The chickpea pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), is a globally distributed, polyphagous pest and a major biotic constraint of chickpea production (Pawar, 1998). It is also considered a major legume pest across Nepal (Manandhar, 1997). Control of *H. armigera* by using chemical insecticides has become ineffective, since this pest has gained a 12-103-fold resistance to the common pyrethroids in Nepal (Armes and Pandey, 1995). The alternative to these chemical insecticides, the mycopesticides have either low or no resistance problem, are host specific, economic and ecologically friendly (Ferron *et al.,* 1991; Mendoca, 1992). *Metarhizium anisopliae* has been applied to control a variety of insect pests

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including *H. armigera*. Likewise, *Beauveria bassiana* has also been reported to be an effective fungus against *H. armigera* and other insect pests under both laboratory and field conditions (Sandhu *et al.,* 2001; Tefera and Pringle, 2004; Ngugen *et al*., 2007; GC *et al*., 2008a, Rijal *et al.*, 2008). In Nepal, *M. anisopliae* (M1) and *B. bassiana* (B3) were identified as being widely distributed and as being the most virulent isolates against several insect pests (GC *et al*., 2008b). Several reports also explained the many opportunities of using indigenous isolates of entomopathogenic fungi such as *M. anisopliae* and *B. bassiana* because of their effectiveness against other caterpillar pests in Nepal (GC. and Keller, 2002; GC. *et al.,* 2004; GC *et al*., 2008a). However, to-date there have been no reports on the evaluation of indigenous *M. anisopliae* and *B. bassiana* isolates against *H. armigera* in Nepal. Therefore, this study is aimed to evaluate the efficacy of native isolates of *M. anisopliae* (M1) and *B. bassiana* (B3) against *H. armigera* under field conditions in Nepal.

Materials and Methods

Field preparation and the sowing of chick pea seeds

All crop residues and weeds were removed and the soil was thoroughly ploughed. Seeds of chickpea, *Cicer arietinum* L. variety "Avrodhi" were sown 5 cm deep at 40 cm spacing between plants and with 10 cm space between rows, and with 20 plants per row. Weeding was done at 20 and 30 days after sowing (DAS).

Preparation of the fungal solution

The fungal isolates were obtained from the stock maintained at the Insect Pathology Laboratory, Department of Entomology, Institute of Agriculture and Animal Sciences, Chitwan, Nepal. The virulent isolates of *M. anisopliae* (M1) and *B. bassiana* (B3) were grown on a selective medium (SM) adapted from Strasser *et al.* (1996) and GC *et al*. (2008b). 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 121°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: 460 g/L) were mixed with other components. To induce growth and sporulation, the fungi were incubated at 25°C and 75% RH for 15 days. The conidia were collected by scraping the contents of each Petri dish.

One mL from the original solution was dropped onto a Thoma haemocytometer, observed under a microscope (TIEFA, Germany) and adjusted to 10^7 conidia/mLl. 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 the conidia was done separately for respective bioassay experiments.

Commercial insecticide preparation

Liquid formulations of a commercial microbial insecticide of HaNPV (*Helicoverpa armigera* nucleopolyhedrovirus) (SOM Phytopharma Limited, Hyderabad, India), a botanical pesticide Margosom (azadirachtin 0.15% w/w) (SOM Phytopharma Limited, Hyderabad, India) and a chemical insecticide "Anumite" (cypermethrin 10% EC) (Anu Products Ltd., Haryana, India) and Biolep (*Bacillus thuringiensis* (Bt) var. *kurstaki* 50000 IU/mg WP) (Biotech International Ltd, New Delhi, India) were diluted to the required concentration for the study. To prepare the desired dilution of the insecticides the following equation was used.

$$I = \frac{C}{\%\ AI} \times 100$$

Where
I = Insecticide / l of water
C = Concentration required
AI = active ingredient

Experimental design

A randomized complete block design with 7 treatments and 3 replications was used in the present study. Treatments included *M. anisopliae* (M1) at 1×10^7 coonidia/mL, *B. bassiana* (B3) at 1×10^7 conidia/mL, HaNPV at 250 larva equivalent (LE)/ha, Biolep (*B. thuringiensis* (Bt) var. *kurstaki* 50000 IU/mg WP) at 2 gm/L, Margosom (azadirachtin 0.15% w/w) 2 mL/L, Anumite (cypemethrin 10 EC) 1 mL/L and water as control were sprayed at 64 DAS for the first application and then sprayed weekly throughout the cropping season.

Insecticide application, observation and data analysis

Three, two and four sprays of insecticide were applied at the vegetative, flowering to pod setting stage and in the pod setting stage and onwards, respectively. Throughout the study, 10 plants were sampled from each treatment for observation. The number of larvae per plant or pod were recorded at 1 and 7 days after treatment (DAT) during the vegetative, the flowering and the pod setting stage of the chick pea. The number of pods damaged or destroyed by *H. armigera* were counted to determine the percentage of pods damages at 98, 108, 115 and 122 DAS. All insect scoring and *H. armigera* larvae population density observations were carried out as described by Lateef and Reed (1983), and the population reduction compared to the control was calculated using the following equation by Fleming and Retnakaran (1985).

$$LP = 1 - \frac{Ta \times Cb}{Tb \times Cb} \times 100$$

Where,
LP = *H. armigera* larvae population reduction (%)
Ta = *H. armigera* larvae population in treatment after spray
Tb = *H. armigera* larvae Population in treatment before spray
Ca = *H. armigera* larvae Population in control after spray
Cb = *H. armigera* larvae Population in control before spray

The weights of dried chickpea grains from each plot were recorded and the yield was converted into yield per hectare. The percent increase in yield over the control was calculated using the following equation.

$$Y = \frac{T - C}{C} \times 100$$

Where,
Y = Chick pea yield increase (%)
T = Chick pea yield from treatment plot
C = Chick pea yield from control plot

Other observed parameters analyzed were pod damage and yield comparison of all treatments. The data analysis was done using MSTAT-C (2002).

Results

Effect of treatments with control agents on *H. armigera* larvae

During the vegetative stage at 1 DAT, a significantly smaller number of *H. armigera* larvae was observed in the HaNPV sprayed plot than in the control plot, however, the number of *H. armigera* larvae observed in the plot sprayed with *M. anisopliae*, *B. bassiana*, HaNPV, Biolep, Anumite and Margosom or *M. anisopliae*, *B. bassiana*, Anumite and in the control were not significantly different (Table 1). Similarly, during the vegetative stage at 7 DAT, a significantly smaller number of *H. armigera* larvae were observed in the plot sprayed with Biolep than in the control

Table 1. Effect of treatments with various control agents against *Helicoverpa armigera* in chickpea during the vegetative stage

Treatments	No. Larvae/10 plants (Mean ± SE)*	
	1 DAT	7 DAT
Metarhizium anisopliae (Strain M1 1×10^7 spores/mL)	8.00 ± 0.67ab	3.67 ± 0.33b
Beauveria bassiana (Strain B3 1×10^7 spores/mL)	8.33 ± 0.33ab	4.33 ± 0.33b
Helicoverpa armigera Nuclear Polyhedrosic Virus (HaNPV 250 LE/ha)	5.67 ± 0.67b	4.33 ± 0.33b
Biolep (*Bacillus thuringiensis* var. *kurstaki* 2 gm/L)	6.67 ± 0.33b	1.33 ± 0.33c
Margosom (azadirichtin 2 mL/L)	7.00 ± 0.88b	5.67 ± 0.88ab
Anumite (cypermethrin 0.1%)	8.00 ± 0.58ab	3.33 ± 0.67b
Control (Water)	9.67 ± 0.58a	6.67 ± 0.58a
CV%	10.25	12.73
CD (P = 0.05)	0.49	0.49
SEm±	0.16	0.16

* Values with the same letter in a column are not significantly different at 5% by DMRT (MSTAT-C, 2002).

Table 2. Effect of treatments with various control agents against *Helicoverpa armigera* in chickpea in the flowering stage

Treatments	No. Larvae/10 plants (Mean ± SE)*	
	1 DAT	7 DAT
Metarhizium anisopliae (Strain M1 1×10^7 spores/mL)	1.00 ± 0.67b	1.67 ± 0.33b
Beauveria bassiana (Strain B3 1×10^7 spores/mL)	2.00 ± 0.58b	2.00 ± 0.00b
Helicoverpa armigera Nuclear Polyhedrosic Virus (HaNPV 250 LE/ha)	0.33 ± 0.58c	0.33 ± 0.33c
Biolep (*Bacillus thuringiensis* var. *kurstaki* 2 gm/L)	0.00 ± 0.00c	0.00 ± 0.00c
Margosom (azadirichtin 2 mL/L)	2.00 ± 0.58b	1.67 ± 0.33b
Anumite (cypermethrin 0.1%)	2.00 ± 0.00b	1.67 ± 0.33b
Control (Water)	7.67 ± 0.67a	8.33 ± 0.58a
CV%	19.69	13.75
CD (P = 0.05)	0.52	0.36
SEm±	0.17	0.12

* Values with the same letter in a column are not significantly different at 5% by DMRT (MSTAT-C, 2002).

plot and plots sprayed with other insecticides. However, the number of *H. armigera* larvae observed in the plot sprayed with *M. anisopliae*, *B. bassiana*, HaNPV, Margosom and Anumite or Margosom and control were not significantly different (Table 1).

During the flowering stage, either at 1 or 7 DAT, a significantly smaller number of *H. armigera* larvae was observed in all treatments other than the control. However, the number of *H. armigera* larvae observed in the plot sprayed with *M. anisopliae*, *B. bassiana*, Margosom and Anumite or HaNPV and Biolep was not significantly different. No *H. armigera* larvae were observed in the plot sprayed with Biolep (Table 2).

During the pod setting stage at 1 DAT, a significantly smaller numbers of *H. armigera* larvae was observed in the plot sprayed with Biolep compared to any other treatment. However, the number of *H. armigera* larvae observed in the plot sprayed with *M. anisopliae* and Margosome or *M. anisopliae*, *B. bassiana* and Anumite

Table 3. Effect of treatments with vrious control agents against *Helicoverpa armigera* in the pod setting stage in chickpea

Treatments	No. Larvae/10 plants (Mean ± SE)*	
	1 DAT	7 DAT
Metarhizium anisopliae (Strain M1 1×10^7 spores/mL)	6.00 ± 0.33bc	5.33 ± 0.33b
Beauveria bassiana (Strain B3 1×10^7 spores/mL)	5.00 ± 0.00cd	3.33 ± 0.67b
Helicoverpa armigera Nuclear Polyhedrosic Virus (HaNPV 250 LE/ha)	3.00 ± 0.58d	1.00 ± 0.58c
Biolep (*Bacillus thuringiensis* var. *kurstaki* 2 gm/L)	1.00 ± 0.33e	0.33 ± 0.33c
Margosom (azadirichtin 2 mL/L)	7.67 ± 0.67b	6.00 ± 0.58b
Anumite (cypermethrin 0.1%)	4.33 ± 0.33cd	4.00 ± 0.33b
Control (Water)	30.67 ± 0.33a	28.67 ± 0.33a
CV%	7.46	15.35
CD (P = 0.05)	0.34	0.60
SEm±	0.11	0.20

* Values with the same letter in a column are not significantly different at 5% by DMRT (MSTAT-C, 2002).

or HaNPV and Anumite was not significantly different (Table 3). Similarly, in the pod setting stage at 7 DAT, a significantly smaller number of *H. armigera* larvae was observed in all treatments other than the control, but the number of *H. armigera* larvae observed in the plot sprayed with *M. anisopliae*, *B. bassiana*, Margosom and Anumite or HaNPV and Biolep was not significantly different (Table 3).

Effect of treatment with control agents on pod damage

At 98 DAS, it was evident that 3.68, 3.33 , 6.19, and 12.59% of the pods were damaged by *H. armigera* larvae on the plot treated with *M. anisopliae*, *B. bassiana*, Margosom and the control, respectively. No pod damage was observed in the plots prayed with HaNPV and Biolep at 98 DAS and Anumite at 98 and 108 DAS (Fig. 1). All treatments had different rates of control of the *H. armigera* larvae populations. At the end of the experiments, 122 DAS, the percentage of pods damaged increased to 17.73, 16.90, 12.65, 10.91, 24.61, 10.73, and 32.38% in the plot treated with *M. anisopliae*, *B. bassiana*, HaNPV, Biolep, Margosom, Anumite and the control, respectively (Fig. 1).

Effect of treatments with control agents on chick pea production

The maximum grain yield was obtained from the cypermethrin treated plot followed by the plots treated with Biolep, HaNPV, *M. anisopliae, B. bassiana,* Margosom and the control plot, respectively. The yield obtained from the plot sprayed with Biolep was significantly higher than the yields obtained from other treated plots. The yields obtained from the plots sprayed with Biolep and Anumite or *M. anisopliae* and *B. bassiana* were not significantly different. However, the yields obtained from the plots sprayed with Nepalese native isolates of *M. anisopliae* and *B. bassiana* were significantly higher than those from the plots sprayed with Margosom or the control plot (Fig. 2).

Discussion

When treated with *M. anisopliae* at 1 and 7 DAT, the number of *H. armigera* larvae was reduced to 9.95 and 29.04% respectively. The *H. armigera* larvae were reduced to 12.07 and 27.62% by *B. bassiana* at 1 and 7 DAT, respectively. There are some new examples of the

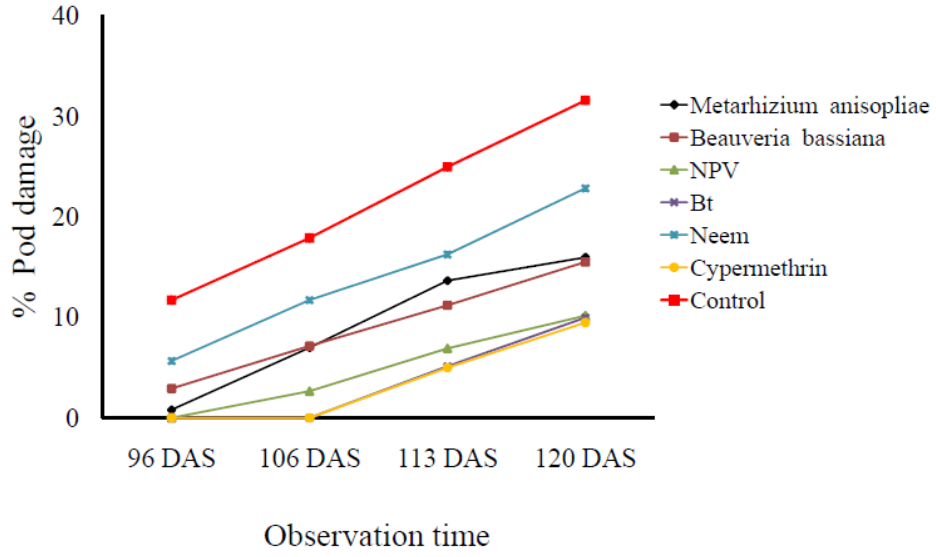

Fig 1. Effect of different treatments with various control agents on pod damage by *Helicoverpa armigera* in Chickpea.

control of *H. armigera* lavae by *B. bassiana* and *M. anisopliae* in other part of the world as well (Nguyen *et al*., 2007). In addition, the *B. bassiana* and *M. anisopliae* had successfuly controled *Chilo partellus* (Lepidoptera: Crambidae) in maize too (Tefera and Pringle, 2004). Naher *et al.* (2004) and Deshpande *et al.* (2000) reported that those microogranisms can effectively control *H. armigera* with different efficicacy rates depending on the different environmental conditions such as temperature, rainfall, RH, and sunshine (Walstad *et al.*, 1970). Another microorganism, HaNPV has also been shown to be highly effective in controlling *H. armigera* in a range of crops (Cherry *et al.*, 2000; GC and Thapa, 2000). The number of *H. armigera* larvae controlled by HaNPV at 1 and 7 DAT were 19.06 and 32.34%, respectively, in the present study. This is in agreement with the studies by other researchers (Praveen *et al.*, 2001; Naher *et al.*, 2003). Neem-based pesticides (Margosom) performed better than the control for controlling the *H. armigera* larvae population and they also reduced the pod damage by 26.15%. This result is in agreement with the findings reported by other researchers (Rao *et al.*, 1990; Sarode *et al.*, 1995; GC and Thapa, 2000). Many synthetic insecticides are effective against *H. armigera*. In this study, the pod damage in Anumite treated plots was only 13.10% and the yield was increased by 40.98%. Neupane and Sah (1988) also reported that Anumite was highly effective against *H. armigera* as is evident in the present study.

In our field experiments, both native entomopathogenic fungi effectively reduced the larval infestation and pod damages. However,it was found that Biolep based pesticides provided a better effective control of *H. armigera* than the rest of the treatments, followed by HaNPV. The larval control rate of *H. armigera* using entomopathogenic fungi was higher at one week after treatment but Anumite, Biolep and HaNPV were effective in the earlier days. The Biolep treatment had the highest grain yield compared to any of the other treatments. In the present study the

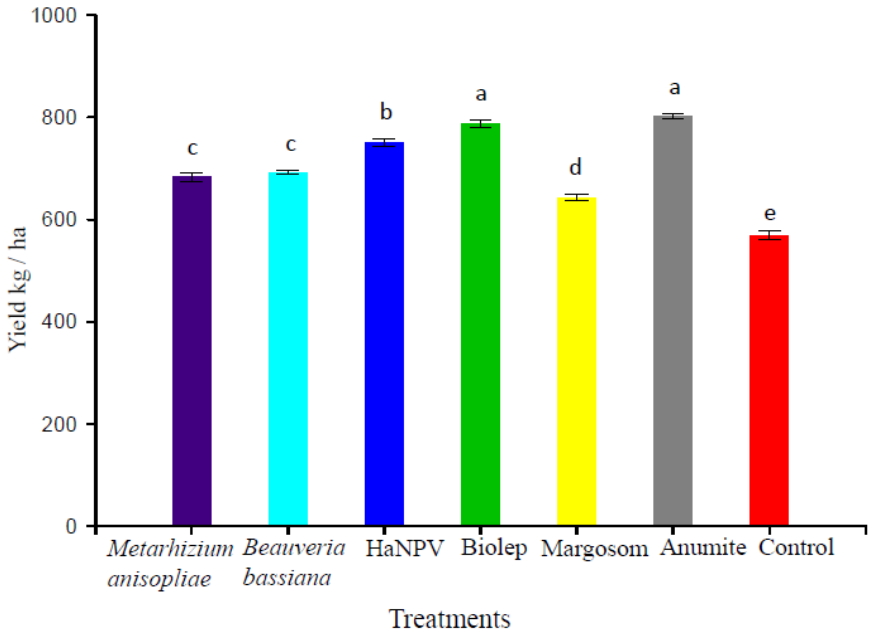

Fig 2. Yield of chick pea with dirrerent treatments against *Helicoverpa armigera* under field conditions in Chitwan, Nepal. Bars with the same letter are not significantly different at 5% by DMRT (MSTAT-C, 2002).

treatment with *B. bassiana* and *M. anisopliae* increased the yield by about 20% over the control. Although, the efficacy of the two native isolates of *M. anisopliae* and *B. bassiana* was not as efficient as that of HaNPV, Biolep and Anumite, it was better than the Margosom product and other control treatments. Therefore, further study is required to develop better formulations and better application methods of both entimopathogenic fungi. Based on the present study, native isolates of the entomopathogenic fungi, *M. anisopliae* (M1) and *B. bassiana* (B3), appear to be potential alternatives for controlling *H. armigera* in the field in Nepal.

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黑殭菌（*Metarhizium anisopliae*）與白殭菌（*Beauveria bassiana*）對鷹嘴豆上蕃茄夜蛾之防治效益

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摘　要

本研究藉由尼泊爾奇旺地區所進行之田間試驗，評估不同藥劑對蕃茄夜蛾的防治效果。所施用的藥劑包括：二種本土蟲生真菌（黑殭菌（*Metarhizium anisopliae*）及白殭菌（*Beauveria bassiana*））分離株、三種商業化生物性農藥與一種化學藥劑。研究結果顯示此二種蟲生真菌之防治效果最佳：無論鷹嘴豆的營養生長期、開花期或是結莢期，蕃茄夜蛾的幼蟲數量皆明顯少於對照組，豆莢所遭受到蕃茄夜蛾危害之比例亦低於施用印楝素或是對照組試驗。經黑殭菌與白殭菌處理後之鷹嘴豆產量明顯高於對照組，但卻低於以蕃茄夜蛾核多角體病毒及蘇力菌之處理組。因此，尼泊爾之二種本土蟲生真菌分離株具有應用於蕃茄夜蛾生物防治上之潛力。

關鍵詞：蕃茄夜蛾、本土白殭菌、黑殭菌、蕃茄夜蛾核多角體病毒、蘇力菌、印楝素。

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