J. Biol. Control, 18(1): 1-8, 2004

Evaluation of indigenous fungal isolates, *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N812 for the control of *Helicoverpa armigera* (Hübner) in pigeonpea field

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ABSTRACT: **The effectiveness of oil-based conidia formulations of indigenous fungal isolates *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N812 were evaluated against *Helicoverpa armigera* (Hübner) infestation on pigeonpea under field conditions. The *M. anisopliae* M34412 conidia in the oil formulation (7:3, diesel: Sunflower oil) were found to be most effective in controlling *H. armigera*. The results were compared with other control agents such as, endosulfan and *Ha*NPV (*H. armigera* Nuclear Polyhedrosis Virus). The per cent efficacies were, *M. anisopliae* 66.74, endosulfan 62.58, *N. rileyi* 60.88 and *Ha*NPV 55.58. *B. bassiana* preparation was found to be relatively less effective (51.25% efficacy). The effectiveness of all the control agents based on per cent pod damage and yield has also been discussed.**

KEY WORDS: *Beauveria bassiana, Helicoverpa armigera, Metarhizium anisopliae, Nomuraea rileyi*, indigenous fungal isolates

INTRODUCTION

The gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a cosmopolitan, polyphagous pest attacking more than 182 host plants belonging to 47 botanical families in the Indian subcontinent (Pawar, 1998). A pulse crop, *Cajanus cajan* (pigeonpea) is heavily infested by *H. armigera*. The loss in yield of pigeonpea, all over world has been estimated to be 45 % (Bhatnagar *et al.*, 1982), while in India, > 60% loss in yield (Anonymous, 1994) has been reported. The mycoinsecticides based on deuteromycetous fungi such as *Metarhizium anisopliae* (Agarwal, 1990), *Beauveria bassiana* (Sandhu *et al.*, 2001), *Nomuraea rileyi* (Tang *et al.*, 1999) have been reported to be useful to control insect pest. In the present study, indigenous isolates of entomopathogenic fungi have been used to determine their effectiveness in the control of *H. armigera* in pigeonpea field.

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MATERIALS AND METHODS

Insect culture

The initial culture of *H. armigera* was established by collecting larval and pupa stages from the fields. The rearing of larvae was done individually in plastic vials on vegetable diet (Okra) disinfected for 10 min with 0.5 per cent sodium hypochlorite as described by Ignoffo *et al.* (1975). The temperature and relative humidity in the insect rearing room were maintained at 25±2 ° C and 65±5 per cent, respectively.

Isolation of insect- pathogenic fungi

The soil samples were collected from the different regions around Pune, Maharashtra. For the isolation of entomopathogenic fungi two different methods, namely, soil dilution method (Goettel and Inglis, 1996) and *Galleria* bait method (Zimmermann, 1986) were used. On the basis of conidial morphology, 22 isolates of *M. anisopliae* and 7 isolates of *B. bassiana* were identified. Fifteen *N. rileyi* strains were isolated from mycosed *Spodoptera litura* (Fabricius) larvae found in sugarbeet fields near Pune. All the *M. anisopliae* and *B. bassiana* isolates were maintained on potato dextrose agar (PDA), while for the maintenance of *N. rileyi* isolates, Sabouraud malt extract yeast extract peptone agar (SMYP= malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, glucose1.0%, agar2%) was used. The stock cultures were maintained at 4°C until used.

Efficacy of fungal isolates against *H. armigera*

To obtain conidia, all the strains were grown on their respective agar media, either PDA or SMYP agar in dark for 14 days at 25°C. For the bioassay, conidial suspensions (1×10^7 conidia/ml) were made in Tween-80 (0.1%) and third instar larvae of *H. armigera* (three replicates of 20 larvae) were dipped in this suspension for 5 seconds. A 10ml spore suspension was used for treating a set of 20 larvae. As a control, 3 batches of 20 larvae each were treated with Tween-80 (0.1%) prepared in sterile distilled water. After treatment, each larva was kept in a separate plastic vial (42x65mm) containing moist Whatman No.1 paper and allowed to feed on okra pieces (disinfected with 0.5% sodium hypochlorite) and was incubated at 25±1°C, relative humidity 70±10 per cent and photoperiod 16:8, L: D. The mortality was recorded up to 14 days. The dead larvae were placed in a sterile Petri-plate containing a moist cotton swab to allow mycelial growth over the cadaver. Mortality estimation was corrected by Abbott's (1925) formula. Based on these results, three best isolates, one from each genus was selected for further studies.

Conidia production

Based on the mortality data, three strains namely, *M. anisopliae* M34412, *B. bassiana* B3301 and *N. rileyi* N812 were selected for the large-scale production of conidia. The mass production of conidia was carried out in unicorn-bags (31x16.7cm, Type 14 with a membrane for gaseous exchange, Unicorn Imp & Mfg Corp., USA) filled with 200g of peeled durum wheat (*Triticum aestivum* (L) soaked overnight in 100 ml distilled water, as a substrate. After autoclaving at 15 lb for 40 minutes, bags were inoculated with 20 ml of biomass grown in YPG (yeast extract 0.3%, peptone 0.5%, glucose1.0 %) medium for *M. anisopliae* and *B. bassiana* and SMYP for *N. rileyi* in shake flasks for 48-72 hours. The inoculated bags were incubated in humidity chamber with 28±2° C and relative humidity 70±10 per cent for 14 days. After 14 days, the sporulated substrate was dried at 30° C for 2-3 days to reduce the moisture content (<20%). The conidia were then harvested with a myco-harvester (a unit specially designed to remove conidia from the biomass under vacuum, CABI Bioscience, UK) and stored at 4°C until used.

Formulation studies

Viability and virulence in terms of per cent mortality of conidia for *M. anisopliae* M34412, *B. bassiana* B3301 and *N. rileyi* N812 was tested in different formulations such as diesel, sunflower oil, diesel: sunflower oil in the ratio 7:3, and Tween-80 (0.1%). For this study, the conidial suspensions (1×10^6 conidia/ml) were prepared in different formulations, kept at room temperature for one hour

and their germination was monitored on YPG agar for *M. anisopliae* M 34412 and *B. bassiana* B 3301 and on SMYP agar for *N. rileyi* N 812 at 28° C for 12-48 hours. The germ tube formation was observed under the microscope (40X) and the per cent germination was then calculated. The virulence studies were carried out by performing the insect bioassay with different formulations as described above with respective formulation without conidia as a control.

Field evaluation

The field evaluation of three strains to control *H. armigera* infestation on pigeonpea (*C. cajan* var. ICPL 87) was carried out in randomized block design with four replications during the *Kharif* season, 2001 at Mahatma Phule Krishi Vidyapeeth (MPKV) College of Agriculture, Pune. The crop was sown during first fortnight of July and was raised by following all normal agronomical practices except plant protection measures. The oil formulation of conidia ($5x10^{12}$ conidia/3L Diesel: Sunflower, 7:3) was sprayed with the Ultra Low Volume (ULV) sprayer (70ml/min; 3L/ha). The endosulfan (2ml/L, 500 L/ha) and *Ha*NPV (250 LE/ha) were sprayed with Hand Compression Knapsack sprayer. The persistence of inoculum in the field was determined by collecting *H. armigera* larvae 0, 3, 5, 7 and 14 days after spraying. These larvae were then kept under observation for a period of 14 days and after death were kept in a plastic vial containing moist filter paper and incubated at 25±1°C and relative humidity 70±10 per cent to observe mycosis. The persistence of the inoculum on the larval population was adjudged based upon the per cent larval mortality data collected from the field after spraying. Field studies were evaluated on the basis of efficacy (Henderson and Tilton, 1955), pod damage and yield, which were determined according to Hassani (2000).

RESULTS AND DISCUSSION

Isolation of insect-pathogenic fungi

The insect-pathogenic fungi *M. anisopliae* (22) and *B. bassiana* (7) were isolated using soil dilution method and *Galleria* bait method while the 15 isolates of *N. rileyi* were isolated from the infected *S. litura* larvae found in sugarbeet field around Pune. In the bioassay, 3 isolates, showing >80 per cent mortality, one from each genus, *M. anisopliae* M34412, *B. bassiana* B3301 and *N. rileyi* N812 were selected for further studies.

Conidia production

Different agricultural residues, cereals, etc. were reported to be used for the conidia production in soild state fermentation (Deshpande, 1999). Vimala Devi (1994) used crushed sorghum for conidia production in *Nomuraea rileyi*. In the present study, conidia production on wheat grains for *M. anisopliae* M34412 was 11.63g ($1.18x10^9$/g), 9.4 g ($1.09x10^9$/g) for *B. bassiana* B3301 and 5.47g ($7.57x10^8$/g) for *N. rileyi* N812 per kg of the substrate.

Formulation studies

The combination of formulation, application and the selection of the strain is one of the key steps for field trials. Use of different oil based formulations for mycoinsecticides has been extensively studied (Lomer and Lomer, 2001). There was significant difference in the percentage of germination and per cent mortality in different oil formulations. Table 1 shows that the conidial germination of *M. anisopliae* M34412 in sunflower oil, diesel: sunflower oil mixture (7:3) and Tween 80 (0.1%) was >90% in 12 hours. In case of *B. bassiana* B3301, as compared to Tween-80 (84.9%), the diesel: sunflower oil mixture showed lower germination (66.7%) in 12 hours. In case of *N. rileyi* N812 the conidial germination was relatively slow. It was >80% in the presence of sunflower oil, diesel: sunflower oil mixture (7:3), diesel: groundnut oil mixture (7:3) and Tween-80 (0.1%) after 36 hours. The less per cent germination was seen in safflower oil, groundnut oil and their combination with diesel which can be attributed to the high viscosity of the oil which aggregated spores and reduced the germination. Similar observations were also reported by Ibrahim *et al.* (1999) for *M. anisopliae*.

Table 1. Effect of different oil formulations on the conidial germination of the selected fungal isolates

Formulation[a]	Conidial germination (%) ± SEM		
	M. anisopliae 34412[b]	*B. bassiana* B3301[b]	*N. rileyi* N812[c]
Diesel	86.7 ± 1.3	47.3 ± 2.2	72.6 ± 2.3
Sunflower	92.7 ± 1.9	55.8 ± 1.8	81.8 ± 1.4
Diesel: Sunflower oil (7:3)	95.9 ± 1.6	66.7 ± 1.7	84.7 ± 1.7
Safflower oil	86.1 ± 2.1	41.8 ± 1.3	79.8 ± 1.7
Diesel: Safflower oil (7:3)	78.1 ± 3.2	52.2 ± 2.1	78.0 ± 2.8
Groundnut oil	88.9 ± 1.8	43.8 ± 2.1	50.7 ± 3.7
Diesel: Groundnut oil (7:3)	84.2 ± 1.5	65.0 ± 6.8	82.0 ± 2.0
Tween-80 (0.1%)	99.4 ± 0.4	84.9 ± 1.0	85.1 ± 1.6

SEM = Standard error of means

a, conidia suspension (1 x 10^6/ml) at room temperature for 1h; b, percent germination after 12h; c, per cent germination after 36h

It has been suggested that oil formulation can prevent conidial dessication, and improve adhesion of conidia to the hydrophobic surface of insect cuticle (Inyang *et al.*, 2000; Vimala Devi and Prasad, 1996). Furthermore, Inyang *et al.* (2000) reported that sunflower oil/ Shellsol T formulations enhanced the infectivity of *M. anisopliae* for mustard beetle, *Phaedon cochleariae*. Figure 1 depicts the per cent mortality of *H. armigera* with the three isolates in different oil-based formulations. In the dip method, though all the tested formulations were found to be effective (>50% mortality with all the isolates), in a diesel: sunflower oil mixture and Tween-80 (0.1%) to 90 per cent mortality with all the three isolates was observed. For the field studies, the conidia were mixed with the formulation just before application in the field.

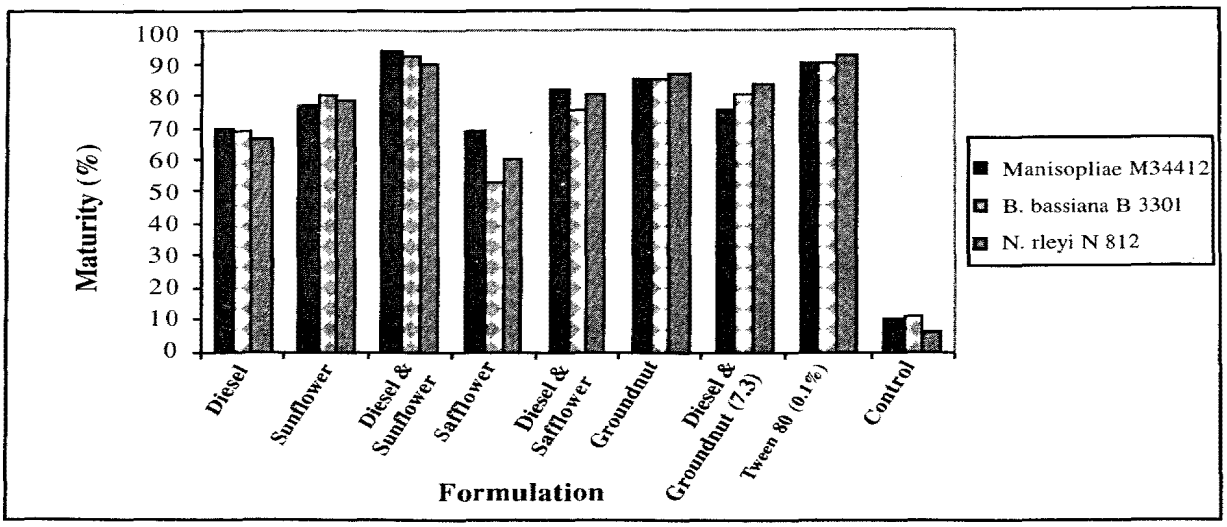

Figure 1. The per cent mortality of *H. armigera* with different oil formulations of the three isolates in bioassay

Field evaluation

The data on per cent efficacy obtained by *M. anisopliae* M34412, *B. bassiana* B3301 and *N. rileyi* N812 are presented in Table 2. In the pooled mean, per cent efficacy from the various treatments after two sprays was found to be in the range from 55.51 to 70.93 against *H. armigera*. The treatment with *M. anisopliae* M34412 was found to be the most effective showing maximum efficacy of 70.93 per cent. Earlier research workers reported 76.7 per cent control of *Rhammatocerus schistocecoides* with *M. anisopliae*, (Magalhaes *et al.*, 2000).

The percent pod damage in the fields sprayed with different preparations is depicted in Figure 2. The pod damage in the *M. anisopliae* M34412 treated plot was found to be least (8.76%). As compared to the control plot (23.63% pod damage) all other treatments showed pod damage in the range 10.24%- 17.27%. The average yield (q/ha) in the control was 7.31 q/ha while it was increased up to 14.04 q/ha in case of *M. anisopliae* M34412 treatment. The treatment with endosulfan showed the yield of 12.78 q/ha while that for *N. rileyi* N 812, *Ha*NPV and *B. bassiana* B 3301 were 11.61, 10.64, and 10.18 q/ha, respectively.

Table 2. Efficacy of different treatments against *H. armigera* infestation on pigeonpea under field conditions

Treatment	Cumulative efficacy (%)*			
	First spraying	Second spraying	Mean ± SEM	Yield (q/ha)
M. anisopliae M34412 ($5x\ 10^{12}$ conidia/ha)	66.74±11.86	75.11 ±9.48	70.93±4.19	14.04
B. bassiana B3301 ($5x\ 10^{12}$ conidia/ha)	51.25±10.37	59.77 ±10.36	55.51±4.27	10.18
N. rileyi N812 ($5x\ 10^{12}$ conidia/ha)	60.88±10.43	65.03 ±10.14	62.95±2.08	11.61
Endosulfan (2 ml/L, 500L/ha)	62.58±4.77	64.28±6.18	63.43±0.85	12.78
*Ha*NPV (250 LE/ha)	55.58±11.54	59.54 ±7.53	57.66±1.98	10.64
Control	–	–	–	7.31

*After Henderson and Tilton (1955)

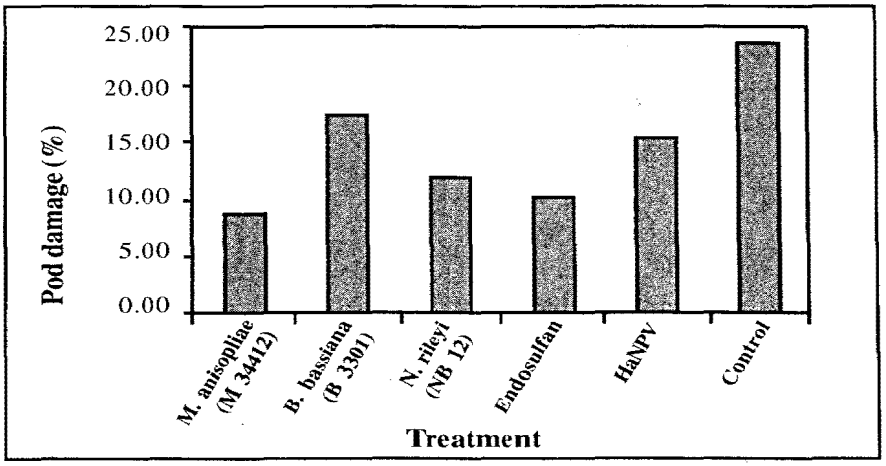

Figure 2. The pod damage by *H. armigera* in the pigeonpea fields sprayed with different treatments

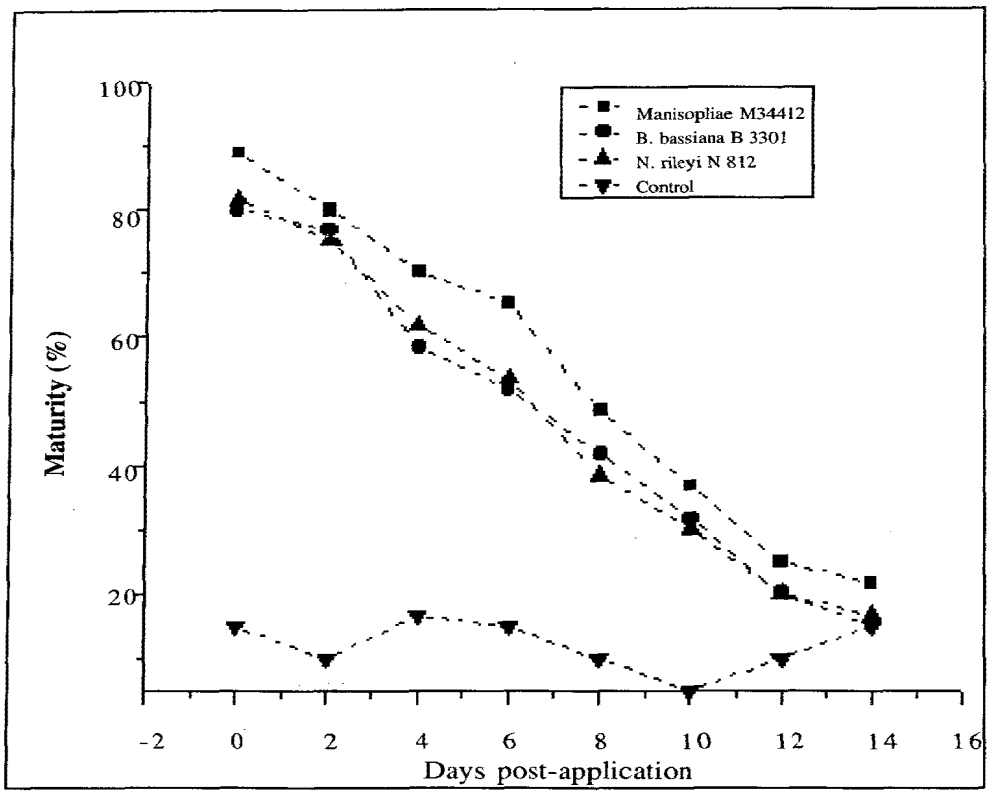

Figure 3. The persistence of insect-pathogenic fungi after first spray in terms of % mortality of *H. armigera* in the field

Figure 3 shows that the mortality after first spraying was highest with *M. anisopliae* M34412 (87.50 %) while with *N. rileyi* N812 and *B. bassiana* it was 85 and 80 per cent, respectively. The inoculum formulated in oils increased the efficacy of pathogen and prolonged viability of conidia (Daoust and Roberts, 1983). The persistence of the inoculum declined (<50 %) after 7th day collection, which suggested that all the three isolates exhibit 50 per cent of viable inoculum up to 8 days, which was proved in terms of mortality. Vimala Devi (1994) recorded that at the end of 6 days, the persistence of *N. rileyi* was less than 50 per cent against *S. litura* on castor under field condition.

Further studies on the effect of conidia formulations on non-target organisms, stability under field conditions, etc. are in progress. Though the oil-based formulation of *M. anisopliae* M34412 showed better performance than endosulfan, the stability of the spore preparation under field conditions is the major concern to make the technology cost effective and viable.

ACKNOWLEDGEMENT

The financial support from Swiss Agency for Development and Cooperation, Berne, Switzerland and Department of Biotechnology, New Delhi to the Project (ISCB-BP1) is gratefully acknowledged.

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