



Research Article

Field evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* against the white grub, *Holotrichia longipennis* damaging soybean in Uttarakhand hills

A. K. PANDEY

College of Forestry & Hill Agriculture, G. B. Pant University of Agriculture & Technology, Hill Campus, Ranichauri, Tehri Garhwal 249 199, Uttarakhand, India.

Corresponding author E-mail: drajay2002@gmail.com

ABSTRACT: Field experiments were conducted at Crop Research Center, College of Forestry and Hill Agriculture, GBPUA&T, Ranichauri during 2006-2009 to evaluate the effectiveness of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* with few insecticides against white grub, *Holotrichia longipennis* damaging soybean. On the basis of average cumulative plant mortality during the study, *M. anisopliae* (5.0×10^{13} spores g^{-1}) was found to be superior compared to other bio-agents by recording lowest plant mortality (21.83%) and 61.58% decrease in grub population followed by *B. bassiana* (5.0×10^{13} spores g^{-1}) where the plant mortality and decrease in grub population was 25.18% and 54.75%, respectively, compared to control. Highest percentage of yield increase (113.41%) was also recorded with *M. anisopliae*. However, among all the treatments, imidacloprid was found to be the most effective in reducing the grub population (82.28%) and thereby increasing the yield 206.86% over control.

KEY WORDS: *Beauveria*, *Metarhizium*, imidacloprid, chlorpyrifos, bio-efficacy, *Holotrichia longipennis*, soybean

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INTRODUCTION

The June beetle, *Holotrichia longipennis* (Coleoptera: Scarabaeidae), which is locally known as “Kurmula and Pagra”, has become a widespread and destructive insect pest of cereals, pulses, vegetable, horticultural crops in Uttarakhand hills. The adults emerge in the month of June following the pre-monsoon rain and feed on the leaves of walnut, apple, apricot, almond, plum and peach besides other wild host plants at night thereby defoliating these valuable plants. The grubs of this pest cause severe damage from August to March by feeding on root zone of almost all agricultural crops resulting in symptoms of wilting and drying of plants. Entomopathogenic fungi such as *Beauveria* and *Metarhizium* have been proved to be useful microbial agents for the management of *Holotrichia* spp. (Sharma *et al.*, 1998; Bhagat *et al.*, 2003; Gupta *et al.*, 2003; Hiromori *et al.*, 2004) which caused a gradual decline in grub population below economic injury level (Fouillaud *et al.*, 2001). Pathogenicity of *Metarhizium anisopliae*, *Beauveria bassiana* and *Heterorhabditis bacteriophora* has been reported by Yadav *et al.* (2004a) who observed that third instars of *H. consanguinea* were highly susceptible to the

entomogenous fungi and nematode. A clear reduction in damage to sugarcane crops coupled with an increased active presence of *B. brongniartii* fungus in most sites has been recorded by Jeuffraut *et al.* (2004).

In the light of above facts, field experiments were carried out to evaluate the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* with few insecticides against *H. longipennis* damaging soybean in Uttarakhand hills.

MATERIALS AND METHODS

Field experiments were conducted at Research Center, College of Forestry and Hill Agriculture, GBPUA&T, Ranichauri for the years 2006-2009 to evaluate the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* in comparison with a few insecticides against the white grub, *H. longipennis* damaging soybean. Fungal formulation of both the fungi was obtained from Project Coordinating Cell. All India Network Project on white grub and other soil arthropods at Agricultural Research Station, Durgapura, Jaipur (Rajasthan). The soybean crop was sown in May under rainfed condition in the plot size

of 5 x 5 m². The line to line and row to row distance was maintained 10 cm and 30 cm, respectively. All the agronomical practices recommended for the crop were followed to grow healthy crop. The experiment was conducted under randomized block design with seven treatments which are as under and each treatment was replicated thrice.

Treatment nos.	Bioagent or insecticide	Dosage (kg ha ⁻¹)
T ₁	<i>Metarhizium anisopliae</i>	(3.0x10 ¹³ spores g ⁻¹)
T ₂	<i>Metarhizium anisopliae</i>	(5.0x10 ¹³ spores g ⁻¹)
T ₃	<i>Beauveria bassiana</i>	(3.0x10 ¹³ spores g ⁻¹)
T ₄	<i>Beauveria bassiana</i>	(5.0x10 ¹³ spores g ⁻¹)
T ₅	Imidacloprid 200 SL at sowing time	80g a.i. ha ⁻¹
T ₆	Chlorpyrifos 20EC	800g a.i. ha ⁻¹
T ₇	Untreated control	–

The bio-agent of each treatment was mixed in compost and chemical insecticide was mixed in pulverized soil, separately, and this was mixed in respective plots just before sowing of soybean. The data related to plant mortality was recorded at 40, 60 and 80 days after treatment (DAT) while data of yield and grub population per square meter at 20 cm depth was taken at the time of harvesting of crop. The data recorded from different treatments were subjected to statistical analysis through MSTAT-C computer program.

RESULTS AND DISCUSSION

On analysing the data of years 2006 to 2009, it was found that there was significant variation in cumulative plant mortality recorded at 40, 60 and 80 days after treatment (DAT), yield and grub population after harvesting of crop.

Among the bioagent treatments the plant mortality was 3.14 to 9.31% at 40 DAT as compared to untreated control where plant mortality was 8.32 to 14.8 percent during the study (2006 to 08). Among all the treatments, the lowest plant mortality of 3.14, 2.86 and 3.45% was recorded in imidacloprid 200SL (@ 0.08 kg a.i. ha⁻¹) during the year 2006 to 2009, respectively, with an average of 3.15 % plant mortality (Table 1). This was at par with chlorpyrifos (@ 0.08 kg a.i. ha⁻¹) where the plant mortality was 3.95, 4.35 and 4.98%, respectively, with an average of 4.42%. *B. bassiana* and *M. anisopliae* were found to be less effective as compared to chemical insecticide. Among the bio agents, *B. bassiana* was found to be the most effective by registering plant mortality of 5.14, 4.60 and 6.84% during the year 2006 to 2009,

respectively, with an average mortality of 5.52% which was non-significantly different from *M. anisopliae* applied (5.0x10¹³ spores g⁻¹) where the average plant mortality was 5.80% at 40 DAT. Both the treatments were significantly at par with lower dosage (3.0x10¹³ spores g⁻¹) of *M. anisopliae* and *B. bassiana*.

The cumulative plant mortality increased with the advancement of crop as recorded at 60 DAT. Among the bio agents, significantly lowest plant mortality of 15.45, 15.60 and 15.99 percent was recorded at higher dosage (5.0x10¹³ spores g⁻¹) of *M. anisopliae* during the years 2006 to 2009, respectively. On the basis of average, higher dosage of (5.0x10¹³ spores g⁻¹) *M. anisopliae* (15.68% cumulative plant mortality) was found to be at par with same dosage of *B. bassiana* (16.06% cumulative mortality), lower dosage (3.0x10¹³ spores g⁻¹) of *B. bassiana* (18.52% cumulative plant mortality) and *M. anisopliae* (18.45% cumulative plant mortality) at 60 DAT. Highest average cumulative plant mortality of 18.52% was recorded with lower dosage of *B. bassiana* followed by lower dosage of *M. anisopliae* where the average cumulative mortality was 18.45%. However, among all the treatments, imidacloprid was found to be most effective insecticide against white grub by registering cumulative plant mortality of 4.32% at 60 days after treatment followed by chlorpyrifos where the plant mortality was 7.30%. Both the treatments were significantly at par with each other.

At 80 DAT, the cumulative plant mortality ranged from 3.71 to 26.84% among the treatments which was significantly superior over untreated control where the average plant mortality was 50.38%. Among the different dosages of bio-agents, average cumulative mortality at 80 DAT indicated that *M. anisopliae* was the most effective at higher dosage (5.0x10¹³ spores g⁻¹) by recording 21.83% average cumulative plant mortality followed by same dosage of *B. bassiana* (25.18% mortality), lower dosage (3.0x10¹³ spores g⁻¹) of *M. anisopliae* (25.19% plant mortality) and *B. bassiana* (26.37% plant mortality). However, compared to higher dosage of *M. anisopliae*, imidacloprid applied @ 0.08 kg a.i. ha⁻¹ was found to be significantly most superior treatment (7.14% cumulative plant mortality) as compared to rest of the treatments except with chlorpyrifos (0.8 kg a.i. ha⁻¹) which was at par by registering plant mortality of 9.86% (Table 1).

Variations in yield among the different treatments were also recorded as presented in Table 2. Among the treatments, the yield ranged from 8.32 to 22.81 q ha⁻¹ which was significantly superior over untreated control where the yield ranged from 6.3 to 7.5 q ha⁻¹ with an average of 6.71 q ha⁻¹. Among the bioagent treatments highest yield was recorded in higher dosage of

Table 1. Effect of entomopathogenic fungi against whitegrub, *Holotrichia longipennis* damaging soybean during different study years

Treatment	Dosage	Mean percent mortality of grubs on different days of treatments in different years											
		2006				2007				2008			
		40	60	80	Av.	40	60	80	Av.	40	60	80	Av.
<i>Metarhizium anisopliae</i>	3.0x10 ¹³ spores g ⁻¹	5.91 (13.62)	17.16 (15.31)	24.50 (26.79)	15.85 (22.73)	8.32 (16.74)	19.80 (26.42)	24.50 (26.79)	17.54 (24.28)	7.29 (15.63)	18.40 (25.38)	26.57 (31.02)	17.42 (24.03)
<i>Metarhizium anisopliae</i>	5.0x10 ¹³ spores g ⁻¹	5.15 (12.87)	15.45 (14.14)	21.19 (25.08)	13.93 (21.22)	6.00 (14.18)	15.60 (23.11)	21.19 (25.08)	14.26 (21.61)	6.25 (14.42)	15.99 (23.56)	23.12 (28.71)	15.12 (22.26)
<i>Beauveria bassiana</i>	3.0x10 ¹³ spores g ⁻¹	6.26 (11.01)	18.78 (15.65)	26.2 (27.95)	17.08 (23.65)	6.27 (14.54)	17.46 (24.73)	26.21 (27.95)	16.64 (23.33)	9.31 (17.74)	19.34 (26.08)	26.70 (31.11)	18.45 (24.98)
<i>Beauveria bassiana</i>	5.0x10 ¹³ spores g ⁻¹	5.14 (12.87)	16.71 (14.14)	24.35 (26.31)	15.40 (22.26)	4.60 (12.39)	14.73 (22.55)	24.35 (26.31)	14.56 (21.50)	6.84 (15.14)	16.75 (24.15)	26.84 (31.19)	16.81 (23.50)
Imidacloprid 200SL at sowing time	0.08 kg a.i. ha ⁻¹	3.14 (14.14)	3.43 (10.86)	3.71 (11.47)	3.42 (10.66)	2.86 (9.81)	3.59 (11.45)	4.72 (12.52)	37.2 (11.06)	3.45 (10.67)	5.96 (14.10)	14.81 (22.62)	8.07 (15.82)
Chlorpyrifos 20E Cat sowing time	0.80 kg a.i. ha ⁻¹	3.95 (8.56)	6.46 (12.35)	7.26 (15.98)	5.89 (13.93)	4.35 (12.25)	7.18 (15.56)	6.46 (15.98)	5.99 (14.10)	4.98 (12.79)	8.26 (16.68)	16.68 (24.09)	9.97 (17.90)
Untreated control	-	9.93 (23.57)	28.96 (19.94)	50.27 (35.66)	29.7 (32.02)	14.8 (22.63)	27.9 (31.88)	50.27 (35.66)	30.99 (33.22)	8.32 (16.74)	27.90 (31.88)	50.61 (45.34)	28.94 (31.33)
SEM (±)		0.96	0.851	0.724	2.51	0.86	0.94	0.724	2.21	0.751	0.525	0.66	1.80
C.D. (5%)	-	2.98	2.62	2.23	5.75	1.82	1.99	2.23	4.83	2.31	1.61	2.06	3.57
CV (%)		12.15	10.07	5.18	18.82	8.65	9.48	5.18	18.02	8.82	3.93	3.79	13.72

Figures in parentheses are angular transformed values

Table 2. Effect of entomopathogenic fungi against whitegrub, *Holotrichia longipennis* damaging soybean during different study years

Treatment	Dosage	Grub population (per square mt.)					Yield (q ha ⁻¹)				
		2006	2007	2008	Average	% decrease over control	2006	2007	2008	Average	% increase over control
<i>Metarhizium anisopliae</i>	3.0x10 ¹³ spores g ⁻¹	9.00	4.70	3.67	5.8	42.67	9.12	9.10	16.04	11.42	70.19
<i>Metarhizium anisopliae</i>	5.0x10 ¹³ spores g ⁻¹	5.00	4.30	2.33	3.9	61.58	10.70	13.50	18.75	14.32	113.41
<i>Beauveria bassiana</i>	3.0x10 ¹³ spores g ⁻¹	8.33	5.30	4.33	6.0	40.69	8.32	9.20	16.21	11.24	67.51
<i>Beauveria bassiana</i>	5.0x10 ¹³ sporesg ⁻¹	8.00	2.70	3.00	4.6	54.75	10.51	10.70	18.29	13.17	96.27
Imidacloprid 200 SL at sowing time	0.08 kg a.i. ha ⁻¹	1.66	1.70	2.00	1.8	82.28	22.81	17.10	21.87	20.59	206.86
Chlorpyrifos 20 EC at sowing time	0.80 kg a.i. ha ⁻¹	3.33	2.30	1.67	2.4	75.94	18.44	16.50	20.00	18.31	172.88
Untreated control	–	13.33	7.30	9.67	10.1	–	6.34	6.30	7.50	6.71	–
SEM (±)		0.25	0.34	0.59	0.80	–	0.66	0.69	0.34	0.73	–
C.D. (5%)		0.13	0.15	0.28	1.48	–	3.35	3.35	1.06	1.59	–
CV (%)		10.0	9.82	15.91	16.5	–	9.40	9.40	3.51	15.6	–

M. anisopliae where the average yield was 14.32 q ha⁻¹ and it was significantly superior over all the treatments of bio-agent except from *B. bassiana* (5.0x10¹³ spores g⁻¹) having the average yield of 13.16 q ha⁻¹. Despite all this insecticidal treatments were found to be most effective as compared to bio-agent treatment as well as control. The significantly highest yield (20.59 qha⁻¹) was recorded with imidacloprid (0.08 kg a.i. ha⁻¹) with 206.86% increase over control followed by chlorpyrifos (0.8 kg a.i. ha⁻¹) where the yield was 18.31 q ha⁻¹ showing 172.88% increase over control (Table 2).

At the end of the cropping season, the grub population per square meter pit was also recorded just after harvesting. Significantly lowest and highest grub populations were recorded with imidacloprid (0.08 kg a.i. ha⁻¹) and with lower dosage of *B. bassiana* with an average of 1.78 and 6.0 grubs per pit, respectively, while in control it was 10.1 grub per pit. Application of *M. anisopliae* @ 5.0x10¹³ spores/g resulted in significant reduction (61.58%) of grub over control. Highest grub (6.0 grubs per pit) population was recorded with lower dosage of *B. bassiana* (3.0x10¹³ spores g⁻¹) followed by *M. anisopliae* where the grub population was 5.8 grubs per pit and was significantly superior over control (10.1 grubs) amounting to 40.69% grub population over control. However, chemical insecticides i.e. imidacloprid (0.08 kg a.i. ha⁻¹) and chlorpyrifos (0.8 kg a.i. ha⁻¹) proved to be most effective and significantly superior to all the treatments as well as control. Both the treatments i.e. imidacloprid (0.08 kg a.i. ha⁻¹) and chlorpyrifos (0.8 kg a.i. ha⁻¹) reduced 82.28 % and 75.9% grub population, respectively, over control but were at par with each other.

The present study revealed that the entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, effectively reduce the population of *H. longipennis* grub under field condition. However, among the bio-agents, *M. anisopliae* and *B. bassiana* did not differ significantly when applied @ 5.0x10¹³ spores g⁻¹ and gave 61.58 and 54.75% grub reduction and 113.41 and 96.27% yield increase, respectively, over control. Yadav *et al.* (2004a) reported that *M. anisopliae* and *B. bassiana* alone caused 40-50% grub mortality and 43.02 and 47.16% reduction in plant mortality over control (Anjana and Bhagat, 2005). Application of *B. brongniartii* @ 10⁹ spores ml⁻¹ resulted in significant decrease of crop damage (2.5%) as compared to untreated plots which showed 74% crop damage (Chirame *et al.*, 2002).

The study revealed that higher dosages (5.0x10¹³ spores g⁻¹) of *B. bassiana* and *M. anisopliae* proved more effective in reducing grub population as compared to lower dosages of same bio-agents. Ansari *et al.* (2004) reported that mortality of grub was dependant on the fungal concentration, exposure time and temperature. The

data of cumulative plant mortality, seed yield and grub population just after the harvesting of crop indicated that *M. anisopliae* was more effective than *B. bassiana* at the same concentration. Present finding is in support of Lin *et al.* (2006), who reported that virulence of *M. anisopliae* on white grub (*Anisoplia austriaca*) was higher than that of *B. brongniartii*. Sharma and Gupta (2003) also reported that *M. anisopliae* and *B. brongniartii* were highly effective against *Holotrichia consanguinea* and *Maladera insanabilis*, however, *B. bassiana* was comparatively weak in controlling *H. consanguinea* and *Maladera insanabilis*. Similarly, Kessler *et al.* (2004) reported that *M. anisopliae* (isolates CLO 53 and CLO 54) caused maximally 90% mortality of *M. melolontha* grub after 10 weeks of post-inoculation. Bhattacharyya *et al.* (2008) reported that *B. bassiana* and *M. anisopliae* applied @ 5x10¹³ conidia ml⁻¹ in combination with imidacloprid 200SL at 48g a.i. ha⁻¹ was found to be effective exhibiting lowest plant mortality (1.66 and 2.28%) and lowest grub population (1.60 and 1.12/pit).

In this study the entomopathogenic fungi were not up to the expected level in reducing the white grub population under field condition. One of the reasons behind this may be due to lower relative humidity resulting from deficient and ill-timed rain during July–August of the study period. As humidity is one of the prerequisite conditions for growth and development of entomopathogenic fungi, lesser humidity might have reduced the virulence of entomopathogenic fungi as Shashi *et al.* (2001) reported that the relative humidity of 53% is most favourable for the growth of entomopathogenic fungi. Yadav *et al.* (2004b) reported that the efficiency of *M. anisopliae* and *B. bassiana* in controlling *H. consanguinea* increased when applied with increasing rates of compost because it provides moisture for germination of fungi resulting in higher mortality of *H. consanguinea*. Scarcity of sufficient moisture might have also reduced the conidial adhesion and germination on grub resulting in lower grub mortality in entomopathogen treated plot as reported by Yaginuma *et al.* (2004) in case of *B. bassiana*. Similar finding has been reported by Chandel (2005) and Benker and Leuprecht (2004) who stated that grub of *Brahmina coriacea* had medium to low susceptibility to *M. anisopliae* and *B. bassiana* and in laboratory and field conditions, both these fungi didn't prove satisfactory in controlling potato white grubs.

It may be concluded that entomopathogenic fungi *B. bassiana* and *M. anisopliae* are effective bio-agents in controlling the white grub. However, among the bio-agents, *M. anisopliae* proved to be most effective at higher dosage against *H. longipennis*. In comparison to chemical insecticide the effectiveness was very low in respect of plant mortality and grub population.

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