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Evaluation of two species of entomopathogenic fungi against white grub, *Holotrichia consanguinea* (Blanchard) infesting potato in Maharashtra, India

M. S. KULYE and D. S. POKHARKAR
Division of Entomology, Mahatma Phule Krishi Vidyapeeth, College of Agriculture, Pune 411 005, Maharashtra, India.

ABSTRACT: The efficacy of two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, against white grub, *Holotrichia consanguinea* infesting potato crop was studied under laboratory and field conditions. The estimated LC_{50} of *M. anisopliae* and *B. bassiana* towards third instar larvae of *H. consanguinea* were 5.76 x 10^5 and 7.50 x 10^5 conidia ml^{-1}, respectively. The LT_{50} of *M. anisopliae* and *B. bassiana* at the concentration of 8 x 10^5 conidia ml^{-1} against third instar larvae of *H. consanguinea* were 4.88 and 6.73 days, respectively. In field experiments, *M. anisopliae* @ 2 x 10^{12} conidia ha^{-1} was found to be effective with an average efficacy of 46.74%, while it was 41.32% in phorate 10G @ 25kg ha^{-1}. The maximum mycosis was recorded with the use of *M. anisopliae* (44.44%). The tuber infestation was statistically lowest (6.91%) and yield of marketable tubers highest (27.6t ha^{-1}) with the use of *M. anisopliae* as against 27.85% and 17.8t ha^{-1} in untreated plot, respectively.

KEY WORDS: *Beauveria bassiana, Holotrichia consanguinea, Metarhizium anisopliae,* potato.

INTRODUCTION

White grubs, *Holotrichia* spp. (Coleoptera: Scarabaeidae), are noxious subterranean pests damaging the root system of several crops (Desai and Patel, 1965; Mishra and Singh, 1993). The white grub species involved are *Holotrichia consanguinea* (Blanchard), *H. serrata* Fabricius, *H. longipennis* Blanchard and *H. fregei* Mittel (Shah and Shah, 1990). Of these, *H. consanguinea* and *H. serrata* occur most abundantly during August-September and pose a serious threat to potato crop in Maharashtra. Although the grubs remain in the soil throughout the crop stage, predominant damage occurs at tuber formation stage (Chandla, 1985). Commonly used insecticides such as phorate applied during planting do not persist in the soil long enough to provide sufficient control of the pest during tuber formation stage. The use of chemical insecticides at tuber formation stage is not recommended due to the possibility of residues in the final produce for export.

Metarhizium anisopliae (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) have been evaluated for the control of a variety of soil inhabiting insects such as corn rootworm (Krueger and Roberts, 1997), Xea beetle (Butt *et al.,* 1997), June beetle, *H. philanthus* (Ansari *et al*., 2004), white grubs, *Cyclocephala signaticollis* Burmeister, *Phyllophaga crinita* Burmeister and *Anomala flavipennis* Burmeister (Beron and Diaz, 2005; Rodriguez *et al.,* 2005). In this study, the locally available aqueous formulations of *M. anisopliae* and *B. bassiana* were tested against *H. consanguinea* to know their effectiveness.

MATERIALS AND METHODS

Source of material

Aqueous formulations of *M. anisopliae* and *B. bassiana* (having 2 x 10^8 cfu ml^{-1}) used in the present study were obtained from M/s Jay Biotech, Pune, and compared with phorate 10G as standard check in the test.

Insect culture

Second instar larvae of *H. consanguinea* were collected from potato fields and reared individually in sterile vials containing pieces of potato disinfected for 10 min in 0.5% sodium hypochlorite solution as diet. The larval culture was maintained at 25 ± 2°C and 65 ± 5% RH.

Determination of median lethal concentration

To determine the median lethal concentration (LC_{50} values) of *M. anisopliae* and *B. bassiana,* the third instar larvae of *H. consanguinea* were employed. The susceptibility of the larvae was determined using larval dip method. Suspensions of *M. anisopliae* and *B. bassiana* were prepared with dilution of fungal formulation in

sterile distilled water. For bioassay, four concentrations of each formulations, *i.e.*, 3, 4, 5 and 6ml lit^{-1} water, were tested to observe the possibility of detecting small changes in virulence and the spore count obtained in resulting suspension was 6 x 10^5, 8 x 10^5, 1 x 10^6 and 1.2 x 0^6 conidia ml^{-1}, respectively. The larvae were dipped in 30ml of conidial suspensions for 5 seconds. A set of 30 larvae with three replications for each concentration of fungal formulation and a control treated with sterile distilled water were maintained. After treatment, individual larva was transferred separately into sterile vials containing sterile soil with 30% moisture at bottom and a piece of disinfected potato as food. The potato pieces were changed every alternate day and the larvae were kept at 25 ± 2°C, 65 ± 5% RH and 16L:8D till death. The dead larvae were then transferred to sterile petri plates containing moist Whatman's No.1 filter paper and kept at 28°C and 70-80% RH for at least 3-7 days to allow mycelial growth and conidia formation over the cadavers. The data on per cent mortality were corrected by Abbott's formula. The estimates of LC_{50} were made using probit analysis (Finney, 1964).

Determination of median lethal time

To determine the median lethal time (LT_{50} value), the mortality data of the treated larvae were recorded daily using 8 x 10^5 conidia ml^{-1} (4 ml formulation lit^{-1}). A set of 30 third instar larvae with three replications were treated with these fungal pathogens and control was treated with sterile distilled water. The individual larva was dipped in 30 ml suspension of *M. anisopliae* and *B. bassiana* for 5 seconds. The same procedure described earlier was followed to maintain the treated grubs and to record the observations after treatment. The data on per cent mortality were corrected by Abbott's formula. The estimates of LT_{50} were carried out as per Throne *et al.* (1995).

Field evaluation

The field experiment was conducted on potato (var. Kufri Jyoti) in the hot spot area in a farmer's field at Kolharwadi, Pune, Maharashtra, for two successive *Kharif* seasons during 2006-2007 and 2007-2008. The experiment was laid out in randomized block design with five treatments and four replications. The plot size was 10 x 10m and plant spacing was 45 x 30cm. The crop was raised following the recommended agronomic practices except plant protection measures. The aqueous formulations of *M. anisopliae* @ 2 x 10^{12} conidia ha^{-1} (10 lit. formulation ha^{-1}), *B. bassiana* @ 2 x 10^{12} conidia ha^{-1} (10lit. formulation ha^{-1}) and *M. anisopliae* @ 1 x 10^{12} conidia ha^{-1} (5lit. formulation ha^{-1}) + *B. bassiana* @ 1 x 10^{12} conidia ha^{-1} (5lit. formulation ha^{-1}) were applied. Before addition of fungal formulations, the farm yard manure (FYM) was solarized. For solarization, the FYM was moistened by sprinkling water over the heap and then spread into a 10cm thick layer, which was covered with a polythene sheet. All sides of the sheet were covered with soil to make it leak-proof. The solarization was done for 3 weeks and the temperature was recorded daily using soil thermometer. For enrichment of FYM with the fungal formulations, a quantity of 100 kg well decomposed FYM was mixed thoroughly with 100ml formulation (2 x 10^{10} conidia) diluted in 20L water and spread as a layer (12.5cm thick) under shade. It was covered with rice straw and water was sprinkled on the top to maintain the FYM moist. It was incubated (25-32°C) for 15 days. Sufficient turning and watering was given to the treated FYM at the interval of 6 days to improve aeration and maintain moisture content. The fungus enriched FYM (100kg having 7.3 x 10^4 cfu $gram^{-1}$) was incorporated in $100m^2$ of the soil before planting and the land was harrowed. Phorate 10G @ 25kg ha^{-1} was also applied in a separate plot at the time of planting to compare the efficacy of the fungal and chemical insecticides. The plot without any treatment served as control.

To record the observations, five spots with one m^2 area per plot were selected randomly and the larval count recorded a day before planting, 30, 60 and 90 days after planting by digging out the soil below plant canopy up to root / tuber zone. The mycosed larvae were also observed to record per cent mycosis. The healthy tuber yield harvested from each treatment plot was recorded separately and converted to per hectare basis. The per cent efficacy of different treatments was worked out using the formula suggested by Henderson and Tilton (1955).

Data analysis

The data on per cent efficacy, mycosis and tuber infestation were transformed into angular values. These transformed data and marketable tuber yield were analyzed statistically using Analysis of Variance (ANOVA) technique. The means were compared using Duncan's Multiple Range Test (DMRT) as per Gomez and Gomez (1984).

Table 1. Median lethal concentration (LC_{50}) values of fungal pathogens against third instar larvae of *H. consanguinea*

Fungal pathogen	χ^2 Value	Regression equation Y = a + bx	LC_{50} (x10^5 conidia ml^{-1})	Fiducial limit (x10^5 - x10^5 conidia ml^{-1})
M. anisopliae	0.33	Y = 2.6806 + 5.0662x	5.76	6.66 – 5.14
B. bassiana	0.98	Y = 2.0367 + 5.1651x	7.50	8.06 – 6.98

Table 2. Median lethal time (LT_{50}) values of fungal pathogens against third instar larvae of *H. consanguinea* at concentration of $8x10^5$ conidia ml^{-1}

Fungal pathogen	χ^2 value	Regression equation Y = a + bx	LT_{50} (Days)	Fiducial limit (Days)
M. anisopliae	12.25	Y = 2.1894 + 4.1492x	4.88	5.17 - 4.61
B. bassiana	1.99	Y = 1.6482 + 4.0650x	6.73	7.09 - 6.39

RESULTS AND DISCUSSION

The median lethal concentration (LC_{50}) for *M. anisopliae* and *B. bassiana* against third instar larvae of *H. consanguinea* at different concentrations are given in Table1. The estimated LC_{50} value for *M. anisopliae* was 5.76 x 10^5 conidia ml^{-1}, while it was 7.50 x 10^5 conidia ml^{-1} for *B. bassiana.* All Chi-square values were not significant (P = 0.05) indicating good fit of regression lines. Beron and Diaz (2005) found that white grub, *Cyclocephala signaticollis* was more susceptible to *B. bassiana* isolate than the strains of *M. anisopliae* and *Paecilomyces lilacinus*. However, the results of the present study are similar to Ansari *et al.* (2004) who reported that *M. anisopliae* strains were more virulent than *B. bassiana* strains against June beetle, *H. philanthus,* with the LC_{50} value of 2.5 x 10^4 to 10^5 conidia g^{-1} soil at 25°C under laboratory conditions.

The median lethal time (LT_{50}) value for *M. anisopliae* was lower (4.88 days) than that for *B. bassiana* (6.73 days) (Table 2). All Chi-square values were not significant (P = 0.05) indicating good fit of regression lines. The present findings are in agreement with Rodriguez *et al.* (2005) who reported that *M. anisopliae* strains were more virulent than *B. bassiana* strains against third instar larvae of *P. crinita* and *A. flavipennis* under laboratory conditions and the median lethal time (LT_{50}) recorded for *M. anisopliae* isolates were 2.9-5.3 days for *P. crinita* and 3.0-7.6 days for *A. flavipennis.*

The average surviving population of *H. consanguinea* larvae m^{-2} in different treatment plots is shown in Fig.1. The larval population m^{-2} before application of treatments in the plots was homogeneous (3.15-3.70). All the treatments

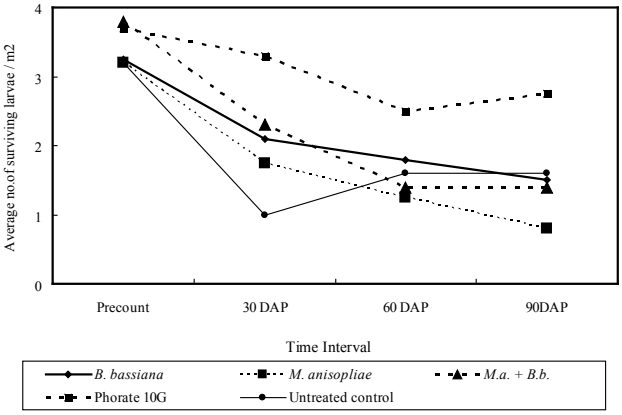

Fig 1. Larval population of *H. consanguinea* in the potato field influenced by fungal pathogens

were superior to control at all the observational periods. The surviving grub population/m^2 was lowest in phorate (1.00) at 30 days, while it was 1.25 at 60 days and 0.90 at 90 days in *M. anisopliae* treated plot. The per cent efficacy of different treatments is shown in Table 3.

Phorate was superior among all the treatments with 63.54 per cent efficacy at 30 days, while *M. anisopliae, B. bassiana* and *M. anisopliae+B. bassiana* were statistically on par with each other. All the treatments were found to be equally effective at 60 days. *M. anisopliae* with 59.44 per cent efficacy was the most effective at 90 days after application, *i.e.*, at the time of harvest. The average per cent efficacy over the entire period of all the treatments were on par.

The per cent mycosis due to *M. anisopliae* and *B. bassiana* in all the treatments was statistically on par at

Table 3. Per cent efficacy of fungal pathogens, tuber infestation and yield of marketable potato influenced by treatments

Treatment	Per cent efficacy at various intervals				Per cent infestation	Marketable tuber yield ($t\ ha^{-1}$)
	30 DAP	60 DAP	90 DAP	Average		
B. bassiana	33.39[a]	22.64[a]	35.92[a]	30.65[a]	14.72[b]	23.0[b]
M. anisopliae	42.40[a]	38.39[a]	59.44[b]	46.74[a]	6.91[a]	27.6[c]
M.a. + B .b.	30.65[a]	42.48[a]	45.57[a]	39.56[a]	9.07[a]	26.4[b]
Phorate 10G	63.54[b]	34.28[a]	26.15[a]	41.32[a]	16.12[b]	25.3[b]
Control	–	–	–	–	27.85[c]	17.8[a]

Means in a column followed by the same letter(s) are not significantly different (P = 0.05) by DMRT; DAP= Days after planting

various intervals. It was, however, observed to be highest (44.44%) due to *M. anisopliae*. The tuber infestation was lowest (6.91%) in the plot treated with *M. anisopliae* and was on par with *M. anisopliae*+*B. bassiana* (9.07%) as against 27.85 per cent in control plot. Similarly, the yield of marketable tubers was highest (27.6t ha^{-1}) in the plots treated with *M. anisopliae*. It was, however, statistically on par with phorate, *B. bassiana* and *M. anisopliae*+ *B. bassiana.*

The successful use of entomopathogenic fungi as microbial control agents will ultimately depend on the use of the right propagule, formulated in an optimal manner and applied at an appropriate dosage and time. The selection of the most virulent species of the entomopathogenic fungus for the management of white grub, *H. consanguinea* in the field was the main objective of this study. The results of this study suggest that *M. anisopliae* could be a potential biocontrol agent.

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