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Research Article

Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against *Plutella xylostella* (L.) under laboratory conditions

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ABSTRACT: Twenty isolates each of *Beauveria bassiana* and *Metarhizium anisopliae* were tested against the second instar larvae of *Plutella xylostella* through laboratory bioassay using larval dip method at the concentration of 1×10^7 conidia/ml. Higher larval mortality of 88.85% and 81.44%, were observed with NBAIR Ma-4 and NBAIR Ma-35 isolates of *M. anisopliae* and 77.36% with NBAIR-Bb-5a isolate of *B. bassiana*. Further studies on dose and time mortality with four isolates indicated lowest LC_{50} value of 2.6×10^4 conidia/ml and LT_{50} of 86.6 hours with NBAIR Ma-35 isolate. These isolates have to be further tested against *P. xylostella* in infested fields of cabbage and cauliflower.

KEY WORDS: *Beauveria bassiana,* Entomopathogenic fungi, *Metarhizium anisopliae, Plutella xylostella*

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INTRODUCTION

Plutella xylostella (L.) (Diamond Back Moth (DBM)) (Lepidoptera: Plutellidae) is widely distributed serious pest of cruciferous crops and causes extensive loss of US$4-5 billion globally (Zalucki *et al.,* 2012; Furlong *et al.,* 2013). In India, cabbage and cauliflower are important cole crops grown in 0.438 million hectares and producing 6.335 million tonnes per annum. The losses in cabbage and cauliflower due to DBM attack in India was estimated at 35 percent even with the use of insecticides and could go upto 90 per cent without following control measures (Mohan & Gujar, 2003). Krishnamurthy (2004) reported 52% yield loss of cabbage due to the attack of diamondback moth in India. This pest is mainly controlled using chemical insecticides and the pest has developed resistance to all categories of chemical insecticides (McCann *et al.,* 2001; Liu *et al.,* 2003) and also to the toxins of the *Bacillus thuringiensis*. Apart from this, chemical insecticides are detrimental to the natural enemies of this pest and also to other living organisms. Hence, alternative control measures are required for safer and cost-effective management of this destructive pest (Bert, 2006).

Entomopathogenic fungi can be considered as one of the alternative approaches as biocontrol agents for insect pests (Sheeba *et al.,* 2001). The present study was taken up to identify promising isolates of *B. bassiana* and *M. anisopliae* against *P. xylostella.*

MATERIALS AND METHODS

Preliminary screening was carried out with twenty isolates each of *Beauveria bassiana* and *Metarhizium anisopliae* on the second instar larvae of *P. xylostella* using laboratory bioassay method for identification of promising isolates.

Insect rearing

A starter culture of DBM moths were obtained from ICAR-National Bureau of Agricultural Insect Resources (NBAIR) for rearing the larvae of *P. xylostella* on mustard seedlings (Liu & Sun, 1984). Mustard seedlings were raised in plastic cups (55.2×68.9×45.1mm) on vermiculate at 25-27° C and 70% RH. Four-days old-mustard seedlings thus raised in cups were placed in acrylic cages (24×24×24") and DBM moths were released in the cages for oviposition. After 24-48 hours, the cups were removed and kept in trays for egg hatch and larval development. Fresh seedlings were again provided for the moths to oviposition. The mustard seedlings containing 2nd instar larvae of *P. xylostella* were used in this study.

Fungal culture

Twenty isolates each of *B. bassiana* and *M. anisopliae* collected from different insect hosts and soils from various geographical areas of India maintained at the culture repository of National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangalore were used for preliminary screening against *P. xylostella*. Fungal culture of each isolate is grown on Sabouraud's Dextrose Yeast extract Broth (SDYB) medium (Dextrose 20g, Mycological peptone 10g, yeast extract 5g, in 1L of distilled water). Conidiated rice was produced by inoculating 4 days old shaker culture to sterilized rice bag and incubated for 15 days at 26 ± 1˚C. Conidial suspension of each isolate was prepared by suspending one gram of 15 days old conidiated rice in sterile distilled water with 0.01% Tween 80. The suspension was filtrated through three layers of muslin cloth to get hyphal-free conidial suspension. The conidial concentration in the suspension was adjusted to 1×10^7 conidia/ml using Neubauer s improved haemocytometer.

Laboratory bioassay

Thirty larvae of *P. xylostella* were dipped in 0.5ml of the spore suspension (1×10^7 conidia/ml) of each of the *B. bassiana* and *M. anisopliae* isolates for 10 seconds and were later transferred to a sterile plastic container, provided with surface sterilized cabbage leaves as a food and placed at 26°C and 70% RH in an incubation chamber. Control group of larvae were treated with distilled water containing 0.01% Tween 80. Larval mortality was recorded for a period of 10 days at 24 h intervals. The percent mortality of the larvae was calculated after deducting the control mortality using Abbott's formula (Abbott, 1925). The data of mortality were subjected to the statistical analysis using analysis of variance SPSS software, Version 20. Based on these studies, promising isolates were identified for further studies (Table 2).

Dose and Time mortality

For dose mortality studies (LC_{50}), five conidial concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7 & 1×10^8 conidia/ml) were prepared and bioassays were carried out as described above. In case of time mortality studies, 1×10^8 conidia/ml concentration was used to determine LT_{50}. The dose and time to kill 50 per cent of the population (LC_{50} & LT_{50}) was determined by probit analysis (Finney, 1971). Statistical analysis was done using SPSS windows version 20.0.

RESULTS AND DISCUSSION

Laboratory bioassay

Among the 20 isolates of *B. bassiana* tested, Bb-5a showed significantly higher mortality (77.36%) and rest of the isolates showed 17.31–51.14% mortality (Table 1). Among the 20 isolates of *M. anisopliae* tested, Ma-4 and Ma-35 showed significantly higher mortality (81.44 & 88.85% respectively) and the rest of the isolates showed 36.95–70.33% mortality (Table 1).

Isolates of *B. bassiana* and *M. anisopliae* tested showed varying percent mortality on second instar larvae of *P. xylostella*. As these isolates were obtained from diverse insect hosts and soils from different climatic conditions, the variability in virulence on *P. xylostella* is expected. According to Maurer *et al.* (1997), the isolates which derived from the same or related host species showed higher virulence. But our studies indicated that higher virulence was observed even with the isolates that have been derived from the soil and non-related host species. Silva *et al.* (2003) reported

Table 1. Effect of *B. bassiana* and *M. anisopliae* isolates on the larvae of *P. xylostella* in the laboratory bioassay

Sl. No.	*Beauveria bassiana* NBAIR Strains	% Mortality	*Metarhizium anisopliae* NBAIR Strains	% Mortality
1.	**Bb-5a**	**77.36**^{a}	**Ma-4**	**81.44**^{ab}
2.	Bb-10	36.07^{bcde}	Ma-7	44.52^{def}
3.	Bb-17	43.56^{bc}	Ma-8	70.33^{abc}
4.	Bb-18	36.07^{bcde}	Ma-10	51.77^{cdef}
5.	Bb-21	39.90^{bcd}	Ma-19	55.72^{cdef}
6.	Bb-22	39.82^{bcd}	**Ma-35**	**88.85**^{a}
7.	Bb-24	43.56^{bc}	Ma-39	44.48^{def}
8.	Bb-26	39.82^{bcd}	Ma-43	36.95^{f}
9.	Bb-27	32.37^{bcde}	Ma-44	59.21^{cde}
10.	Bb-28	17.31^{de}	Ma-45	48.19^{def}
11.	Bb-29	43.56^{bc}	Ma-46	37.07^{f}
12.	Bb-30	47.35^{bc}	Ma-47	51.85^{cdef}
13.	Bb-40	32.33^{bcde}	Ma-48	55.55^{cdef}
14.	Bb-43	25.47^{cde}	Ma-49	62.92^{bcd}
15.	Bb-45	51.14^{b}	Ma-51	55.63^{cdef}
16.	Bb-51	39.78^{bcd}	Ma-53	48.19^{def}
17.	Bb- 55	47.35^{bc}	Ma-54	55.51^{cdef}
18.	Bb-57	51.10^{b}	Ma-55	55.51^{cde}
19.	Bb- 60	47.44^{bc}	Ma-56a	40.86^{ef}
20.	Bb-74	28.50^{bcde}	Ma-57	55.51^{cdef}
21.	Control	11.33^{e}	Control	10.00^{g}
	F- value	3.428	**F- value**	5.930
	P-value	0.072	**P-value**	0.019

*Values in columns followed by the different letter are significantly different with each other according to Tukeys test ($P < 0.01$)

78–90% mortality of second-instar larvae of diamondback moth when treated with *B. bassiana* isolates at concentration of 10^8 conidia/ml. Valda *et al.* (2003) reported 26–96% larval mortality of DBM with *B. bassiana* and *M. anisopliae* isolates at 10^8 conidia/ml. In our study, *M. anisopliae* isolates showed higher mortality of 81-88% than *B. bassiana* isolates (51-77%) at 10^7 conidia/ml concentration. Suwannakut *et al.* (2005) reported that variation in virulence can be attributed due to genetic diversity of the isolates, based on insect host and geographical region. This laboratory studies helped in identifying the local promising isolates for further field testing against *P. xylostella* in crucifer's crops.

Dose and Time mortality

Among the four isolates tested, NBAIR Ma-35 showed lowest LC_{50} at 2.6×10^4 condia/ml and LT_{50} at the 86.6 hours against *P. xylostella*. NBAIR Ma-4 with LC_{50} at 5.3×10^4 and LT_{50} at 121.30 hours, NBAIR Bb-5a with LC_{50} at 1.3×10^5 and LT_{50} at the 100.3 hours and NBAIR Bb-45 with LC_{50} at 1.9×10^6 and LT_{50} at the 156.6 hours (Table 3&4).

Chui-Chai *et al.* (2012) reported LT_{50} ranging from 25-145 hours with *Beauveria* spp. and *Metarhizium* spp. and LC_{50} of 2.66×10^6 and 3.11×10^5 conidia ml^{-1} respectively. *B. bassiana* and *M. anisopliae* isolates showed LT_{50} ranging from 0.7-5.8 days and LC_{50} of 1.2×10^6 and 8.6×10^6 conidia ml^{-1} (Valda *et al.,* 2003). In present study, the *Metarhizium anisopliae* isolates showed high LC_{50} value at $2.6\text{-}5.3\times10^4$ conidia/ml and LT_{50} of 86-121 hours. Duarte *et al.,* 2016 stated that mortality at lower conidial concentrations is important from economic point of view which helps in product development and marketing of entomopathogenic microbes. Our results also showed 50% mortality at 10^4 conidia/ml and within 3.5 days with NBAIR Ma-35 isolate of *M. anisopliae* against *P. xylostella*.

CONCLUSION

Based on the laboratory bioassay studies, three promising isolates of entomofungal pathogens viz. NBAIR Ma-35 & NBAIR Ma-4 of *M. anisopliae* and NBAIR Bb-5a of *B. bassiana* were identified against *P. xylostella* which can be further tested in the infested fields of cabbage and cauliflower.

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Table 2. Details of promising isolates of *B. bassiana* and *M. anisopliae* against *P. xylostella*

Sl. No.	Isolate Code	Genbank Accession number	Source	Location India
1.	NBAIR Bb5a	JF837134	*Hypothenemus hampei* (Coffee berry borer)	Karnataka
2.	NBAIR Bb45	JF837094	Rhizosphere soil of Carrot	Tamil Nadu
3.	NBAIR Ma4	JF837157	*Plocaederus ferrugineus* (Cashew Stem Borer)	Karnataka
4.	NBAIR Ma35	JQ518481	Soil (Fallow land)	Gujarat

Table 3. Dose mortality response of entomopathogenic fungi against *P. xylostella*

Isolates	LC_{50} conidia/ ml	95% fiducial limit (conidia/ml)	Slope±SE	X^2	P value	df
Metarhizium anisopliae ICAR-NBAIR Ma-35	$2.6\ x10^4$	$4.6\ x10^3$-$7.6\ x10^4$	0.572±0.106	1.281	0.734	3
Metarhizium anisopliae ICAR-NBAIR Ma-4	$5.3\ x10^4$	$1.1\ x10^4$-$1.5\ x10^5$	0.525±0.095	0.453	0.929	3
Beauveria bassiana ICAR-NBAIR Bb-5a	$1.3\ x10^5$	$3.1\ x10^4$- $3.6\ x10^5$	0.500±0.087	0.903	0.825	3
Beauveria bassiana ICAR-NBAIR Bb-45	$1.9\ x10^6$	$5.7\ x10^5$- $7.7\ x10^6$	0.415±0.081	0.574	0.902	3

SE- standard error, X^2- Chi square, df- degree of freedom

Table 4. Time mortality response of entomopathogenic fungi against *P. xylostella*

Isolates	LT_{50} hours	95% fiducial limit (hours)	Slope±SE	X^2	P value	df
Metarhizium anisopliae ICAR-NBAIR Ma-35	86.6	71.91-119.36	4.105±0.958	1.165	0.761	3
Metarhizium anisopliae ICAR-NBAIR Ma-4	121.30	92.39-214.83	2.928±0.705	10.309	0.172	7
Beauveria bassiana ICAR-NBAIR Bb-5a	100.32	84.04-132.67	3.623±0.605	4.038	0.846	5
Beauveria bassiana ICAR-NBAIR Bb-45	156.64	123.16-271.01	3.276±0.800	1.167	0.948	5

SE- standard error, X^2- Chi square, df- degree of freedom

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