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Effect of endophytic isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage

Poornesha Bathina^{1,2*} and Ramanujam Bonam¹

Abstract

Background: The diamondback moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) is one of the major pests in cabbage which causes severe loss to the cruciferous crops. Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have been established as endophytes in cabbage plants by seed treatment/root inoculation/ foliar application methods in glasshouse conditions.

Main body: A glasshouse experiment was conducted to study the effect of endophytic isolates of *B. bassiana* (NBAIR Bb-5a and NBAIR Bb-45) and *M. anisopliae* (NBAIR Ma-4 and NBAIR Ma-35) on *P. xylostella* in cabbage using detached leaf bioassay method. The isolates were applied through seed treatment/root inoculation/foliar application at the concentration of 1×10^8 spores/ml and evaluated at 15, 30, 45, and 60 days after treatment (DAT) in 2017 and 2018. These isolates were colonized in cabbage leaf tissues from 15 to 60 DAT. All 4 isolates showed different mortality percentages during 15–30 DAT, and no mortality was observed after 45 and 60 DAT in different inoculation methods during both years. Among the application methods tested, foliar application method gave the highest mortality of 70–80% at 15 DAT and 12–58% at 30 DAT mortality of 2nd instar larvae of *P. xylostella* in cabbage. Among the isolates tested, NBAIR Ma-35 showed the highest mortality (35–79%) in all the 3 inoculation methods tested.

Conclusion: Endophytic isolates of *B. bassiana* and *M. anisopliae* suppressed the population of *P. xylostella* on cabbage leaves in three inoculation methods tested. Among all the methods tested, foliar application method showed highest mortality. These promising isolates have to be further tested under field conditions for management of *P. xylostella* in cabbage.

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, *Plutella xylostella*, Cabbage, Endophytic

* Correspondence: poorniintbiotech@gmail.com

¹Division of Germplasm, Conservation and Utilisation, ICAR-National Bureau of Agricultural Insect Resources, Post Bag No. 2491, H.A. Farm Post, Bellary Road, Bengaluru, Karnataka 560024, India

²Department of Biotechnology, Center for Post Graduate Studies, Jain University, Jayanagar, Bengaluru, Karnataka 560011, India

Background

Cabbage, *Brassica oleracea* var. *capitata* L., is one of the extensively cultivated cruciferous crops in the world. India is the second largest producer of cabbage cultivating in an area of 0.40 million ha with a production of 9.04 million ton and average productivity of 22.56 ton/ha (Anonymous 2018). The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the major pests in cabbage. Its larvae feed extensively on cabbage leaves leading to drying, defoliation, and stunting of cabbage heads (Gujar 1999). The chemical insecticides are being used for management of *P. xylostella* in cabbage for a long time. Resistance development in the pest species was reported against most of the chemical insecticides and to the Bt-based products (Sayyed et al. 2008). Considering this as a significant concern, there is a need for an alternative safe and ecofriendly pest control method.

Utilization of entomopathogenic fungi (EPF) is one of the alternative biological control methods which play a key role on sustainable pest management programs. Cost effectiveness, simple mass production procedure, and safety to non-target organism and to the environment are some of the advantages of using these as biocontrol agents (Charnley and Collins 2007; and Lacey 2016). Apart from this, recently EPF species have been reported to colonize the tissues of certain crop plants by artificial inoculation for pest management programs (Vega 2018). This novel method of establishing them as endophytes in crop plants will help to overcome the dry weather conditions by giving them better effectiveness against insect pests. Thus, endophytically established isolates of *Beauveria bassiana*, *Metarhizium* spp., *Lecanicillium* spp., and *Isaria* spp. showed effectiveness against various arthropod pests in different crops (Mantzoukas and Eliopoulos 2020).

In earlier studies, four indigenous isolates of EPF, *B. bassiana* (NBAIR Bb-5a and NBAIR Bb-45), and *M. anisopliae* (NBAIR Ma-4 and NBAIR Ma-35) were found effective against *P. xylostella* in the laboratory bioassay and were also established as endophytes in cabbage by artificial inoculation of conidial suspensions. Hence, the present study aimed to evaluate the EPF endophytically established isolates against *P. xylostella* in cabbage under glasshouse conditions.

Materials and methods

The glasshouse experiment was conducted using NBAIR Bb-5a and NBAIR Bb-45 isolates of *B. bassiana* and NBAIR Ma-4 and NBAIR Ma-35 isolates of *M. anisopliae* using detached leaf bioassay method. The experiment was carried out in 2017 and 2018 to check the efficacy of these isolates.

Insect culture

Second instar larvae of *P. xylostella* were obtained from mass production unit of ICAR-NBAIR, Bangalore, India, for this study.

Fungal culture

Fungal cultures were obtained from Entomofungal Repository of National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangalore, which were isolated from different agro-climatic zones of India (Table 1). Each fungal culture was grown on Sabouraud's dextrose yeast extract broth (SDYB) medium (dextrose 20 g, mycological peptone 10 g, yeast extract 5 g, in 1 l of distilled water) for mass production of this fungus on broken rice. Four-day-old shaker culture was added to sterilized rice bag and incubated for 15 days at 26 ± 1 °C. The conidiated rice was used for preparation of conidial suspension. The conidial suspension was prepared by suspending 1 g of 15-day-old conidiated rice in sterile distilled water with 0.01% Tween 80. The conidial suspension was filtrated through three layers of muslin cloth to get hyphal-free suspension. The conidial concentration in the suspension was adjusted to 1×10^8 spores/ml using Neubauer's improved hemocytometer.

Pot culture studies

Cabbage var. Unnati was used for pot culture studies. As per the protocols of Tefera and Vidal 2009 and Russo et al. 2015 with slight modifications, seed treatment was given by dipping the cabbage seeds in conidial suspension (1×10^8 conidia/ml) of each isolate individually for 1 h and were later sown into the sterilized coir pith trays. The seeds were dipped in sterile water containing 0.01% Tween 80 as untreated control. For root inoculation method, roots of 30-days-old cabbage seedlings were dipped into the conidial suspension (1×10^8 conidia/ml) for 1 h and later transplanted into the pots containing sterile soil and untreated control plants were dipped in sterile water containing 0.01% Tween 80 (Tefera and Vidal 2009; Russo et al. 2015). In foliar application method, conidial suspension of each isolate (1×10^8 conidia/ml) was sprayed on 30-day-old plants using hand atomizer. Untreated control plants were sprayed with sterile water containing 0.01% Tween 80. Completely randomized block design with 5 replications were maintained with 10 plants per replication for each isolate and for each method.

Detached leaf bioassay

As per the Qayyum et al. (2015) with slight modifications, the leaf samples treated with the isolates of *B. bassiana*, *M. anisopliae* and the untreated control were collected at 15, 30, 45, and 60 days after treatment for bioassay studies against *P. xylostella*. The leaf samples

Table 1 Details of promising isolates of *Beauveria bassiana* and *Metarhizium anisopliae*

Isolate code	NCBI Genbank accession number	Source	Location
NBAIR Bb5a	JF837134	<i>Hypothenemus hampei</i> (Coffee berry borer)	Karnataka
NBAIR Bb45	JF837094	Rhizosphere Soil of Carrot	Tamil Nadu
NBAIR Ma-4	JF837157	<i>Plocaederus ferrugineus</i> (cashew stem borer)	Karnataka
NBAIR Ma-35	JQ518481	Soil (fallow lands)	Gujarat

were surface sterilized by sodium hypochlorite (1%) for 3 min and then with ethanol (70%) for 30 s. The surface sterilized samples were then rinsed in sterile distilled water for three times and dried on sterile blotting paper for 3 min in a laminar flow. The surface sterilization was done to eliminate surface microbial contaminants on the leaf surface. Each of the surface sterilized leaf was placed in a sterile plastic container and 2nd instar larvae of *P. xylostella* were released. Five replications (10 larvae per replication) were maintained for each isolate and untreated control. Fresh surface sterilized leaves of each treatment were provided to the larvae at 24 h interval. 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's 1925).

$$\text{Corrected\%mortality} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \right) \times 100$$

Where : n = Insect population, T = treated, Co = control

Statistical analysis was mainly carried out among the treatments to find out the significant differences. Data of mortality were subjected to the ANOVA (analysis of variance) by statistical analysis using SPSS software 20 version.

Results and discussion

Detached leaf bioassay

All the 4 isolates showed different mortality percentages during 15–30 days after treatment (DAT) in different inoculation methods in both years (Tables 2, 3, and 4).

In seed treatment during 2017, the 4 isolates showed 10–38% mortality of *P. xylostella* at 15 DAT and the mortality ranged between 20 and 44% by the 3 isolates (NBAIR Bb-45, Ma-4, and Ma-35) at 30 DAT. In 2018, the 3 isolates (NBAIR Bb-5a, Bb-45, and Ma-35) caused *P. xylostella* mortality between 28.9 and 48.9% at 15 DAT, while the 4 isolates showed 28–48% mortality during 30 DAT. When the average mortality rates of the 2 years were taken into consideration, NBAIR Bb-45 and NBAIR Ma-35 isolates showed the highest mortality rates of 40.1 and 42.4%, respectively, at 15 and 30 DAT, while NBAIR Ma-4 and NBAIR Ma-35 showed a high mortality rate of 36% each.

In root inoculation method during 2017, the 2 isolates (NBAIR Ma-4 and NBAIR Ma-35) showed 12 and 30% mortality rates of *P. xylostella*, respectively, at 15 DAT while the mortality ranged between 18 and 50% by the 3 isolates (NBAIR Bb-45, Ma-4, and Ma-35) at 30 DAT. In 2018, 3 isolates (NBAIR Bb-45, Ma-4, and Ma-35) showed 20–50% mortality rates of *P. xylostella* at 15 DAT, while NBAIR Bb-45 and NBAIR Ma-35 showed 10 and 20% mortality rates, respectively at 30 DAT. When the mortality rates of the 2 years taken into consideration, NBAIR

Table 2 Effect of endophytically established isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on *Plutella xylostella* in cabbage (seed treatment method)

Isolate	% mortality-2017		% mortality-2018		Mean mortality of 2017 and 2018 (%)	
	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT
NBAIR Bb-5a	26.0 ± 5.09 ^a	0.0 ± 0.00 ^c	28.9 ± 5.66 ^b	42.0 ± 3.74 ^a	27.4 ± 5.22 ^b	21.0 ± 1.87 ^b
NBAIRBb-45	38.0 ± 3.74 ^a	20.0 ± 3.16 ^b	42.2 ± 4.17 ^{ab}	30.0 ± 3.16 ^b	40.1 ± 3.18 ^a	25.0 ± 2.24 ^b
NBAIRMa-4	10.0 ± 3.16 ^b	24.0 ± 5.09 ^b	0.0 ± 0.00 ^d	48.0 ± 3.74 ^a	5.0 ± 9.64 ^c	36.0 ± 2.45 ^a
NBAIRMa-35	36.0 ± 5.09 ^a	44.0 ± 5.09 ^a	48.9 ± 5.68 ^a	28.0 ± 3.74 ^b	42.4 ± 9.84 ^a	36.0 ± 4.0 ^a
Control	0.0 ± 0.00 ^c	0.0 ± 0.00 ^c	10.0 ± 3.16 ^c	0.0 ± 0.00 ^c	5.0 ± 9.38 ^c	0.0 ± 0.00 ^c
F value	18.026	27.484	23.530	32.962	35.532	35.787
df	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20
LSD (<i>P</i> < 0.01)	15.456	13.886	17.655	13.258	11.849	9.732

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

Table 3 Effect of endophytically established isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on *Plutella xylostella* in cabbage (root inoculation method)

Isolate	% mortality-2017		% mortality-2018		Mean mortality of 2017 and 2018 (%)	
	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT
NBAIR Bb-5a	0 ± 0.00 ^c	0 ± 0.00 ^d	0 ± 0.00 ^c	0 ± 0.00 ^c	0 ± 0.00 ^d	0 ± 0.00 ^c
NBAIR Bb-45	0 ± 0.00 ^c	18 ± 3.74 ^c	20 ± 4.47 ^b	10 ± 0.00 ^b	10 ± 2.24 ^b	23 ± 1.87 ^b
NBAIR Ma-4	12 ± 2.00 ^b	32 ± 5.83 ^b	46 ± 5.09 ^a	0 ± 0.00 ^c	35 ± 3.32 ^c	16 ± 2.92 ^b
NBAIR Ma-35	30 ± 3.16 ^a	50 ± 3.16 ^a	50 ± 4.47 ^a	20 ± 3.16 ^a	40 ± 2.74 ^a	35 ± 2.74 ^a
Control	0 ± 0.00 ^c	0 ± 0.00 ^d	0 ± 0.00 ^c	0 ± 0.00 ^c	0 ± 0.00 ^d	0 ± 0.00 ^c
F value	61.714	39.828	44.030	40.000	68.766	53.333
df	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20
LSD (<i>P</i> < 0.01)	7.504	13.701	16.105	5.842	9.862	8.132

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

Ma-35 showed the highest mortality rate of 40 and 35%, respectively, at 15 and 30 DAT.

In foliar application method, all the 4 isolates caused 72–78% mortality of *P. xylostella* at 15 DAT and 12–58% at 30 DAT. In 2018, the 4 four isolates recorded 75–80% mortality rate of *P. xylostella* at 15 DAT and 13.3–55.6% at 30 DAT. When the average mortality of 2 years was taken into consideration, NBAIR Ma-35 showed the highest mortality of 79% at 15 DAT and 56.8% at 30 DAT.

No mortality was observed at 45 and 60 DAT in all the inoculation methods. No mycosis was observed on the dead larvae in all methods. The dead larvae which their color turned to brown were considered for mortality.

Among the three methods tested, foliar application gave the highest mortality rate of *P. xylostella*. Among the isolates tested, NBAIR Ma-35 showed the highest mortality rate in all the inoculation methods tested.

EPF as endophytes are reported as biocontrol agents showing effective protection to host plants from pests. There are reports of significant pest reduction and plant

damage in certain crops by endophytically established EPF (Mantzoukas and Eliopoulos 2020). Also, there are reports of poor performance of endophytic EPF due to complexity of these EPF insect–plant interactions (Powell et al. 2009; Akello and Sikora 2012; Clifton et al. 2018; and Jensen et al. 2019).

M. anisopliae when inoculated into the plants of *Brassica napus* through foliar spray showed 63.3% mortality of *P. xylostella* larvae after 4 weeks of endophytic establishment (Batta 2013). Gautam et al. 2016 reported non-survival of *P. xylostella* larvae on *B. bassiana* sprayed leaves, which were attributed to production of certain volatile compounds produced due to the interaction of *B. bassiana* with the host plant and no mycosis was observed on the dead larvae. In the present study also, no mycosis was observed on the dead larvae.

A fungal endophyte *Acremonium alternatum*, when applied to the roots of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) reduced DBM larval feeding and its growth rate (Raps and Vidal 1998). In the present study on cabbage, *B. bassiana* and *M. anisopliae* isolates when

Table 4 Effect of endophytically established isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on *Plutella xylostella* in cabbage (foliar application method)

Isolate	% mortality-2017		% mortality-2018		Mean mortality of 2017 and 2018 (%)	
	15DAT	30DAT	15DAT	30DAT	15DAT	30DAT
NBAIR Bb-5a	76 ± 4.0 ^a	12 ± 2.0 ^d	75.0 ± 3.95 ^a	13.3 ± 2.22 ^c	75.5 ± 3.76 ^a	12.7 ± 1.29 ^c
NBAIR Bb-45	72 ± 3.74 ^a	30 ± 3.16 ^c	77.5 ± 2.5 ^a	37.8 ± 5.68 ^b	74.8 ± 1.15 ^a	33.9 ± 4.01 ^b
NBAIR Ma-4	74 ± 2.45 ^a	42 ± 3.74 ^b	70.0 ± 8.48 ^a	48.9 ± 4.46 ^{ab}	72.0 ± 5.31 ^a	45.5 ± 2.71 ^a
NBAIR Ma-35	78 ± 5.83 ^a	58 ± 3.74 ^a	80.0 ± 3.06 ^a	55.6 ± 3.53 ^a	79.0 ± 3.48 ^a	56.8 ± 1.96 ^a
Control	0 ± 0.00 ^b	0 ± 0.00 ^e	20.0 ± 3.16 ^b	10.0 ± 3.16 ^c	10.0 ± 1.58 ^b	5.0 ± 1.58 ^d
F value	80.714	63.667	27.956	26.758	73.727	75.201
df	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20
LSD (<i>P</i> < 0.01)	14.310	12.932	19.513	16.597	12.638	10.293

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

applied through foliar spray, showed 72–80% mortality of *P. xylostella* larvae after 15 DAT indicating their efficacy. In earlier studies, extent of endophytic colonization of EPF in cabbage leaf was studied in seed treatment/root inoculation/foliar spray through plating technique and PCR studies which indicated a high percent colonization in foliar application. The highest mortality of *P. xylostella* larvae observed in these studies may be due to the highest colonization of these isolates in cabbage leaves during that period. The mortality rate is mainly dependent on the internal colonization of the fungus and production of secondary metabolites in crop plants (Gautam et al. 2016). The larval mortality was mainly depended on the application method of the fungus as endophytes in the crop plants (Qayyum et al. 2015). Qayyum et al. (2015) reported that, *B. bassiana* successfully established as endophyte by root dipping method compared to the other inoculation methods (injection, solid substrate, and direct foliar application) and found effective against *Helicoverpa armigera* in tomato plants. Endophytic colonization of *B. bassiana* in cotton by seed treatment showed negative effect on the aphid species, *Aphis gossypii* (Lopez and Sword 2015) and reduction in survival of *Helicoverpa zea*. Earlier results on colonization indicated higher colonization and longer persistence in cabbage by foliar application method compared to root inoculation and seed treatment methods which attributed the mortality of *P. xylostella*. No mortality was observed at 45 and 60 DAT, which may be due to less/no colonization of the fungus in the cabbage leaves in all the inoculation methods. Few reports showed mycosis on endophytic screening of insects' viz., *Cosmopolites sordidus*, *H. zea*, *H. armigera*, and *Tuta absoluta* (Vega 2018) whereas in the present study no mycosis was observed.

The negative effect of fungal endophytes on the survival and performance on insect herbivores may be due to production of secondary metabolites and superoxides, changes in the phytosterol profile of plants or by inducing an indirect systemic defense response in the plants leading to resistance in insect feeding (Hartley and Gange 2009; and White and Torres 2010). The secondary metabolites of the endophytic EPF in plant tissues with anti-feeding properties and insecticidal activity might have led to the pest suppression through antibiosis or feeding deterrence (Vega et al. 2008; and Gurulingappa et al. 2011). Fungal toxins like destruxins were reported to be produced by endophytic *Metarhizium robertsii* in cowpea (Golo et al. 2014) and *M. brunneum* in melon and potato (Garrido-Jurado et al. 2017). The exact mechanisms on the effect of endophytic EPF on the herbivorous insects are yet to be studied in detail.

Conclusion

Endophytically established isolates of *B. bassiana* and *M. anisopliae* suppressed *P. xylostella* on cabbage leaves in three methods tested. However, foliar application method showed highest larval mortality. These isolates have to be further tested under field conditions to confirm their efficacy against *P. xylostella* in cabbage.

Abbreviations

NBAIR: National Bureau of Agricultural Insect Resources; NCBI: National Center for Biotechnology Information; Bb: *Beauveria bassiana*; Ma: *Metarhizium anisopliae*; DAT: Days after treatment; EPF: Entomopathogenic fungi; DBM: Diamond back moth; SDYB: Sabouraud's dextrose yeast extract broth; Bt: *Bacillus thuringiensis*

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Authors' contributions

BP performed the experiments on bioassay and analyzed the data. The manuscript was prepared by BP and BR. All the authors read and approved the manuscript.

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All data are available in the article and the materials used in this work are of high quality and grade.

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The authors declare that they have no competing interests.

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