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Screening of endophytic isolates of *Metarhizium anisopliae* (Metchnikoff) Vuillemin and *Beauveria bassiana* (Balsamo) against *P. xylostella linnaeus*

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Abstract
An experiment was conducted to discover the promising endophytic fungal isolates against the target pest, diamondback moth, *Plutella xylostella* using the larval dip method in a laboratory bioassay at Biological Control Research Laboratory, ICAR Unit-9, Anand Agricultural University, Anand (Gujarat). We tested five of each species, *M. anisopliae*, and *B. bassiana*, isolated from diverse soil and insect hosts from distinct geographical locations in Gujarat against *P. xylostella* larvae in their second and third instars. With a high mortality rate of 89.97 and 86.96, AAUBC *Bb*-5a and AAUBC *Ma*-26 were the two promising isolates chosen for second-instar larvae, showing a high mortality rate. This was corroborated by data on third-instar larvae, with 86.95 and 79.95 percent mortality for AAUBC *Bb*-5a and AAUBC *Ma*-26, respectively which was a somewhat reduced mortality rate when paralleled with second-instar larvae. The results of dosage mortality experiments at different concentrations showed that *M. anisopliae* AAUBC *Ma*-26 had a larger LC_{50} value of 2.8×10^6 and 1.2×10^7 in the second and third instars, respectively. In comparison *B. bassiana* isolates, AAUBC *Bb*-5a had lower LC_{50} values of 1.1×10^6 and 2.1×10^6 in their second and third instars, respectively.

Keywords: *B. bassiana, M. anisopliae, Plutella xylostella*, endophytes, bioassay

Introduction
The most widely grown cruciferous crop in the world is cabbage. The cabbage plant, *Brassica oleracea* var. capitata L., belongs to the *Brassica* genus of Brassicaceae family. With 48% of the global production, China is the world's top producer of cabbage. With 10.25 million metric tonnes of output and an average productivity of 23.5 tonnes per hectare over 436 thousand hectares, India stands the second-largest producer of cabbage in the world [1]. However, among several factors, the biotic pressure from diseases and insect-pests restricts its output. Various insect species have been documented to cause harm to cabbage at different phases of growth. Of these, the most dangerous one globally is the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), the most devastating pest of cabbage [2]. This insect has developed resistance to many insecticides and causes 50 to 80% losses in marketable yield. The first instar larvae typically mine the spongy mesophyll tissues after hatching from the eggs [3]. In their second, third, and fourth instars, larvae consume leaves, buds, flowers, siliques, the green outer layer of stems, and developing seeds inside older siliques [4].
Over the years, the use of pesticides has proved ineffective because even 15 to 20 insecticide applications during a crop cycle have not reduced the losses caused by *P. xylostella* [5]. Due to the limited effectiveness of existing management measures, more profitable and environmentally and human health-conscious alternatives must be developed. A variety of serious problems, including resurgence, secondary pest outbreaks, loss of natural enemies, environmental harm, and severe health issues in humans, have been brought on by the ongoing and needless use. Contamination from pesticides must so be controlled. One environmentally sustainable alternative to pesticides for controlling insects is the possible use of entomopathogenic fungus (EPF) as biocontrol agents against insect-pests [6]. However, there are some restrictions on the use of fungi as insecticides. These include the short shelf life of the inoculum, the necessity for a

period during which no fungicide is applied, and the requirement of high relative humidity during application hours. EPF species have been known to artificially inoculate specific crop plants with their tissues to colonize them for pest management purposes [7].

The term "endophyte" refers to fungi or bacteria that occur inside asymptomatic plant tissues. It also refers to all organisms inhabiting plant organs at certain stages of their life cycles, which can colonize internal plant tissues without causing apparent harm to the host [8, 9]. The most extensively studied fungal entomopathogen as an endophyte is *B. bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) are common terrestrial entomopathogens [10]. The fungus, *Metarhizium* is a common soil habitant that has been investigated and employed as an insect pathogen for biological control [11]. For instance, *Metarhizium* spp. has been identified as endophytes from several plants. The results of this study have allowed the most promising isolates from both fungal species to be identified, and these isolates have been chosen for further investigation in the current work. More precisely, the goal of this study was to find the fatal concentration (LC_{50}) for both fungal species as well as to evaluate the effectiveness of *B. bassiana* and *M. anisopliae* against different larval stages of *P. xylostella*.

Materials and Methods

Preliminary screening was carried out at Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand with *M. anisopliae* and *B. bassiana* isolates against *P. xylostella* (2nd and 3rd instar larvae) for identification of promising isolates using laboratory bioassay method.

Mass rearing of *P. xylostella* larvae

To rear the diamondback moth (DBM) larvae, initial culture of *P. xylostella* was collected from farmers' field for mass rearing. During *rabi*, 2023-24 survey was conducted on farmers' fields such as Navli, Mansa, Prantij for larvae of *P. xylostella* infesting cruciferous crops such as cabbage and cauliflower. Mustard seedlings were raised in plastic cups with having height 10.5 cm and a diameter 8.5 cm containing vermiculite to maintain DBM culture. After the emergence of adults, 5-day-old mustard seedlings were provided for oviposition in acrylic cages of 30 × 30 cm and adults were released. The egg hatches in about 3-4 days and the first instar larvae feed on the leaves initially by mining and later on the entire leaves leaving the petiole portion of mustard seedlings which were kept in other rearing cages made of plastic. These petioles along with the larvae were cut with the help of scissors and kept on mesh over fresh mustard seedlings to transfer them. Within 24 hours larvae migrated to the fresh seedlings. Plastic cups with fresh mustard seedlings were replaced at every two-day interval. The second instar larvae of *P. xylostella* were collected from mustard seedlings and used for further study.

Entomopathogenic Fungal Culture

A total of ten fungal isolates, five of each *M. anisopliae* and *B. bassiana* isolated from different soil and insect hosts from various geographical locations of Gujarat maintained at the culture repository of Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand was used for the initial screening of *P. xylostella*. The fungal culture of each isolate was grown on Sabouraud's Dextrose Yeast Extract Broth (SDYB). The fungal conidial suspension was prepared by putting one gram of 15-day-old fungal-grown rice in sterile distilled water containing 0.01% Tween 80 and mixing it thoroughly to release the conidia into the water. The conidial suspension was passed through three layers of muslin cloth to get a hyphal-free suspension.

Laboratory Bioassay Method

Ten-second instar larvae of *P. xylostella* were dipped in 0.5 mL of conidial suspension (1×10^8 conidia/mL) for 10 seconds in each of *M. anisopliae* and *B. bassiana* isolates and transferred to a sterile plastic container. The surface sterilized cabbage leaves were provided as food and larval mortality was recorded at 24 h intervals for 10 days. The percent mortality of the larvae was calculated after removing the control mortality by using Abbott's formula [12]. The experiment was conducted by using a Completely Randomized Design and data were analyzed using suitable statistical methods. The two promising isolates were utilized for further dosage mortality experiments (LC_{50}) and five conidial concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} conidia/mL), and bioassays were conducted. Probit analysis was used to determine the dosage and duration of mortality required to kill 50% of the larval population (LC_{50}). Statistical analysis was carried out using SPSS software, Version 21.

Results and Discussion

The isolate *B. bassiana* AAUBC *Bb*-5a was the most promising out of ten examined isolates against second-instar larvae of *P. xylostella* (Table 1), with 89.97 percent larval mortality. Nonetheless, it was determined to be on par with AAUBC *Ma*-26 with 86.96 percent larval mortality. With a mortality rate of 76.78 percent, isolate AAUBC-*Ma* 15 came in second place. This larval mortality rate was comparable to that of isolates AAUBC-*Bb* 53, AAUBC-*Ma* 7, AAUBC-*Bb* 2, and AAUBC-*Ma* 21 (73.44, 69.97, 66.71, and 66.71 percent, respectively). On the other hand, the isolate AAUBC-*Ma* 22 recorded the lowest larval mortality rate of *P. xylostella* (60.11%).

This was also supported by the data, which showed that the mortality rates for third-instar larvae were in accordance with the second instar, with the highest mortality rate for AAUBC *Bb*-5a at 86.95 percent and AAUBC Ma-26 at 79.95 percent which were statistically equivalent to each other. However, the isolate AAUBC *Ma*-15 stood next with 69.97 percent mortality. While the remaining isolates registered larval mortality between 63.36 to 49.97 percent for third-instar larvae of *P. xylostella.*

The findings of this study revealed that *B. bassiana* induced higher larval mortality (percent) which ranged from 63.33 to 89.97 percent on DBM larvae in comparison to the isolates of *M. anisopliae* where the mortality (percent) varied between 60.11 to 86.96 percent at a concentration of 10^8 conidia/mL. The results were also similar for 3rd instar larvae in which higher mortality (percent) was induced by the *B. bassiana* isolates in comparison with *M. anisopliae.* The cabbage-feeding bioassay revealed that *B. bassiana* isolates killed over 80% of *P. xylostella* [13]. The treatments using isolates of *B. bassiana* when given as foliar spray and by leaf dip method, it was more pathogenic to *P. xylostella* (77%) larvae than *M. anisopliae* (70%) larvae [14].

Isolates of *B. bassiana* and *M. anisopliae* were tested and percent mortality of second instar larvae of *P. xylostella* was found to be 87.3 percent for *M. anisopliae* and 82.7 percent for *B. bassiana* [15]. The percentage mortality of *P. xylostella* larvae varied across the isolates of *M. anisopliae* and *B. bassiana* that were investigated. The mortality (%) of best isolates of *M. anisopliae* have shown similar results with 88.85 and 81.44 percent. In the case of *B. bassiana* the best isolates had a mortality rate of 77.36 and 51.14 percent which were deviating [16]. These deviations or differences in their virulence against *P. xylostella* observed could be attributed to the fact that these isolates came from various soil sources and pathogenic insect

hosts from various agroclimatic areas and also the virulence of insect-pests can vary depending on factors such as genetic diversity, the fungus's source, and geographic location based on these factors there will be different larval mortality.

Table 1: Efficacy of *M. anisopliae* and *B. bassiana* isolates against diamondback moth, *P. xylostella* under laboratory bioassay

Sr. No.	AAU Isolates	Larval Mortality (%)	
		Second instar	Third instar
1	AAUBC-*Ma* 7	56.77 ^{bcd} (69.97)	48.82 ^{de} (56.65)
2	AAUBC-*Ma* 15	61.19 ^{b} (76.78)	56.77 ^{bc} (69.97)
3	AAUBC-*Ma* 21	54.76 ^{bcd} (66.71)	46.90 ^{de} (53.31)
4	AAUBC-*Ma* 22	50.83 ^{d} (60.11)	44.98 ^{e} (49.97)
5	AAUBC-*Ma* 26	68.83 ^{a} (86.96)	63.40 ^{ab} (79.95)
6	AAUBC-*Bb* 2	54.76 ^{bcd} (66.71)	52.75 ^{cd} (63.36)
7	AAUBC-*Bb* 5a	71.54 ^{a} (89.97)	68.83 ^{a} (86.95)
8	AAUBC-*Bb* 21	52.75 ^{cd} (63.36)	50.83 ^{cde} (60.11)
9	AAUBC-*Bb* 53	58.98 ^{bc} (73.44)	50.75 ^{cde} (59.97)
10	AAUBC-*Bb* 54	52.75 ^{cd} (63.36)	48.82 ^{de} (56.65)
	S.Em.±	2.12	2.12
	C.D. at 5%	6.25	6.27
	C.V. (%)	6.29	6.90

Note:
1. Figures outside the parentheses are arcsine transformed values, those inside are retransformed values
2. Treatment mean(s) with letter(s) in common are not significant by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance

Dose Mortality

The LC_{50} values for second and third larval instars of *P. xylostella*, calculated at different concentrations of the promising endophytic isolates *i.e.*, *M. anisopliae* AAUBC *Ma*-26, and *B. bassiana* AAUBC *Bb*- 5a (1×10^4 to 1×10^{10} conidia/mL), are illustrated in Table 2.

Larval mortality was revealed to be higher in early instars of the experiment and to be lower in late instar larvae when exposed to different concentrations. Out of two promising isolates evaluated for dosage mortality, *B. bassiana* AAUBC *Bb*- 5a isolate had the highest mortality against *P. xylostella* for the second and third larval instars, the estimated LC_{50} values were 1.1 $\times 10^6$ and 2.1 $\times 10^6$ conidia/mL, respectively. This was followed by *M. anisopliae* AAUBC *Ma*-26 with an LC_{50} of 2.8 $\times 10^6$ and 1.2 x 10^7 conidia/mL for the second and third larval instars, respectively. When exposed to different concentrations, the maximum mortality rate (100%) was noted for *P. xylostella* larvae in their second instar, while the mortality rate (96.67%) was noted for those in their third instar.

The LC_{50} values for *Bb-Taif1* and *Bb-Taif2* were 6.0×10^4 and 3.2×10^5 spores/mL, respectively. The results demonstrated that the *Bb*-Taif1 isolate had greater virulence and effectiveness than the *Bb-Taif2* isolate [17]. The isolates of *M. anisopliae* and *B. bassiana* had LC_{50} of 1.2 and 8.6 $\times 10^6$ conidia/mL when tested against *P. xylostella* larvae [18]. The results of the study conducted by [16]. Bathina (2023) revealed that 50% of mortality was caused by NBAIR *Ma*-35 isolate of *M. anisopliae* at a dose of 10^4 conidia/mL [16].

Table 2: Dose mortality (LC_{50}) of *M. anisopliae* AAUBC-*Ma* 26 and *B. bassiana* AAUBC-*Bb* 5a on diamondback moth, *P. xylostella*

Isolates	Instar	LC_{50} (conidia /ml)	Fiducial limit 95%	
			Lower	Upper
M. anisopliae AAUBC-*Ma* 26	Second	2.8×10^6	1.6×10^5	2.3×10^7
	Third	1.2×10^7	1.8×10^6	8.4×10^7
B. bassiana AAUBC-*Bb* 5a	Second	1.1×10^6	1.2×10^5	1.4×10^7
	Third	2.1×10^6	1.7×10^5	1.5×10^7

Conclusion

The preliminary screening utilizing isolates of *M. anisopliae* and *B. bassiana* against *P. xylostella* (2nd and 3rd instar larvae) discovered that AAUBC *Bb*-5a was found to be the most effective against *P. xylostella* and this was followed by *M. anisopliae* AAUBC Ma-26 among all the evaluated isolates. The isolate AAUBC *Bb*-5a showed the highest efficacy against *P. xylostella* second instar larvae with a significantly higher mortality rate of 89.97 and 86.95 percent for second and third instar larvae, respectively and AAUBC *Ma*-26 showed the most promising result out of the five isolates studied in the case of *M. anisopliae* for second instar larvae, with substantial mortality of 86.96 percent. This was further confirmed by the studies using third-instar larvae for AAUBC *Ma*-26, which had the greatest mortality rate of 79.95 percent. The LC_{50} values for the two promising isolates *M. anisopliae* AAUBC *Ma*-26 and *B. bassiana* AAUBC *Bb*-5a isolate, were found to be 2.8 $\times 10^6$ and 1.1 $\times 10^6$ conidia/mL respectively for the second instar larvae of *P. xylostella*. In the case of bioassays conducted for third instar larvae the LC_{50} values were found as 1.2 x 10^7 conidia/mL for *M. anisopliae* AAUBC *Ma*-26 and 2.1 x 10^6 conidia/mL for *B. bassiana* AAUBC *Bb*-5a. Overall, the results revealed that *B. bassiana* AAUBC *Bb*-5a was potentially more toxic and caused higher mortality in *P. xylostella* larvae in comparison to *M. anisopliae* AAUBC *Ma*-26.

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