Original Research Article

Virulence of Native Isolates of *Metarhizium anisopliae*(Metschnikoff) Against *Helicoverpaarmigera* (Lepidoptera: Noctuidae) in Western Uttar Pradesh, India

Abstract

The chickpea pod borer *Helicoverpaarmigera* (Hubner) (Lepidoptera: Noctuidae) is a destructive pest of chickpeas that is difficult to control using conventional methods. We isolated and evaluated the virulence of five isolates of *Metarhizium anisopliae* against larvae of *H. armigera*. All isolates of *M. anisopliae*, SVPUAT 1, SVPUAT 2, SVPUAT 3, SVPUAT 4, and SVPUAT 5, were most effective against the second instar *H. armigera* at 2×10^8 conidia/ml. Among all isolates, SVPUAT 1Accession no. ON183248 had the highest virulence 100 % mortality, whereas LT_{50} and LT_{90} were 3.16 and 5.16 days.

Keywords: isolate, *H. armigera*, *Metarhizium ansopliae* and Mortality.

Introduction

In recent years, sustainable management of agricultural pests has become an imperative focus of research and development globally (*Crews et al.,* 2018). *Helicoverpaarmigera*, commonly known as the cotton bollworm, is one of the most destructive and economically significant insect pests, causing extensive damage to various crops, particularly cotton, in various parts of the world (*Riaz et al.,* 2021). Western Uttar Pradesh, India, with its fertile lands and diverse agricultural practices, has been facing persistent challenges posed by this voracious pest.

In the pursuit of ecologically friendly and cost-effective solutions to manage pest infestations, biopesticides have emerged as promising alternatives to synthetic chemical insecticides. Among these, entomopathogenic fungi have gained attention for their potential to control various insect pests without causing harm to non-target organisms or the environment. *Metarhizium anisopliae* (Metschnikoff), a well-studied entomopathogenic fungus, has a remarkable ability to infect and kill a wide range of insect hosts, including *H. armigera* larvae (Shrma*et al.,* 2023; Shanker *et al.,* 2023).

While the virulence of *Metarhizium anisopliae* has been extensively studied against various insect pests across different geographic regions, the efficacy of native isolates against *H. armigera* in the specific agroclimatic conditions of Western Uttar Pradesh remains relatively unexplored. Factors such as temperature, humidity, and the prevailing agricultural practices in

this region can influence the performance of entomopathogenic fungi in controlling pest populations. Therefore, a comprehensive assessment of the virulence of native isolates of *Metarhizium anisopliae* against *H. armigera* in this region is essential to determine their potential as viable biocontrol options.

This study aims to fill this critical knowledge gap by investigating the virulence of native isolates of *Metarhizium ansopliae* against *H. armigera* in western Uttar Pradesh, India. By evaluating factors such as fungal pathogenicity, environmental conditions, and agronomic practices, this study intends to provide valuable insights into the feasibility of using native *Metarhizium* isolates as a biocontrol strategy to mitigate the impact of *H. armigera* infestations. The findings of this research could offer significant contributions to the field of integrated pest management, fostering sustainable agricultural practices, and reducing the reliance on synthetic pesticides.

Materials and Methods

Collection of soil samples

Several soil samples (Table 1) were collected in late winter 2021 from various fields in Western Uttar Pradesh, India, by gathering topsoil down to 40 cm depth with a metal shovel. Samples from each site were packed in sterile plastic bags, transported to the laboratory, and stored at 4–8 °C until use.

Isolation, purification, and identification of the isolates

The insect bait technique recommended by Zimmermann (1986) was adopted to screen and isolate the native species of EPF using larvae of the wax moth, *Galleria mellonella*. Larvae were treated with warm water to avoid extensive webbing in the soil (Meyling and Eilenberg 2006). Soil samples were moistened and stored in Petri dishes. Twenty medium-sized larvae were used for each soil sample. Samples were incubated at 25±2°C in the dark and inverted every day. Soil samples were examined after 5 days; departed bait larvae were collected and surface sterilized with 1% Na-hypochlorite to prevent external saprophytic fungi from growing on the dead cadaver. Dead larvae were placed in Petri dishes lined with a single layer of wet foil paper until signs of green muscardine were observed. Fungal spores were grown on

Sabourauddextrose yeast agar (SDAY) medium. The Petri dishes (5 cm×1 cm) were incubated at 28 °C for 3–7 days. For extra refinement, single-spore cultures were plated from multispore cultures. Fungal strains showing good growth and spore production traits were selected, purified, and identifed according to microscopic observations following the taxonomic keys, using a color atlas of pathogenic fungi for the Metarhizium genus (Frey *et al.*, 1979; Webster and Weber 2007).

***Helicoverpaarmigera* (Hub.) rearing**

The *H. armigera* larvae were brought to the lab from tomato, chickpea, and pigeon pea crops that are shown in the experimental field of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. According to the procedure provided by Wakil *et al. (2011), the rats were* raised on an artificial diet containing chickpea four, red kidney beans, canned tomato paste, yeast, agar, and vitamin mixture mixed in distilled water. The rearing condition was maintained at a temperature of 27±2 °C, 75±5% RH.

Selection of the most virulent isolate

Selection of the most virulent strain among five locally isolated *M. anisopliae* and isolates was performed against *H. armigera* by the dipping assay method (Khaskheli, *et al.*, 2019). Conidia of different isolates were produced in Petri dishes (9 cm) containing Sabouraud Dextrose Agar (SDA) and incubated at 25±1^{0}C. After ten days, conidia were harvested using peanut oil and a spatula and transferred to conical flasks (250 ml) containing 100 ml sterilized distilled water with 0.02% speeder sticker (tween, 80). The conidial concentrations in the suspensions were quantified directly under an optical microscope using a hemocytometer. The suspensions were then standardized until a direct concentration of 2×10^8 spores/ml was obtained. The 2rd instars larvae of *H. armigera* were dipped in prepared suspension and placed in Petri dishes with fresh food. The control was treated with distilled water. Each treatment was replicated three times along with the control. Percent mortality was calculated according to Abbott formula (Abbott, 1925). The experiment was carried out under laboratory condition at 26^{0}C±2 and 60-70 % RH.

Results:

Isolation of the native isolates of the entomopathogenic fungi *M. anisopliae* was performed. Among the 207 soil samples examined from four districts, viz. Meerut, Muzaffarnagar, Saharanpur, and Shamali in the western plain zone of Uttar Pradesh, only five samples were positive for *M. anisopliae* from the districts Meerut, Muzaffarnagar, Saharanpur, and Shamali, as presented in Table 1. The radial growth of *M. ansopliae* was high when it was grown on Sabouraud Dextrose Agar (SDA). The initial colonies on SDA have a white mycelial margin with clumps of conidiophores that become colored with the development of spores, varying from olivaceous buff to yellow/green to olivaceous/dark herbage green. Phialides was 6.3 – 13.5 × 1.8 - 3.6 µm. Conidia usually 5-8 µm long by 1.5–3.5 unwire var. *anisopliae*.

Five isolated local strains of *M. anisopliae* were examined to test their pathogenicity against second instar larvae of *H. armigera* (Table 2). Pathogenicity of local fungus of *M. ansopliae* isolate SVPUAT 1 Accession no. ON183248 was highest (100 per cent) after seven days of treatment, followed by isolates SVPUAT 5 (71.11 per cent), SVPUAT 4 (57.77 per cent) and SVPUAT 2 (44.44 per cent). Minimum mortality per the cent recorded in isolate SVPUAT 3 (31.11 per the cent). The lowest LT_{50} and LT_{90} values were also recorded in isolate SVPUAT 1 at 3.16 and 5.16 days, respectively.

Discussion

Many species of EPF are used to regulate insect pests in glasshouse and field crops, orchards, ornamentalplants,stored products, and forest areas. These biological control agents are also used to reduce pests and vector insects of veterinary and medical importance (*Lacey et al.*, 2001).*Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) Sorokin are the most common EPF found and grow naturally in soils throughout the world and act as a parasite on various insect species (Kilingen I, 2006 and Kilic *et al.*, 2019).

This study revealed thatthe results from the screening of five native EPF isolates showed that M. anisopliae was virulent to the second larval instar of *H. armigera*. Most isolates caused >40% larval mortality, demonstrating that these isolates are capable of causing mortality against *H. armigera* larvae. Among the tested isolates, SVPUAT 1 was more virulent than the other isolates under laboratory conditions. In agreement with this studybyFite *et al.* (2019).

Recently, Kalvnadi*et al.* (2018) and Jarrahi and Safavi (2016) reported that *M. anisopliae* is virulent, causing high larval mortality and adverse effects on the biological parameters of *H. armigera* The *M. ansopliae* fungal isolate SVPUAT 1 had the highest virulence against the second larval instar of *H. armigera* because it had lower LT_{50} and LT_{90}. These results were comparable with those of Fite *et al*., 2019 and Nguyen *et al.* (2007), who reported the lowest LT_{50} values of 6.20 days and 3 days at 1×10^9 and 1×10^7 spores/ml respectively.

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Table 1: Details of soil samples collected for isolation of *M. ansopliae* in western Uttar Pradesh

Locations	Geographical location (the lat. N, long. E)	No. of soil samples collected	Standing crops	Soil type	Positive/ Negative sample
Meerut					
SVP Orchard	The n. Lat. 28^0 57´ E. Long.77^0 40´	15	Mango, Guava, Pomegranate, and Litchi	Sandy	-
Daurala		15	Mango	Loamy	-
Mavana		10	Mango, Guava	Sandy Loam	-
Lawar		09	Mango, Citrus	Sandy Loam	-
Mauhmadpur		12	Mango	Loamy	+
Muzaffarnagar					
Jansath	The n. Lat. 29^0 97´ E. Long. 77^0 55´	09	Cucurbitaceous Crops	Loamy	+
Khatauli		14	Mango, Papaya	Loamy	-
Shadpur		10	Mango	Sandy Loam	-
Khanpur		16	Mango, Papaya, and Litchi	Sandy Loam	+
Jawan		05	Mango, Guava	Sandy Loam	-
Saharnpur	The n. Lat. 29^0 45´				

Umarikala	E. Long. 77^0 55´	12	Mango	Sandy Loam	-
Badgaov		14	Mango, Popular	Sandy	+
Bidvi		10	Mango, Citrus	Loamy	-
Jhabhirun		13	Mango, Popular	Loamy	-
Shamali	N. Lat. 29^0 45´ E. Long. 77^0 32´				
Jasala		10	Guava, Mango	Sandy Loam	-
Gageru		20	Mango, Chilly	Loamy	+
Kandhala		13	Mango, Papaya, Popular	Loamy	-
Total		**207**			

Negative symbol (-) = *M. ansopliae* absent

Positive symbol (+) = *M. ansopliae* present

Table 2: Mortality of *Helicoverpaarmigera* after exposure to indigenous *Metarhizium ansopliae* isolates

Isolates	% mortality (Mean ± SE)	LT_{50} (95 % CI)	Slope (±SE)	LT_{90} (95 % CI)	Slope (±SE)
SVPUAT 1	100.0 ± 0.00a	3.16	5.97 ± 1.07	5.16	5.97 ± 1.11
SVPUAT 2	44.44 ± 3.84e	6.00	2.70 ± 1.20	17.83	2.70 ± 1.60
SVPUAT 3	31.11 ± 3.85d	9.50	2.09 ± 1.48	39.16	2.09 ± 2.49
SVPUAT 4	57.77 ± 7.69c	4.66	3.05 ± 1.13	12.25	3.05 ± 1.38
SVPUAT 5	71.11 ± 3.85b	4.16	3.78 ± 1.10	9.15	3.78 ± 1.24

Mortality of *H. armigera* at 6 days post-treatment. Each point is the mean of three replicates. Value in the same column followed by different superscripts is highly significantly different ($p< 0.05$) according to Tukey's HSD test. The LT_{50} and LT_{90} (in days) with 95 % confidence intervals (CI) and the slope are also indicated.