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Virulence of Native Isolates of *Metarhizium anisopliae* (Metschnikoff) against *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Western Uttar Pradesh, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Chickpea pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a destructive pest of chickpeas that has proven difficult to control using conventional methods. We isolate and evaluate the virulence of five isolates of *M. anisopliae* against larvae of *H. armigera*. All isolates of

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M. anisopliae, SVPUAT 1, SVPUAT 2, SVPUAT 3, SVPUAT 4 and SVPUAT 5 were most effective against second instar, *H. armigera* at 2 ×10^8 conidia/ml. Among the all isolates, SVPUAT 1 Accession no. ON183248 had the highest virulence 100 percent mortality; whereas, LT_{50} and LT_{90} were 3.16 and 5.16 days.

Keywords: Isolate; H. armigera; M. anisopliae; mortality.

1. INTRODUCTION

In recent years, the sustainable management of agricultural pests has become an imperative focus of research and development globally [1]. The *Helicoverpa armigera*, commonly known as the cotton bollworm, stands as one of the most destructive and economically significant insect pests, causing extensive damage to a wide range of crops, particularly cotton, in various parts of the world [2]. Western Uttar Pradesh, India, with its fertile lands and diverse agricultural practices, has been facing the persistent challenge posed by this voracious pest.

In the pursuit of ecologically friendly and cost-effective solutions to manage pest infestations, biopesticides have emerged as a promising alternative to synthetic chemical insecticides. Among these, entomopathogenic fungi have gained attention for their potential to control a variety of insect pests without causing harm to non-target organisms or the environment. *M. anisopliae* (Metschnikoff), a well-studied entomopathogenic fungus, has exhibited a remarkable ability to infect and kill a wide range of insect hosts, including *H. armigera* larvae [3,4].

While the virulence of *M. 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 *M. anisopliae* against *H. armigera* in this region is essential to determine their potential as a viable biocontrol option.

This research aims to fill this critical knowledge gap by investigating the virulence of native isolates of *M. anisopliae* 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 utilizing native *M. anisopliae* 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.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

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

2.2 Isolation, Purification and Identification of Isolates

Insect bait technique recommended by [5] was adopted to screen and isolate the native species of EPF, using larvae of the wax moth, *Galleria mellonella*. Larvae were treated by warm water to avoid extensive webbing in the soil [6]. Soil samples were moistened and kept 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 dish lined with a single layer of wet flter paper until signs of green muscardine were perceived. The fungal spore was grown on Sabouraud dextrose 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 out from multispore cultures. Fungal strain showing good growth and spore production traits was selected, purified and

identified according to microscopic observations following the taxonomic keys, using color atlas of pathogenic fungi for Metarhizium genus [7,8].

2.3 *Helicoverpa armigera* (Hub.) Rearing

The *H. armigera* larvae were brought to the lab from the tomato, chickpea and pigeon pea crop that is showed 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.,* [9], it was raised on an artificial diet containing chickpea four, red kidney beans, canned tomato paste, yeast, agar, vitamin mixture mixed in distilled water. The rearing condition maintained at a temperature of 27±2 °C, 75±5% RH.

2.4 Selection of Most Virulent Isolate

Selection of the most virulent strain between five locally isolated *M. anisopliae* and isolates were performed against *H. armigera* by dipping assay method [10]. 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 spatula and transferred to conical flasks (250 ml) containing 100 ml sterilized distilled water with 0.02% the speeder sticker (tween, 80). The conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemocytometer. Then the suspensions were standardized until the direct concentration of 2 × 10^8 spores/ml was obtained. The 2rd instars larvae of *H. armigera* 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 control. Per cent mortality was calculated according Abbott formula [11]. The experiment was carried out under laboratory condition at 26^{0}C±2 and 60-70 % RH.

3. RESULTS

Isolation of the native isolates of entomopathogenic fungi *M. anisopliae* was carried out. Among the 207 soil samples examined from four districts *viz*. Meerut, Muzaffarnagar, Saharanpur and shamali in western plain zone of Uttar Pradesh, revealed only five samples were positive with *M. anisopliae* from districts Meerut, Muzaffarnagar, Saharanpur and shamali which is presented in Table 1. The radial growth of *M. anisopliae* was high when it grew on Sabouraud Dextrose Agar (SDA). The initial colonies on SDA have a white mycelial margin with clumps of conidiophores which become coloured 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*.

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

4. DISCUSSION

Many species of EPF (Entomopathogenic Fungi) are used to regulate insect pests in field crops, orchards and forest area. These biological control agents are also practiced for reduction of pest and vector insect of veterinary and medical importance [12]. *Beauveria bassiana* (Bals.) and *M. anisopliae* (Metsch.) Sorokin are the most common EPF found and grow naturally in soils throughout the world and act as a parasite on various insects species [13 and 14].

The present study revealed that, results from the screening of five native EPFs isolates shown M. anisopliae was virulent to the second larval instar of *H. armigera*. Most isolates caused >40% larval mortality, which demonstrated 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 study, Fite *et al.* [15].

Recently, Kalvnadi *et al.* [16] and Jarrahi and Safavi [17] reported that *M. anisopliae* is virulent, causing high larval mortality and having adverse-effects on the biological parameters of *H. armigera*, respectively. The *M. anisopliae* fungal isolate SVPUAT 1had the highest virulence against the second larval instar of *H. armigera* because it had a lower LT_{50} and LT_{90}. These results were comparable with those of Fite *et al*., [15] and Nguyen *et al.* [18], who reported the lowest LT_{50} value 6.20 days and 3 days at 1 × 10^9 and 1 × 10^7 spores/ml respectively.

Table 1. Details of the soil samples collected for isolation of *M. anisopliae* in Western Uttar Pradesh

Locations	Geographical location (lat. N, long. E)	No. of soil samples collected	Standing crops	Soil type	Positive/Negative sample
Meerut	N. Lat. 28^0 57´				
SVP Orchard	E. Long.77^0 40´	15	Mango, Guava, Pomegranate, Litchi	Sandy	-
Daurala		15	Mango	Loamy	-
Mavana		10	Mango, Guava	Sandy Loam	-
Lawar		09	Mango, Citrus	Sandy Loam	-
Mauhmadpur		12	Mango	Loamy	+
Muzaffarnagar	N. Lat. 29^0 97´				
Jansath	E. Long. 77^0 55´	09	Cucurbitaceous Crops	Loamy	+
Khatauli		14	Mango, Papaya	Loamy	-
Shadpur		10	Mango	Sandy Loam	-
Khanpur		16	Mango, Papaya, Litchi	Sandy Loam	+
Jawan		05	Mango, Guava	Sandy Loam	-
Saharnpur	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´				
Jasala	E. Long. 77^0 32´	10	Guava, Mango	Sandy Loam	-
Gageru		20	Mango, Chilly	Loamy	+
Kandhala		13	Mango, Papaya, Popular	Loamy	-
Total		**207**			

Negative symbol (-) = M. anisopliae absent; Positive symbol (+) = M. anisopliae present

Table 2. Mortality of *Helicoverpa armigera* after exposure to indigenous *M. anisopliae*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

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

5. CONCLUSION

Numerous side effects of chemical pesticides such as development of resistance and adverse impact on the environment has encouraged several scientists to explore another control methods on imperative agricultural pests. In consequence, the development of biopesticides that are effective, recyclable and no detrimental side effect on the environment, turn out to be priority of these studies. Based on our studies, all the isolates of *M. anisopliae* were infectious to *H. armigera* larvae under laboratory condition. Furthermore, detail studies will be directed to test the control efficiency of these fungal isolates in lab and field condition against different instar of this insect.

CONSENT

In written informed consent was obtained from all individual participants included in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Crews TE, Carton W, Olsson L. Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. Global Sustainability. 2018;1:e11.
2. Riaz S, Johnson JB, Ahmad M, Fitt GP, Naiker M. A review on biological interactions and management of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Journal of Applied Entomology. 2021;145(6):467-498.
3. Sharma A, Sharma S, Yadav PK. Entomopathogenic fungi and their relevance in sustainable agriculture: A review. Cogent Food & Agriculture. 2023;9(1):2180857.
4. Shanker R, Prajapati MR, Singh RP, Singh R, Singh J, Kumar P. Isolation, molecular characterization of indigenous *M. anisopliae* (Metchnikoff) isolate, using ITS-5.8 s rDNA region, and its efficacy against the *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control. 2023;33(1):23.
5. Zimmermann G. The 'Galleria bait method' for detection of entomopathogenic fungi in soil. J Appl Entomol. 1986;102(2):213–215.
6. Meyling NV, Eilenberg J. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric Ecosyst Environ. 2006;113(1–4):336–341.
7. Frey D, Oldfeld RJ, Bridger RC. A colour atlas of pathogenic fungi. Wolfe Medical Publications Ltd Wolfe House 3–5 Conway Street, London W1P 6HE; 1979.
8. Webster J, Weber R. Introduction to fungi. Cambridge University Press White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In: Innis MA, Gelfand; 2007.
9. Wakil W, Ghazanfar MU, Sahi ST, Kwon YJ, Qayyum MA. Effect of modified meridic diet on the development and growth of tomato fruit worm *Helicoverpa armigera* (Lepidoptera: Noctuidae). Entomol Res. 2011;41(3):88–94.
10. Khaskheli XC, Gong G, Poussio GB, Otho SA. The use of promising entomopathogenic fungi for eco-friendly management of *Helicoverpa armigera* Hubner in chickpea. Int. J. Environ. Agric. Biotechnol. 2019;4:704-712.
11. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of economic Entomology. 1925;18(2): 265-267.
12. Laracy LA, Frutos R, Kaya KH, Vails P. Insect pathogens as biological control agents: Do they have a future. Biol Control. 2001;21(3):230-248.
13. Klingen I, Haukeland S. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. In Eilenberg J and Hokkanen HMT (Eds): An ecological and societal approach to biological control. Series: progress in Biological Control. 2006;2:145-211.
14. Kilic E, Yazici A, Ortucu S. Isolation, molecular characterization and

pathogenicity of *M. anisopliae* (Metsch.) sorokin (Hypocreales: Clavicipitaceae) from soil in Erzincan province, Turkey. Applied Ecology and Environmental Research. 2019;17(3):5401-5408.

15. Fite T, Tefera T, Negeri M, Damte T, Sori W. Evaluation of Beauveria bassiana, Metarhizium anisopliae, and Bacillus thuringiensis for the management of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions. Biocontrol Science and Technology. 2019;30(3):278-295.
16. Kalvnadi E, Mirmoayedi A, Alizadeh M, Pourian H. Sub-lethal concentrations of the entomopathogenic fungus, Beauveria bassiana increase fitness costs of *Helicoverpa armigera* (Lepidoptera: Noctuidae) offspring. Journal of Invertebrate Pathology. 2018;158:32–42.
17. Jarrahi A, Safavi SA. Fitness costs to *Helicoverpa armigera* after exposure to sub-lethal concentrations of *M. anisopliaesensu* lato: Study on F1 generation. Journal of Invertebrate Pathology. 2016;138:50–56.
18. Nguyen TH, Borgemeister C, Poehling MH, Zimmermann G. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) Larvae and Pupae. Biocontrol Science and Technology. 2007;8 (17):1360-0478.

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