Efficacy of *Metarhizium anisopliae* (Metschnikoff) Sorokin SBI-Ma-SF 5 strain against tomato fruit borer *Helicoverpa armigera* Hubner (Lepidopetra: Noctuidae)

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ABSTRACT

The polyphagous tomato fruit borer *Helicoverpa armigera* (Hubner) causes yield loss of 40 – 60 per cent under favourable conditions in Tomato. The farmers rely upon chemical insecticides for its management and its injudicious use leads to unwarranted problems. The use of bio pesticides as a component of integrated pest management is one of the important factor to overcome the pesticides related issue. Among the bio-pesticides entomopathogenic fungi proved their ability against many Lepidopterans. The pathogenicity of *Metarhizium anisopliae* (Metschnikoff) Sorokin SBI SF Ma 5 strain was studied against tomato fruit borer *H. armigera*. The median concentration (LC_{50}) of *M. anisopliae* SBI Ma SF 1 strain was 3.1 x 10^8 conidia/ml with fiducial limits 2.2 x 10^7 to 4.2 x 10^9 conidia/ml. The median lethal time (LT_{50}) value was to be 6.53 days. The SBI SF Ma 5 strain caused 88.83 per cent mortality in second instar *H. armigera* at 1 x 10^9 conidia/ml concentration. The decrease in conidial concentration reduced the efficacy of *M. anisopliae* strain. This strain can be used in the *H. armigera* management after field evaluation.

Key words: *H. armigera, M. anisopliae, pathogenicity, insecticides*

1. INTRODUCTION

Tomato fruit borer, *Helicoverpa armigera* (Hubner) is a destructive and polyphagous pest having the potential to cause damage to 60 species of plants belonging to 67 host families (Krishnamoorthy and Mani 1996; Pogue 2004). It causes damage to economic crops *viz*., tomato, cotton, maize, pulses, flowers, ornamentals etc at both vegetative and reproductive stage. The worldwide annual crop loss due to *H. armigera* damage is estimated to be approximately 5 billion US dollars (Sharma 2001). The farmers mostly rely to chemical management due to its rapid damage potential and polyphagous nature. However, the complete reliance on chemical management apart from increasing plant protection cost leads to unwarranted effects *viz*., environmental pollution, effect on non- targets, resistance development and ecological imbalance (Macharia, 2015). Moreover, the injudicious use of insecticides particularly in vegetable ecosystem for better profits helps the target insect to develop resistance apart from leaving considerable residues in the produce. *H. armigera* developed resistance to commonly used conventional insecticides (Qayyum *et al.* 2015).

Therefore, apart from managing the pest effectively, the unwarranted impacts on environment and non targets also have to be reduced. The inclusion of biopesticides in the integrated pest management (IPM) is one of the important strategies to reduce the selection pressure in target insects (Ai *et al*., 2018). The entomopathogenic bacteria has proved its ability against lepidopterans as a alternative to chemical insecticides (Lacey *et al.* 2015). *Beauveria bassiana* (Bats). Vuill., *Metarhizium anisopliae* Metch. (Sorokin), *Isaria fumosorosea* (Wize) and *Lecanicillium leccanii* (Zimm.) are the important entomopathogenic fungus employed against most of the insect pests (Kulkarni *et al.* 2008; Yasin *et al.* 2019). Though Entomopathogenic fungai are promising pest management options and various factors influences their efficacy against target insects. The use of indigenous native isolates against target insects has edge over commonly available isolates and the same has been proven against various agricultural insect pests (Hanen *et al.,* 2016). Therefore, the present study was conducted to study the pathogenicity of native *Metarhizium anisopliae* strain SBI SF Ma 5 against *Helicoverpa armigera* under laboratory conditions.

2. MATERIAL AND METHODS

2.1. Fungal culture

The fungal strain SBI SF Ma 5 for this study was obtained from the Sugarcane Breeding Institute, Coimbatore repository. The isolate was inoculated on *H. armigera* larvae for re-initiation. After conidial inoculation the dead larvae were transferred to petri dish with moistened filter paper and incubated at room temperature for fungal growth. After that isolate was grown on potato dextrose agar (PDA) and incubated at room temperature and 60-70 per cent relative humidity for two weeks. The conidia of the *M. anisopliae* strain were harvested and added to 10 ml sterilized water in a test tube. Then the suspension was filtered using muslin cloth and shaken using vortex mixture to homogenous the spore suspension. The spore concentration was determined using Neubauer's haemocytometer Alves and Moraes (1998).

2.2. Maintenance of *Helicoverpa armigera* culture

The *H.* armigera larvae were collected from farmer's fields at Dharmapuri district were used as nucleus culture. The culture was maintained for two generations on artificial diet to get homogenous population. The larvae were reared on a semi-synthetic artificial diet as described by Krishnareddy and Hanur (2015). The early instars (I & II instars) were reared in group and afterwards transferred into individual 30 x 40 x 40 mm plastic containers with perforated lids to ensure optimal ventilation for the larvae. Diet cubes were frequently replaced to provide fresh nourishment. The larvae were reared at room temperature of 28±2°C and 60 – 70 per cent relative humidity. After pupation, the pupa were relocated to the oviposition chambers and a black linen was placed above each chamber to serve as the oviposition substrate for the adults. Adult moths were fed with 1:1 solution of honey and water.

2.3. Preparation of conidial suspension

Completely sporulated cultures of *M. anisopliae* SBI SF Ma-5 isolate (12-day-old) were used to study pathogenicity on *H. armigera* Batta (2003). First, spores were scraped with a sterile scalpel and mixed with 10 ml of sterile distilled water containing 0.001% Tween 80, which acts as a wetting agent and mixed well using vortex mixture. The spore concentration was determined using a Neubauer haemocytometer.

2.4. Pathogenicity testing of SBI SF Ma- 5 isolate of *M. anisopliae*

The second instar *H. armigera* larvae were starved for twelve hours. Tomato leaf discs of 1.5cm diameter was prepared and dipped in the spore suspensions ranging from 1 x 10^9 to 1 x 10^4 conidia/ml, which had been thoroughly mixed with 0.001% Tween 80 using a vortex mixture. After 5 minutes, the leaves were removed and set aside to dry. The treated leaf discs were then placed inside the bioassay trays (8.5 x 12.7 x 2 cm) and one larvae per well was released. For each concentration ten second instar larvae were released and replicated three times. The mortality rate was recorded at 4, 7, and 11 days after treatment. To confirm the larval mortality due to *M. anisopliae* infection the larval cadavers were placed on moistened filter paper in petri dish after surface sterilizing using 70 per cent ethanol.

2.5. Statistical Analysis

The data on percentage mortality from three replications were pooled to get average mortality and corrected using Abbott's formula (Abbott 1925). Analysis of variance was employed to examine the disparities in mortality between the treatment and control groups (ANOVA). Treatment means were compared using Duncan Multiple Range Test (DMRT). The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) along with fiducial limits were calculated using SPSS software version 26.0

3. RESULTS AND DISCUSSION

The response of *H. armigera* on second instar larvae to *M. anisopliae* strain SBI SF Ma 5 was presented in Table 1. The results clearly indicated that the susceptibility of the *H. armigera* to SBI SF Ma-5 *M. anisopliae* strain under laboratory conditions. The *H. armigera* doesn't show much difference in their response to *M. anisopliae* on 4th day, but gradually the difference was observed from 7th day after treatment. The highest concentration 1 x 10^9 conidia/ml recorded 57.17 per cent mortality at 7 DAT and 88.83 per cent mortality at 11 DAT (Table 1).

The difference in mortality between highest and lowest concentration was 46. 66 per cent. The concentrations 1 x 10^5 conidia/ml and 1 x 10^4 conidia/ml were not statistically significant in the present study, whereas the other concentrations (1 x 10^6,1 x 10^7 and 1 x 10^8 conidia/ml) were statistically significant. The mortality response of *H. armigera* to *M. anisopliae* SBI SF Ma-5 strain was given in Table (2). The median concentration (LC_{50}) and median lethal time (LT_{50}) of *M. anisopliae* SBI SF Ma-5 strain ware 3.1 x 10^8 conidia/ml and 6.53 days respectively (Table 2., Fig. 1 & Fig.2).

Table 1. Pathogenicity of *Metarhizium anisopliae* SBI SF Ma-5 strain against *Helicoverpa armigera* during 2021-22

S.NO	Treatment details	Per cent mortality %		
		4 DAT	**7DAT**	**11DAT**
1.	T1 (1 x 10^9 conidia/ml)	23.83 $(29.22)^{a}$	57.17 $(49.12)^{a}$	83.83 $(66.32)^{a}$
2.	T2 (1 x 10^8 conidia/ml)	17.17 $(24.48)^{b}$	43.83 $(41.46)^{b}$	73.83 $(59.24)^{b}$
3.	T3 (1 x 10^7 conidia/ml)	10.50 $(18.91)^{c}$	33.83 $(35.57)^{c}$	60.50 $(51.06)^{c}$
4.	T4 (1 x 10^6 conidia/ml)	7.17 $(15.53)^{d}$	27.17 $(31.41)^{d}$	53.83 $(47.20)^{d}$

5.	T5 (1 x 10^{5} conidia/ml)	10.50 (18.91)^{c}	17.17 (24.48)^{e}	43.83 (41.46)^{e}
6.	T6 (1 x 10^{4} conidia/ml)	7.17 (15.53)^{d}	17.17 (24.48)^{e}	37.17 (37.56)^{f}
7	T7 (control)	0.00 (4.0548)^{e}	0.00 (4.0548)^{f}	0.00 (4.0548)^{g}
	S. Ed	0.2134	0.4328	0.4328
	CD(.05)	0.4578	0.9283	0.9283

*No. of insects per replication: 30
*Values presented are arcsine transformation values
*Values sharing same alphabets in superscript statistically on par DMRT

Table 2. Mortality response of SBI SF Ma-5 isolate of *M. anisopliae*

Regression equation	LC_{50}	LT_{50}	Fiducial limit
y = 0.2362x + 2.9666	3.1 x 10^{8} conidia/ml	--	2.2 x 10^{7} to 4.2 x 10^{9} conidia/ml
y = 3.**79**43x + 2.0018	--	6.53 (Days)	5.19 to 8.21 days

Fig. 1. Dose mortality response **Fig. 2. Time mortality response**

This investigation demonstrates the pathogenicity of *M. anisopliae* SBI SF Ma-5 strain against *H. armigera*. Phukon *et al.* 2014 in their field study recorded 87.01per cent damage reduction in tomato fruits sprayed with *M. anisopliae* strain. Gebremariam *et al.* (2021) screened five *M. anisopliae* isolates against *Galleria mellonella* and recorded 86.67 - 100% mortality under laboratory conditions. Vijayavani *et al.* (2010) studied the efficacy of few *M. anisopliae* strains *viz*., SBT 27 and SBT 29 against *H. armigera* and recorded 98 - 100 percent and 90 - 92 percent mortality after 8 days, respectively. But In the present investigation 11 days after treatment 83.33 percent mortality was recorded and it may require another 3 - 4 days for 100 per cent mortality for SBI SF Ma-5 strain. This may be due to the fact that the SBI SF Ma-5 strain was isolated from *Spodoptera fugiperda*, whereas SBI 27 and SBI 29 strains were isolated from *H. armigera*.

The present results concur with the findings of Fite *et al.* (2020) and Kalvnadi *et al.* (2018) as they revealed that *M. anisopliae* causes larval mortality and adverse impact on the biological parameters of *H. armigera*. The increased conidial concentration increases the mycosis and mortality in wire worm, *Agriotes obscurcus* (L.) (Coleoptera; Elateridae) (Rogge et al. (2017). Alikhani *et al.* (2019) revealed that increased *M. anisopliae* conidial concentration decreased the intrinsic and finite rates of increase in tomato pin worm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Tahir *et al.* (2019) revealed that the pathogenicity of *M. anisopliae* against *H. armigera* was dependent on the spore concentration. In the present investigation decreased spore concentration reduces the efficacy of *M. anisopliae*.

The conidiogenesis is one of the important factors which determine the efficacy of entomopathogenic fungi by Inglis *et al.* (2001) In the present investigation also, the mycosis was more in higher concentration as described by Tahir *et al.* (2019). The effectiveness of entomopathogenic fungi to target insect depends upon the virulence factors assemblage, which is adopted for single or broad host (Valero-Jiménez *et al.* 2016). Boston *et al.* (2020) revealed that the pathogenicity also depends upon the ability to overcome the host defense mechanisms.

Taliyan *et al.* (2020) revealed that first instar *H. armigera* was most susceptible to *M. anisopliae* at a spore concentration of 1.8 x 109 conidia/ml followed by second instar, which recorded 92.19 per cent mortality 12 days after treatment. These findings corroborate with present results. The lower susceptibility of higher instars might be due to melanism in the cuticle. Wilson *et al*., 2001 recorded that melanisation in insect cuticle prevents the penetration by pathogens.

4. CONCLUSION

The present investigation confirms the potential of *M. anisopliae* SBI SF Ma 5 strain against *H. armigera* under laboratory conditions. Though many strains of *M. anisopliae* were available, they are not ideal for different ago-ecological conditions and their continuous application reduces the efficacy against target insect pests. Hence this native strain can be integral component of *H. armigera* management after evaluation at field level.

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DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

COMPETING INTRESTS

Authors declared that no competing interests exists

REFERENCES

1. Abbott, WS. 1925. "A method of computing the effectiveness of an insecticide." *Journal of Economic Entomology* 18 (2):265-267.
1. Ai, X., Wei, Y., Huang, L., Zhao, J., Wang, Y., & Liu, X. 2018. Developmental control ofHelicoverpa armigera by ingestion of bacteria expressing dsRNA targeting an arginine kinase gene. Biocontrol Science and Technology, 28(3), 253–267.
2. Alikhani, M, SA Safavi, and S Iranipour. 2019. "Effect of the entomopathogenic fungus, Metarhizium anisopliae (Metschnikoff) Sorokin, on demographic fitness of the tomato leaf miner, Tuta absoluta (Meyrick)(Lepidoptera: Gelechiidae)." *Egyptian Journal of Biological Pest Control* 29 (1):1-7.
3. Alves, SB, and S Moraes. 1998. "Quantificação de inóculo de patógenos de insetos." *Controle microbiano de insetos*. Spanish
4. Batta, Y. 2003. "Production and testing of novel formulations of the entomopathogenic fungus Metarhizium anisopliae (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes)." *Crop Protection* 22 (2):415-422.
5. Boston, W, D Leemon, and JP Cunningham. 2020. "Virulence screen of *Beauveria bassiana* isolates for *Australian Carpophilus* (Coleoptera: Nitidulidae) beetle biocontrol." *Agronomy* 10 (8):1207.
6. Fite, T, T Tefera, M Negeri, T Damte, and W Sori. 2020. "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* 30 (3):278-295.
7. Gebremariam, A, Y Chekol, and F Assefa. 2021. "Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, *Beauveria bassiana* (Balsam)

Vuillemin, and *Metarhizium anisopliae* (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide." *Heliyon* 7 (5):e07091.

8. Inglis, G, M Goettel, T Butt, H Strasser, J Jackson, and N Magan. 2001. "Use of hyphomycetous fungi for managing insect pests, pp. 23–69." *In TM Butt, C. Jackson, and N. Magan (eds.). Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Oxfordshire, UK*.
9. Kalvnadi, E, A Mirmoayedi, M Alizadeh, and H-R Pourian. 2018. "Sub-lethal concentrations of the entomopathogenic fungus, *Beauveria bassiana* increase fitness costs of *Helicoverpa armigera* (Lepidoptera: Noctuidae) offspring." *Journal of Invertebrate Pathology* 158:32-42.
10. Krishnamoorthy, A, and M Mani. 1996. "Biosuppression of *Helicoverpa armigera* (Hubn.) on tomato using two egg parasitiods, *Trichogramma brasiliensis* (Ashm.) and *T. pretiosum* (Riley)." *Journal of Entomological Research* 20 (1):37-41.
11. Krishnareddy, B, and VS Hanur. 2015. "Enhanced synthetic diet for rearing *Helicoverpa armigera* under laboratory conditions." *Journal of Entomology and Zoology Studies* 3 (1):165-167.
12. Kulkarni, N, S Paunikar, S Hussaini, and K Joshi. 2008. "Entomopathogenic Nematodes in insect pest management of forestry and plantation crops: An appraisal." *Indian Journal of Tropical Biodiversity* 16 (2):155-166.
13. Lacey, L, D Grzywacz, D Shapiro-Ilan, R Frutos, M Brownbridge, and M Goettel. 2015. "Insect pathogens as biological control agents: back to the future." *Journal of Invertebrate Pathology* 132:1-41.
14. Macharia, I. 2015. Pesticides and health in vegetable production in Kenya. BioMed Research International, 2015, 1–10. Article ID 241516.
15. Phukon, M, I Sarma, R Borgohain, B Sarma, and J Goswamp. 2014. "Efficacy of *Metarhizium anisopliae*, *Beauveria bassiana*." *Asian Journal of Bio Science* 9 (2).
16. Pogue, MG. 2004. "A new synonym of *Helicoverpa zea* (Boddie) and differentiation of adult males of *H. zea* and *H. armigera* (Hübner)(Lepidoptera: Noctuidae: Heliothinae)." *Annals of the Entomological Society of America* 97 (6):1222-1226.
17. Qayyum, MA, W Wakil, MJ Arif, ST Sahi, NA Saeed, and DA Russell. 2015. "Multiple resistances against formulated organophosphates, pyrethroids, and newer-chemistry insecticides in populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan." *Journal of Economic Entomology* 108 (1):286-293.
18. Rogge, SA, J Mayerhofer, J Enkerli, S Bacher, and G Grabenweger. 2017. "Preventive application of an entomopathogenic fungus in cover crops for wireworm control." *BioControl* 62 (5):613-623.
19. Sharma, H. 2001. "Crop protection compendium: *Helicoverpa armigera*." *Electronic compendium for crop protection*.
20. Tahir, M, W Wakil, A Ali, and ST Sahi. 2019. "Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against larvae of the polyphagous pest *Helicoverpa armigera*." *Entomologia Generalis* 38:225-242.
21. Taliyan, A, A Kumar, R Singh, R Rana, and S Rana. 2020. "Pathogenicity of *Metarhizium anisopliae* on *Helicoverpa armigera* larvae instars." *International Journal of Agricultural Research, Innovation and Technology* 5:105-109.
22. Valero-Jiménez, CA, H Wiegers, BJ Zwaan, CJ Koenraadt, and JA van Kan. 2016. "Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*." *Journal of Invertebrate Pathology* 133:41-49.
23. Vijayavani, S, K Reddy, and G Jyothi. 2010. "Identification of virulent isolate of *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) for the management of *Helicoverpa armigera* (Hubner)." *Journal of Biopesticides* 3 (3):556.
24. Yasin, M, W Wakil, MU Ghazanfar, MA Qayyum, M Tahir, and GO Bedford. 2019. "Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium*

anisopliae against red palm weevil, *Rhynchophorus ferrugineus* (Olivier)." *Entomological Research* 49 (1):3-12.