



## Pathogenecity of *Metarhizium anisopliae* on *Helicoverpa armigera* larvae instars

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### ABSTRACT

Studies on pathogenicity of *M. anisopliae* against I<sup>st</sup> - VI<sup>th</sup> instar *H. armigera* larvae revealed that larval mortality. The effect of *Metarhizium anisopliae* spore/ml against *Helicoverpa armigera* larval instars was significantly higher recorded showed that in the results revealed that all the treatments were significantly larval mortality. The results revealed that all the treatments were significantly higher effective in larval mortality as compared to other larval instars. The data recorded on 4, 6, 8 11 and 14 day after pathogenicity of various larval instars in the I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the best I<sup>st</sup> instars larva *H. armigera* per cent mortality by *M. anisopliae* up to (35.63) followed by 73.39 (85.69) (98.37) and (100.0) production was significantly recorded.

### KEYWORDS

Pathogenecity, Mass Multiplication, *Metarhizium anisopliae*, *Helicoverpa armigera*, Different Media

### HOW TO CITE THIS ARTICLE

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The increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also has resulted in adverse effects on the environment and non-target organisms. In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. The first ground-breaking field trials with EPF started with a Russian microbiologist, Elie Metchnikoff in 1888, who later became a Nobel Prize winner and named *Metarhizium anisopliae* more recently named is *M. brunneum* (Vega *et al.*, 2009). Metchnikoff mass produced fungal conidia on sterilized brewer's mash and combine cultures with sand granules for

spreading on field crops. Though results were inconsistent, the work of Metchnikoff ignited curiosity around the world and led to programs in Europe and United States for experimentation with "friendly fungi" against insect pests (Lord, 2005). Studies on EPF was quiet after the World war II when affordable synthetic chemical insecticides became available for insect pests control. Recent developments on EPF show that they can serve as an integral part of integrated pest management strategy. Many insect pathogenic fungi based bio-insecticides have been formulated and commercially manufactured (Hafiza *et al.*, 2014). Entomopathogenic fungi infect insects of almost all orders, most common are Hemiptera, Diptera,

Coleoptera, Lepidoptera, Orthoptera and Hymenoptera (Ramanujam, 2014). Some fungi have restricted host ranges, e.g. *Aschersonia aleyrodis* infects only whiteflies and *Nomuraea rileyi* infects only lepidopteran larvae, while others like *B. bassiana* and *M. anisopliae* infect more than 700 species in several insect orders. However, most of the pathogenicity tested is against aphids, whiteflies, thrips and few against lepidopteran and coleopteran pests. *Metarhizium* causes a disease known as 'green muscardine' in insect hosts because of the green colour of its conidial cells. In 1883, Metschnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metschnikoff) Sorokin is the second most widely exploited entomopathogenic fungus in biocontrol trials.

It is known to attack over 200 species of insects belonging to orders Coleoptera, Dermoptera, Homoptera, Lepidoptera and Orthoptera and more than 55 per cent of total insecticide used in the country is being utilized in cotton pest management with a special emphasis on *H. armigera*, where in, the crop occupies just about 5 per cent of total cultivable area in India (Puri, 1995). Even though pesticides form the first line of defence against the pest preventing the economic damage due to its rapid curative action, wide spread report of insecticide resistance in *H. armigera* to almost all popular insecticides has forced the farmers to think about better alternatives. Taking into consideration the encouraging results obtained by using microbial insecticides, studies were initiated on *Metarhizium anisopliae* (Metsch) Sorokin, a green muscardine soil inhabiting fungus to assess the effect of temperature and media on growth and sporulation of this fungus with a special emphasis to track down a cheaper formulation along with its pathogenicity against *H. armigera* larvae.

## Materials and Methods

The present investigations storability of *Metarhizium anisopliae* (Metschnikoff) was studied at two conditions, under room temperatures/ambient and freezing at 4°C with two solutions i.e. liquid solution and of wettable powder in laboratory at Biocontrol Research Laboratory, Department of Entomology, Sardar Vallabhbhai Patel University Agriculture and Technology, Meerut, U.P. India.

The material and methods employed for these studies are presented in this chapter.

## Medium and Equipments for Multiplication

The medium used for multiplication of the fungus was autoclaved potato dextrose agar (PDA) broth medium. Aluminium trays, inoculation needles, laminar flow cabinet, petridishes, glass slides, plastic tubs, glass jars, tissue paper, sterilized cotton, test tubes, pipettes, B.O.D incubator, polythene bags, pins, ocular and stage micrometer, binocular microscope, binocular microscope with photographic facility, spirit, spirit lamp, and disinfectants were made available in Biocontrol Laboratory of Department of Entomology, Sardar Vallabhbhai Patel University Agriculture and Technology., Meerut (U.P.) India.

## Experimental Details

To evaluate the different substrate for Mass production of entomopathogenic fungus *Metarhizium anisopliae* on solid and liquid substrates a plan of field experiment was carried out for the year 2018-19 with 11 treatments, three replication and CRD design.

## Nucleus Culture

The nucleus culture of *Metarhizium anisopliae*, was thereafter maintained on Sabouraud Dextrose Broth (SDB) medium as per the procedure of (Prasad *et al.*, 2014). Briefly, SDB was prepared and sterilized at 121°C (15 lbs) for 20 minutes. Then cooled and poured in presterilized petri plates and *Metarhizium anisopliae* from stock culture was inoculated aseptically. The Petri plates were incubated at 25 ± 1°C in BOD incubator for two weeks to harvest the inoculums culture. Solution of *M. anisopliae* for the isolation of the entomopathogen, a number of diseased *H. armigera* larvae with dense coating of dark green fungal growth were collected from cotton fields at the bio-control laboratory, Meerut in rabi 2019-20. Isolation was done by the standard method on potato dextrose agar. Out of 19 samples only one showed the mycelial growth which was later, identified as *M. anisopliae*. Identification was done on the basis of external symptoms, morphology of spores and sporulating structures (Radha *et al.*, 1955, Gopalakrishnan and Narayanan, 1988).

## Formulation

Fifteen days after inoculation, the spent medium with fungal biomass of *M. anisopliae* was blended in electric mixer for 1-2 minutes to homogenize the slurry. It was filtered through muslin cloth to remove debris under aseptic condition with spore count maintained at 107 spores/ml. The slurry of known microbial strength along with sticker Nirma soap @ 0.23 g/litre (Puzari *et al.*, 1991) was packed in already sterilized polypropylene bags with proper label and instructions. Bags were stored in the refrigerator and were subjected to the counting of colony forming units after 45 days. *H. armigera* culture Rearing of *H. armigera* culture was done at the insectary unit, Department of Entomology, College of Agriculture, Nagpur under standard conditions (28±2°C temperature, 75 per cent relative humidity, 12: 12h light and dark period). Stage wise larval culture was procured as per the experimental requirement.

## Spore Counting

A drop of conidial suspension of *Metarhizium anisopliae*, (obtained from the growing media by filtering through muslin cloth) was placed on the hemocytometer. The cover glass was put over the grid carefully so that no air bubble entered between cover glass and slide. The conidia of entomopathogenic fungi were counted under Olympus microscope.

## Preparation of Fungi

The cultured in SDA medium and kept 10 days at 25±1°C, 100% RH and 12:12 (L:D) photoperiod, Then conidia were harvested and suspended in Tween 80 (0.2 ml/L) in sterile distilled water and vortexes for 3 min to produce a homogenous suspension. Then the suspension was filtered through several layers of cheesecloth to remove mycelia and debris. By using a Haemocytometer, the spore concentration was determined and adjusted 10<sup>4</sup> conidia/ml-1.

## Morphological and Biometrical Characters of *M. anisopliae*

Mycelial, conidiophore and conidial characters were studied by observing the petriplate culture under binocular microscope in laboratory conditions. The culture was grown under ambient

condition (average temperature of 27 ± 2°C with RH of 85 per cent) in laminar flow cabinet. Autoclaved potato dextrose agar in petri plates was inoculated with *M. anisopliae* culture. The inoculation was made in the centre of the petridishes and incubated at 20°C in the incubator. In the same manner 10 petridishes were prepared and utilized for recording the biometrical and morphological characters of *M. anisopliae*.

## Viability of CFUs

The method used for the purpose was as suggested by (Ming Guage Feng, *et al.*, 1990). The autoclaved potato dextrose agar medium in petridishes (100 mm diameter) was inoculated with the help of micropipette by releasing 0.1 ml *M. anisopliae* suspension, other petridishes with medium were prepared in similar manner and inoculated with various dilution in the series (10<sup>1</sup> to 10<sup>10</sup> CFU/ml) and incubated at 27±1°C. After 48 hrs colony count was recorded.

## Statistical Analysis of Data

The data recorded during the course of investigation were subjected to statistical analysis by using analysis of variance technique (ANOVA) for randomized block design as suggested by (Panse and Sukhatme, 1978).

## Results and Discussion

### Pathogenicity *M. anisopliae* on the 5th Instars of *H. armigera* Larva

Studies on pathogenicity of *M. anisopliae* against 1<sup>st</sup> - 5<sup>th</sup> instar *H. armigera* larvae revealed that larval mortality. The effect of *Metarhizium anisopliae* spore/ml against *Helicoverpa armigera* larval instars was significantly higher recorded showed in table 1. The results revealed that all the treatments were significantly higher effective in larval mortality as compared to other larval instars. The pathogenicity on 4 day after pathogenicity of various larval instars in the (table 1). T<sub>1</sub> 1<sup>st</sup> instar of *H. armigera* with 1.8 x 10<sup>9</sup> spores suspension, was the best 1<sup>st</sup> instars larva *H. armigera* per cent mortality by *M. anisopliae* up to (35.63) followed by T<sub>2</sub> 1<sup>st</sup> instar of *H. armigera* with 1.8 x 10<sup>9</sup> spores suspension, was the per cent mortality (32.34), T<sub>3</sub> 1<sup>st</sup> instar of *H. armigera* with 1.8 x 10<sup>9</sup> spores

suspension, was the per cent mortality (30.13), T<sub>4</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the (27.36) per cent mortality, T<sub>5</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the per cent mortality (18.31) and T<sub>6</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension respectively. Observation recorded on 6<sup>th</sup>

day pathogenicity of *M. anisopliae* against I<sup>st</sup> - VI<sup>th</sup> instar *H. annigera* larvae revealed that larval mortality to the extent of 73.39, 63.15, 54.13, 44.31, 19.15 and 13.34 per cent, respectively, on 8th day with significant differences between them when sprayed with fungal suspension of  $10^8$  spores/ml (table 1) cent percent larval mortality respectively.

**Table 1. Pathgenicity *M. anisopliae* on the six instars of *H armigera* larva**

Larvae instars	No of Treated Larvae	Per cent Larvae Mortality at 25°C and Day Wise				
		4 Day	6 Day	8 Day	10 Day	12 Day
T <sub>1</sub> I <sup>st</sup> Instars Larva	50	35.63 (38.12)	73.39 (56.37)	85.69 (67.55)	98.37 (82.34)	100.0 (90.13)
T <sub>2</sub> II <sup>nd</sup> Instars Larva	50	32.34 (34.18)	63.15 (57.36)	83.36 (63.89)	89.43 (67.33)	92.29 (72.16)
T <sub>3</sub> III <sup>rd</sup> Instars Larva	50	30.13 (36.18)	54.13 (48.34)	78.15 (63.15)	81.33 (63.15)	78.17 (69.32)
T <sub>4</sub> IV <sup>th</sup> Instars Larva	50	27.36 (31.67)	44.31 (40.34)	58.19 (53.13)	59.63 (53.36)	63.15 (51.33)
T <sub>5</sub> V <sup>th</sup> Instars Larva	50	18.31 (13.15)	19.15 (17.18)	31.36 (19.15)	23.15 (21.34)	25.30 (23.18)
T <sub>16</sub> VI <sup>th</sup> Instars Larva	50	13.34 (11.34)	12.13 (11.18)	14.15 (13.18)	16.13 (15.32)	17.53 (16.19)
Control		0.00 (6.37)	0.00 (6.37)	0.00 (6.37)	0.00 (6.37)	0.00 (6.37)
SEM±		2.543	3.736	4.734	4.596	4.869
CD at 5%		5.370	6.236	9.236	9.191	12.361

Same trends after 8 day after the day after pathogenicity of various larval instars in the T<sub>1</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the best (85.69) per cent mortality followed by (83.36) followed by (78.15) per cent mortality followed by (58.19) followed by (21.36) and (14.15) per cent mortality, respectively. Observation recorded on tenth day pathogenicity of various larval instars in the T<sub>1</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the best I<sup>st</sup> instars larva *H. armigera* per cent mortality by *M. anisopliae* up to (98.37) followed by T<sub>2</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  was the per cent mortality (89.43), T<sub>3</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was t per cent mortality (81.33), T<sub>4</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores, was the (59.63) per cent mortality, T<sub>5</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the per cent mortality (23.15) and T<sub>6</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (16.13) suspension respectively.

Observtions on tenth day observe that pathogenicity of various larval instars in the T<sub>1</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the best I<sup>st</sup> instars larva *H. armigera* per cent mortality by *M. anisopliae* up to (98.37) followed by T<sub>2</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$ , was the per cent mortality (89.43), T<sub>3</sub> I<sup>st</sup> instar of *H. armigera* with

$1.8 \times 10^9$  spores suspension, was the % mortality (81.33), T<sub>4</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores, was the (59.63) per cent mortality, T<sub>5</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the per cent mortality (23.15) and T<sub>6</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (16.13) suspension respectively. Twelfth day observe that pathogenicity of various larval instars in the T<sub>1</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the best I<sup>st</sup> instars larva *H. armigera* per cent mortality by *M. anisopliae* up to (100.0) followed by T<sub>2</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores, was the per cent mortality (92.29), T<sub>3</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores, was the % mortality (78.17), T<sub>4</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension, was the (63.15)% mortality, T<sub>5</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores suspension was the per cent mortality (25.30), and T<sub>6</sub> I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (17.53) suspension respectively.

Overall result showed that among the media tested, *Metarhizium anisopliae* spore/ml I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (100.0) followed by II<sup>nd</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (17.53) production was significantly recorded. The present results are in conformity with the findings of (Gopalakrishnan and

Narayanan, 1988, Abida Nasreen and Ghulam Mustafa, 2000, Wadyalkar S. R., *et al.*, 2003, Vinod Kumar and P. N. Chowdhry, 2004, N. Revathi *et al.* and Mehinto Joelle Toffa *et al.*, 2014, Muhammad Tahir *et al.*, 2018, Muhammad Tahir *et al.*, 2019).

## Summary and Conclusion

Overall result showed that among the media tested, *Metarhizium anisopliae* spore/ml I<sup>st</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (100.0) followed by II<sup>nd</sup> instar of *H. armigera* with  $1.8 \times 10^9$  spores per cent mortality (17.53) production was significantly recorded.

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