The Pharma Innovation Journal 2022; SP-11(10): 1726-1728

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 1726-1728

www.thepharmajournal.com
Received: 01-08-2022
Accepted: 05-09-2022

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Effect of temperature on the biological attributes of promising isolates of *Metarhizium anisopliae* collected from three agro-climatic zones of Telangana

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Abstract
A study was conducted at Department of Entomology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad during *rabi* 2021 to evaluate effect of temperature on the biological attributes of promising isolates of *Metarhizium anisopliae.* The samples were collected from farmer fields of intense maize growing districts from three zones of Telangana *viz.*, Northern Telangana Zone (NTZ), Central Telangana Zone (CTZ) and Southern Telangana Zone (STZ). The isolates were exposed to different levels of temperature *viz.*, 20 °C, 25 °C, 30 °C, 40 °C and 45 °C.

Keywords: *Metarhizium anisopliae*, isolate, temperature

1. Introduction
Maize (*Zea mays*. L) is one of the most important cereal crops next to wheat and rice in the world. Among the maize growing countries, India ranks 4th in area and 7th in production, representing around 4 per cent of the world maize area and 2 per cent of total maize production (DACNET 2020). In India, during 2020-21 maize is grown in 9.89 million ha with production of 31.6 million tonnes with productivity of 3199 kg/ha (Indiastat – 2020-21). In Telangana state, maize was grown in 2.59 million ha with production of 17.56 million tonnes with productivity of 6782 kg/ha (Indiastat – 2020-21).

A new invasive pest fall armyworm (FAW), (J. E. Smith) (Lepidoptera: Noctuidae) recently invaded India, and is reported in the states of Telangana, Andhra Pradesh, Gujarat, Karnataka, Maharastra and Tamil Nadu infesting maize crop. It is a serious pest of corn and is native to tropical and subtropical regions of America. This insect has high dispersal ability, high fecundity and wide host range makes it one of the most severe economic pests. Larvae are voracious feeders and can destroy the whole plant in a short time. Studies in Africa indicated that infestation of *Spodoptera frugiperda* on maize exceeds 94% with damage levels ranging between 25% and 50%.

Fall armyworm can be managed by various measures, of which chemical measures are important owing to the economic damage caused. Despite effective, the extensive use of chemicals lead to several implications like environmental pollution, residue problems and development of resistance in insects.

For eco-friendly management of the pests, biocontrol agents are decisive components which include natural enemies *viz.*, parasitoids, predators and insect pathogens (fungi, bacteria, virus, protozoa and nematodes). Among these microbial based biopesticides offers an ecofriendly, sustainable and cost-effective management of insect pests. Among those biopesticides entomopathogenic fungi offer a larger scope as plant protection agents. Entomopathogenic fungal species, especially isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria farinosa* have emerged as potential biocontrol agents in many important crops of Telangana. Among these EPF's *M. anisopliae* have been most intensively investigated as insecticide in the crop pest control.

M. anisopliae is one of the most potential fungal pathogens and has been used successfully for the control of fall armyworm. The efficacy of entomopathogenic fungi (EPF) as biocontrol agents is affected by many biotic and abiotic factors in their environment (Roy *et al.*, 2006) [7] which is one of the major bottlenecks in their usage.

Temperature is one such factor affecting the entomopathogenic fungi (EPF) in nature (Ouedraogo *et al.*1997) [6]. It affects the rate of conidial germination and mycelial development of the fungal pathogens which in turn affect the rate of infection and virulence of these

pathogens against the target pests (Nussenbaum *et al.*, 2013) [5]. The ability of certain isolates of EPF to grow and sporulate under a wide range of environmental conditions is very useful in their application as biological control agents particularly in the semi-arid climates.

2. Material and Methods

The present study was carried out at Department of Entomology, College of Agriculture, Rajendranagar, PJTSAU Hyderabad, during *rabi* 2021.

2.1 Effect of temperature on biological attributes of *M. anisopliae*:

For the preparation of media, 100ml portion of the Potato Dextrose Agar (PDA) was dispensed into a 250 ml conical flask and autoclaved at 121 ˚C for 30 minutes. It was then cooled to about 45 ˚C. Just before solidification it was poured evenly into sterile petri dishes. After solidification, the petri plates were inoculated with circular discs of fungal mat from actively growing cultures of *M. anisopliae* separately by using a sterilized cork borer. The collected isolates of *M. anispoliae* were then tested for their growth, sporulatio n and other parameters at five different temperatures, *viz.*, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C and the following observations were recorded.

2.1.1 Radial mycelial growth

Radial growth of the isolates at different temperatures was determined by fresh inoculation from a 5mm diameter circular agar disc, cut using a cork borer, from 14-day old culture onto a fresh PDAY medium plate (Bugeme *et al.*, 2008) [8]. Five replicate plates were kept for each isolate and at each temperature. The petri plates were placed in inverted position and incubated at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C in respective incubators under dark conditions. The diameter of the colony was measured at 7th, 14th, and 21st day from the day of inoculation of the disc. The measurement was done along the same axis each time.

2.1.2 Conidial yield

The circular discs of 10 mm diameter were cut randomly from 2 weeks old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml of distilled water. The spores present in the discs were allowed to disperse uniformly in the water by rotating the test tube on a vortex for one minute. Proper care was taken to avoid spillage of the suspension while rotation. The suspension was serially diluted and the spores were counted with the help of an improved haemocytometer under a compound microscope at 40 X magnification and number of spores present per millilitre was calculated using the below mentioned formula suggested by Aneja (1996) [1].

2.1.3 Conidial viability

Conidial viability was assessed using slide method. Conidial suspension of the isolate was spread on a thin layer of PDAY on glass slide kept in petri plate layered with moist filter paper. The plates were kept at different incubation temperatures and the time taken for 50% germination was estimated after 6 h till 72 h. The germination of conidia was recorded after 24 h of incubation and the per cent spore germination was calculated using the formula. A conidium was considered to be germinated when a distinct germ tube projected from it, and was at least twice the diameter of the conidium.

3. Results and Discussion

All the three isolates showed maximum radial growth of 62.3 mm, 58.8 mm and 53.2 mm for MaE20-9-23, MaE20-9-9 and MaE20-9-19, respectively at 30°C followed by maximum growth at 25 °C and 35 °C. No growth of the pathogen was observed at 40 °C and 45 °C for all the three isolates. Among the isolates, MaE20-9-23 showed maximum growth followed by MaE20-9-9 and the least growth was observed in MaE20-9-19 at all tested temperatures (Table 1). Ekesi *et al.* (1999) stated that EPF in general, have maximum radial growth at temperatures between 25 °C and 30 °C. The optimum temperature for the growth and conidial germination of six different isolates of *M. anisopliae* was 25°C (Dimbi *et al.*, 2004).

Table 1: Effect of temperature on radial growth of three different strains of *M. anisopliae*

Treatments	Radial growth (mm)		
	MaE20-9-9	MaE20-9-19	MaE20-9-23
25 °C (T_1)	53.1	49.2	58.3
30 °C (T_2)	58.8	53.2	62.3
35 °C (T_3)	16.6	14.5	18.2
40 °C (T_4)	0.00	0.00	0.00
45 °C (T_5)	0.00	0.00	0.00
CD (P = 0.05%)	7.03	8.01	8.03

The conidial yield of the pathogen was found to be maximum at 30 °C *viz.*,15.1, 9.6 and 6.3 for MaE20-9-19, MaE20-9-9 and MaE20-9-23, respectively followed by higher growth at 25 °C and 35 °C. No conidial yield was observed at 40 °C and 45 °C. Among the isolates MaE20-9-19 showed highest conidial yield than other two isolates (Table 2). Isolates that produce more conidia or reproduce more quickly are potentially better bio control agents than isolates that may be more pathogenic but reproduce relatively less. Both germination and growth declined steeply above 25 °C and ceased above 30 °C (Burges, 1981).

Table 2: Effect of temperature on conidial yield of three different strains of *M. anisopliae*

Treatments	Conidial yield (No. of conidia/ml)		
	MaE20-9-9	MaE20-9-19	MaE20-9-23
25 °C (T_1)	5.7	4.9	5.1
30 °C (T_2)	9.6	15.1	6.3
35 °C (T_3)	1.0	0.4	0.8
40 °C (T_4)	0.00	0.00	0.00
45 °C (T_5)	0.00	0.00	0.00
CD (P = 0.05%)	0.15	0.19	0.16

The time taken for 50% germination of the pathogen was found to be least at 30 °C *viz.*, 6.66, 8.33 and 15.50 for MaE20-9-19, MaE20-9-9 and MaE20-9-23, respectively followed by 25 °C and 35 °C. No germination of the conidia was observed at 40 °C and 45 °C. Among the isolates least time for 50% germination was observed for MaE20-9-19 (Table 3). Hywel-Jones and Gillespie (1990) studied the spore germination of *M. anisopliae* and *B. bassiana* at 20-30 °C.

Table 3: Effect of temperature on time taken for 50% germination of three different strains of *M. anisopliae*

Treatments	Time taken for 50% germination(hours)		
	MaE20-9-9	**MaE20-9-19**	**MaE20-9-23**
25 °C (T_1)	10.66	8.50	16.66
30 °C (T_2)	8.33	6.66	15.50
35 °C (T_3)	16.66	18.66	21.33
40 °C (T_4)	0.00	0.00	0.00
45 °C (T_5)	0.00	0.00	0.00
CD (P = 0.05%)	0.6	0.4	0.8

4. Conclusion

The growth of all the three isolates *viz.*, MaE20-9-9, MaE20-9-19 and MaE20-9-23 in terms of radial mycelial growth(mm), conidial yield (No. of conidia/ml) and time taken for 50% germination(hours) was found to be maximum at 30 °C followed by 25 °C and 35 °C. No growth of the pathogen occurred at 40 °C and 45 °C. Among the three isolates tested highest radial growth was observed in MaE20-9-23 and the other two growth attributes *viz.*, conidial yield and time taken for 50% germination was found out to be best in MaE20-9-19.

5. References

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