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Natural incidence of *Metarhizium anisopliae* (Metchnikoff) Sorokin on South American tomato moth, *Tuta absoluta* (Meyrick) from India

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ABSTRACT: South American tomato moth, *Tuta absoluta* is one of the major destructive invasive pest globally. The pest was reported from India during 2014 and is spreading to various states very rapidly causing extensive damage on solanaceous crops, particularly on tomato. Natural incidence of green muscardine fungus, *Metarhizium anisopliae* infecting *T. absoluta* larva on tomato was observed for the first time in India causing up to 35 % mortality. A laboratory study was carried out to assess the pathogenicity and effectiveness of *M. anisopliae* against *T. absoluta* during 2016-17, using the leaf immersion method. Hundred per cent mortality was recorded at a concentration of $1x10^9$spores/ml at 120 hours after treatment with $LC_{50}$of 9.03×10^5spores/ml.

Keywords: Natural incidence, *Tuta absoluta*, *Metarhizium anisopliae*, dose-mortality relationship, LC_{50}

INTRODUCTION

The South American tomato leaf miner or South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is native to South America, causing devastating damage on tomato, *Solanum lycopersicum*L. (Gontijo *et al.,* 2013). *T.absoluta* is considered a typical invasive species, because of its quick development under suitable agro-ecological conditions and rapid spread into new areas causing economic damage (Desneux *et al*., 2010).In India, it was first reported from Karnataka (Sridhar *et al*., 2014),where this pest is becoming a serious threat to tomato production in both greenhouse and outdoor crops (Sridhar *et al*., 2015) and in recent past the pest is spreading across the states like Maharashtra, Tamil Nadu, Andhra Pradesh, Telangana, Gujarat, Delhi, Chhattisgarh *etc*. (Shashank *et al.,* 2016; Taram *et al.,* 2016).

It poses a severe threat to future cultivation of tomato and other solanaceous crops like potato, brinjal *etc*. Though chemicals are available for its control, in order to reduce the ill effects posed by them like residues and resistance development in insect pests, use of entomopathogenic fungi will be the best practical means for effective and sustainable management of this invasive insect pest in an eco-friendly manner.

MATERIALS AND METHODS

During the regular pest and natural enemies' surveillance on tomato under polyhouse conditions, larvae of *T.absoluta* were observed for natural incidence of entomopathogens. Based on the number of dead and live insects per cent mortality due to fungal infection was worked out. The infected insects were separated and kept for sporulation under the humid chamber. The fungus was isolated and sub-cultured on potato dextrose agar slants and petri-dish for morphological identification and further confirmation through molecular tools.

Bioassay studies

In-vitro studies were carried out to study the pathogenicity and effectiveness of *M. anisopliae* against *T.absoluta* during 2016-17.

T. absoluta culture

Colony of *T. absoluta* was established in the laboratory from the pupae collected from tomato field and were further reared on tomato plants. For the bioassay purpose, newly emerged adults were collected using an aspirator and provided with 10 % honey as food source and terminal twigs of tomato were provided for egg laying (Hussein *et al*., 2014 and Abdel *et al*., 2015) for further culturing.

M. anisopliae concentrations

Efficacy of *M. anisopliae* on 2nd instar larva of *T. absoluta* was assessed by using five concentrations *i.e.,*1 x 10^5, 1 x 10^6, 1 x 10^7, 1x 10^8 and 1x 10^9 spores/ml along with a control. All the treatments were replicated four times.

Fresh leaves of tomato were kept in petriplates and ten second instar larvae were released per each replication and allowed to feed on them. The *T. absoluta* mined leaves were dipped in different concentrations of *M. anisopliae* as mentioned above and were transferred to petri dishes with filter paper for maintaining sufficient moisture. A set of untreated mined leaves were kept as control. Untreated fresh leaves were fed for further feeding by the larvae. The per cent mortality of larvae was observed at 24 hours interval for five days. The corrected mortality was obtained using Abbott's formula (Abbott, 1925).

Corrected percent mortality= ((Pt-Pc) / (100-Pc)) x 100

Where,

P_t - Observed mortality in treatment; P_c - Observed mortality in untreated check

The median lethal concentration (LC_{50}) values were obtained using probit analysis (Finney, 1962). Further, the mortality data obtained at different concentrations and time intervals was analyzed using one way ANOVA after transforming the per cent mortality data into arc sine transformations (Gomez and Gomez,1984).

RESULTS AND DISCUSSION

The entomopathogenic fungus infecting *T. absoluta* larva was identified as *Metarhizium anisopliae* (Metchnikoff) Sorokin based on morphological characters *i.e,* the colour of the fungal colony that varied from buff cream to dark green colour (Plate 1), structure and shape of conidia which was confirmed with the help of dichotomous keys (Ghayedi and Abdollahi, 2013). These findings were in accordance with the observations of France *et al.* (2000) who reported similar symptoms of mycosis with external buff to dark green colour sporulation. The identification of the pathogen was further confirmed through molecular tools (Gen Bank accession Nos. SUB4703842 *Metarhizium* MK101209 and SUB4703864 *Metarhizium* MK101224).

Green colour spores were observed within three to four days on the infected insect cadavers (Plates-2 and 3). Up to 35 % natural incidence of *M. anisopliae* was observed on *T. absoluta* during September – October, 2016 at IIHR, Bengaluru. During the peak incidence of the fungal infection (39th standard meteorological week (SMW) on *T. absoluta* various meteorological parameters observed were 19 - 25 °C minimum temperature, 24-26 °C maximum temperature, highest relative humidity of 75-91 % and lowest relative humidity of 51-64 per cent.

Plate -1. Pure culture of *M.anisopliae*

Plate -2

Plate -3

Plate 2 & 3. *T. absoluta* larva infected with *M. anisopliae*

Bioefficacy of *M. anisopliae*

There was a significant difference among the doses of *M. anisopliae* spores and duration against *T. absoluta i.e.*, mortality of the larvae was proportionate to the spore concentration levels. With the increase in duration, there was a gradual increase in the mortality of *T. absoluta* in all the concentrations tested. At highest concentration, the observed mortality ranged from 25 % (24 hours) to

Table 1. Efficacy of *M. anisopliae* on *T. absoluta* (treated on early second instar)

Spore concentration	Mortality of *T. absoluta* larvae (%) after				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
1x 10^5spores/ ml	2.50 (7.77)	5.00 (11.48)	10.00 (17.20)	22.50 (28.22)	37.50 (37.96)
1x 10^6 spores/ ml	2.50 (7.77)	7.50 (13.48)	22.50 (28.57)	30.00 (33.37)	52.50 (46.80)
1x 10^7 spores/ ml	7.50 (13.48)	20.00 (26.06)	40.00 (39.46)	47.50 (43.79)	67.50 (55.82)
1x 10^8 spores/ ml	12.50 (20.91)	22.50 (28.57)	52.50 (46.73)	62.50 (52.64)	85.00 (70.74)
1x10^9 spores/ ml	25.00 (30.22)	40.00 (39.25)	60.00 (51.43)	90.00 (74.47)	100.00 (90.00)
Control	0.00 (4.05)	5.00 (11.48)	5.00 (11.48)	7.50 (13.48)	7.50 (15.19)
S.Em (±)	3.36	4.36	3.82	4.15	4.10
CD(p=0.05)	9.84	12.80	11.21	12.16	12.02

Note:*Figures in parentheses are arcsine transformed values
Average of four replications

Table 2. Dose - mortality response of *T. absoluta* to *M. anisopliae*

Hours after treatment	Heterogeneity ($\chi 2$)	Regression equation	LC_{50} (spores ml^{-1})	Fiducial limits	
				Lower	**Upper**
24	0.483	y = 0.388x + 1.196	9.64×10^{10}	1.02×10^{9}	9.07×10^{12}
48	1.125	y = 0.412x + 0.992	5.72×10^{9}	4.72×10^{8}	6.93×10^{10}
72	0.467	y = 0.524x + 0.865	6.91×10^{7}	2.32×10^{7}	2.06×10^{8}
96	2.806	y = 0.538x + 1.159	1.58 x10^{7}	6.76 $\times10^{6}$	3.70×10^{7}
120	0.961	y = 0.564 x + 1.634	9.03 x 10^{5}	3.30 x 10^{5}	2.47 x 10^{6}

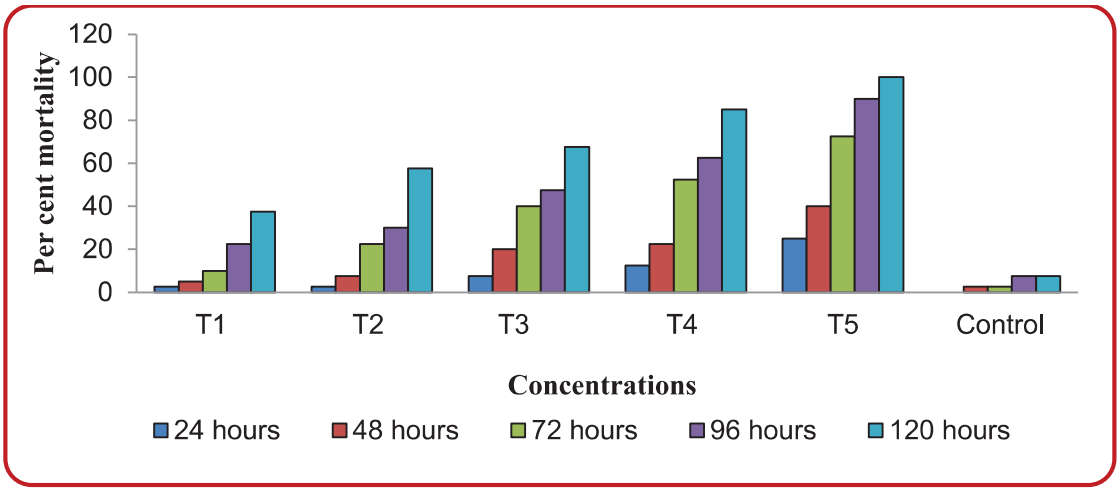

Fig 1. Mortality of *T. absoluta* larva at different concentrations of *M. anisopliae*

100.00% (120 hours) after treatment (Table 1 and Figure 1). The LC_{50} values for *M. anisopliae* were assessed as 9.64×10^{10}, 5.72×10^{9}, 6.91×10^{7}, 1.58×10^{7} and 9.03×10^{5} spores/ml at 24, 48, 72, 96 and 120 hours after treatment against 2nd instar larvae of *T.absoluta,* respectively (Table 2). İnanlı *et al*., (2012) reported similar efficacy of *M. anisopliae* against eggs and first in star larvae of *T. absoluta*. Similar findings on efficacy of *M. anisopliae* on *T. absoluta* were reported by Contreras *et al*., (2014) and Sabbour and Singer (2014).

Further investigations are being carried out for utilization of the fungus for the management of different stages of *T. absoluta.*

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