

Journal of Biological Control, 39(2): 196-203, 2025, DOI: 10.18311/jbc/2025/48307

Research Article

Impact of *Beauveria bassiana* and sub-lethal doses of azadirachtin and chlorantraniliprole on *Pthorimaea absoluta*

KHUSHBOO KUMARI[1], SUBHASH C. VERMA[1], PREM L. SHARMA[1], RAJESHWAR S. CHANDEL[1], VISHAV G. S. CHANDEL[1], NIKITA CHAUHAN[2*], VIBHUTI SHARMA[1], LALIT KALIA[1], ANSHUMAN SEMWAL[1], CHANDER SINGH[1], ADARSH SHARMA[1] and NIKSHUBHA SHARMA[1]

[1]*Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan – 173230, Himachal Pradesh, India*

[2]*School of Agriculture, Graphic Era Hill University, Dehradun – 248002, Uttarakhand, India*

**Corresponding author E-mail:* nikitacjm@gmail.com

ABSTRACT: Entomopathogenic fungi are the most versatile fungi, which cause infection in various insects. In the current study, *Beauveria bassiana* at the concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml, sub-lethal doses of azadirachtin (0.92 and 1.11 ppm) and chlorantraniliprole (0.61ppm) were assessed against the 1st, 2nd, 3rd and 4th larval instars of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in laboratory conditions. *Beauveria bassiana* at a concentration of 10^6 conidia/ml applied against 1st and 2nd larval instars of *T. absoluta* resulted in a maximum mortality of 86.67% and 83.33%, respectively. The maximum mortality of the 3rd and 4th larval instars of *T. absoluta, i.e.*, 86.67% and 80.00%, respectively, was observed with 10^8 conidia/ml of *B. bassiana*. Furthermore, it was also observed that *B. bassiana* mixed with chlorantraniliprole (0.61 ppm) and azadirachtin (0.92 and 1.11 ppm) suppressed the growth of *B. bassiana,* but the lethal effect against *T. absoluta* remained almost similar.

KEYWORDS: Azadirachtin, *Beauveria bassiana*, entomopathogenic fungus, chlorantraniliprole, *Tuta absoluta*

(Article chronicle: Received: 28-12-2024; Revised: 10-04-2025; Accepted: 12-04-2025)

INTRODUCTION

The South American Pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera) is native to Peru (South America) and is one of the most destructive pests of solanaceous crops, including tomato in the world (Biondi *et al.*, 2018; Han *et al.,* 2019). It is highly invasive and has spread to over 100 countries since 2006 (Biondi *et al.,* 2018; Han *et al.,* 2019). High spreading ability, ineffectual surveillance, poor quarantine and increased trade are the major factors contributing to the rapid International spread of the pest (Han *et al.*, 2019). This pest has been observed to spread quickly within the country once it gains initial entry. Sridhar *et al.* (2014) reported *T. absoluta* for the first time from Pune, in western India, during October 2014 on tomato plants grown in polyhouses and fields. In contrast, in 2015, it was observed for the first time in Himachal Pradesh, in Northern India (Sharma & Gavkare, 2017). The larval stage of this pest is most obnoxious as it attacks almost all parts of the plant, including leaves, fruits, flowers, buds, apical shoots, *etc*. (Sharma & Gavkare, 2017). In the absence of appropriate control measures, this pest can cause 100 per cent crop damage (Campos *et al.*, 2017; Biondi *et al.,* 2018). Farmers rely heavily on the application of insecticide for the management of this pest (Roditakis *et al.,* 2013), despite the availability of eco-friendly management strategies. The high resistance capability of this insect to synthetic insecticides, as well as its concealed feeding behaviour, makes its management a daunting task (Guedes & Picanço, 2012). Besides, the indiscriminate use of chemical insecticides may lead to serious environmental consequences, *viz*., pest resurgence (Kumar *et al.,* 2021), secondary outbreaks of pests, insecticide resistance (Guedes, 2017; Abdel-Baky *et al.,* 2019), loss of beneficial insects like pollinators, predators, and parasitoids (Torres & Bueno, 2018) and pesticide residues (Ahmed *et al.,* 2011; Neme & Satheesh, 2016). As a result, alternative tools for its management need to be developed. The use of Entomopathogenic Fungi (EPFs) can be an effective alternative to chemical pesticides due to their eco-friendly properties, safety for non-target organisms, and ability to prevent pesticide resistance. Due to their wide host range, ability to infect insects at different developmental stages and ability to penetrate the cuticle of their hosts, EPF are the most versatile (Vega *et al.,*2012. *Among EPFs,*

Beauveria bassiana, which causes white muscardine disease in insects, has been known for its entomopathogenic properties for a long time. *B. bassiana* (Hypocreales: Cordycipitaceae) was previously known as *Tritirachium shiotae*. It is a Deutomycete fungus with a cosmopolitan occurrence and reported on a wide range of insect hosts. Hall and Papierok (1982) recorded it from more than 500 host species.

The application of entomopathogenic fungi is occasionally conducted along with synthetic chemical pesticides for pest management by the farmers. Consequently, it becomes pertinent to investigate the effect of synthetic chemical insecticides on *B. bassiana* and determine if they are compatible. The compatibility of EPF in combination with pesticides has been studied by many authors (Kachhadiya *et al.*, 2014; Sain *et al.,* 2019;; Halder *et al.,* 2021; Khun *et al.*, 2021; Sain *et al.*, 2022). However, very limited information is available on the combined effect of sub-lethal concentrations of pesticides and *B. bassiana*. So, the current study aimed to evaluate the susceptibility of *T. absoluta* larvae to *B. bassiana* impregnated with sub-lethal doses of chlorantraniliprole and azadirachtin under laboratory conditions.

MATERIALS AND METHODS

Insect culture rearing

Larvae of American pinworm, *T. absoluta* were collected from the tomato field of the Department of Entomology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan and maintained in vitro at 25±0.5ºC, 70±5 per cent RH and 14L:10D. The larvae were provided with tomato (var. Solan lalima) leaves and reared individually in a glass vial (10 ml) with good ventilation. Food was provided to the larvae as per requirement until pupation. After pupation, the obtained pupae were shifted to glass chimneys for the emergence of adults. For mating and egg laying, adults were transferred to the rearing cages. 30 per cent honey solution as food was provided to them as food, along with filter paper strips for egg laying purposes. Rearing of these insects for two generations was done before using them for experiments.

Culturing of *B. bassiana* and treatment of *T. absoluta*

The nucleus culture of *B. bassiana* was acquired from the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, for the experiments and its multiplication was done by using Potato Dextrose Agar (PDA). Freshly prepared slants of *B. bassiana* were incubated at 25±0.5°C and 70±5% RH. Conidia were obtained from the 15-day-old well-sporulated culture in tubes by pouring 10 ml of sterilised, emulsified (0.5% Tween 80) distilled water into each tube. Neubauer Haemocytometer was used to determine the conidial concentration in the suspension, and subsequently, the conidial suspension was adjusted to a concentration of 10^8 or 10^7 conidia/ml, depending on the harvesting of conidia. Test concentrations of 10^6, 10^5, 10^4, 10^3 and 10^2 conidia/ml were prepared after serial dilution of conidial suspension in a 1:9 ratio using sterilised emulsified distilled water. Fortification of test concentrations of conidial suspension was done with different concentrations of azadirachtin (Neema vati 0.15% EC) (0.92 and 1.11 ppm) and chlorantraniliprole (Coragen 18.5% w/w) (0.61 ppm). These suspensions were evaluated on 1st, 2nd, 3rd and 4th larval instars of *T. absoluta*. The larval dip method was used for treating *T. absoluta* for 10 seconds, and data were recorded after every 24 h of larval dip for 7 days.

Data analysis

Probit analysis of mortality data was done according to Finney (1952), and for probit analysis, the range of 20–80 per cent mortality was used. Subsequently, IBM SPSS Statistics 16 was used to calculate LC_{50}/ LC_{90} values.

Corrected mortality was calculated using the following formula by Abbott (1925)

Corrected Mortality (%) = [(% Mortality in Treated - % Mortality in Control)/(100 - % Mortality in Control)] * 100

Effect of pesticides on the growth of *B. bassiana*

Botanical and pesticides used were:

(i) Azadirachtin @ 0.92 ppm,

(ii) Azadirachtin @ 1.11ppm and

(iii) Chlorantraniliprole @ 0.61 ppm

For studying the effect of pesticide on the growth of *B. bassiana*, 250 ml of growth media (PDA) was fortified with sub-lethal doses of azadirachtin (0.92 ppm and 1.11 ppm) and chlorantraniliprole (0.61 ppm), and *B. bassiana* was allowed to grow on this medium. Control treatment (medium without pesticides) was also maintained for comparison.

Fortification of growth medium with pesticides

Before mixing the pesticides into the medium, a stock solution of azadirachtin 15 ppm (10 ml of V-neem azadirachtin 1500 ppm was mixed in 990 ml sterilised distilled water) and chlorantraniliprole 185 ppm (1 ml of Coragen was mixed in 999 ml sterilised distilled water) was prepared under aseptic conditions. Four sets of growth medium having the following composition were prepared by following the same procedure as described earlier.

Just before pouring the medium into plates, in set I and set II, 15.34 and 18.5 ml of stock solution of azadirachtin

(15 ppm) was added and mixed thoroughly under aseptic conditions to get fortification levels of 0.92 and 1.11 ppm, respectively. Similarly, in set III, 0.81 ml of stock solution of chlorantraniliprole (185 ppm) was added to get a fortification level of 0.606 ppm. Set IV was kept as a control. The medium was poured into a Petri plate (9 cm dia) and allowed to solidify under a laminar air flow cabinet. For inoculation of fungus agar disc along with mycelium mat of pure culture of *B. bassiana* was cored from 15 days old culture by 5 mm diameter cork borer and inoculated in the centre of the Petri plate. Growth medium without pesticides but inoculated with a mycelium fungal disc serves as an untreated check. The plates were sealed with parafilm and incubated at 25±0.5ºC, 70±5 per cent RH and 14L:10D photoperiod. Each treatment was replicated 5 times. The radial growth of the fungi in the Petri plate was measured after 7 days of inoculation. Per cent growth inhibition of the fungus by each treatment over the control was calculated as per Hokkanen and Kotiluoto (1992).

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

Effect of chlorantraniliprole and azadirachtin on the growth and efficacy of *B. bassiana*

The effect of azadirachtin and chlorantraniliprole on the efficacy of *B. bassiana* against *T. absoluta* was studied by adding azadirachtin (0.92 and 1.11 ppm) and chlorantraniliprole (0.61ppm) into different conidial suspensions of the fungus. Fungal suspensions of different concentrations fortified with the above pesticides were evaluated against 2nd and 3rd-instar larvae of *T. absoluta* using the larval-dip method. Larvae were observed daily, and mortality was subjected to probit analysis and compared with toxicity caused by the fungus in the absence of pesticides to determine synergistic/antagonistic effect on *B. bassiana.*

Data analysis

Mortality data were subjected to probit analysis as per Finney (1952). The mortality data falls in the range of 20 to 80 per cent or close to it, was subjected to probit analysis, and LC_{50}/LC_{90} values were calculated by IBM SPSS Statistics 20. Mortality data recorded at different time intervals for effective concentration (Mortality 80% or high) were also subjected to probit analysis for calculating LT_{50}/LT_{90} values. The inhibition per cent data was subjected to using online OPSTAT software developed by Sheoran *et al.* (1998), CCS Haryana Agriculture University, Hissar.

RESULTS

Mortality response of different larval instars

Different test concentrations (10^2-10^8 conidia/ml) of *B. bassiana* were evaluated against different instars of *T. absoluta* to determine the mortality response (Table 1) The first instar *T. absoluta* larvae had the highest corrected mortality (100%) at 10^7 conidia/ml, while the lowest mortality (20.00%) was observed at 10^2 conidia/ml. Likewise, for the second instar larvae of *T. absoluta,* the maximum mortality of 96.67% at 10^7 conidia/ml and the minimum mortality of 16.67% at 10^2 conidial/ml were observed. When third and fourth-instar larvae of *T. absoluta* were treated with *B. bassiana* at a concentration of 10^2 conidia/ml, there was no mortality observed. The highest mortality (86.67%) in the third instar was recorded at 10^8 conidia/ml, while the least mortality (23.33%) was observed at 10^3 conidia/ml. Similarly, when *B. bassiana* was applied at a concentration of 10^3 conidia/ml against fourth instar larvae, it led to a mortality rate of 20.00% and at a concentration of 10^8 conidia/ml, the mortality was 80.00%. The findings indicated that as the larval instars progressed, mortality decreased even when exposed to high concentrations.

The pathogenicity parameters and LC_{50}/LC_{90} values

The pathogenicity parameters of *B. bassiana* against *T. absoluta* were evaluated, and calculations for LC_{50} and LC_{90} values are represented in Table 2. For the first instar, the LC_{50} was 4.9×10^3 with fiducial limits (95%) 1.5×10^3 and 1.4×10^4 conidia/ml whereas, LC_{90} value was 2.4×10^6 conidia/ml with fiducial limits (95%) 4.3×10^5 and 6.5×10^7 conidia/ml on 7 DAT (Day After Treatment). Probit kill correlated linearly with log concentration, as evident from the regression equation Y = 0.48X - 1.76. The data were homogeneous as the χ^2_{cal} (0.21) was less than χ^2_{tab} (7.81) at a 5 per cent level of significance and 3 degrees of freedom. The LC_{50} and LC_{90} values were 1.3×10^4 conidia/ml (95% fiducial limits: 3.5×10^3 and 3.6×10^4 conidia/ml) and 5.8×10^6 conidia/

Table 1. Mortality response of *B. bassiana* to different larval instars of *T. absoluta* after 7 days of treatment

Larval instars	Conidial/ml						
	10^8	10^7	10^6	10^5	10^4	10^3	10^2
I	-	-	86.67	73.33	53.33	40.00	20.00
II	-	_-	83.33	63.33	46.67	30.00	16.67
III	86.67	73.33	63.33	43.33	33.33	23.33	-
IV	80.00	73.33	60.00	46.67	30.00	20.00	

ml (95% fiducial limits: 9.6×10^5 and 2.9×10^8 conidia/ml) for the second instar larvae. The χ^2 test indicated that the data were homogeneous, as calculated and tabulated values of χ^2. The regression equation for probit kill (Y) showed a linear dependence on log concentrations (X), expressed as Y = 0.48X - 2.0. Similarly, for the third instar larvae, Log concentration and probit kill were linearly related (Y = 0.37X - 1.89), the χ^2 test indicated that the data were homogeneous (χ^2_{cal}: 0.41, χ^2_{tab}: 9.48 at p=0.05 and 4 df). LC_{50} (median lethal concentration) and LC_{90} values were 1.4×10^5 conidia/ml (95% fiducial limits: 3.7×10^4 and 5.3×10^5 conidia/ml) and 4.7×10^8 conidia/ml (95% fiducial limits: 5.4×10^7 and 2.7×10^{10} conidia/ml). Likewise, for the fourth instar, the concentration required to kill 50% test population (LC_{50}) was 2.3×10^5 conidia/ml with fiducial limits (95%) of 5.6×10^4 and 8.7×10^5 conidia/ml. Similarly, the concentration required to cause 90% mortality (LC_{90}) was 1.1×10^{10} conidia/ml with fiducial limits (95%) 9.9×10^7 and 9.9×10^{10} conidia/ml. The regression equation for the relationship between the probit kill (Y) and the log concentration (X) was Y = 0.35X - 1.88. The data was homogeneous at a 5 per cent level of significance and 4 degrees of freedom, χ^2_{cal} (0.23) was less than χ^2_{tab} (9.48).

Effect of chlorantraniliprole and azadirachtin on the growth of *B. bassiana*

Different concentrations of chlorantraniliprole and azadirachtin were tested against *B. bassiana* to determine the effect of these pesticides on the growth of the fungus. As evident from Table 3, both pesticides inhibited the growth of the fungus over the control. Azadirachtin (0.92ppm) mixed media showed significantly maximum growth (1.68 cm^2) among pesticidal treatments while, *B. bassiana* radial growth on cultures containing azadirachtin (1.11 ppm) and chlorantraniliprole (0.61 ppm) was lower, *i.e.*, 1.05 and 0.79 cm^2, respectively against the significantly higher growth in control culture (2.96 cm^2). Chlorantraniliprole (0.61 ppm) showed a maximum inhibitory effect (73.31%), followed by azadirachtin at 1.11 ppm (64.52%) and azadirachtin at 0.92 ppm (48.64%).

Mortality response of *T. absoluta* larvae to *B. bassiana* mixed with azadirachtin

Different concentrations of conidial suspension (10^2 -10^8) fortified with two concentrations of azadirachtin (0.92 and 1.11 ppm) were applied against second and third-instar larvae of *T. absoluta* to determine the concentration mortality response and calculate the LC_{50} and LC_{90} values (Tables 4 and 5). The corrected mortality after 7 days of treatment for the second instar larvae applied with *B. bassiana* at 10^2, 10^3, 10^4, 10^5 and 10^6 conidia/ml mixed with azadirachtin (0.92 ppm) was 10.71, 24.99, 49.99, 71.42 and 89.26 per cent, respectively (Table 4). The LC_{50} was 1.09 x 10^4 (fiducial limits: 2.57 x 10^3 and 1.76 x 10^4) and LC_{90} was 1.27 x 10^6 (fiducial limits: 3.10 x 10^5 and 1.57 x 10^7). The data were homogenous since χ^2_{cal} (0.05) was less than χ^2_{tab} (7.81) at a 5% level of significance with 3 degrees of freedom. Probit kill and Log concentration were linearly related as Y = 0.62X - 2.51 (Table 5).

Likewise, *B. bassiana* blended with azadirachtin (1.11 ppm) at concentrations of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8

Table 2. Pathogenicity of *B. bassiana* to larval instars of *T. absoluta* after 7 days of treatment

Larval instars	LC_{50}	95% fiducial limits (LC_{50})	LC_{90}	95% fiducial limits (LC_{90})	Regression equation	χ^2 calculated	χ^2 tabulated
I	4.9×10^3	1.5×10^3 and 1.4×10^4	2.4×10^6	4.3×10^5 and 6.5×10^7	Y=0.48X - 1.76	0.21	7.81
II	1.3×10^4	3.5×10^3 and 3.6×10^4	5.8×10^6	9.6×10^5 and 2.9×10^8	Y=0.48X - 2.00	0.5	7.81
III	1.4×10^5	3.7×10^4 and 5.3×10^5	4.7 x 10^8	5.4×10^7 and 2.7×10^{10}	Y=0.37X - 1.89	0.41	9.48
IV	2.3×10^5	5.6×10^4 and 8.7×10^5	1.1×10^{10}	9.9×10^7 and 9.9×10^{10}	Y= 0.35X - 1.88	0.23	9.48

Table 3. Effect of chlorantraniliprole and azadirachtin on the growth of *B. bassiana*

Pesticides	Conc. (ppm)	Mean growth (cm^2) and inhibition (%) over control	
		Growth	Inhibition
Azadirachtin	0.92	1.68	48.64
	1.11	1.05	64.52
Chlorantraniliprole	0.61	0.79	73.31
Control	-	2.96	-
CD (p = 0.05)	-	0.35	-

Table 4. Mortality response of different larval instars of *T. absoluta* to *B. bassiana* mixed with azadirachtin and chlorantraniliprole

Concentration (ppm)		Larval instar	Conidial suspension/ml							Control (Water)
		10^8	**10^7**	**10^6**	10^5	10^4	10^3	10^2	0	
		Larval mortality								
Azadirachtin	0.92	II	-	-	89.26	71.42	49.99	24.99	10.71	0.00
	1.11	III	89.26	78.57	64.28	42.85	24.99	14.28	-	0.00
Chlorantraniliprole	0.61	II	-	-	85.71	67.85	46.64	35.71	14.28	0
	0.61	III	85.71	75.00	57.14	39.27	28.56	21.42	-	0

Table 5. Pathogenicity of *B. bassiana* mixed with azadirachtin to different larval instars of *T. absoluta* at 7 days of treatment

Parameters	**Azadirachtin (0.92 ppm) (II instar larvae)**	**Azadirachtin (1.11 ppm) (III instar larvae)**	**Chlorantraniliprole (0.61 ppm) (II instar larvae)**	**Chlorantraniliprole (0.61 ppm) (III instar larvae)**
Reg. Equation	Y = 0.62X - 2.51	Y = 0.59X - 2.98	Y = 0.50X - 2.01	Y = 0.38X - 2.07
χ^2 calculated	0.05	2.30	0.62	0.7
χ^2 tabulated	7.81	9.48	7.81	9.48
LC_{50} (Conidia/ml)	1.09×10^4	1.16×10^5	9.96×10^3	2.49×10^5
Fiducial limits (Conidia/ml)	2.57×10^3 and 1.76×10^4	2.72×10^4 and 1.73×10^5	1.7×10^3 and 1.8×10^4	3.09×10^4 and 4.75×10^5
LC_{90} (Conidia/ml)	1.27×10^6	1.74×10^7	3.52×10^6	5.32×10^8
Fiducial limits (Conidia/ml)	3.10×10^5 and 1.57×10^7	4.55×10^6 and 1.85×10^8	5.6×10^5 and 1.2×10^7	5.53×10^7 and 3.49×10^{10}

conidia/ml resulted in corrected mortality of 14.28, 24.99, 42.85, 64.28, 78.57 and 89.26 for third instar larvae after 7 days of treatments (Table 4). Median lethal concentration (LC_{50}) and LC_{90} values were 1.16×10^5 conidia/ml (fiducial limits: 2.72×10^4 and 1.73×10^5) and 1.74×10^7 conidia/ml (fiducial limits: 4.55×10^6 and 1.85×10^8). The relationship between probit kill and log concentration followed a straight linear curve; Y = 0.59X - 2.98. The χ^2 test proved that the data were homogeneous (χ^2 cal = 2.30; χ^2 tab = 9.48 at p = 0.05 and 4 df) (Table 5).

Mortality response of *T. absoluta* larvae to *B. bassiana* mixed with chlorantraniliprole

Mortality response of *T. absoluta* by application of *B. bassiana* at different conidial concentrations fortified with chlorantraniliprole was also observed for the second and third instar larvae, and LC_{50} and LC_{90} values were determined. *B. bassiana* at 10^2, 10^3, 10^4, 10^5 and 10^6 conidia/ml blended with chlorantraniliprole (0.61 ppm) applied against second instar larvae of *T. absoluta* showed 14.28, 35.71, 46.64, 67.85 and 85.71 per cent corrected mortality, respectively, after 7 days of treatments (Table 4). The LC_{50} and LC_{90} values were 9.96×10^3 conidia/ml (95 % fiducial limit: 1.7×10^3 and 1.8×10^4 conidia/ml) and 3.52×10^6 conidia/ml (95% fiducial limit: 5.6×10^5 and 1.2×10^8 conidia/ml), respectively. The χ^2 indicated that data were homogenous as χ^2_{cal} (0.62) was less as compared to χ^2_{tab} (7.81) at a 5 per cent level of significance and 3 degrees of freedom. The linear relationship between probit kill and log concentration was Y = 0.50X - 2.01 (Table 5).

Similarly, the concentration-dependent mortality response of third instar larvae of *T. absoluta* to *B. bassiana* at concentration of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 impregnated with chlorantraniliprole (0.61 ppm) recorded 21.42, 28.56, 39.27, 57.14, 75.00 and 85.71 per cent corrected mortality, respectively, following 7 days of treatment. LC_{50} and LC_{90} were 2.49×10^5 conidia/ml (fiducial limits: 3.09×10^4 and 4.75×10^5 conidia/ml) and 5.32×10^8 conidia/ml (fiducial limits: 5.53×10^7 and 3.49×10^{10}conidia/ml), respectively. The χ^2 test showed that the data were homogeneous at a 5 per cent level of significance and 4 degrees of freedom, since χ^2_{cal} (0.70) is less than χ^2_{tab} (9.48). Probit kill followed a straight-line curve with log concentration; Y = 0.38X – 2.07 (Table 5).

DISCUSSION

In the current study, the early instar larvae of *T. absoluta* showed a high mortality rate, which may be attributed to the larvae's delicate and thin cuticle. This thin outer layer made it easier for the germ tube of the *B. bassiana* conidia

to penetrate, germinate, and subsequently grow mycelium, leading to the mortality of the larvae. During the current studies, the first and second larval instars had a 100 per cent mortality at a 10^7 conidial concentration of *B. bassiana*. These results conform with Abdel-Raheem *et al.* (2015), who reported 100 per cent mortality to first instar larvae due to *B. bassiana* with concentrations of 10^7 conidia/ml. The mortality of third and fourth instars at a conidial concentration of 10^8 during this study was 86.67 and 80.00 per cent. Similar findings were observed by Tadele and Emana (2017), who tested *B. bassiana* isolates on third instar larvae and found larval mortality of 95.83 per cent at a concentration of 2.5 x 10^9 spores/ml. In a study conducted by Ndereyimana *et al.* (2019), it was found that the application of *B. bassiana* against third instar *T. absoluta* larvae resulted in 82.80 per cent larval mortality. The current results align with the findings of Karaca *et al.* (2022), who reported a mortality rate of 87.65 per cent in third instar larvae of *T. absoluta* at a concentration of 2×10^8 conidia/ml of *B. bassiana*. Similarly, Dabsu and Kovanci (2022) reported 84.61 per cent mortality of third instar larvae of *T. absoluta* due to *B. bassiana* at a concentration of 10^8 conidia/ml. Youssef (2015) reported 86.70 per cent mortality of fourth instar larvae of *T. absoluta* at a concentration of 10^8 conidia/ml of *B. bassiana*.

It has been observed in the present studies that larval mortality increased with the increase in concentration of conidia. The present outcomes are in line with the research conducted by Abdel-Raheem *et al.* (2015), which revealed that there is a direct relationship between the concentration of *B. bassiana* and the mortality of *T. absoluta* larvae. In simpler terms, the higher the concentration of *B. bassiana*, the greater the mortality of *T. absoluta* larvae. In addition, Tsoulnara and Port (2016) conducted a study to evaluate the efficacy of *B. bassiana* against third instar larvae of *T. absoluta* and reported that 90.00 per cent mortality at a higher concentration. Different isolates of *B. bassiana* may lead to a varying mortality response among the larvae of *T. absoluta*. Ayele *et al.* (2020) reported 48.47 to 90.00 and 50.00 to 95.66 per cent mortality of second and third instar larvae of *T. absoluta,* respectively, when treated with different isolates of *B. bassiana* at a concentration of 1 x 10^7 spores/ml.

During the course of the current research, it has been observed that both azadirachtin and chlorantraniliprole increased *B. bassiana*'s lethal effect at least for the second instar larvae, as evident from the LC_{50} and LC_{90} values. This may be due to the fact that with increased susceptibility of larvae, the *B. bassiana* showed increased efficacy when combined with azadirachtin and chlorantraniliprole. The difference in the pathogenicity of *B. bassiana* mixed with different pesticides may be attributed to various reasons. In the current studies, it has been observed that *B. bassiana* with chlorantraniliprole (0.61 ppm) performed better compared to *B. bassiana* with azadiracthin (0.92 ppm) against *T. absoluta* second instar larvae. The superiority of chlorantraniliprole may be attributable to the fact that it causes the larvae more stress as compared to azadirachtin. On the other hand, the slightly better performance of *B. bassiana* with azadirachtin (1.11 ppm) in comparison to *B. bassiana* mixed with chlorantraniliprole (0.61 ppm) in third instar larvae of *T. absoluta* may be because neem (azadirachtin) interferes with insect development, cuticle formation, or the moulting process (Staal, 1987; Rembold, 1989). According to Zimmermann (2007), if the deposition, hardening, and tanning processes involved in new cuticle formation are impacted, it will lessen the fungus's ability to blockade, consequently, mycosis risk could rise.

CONCLUSION

From the above study, it is clearly evident that the susceptibility of *T. absoluta* larvae decreased with the increase in larval instar stage. Also, the studies revealed that *B. bassiana* at 10^7 conidia/ml, exclusively or mixed with azadirachtin (0.92 and 1.11 ppm) or chlorantraniliprole (0.61 ppm), produced almost similar mortality to *T. absoluta* larvae as in case of chemical insecticides (all treatments were statistically on par). Therefore, *B. bassiana* can be recommended as a component in an Integrated Pest Management (IPM) strategy for the management of *T. absoluta*.

ACKNOWLEDGEMENT

The authors thank the Department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, for their assistance in providing the essential resources for smooth conduct of the research.

REFERENCES

Abdel-Baky, N. F., Alhewairini, S. S., and Bakry, M. M. S. 2019. Evaluation the efficiency of Emamectin Benzoate against *Tuta absoluta* and *Spodoptera littoralis* larvae in Qassim region, KSA. *Pak J Agri Sci,* **56**: 801-808.

Abdel-Raheem, M., Ismail, I. A., Abdel-Rahman, R. S., Farag, N. A., and Abdel-Raheem, I. E. 2015. Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) as biological control agents on some stored product insects. *J Entomol Zool Stud,* **3**: 316-320.

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol*, **18:** 265-267. https://doi.org/10.1093/jee/18.2.265a

Ahmed, A., Randhawa, M. A., Yusuf, M. J., and Khalid, N. 2011. Effect of processing on pesticide residues in food crops: A review. *J Agri Res,* **49:** 379-390.

Ayele, B. A., Muleta, D., Venegas, J., and Assefa, F. 2020. Morphological, molecular, and pathogenicity characteristics of the native isolates of *Metarhizium anisopliae* against the tomato leafminer, *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) in Ethiopia. *Egypt J Biol Pest Control*, **30**: Article 59. https://doi.org/10.1186/s41938-020-00261-w

Biondi, A., Narciso, R., Guedes, C., Wan, F. H., and Desneux, N. 2018. Ecology, worldwide spread, and management if invasive South American Tomato pin worm, *Tuta absoluta*: Past, present and future. *Annu Rev Entomol*, **63**: 239-258. https://doi.org/10.1146/annurev-ento-031616-034933 PMid:28977774

Campos, M. R., Biondi, A., Adiga, A., Guedes, R. N. C., and Desneux, N. 2017. From the Western Palaearctic region to beyond: *Tuta absoluta* ten years after invading Europe. *J Pest Sci*, **90:** 787-796. https://doi.org/10.1007/s10340-017-0867-7

Dabsu, T. K., and Kovanci, O. B. 2022. Evaluation of the pathogenicity of some entomopathogenic fungi against Tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae. *Türk Biyol Mücadele Derg*, **13:**103-117. https://doi.org/10.31019/tbmd.1112324

Finney, D. J. 1952. *Probit analysis: A statistical treatment of the sigmoid response curve*. Cambridge University Press, Cambridge.

Guedes, R., and Picanço, M. 2012. The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. *Eur Pub Pros Office Bull* **42**: 211-216. https://doi.org/10.1111/epp.2557

Guedes, R. N. C. 2017. Insecticide resistance, control failure likelihood and the first law of geography. *Pest Manag Sci*, **73:** 479-484. https://doi.org/10.1002/ps.4452 PMid:27714951

Halder, J., Majumder, S., and Rai, A. B. 2021. Compatibility and combined efficacy of entomopathogenic fungi and neonicotinoid insecticides against *Myzus persicae* (Sulzer): An eco-friendly approach. *Entomol. Hell*, **30:** 24-32. https://doi.org/10.12681/eh.25417

Han, P., Bayram, Y., Shaltiel-Harpaz, L., Sohrabi, F., Saji, A., Esenali, U. T., …, Desneux, N. 2019. *Tuta absoluta* continues to disperse in Asia: Damage, ongoing management and future challenges. *J Pest Sci*, **92**: 1317-1327. https://doi.org/10.1007/s10340-018-1062-1

Hokkanen, H.M.T. and Kotiluoto, R. 1992. Bioassay of the side effects of pesticides on Beauveria bassiana and Metarhizium anisopliae: Standardized sequential testing procedure. IOBC/WPRS *Bulletin,* **11:** 148-151.

Kachhadiya, N. M., Kapadia, M. N., and Jethva, D. 2014. Bio-efficacy of *Nomuraea rileyi* (Farlow) Samson alone and in combination with insecticides against *Spodoptera litura* (Fabricius) on groundnut under laboratory condition. *Pestol*, **38:** 19-21.

Karaca, G., Erol, A. B., Çığgın, B. A., Acarbulut, and Karaca, İ. 2022. Efficacy of some entomopathogenic fungi against tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egy J Biol Pest Cont*, **32**: 1-7. https://doi.org/10.1186/s41938-022-00577-9

Khun, K. K., Ash, G. J., Stevens, M. M., Huwer, R. K., and Wilson, B. A. 2021. Interactions of fungal entomopathogens with synthetic insecticides for the control of *Kuschelorhynchus macadamiae* (Coleoptera: Curculionidae). *J Appl Entomol*, **145**(6): 553-566. https://doi.org/10.1111/jen.12879

Kumar, J., Ramlal, A., Mallick, D., and Mishra, V. 2021. An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, **10**: Article 1185. https://doi.org/10.3390/plants10061185 PMid:34200860 PMCid:PMC8230470

Ndereyimana, A., Nyalala, S., Murerwa, P., and Gaidashova, S. 2019. Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyp J Biol Pest Control*, **29:** 1-5. https://doi.org/10.1186/s41938-019-0184-y

Neme, K., and Satheesh, N. 2016. Review on pesticide residue in plant food products: Health impacts and mechanisms to reduce the residue levels in food. *Arch Appl Sci Resi,* **8:** 55-60.

Rembold, H. 1989. Azadirachtins: their structure and mode of action. In: J. Arnason (ed), *Insecticides of plant origin*, Vol. 387 (pp. 150-163). Symposium series of American Chemical Society, Washington, DC. https://doi.org/10.1021/bk-1989-0387.ch011

Roditakis, E., Skarmoutsou, C., and Staurakaki, M. 2013. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Manag Sci*, **69**: 834-840.

Sain, S. K., Monga, D., Kranthi, S., Hiremani, N. S., Nagrale, D. T., Kumar, R., … Prasad, Y. G. 2022. Evaluation of the bioefficacy and insecticide compatibility of entomopathogens for management of

whitefly (Hemiptera: Aleyrodidae) on upland cotton under laboratory and polyhouse conditions. *Neotrop Entomol*, **51**: 600-612. https://doi.org/10.1007/s13744-022-00964-9 PMid:35680781

Sain, S. K., Monga, D., Kumar, R., Nagrale, D. T., Hiremani, N. S., and Kranthi, S. 2019. Compatibility of entomopathogenic fungi with insecticides and their efficacy for IPM of *Bemisia tabaci* in cotton. *J Pestic Sci*, **44**(2): 97-105. https://doi.org/10.1584/jpestics.D18-067 PMid:31148937 PMCid:PMC6529749

Sharma, P. L., and Gavkare, O. 2017. New distributional and record of invasive pest, *T. absoluta* (Meyrick) in north western Himalayan region of India. *Natl Acad Sci Lett*, **40**, 217-220. https://doi.org/10.1007/s40009-016-0526-1

Sheoran, O. P., Tonk, D. S., Kaushik, L. S., Hasija, R. C., and Pannu, R. S. 1998. Statistical Software package for agricultural research workers. In D. S. Hooda, & R.C. Hasija (eds), *Recent Advances in information theory, Statistics and Computer Application* (pp. 139-143).

Sridhar, V., Chakravarthy, A. K., Asokan, R., Vinesh, L. S., Rebijith, K. B., and Vennila, S. 2014. New records of invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India. *Pest Manag Horticul Ecosys,* **20**: 148-154.

Staal, G. B. 1987. Juvenoids and anti-juvenille hormone agents as IGRs. In V. Delucchi (ed.) *Proceedings Symposium on Insect Pest Management: An International Perspective*, Geneva, Switzerland.

Tadele, S., and Emana, G. 2017. Entomopathogenic effect of *Beauveria bassiana* (Bals.) and *Metarrhizium* anisopliae (Metschn.) on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae under laboratory and glasshouse conditions in Ethiopia. *J Plant Pathol Microbiol*, **8**: Article 411.

Torres, J. B., and Bueno, A. D. F. 2018. Conservation biological control using selective insecticides – A valuable tool for IPM. *Biol Cont*, **126:** 53-64. https://doi.org/10.1016/j.biocontrol.2018.07.012

Tsoulnara, D., and Port, G. 2016. Efficacy of a *Beauveria bassiana* strain, *Bacillus thuringiensis* and their combination against the tomato leafminer *Tuta absoluta*. *Entomol Hell*, **25**: 23-30. https://doi.org/10.12681/eh.11548

Vega, F. E., Meyling, N. V., Luangsa-ard, J. J., and Blackwell, M. 2012. Fungal entomopathogens. *Insect pathology* (2nd ed) (pp. 171-220). Academic Press, UK, USA. https://doi.org/10.1016/B978-0-12-384984-7.00006-3

Youssef, N. A. 2015. Efficacy of the entomopathogenic nematodes and fungi for controlling the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Arab Univ J Agric Sci*, **23**: 591-598. https://doi.org/10.21608/ajs.2015.14599

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Sci Technol*, **17**: 553-596. https://doi.org/10.1080/09583150701309006