



Compatibility of insecticides and *Elachertus inunctus* Nees (Hymenoptera: Eulophidae) for controlling *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in greenhouse condition

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Abstract

South American Tomato Pinworm (SATP), *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is one of the most devastating pests in tomato greenhouses. Efficacy of some chemical and biorational insecticides, namely chlorfenapyr, thiocyclam, azadirachtin and *Bacillus thuringiensis* (Bt), in controlling SATP and their compatibility with *Elachertus inunctus*, a new SATP larval ectoparasitoid, were studied under greenhouse conditions. For this purpose, larval mortality, leaf and fruit damage and parasitism following the different insecticide treatments and after various days from the treatment (DAT) were recorded and compared to untreated control. Results showed that chlorfenapyr had suitable effects on SATP larvae. Although the experiments indicated that the short-term effects of azadirachtin, thiocyclam and Bt were not detrimental to SATP larvae, but their residual effects were significant in the long term. Among the tested insecticides, Bt was more compatible with *E. inunctus* release. Overall, the results suggest that the integration application of Bt with early inundate release of *E. inunctus* can be recommended for suitable and environmentally safe control of SATP in greenhouse tomato.

Keywords Parasitic wasps · Bio-insecticides · Tomato leaf miner · IPM

Introduction

The South American Tomato Pinworm (SATP), *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is an invasive pest native to South America, where it is considered one of the most dangerous pests in tomato greenhouse production and in the open field (Yankova 2012). In the last 10 years, SATP has spread and expanded to many regions of the world including Europe, Africa and Asia, causing extensive damage to international tomato trade (Biondi et al. 2018; Campos et al. 2017; Sankarganesh et al. 2017;

Sylla et al. 2017; Xian et al. 2017). The presence of *T. absoluta* was reported in Iran (Baniamერი and Cheraghian 2012), in Sub-Saharan Africa (Sylla et al. 2017), recently in northern India (Sankarganesh et al. 2017), and now threatening China (Xian et al. 2017), i.e. the biggest tomato world producer. The female usually lays eggs on leaves, stems, and to a lesser extent on fruits. The young larvae mine the leaves or stems producing large galleries and burrow into the fruit. On leaves, the larvae feed only on mesophyll cells, leave the epidermis intact and make irregular leaf mines, which may later become necrotic and affect photosynthesis in the plant (Desneux et al. 2010; Biondi et al. 2018). Damage from this pest throughout the entire growing cycle of tomatoes can significantly reduce both yield and fruit quality by the direct feeding of *T. absoluta* and secondary pathogens that may enter through the wounds made by the insect. In the absence of control strategies, larval feeding damage can reach up to 100% (Desneux et al. 2011; Yankova 2012; Biondi et al. 2018). Chemical control using synthetic insecticides is an effective management tactic for this pest (Guedes and Siqueira

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2012). Also, the general entophytic behaviour of the larval instars makes it difficult to conduct effective control practices against this pest (Lietti et al. 2005; Guedes and Picanço 2012). Application of the chemicals causes many problems including incensement in production costs, side effects on natural enemy populations, harmful pesticide residues in fruits (Braham and Hajji 2012) and pest resistance to the chemicals (Desneux et al. 2007; Biondi et al. 2018).

Biopesticides in the plant protection systems have the potential to aid the management of the pest (Copping and Menn 2000). Some vegetal products were assessed for potential use in the leaf miner control and can provide a safe control method under organic agricultural conditions. Products with active ingredient azadirachtin possess specific antifeedant and deterrent activities, suppressing and stopping the feeding, reduction of moulting and deformations in pupae and imago, and decrease fecundity in the females (Kleeberg 2001; Isman 2015). Chlorfenapyr is applied against lepidopteran pests. The insecticides were introduced during the 1990s as an alternative to synthetic pyrethroids because of its low toxicity to mammalian and aquatic organisms (Raghavendra et al. 2011). Thiocyclam (Evisect®) is highly efficient in controlling the pest outbreaks (Lietti et al. 2005).

The mixture of spores and protein crystals from pathogen *Bacillus thuringiensis* subsp. *krustaki* Berliner (Bt) is the most widely used microbial insecticide against lepidopteran pests (Wilcox et al. 1986; Peralta and Palma 2017). Bt is not toxic to humans, most beneficial insects, and other nontarget organisms; therefore, it does not cause the serious environmental and safety problems associated with conventional synthetic insecticides (Peralta and Palma 2017).

Genus of *Elachertus* (Hymenoptera: Eulophidae) is primary parasitoids of a variety of lepidopteran larvae. Some species are polyphagous attacking hosts belonging to several different families (Schauff 1985). The parasitoid wasp of *Elachertus inunctus* Nees was originally collected as larval ectoparasitoids of *T. absoluta* on greenhouse tomato in Khuzestan province in the south-west of Iran (Yarahmadi et al. 2016). This parasitoid wasp naturally suppressed SATP larvae on tomato in greenhouse and field conditions in Khuzestan province, south-west of Iran.

The first step in application of a control strategy (chemical control, biological control, etc.) in integrated pest management programme is evaluation of its effectiveness (Pedigo 2002). Also in IPM programmes, biological control agents and the applied pesticides must be compatible with each other (Stark et al. 2007; Desneux et al. 2007). The compatibility of natural enemies to biorational or synthetic insecticides is obviously variable according to the type of the insecticide and species of the

natural enemies. Biorational insecticides are efficient to control varied greenhouse pests. Biorational insecticides are also more likely compatible with natural enemies. Compatibility of biorational pesticide with natural enemies is necessary for developing IPM programme (Pedigo 2002).

There has not been any effort to determine efficacies of the bio-insecticides chlorfenapyr, azadirachtin, Bt and *E. inunctus* in control of *T. absoluta* for greenhouse crops. Therefore, the objective of this study was to evaluate the bio-insecticides and parasitoid efficacies against SATP and their integration potential.

Materials and methods

Experimental design

The experiment was performed at a commercial greenhouse, with 3000 m² area, located in Kute Seied Soltan, Ahvaz, Khuzestan province, south-west of Iran (31°27'24"N 48°49'34"E). The greenhouse was cultivated with greenhouse tomato seedlings, Cherry® variety. No insecticide applications were carried out except in experimental treatment. In addition, no herbicides or fungicides were applied in the experimental greenhouse. All growers' practices (growing, fertilizer application, weeding and irrigation of tomato) were used in accordance with the recommendations of the Khuzestan agricultural organization. The experiment was arranged in a randomized complete block design with four replications (125 m²) and 3-metre-wide ridges were made in each. Also, each replication was separated from others by Nylon insect screen (mesh 50, Green-tek®, Canada). The active ingredients, the trade name and doses of the experimental insecticides are given in Table 1. Control was sprayed with water. Treatments were applied using an electronic backpack sprayer (Matabi®, Taizhou Kaide Machinery Co., Ltd.) using the hollow cone, solid spray tip type of nozzle (TXVK-10).

Parasitoid wasp

The parasitized larvae of *E. inunctus* were collected from infested tomato field of the Veis region, Ahwaz, Khuzestan province (31°30'28"N 48°50'37"). The larvae were transferred to the laboratory and reared in an incubator at temperature 25 ± 1 °C, RH 60% and photoperiod 16:8 (L:D). The parasitoid wasps—five female and five male adults (2 days old) per block—were released 2 weeks after the first observed SATP contamination.

Table 1 Insecticides, mode of action, trade name and doses of application experimented under tomato greenhouse in 2015

No.	Treatment	Trade name	Formulation	Mode of action	Applied rate per hectare
1	Azadirachtin ^a (Az)	Neemarin 1500 PPM [®]	1% EC	Insect growth regulators	1 lit
2	Chlorfenapyr ^b	Crown [®]	24% EC	Disrupting the production of adenosine triphosphate	400 ml
3	Thiocyclam ^c	Evisect [®]	50% SC	Nicotinergic acetylcholine blocker	500 g
4	<i>Bacillus ssp. thuringiensis kurstaki</i> ^b (Bt)	Belthirul [®]	32,000 spore/gr WP	Its delta- endotoxin act as digestive toxin	0.5 kg

^aBiotech international Ltd

^bShijiazhuang Awiner Biotech Co., Ltd, China

^cBiotech international Ltd

Sampling

The samplings were performed before treatment and 1, 6, 10 and 14 days after treatment (DAT). At each sampling date, six randomly selected plants were checked by travelling in a X-shaped pattern through each plot. Per sampling, 10 leaves were randomly selected from the upper one-third of the plant. The leaves were taken to the laboratory, and alive, dead and parasitized numbers of larvae, as well as the larval mines, were separately recorded under a stereomicroscope. Also, three fruits were randomly picked from each selected plant and numbers of damaged fruits (with wounds made by SATP larvae) were separately recorded in each treatment.

Data analysis

The mean number of live larvae, leaf mines or damaged fruits per leaf was tested for normality assumption by Kolmogorov–Smirnov test; the data were then arcsine-transformed. The data were statistically analysed by ANOVA. Duncan's multiple range test was used for means separation. All analyses were done using SPSS (Version 16) software (Chicago, IL, USA).

Results

The efficacy of azadirachtin, chlorfenapyr and thiocyclam against *T. absoluta* in tomato plants under greenhouse conditions with respect to larval mortality, numbers of leaf mines and fruit wounds are presented in Tables 2, 3 and 4.

The highest larval mortality of SATP was recorded in the chlorfenapyr treatment (52.8%) at 1 DAT, while in the other treatments, no significant difference was recorded at 1 DAT (Table 2). Similar results were observed at 6 DAT. However, the SATP larvae mortality in thiocyclam,

azadirachtin and Bt treatment was dramatically increased and reached 60, 78.1 and 83.3% respectively, with all of them being significantly higher than control. The data implicated that the azadirachtin, thiocyclam and Bt do not have any significant short-term effects against the pest, but their slower (chronic) effects were sufficient as well as in the case of chlorfenapyr.

The least protective effect against larval leaf damages was observed in thiocyclam, azadirachtin and Bt treatments (Table 3). The leaf mine numbers of the pest were higher than thiocyclam and azadirachtin. These data implicated that thiocyclam and azadirachtin treatments not only had no preventive effect on leaf damages by the pest larvae, but also had an adverse effect on the parasitism and natural control of the pest by *E. inunctus*. Chlorfenapyr significantly reduced the leaf mines at 10 and 15 DAT (34–76%).

All treatments cause significant reduction (83.5–100%) in tomato fruit damages by the larvae (Table 4). However, Bt and azadirachtin (no observed damage) had less damage than chlorfenapyr and thiocyclam (0.2 larval wound per fruit) at 15 DAT.

The larval parasitism of *T. absoluta* by *E. inunctus* ranged between 0 and 19%. The parasitism of SATP larvae was not significantly different at 1 and 6 DAT. But the parasitism ratio was suddenly reduced and reached zero in chlorfenapyr, thiocyclam and azadirachtin treatments at 10 and 14 DAT. The highest activity of the parasitoid wasp was observed in control and Bt treatments (Table 5).

Discussion

Use of biocontrol agents against pest is an alternative pest management tactic in IPM programmes. But some natural enemies may not always provide economically acceptable biological control for the pest in greenhouses. Therefore, the concurrent use of natural enemies and

Table 2 Mortality percentages *Tuta absoluta* larvae in the different experimental treatments

Sampling date	Mean \pm SE					P value	$F_{(df)}$
	Chlorfenapyr	Thiocyclam	Azadirachtin	Bt	Control		
1DAT	52.8 \pm 7.1b*	10.7 \pm 2.9a	3.5 \pm 2.1a	0 \pm 0a	4.3 \pm 2.6a	<0.001	33 _(4,25)
6DAT	62.8 \pm 16.8b	14.3 \pm 5.1a	20.7 \pm 7.5a	25.8 \pm 11.2a	10 \pm 10a	0.02	3.729 _(4,25)
10DAT	70 \pm 20b	60 \pm 18.7b	78.1 \pm 6.9b	83.3 \pm 16.6b	15.2 \pm 11.8a	0.05	2.797 _(4,25)
15DAT	0 \pm 0a	27.3 \pm 18.6a	13.3 \pm 9.7a	0 \pm 0a	0 \pm 0a	0.194	1.68 _(4,25)

* Means followed by the same letter within a row are not significantly different (Duncan test; $P < 0.05$)

Table 3 Number of leaf mines (per three leaves) in the different experimental treatments

Sampling date	Mean \pm SE					P value	$F_{(df)}$
	Chlorfenapyr	Thiocyclam	Azadirachtin	Bt	Control		
1DAT	0.45 \pm 5.4a*b	0.68 \pm 6.1c	0.41 \pm 2.3a	0.41 \pm .3a	0.43 \pm 4.3ab	<0.001	10.3 _(4,20)
6DAT	0.5 \pm 3.06ab	0.99 \pm 4.2b	0.74 \pm 3.4ab	0.74 \pm 3.4ab	0.5 \pm 2.9a	0.002	6.5 _(4,20)
10DAT	0.3 \pm 2.7a	0.7 \pm 3.9b	0.4 \pm 1.2a	0.4 \pm 1.2a	0.4 \pm 4.9a	<0.001	8.2 _(4,20)
15DAT	0.26 \pm 0.66a	0.4 \pm 3.6ab	0.5 \pm 2.2b	0.5 \pm 2.2b	0.3 \pm 3.3ab	<0.001	13.5 _(4,20)

Means followed by the same letter within a row are not significantly different (Duncan test; $P < 0.05$)

Table 4 Mean of larval wounds (per fruit) in the different experimental treatments

Sampling date	Larval mortality \pm SE					P value	$F_{(df)}$
	Chlorfenapyr	Thiocyclam	Azadirachtin	Bt	Control		
1DAT	0.4 \pm 6a*	0 \pm 0a	0 \pm 0a	0 \pm 0a	0 \pm 0a	0.19	1.7 _(4,20)
6DAT	0 \pm 0b	0 \pm 0b	0 \pm 0b	0 \pm 0b	0.4 \pm 0.6a	0.002	6.5 _(4,20)
10DAT	0 \pm 0a	0 \pm 0a	0 \pm 0a	0 \pm 0a	0.4 \pm 0.4a	0.43	1 _(4,20)
15DAT	0.2 \pm 0.2b	0.2 \pm 0.2b	0 \pm 0b	0 \pm 0b	0.8 \pm 1.2a	0.003	5.2 _(4,20)

Means followed by the same letter within a row are not significantly different (Duncan test; $P < 0.05$)

Table 5 Ratios of parazited larvae (per leaf) by *E. inunctus* in the different experimental treatments

Sampling date	Larval mortality \pm SE					P Value	$F_{(df)}$
	Chlorfenapyr	Thiocyclam	Azadirachtin	Bt	control		
1DAT	0 \pm 0a	0.04 \pm 0.04a	0 \pm 0a	0 \pm 0a	0 \pm 0a	0.431	1 _(4,20)
6DAT	0.07 \pm 0.04a	0.08 \pm 0.02a	0.07 \pm 0.03a	0.1 \pm 0.06a	0.13 \pm 0.01a	0.766	0.458 _(4,20)
10DAT	0 \pm 0a	0 \pm 0a	0 \pm 0a	0.09 \pm 0.2b	0.19 \pm 0.27b	0.163	1.82 _(4,20)
15DAT	0 \pm 0a	0 \pm 0a	0 \pm 0a	0.03 \pm 0.03ab	0.11 \pm 0.27b	0.007	4.88 _(4,20)

Means followed by the same letter within a row are not significantly different (Duncan test; $P < 0.05$)

pesticides and their compatibility has studied (Pedigo 2002). A new crop/pest/parasitoid system, i.e. tomato/*T. absoluta*/*E. inunctus*, was used to investigate how the four insecticides (azadirachtin and Bt as biorational and chlorfenapyr and thiocyclam as synthetic insecticides) may

affect the pest population, the plant, leaf and fruit damages and activity of the new reported larval parasitoid.

Our greenhouse study showed that chlorfenapyr had suitable acute and chronic effects on SATP larvae. Although it was indicated that acute effects of azadirachtin,

thiocyclam and Bt were not detrimental to SATP larvae, but their residual effects were significant in the long term.

Although the product azadirachtin is of vegetable origin, it shows specific effects on the pests. It causes disturbances in basic processes—feeding, metamorphosis and fecundity, followed by lethality (Yankova et al. 2014). Neem-extracted insecticides are known as slow-acting insecticides which is one of the major limitations of these products. The insecticides do not produce an immediate ‘knock-down’ effect (Isman 2015). Lowerey and Isman (1994) showed that observation time is an important variable in toxicity assessment of neem-extracted products because azadirachtin is a slow-acting insecticide and its antifeedant or growth-inhibitory properties vary depending on the concentration and species of target pest. Similarly, the delayed effect of azadirachtin on *T. absoluta* was previously demonstrated (Braham et al. 2012; Nazarpour et al. 2016). Toxicity of neem-based insecticides against some lepidopteran larvae such as *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Liang et al. 2003), *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Singh et al. 2007), *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Zabel et al. 2002), *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) (Greenberg et al. 2005; Yee and Toscano 2014) and *T. absoluta* (Arnò and Gabarra 2011; Braham et al. 2012; Nazarpour et al. 2016) were reported by various researchers.

Bacillus thuringiensis has been used as an alternative to synthetic insecticides for decades (Peralta and Palma 2017). The efficacy of the bacterium spores against *T. absoluta* was evaluated in a few studies. For example, the significant effect of *B. thuringiensis* subs. *krustaki* against SATP larvae in tomato fields was previously documented (Nazarpour et al. 2016). Moreover, the efficacy of Bt against SATP larvae and its damage were documented by Gozalez-Caberera et al. (2001), Sabbour and Soleiman (2012), and Nazarpour et al. (2016). In all this research, a lag time was observed between Bt spray and SATP population decrease.

The findings agree with the laboratory experiment results of Silvério et al. (2009), who showed that chlorfenapyr is a good candidate for chemical control of SATP. Also, their study demonstrated that thiocyclam at dosage 60 g L⁻¹ showed intermediate efficacy (34% mortality) for SATP control in laboratory condition (Braham et al. 2012). In contrast to our findings, the result of LD₅₀ values indicated that chlorfenapyr (3.165%) was less toxic for SATP larvae in comparison with emamectin benzoate (0.461%), imidacloprid (0.621%), indoxacarb (0.753%), profenofos (0.643%), pyridalyl (0.511), methomyl (0.468) and teflubenzuron (1.054%) (Soleiman et al. 2013). This conflict in results may be related to different chemicals used in

laboratory experiments of Soleiman et al. (2013) and different methodology of these studies.

The parasitoid wasp, *E. inunctus*, could parasitize up to 19% of SATP larvae. Therefore, the parasitoid wasp alone is not sufficient to control the SATP population in a greenhouse. Possibly the greater release rate of the parasitoid wasp is required for more efficient biological control of the pest in greenhouse tomato. Certainly, integrated application of insecticide (biorational or chemical) with inundate releases of *E. inunctus* is required for sufficient SATP population suppression.

Several commercially available biorational insecticide companies claim that their products are not disruptive to beneficial arthropods. However, research conducted worldwide has shown that biorational insecticides may, in fact, be harmful to certain natural enemies (Biondi et al. 2012a, b, 2013). Although biorational insecticides may not be directly toxic to a particular natural enemy, there may be indirect effects such as delayed development of the host and natural enemy inside, delayed adult emergence and/or decreased natural enemy survivorship (Croft 1990). There is variability apparent in the compatibility of natural enemies to biorational insecticides based on the type of biorational pesticide, whether the natural enemy is a parasitoid or predator, and their developmental stage. Biorational insecticides are effective to control many different types of greenhouse pests (Glare et al. 2012). However, it is important to know which biorational insecticide/miticide is compatible or not compatible with natural enemies in order to avoid disrupting successful biological control programmes (Biondi et al. 2012a, b).

Our results indicated that chlorfenapyr, thiocyclam and azadirachtin have adverse effects on parasitism of SATP larvae by *E. inunctus*. The larval parasitism significantly in Bt treatment was more than other insecticide treatments. Therefore, our finding showed that Bt is the best candidate for integrated control of SATP infestation with *E. inunctus* release. A few studies were conducted on the integrated use of natural enemies and biorational or synthetic insecticides against *T. absoluta* (Mollá et al. 2011; Arnò and Gabarra 2011; Zappalà et al. 2012; Biondi et al. 2013). Similarly, high toxicity of chlorfenapyr on some parasitoid wasps was previously reported (Pietrantonio and Benedict 1999, 1999; Haseeb and Amano 2002; Kapuge et al. 2003; Haseeb et al. 2005; Wang et al. 2014). Also, it was demonstrated that thiocyclam was highly toxic for parasitoids of citrus leaf miner, *Phyllocnistis citrella* Stainton. In contrast to our findings, Ibrahim and Kim (2008) prove that thiocyclam had less toxicity for *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae). The different parasitic wasp may be a major reason of the conflict in results. In the other hand, *D. isaea* is a widely used biocontrol agent (Saad et al. 2007; Ibrahim and Kim 2008; Abou-Fakhr Hammad and

Mc Auslane 2010) and it may have developed resistance to some chemicals.

Effects of azadirachtin-based insecticides on eulophid parasites were studied by some authors. For example, azadirachtin considered as a harmless insecticide for *Tamarixia triozae* Burks (Hymenoptera: Eulophidae) (Luna-Cruz et al. 2015), *D. mollipla* Holmgren (Akol et al. 2001), *Colpoclypeus florus* Walker (Brunner et al. 2001), *Diglyphus isaea* (Saad et al. 2007) and *D. isaea* (Abou-Fakhr Hammad and Mc Auslane 2010). In agreement with our results, a high toxic effect of azadirachtin on the other species of the family, *Tamarixia radiata* Waterston, was reported by Santos et al. (2015). Biondi et al. (2013) showed that azadirachtin did not have a lethal impact on females of *Bracon nigricans* Szépligeti. However, it causes important delays in population growth.

Hamel (1977) showed that parasitism by *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae) and *Glypta fumiferanae* Vier. (Hymenoptera: Ichneumonidae), parasitoid wasps of first instar larvae of *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), was significantly higher in treatment blocks following Bt application. But parasitism by *Phaenogenes hariolus* Cresson (Hymenoptera: Ichneumonidae) and *Ceromonia auricaudata* Tns. (Hymenoptera: Ichneumonidae)—parasitoids of late instars and pupae of the pest—was significantly lower following treatment (Hamel 1977). Chilcutt and Tabashnik (1999) showed that there was no effect of Bt treatment on oviposition by *C. plutella*. Similar to our findings, Brunner et al. (2001) demonstrated that Bt products cause no toxicity to *C. florus*. Brunner et al. (2001) suggested that Bt products cause no toxicity to *C. florus*. Similarly, Haseeb et al. (2004) proved that field dosage of Bt causes low adult mortality of *C. plutella*.

Several biocontrol agents and integrated pest management programmes (IPMs) have been recently evaluated for control of SATP (Zappalà et al. 2012). Biocontrol agents (predators, parasitoids or pathogens) are considered one possible solution to the *T. absoluta* crisis (Desneux et al. 2010). This strategy offers a more sustainable and less expensive alternative to chemicals (Urbaneja et al. 2013).

In conclusion, chlorfenapyr was the best insecticide for control of SATP larvae and its damage to tomato leaves and fruits. But the insecticide was not compatible with parasitoid wasp, *E. inunctus*. Among the insecticides tested, Bt was more compatible with *E. inunctus* release. Also, Bt had no significant short-term effect on SATP population. Therefore, integrated application of Bt with early inundate release of *E. inunctus* was recommended for suitable and environmentally safe control of SATP in greenhouse tomato. The results of this study can be used in the IPM programme of tomato greenhouses.

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