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Impact of combine releases of the egg parasitoid, *Trichogramma euproctidis* (Girault) and the entomopathogenic nematode, *Heterorhabditis bacteriophora* to control *Tuta absoluta* (Meyrick) in tomato greenhouses in Egypt

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Abstract

In greenhouses, tomato plants are subject to attack by several pest species. The present study aimed to investigate the compatibility of releasing the egg parasitoid, *Trichogramma euproctidis* (Girault) (Hymenoptera: Trichogrammatidae), and the entomopathogenic nematode, *Heterorhabditis bacteriophora*, strain HP88 against the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in commercial tomato greenhouses (Cherry and Bushra varieties) at winter plantation of 2018–2019 in Egypt. Three commercial plastic greenhouses were used. The first plastic greenhouses were treated by the two tested biological control agents (BCG), the second sprayed by certain recommended pesticides (PG), and the third used for control. Pheromone traps were used for monitoring the appearance of *T. absoluta* moths. The combined use of *T. euproctidis* and *H. bacteriophora* resulted to reduce the population density of the *T. absoluta* gradually until the end of the season in BCG. In the 17th week of treatments by *T. euproctidis* and *H. bacteriophora*, the population density of *T. absoluta* was estimated as leaf mines/plant (0.8 and 1.26 mines/leaf, in Cherry and Bushra varieties, respectively) in BCG. Also, in the 17th week of treatments by recommended pesticides, the population density of *T. absoluta* reached 12.73 mines/leaf and 18.33 mines/leaf, in CG. Early use of pesticides, by the appearance of *T. absoluta* infestation, could not suppress its population density that continued to increase until the end of the season in PG. Results revealed that the combination of the tested biological control agents against *T. absoluta* is recommended to be a main part in pest management practices in tomato greenhouses.

Keywords: Biological control, Pesticides, Tomato, *Trichogramma euproctidis*, *Heterorhabditis bacteriophora*, *Tuta absoluta*, Greenhouses

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Background

Tomato crop (*Solanum lycopersicum* L.) is one of the economical vegetable crops in Egypt. Tomato plants are attacked by several pests causing losses in tomato quality and quantity. The tomato leaf minor, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), the tobacco whitefly, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae), and the red spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae) are the major economic pests attacking vegetable crops in greenhouses. *T. absoluta* has become one of the major devastating insect pests attacking tomato in many of the tomato-producing regions worldwide (Desneux et al. 2010). It is originated from South America and rapidly invaded various European countries and spread along the Mediterranean Basin including Egypt (EPPO 2017). The pest causes several damages by larvae feeding on leaves, flowers, stalks, and fruits of tomato plants, causing economic losses 80–100% (Desneux et al. 2010). Females of *T. absoluta*, usually, deposit their eggs on leaves. Its four larval instars are living in the mesophyll tissue of leaves making tunnels where they feed and develop. Pupae are principally found in soil but may also occur on the plants (Urbaneja et al. 2007).

Pesticides are frequently used for controlling the greenhouses' pests, but the risk of developing resistance has been demonstrated by various studies (Campos et al. 2014). To solve this problem, integrated pest management (IPM) is better to be emphasized, by using different methods such as biological control agents; the egg parasitoids *Trichogramma* spp. and the entomopathogenic nematodes (EPNs) for insect's control (Agamy 2003; Koppenhöfer and Kaya 1998).

Entomopathogenic nematodes (EPNs) are usually located in the soil around their hosts in response to their carbon dioxide, insect movement, and insect feces (Kaya and Gaugler 1993). Two families (Heterorhabditidae and Steinernematidae) have been effectively used as biopesticides in IPM programs (Grewal et al. 2005). The infective juvenile stage (IJ) is the only free living stage of EPNs. The 2 families are mutualistic associated with bacteria of the genus *Photorhabdus* and *Xenorhabdus*, respectively, that are responsible for killing their target insects within 24–48 h by septicemia (Ferreira and Malan 2014).

Using of more than one bioagent is also a recent topic in biological control, because the crop can be affected by several pests simultaneously. The combining use of bioagents is expected to increase the efficacy of their role (Lucas and Alomar 2002).

The present study was designed to evaluate the impact and compatibility of releasing the egg parasitoid, *Trichogramma euproctidis* (Girault 1911), with the application of heterorhabditis nematode, *Heterorhabditis bacteriophora*, strain HP88 against *T. absoluta* in commercial tomato greenhouses.

Materials and methods

Greenhouses

The experiment was carried out at the protected cultivation Experimental Station of the Ministry of Agriculture at Dokki, Giza, Egypt, using 3 commercial plastic greenhouses. Each greenhouse measured 400 m^2 (50 m × 8 m) each was planted on the 5th of October 2018, as tomato winter plantation. Almost 1000 tomato seedlings were planted (Cherry variety on 2 rows and Bushra variety on 3 rows). One of these greenhouses was used for releasing biological control agents (BCG). The second was for spraying commercial-recommended pesticides (PG). The third was the control, without any treatment (CG). Daily data of minimum and maximum temperatures and relative humidity were obtained from the Central Laboratory for Agriculture Climate (CLAC), Dokki, Giza, Egypt.

Sources of the parasitoid and nematode

The egg parasitoid, Trichogramma euproctidis

The egg parasitoid, *T. euproctidis*, was obtained from the Mass Rearing Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. *T. euproctidis* was reared on sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in a controlled climatic room at 25 ± 2 °C, 70% R.H., and 16:8 L:D photoperiod.

Entomopathogenic nematode, Heterorhabditis bacteriophora

The EPN species, *H. bacteriophora*, strain HP88 was obtained from Randy Gaugler, Rutgers University, New Brunswick NJ, USA. The strain was reared in vivo on the full-grown larvae of the greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) following the method of Dutky et al. (1964). The rearing of *G. mellonella* was carried out in accordance with the methodology adapted by Dutky et al. (1964), using an artificial diet modified by Huang et al. (2010).

Experimental design

In the BCG, the release of the egg parasitoid, *T. euproctidis*, and the nematode, *H. bacteriophora*, alternated weekly. Each release of *T. euproctidis* included 40 hangable packs hanged on the branches. Each displayed 250 parasitized *E. kuehniella* eggs/rectangle. These packs had a 48 h different age, in order to obtain 2 waves of emerging parasitoids on the tomato plant.

The releasing rate of *H. bacteriophora* (strain HP88) was 100 IJs/ml sprayed on the whole plant and soil at each release. The application of *H. bacteriophora* on the soil would control last larval instar, when they slide down from the leaves to pupate in soil, as well as emerging adults from the buried pupae. This strain was selected because of its tolerance to high temperatures inside the greenhouse (Nouh et al. 2016).

After releasing the EPN, some of the infected *T. absoluta* larvae were collected. The diseased larvae were those gave the characteristic dark color confirming nematode infection by the released nematodes. Infective juveniles were harvested, using White traps according to White (1927).

In the PG, the recommended pesticides were applied according to the application program. Three pesticides: Vertimec 1.8% EC, Micronized Sulfur and Mospilan 20% SP were applied 8 times throughout the season.

In the CG, for control, no treatments were used.

Sampling

Sampling took place, using pheromone traps to capture the male moths of *T. absoluta* and monitoring the presence of the pest in the greenhouse. The pheromone traps were exchanged every 45 days. The number of captured moths was recorded weekly. Population densities of *T. absoluta* (represented by larval mines), starting the 3rd week post-planting date. Thirty random plants were inspected/variety/sampling date. The population of each pest species was estimated at 3 leaves represented (top, middle, and low level)/plant. A pre-count of studied pest was estimated in both greenhouses (BCG and PG) before treatments.

The samples of the leaves infested with *T. absoluta* larvae were collected regularly and transferred to the laboratory for dissection and confirmation of mortality caused by the presence of the nematode.

Statistical analysis

Data were statistically analyzed using two-way ANOVA. Tukey's HSD test at 0.05 level was preformed to analyze the significance between means. The data was statistically analyzed by correlation analysis between weather parameters and pests' population.

Results and discussion

In the greenhouses, overall weekly means of maximum and minimum temperatures and RH ranged 16.76–26.37 °C for maximum and 4.91–11.9 °C for minimum temperatures and 49.75–84.29% for RH. Maximum temperature showed a negative correlation (r = – 0.59, – 0.67, – 0.87, and – 0.81) with *T. absoluta* population, in BCG, PG, and CG, respectively.

Minimum temperature showed, also, a negative correlation (r = – 0.014, – 0.23, – 0.131, and – 0.077) with *T. absoluta*, in BCG, PG, and CG, respectively.

On the contrary, the relative humidity showed positive correlation (r = 0.502, 0.607, 0.511, and 0.479) with *T. absoluta*, in BCG, PG, and CG, respectively.

Pheromone traps

Pheromone traps were used to monitor the appearance of moths to start control. The number of male moths of *T. absoluta* caught per pheromone trap in the greenhouses fluctuated throughout the experimental period (Fig. 1). The number of male moths was captured at the

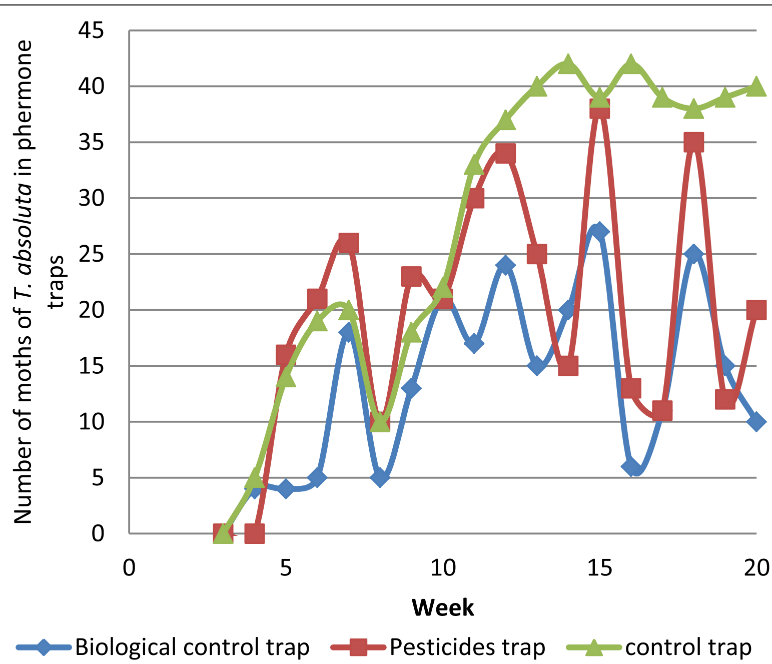

Fig. 1 Number of male moths of *Tuta absoluta* in pheromone traps in tomato greenhouses in the winter plantation, 2018–2019

beginning of the crop growing season as low population. The first appearance was by 4, 0, and 5 moths/trap in the 4th week in BCG (*T. euproctidis* and *H. bacteriophora*) and CG, respectively, and 16 moths/trap in the 5th week for PG.

The number of moths in the traps increased to reach its maximum in the 15th week in BCG and PG and in the 14th week in CG, where it reached 27, 38, and 42 moth/trap, respectively.

ANOVA revealed significant differences among the numbers of moths in the traps (F = 15.54, $p > 0.05 = 1.16 \times 10^{-05}$). The significant difference was between BCG and CG.

Sex pheromone is usually used as indicator to start the control of the pest. Prasad and Prabhakar (2012) confirmed the importance of using the sex pheromones monitor, forecast, or control of the moth population pests.

Also, Goda et al. (2015) recorded a high reduction of infestation with *T. absoluta* on tomato crop in Egypt when used pheromone traps for monitoring mass trapping and release of the egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hym.: Trichogrammatidae). Awad et al. (2018) reported that the population of *T. absoluta* moths in pheromone traps increased in warm climates in winter evergreen tomato during October and November (19–22 °C) and in the summer evergreen during May and June (23–26 °C).

Control of the tomato leaf miner, *T. absoluta*

In BCG, after occurrence of the moths in the pheromone traps, the release of the parasitoid, *T. euproctidis*, and the nematode, *H. bacteriophora*, was alternated weekly starting from the 5th week to the 17th week on both Cherry and Bushra varieties.

The maximum mean number of mines in the BCG was 6.13 and 7.4 mines/leaf in the 13th and 14th weeks in Cherry and Bushra varieties, respectively. After that, the population density of *T. absoluta* started to decrease gradually until the end of the season. In the 17th week, the population density reached 0.8 and 1.26 mines/leaf, in the mentioned varieties, respectively (Fig. 2).

The collected *T. absoluta* larvae, which are infected with the EPN, were placed in white traps to produce the infective juveniles of *H. bacteriophora*.

In PG and CG greenhouses, *T. absoluta* occurrence started from the 5th week in a low population. The population density reached 0.67 and 0.433 mine/leaf in PG and CG respectively. While the application of the pesticides started from week 2. Three pesticides: Vertimec 1.8% EC, micronized sulfur, and Mospilan 20% SP were applied 8 times for pests' control (Table 1). The population density of *T. absoluta* continued to increase gradually up to the end of the season in both greenhouses for PG and CG, where the population density of the pest represented by 12.73 and 18.33 mines/leaf, respectively, in the 17th week (Fig. 2).

The combination between *T. euproctidis* and *H. bacteriophora* succeeded to reduce the population density of the *T. absoluta* gradually until the end of the season. However, in spite of the early use of the pesticides before the appearance of the *T. absoluta*, the population density continued to increase up to the end of the season.

ANOVA revealed significant differences among the mean number of mines of *T. absoluta* in the 3 greenhouses (F = 12.76, $p > 0.05 = 1.73 \times 10^{--06}$). The

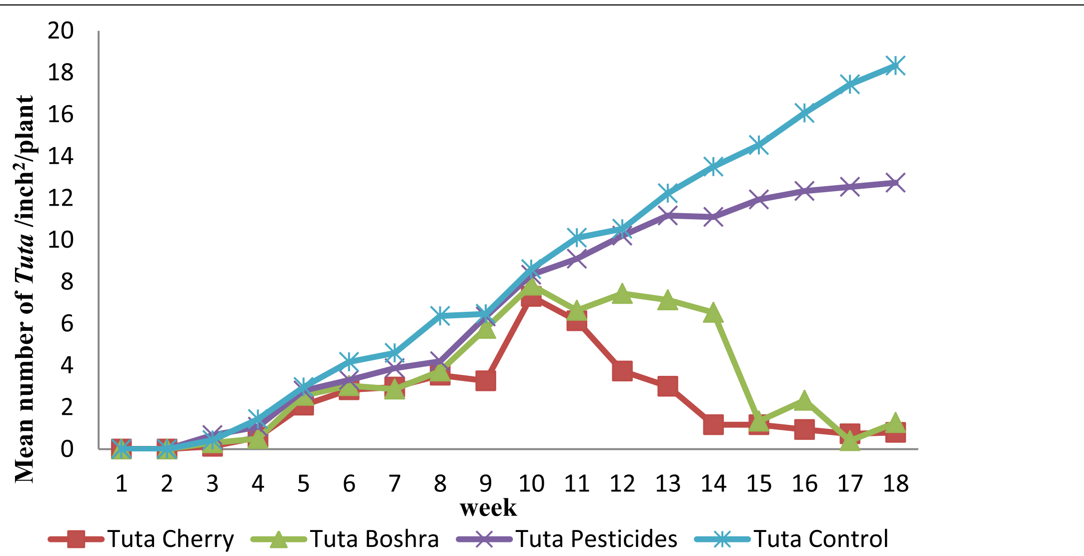

Fig. 2 Mean number of mines of *Tuta absoluta* larvae in cherry, Boshra, pesticides and control greenhouses in the winter plantation, 2018–2019

Table 1 Weekly releases and rates of biological control agents in biological greenhouses and pesticides application in chemical greenhouse and their rates, in tomato winter plantation 2018–2019

Week	Biological control		Chemical control	
	Release	Rate	Pesticide	Rate
2	-	-	Vertimec 1.8% EC	25 cm/100 l
3	-	-	Micronized Sulfur	250gm/100 l
4	-	-	-	-
5	*T. euproctidis*	50 parasitoids/m^2	Mospilan 20% SP	25gm/100 l
6	*H. bacteriophora*	100 IJs/ml	Vertimec 1.8% EC + micronized sulfur	100 cm/100 l + 250gm/100 l
7	*T. euproctidis*	50 parasitoids/m^2		
8	*H. bacteriophora*	100 IJs/ml	Mospilan 20% SP	25gm/100 l
9	*T. euproctidis*	50 parasitoids/m^2	Mospilan 20% SP	25gm/100 l
10	*H. bacteriophora*	100 IJs/ml		
11	*T. euproctidis*	50 parasitoids/m^2	Vertimec 1.8% EC	100 cm/100 l
12	*H. bacteriophora*	100 IJs/ml		
13	*T. euproctidis*	50 parasitoids/m^2	Vertimec 1.8% EC	100 cm/100 l
14	*H. bacteriophora*	100 IJs/ml		
15	*T. euproctidis*	50 parasitoids/m^2		
16	*H. bacteriophora*	100 IJs/ml		
17	*T. euproctidis*	50 parasitoids/m^2		
18	-	-		

significant differences were found between Cherry and PG, Cherry and CG, and Bushra and CG.

In a survey study, Oliveira et al. (2017) found that *Trichogramma achaeae* caused natural rate of parasitism reached to 2.5% of *T. absoluta*. The same authors studied the rate of parasitism with *T. achaeae* and *T. cordubensis* Vargas & Cabello against *T. absoluta* in caged tomato plants (microhabitats). Their results revealed that *T. achaeae* had a high parasitism and emergence rates (29.6 and 65.9%, respectively), relative to those rates observed by *T. cordubensis* (6.1 and 39.3%, respectively).

The present study results agree with the previous studies which revealed that *T. euproctidis* when released at high rate can control *T. absoluta.*

El-Arnaouty et al. (2014) compared the efficiency of 2 different *Trichogramma* species. The indigenous, *T. euproctidis* and the cosmopolitan *T. achaeae* Nagaraja & Nagarkatti, for the biological control of *T. absoluta* in protected tomato cultivations in Egypt (growing seasons: 2011/12 and 2012/13). Both *Trichogramma* species were significantly efficient, especially at high rate (75 parasitoids/m^2), keeping low *T. absoluta* mines during both experimentation years. Kortam et al. (2017) indicated that both parasitoids *T. euproctidis* and *T. achaeae* were, significantly, efficient in keeping down the tomato leaf miner *T. absoluta*, especially at high relative humidity. Asma et al. (2019) found that the species *T. cacoeciae* (Marchal) is a promising, ecofriendly management tactic against *T. absoluta* in the largest tomato-producing area in Tunisia. Results indicated that 20 *Trichogramma* species/plant was the most effective release rate that caused significantly decreasing the pest's life stage densities.

The decrease of the *T. absoluta* population by using of the 2 bioagents *T. euproctidis* and *H. bacteriophora* compatible with the previous studies which confirmed that the multi-use of natural enemies has showed an increase in their efficacies for controlling specific pests and for reducing the risk of virus infection (Perdikis et al. 2008).

Mustapha et al. (2018) evaluated the efficacy the biopesticides: azadirachtin, *Bacillus thuringiensis*, *Steinernema feltiae*, and *Beauveria bassiana* individually and in combination against *T. absoluta* under laboratory and greenhouse conditions. Their results indicated that the biopesticides, except *S. feltiae*, can contribute in *T. absoluta* control in greenhouse tomato crops, in particular, the combined use of Azadirachtin with *B. thuringiensis* or *B. bassiana* provided the highest level of control of the pest. The potential for including these biopesticides in an overall sustainable integrated pest management programme for *T. absoluta* was discussed.

Sabino et al. (2018) investigated the susceptibility of combined EPN, *H. amazonensis* JPM4 with different percentages of insecticides to control *T. absoluta* in tomato crop under laboratory conditions and in the greenhouse. Their results suggested that the preventive use of systemic insecticides applied on the soil combined with applications of *H. amazonensis* can be a strategy to control

T. absoluta, aiming to reduce the inappropriate use of insecticides.

Conclusion

Using pheromone traps to monitor pest occurrence and releases of combining specific bioagents (*T. euproctidis* and *H. bacteriophora*) succeeded to decrease the population density of *T. absoluta* infesting tomato plants under greenhouse conditions. Using and combining biological control agents to control certain pest such as *T. absoluta* is recommended to be a main part in pest management practices in tomato greenhouses.

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Author's contribution

All of the authors of this manuscript contributed equally to the design and/ or execution of the experiments described in the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

All data are available in the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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