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BIOLOGICAL CONTROL

Humic Fertilizer and Vermicompost Applied to the Soil Can Positively Affect Population Growth Parameters of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) on Eggs of *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Keywords

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Abstract

The tomato leaf miner, *Tuta absoluta* (Meyrick), is a devastating pest of tomato worldwide. One of the control measures of *T. absoluta* is the use of biological control agents, such as *Trichogramma* wasps. Interactions between natural enemies and insect pests may be affected by application of fertilizers, because changes in plant quality through the fertilizer application may therefore affect herbivore characteristics and suitability of them to parasitism. Laboratory tests were carried out to evaluate the life table parameters of *Trichogramma brassicae* Bezdenko on *T. absoluta* eggs reared on tomato plants treated either with vermicompost (40%), humic fertilizer (2 g/kg soil), or control (suitable mixture of field soil and sand). Population growth parameters of *T. brassicae* were affected by fertilizer treatments. Significant differences were found for immature life period and total fecundity of *T. brassicae* on the treatments. Differences of intrinsic rate of natural increase (r_m), finite rate of increase (λ), net reproductive rate (R_o), mean generation time (T), and doubling time (DT) of *T. brassicae* among treatments were also significant. The lowest values of r_m, λ, and R_o were recorded for *T. brassicae* developed on *T. absoluta* eggs on control treatment, whereas the highest values of these parameters were observed on 2 g/kg humic fertilizer. Furthermore, *T. brassicae* had the shortest T and DT values on 2 g/kg humic fertilizer and 40% vermicompost treatments. Our results showed that application of humic fertilizer and vermicompost could positively affect population growth parameters of *T. brassicae* on eggs of *T. absoluta* fed on tomato plants.

Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the major pests that attack tomato plants both in greenhouse and open field cultivations (Urbaneja *et al* 2007, Desneux *et al* 2010). It was originally widespread in South America and rapidly invaded Southern Europe and North Africa (Urbaneja *et al* 2007, Potting 2009, Desneux *et al* 2010). In Iran, it was first reported in 2010 and now is a key pest of tomato (Baniameri and Cheraghian 2011). It also infests other solanaceous crops such as eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), sweet pepper (*Solanum muricatum* L.), and tobacco (*Nicotiana tabacum* L.), as well as non-cultivated Solanaceae plants (Desneux *et al* 2010). Economic losses caused by *T. absoluta* are due to direct damage by larval feeding on leaves, terminal buds, flowers, and fruits and indirect damage to fruits by scorching after defoliation (Souza and Reis 1992).

Management of *T. absoluta* is quite challenging because the larval instars live inside leaves, fruits, and stems. Furthermore, due to its higher reproductive capability and short generations, it has developed resistance to many insecticides (Lietti *et al* 2005, Silva *et al* 2011). Extensive and improper use of insecticides by tomato growers led to a multitude of undesired side effects on ecosystem and non-target organisms (Biondi *et al* 2012). There is a real need to improve crop protection against *T. absoluta* and in the meanwhile reducing the use of synthetic insecticides. Several biological control agents and integrated pest management (IPM) programs have recently been evaluated to control of *T. absoluta* (Chailleux *et al* 2013, Zappalà *et al* 2013, Abbes *et al* 2015, Jaworski *et al* 2015, Sylla *et al* 2016). Biological control, as an environmentally and economically sound method, may be effective to control this invasive pest (Urbaneja *et al* 2012). Parasitoids are a very important component of the natural enemy complex of *T. absoluta* and have been the most common biological control agent of this pest (Ghoneim 2014). Parasitoids belonging to the *Trichogramma* genus (Hymenoptera: Trichogrammatidae) have been used in tomato crops against *T. absoluta* through inundative releases mainly *Trichogramma pretiosum* Riley (Parra and Zucchi 2004, Pratissoli *et al* 2005) and *Trichogramma achaeae* Nagaraja and Nagarkatti (Cabello *et al* 2012, Calvo *et al* 2012). The most widespread *Trichogramma* species in Iran is *Trichogramma brassicae* Bezdenko (Azema and Mirabzadeh 2005), which has been used worldwide for the control of lepidopterous pests such as *T. absoluta* (Potting *et al* 2013, Ghoneim 2014, Ahmadipour *et al* 2016).

Host selection by parasitoids may be influenced by the characteristics of both the host and its food plant (Chau and Mackauer 2001, Desneux and Ramirez-Romero 2009). The performance and preference of parasitoids can be influenced by plant quality as a direct response to plant chemistry and also as an indirect response to changes in herbivore size or survival (Fox *et al* 1996, Dicke *et al* 2003). Nutritional or allelochemical variation in hosts or their food plants may modify the dynamics of parasitoid-host interactions by influencing growth and survivorship of immature parasitoids (Barbosa 1988) and oviposition choices of adults (Williams *et al* 1988). It is known that soil fertility management may affect plant quality and may therefore affect pest abundance (Arancon *et al* 2005, 2007; Yildirim and Unay 2011, Razmjou *et al* 2011, 2012). Organically fertilized crops generally exhibit lower densities of several insect herbivores, but the direct linkage to fertility is confounded by the increased abundance of natural enemies compared with conventional practices (Altieri *et al* 2005). Fertilizer type may alter aspects relating to the suitability of a host for a parasitoid to survive and develop in, such as the host size and nutritional value, its immune and defense responses or its ability to survive an initial attack (Pope *et al* 2012). The use of organic amendments to soil can supply a more balanced source of nutrition for plant growth, since the organic matters gradually degrade by microorganisms and the available nutrients of these materials are released with lower mineralization (Patriquin *et al* 1995, Zink and Allen 1998).

Vermicompost is finely organic material and contains readily available nutrients for plants, plant growth hormones, and humic acids; therefore, it can improve plant health and tolerance to pests and diseases (Atiyeh *et al* 2000, Arancon *et al* 2004, 2005, 2007). Humic substances as the major component of soil organic matter have beneficial effects on physical, chemical, and microbiological properties of the soil and can improve growth of a variety of crops (Varanini and Pinton 1995, Chunhua Liu *et al* 1998, Arancon *et al* 2003), as well as enhance the resistance of plants to insect attacks (Jackson 1993, Yildirim and Unay 2011). Vermicompost and humic fertilizer positively contribute to plant quality. Vermicomposts augment the quality of plants by providing greater availability of mineral nutrients, and humic fertilizers help plants to absorb other minerals (Arancon *et al* 2003, Yildirim and Unay 2011). Changes in plant nutritional quality may therefore affect herbivore characteristics and suitability of them to parasitism. Some researches reported a high parasitoid population and parasitism level on plants amended by vermicompost (Suryawan and Reyes 2006) and other organic fertilizers (Ponti *et al* 2007, Pope *et al* 2012).

The aim of the present study was to evaluate the effect of organic substrates (vermicompost and humic fertilizer) on the biocontrol potential of *T. brassicae* on *T. absoluta* through the study of the population growth attributes of the parasitoid. We hypothesized that the use of such fertilizers would positively affect parasitoid populations on *T. absoluta*.

Material and Methods

The experiments were conducted during 2016 (July–September) in the greenhouse and in the laboratory of Plant Protection Department, Faculty of Agriculture, Miyaneh Azad University, East Azarbaijan province, Iran. The cattle manure vermicompost was obtained from AnoosheAaraab Co. Ltd., Tehran, Iran. The chemical properties and nutrient composition of vermicompost used in this study are shown in Table 1. Also, the trade humic composition named Perl Humus (containing 60% humic acid, 1% N, 0.2% P, and 0.3% K) was obtained from Bazargankala Company, Tehran, Iran. The soil used to prepare potting media in control treatment or in combination with vermicompost and humic fertilizer was collected from a tomato field located at Miyaneh, East Azarbaijan province, Iran. Chemical properties and nutrient composition of soil were determined (PH = 7.17, EC = 4.86 (ds/m), 0.08% N, 0.0058% P, 0.0676% K).

Table 1 Chemical properties and nutrient measurement of vermicompost used in the experiments.

PH	EC (ds/m)	N%	P%	K%	Ca%	Mg%	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Pb mg/kg	C/N	OM%	OC%
7.64	1.12	1.55	0.4	0.4	2.73	0.95	5000	275	20	110	19	21.25	56.8	32.9

Analysis of vermicompost samples was conducted by Soil and Water Research Institute of Karaj, Iran.

Host plant

The seeds of tomato, *Lycopersicon esculentum* Mill var. Urbana 9090, were planted in plastic pots (8 cm diameter × 10 cm height) filled with soil, according to the treatments. The plants were kept in a greenhouse at 19–28°C, 50–60% RH, and the natural photoperiod before using in the experiments. Tomato seedlings at the six- to eight-leaf stages were used for experiments.

Insects

The laboratory colonies of *T. absoluta* were established with larvae collected from a commercial tomato (var. Super Chief) plantation located at Miyaneh, East Azarbaijan province, Iran. The colony of *T. absoluta* was reared in the laboratory at 25 ± 2°C, 65 ± 5% RH, and 16:8 h (L/D) on tomato seedlings. The larvae were added with tomato seedlings in cages. Pupae were collected from leaves of tomato plants and were housed in plastic cages (8 × 6 × 4 cm) until the emergence of adults. When at least five pairs of adults had emerged, they were put in clear plastic cage (20 × 20× 30 cm) prepared with tomato seedlings as egg substrates. A piece of cotton saturated in a 10% sugar solution was placed in each cage for feeding the adult moths. The eggs on the tomato seedling were reared until pupation.

Population of *Trichogramma* wasps was collected from the same commercial tomato (var. Super Chief) plantation that we collected *T. absoluta* larvae (Miyaneh, East Azarbaijan province, Iran) by using trap cards with eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). Based on morphometric analysis such as genitalia shape, collected wasps were identified as *T. brassicae*. Antennal characters were used to separate female and male individuals. The colony of *T. brassicae* was reared in glass vials (15 × 150 mm) on eggs of *S. cerealella* as factitious host that were obtained in commercial insectary in Guilan province, Iran. Adults of *T. brassicae* were fed on droplets of 20% honey/water. Eggs of *S. cerealella* were glued on a piece of cardboard (10 by 50 mm) with 10% Arabic gum (Pizzol *et al* 2010). Parasitoids were maintained for at least three generations at 25 ± 2°C, 65 ± 5 RH, and a photoperiod of 16:8 (L/D) on *S. cerealella* eggs before starting experiments.

Experiments

To study the development and fecundity of *T. brassicae* on eggs of *T. absoluta*, the experiments were conducted in a growth chamber at 25 ± 2°C, 65 ± 5 RH, and a photoperiod of 16:8 h (L/D) by using a randomized complete block design with three treatments. Treatments were tomato grown in the soil amended with 40% vermicompost, tomato grown in the soil contained 2 g/kg humic fertilizer, and control plants grown in a mixture of soil (2:1 field soil and sand, respectively) without any fertilizer. In previous study, we found that the addition of 2 g/kg humic fertilizer and 40% vermicompost to the growing soil of tomato plants improved growth parameters of tomato seedlings (plant height, wet weight, and dry weight) compared with other rates of these fertilizers and control treatment. Also, these rates of fertilizers negatively effected *T. absoluta* populations compared with other treatments (decreased R_0, r_m, λ and increased T, DT values) (Mohamadi *et al* 2017).

The experimental units were plastic box (8 × 6 × 4 cm) with a hole (2 cm diameter) in the lid, which was covered by fine-mesh net for ventilation. In each box, one leaf (3 cm diameter), with its petiole maintained in moist cotton wool, was placed in center. Each leaf surface contained ten eggs (0–24 h old) of *T. absoluta*. For each experimental unit, one 24-h-old mated female wasp (fed on drop of 20% honey/water on the inner walls) was released from the colony on *T. absoluta* eggs on tomato leaves and left for 12 h inside a ventilated box. This box was then sealed with Parafilm and placed in the growth chamber. At the end of that period, the females were withdrawn with the aid of stereoscopic microscope from experimental units and each set of experimental unit was transferred to growth chamber. Parasitized eggs were monitored daily and female emergence date was checked precisely. We used at least 100 parasitized eggs for each treatment, and the parameters were duration of egg-adult period, viability, and sex ratio (% of female) of *T. brassicae* on eggs of *T. absoluta*.

Twenty females (per treatment) that were developed on *T. absoluta* eggs were separately released in experimental units sealed with Parafilm containing honey droplets on the inner walls to feed the adults. One male was released in each experimental unit for mating. Tomato leaves with 30 *T. absoluta* eggs were introduced into the boxes daily for each individual female. To determine the number of parasitized eggs, the number of black eggs was counted after 5 days of exposing them to the female parasitoid. The following parameters were evaluated: number of daily parasitized eggs, total number of eggs parasitized by female, and female longevity. Life table parameters of *T. brassicae* on eggs of *T. absoluta* at three treatments were determined using the data obtained from biology and parasitism capacity.

Statistical analysis

Normality of data was tested by the Kolmogorov-Smirnov method. All data of survivorship, duration of immature stages, ovipositon period, pre-and post-oviposition period, adult longevity, and fecundity of *T. brassicae* were analyzed by one-way analysis of variance (ANOVA) using the SPSS ver.16.0 (SPSS 2007) statistical software. When differences among treatments were significant, comparison among means were conducted using Tukey's test at $\alpha = 0.05$. Hatching percentage and sex ratio were compared by the chi-square test using the SPSS ver.16.0 (SPSS 2007) statistical software. Differences in stable population growth parameters including intrinsic rate of increase (r_m), net reproductive rate (R_o), mean generation time (T), doubling time (DT), and finite rate of increase (λ) values and their standard errors were tested through the Jackknife procedure (Maia *et al* 2000) using the SAS System Software ver.8.2 (SAS Institute 2001). The mean values were compared by Tukey's test with the SPSS ver.16.0 (SPSS 2007) statistical software.

Results

Effect of fertilizer treatment on developmental period and adult longevity of T. brassicae

There were significant differences for immature life period of *T. brassicae* on eggs of *T. absoluta* from tomato plants grown with different fertilizer treatments ($F = 9.882$; df = 2, 203; $P = 0.000$). The longest values of this parameter was recorded for *T. brassicae* on eggs of *T. absoluta* reared on control plants, and the shortest values were observed for *T. brassicae* on eggs of *T. absoluta* reared on plants grown in the soil contained 2 g/kg humic fertilizer and 40% vermicompost. No pre-reproductive period was observed and reproduction starts at the first day for all treatments. Reproductive period ($F = 0.522$; df = 2, 57; $P = 0.596$) and post-reproductive period ($F = 0.068$; df = 2, 57; $P = 0.934$) of *T. brassicae* on eggs of *T. absoluta* were not significantly different among treatments. There were also no significant differences for female longevity ($F = 0.288$; df = 2, 57; $P = 0.751$) and female lifespan ($F = 1.285$; df = 2, 57; $P = 0.285$) of *T. brassicae* on eggs of *T. absoluta* on different treatments (Table 2).

Effect of fertilizer treatment on fecundity table parameters of T. brassicae

Effects of fertilizer treatments were not significant for hatching percentage ($\chi^2 = 0.184$; df = 2; $P = 0.912$) and sex ratio ($\chi^2 = 0.144$; df = 2; $P = 0.931$) of *T. brassicae* on eggs of *T. absoluta* (Table 3). Significant differences were observed for the total fecundity (total number of *T. absoluta* eggs parasitized by female) ($F = 16.203$; df = 2, 57; $P = 0.000$) of *T. brassicae* on eggs of *T. absoluta* on plants grown with different treatments, but the mean daily fecundity (number of *T. absoluta* eggs parasitized daily by female) of *T. brassicae* was not significant among treatments ($F = 2.333$; df = 2, 57; $P = 0.106$) (Table 3).

Effect of fertilizer treatment on life table parameters of T. brassicae

The influence of different fertilizer treatments on the population growth parameters of *T. brassicae* on eggs of *T. absoluta* is presented in Table 4. There were significant differences for population growth parameters of *T. brassicae* including: net reproductive rate (R_o) ($F = 45.761$; df = 2, 57; $P = 0.000$), intrinsic rate of natural increase (r_m) ($F = 62.319$; df = 2, 57; $P = 0.000$), and finite rate of increase (λ)

Table 2 Mean (±SE) developmental period and adult longevity (days) of *Trichogramma brassicae* on *Tuta absoluta* eggs reared on tomato plants with different fertilizer treatments.

Period	Treatment		
	Control	Vermicompost (40%)	Humic substances (2 g/kg)
Immature life	13.02 ± 0.08a[a]	12.72 ± 0.08b	12.55 ± 0.06b
Reproductive	2.90 ± 0.12a	3.00 ± 0.10a	3.05 ± 0.09a
Post-reproductive	0.35 ± 0.11a	0.40 ± 0.11a	0.35 ± 0.11a
Adult longevity	3.25 ± 0.14a	3.35 ± 0.15a	3.40 ± 0.13a
Total lifespan	16.35 ± 0.21a	16.05 ± 0.17a	15.95 ± 0.17a

Within the rows, means followed by different letters are significantly different ($P < 0.01$).

[a] For each parameter, differences among treatments were determined by Tukey's test.

Table 3 Mean (±SE) fecundity table parameters of *Trichogramma brassicae* on *Tuta absoluta* eggs reared on tomato plants with different fertilizer treatments.

Parameter	Treatment		
	Control	Vermicompost (40%)	Humic substances (2 g/kg)
Mean total fecundity (eggs/female)	17.55 ± 0.45b[a]	19.80 ± 0.46a	21.15 ± 0.45a
Mean daily fecundity (eggs/female/day)	6.26 ± 0.31a	6.71 ± 0.22a	7.02 ± 0.20a
Hatching percentage	66	71	69
Sex ratio	57.58	60.56	62.31

Within the rows, means followed by different letters are significantly different ($P < 0.01$).

[a] Differences among treatments for mean total fecundity and mean daily fecundity were determined by Tukey's test. Hatching percentage and sex ratio differences among treatments were determined by the chi-square test.

($F = 62.293$; df = 2, 57; $P = 0.000$) among treatments. The highest and lowest values of these parameters were recorded for 2 g/kg humic fertilizer and control treatment respectively. Furthermore, *T. brassicae* had significantly the shortest mean generation time (T) ($F = 3.437$; df = 2, 57; $P = 0.039$) on *T. absoluta* eggs treated with humic fertilizer and significantly the shortest DT ($F = 59.514$; df = 2, 57; $P = 0.000$) on *T. absoluta* eggs treated with humic fertilizer and vermicompost.

Discussion

The possible reason for changes in immature life duration, total fecundity, and some life table parameters of *T. brassicae* on eggs of *T. absoluta* reared on different fertilizer treatments is that parasitoids responded to characteristics of *T. absoluta* eggs that were influenced by plant quality, to properties of the food plants themselves, or to both. Life table parameters are related to the life history parameters such as survival and fecundity. In fact, a life and fertility life table (or fecundity schedule) may be constructed by preparing a life table with x (age), l_x (age-specific survival), and m_x (age-specific fertility) columns (Southwood and Henderson 2000). Therefore, in the current study, shorter immature life duration and higher fecundity in vermicompost and humic fertilizer treatments positively affected the age-specific fertility and then some life table parameters (increased r_m, R_o, λ, and decreased DT values) compared with control treatment. On the basis of parameters that were evaluated in this research (r_m, R_o, λ, and DT were different among treatments), populations of *T. brassicae* on *T. absoluta* eggs were different on plants grown with tested fertilizers compared with control. Life table parameters allow the comparison of the biological performance of an insect in different conditions (Parra 1994). The effectiveness of *Trichogramma* as a biological control agent may depend not only on the biological characteristics of the parasitoid species or strains used but also on their interactions with a specific pest-plant system (Tabone *et al* 2010, Andrade *et al* 2011, Yuan *et al* 2012).

According to the results of this study, population growth of *T. brassicae* increased on *T. absoluta* eggs on plants grown with vermicompost compared with control treatment. Similar results were obtained by Suryawan and Reyes (2006) that reported the highest population and parasitism level of parasitoids of *Liriomyza huidobrensis* Blanchard on potato plants with vermicompost and with vermicompost plus reflective plastic mulch treatments compared with other treatments including standard cultural practice, farmer's practice, and their combinations. The exact reason for

Table 4 Mean (±SE) population growth parameters of *Trichogramma brassicae* on *Tuta absoluta* eggs reared on tomato plants with different fertilizer treatments.

Parameter	Treatment		
	Control	Vermicompost (40%)	Humic substances (2 g/kg)
R_o (eggs)	6.67 ± 0.17b[a]	8.51 ± 0.20a	9.09 ± 0.19a
r_m (day^{-1})	0.145 ± 0.002c	0.167 ± 0.002b	0.173 ± 0.002a
T (day)	13.10 ± 0.12a	12.84 ± 0.09ab	12.73 ± 0.10b
DT (day)	4.78 ± 0.07a	4.15 ± 0.04b	4.00 ± 0.04b
λ (day^{-1})	1.16 ± 0.003c	1.18 ± 0.002b	1.19 ± 0.002a

Within the rows, means followed by different letters are significantly different ($P < 0.01$).

[a] For each parameter, differences among treatments were determined by Tukey's test.

positive effects of vermicomposts on parasitoid population reported here remains unknown, but according to the substantial variation in chemical composition among the soil and the vermicompost, it is possible that organic matter in vermicompost improved plant quality and therefore affected herbivore nutritional value or other characteristics that may change the performance of parasitoid. Even in previous study, we found that vermicomposts improved growth parameters of tomato plants compared with control treatment (Mohamadi *et al* 2017). There is not a lot of research about effects of vermicomopsts on populations of natural enemies (specially on parasitoids), and only a small number of studies have investigated the effects on performance of predators (Mottaghinia *et al* 2016, Alizamani *et al* 2017).

Humic substances increased population growth of *T. brassicae* on *T. absoluta* eggs compared with other treatments. It may be attributed to the higher plant quality in response to promoted nutrient uptake of plant that may affect insect pest suitability for parasitoid. In fact in previous study, we found that humic fertilizers improved growth parameters of tomato plants compared with vermicompost and control treatment (Mohamadi *et al* 2017). However, there are no researches about effects of humic substance on the third trophic level especially on parasitoids, but some studies showed that organic fertilizers affect the performance of natural enemies (Ponti *et al* 2007, Pope *et al* 2012).

The type of fertilizer supplied to a crop can influence the nitrogen levels of plant leaves. Fox *et al* (1990) reported that the parasitism rates of *Plutella xylostella* L. by *Diadegma insulare* Cresson were higher on high N plants than unfertilized plants. Application of nutrients in a plant may influence the parasitoid-host interactions by altering morphological and chemical features of that plant (Desneux and Ramirez-Romero 2009). In conclusion, the present study shows that application of humic fertilizer and vermicompost to the growing soil of tomato plants can positively affect population growth parameters of *T. brassicae* as a natural enemy of *T. absoluta*. These findings will be helpful to provide relatively effective biological control of *T. absoluta* when planning integrated management strategy of this pest.

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