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# Parasitization Capability of Four Trichogrammatid Species against the Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Under Different Releasing Regimes

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## ABSTRACT

Tomato leaf miner (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest causing significant loss to tomato production in Egypt. The success of biological control programs against this serious threat in tomato fields using trichogrammatid species release is a challenge, and depends basically on the number of released parasitoids and spacing among release points. In this study, cage and greenhouse experiments were conducted to evaluate the effect of release density and distance from releasing point on the parasitization capability of four trichogrammatid species (*Trichogramma cacoeciae, T. evanescens, T. pretiosum* and *Trichogrammatoidea bactrae*) against TLM. Results indicated that *T. bactrae* was the most effective parasitoid as indicated by higher percentages of parasitism, followed by *T. evanescens, T. cacoeciae* and *T. pretiosum*. In cage experiment, percentage of parasitism increased gradually with the increase of releasing density and reached its maximum at 256 parasitoid females/ cage. *T. bactrae* showed the greatest overall average across all releasing densities at 51.82%, followed by *T. cacoeciae, T. evanescens* and *T. pretiosum* with 34.32, 32.58 and 12.45%, respectively. In greenhouse experiment, the percentages of parasitism decreased with increasing distance from the releasing point. *T. bactrae* was also superior in parasitization compared to the other species at all tested distances, where the percentages of parasitism by *T. bactrae* were 77.08, 65.00, 40.00 and 38.58% at the distances of 1.5, 3, 4.5 and 6 m from releasing point, respectively.

**Key words:** Biological control, Trichogrammatid species, *Tuta absoluta*, Releasing density and distance.

## INTRODUCTION

The tomato leaf miner (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of most devastating insect pests attacking tomato (*Solanum lycopersicon*) in many of the tomato-producing regions worldwide (EPPO, 2005). It is currently considered as a key agricultural threat to tomato production in European and Mediterranean Basin countries (Germain *et al.*, 2009). In Egypt, since the detection of this new pest at the end of 2009, TLM became a serious threat to greenhouse and open-field tomato production (Mohammed, 2010). Severe infestation of TLM by feeding on all aerial parts of tomato plants may result in a significant damage causing economic losses up to 80-100%, if the pest is not managed properly (Desneux *et al.*, 2010). Due to its biology and mine-feeding behavior of larvae, it becomes a very challenging pest to control (Lietti *et al.*, 2005).

Control of TLM relies mainly on the synthetic insecticides, which led to resistance to the most commonly used pesticides (Siqueira *et al.*, 2000 and Lietti *et al.*, 2005), toxicity to natural enemies (Consoli *et al.*, 1998), build-up of insecticide residues in tomato fruits (Walgenbach *et al.*, 1991), as well as environmental contamination (Siquira *et al.*, 2000 and Lietti *et al.*, 2005). Biological control would be the concerted use as a major component of integrated management for TLM. More than 20 species of parasitic wasps, especially the oophagous parasitoids, have proven to be effective against it (Parra and Zucchim, 2004).

Trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) are the most widely used natural enemies in biological control strategies worldwide. They are effective biocontrol agents for suppression and control of lepidopterous pests on many economic crops (Agamy, 2003). In this respect, the most important factors influencing parasitization performance of different *Trichogramma* species are introduction rate, frequency and density among release points (Smith, 1996 and Wang and Ship, 2004).

Therefore, this study was conducted to evaluate the efficacy of four trichogrammatid species at different release densities against TLM in cages under laboratory conditions as well their dispersal ability at different distances from releasing point under greenhouse conditions. The effect of host egg site, at two different height levels, on the parasitization capability of the tested species was also analyzed.

## MATERIALS AND METHODS

The experiments were performed under laboratory and glasshouse conditions in the Public Service Center of Biological Control (PSC-BC), Suez Canal University, Ismailia, Egypt (PSCBC, SCU) during the period from March to August, 2012.

### Tomato plants

Tomato seedlings, *Solanum lycopersicum* L. cv. GS12 were cultivated in plastic pots (15 cm diameter and 20 cm height) under glasshouse conditions (25±2°C, 70±10% RH and 14L: 10D). Chemical pesticides were strictly avoided, while fertilizer was applied whenever needed. Plants, at the beginning of the flowering stage, approximately 30 cm height, were used for the experiments.

**Maintenance of TLM**

Eggs of TLM were obtained from the adults' stock culture reared in PSC-BC on tomato seedlings placed in a rearing cage (40×60×80 cm). TLM was reared and maintained for at least 3 generations before used in the intended experiments. Newly laid TLM eggs (≤ 24 h. old) were glued onto small cartoon cards, each containing 10 eggs. These egg cards were prepared one hour prior to use in the experiment.

Four trichogrammatid egg parasitoid species were evaluated for their parasitization capability. *Trichogramma evanescens* (West.) was obtained from the stock colony kept in the PSCBC, SCU, *T. cacoeciae* Marchal and *Trichogrammatoidea bactrae* (Nagaraja) were kindly provided by Profs. E. Agamy (Cairo University) and A. El-Heneidy (Agricultural Research Center), respectively. As for *T. pretiosum* (Riley), it was obtained from Berkeley, California University, USA. Stock colonies of the selected parasitoid species were reared on eggs of *Sitotroga cerealella* (Olivier), (Lepidoptera: Gelechiidae) and maintained for several generations under laboratory conditions. One-day-old females of these parasitoids were used in the experiments.

**Cage experiment**

In this experiment, a little modification on the technique proposed by Agamy (1994) was used. The experimental unit consisted of aluminum screen cage similar to that used in TLM rearing. Each cage had four tomato seedlings (each of about 30 cm height) growing in the plastic pots as mentioned before. For each plant, 2 egg cards containing 10 TLM eggs/card were installed on the upper surface of the leaves, one on the basal half of the plant and the other on the distal half. Adults of each parasitoid species were separately released at the center of the cage (sex ratio is about 1: 1). Thus, each egg card was about 20 cm away from the level of the releasing point of parasitoids. Release of parasitoid individuals was performed in the experimental cages by launching the parasitoid at the tested release density of 8, 16, 32, 64, 128 and 256 individuals/ cage. Each treatment of the tested releasing densities was replicated 4 times. One day post release, TLM egg cards were collected from the cages and each card was kept in a small glass vial (2×7 cm), covered with a piece of cotton cloth and held in place by a rubber band under the above-mentioned laboratory conditions till adults' emergence, then the rate of parasitism was recorded (Agamy, 1994).

**Glasshouse experiment**

Dispersal ability of each of the four trichogrammatid species to allocate and parasitize TLM eggs on tomato plants under semi field conditions (glasshouse) was studied in a small glasshouse (4×5×7 m). Twelve tomato seedlings (30 cm in height), grown in plastic pots (15 cm in diameter), were introduced into the glasshouse and arranged into four rows of three plants at 1.5 m apart. All pots were labeled and then placed on the floor of the glasshouse. Each treatment was repeated four times.

Two cards of freshly deposited eggs of TLM containing 10 eggs/ card were fixed on each plant in two levels, one at the basal half of plant and the other at the distal half. Introduction and release of the parasitoid was performed at the rate of 1 female/TLM egg (10 parasitoids / egg card); *i.e.* 240 individuals for each tested parasitoid species. Releasing point of the parasitoids was conducted at a distance of 1.5 m away from the plants of the first row. One day after release, all egg cards were collected and kept inside small glass vials (2×7 cm), covered with pieces of cotton cloth till adults' emergence. Each experiment was repeated four times. Rates of parasitism and emergence were calculated as follows:

$$\text{Parasitism (\%)} = \frac{\text{No. of parasitized eggs (Black eggs)}}{\text{Total no. of exposed host eggs}} \times 100$$

$$\text{Emergence (\%)} = \frac{\text{No. of parasitized eggs with emergence holes}}{\text{Total no. of parasitized eggs (Black eggs)}} \times 100$$

**Statistical analysis**

All data were examined for normality with the Shapiro-Wilk test before analysis. When a Shapiro-Wilk test indicated that data were normally distributed, data were analyzed by parametric analysis of variance (ANOVA) and then the Holm-Sidak or Student-Newman-Keuls Methods were used for all pairwise multiple comparisons. When data were not normally distributed, a nonparametric Kruskal–Wallis ANOVA on ranks (H test) was used and Tukey's or Dunn's tests were used to compare treatment means at a 0.05 level of significance. Proportional data were square root transformed before analysis to improve the normality of residuals and to reduce the impact of any extreme values. Data were analyzed using Sigma-Plot 12.3 (Systat Software Inc., 2011).

## RESULTS AND DISCUSSION

**Influence of parasitoid release density on parasitism performance**

Obtained results showed that percentages of parasitism varied significantly among the four tested parasitoid species and among their releasing densities in the cage experiment (Fig 1). Data revealed that the percentage of parasitism increased gradually with the increase of releasing density and reached their maxima at the density of 256 parasitoid individuals /cage (Fig. 1). While, as the number of parasitoid increased 32 folds (8 vs. 256 parasitoids), the increase in parasitization rate was approximately 8 folds only (9.02 vs. 70.9%). *T. bactrae* had the highest percentage of parasitism at all the tested releasing densities, followed by *T. cacoeciae*, *T. evanescens* and *T. pretiosum*. Parasitization rate of *T. bactrae* at the release density of 8 parasitoids /cage was 17.97%, increased gradually to 29.06, 38.44, 60.94, 76.56 and 87.97% at the densities of 16, 32, 64, 128 and 256 parasitoid females/ cage, respectively. Correspondent percentages of parasitism were 9.22, 14.06, 23.91, 36.88, 44.53 and 77.34% for *T. cacoeciae*; 7.97, 12.97, 23.75, 35.94, 45.16 and 69.69% for *T. evanescens* and 0.94, 2.03, 5.63, 7.66, 17.50 and 48.60% for *T. pretiosum* (Fig. 1).

Statistical analysis showed that there were significant differences among the tested parasitoid densities in the percentages of parasitism by *T. bactrae* ($F_{5, 18}$= 263.70; $P$= 0. 001), *T. cacoeciae* ($F_{5, 18}$= 502.96; $P$= 0.001), *T. evanescens* ($F_{5, 18}$= 426.02; $P$= 0.001) and *T. pretiosum* ($F_{5, 18}$= 239.57; $P$= 0.001). Furthermore, statistical analysis indicated significant differences among the tested parasitoids in the mean numbers of parasitized eggs of TLM at releasing density of 8 parasitoids /cage ($F_{3, 12}$= 57.03; $P$= 0.001), 16 parasitoids / cage ($H_3$= 13.30; $P$= 0.004), 32 parasitoids /cage ($H_3$= 12.79; $P$= 0.005), 64 parasitoids /cage ($F_{3, 12}$= 202.05; $P$= 0.001), 128 parasitoids /cage ($F_{3, 12}$= 319.14; $P$= 0.001) and 256 parasitoids /cage ($F_{3, 12}$= 136.71; $P$= 0.001).

**Interacting effects of the parasitoid-release-density-dependence**

This tryout was designed in cage experiment to evaluate how parasitism rate of four parasitoid species interacts with the six different densities of parasitoid release and the two levels of host-egg sites. The results provided strong evidence that the parasitism rate was affected by the species, release density and height of host egg card on tomato plant. The densities of the tested species and height level of TLM egg card effects appeared to be additive. When considering the effect of each parameter separately, rate of parasitism differed significantly (species: $F_{3, 191}$ = 920.33, $P$<0.001; density: $F_{5, 191}$ = 1328.62, $P$<0.001; height: $F_{1, 191}$ = 525.65, $P$<0.001). On the other hand, when accounting the differences in rate of parasitism that based on the interaction among all tested parameters, a significant statistical difference was found ($F_{15,191}$ = 6.579, $P$<0.001), indicating that the rate of parasitism of the tested species depended on both density and host-eggs height (Table 1).

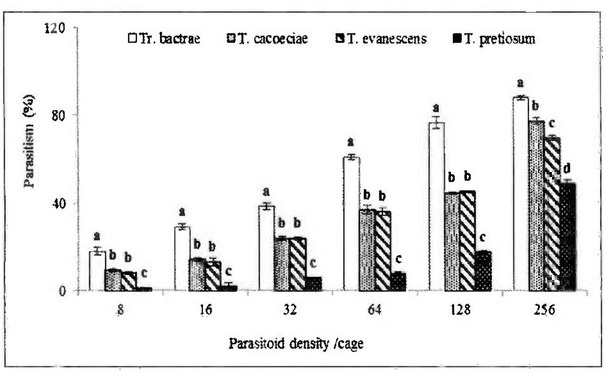


Fig. (1): Mean percentages of parasitism (%±SE) in eggs of TLM on tomato plants under different release parasitoid densities in cages under laboratory conditions.

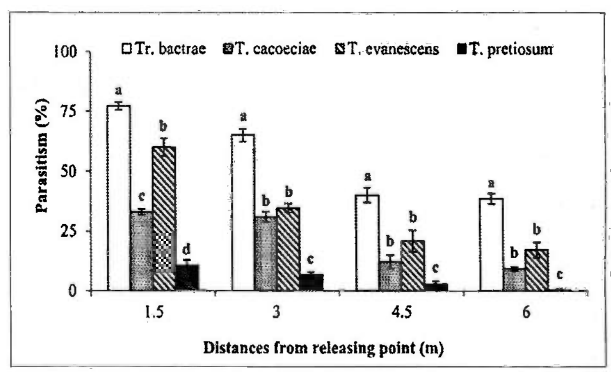


Fig. (2): Mean percentages of parasitism (%±SE) in eggs of TLM placed on tomato plants at different distances from the releasing points by four trichogrammatid species under glasshouse conditions.

*Bars with different letters in the same treatment indicate significant differences (P<0.05).

Table (1): Three-way ANOVA data indicating the effects of density and host-egg height on parasitism rate by different trichogrammatid species on *T. absoluta* in cages under laboratory conditions

| Source of Variation | *Df* | SS | MS | F | *P* |
|---|---|---|---|---|---|
| Species | 3 | 22356.354 | 7452.118 | 920.330 | <0.001 |
| Density | 5 | 53790.854 | 10758.171 | 1328.625 | <0.001 |
| Height | 1 | 4256.333 | 4256.333 | 525.654 | <0.001 |
| Species × Density | 15 | 3569.271 | 237.951 | 29.387 | <0.001 |
| Species × Height | 3 | 211.042 | 70.347 | 8.688 | <0.001 |
| Density × Height | 5 | 421.042 | 84.208 | 10.400 | <0.001 |
| Species × Density × Height | 15 | 799.083 | 53.272 | 6.579 | <0.001 |
| Residual | 144 | 1166.000 | 8.097 | | |
| Total | 191 | 86569.979 | 453.246 | | |

Table (2): Three-way ANOVA data indicating the effects of both distance and height of host-egg on parasitism rate of different trichogrammatid species on *T. absoluta* under glasshouse conditions

| Source of Variation | *df* | SS | MS | F | P |
|---|---|---|---|---|---|
| Species | 3 | 3669.750 | 1223.250 | 317.384 | <0.001 |
| Distance | 3 | 1642.563 | 547.521 | 142.059 | <0.001 |
| Height | 1 | 946.125 | 946.125 | 245.481 | <0.001 |
| Species × Density | 9 | 413.938 | 45.993 | 11.933 | <0.001 |
| Species × Height | 3 | 228.375 | 76.125 | 19.751 | <0.001 |
| Density × Height | 3 | 60.813 | 20.271 | 5.259 | 0.002 |
| Species × Density × Height | 9 | 82.438 | 9.160 | 2.377 | 0.018 |
| Residual | 96 | 370.000 | 3.854 | | |
| Total | 127 | 7414.002 | 58.378 | | |

Obtained results revealed also that releasing density of these oophagous parasitoids can play a crucial role for management of TLM. An increase in parasitism percentage was observed by increasing wasp release densities of all tested parasitoids against TLM eggs in cage experiments. These findings are in conformity with those reported earlier by Thorpe and Dively (1985) on *Heliothis virescens* by *T. minutum*, *T. pretiosum* and *T. exiguum* in cage experiments and by Agamy (1994) on *Ostrinia nubilalis* (Hübner) eggs by *T. ostriniae, T. brassicae* and *T. evanescens*.

Under low levels of release density of 8 or 16 parasitoids /cage, the mean number of parasitized TLM eggs/egg card was very low, regardless to parasitoid species. On contrast, Pratissoli *et al.* (2005) argued that the rate of 16 parasitoids (*T. pretiosum*) per TLM egg was optimum to be released to provide a satisfactory control of TLM under greenhouse conditions.

**Influence of long distance of releasing point on the parasitism performance**

As shown in Fig. (2), percentages of parasitism decreased as distance from releasing point increased, irrespective of the parasitoid species. Obviously, *T. bactrae* manifested greatest rates of parasitism as compared to the remaining tested parasitoid species, irrespective of distance from releasing point. Percentages of parasitism by *T. bactrae* were 77.08, 65, 40 and 38.58% at 1.5, 3, 4.5 and 6 m from releasing point, respectively. Respective percentages were 32.92, 30.83, 12.08 and 9.17% for *T. cacoeciae*; 60, 34.85, 20.83 and 17.08% for *T. evanescens,* and 10.42, 6.67, 2.92 and 0.42% for *T. pretiosum*. Subsequently, the overall averages of percentage of parasitism for each parasitoid species across all the studied distances from releasing point attained 55.17, 33.13, 21.25 and 5.11% for *T. bactrae*, *T. evanescens*, *T. cacoeciae* and *T. pretiosum*, respectively (Fig. 2).

Statistical analysis showed that significant differences existed in rates of parasitism among the tested parasitoid species under glasshouse conditions at 1.5 m from the release point ($F_{3,12}$= 146.26; *P*= 0.001), 3 m ($F_{3,12}$= 137.93; *P*= 0.001), 4.5 m ($H_3$= 12.90; *P*= 0.005) and 6 m ($H_3$= 13.93; *P*= 0.003). Likewise, there were significant differences ($P \leq 0.05$) among different distances from releasing point for *T. bactrae* ($F_{3,12}$= 69.62; *P*= 0.001), *T. cacoeciae* ($F_{3,12}$= 42.90; *P*= 0.001), *T. evanescens* ($F_{3,12}$= 30.17; *P*= 0.001) and *T. pretiosum* ($H_3$= 5.98; *P*= 0.113).

**Interacting effects of the parasitoid-release-distance-dependence**

In a greenhouse assay, the results provided strong evidence that the parasitism rate was affected by the trichogrammatid wasp species, long distance of the parasitoid release point and more specifically by the height level of host egg cards on tomato plants. The long distance of the release point of the tested trichogrammatid species and height level of host egg card effects appeared to be additive: there was a strong statistical evidence that they interacted. The same tendency of interaction effect was also reported that the rate of parasitism of different species depended on both distance and height of host-eggs ($F_{9,127}$ = 2.377; *P*= 0.018). Likewise the cage experiment, taking into account each parameter merely, parasitism rate of tested species was drastically influenced by host-egg height ($F_{1,127}$ = 245.48; *P*<0.001), distance ($F_{3,127}$ = 547.52; *P*<0.001) and parasitoid species ($F_{3,127}$ = 317.38; *P*<0.001) (Table, 2).

Obtained results indicated that the parasitization rate for all the parasitoid species decreased with the increase of distance from the releasing point in the glasshouse. Similar results were obtained by Agamy (1994) who reported that egg batches of *O. nubilalis* at the nearest distance from point of release (1.5 m) received the highest rates of parasitism, followed by 3 and 6 m. Nevertheless, high rate of parasitism (20-50%) by *T. ostriniae* on eggs of *O. nubilalis* was observed at the distance of 45 m, but the parasitism rate decreased gradually with increasing distance from the release point (Chapman, 2007). The same trend was also recorded for *T. platneri* in apple orchards (McDougall and Mills, 1997) and *Trichogramma* spp. on *Cydia pomonella* (Linnaeus) eggs (Sakr, 2003).

In the present study, *T. evanescens* showed low dispersal ability and in turns lower rates of parasitism as compared to the other parasitoid species. This finding is consistent with that reported by Agamy (1994) who reported that *T. evanescens* was less effective than *T. ostriniae* and *T. brassicae* at distances similar to those used in the current study. Also, Atwa (2009) stated that *T. evanescens* showed poor dispersal ability when searching for PTM eggs in potato stores. Interestingly, the most efficient strains of *T. evanescens, T. euproctidis, T. achaeae, T. pretiosum* and *T. cordubensis* were all arrhenotokous ones. Indeed, 2 out of 9 strains of *T. evanescens* were as efficient as *T. achaeae* in parasitizing TLM eggs (Chailleux *et al.*, 2012).

*T. pretiosum* is the most frequent egg parasitoid used for controlling TLM in South and Latin America (Pratissoli *et al.*, 2006a and b). Therefore, it was introduced from the USA to compare its efficacy with other trichogrammatids against TLM. Unfortunately, *T. pretiosum* showed the lowest rates of parasitism in the concurrent study either in the cage or in the glasshouse experiments. This could be attributed to: 1) different strains of *T. pretiosum,* which showed different behaviors and rates of parasitism (Pratissoli *et al.*, 2006a and Chailleux *et al.*, 2012), and 2) slower locomotion and flight inability of this wasp as compared to the other tested *Trichogramma* spp.

In conclusion, as idiobionts, oophagous wasps kill their hosts before they reach the plant-feeding stage (Agamy, 2003; Sarhan, 2004 and Mandour *et al.*, 2012). However, the most important factors on which the success of the biological control program of TLM with trichogrammatids depends are the parasitoid releasing density, host egg height and long distance of releasing point. Indeed, under low levels of release density, the mean numbers of parasitized TLM eggs were very low, regardless to parasitoid species. In contrast, release distances had considerable direct influence on the decrease of parasitism rate for all tested parasitoid species. *T. bactrae* had highest parasitism rates as compared to other species at all tested densities and distances. Thus, when introducing such potential bioagents in IPM protocols for controlling TLM in tomato fields, it has to be taken into consideration the potential of the selected parasitoid species.

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