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Biological parameters of three *Trichogramma pretiosum* strains (Riley, 1879) (Hymenoptera: Trichogrammatidae) on eggs *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)

Gabriel dos Santos Carvalho*, Luciana Barboza Silva, Maisa Sousa Veras, Eliane Carneiro Bueno dos Santos, Mayra Layra dos Santos Almeida and Soislan Sousa Reis

*Programa de Pós-graduação em Agronomia Fitotecnia, Universidade Federal do Piauí, Campus: Professora Cinobelina Elvas, BR-135, km 3, Bairro Planalto Horizonte, 64900-000, Bom Jesus, Piauí, Brazil. *Author for correspondence. E-mail: gabrieldossc@hotmail.com*

ABSTRACT. This project has an objective to study the biological parameters of *Trichogramma pretiosum* on eggs of *H. armigera*. The three strains of *T. pretiosum* were evaluated: TM, TMC and TLEM, from different places. The experimental establishing was completely randomizing with twenty repeats per treatment, being used by a female parasitoid by repetition and later on they were offered 20 *H. armigera* eggs. The experiment was kept on BOD cameras to 25 ± 2° C, UR 60 ± 10% and photo phase of 14 hours. Evaluating the following biological parameters: parasitism percentage, emergency percentage, number of adults emerged per egg, sex ratio, viability, and longevity and cycle duration. The parasitism percentage, viability and number of individuals per egg were higher for TM and TMC. The TMC strain presented a larger sex ratio proportion, not being different statistically from the TM stream. The longevity of the strain TM was different regarding the others. There was no variation regarding the cycle duration. Therefore, according with the evaluated biologic parameters, it is concluded that *Trichogramma pretiosum* strain TM obtained a better egg development of *H. armigera* on laboratory conditions.

Palavras-chave: Controle biológico, lagarta da soja, parasitoide de ovos.

Parâmetros biológicos de três linhagens de *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) sobre ovos de *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)

RESUMO. Este trabalho teve como objetivo estudar os parâmetros biológicos de *Trichogramma pretiosum* em ovos de *H. armigera*. As três linhagens de *T. pretiosum* avaliadas foram: TM, TMC e TLEM, oriundas de diferentes localidades. O delineamento experimental foi inteiramente casualizado com vinte repetições por tratamento, sendo utilizada uma fêmea do parasitoide por repetição e posteriormente foram oferecidos 20 ovos de *H. armigera*. O experimento foi mantido em câmaras BOD a 25 ± 2° C, UR 60 ± 10% e fotofase de 14 horas. Avaliou-se os seguintes parâmetros biológicos: porcentagem de parasitismo, porcentagem de emergência, número de adultos emergidos por ovo, razão sexual, viabilidade, longevidade e duração do ciclo. A porcentagem de parasitismo e viabilidade e número de indivíduos por ovo foram superiores para as linhagens TM e TMC. A linhagem TMC apresentou a maior proporção para razão sexual, não diferindo estatisticamente da linhagem TM. A longevidade da linhagem TM se destacou em relação às demais. Não houve variação com relação a duração do ciclo. Assim de acordo com os parâmetros biológicos avaliados, conclui-se que *Trichogramma pretiosum* linhagem TM obteve o melhor desenvolvimento nos ovos de *H. armigera* em condições de laboratório.

Keywords: Biological control, soy caterpillar, parasitoid eggs.

Introduction

Among the insect pests that are able to limit the production of soybean, we emphasize the (Hemiptera: Pentatomidae) bugs and the complex of defoliating caterpillars. Recently this situation has worsened with the recording of the occurrence of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) in Brazil (Czepak, Albernaz, Vivan, Guimarães, & Carvalhais, 2013; Tay et al., 2013; Gómez et al., 2016), where it was classified as an A1 quarantine pest and it is currently found in the states of Goiás, Bahia, Mato Grosso and Piauí mainly associated to soybean, cotton and tomato crops (Czepak et al., 2013; Gómez et al., 2016).

This fact has led to high adoption of chemical controls, to contain this pest, causing serious consequences that entail disadvantages due to high use of toxic substances, being unselective and with slow environmental degradation, which leads to imbalance in the agricultural ecosystems and damage to farmers (Saber, 2011; Wang, Chen, Ma, Zhu, & Lei, 2015; Amichot, Curty, Benguettat-Magliano, Galleti, & Wajnberg, 2016).

Thus, alternative measures must be adopted and biological control using parasitoids of *Trichogramma* eggs, is an important measure because they are key species in the regulation of the populations of several species of Lepidoptera pests, however, there are still insufficient studies involving the use of *Trichogramma* spp., on *H. armigera* (Bueno, Parra, Bueno, & Haddad, 2009; Molnár, López, Gámez, & Garay, 2016).

However, the successful use of *Trichogramma* in biological control, depends on the knowledge of bio-ecological characteristics of the parasitoid and the interaction with the target host (Bourchie & Smith, 1996). Thus, it is necessary to understand the bio-ecology of the parasitoid in the target pest, because the efficiency has shown to differ between species and/or strains of *Trichogramma*, making it necessary to study the performance of different strains of *Trichogramma*, collected in different ecosystems on the target pest (Hassan, Kolher, & Rost, 1998; Bueno et al., 2009). Such knowledge enables selecting strains that are well adapted to the pest to be controlled (Bezerra & Parra, 2004).

Thus, this study aimed to evaluate the biological parameters of three strains of *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) and indicate the most efficient for *Helicoverpa armigera* control in laboratory conditions.

Material and methods

Location of the experiment

The experiment was conducted in the Plant Science Laboratory of the *Universidade Federal do Piauí*, kept in BOD with constant temperature of 25 ± 2° C, relative humidity 60 ± 1% of the Campus: Professor Cinobelina Elvas - CPCE.

Raising of *Helicoverpa armigera*

The used population of *H. armigera* originated from the insect breeding laboratory, where they were kept in an artificial diet adapted from Kasten Jr., Precetti, and Parra (1978). Neonate larvae (< 24 hours old) were individualized and transferred to plastic containers with a lid, containing 100 ml of artificial diet until they reached the pupal stage. When they became adults, they were transferred to PVC cages (40 cm H x 30 cm Ø), internally coated with bond paper sheets for oviposition. The moths were fed with a honey-based solution (10%) and kept under controlled conditions (25 ± 5° C, 60 ± 10% RH, 12:12). The eggs were collected and stored in pots and kept in laboratory conditions until the hatching of the caterpillars, where one half was intended for experiments and the other for maintenance of the creation in the laboratory.

Strains of *Trichogramma pretiosum* for *Helicoverpa armigera* control

The selection was made from strains of *Trichogramma pretiosum*. The LEM strain was obtained from the Insect Biology Laboratory, Department of Entomology and Acarology, "Luiz de Queiroz" Agricultural College (ESALQ/USP); and the TMC and TM strains were collected in traps, with eggs of the alternative host *A. kuehniella* and *H. armigera* eggs, respectively. Samples were collected in soybean commercial plantations at Cerrado (Brazilian savannah), of the municipality of Baixa Grande do Ribeiro-Piauí, Brazil. This municipality has sub-humid tropical climate, with temperatures between 26 to 36°C and average rainfall of 700mm to 1200mm between the months of December to May (Companhia de Pesquisa de Recursos Minerais [CPRM], 2004). Three strains of *T. pretiosum* were established (Table 1).

Before the installation of the experiments, three strains of *Trichogramma pretiosum* were maintained for one generation, in *H. armigera* eggs in order to eliminate a possible pre-imaginal conditioning by raising them in an alternate host (*A. kuehniella*). The species was identified by morphological characteristics according to (Querino & Zucchi, 2003).

Table 1. Strains of *Trichogramma pretiosum* with their respective collection origins used in the screening test for the control of *Helicoverpa armigera*.

Treatments	Species/Strains	Origin	Host
T1-TLEM	*T. pretiosum*/ TLEM	Luiz Eduardo Magalhães, BA/ESALQ	-
T2-TMC	*T. pretiosum*/ TMC	(Area1) (S08°40'58.7") (W045°05'39.2") Baixa grande do Ribeiro, PI	*H. armigera*
T3-TM	*T. pretiosum*/ TM	(Area2) (S08°40'56.4") (W045°05'39.2") Baixa grande do Ribeiro, PI	*H. armigera*

Raising and maintenance of *Trichogramma pretiosum* strains

The raising of *Trichogramma* was performed according to the methodology of (Stein & Parra, 1987), eggs of the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). The diet of the alternative host was placed in plastic trays containing corrugated cardboard strips, pre-cut that aim to provide support and also serve as a place for pupation.

In each tray, 0.35 g of *A. kuehniella* eggs was placed and each tray was covered with a plastic bag with an opening covered with "voile" type tissue allowing internal ventilation. This procedure was performed to prevent an attack of the parasitoid *Bracon hebetor* (Say, 1857) (Hymenoptera: Braconidae) that parasites *A. kuehniella* caterpillars in the final instar of the host. Then the trays were placed in a larval development room, where they remained until the beginning of adult emergence. After the emergence of the first adults, their diet, hardened due to the formation of webs by the caterpillars, was moved and fixed inside the adult collection boxes.

For the collection of the adult *A. kuehniella* that had emerged, a vacuum cleaner that was adapted to suck the insects inside the creation of boxes was utilized and later the adults were transferred to plastic containers (2.5 L). At the end of the process, the eggs were collected from the plastic containers through a fine mesh screen that stays in the bottom where the eggs fall.

Once collected, the eggs of the factitious host were fixed in blue cardboard pieces (8.0 x 2.0 cm) with diluted gum arabic in water (50%), and then were subjected to the unviability process through exposure of the eggs to ultraviolet germicidal light for a period of 50 minutes and at a distance of 15 cm from the light source. At the extremities of the cartouches were recorded the date of parasitism and the strain identification code, allowing control of *Trichogramma pretiosum* strains.

Experiment execution

The experiment was carried out in climatic chambers regulated at 25 ± 2° C, RH 70±10% and photoperiod of 14 hours in a completely randomized design with the treatments consisting of the three strains, using twenty repetitions with a female parasitoid by repetition.

Females of each strain were individualized, with 24 hours of age in glass tubes (12 mm diameter and 75 mm height) capped with PVC plastic film (Magipack™) being fed with a droplet of pure honey. Female parasitoids were identified through sexual dimorphism displayed by the antennas (Bowen & Stern, 1966), with the help of a stereoscopic microscope.

For each strain, 20 cartouches containing 20 eggs of *H. armigera* with 24 hours of age were used. After 24 hours, the females were removed from the glass tubing with each strain (treatment) containing 20 replicates.

We evaluated the following biological parameters of *T. pretiosum*: **parasitism percentage** by counting the darkened eggs; **emergence percentage**, emergence percentage by counting of adults; **viability percentage** acquired by counting the host eggs that had outlet orifice of the adults viewed under a stereoscopic microscope; **number of adults emerged per egg** calculated using the formula:

$$\text{Adults emerged per egg} = \frac{\text{N}^{\underline{o}} \text{ of females} + \text{N}^{\underline{o}} \text{ of males}}{\text{N}^{\underline{o}} \text{ of parasitized eggs}}$$

For **sex ratio** based on the methodology proposed by (Bowen & Stern, 1966), calculated using the formula:

$$\text{sr} = \frac{\text{N}^{\underline{o}} \text{ of females}}{\text{N}^{\underline{o}} \text{ of females} + \text{N}^{\underline{o}} \text{ of males}}$$

Longevity, accomplished through daily observations, always at the same time, with 24 hours intervals for mortality accounting; **cycle duration** (egg-adult), conducted through daily observations, always at the same time, with 24 hours intervals. The determination of the number of individuals per egg was calculated by dividing the total number of adults by the total number of holes observed in 20 *H. armigera* eggs in each tube. The results were submitted to analysis of variance and averages were compared by the Tukey test ($P \leq 0.05$). The data was analyzed using the statistical program R version 3.0.3.

Results

When analyzing the performance of the three strains of *Trichogramma pretiosum*, there were differences in the evaluated parameters. For parasitism, we observed differences in the aggressiveness of the strains evaluated on the *H. armigera* eggs being TM and TMC the superior strains in relation to TLEM, respectively (Figure 1A).

The emergence percentage of the studied *T. pretiosum* strains differ significantly where the TM and TMC strains showed the highest percentages of 84.4 and 73.9%, respectively (Figure 1B).

For the viability percentage, the TMC and TM strains showed significantly higher values than the TLEM strain (Figure 1C).

The number of adults emerged per egg was greater for the TM strain when compared to TMC and TLEM with 1.19, 0.98, and 0.94 individuals per egg, respectively (Figure 1D).

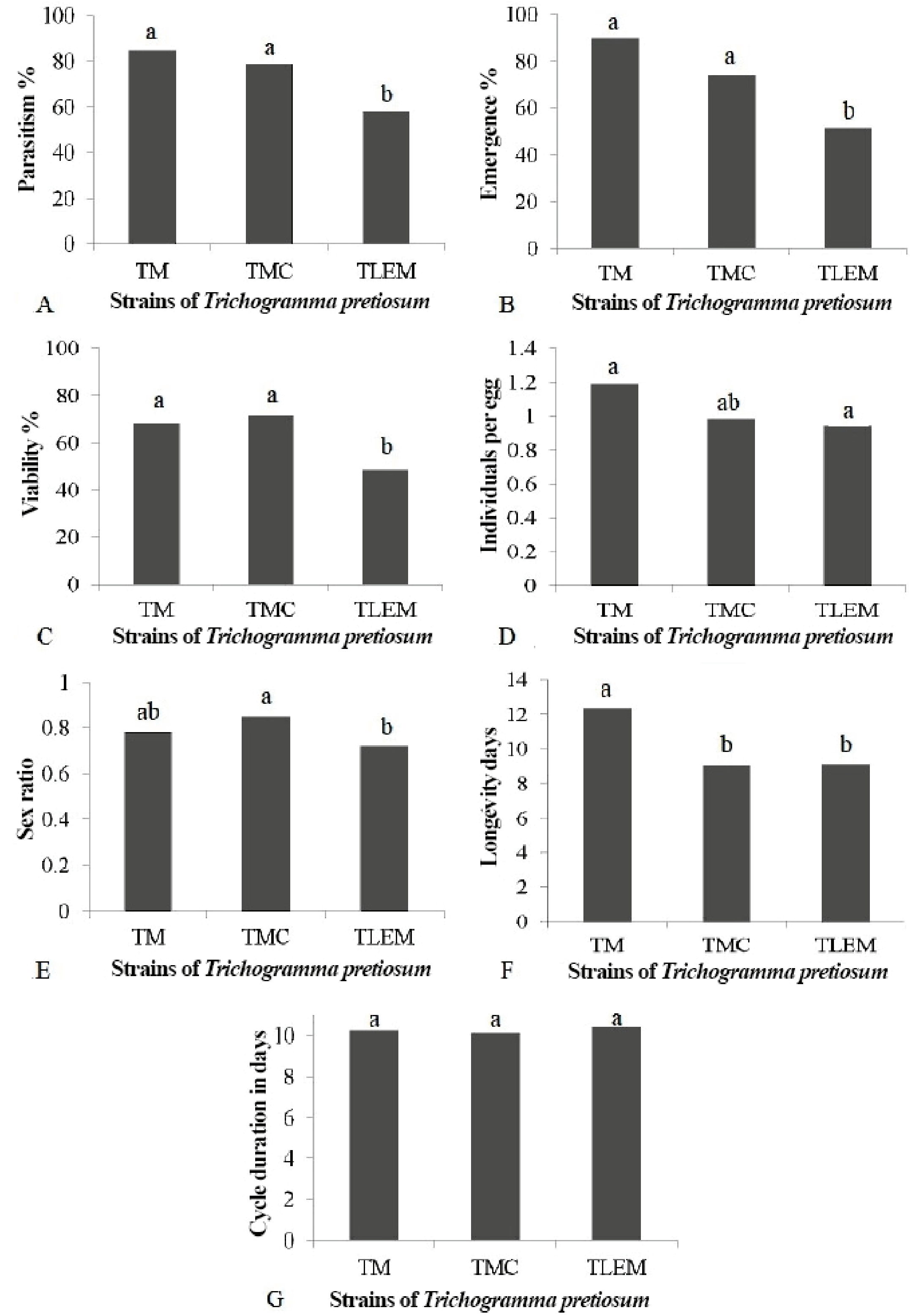

Figure 1. (A) Parasitism percentage, (B) Emergence percentage (C) Viability percentage, (D) Individuals per egg (E) Sex ratio (F) Adult longevity, (G) Cycle duration of the three strains of *Trichogramma pretiosum*: TM, TMC and TLEM created in *Helicoverpa armigera* eggs. Temperature of 25±1°C, RH 70±10% and photoperiod of 14 hours. Averages of the bars followed by the same letter do not differ by Tukey's test at 5% probability.

By analyzing the sex ratio, there was no significant difference between the TMC and TM strains, having the highest proportions of females compared to the TLEM strain with the lowest proportion (Figure 1E).

The longevity of the descendants of the *T. pretiosum* TM strain when developed in *H. armigera* eggs was greater with 12.35 days of adult survival in comparison to TMC and TLEM whose strains lasted 9.05 and 9.10 days, thus, the TM strain according to the results, produces females have a greater survival time (Figure 1F).

The duration of the cycle, did not vary between the strains evaluated, with an average of 10 days when subjected to the host in the study (Figure 1G).

Discussion

The parasitism percentage of *H. armigera* eggs by the strains TM (31.5%) and TMC (25.9%) was higher compared to the TLEM strain. The parasitism efficiency reveals a variation in the characteristics of each strain of *T. pretiosum* studied. This factor may be associated with acceptance of the parasitoid by the host strains studied, as a result of a physicochemical characteristic used to recognize *H. armigera* eggs. Similar percentages of parasitism, between 81 and 56% were obtained by (Bueno, Parra, & Bueno, 2012), By evaluating 13 *Trichogramma* strains in *Chrysodeixis includens* (Walker, 1958) eggs (Lepidoptera: Noctuidae). Ko et al. (2014) had results that were similar to the present study, when evaluating a strain of *T. pretiosum* on eggs of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). Öztemiz (2008) observed an efficiency between 66 and 90% in the parasitism of *H. armigera* using *T. euproctidis* (Giralt, 1911) (Hymenoptera: Trichogramatidae) with cotton cultures in field conditions.

Parasitoids of the *Trichogramma* genus can be easily multiplied in a laboratory with high efficiency and low cost (Öztemiz & Kornosor, 2007). However, the release in cultures after several generations of laboratory breeding can reduce their effectiveness due to inbreeding and genetic erosion, which reduces the potential foraging and parasitism rate (Pratissoli et al., 2005), which may justify the performance inferiority of the TLEM strain compared to the TM and TMC strains that were collected in the field. This shows that *H. armigera* is an excellent host according to the results found, especially for the TM and TMC strains, having suitable characteristics for the development of the parasitoids.

The high emergence rate of 89.4 and 73.9% presented by the TM and TMC strains respectively, indicates the nutritional quality of *H. armigera* eggs. These numbers are acceptable in pest control programs (Nava, Takahashi, & Parra, 2007, Dias, Parra, & Lima, 2008). The concomitant emergence rate of parasitism by the TM and TMC strains may represent an *H. armigera* control efficiency, which means the mass presence of the parasitoid in the area and consequently reduced pest population density.

As for the number of emerged individuals per egg, there was no difference between strains, which was not observed in the study by Altoé et al. (2012) who studied the *T. pretiosum* on *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae), which observed variation in the number of individuals per egg, showing that this parameter is influenced by host characteristics. Studies by Bueno et al. (2009) reported that egg size can influence the number of offspring, a characteristic that was also observed in *H. armigera* eggs, where it presented high quality, for the percentages presented in the three strains.

For sex ratio, all the results were favorable as the required standard for the parasitoid. For Navarro (1998), the ideal sex ratio should be less than 0.5 for use in biological control programs. The TMC strain showed a better ratio but did not differ statistically from the TM line. These results favor its use in the field, by providing a high number of parasitoid females, required in *Trichogramma* quality control.

The TM strain showed the highest average with 12.5 days, representing 3.3 and 3.25 days more longevity compared to TMC and TLEM. Similar results were found by Nava et al. (2007) to evaluate the longevity of *T. pretiosum* L4 strain in *Stenoma catenifer* eggs, 11.5 days. This feature is important in biological control programs, for longer-lived parasitoids created in *H. armigera* eggs may have greater efficiency in the field and be able to parasitize for a longer period of time.

The biological control, using parasitoids of the *Trichogramma* genus as a tactic of the Integrated Pest Management (IPM) has been successfully implemented by cotton producers in Asia and North Africa who have suffered great losses caused by *H. armigera*. (Heydari & Gharedaghli, 2007). Programs like these should be seen as an example for further studies for the implementation of this tool in integrated pest management for the soybean crop-producing areas of Brazil.

Studies have shown variations between species and/or strains on the search behavior, host preference, response to environmental conditions, among other features, emphasizing the importance of choosing an appropriate strain for use in biological control programs, because these variations may affect the successful use of *Trichogramma* spp. (Beserra, Dias, & Parra, 2003; Coelho Jr, Rugman-Jones, Reigada, Stouthamer, & Parra, 2016).

In the present study the TM strain was the one with the greatest potential for use *in H. armigera* control programs, not only having shown high parasitism (%) it stood out for having the greatest longevity among the evaluated strains, which may reflect a greater foraging period in the field and greater dispersion.

Conclusion

According to the biological parameters evaluated in this study, we can say that the *T. pretiosum* strain TM presents the best *H. armigera* control potential in laboratory conditions, standing out due to the high percentage of parasitism presented and especially the highest longevity among the strains studied, and these factors are considered important in biological control. It is important to note that more studies are needed in field conditions for its implementation within the integrated pest management.

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