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Efficacy of the parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) egg masses

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Abstract

Background: The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the major insect pests, causing a significant damage on different cultivated agricultural crops. Developing an alternative non-chemical tool, an effective and environmentally friendly method to suppress pest's infestation is essentially needed. Therefore, biological control by releasing the egg parasitoids could be the most promising tool for integrated pest management.

Results: This study was designed to evaluate the efficacy of the egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) as a bio-control agent against *S. littoralis* egg masses with different physical characteristics (number of egg layer and degree of scale density) in a no-choice and choice tests, under laboratory conditions. Also, the parasitoids' fitness in terms of parasitism percentage, developmental period, adults' emergence percentage, female offspring percentage, and longevity were investigated. The results revealed that *T. bactrae* wasps had a great ability to parasitize *S. littoralis* egg masses, but with different rates, related to their layers and scales' thickness in both tests. The highest parasitism percentage was observed on one-layer eggs, followed by two layers. However, 3-layer eggs were the least preferable one. High numbers of adult emergencies (> 80%) were observed in all tested egg masses, except in the case of 3 layers with high scales. Furthermore, female-biased sex ratios were noticed at all examined eggs, with only the exception of high-scaly eggs with a single layer that recorded the lowest rate (≤ 45%). Besides, the survival of adult female parasitoids was not significantly affected in both tests.

Conclusions: *T. bactrae* could be used as a bio-control agent against *S. littoralis* egg masses with different physical characteristics based on the achieved results.

Keywords: *Trichogrammatoidea bactrae*, *Spodoptera littoralis*, Physical characteristic, Biological control, Parasitism

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Background

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is one of the major phytophagous insect pests in Egypt as well as in many other countries (Pineda et al. 2004), causing significant damages on different cultivated agricultural crops such as cotton, corn, clover, soybeans, peanuts, flowers, fruits, and vegetables (Kandil et al. 2003). Management of this polyphagous pest is still dependent upon chemical insecticides (Dahi et al. 2016). However, chemical control has not provided a long-term solution for this pest problem because of the high costs, environmental pollution, and hazards to human's health. Besides, insecticide-resistance insect strains, drastic effects on natural enemies, resurgence, and outbreaks of secondary pests are well known (El-Heneidy et al. 2015). Developing an alternative non-chemical tool, an effective and environmentally friendly method to suppress pest infestation is essentially needed. Therefore, the biological control by releasing the egg parasitoids could be the most promising tool for integrated pest management. In this context, the potential of different *Trichogramma* species against different *Spodoptera* spp. have been studied by several researchers worldwide (Toonders and Sánchez 1987; Beserra et al. 2002 and 2005; Beserra and Parra 2005; Brotodjojo and Walter 2006; Bueno et al. 2010; Díaz et al. 2012; Dequech et al. 2013; Puneeth and Vijayan 2013; Figueiredo et al. 2015; Saljoqi et al. 2015; Jaraleño-Teniente et al. 2020). However, the efficiency of these parasitoids showed variation in their range related to the species of *Trichogramma*. Moreover, this variability may be due to the egg masses' layers deposited by *Spodoptera* females covering with the dense scales, which can alter the parasitism behavior (Toonders and Sánchez 1987; Beserra et al. 2005). Up to date, there is no detailed information on the performance of the *Trichogrammatoidea* species towards any *Spodoptera* spp. This study is an attempt to assess the efficacy of egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja as a bio-control agent against *S. littoralis* eggs with different physical characteristics (number of egg layers and degree of scale density) in a no-choice and choice tests, under laboratory conditions. As far as we know, this study is the first to evaluate this parasitoid species against the target pest.

Methods

Cultures of the cotton leafworm, *S. littoralis* and the egg parasitoid, *T. bactrae* were reared under standard rearing conditions of (25 ± 2 °C and > 60% RH with 14 L: 10D cycle) at the mass rearing unit of *Trichogramma*, Plant Protection Research Institute, Agricultural Research Center (ARC), Assiut Governorate, Egypt.

S. littoralis rearing

Initial culture of *S. littoralis* egg batches used in the study was obtained from the experimental farm of Plant Protection Department, Faculty of Agriculture, Assiut University. The insect rearing technique was conducted according to the methodology described by Dahi (1997). Newly hatched larvae were reared on fresh caster bean leaves (*Ricinus communis* L.) and supplied daily until pupation. The pupae were collected and transferred to new wooden boxes (35 × 35 × 35 cm^3), covered with a layer of fine wood dust to keep moisture until adult moth emergence. Adults were fed on 10% sugar solution hanged inside the cage. The inner walls of the cage were covered with sheets of (A_4) white copy paper, and branches of oleander (*Nerium oleander* L.) as the ovipositional site. Newly laid egg masses were collected daily to start the experiments, and the others were used to maintain the insect colony.

T. bactrae rearing

The parasitoid used in this study was imported for the first time from Australia in 1992 by Dr. A. H. El-Heneidy (ARC, Egypt) and was established under Egyptian environmental conditions (Mohamed and El-Heneidy 2020). This parasitoid species was mass-reared on eggs of the Angoumois grain moth *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae) for 4 successive generations, under standard rearing conditions.

Experimental design

The deposited egg masses (≤ 24 h) (approximately 150 ± 50 eggs) according to their physical characteristics (no. of egg layers and degree of scale density) were divided into 9 treatments as one layer of eggs with no scales, with low scale density, with mid scale density, and with high scale density; two layers of eggs with low scale density, with mid scale density, and with high scale density; and three layers of eggs with low scale density and with high scale density. The degrees of scales were divided according to Brotodjojo and Walter (2006). Thirty newly emerged *T. bactrae* wasps without any previous oviposition experience were exposed to each type of the egg masses in plastic jars (500 ml) for 24 h to avoid super-parasitism in 2 types of tests separately. No-choice test, by offering only one type of egg masses individually, and for choice test, by offering all tested egg masses simultaneously to the adult parasitoids. After 24 h of exposure to the parasitoid, parasitized egg cards from each treatment were collected and maintained in new plastic jars (250 ml) under standard rearing conditions. The egg masses were replaced by new ones for 3 days. The length of the experiment was decided depending on Doyon and Boivin (2005) that more than 70% of parasitization occurred within the first 3 days after adult emergence. For each treatment (egg masses), 30 replicates were used.

Biological parameters

The fitness of the egg parasitoid, *T. bactrae* on *S. littoralis* egg masses with different physical characteristics was assessed by measuring the following biological variables in both no-choice and choice tests as parasitism percentage (no. of parasitized eggs (blackened eggs)/total no. of eggs exposed). The total number of eggs on each egg mass was determined by counting (no. of hatched larvae + unhatched eggs (dead larvae) + blackened parasitized eggs) (Dequech et al. 2013). Besides, developmental period (days) (the average time till adult emergence), adult emergence rate (no. of all emerged adults/no. of parasitized eggs), and females' ratio by examining dead adults under a stereomicroscope (no. of emerged adult females/total individuals) were determined. The longevity of adults was recorded daily by keeping 50 emerged adults individually from each examined egg masses into glass vials (2 cm diameter × 4 cm height) till mortality. For parasitoid nutrition, few droplets of 10% sugar solution were provided daily until the wasps died.

Statistical analysis

Obtained data were subjected to one-way analysis of variance (ANOVA). Data were arcsine √proportions transformed before analysis to meet normality. Means were separated by *t* test at $P \leq 0.05$ level. All calculations and graphs were used by Microsoft Excel® software according to Fowler et al. (1998).

Results

Parasitism percentage

The *T. bactrae* wasps were able to parasitize the *S. littoralis* egg masses, but with different rates (Fig. 1) according to their different layers and degrees of scales' thickness, in both no-choice and choice tests (Table 1). Regardless the degree of scales, the highest parasitism percentage was significantly ($P < 0.001$) recorded in one layer eggs (66.67, 60.53%), followed by two layers (58.23, 48.53%), while the least preferable one was three layers egg masses (29.03, 22.67%) in both tests, respectively. In addition, the thick scaly eggs with three layers had significantly (P = 0004, 0.007) the least parasitism rate (< 20%) in all egg masses in both tests, respectively. When the parasitoids had no choice on egg masses, the rates of parasitism were more prominent than when those were given choice among different eggs. The percentage of parasitism was highly significantly differed among all tested egg masses (ANOVA, $F_{8,168}$ = 33.421, 73.069; $P < 0.001$) in no-choice and choice tests, respectively.

Developmental period

The average time required to emerge the parasitoid was non-significantly changed among the egg masses with different physical characteristics (ANOVA, $F_{8,72}$ = 0.337; P = 0.949) in the case of no-choice test (Fig. 2). In contrast, when the parasitoids were allowed to choose between all examined eggs at a time, the developmental period varied significantly (ANOVA, $F_{8,72}$ = 2.625; P = 0.013). These differences were associated only with the degree of scales covering the eggs, not the layers.

Adults' emergence percentage

High numbers of adult emergency were observed in all egg masses in both tests (Table 2). The percentage of emergence was significantly changed ($F_{8,126}$ = 5.385; P < 0.001) as a result of the scales' thickness only in the case of no-choice test. On the other hand, both of the layer's number and the degree of scales affected significantly on the emergence rate of the parasitoid in choice test ($F_{8,126}$ = 17.218; $P < 0.001$). The lowest emergence was recorded on high-scaly three-layer eggs in both tests.

Females' percentage

The overall sex ratio of emerged adults was female-biased, but with different rates, regardless of the number of egg layers as well as the scale density (Table 3). The only exception was noticed in the case of one-layer eggs covering with thick scales; the percentage of females was decreased dramatically in both tests and recorded the lowest rate (≤ 45%). The female percentage was highly significantly influenced by the different physical characteristics of eggs in no-choice and choice tests (ANOVA, $F_{8,92}$ = 17.946, 13.779; $P < 0.001$), respectively.

Longevity of adults

The survival of fed female parasitoid at 25 °C was non-significantly affected by the egg layers and the scale density in both tests (ANOVA, $F_{8,125}$ = 0.385, 1.455; P = 0.927, 0.185), respectively (Fig. 3).

Discussion

There is an urgent need for minimizing the dependence of chemical pesticides in the management of insect pests. Therefore, in view of the problems based on the chemical, alternative environmentally friendly methods are essentially needed. Most of the researches focused on different *Trichogramma* species against different *Spodoptera* species and none of them studied *T. bactrae* and its association with *S. littoralis*. It is important to bear in mind that understanding and knowing the factors affecting the efficacy of *T. bactrae* wasps against *S. littoralis* eggs with different physical characteristics is crucial to improve the control program against this pest in the field. Host preference is an important parameter for biological control programs as more than one pest species of different physical characteristics may occur in the field (Goulart et al. 2011). Meanwhile, host

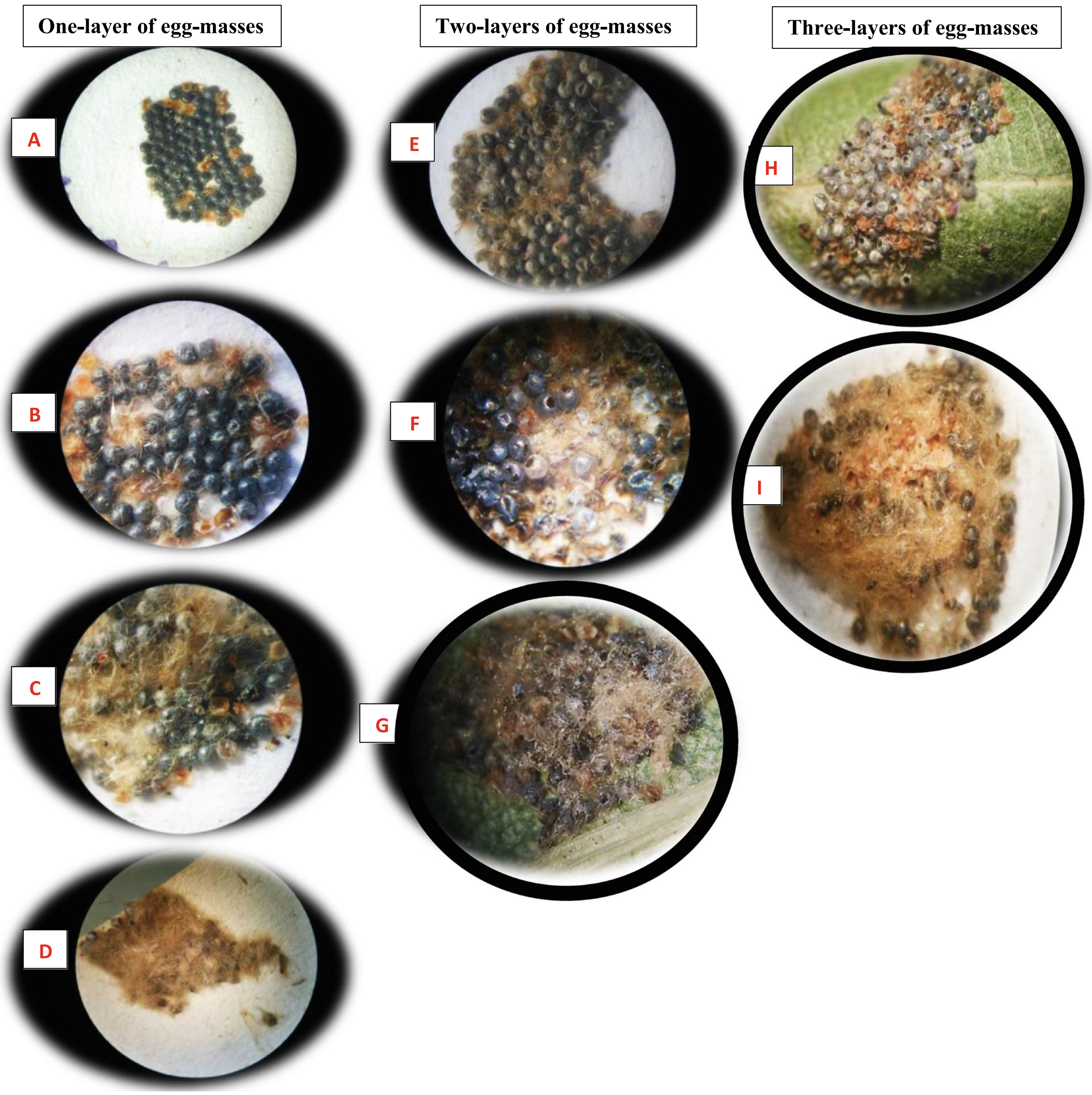

Fig. 1 Parasitism of *T. bactrae* on *S. littoralis* egg masses with different physical characteristics: **a** 1 layer with no scales, **b** with low scale density, **c** with mid scale density, **d** with high scale density, **e** 2 layers with low scale density, **f** with mid scale density, **g** with high scale density, **h** 3 layers with low scale density, **i** with high scale density

recognition and acceptance are mainly driven by physical and chemical cues, which are mostly detected by the sensilla on the antennae and the ovipositor; these assess the suitability of a host for parasitization (Schmidt 1994). Parasitism capacity is an important indicator for the parasitoid efficiency, reproduction, and flourishing as a bio-control agent (Nurindah and Cribb 1997). Based on the present results, the higher parasitism rates of *T. bactrae* were observed on the single-layer eggs, maybe due to the parasitoid was able to access and examine all the eggs deposited in the mass, followed by the two-layers. However, three-layer eggs had the least rate in both no-choice and choice tests, because the parasitoid was only able to arrive and parasitize the outer, the edge, and/or the exposed eggs, but unable to parasitize the lower layer. Based on our observations, scales with low and mid thickness degree covering the eggs seemed to be as a chemical stimulus and attracted the female parasitoid of *T. bactrae* to parasitize, but with different rates related to the overlapping layers. The findings are mostly

Table 1 Percentages of parasitism of *T. bactrae* on *S. littoralis* egg masses with different physical characteristics in a no-choice and choice tests

Egg masses	No-choice		Choice	
	Parasitism%	Overall average	Parasitism%	Overall average
One layer with no scales	70.73 ± 1.84 Ab (50–96.82)	**66.67± 2.22[a]**	71.76 ± 2.86 Aa (57.14–100)	**60.53 ± 2.49[a]**
One layer with low scale density	77.08 ± 1.62 Aa (60–100)		70.85 ± 2.61 Ba (53.33–88.37)	
One layer with mid scale density	71.10 ± 2.46 Ab (36–87.04)		54.38 ± 2.12 Bc (33.33–78.20)	
One layer with high scale density	47.71 ± 2.97 Ae (15–69)		45.12 ± 2.37 Ad (30–58.62)	
Two layers with low scale density	64.50 ± 1.97 Ac (48.19–81.03)	**58.23± 2.06[b]**	61.81 ± 1.03 Ab (55–68.54)	**48.53 ± 1.31[b]**
Two layers with mid scale density	56.03 ± 2.35 Ad (36.58–82.83)		48.81 ± 2.64 Bcd (28.95–58.67)	
Two layers with high scale density	54.17 ± 1.86 Ade (38.09–71.95)		35.00 ± 0.28 Be (32-36.67)	
Three layers with low scale density	38.84 ± 2.69 Af (30–54.61)	**29.03±3.11[c]**	25.63 ± 0.08 Bf (25–26.65)	**22.67 ± 2.29[c]**
Three layers with high scale density	19.23 ± 3.52 Ag (8–36)		19.72 ± 3.13 Af (5–36.57)	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows are statistically insignificant
$P > 0.05$. Numbers in parentheses represent the range

in accordance with those reported by other researchers on different *Trichogramma* species as mentioned by (Beserra and Parra 2005) that the parasitism percentage on *S. frugiperda* egg masses with one, two, and three layers were (66.24, 45.20, and 40.10%), respectively. Besides, Beserra et al. (2005) recorded 63.2% parasitism by *T. atopovirilia* on single-layer eggs of the same pest. As a result of releasing *T. pretiosum* parasitoid against this pest on maize fields, the rates increased according to the number of releases (1–3) (69.8, 79.2, and 68.75%), respectively (Figueiredo et al. 2015). On the other hand, *T. pretiosum* and *T. minutum* wasps were parasitized on (44.8 and 51.6%) of *S. exigua* eggs, respectively (Greenberg et al. 1998). On *S. litura* eggs, the parasitism rate by *T. chilonis* reached 80.31% (Puneeth and Vijayan 2013). Obtained results on the high-scaly eggs seemed to be less attractive or unpreferable host and had a negative impact on overall behavior. This may be because of different females of *Trichogramma* species reabsorbed their oocytes during unsuitable conditions or hosts' deprivation, causing a reduction in parasitism percentage and the period of fertility (Hougardy et al. 2005). The parasitism rates on *S. frugiperda* egg layers with thick scales were very low (≤ 10%) as reported by Toonders and Sánchez (1987) and Beserra et al. (2002, 2005) in Brazil and Mexico. This may be due to the females avoided parasitism on high-scaly eggs that prevents their progeny, searching for new preferable ones. Another aspect as reported by Noldus (1989), *S. frugiperda* eggs are rarely attacked by *T. pretiosum* because this parasitoid does not respond to its semiochemicals and thus led to low parasitism.

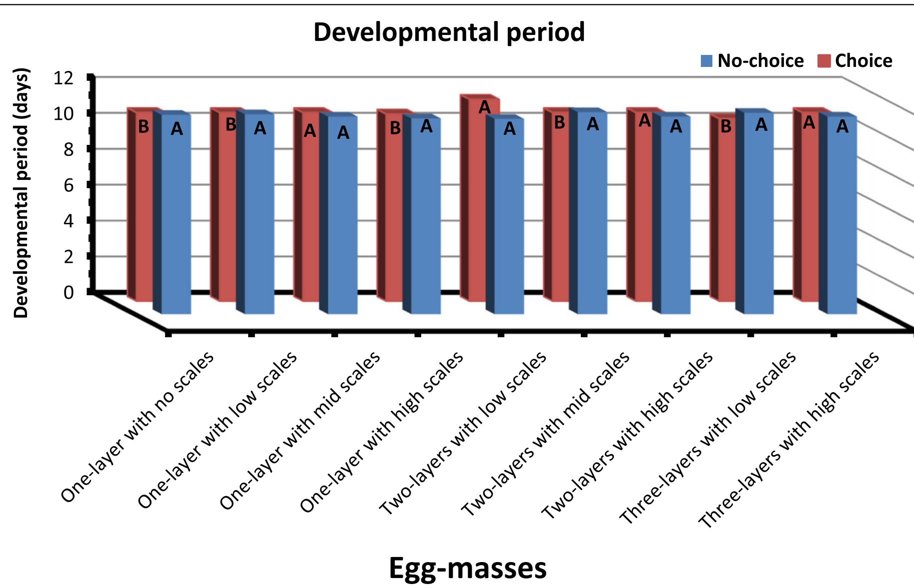

Fig. 2 Developmental period (days) of *T. bactrae* from *S. littoralis* egg masses with different physical characteristics in a no-choice and choice tests. Means denoted different letters between both tests are significantly different ($P < 0.05$)

Table 2 Percentages of adults' emergences of *T. bactrae* from *S. littoralis* egg masses with different physical characteristics in a no-choice and choice tests

Egg masses	No-choice		Choice	
	Adult emergence percentage	Overall average	Adult emergence percentage	Overall average
One layer with no scales	85.54 ± 1.81 Abc (60–100)	**86.99 ± 1.92^{a}**	83.03 ± 4.88 Aab (50-100)	**84.41 ± 2.92^{b}**
One layer with low scale density	89.61 ± 1.38 Aab (66.68–100)		89.42 ± 2.60 Aa (66.67–100)	
One layer with mid scale density	87.02 ± 3.34 Aabc (60–96)		82.53 ± 4.04 Ab (66–100)	
One layer with high scale density	85.79 ± 1.17 Abc (80–93.94)		82.65 ± 0.14 Ab (82–83.33)	
Two layers with low scale density	91.43 ± 1.98 Aa (70–100)	**87.28 ± 2.50^{a}**	91.14 ± 1.26 Aa (84.21–97.33)	**88.99 ± 1.76^{a}**
Two layers with mid scale density	87.86 ± 2.02 Aabc (61.6–99.04)		86.11 ± 2.31 Aab (73.91–97.14)	
Two layers with high scale density	82.55 ± 3.49 Abc (50–96.66)		89.71 ± 1.73 Aa (84–100)	
Three layers with low scale density	82.22 ± 4.10 Ac (70–95.52)	**75.3 ± 8.11^{a}**	70.20 ± 2.10 Bc (60–85)	**59.51 ± 3.90^{c}**
Three layers with high scale density	75.63 ± 6.22 Ad (50–94.44)		52.82 ± 4.50 Bd (25–83.33)	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows are statistically insignificant
$P > 0.05$. Numbers in parentheses represent the range

Occasionally, *T. bactrae* parasitoid tried to remove the scales by moving on the egg mass and passing the front legs over the antennae, also rubbing the hind legs with each other's, cleaning the scales adhered to the body. Similar behavior was observed by the egg parasitoid, *Telenomus remus* Nixon females on *S. frugiperda* egg masses (Carneiro and Fernandes 2012).

Ultimately, the variability in the number of parasitized eggs noticed on the previous and the present studies may be related to the different *Spodoptera* species, also to the geographical origin, and the parasitoid strain. Therefore, an adaptation of a specific parasitoid strain from a specific region against the target pest must be taken into consideration for the success of the biological control program.

Afterwards, high adult emergence rates were recorded in all examined egg masses, with exception of high-scaly three-layer eggs. This finding was in similar line with that of Bueno et al. (2010) who recorded the emergence rate of *T. pretiosum* from *S. frugiperda* eggs was higher than 88% in all tested temperatures (18–32 °C). Another important parameter in the biological control programs is sex ratio. Higher rates of females in their progeny are vital in mass production and responsible for the direct suppression of the target pest when released in the field (Bueno et al. 2009). With the following mating, the female wasps store their sperms in the spermatheca, and through parasitization, it can decide the sex ratio of their progeny by controlling the entry of sperm to the egg (Suzuki et al. 1984). At the present experimental conditions, the overall sex ratio of emerged adults among all examined egg masses was female-biased. Contrary to this prediction, on high-scaly eggs with a single layer, the percentage of females decreased significantly (< 45%) in both no-choice and choice tests. This may be due to the presence of densely scales covered the eggs, making them as unsuitable or unattractive hosts for the decision to deposit fertilized eggs and thus decreased the number

Table 3 Percentages of *T. bactrae* females emerged from *S. littoralis* egg masses with different physical characteristics in a no-choice and choice tests

Egg masses	No-choice		Choice	
	Female percentage	Overall average	Female percentage	Overall average
One layer with no scales	69.33 ± 2.47 Ab (60–81)	**65.73 ± 1.98^{a}**	72.00 ± 2.81 Aa (60–90)	**60.6 ± 2.42^{b}**
One layer with low scale density	71.67 ± 1.23 Ab (59–82)		63.80 ± 1.28 Bb (55–70)	
One layer with mid scale density	76.28 ± 1.04 Aa (74–80)		64.80 ± 2.99 Bab (60–90)	
One layer with high scale density	45.62 ± 3.19 Ad (30–55)		41.80 ± 2.69 Ac (30–55)	
Two layers with low scale density	67.91 ± 2.00 Ab (59–79)	**66.7 ± 1.70^{a}**	66.77 ± 2.03 Aab (50–73)	**66.62 ± 1.80^{a}**
Two layers with mid scale density	71.18 ± 1.97 Ab (60–82)		66.00 ± 1.94 Aab (60–75)	
Two layers with high scale density	61.00 ± 1.14 Bbc (55–65)		67.10 ± 1.42 Aab (60–72)	
Three layers with low scale density	64.43 ± 1.72 Abc (55–70)	**62.57 ± 1.71^{b}**	68.80 ± 2.92 Aab (55–90)	**65.9 ± 2.22^{ab}**
Three layers with high scale density	60.71 ± 1.70 Ac (55–65)		63.00 ± 1.53 Ab (55–70)	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows are statistically insignificant
$P > 0.05$. Numbers in parentheses represent the range

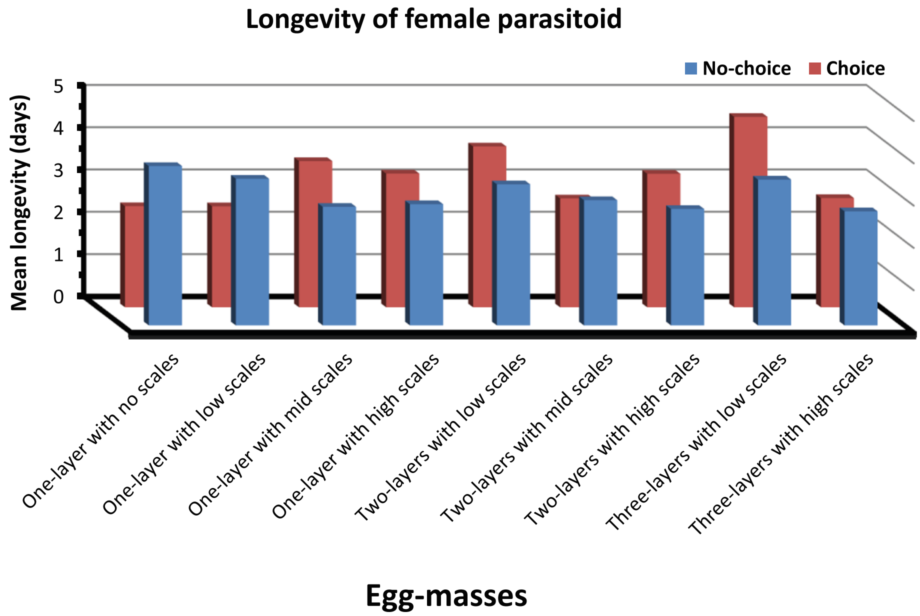

Fig. 3 Mean longevity (days) of female *T. bactrae* adults emerged from *S. littoralis* egg masses with different physical characteristics in a no-choice and choice tests. No significant differences were found between both tests

of produced females in the progeny. In addition, as reported by Houseweart et al. (1983), the sperm depletion or decrease in the females' spermatheca led to a decrease in the number of fertilized eggs, so it forced to parasitize but produced unfertilized eggs (males).

Certainly, this laboratory study is necessary to improve the biocontrol program against this pest. Moreover, further field studies of the efficacy of this parasitoid toward different *Spodoptera* species are needed.

Conclusions

The results proved that *T. bactrae* is an important parasitoid species with a great ability to parasitize *S. littoralis* egg masses related to their layers and the degree of scales' thickness, in choice and no-choice tests. The most preferable egg mass to this parasitoid was one layer, followed by two layers. Highest numbers of adult emergency were observed in all examined egg masses, except in the case of the 3 layers with high-scaly eggs. *T. bactrae* could be used as a bio-control agent against *S. littoralis* egg masses with their different physical characteristics.

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Author's contributions
HOM suggested the aim of study, conducted the experiments, and prepared and wrote the manuscript (single author). The author read and approved the final manuscript.

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Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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