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Impact of egg mass layers and scale thicknesses of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), on the parasitic performance of *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae)

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Abstract

Background Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the new alien destructive pests of maize and other 350 economic crops. The majority of farmers are still depended upon chemical insecticides to suppress the pest, but *S. frugiperda* has succeeded to develop resistance against most of the chemical families. Improving an effective environmentally-friendly approach is highly recommended. Therefore, the egg parasitoids are the best weapon for managing the FAW in the early egg stage due to the feeding behavior of their larvae. In this regard, the impact of FAW egg mass layers and scale thicknesses, as physical barriers, on the parasitic performance of the egg-parasitoid species, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae), in non-choice and choice tests was assessed. Besides, the efficacy of FAW-produced adult wasps on the next generation based on the rates of parasitism, adults' emergence, and female progeny was determined.

Results Obtained results exhibited that *T. bactrae* was able to parasitize all exposed FAW egg masses but with different rates related to the layers' number and scales' thicknesses in both tests. One-layer (83.18, 78.24%) and two-layer egg masses (65.99, 76.42%) had significantly the highest parasitism rate, while three layers (42.15, 46.05%) was the least one, in both tests, respectively. All parasitoids emerged after 10–12 days with high rates (~ 88–98%) from all the tested egg masses, and the majority offspring were female-biased in both tests. Furthermore, parasitic performance in F_1 progeny was similar with that recorded in parental generation in terms of parasitism rate, high parasitoid emergency (~ 87–95%), and strongly female-biased (~ 68–76%) in all the exposed egg masses.

Conclusions The egg parasitoid, *T. bactrae,* could be an efficient and recommended bio-control agent against FAW as its greatest ability to overcome the layers' number and scales' thickness.

Keywords Biological control, Egg parasitoid, Fall armyworm, Physical barriers, Parasitism

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Introduction

The invasion of insect pests to any new ecosystem through the agricultural products movement and international trade, conventional quarantine programs, climatic conditions, human practices (Paini et al. 2016), and other unidentified factors is directly causing serious threats to the food security, livelihood of many millions of farmers and consumers, agricultural production, and subsequently national economy and income level decreased (Sharanabasappa et al. 2018). One of these new alien invasive pests is the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), which feeds on the vegetative and reproductive parts of maize as its preferred host (FAO 2017), and other 350 economical crops (CABI 2020). The major economic damage of FAW is related to its ability to disperse approximately 500 km before oviposition, high reproductive and fecundity rate (> 2000 eggs), feeding behavior, and adaptability to different climatic conditions rather than its continuous generation per year (Mohamed 2022). The first confirmed documentation of the FAW invasion in Africa was published in early 2016 (Goergen et al. 2016), and then, it continued its spread reaching Upper Egypt for the first time by May 2019 (Dahi et al. 2020). Afterward, this migratory pest invaded most of the Egyptian governorates by late 2021 (Mohamed 2022).

Currently, different control techniques have been used to manage FAW outbreaks, but chemical control is still the most common one applied by most farmers. Over-use applications of these insecticides with different classes have multiple negative side effects on natural enemies, non-target organisms, environmental contamination, human health, and secondary pests' outbreaks (Akhtar and Farooq 2019). Besides, *S. frugiperda* has developed resistance against most of the chemical families and it is ranked on the top 15 most resistant arthropods as listed in the "Arthropod Pesticide Resistance Database" (Hilliou et al. 2021). Moreover, their resistance increased against *Bacillus thuringiensis* (*Bt*) proteins (Murŭa et al. 2019) such as Cry1A and Cry1F. In response to the above concerns, it is highly necessary to improve an effective environmentally-friendly approach rather than chemicals for the sustainable control of this quarantine pest. Among these non-chemical techniques, biological control using natural enemies is an essential tool of integrated pest management (IPM) and more suitable for farmers who lack the financial resources to purchase the costly insecticides to suppress this pest (Abate et al. 2000). As FAW females moths laid their eggs on the leaves of different host plants, the egg parasitoids are the most appropriate and effective tool for managing them in this early egg stage (Parra and Coelho Jr 2019), thereby preventing the destructive damage caused to the crops. Since the feeding behavior of FAW larvae was hidden within the plant leaves and pupae were in the soil, both of these two developmental stages were under protected conditions.

Since the last century, the egg-parasitoid wasps of *Trichogramma* and *Trichogrammatoidea* species are considered the most recommended biological control agents against eggs of different lepidopteran pests on different economic crops (Haoxiang et al. 2023). In this context, the efficiency of *Trichogramma* species against this invasive pest has been investigated in different regions as reported previously by Dong et al. (2021), Hou et al. (2022), Li et al. (2023). All of these tested egg-parasitoid species revealed variations in their parasitic behavior and these may be related to the parasitoid species efficacy and/or the FAW female deposited its egg mass in different numbers of layers (one to > 3 egg layers) covering with various degrees of scale thicknesses, which can affect the parasitism rates. Until now, limited information is available on the parasitic potential of the *Trichogrammatoidea* species against *S. frugiperda* egg masses without considering the different morphological characteristics (layers and scales). In this response, this study highlighted the impact of egg mass layers and degrees of scales' thicknesses of *S. frugiperda* as physical limitation barriers on the parasitic performance of *T. bactrae* in non-choice and choice tests, under standard laboratory conditions. In addition, their efficiencies on F_1 progeny in terms of parasitism rate, adult emergence rate, and female ratio were evaluated. This study has provided a detailed picture on the efficacy of *T. bactrae* wasps against FAW egg masses with different layers and scales for the first time to improve the biological control strategy in the field.

Methods

Insect culture

The culture of *S. frugiperda* larvae was initially collected from infested maize fields, El Fath District (Bani Murr), Assiut Governorate (27° 13′ 07″ N, 31° 11′ 46″ E, 52 m), Egypt. The rearing technique of FAW was carried out in accordance with the methodology defined by Mohamed (2022). Larvae were divided in a clean plastic container (20 cm length × 15 cm width × 20 cm height) provided with fresh maize leaves for feeding until pupation. Pupae were collected and placed in rearing wood cages (35 × 35 × 35 cm) until the adult moth emergence. Moths were fed on 10% honey solution and renewed daily until their death. The rearing cage was supplied with zigzag sheets of white copy paper (A_4 size) and branches of oleander (*Nerium oleander* L.) to allow oviposition. Deposited egg masses were collected daily and neonates were reared on fresh castor oil leaves (*Ricinus communis* L.) until pupation and transferred to new cages for emergence of adult and egg deposition. Samples of egg masses were used either to begin the experiments or to maintain the rearing of insect colonies.

Parasitoid species

The parasitic wasp, *T. bactrae,* was imported from Australia for the first time by Dr. A. H. El-Heneidy (ARC, Egypt), in 1992. It was mass reared at the Center of Bio-organic Agricultural Services (CBAS), in Aswan Governorate, Egypt, and established under Egyptian environmental conditions (Mohamed and El-Heneidy 2020). In 2014, it was transferred to the mass rearing unit of *Trichogramma*, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Assiut Governorate, Northern Upper Egypt. For 3 successive generations before the experiment started, the parasitoids were maintained on eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae) (Mohamed et al. 2016), under standard rearing conditions (26 ± 1 °C; > 60% RH with a photoperiod of 14L: 10D). Voucher specimens were deposited at the Biological Control Department, Plant Protection Research Institute, Giza, Egypt.

Experimental protocol and biological parameters assessment

Newly laid FAW egg masses (≤ 24 h old) (250–300 eggs) were divided morphologically based on the egg mass layers' number and degrees of scales' thickness into 10 treatments. These egg mass treatments are: one layer of eggs without scales, one layer with low scales, one layer with mid-scales, one layer with thick scales, two layers with low scales, two layers with mid-scales, two layers with thick scales, three layers with low scales, three layers with mid-scales and three layers with thick scales. The scales' thicknesses were distinguished as low scales referred to a thin coverage layer of scales but thick scales to dense or full-scale coverage. Freshly 40 mated *T. bactrae* females (≤ 24 h old) from *S. cerealella* without any prior ovipositional experience on FAW in plastic jars (500 ml) were exposed to the examined egg masses (10 treatments) in 2 types of tests (non-choice and choice tests), for 24 h to prevent super-parasitism. Non-choice test, by permitting only one type of egg masses separately to the adult parasitoids, while choice test by allowing all examined eggs at a time to the parasitoids. The parasitized FAW eggs of each treatment after 24 h of exposure were removed and kept in new jars, under the above standard rearing conditions. After three days post-parasitism, newly hatched FAW larvae (neonates) emerged from un-parasitized egg mass were gently removed by a fine brush to prevent them from feeding on parasitized eggs. These experiments were repeated 15 times for each tested egg mass.

For biological assessment of *T. bactrae* on *S. frugiperda* egg masses with various morphological characteristics, parasitism %, developmental duration (days), adult emergence % and female offspring % were measured in both tests. After six days of exposure, all examined FAW egg masses were checked under a stereomicroscope to calculate parasitism % (number of blackened parasitized eggs / overall number of exposed eggs) × 100. The exposed egg mass was counted as (number of hatched larvae (neonates) + un-hatched eggs (dead larvae) + blackened parasitized eggs). Developmental duration (d) (the average number of days from egg mass exposure to the parasitoid until adult parasitoid emergency) and adult emergence rate (number of parasitized eggs with exit holes / all parasitized eggs) × 100 were recorded. Adult female % was assessed as (number of adult emerged females / all individuals) × 100, using a stereomicroscope to examine dead adults based on the morphology of antennae as male with long terminal antennal segment and dense curved hairs, while female with tiny hairs and a leaf appearance terminal segment (Fig. 1). In addition, the efficiency of the produced parasitoids from FAW egg mass on their next progeny in terms of parasitism %, adults' emergence %, and female % was evaluated with the same method as calculated above, under the aforementioned rearing conditions. Samples of FAW egg masses (10 treatments) were exposed for 24 h to the produced parasitoid and were repeated 10 times for each tested egg mass.

Data analysis

Obtained data from parental parasitoid and F_1 progeny were analyzed using one-way analysis of variance (ANOVA). Before analysis, data were subjected to arcsine $\sqrt{\text{proportion}}$ transformed to meet normality. When significant differences were observed, means were separated by t test at $P \leq 0.05$ level for comparisons. The statistical analysis and graphs were aided by Microsoft Excel® software, according to Fowler et al. (1998).

Results

Parental generation

Parasitism percent

The egg parasitoid, *T. bactrae*, was capable to parasitize with different rates all exposed *S. frugiperda* egg masses depending on the number of layers and scale thicknesses (Fig. 2) in non-choice and choice tests (Table 1). Regarding egg mass layers, one-layer (83.18, 78.24%) and two-layer egg masses (65.99, 76.42%) had significantly ($P < 0.05$) the highest mean of parasitism %. The three layers (42.15, 46.05%) were the least preferable one, in both tests, respectively (Fig. 3). High scaly three-layer egg mass had effectively limited parasitism rate by the parasitoid ($P = 0.001$, 0.004), reaching the least rate (≤ 40%) than all

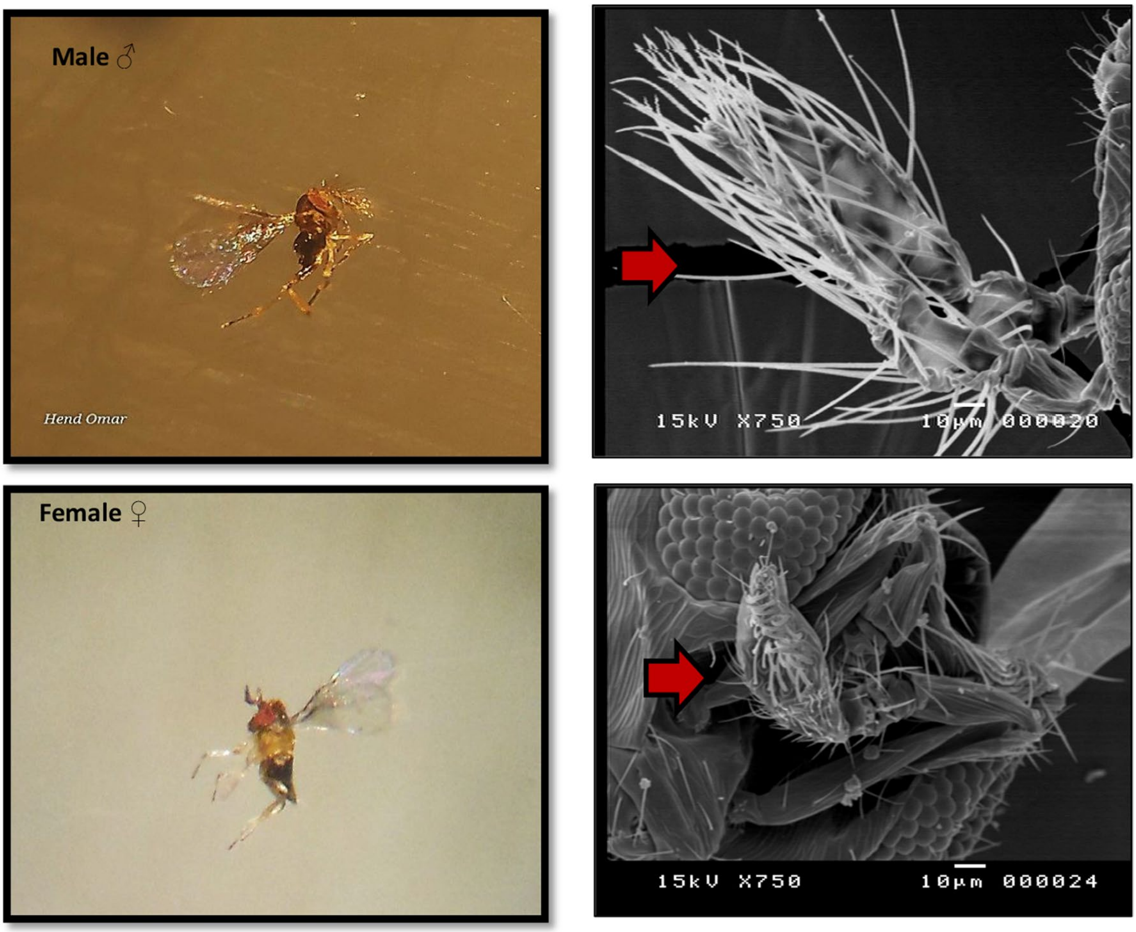

Fig. 1 Male and female egg parasitoid, *Trichogrammatoidea bactrae* showing the male antenna as long terminal antennal segment with dense curved hairs, while female with tiny hairs and a leaf appearance terminal segment under scanning electron microscope (SEM)

exposed egg masses in both tests, respectively. The parasitism rates were more prominent when the parasitoids were permitted to choose among all tested eggs masses at the same time (choice test) than when those were offered individually, particularly in the two-layer egg masses. The only exception was on one-layer egg mass with a thick scale; the parasitoid was able to parasitize on (~73%) of the exposed eggs when forced in a non-choice test than (~55%) in choice one (Table 1). The physical characteristics of FAW egg masses (layers and scales) exhibited a remarkable effect on the parasitism percent of *T. bactrae* ($F_{9,150}$ = 73.729, 56.161; P < 0.0001) in non-choice and choice tests, respectively. Regardless of scale thicknesses, non-significant changes were found between both tests based on the egg mass layers (Fig. 3).

Developmental duration

The mean developmental duration needed for the adult parasitoid emergence was not affected by the variations in layers or scale thicknesses on the egg masses ($F_{9,50}$ = 1.222, 0.495; P = 0.303, 0.871) in non-choice and choice tests, respectively (Fig. 4). All parasitoid emerged after 10–12 days from all examined FAW eggs in both two tests.

Adults' emergence %

The proportion of adults' parasitoid emergency from *S. frugiperda* egg masses was higher (~88–98%) in all 10 treatments, in both tests (Table 2). Besides, it was directly varied ($F_{8,126}$ = 4.456; P < 0.0001) by the degree of scale thicknesses only not the egg layers in the non-choice test (Fig. 5). However, the number of egg mass layers and scale's coverage influenced significantly on the parasitoid emergence % in the choice test ($F_{8,126}$ = 17.218; P < 0.001). Except for the significant change (P < 0.01) in emergence rate of *T. bactrae* wasps from the egg mass without or with low scale level when considering the number of egg layers between non-choice and choice tests, there were no variations among other exposed egg masses in both tests (Table 2). Regardless of the scales' level, egg mass

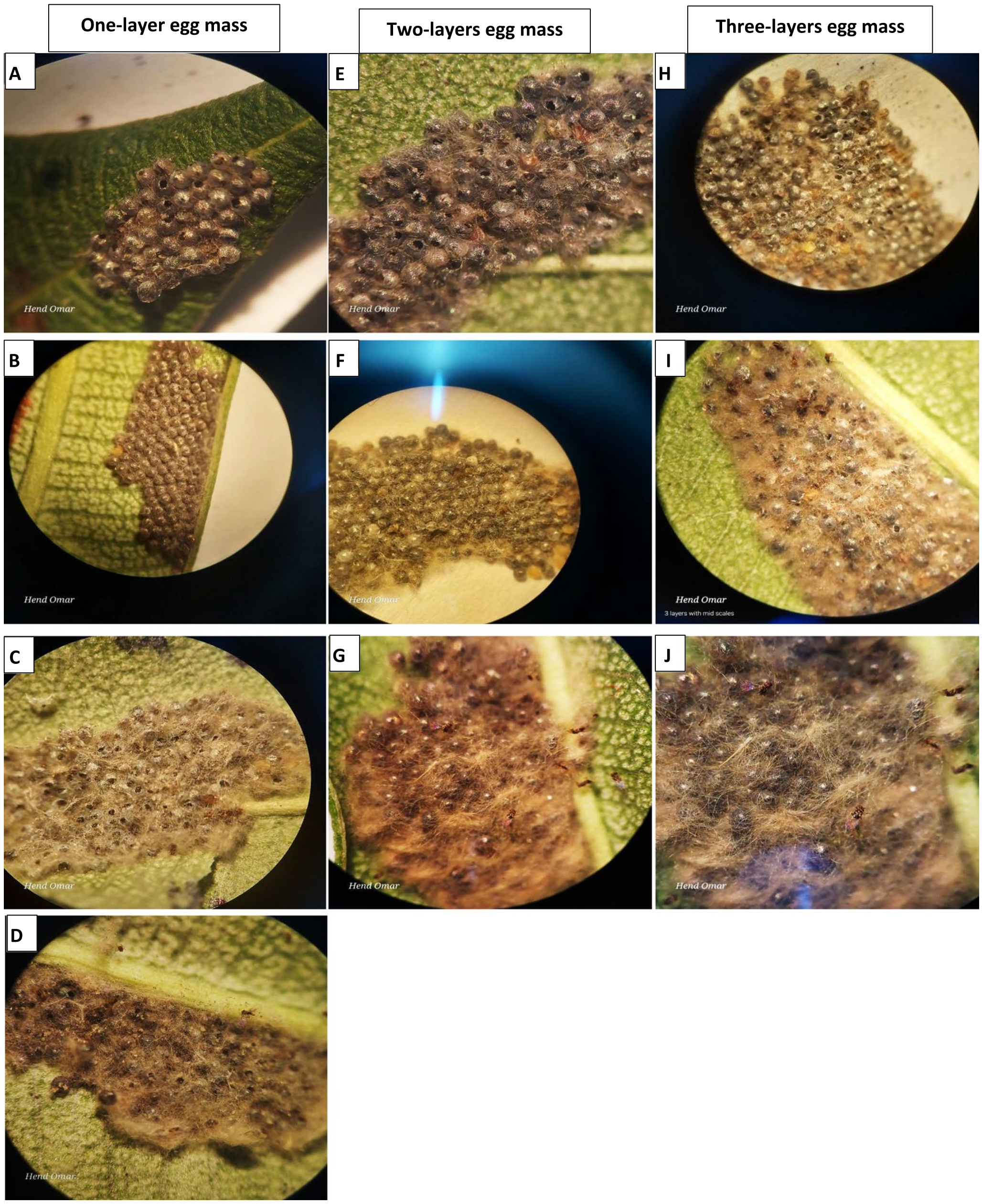

Fig. 2 Parasitization of *Trichogrammatoidea bactrae* on *Spodoptera frugiperda* egg masses of different layers' number and scales' thickness: **A** one-layer eggs without scales, **B** one layer with low scales, **C** one layer with mid-scales, **D** one layer with thick scales, **E** two layers with low scales, **F** two layers with mid-scales, **G** two layers with thick scales, **H** three layers with low scales, **I** three layers with mid-scales, **J** three layers with thick scales

layers did not affect negatively ($P=0.285$) the emergence % between non-choice and choice tests (Fig. 5).

Females' percentage

The majority of adult parasitoids that emerged from all exposed FAW egg masses were female-biased in both tests (Table 3). The female ratio (62–74.5%) was directly changed by the number of egg mass layers and the scale thicknesses in a non-choice test ($F_{9,100}=3.865$; $P=0.0003$) (Table 3; Fig. 6). However, non-significant variation ($P=0.574$) was recorded in the female percent (>73%) when the parasitoid was offered simultaneously to all egg masses according to the egg layers (Fig. 6), while the significant variation (~67–77%) was only related to the degree of scale's density ($F_{9,100}=2.583$; $P=0.01$) (Table 3). Statistically, non-significant differences were

Table 1 Percentages of parasitism of *Trichogrammatoidea bactrae* on *Spodoptera frugiperda* egg masses on different layers' and scales' thickness in a non-choice and choice tests

Egg masses	[a]Exposed eggs	Parasitism (%)		*t*-test
		Non-choice test	Choice-test	*P*-value
One layer without scales	265.64	88.98 ± 0.91^{aA}	86.26 ± 1.25^{aA}	0.094
One layer with low scales' density	269.25	88.74 ± 1.56^{aA}	88.70 ± 1.37^{aA}	0.985
One layer with mid-scales' density	270.55	81.50 ± 2.35^{bA}	82.18 ± 2.31^{abA}	0.192
One layer with thick scales' density	268.8	73.48 ± 1.27^{cA}	55.82 ± 5.36^{eB}	0.008
Two layers with low scales' density	279.37	71.62 ± 1.78^{cdB}	81.47 ± 1.68^{bcA}	0.0005
Two layers with mid-scales' density	279	67.99 ± 2.09^{dB}	76.11 ± 2.91^{cdA}	0.035
Two layers with thick scales' density	275.5	58.35 ± 3.19^{eB}	71.68 ± 1.18^{dA}	0.0009
Three layers with low scales' density	278.86	46.57 ± 2.58^{fB}	53.28 ± 0.41^{eA}	0.021
Three layers with mid-scales' density	280.5	43.83 ± 2.75^{fA}	44.79 ± 1.91^{fA}	0.778
Three layers with thick scales' density	280.94	36.05 ± 1.29^{gA}	40.07 ± 2.43^{fA}	0.163
$F_{(9,150)}$		73.729	56.161	
P_{ANOVA}		< 0.0001	< 0.0001	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows between tests are statistically insignificant ($P > 0.05$)

[a] Mean number of exposed eggs

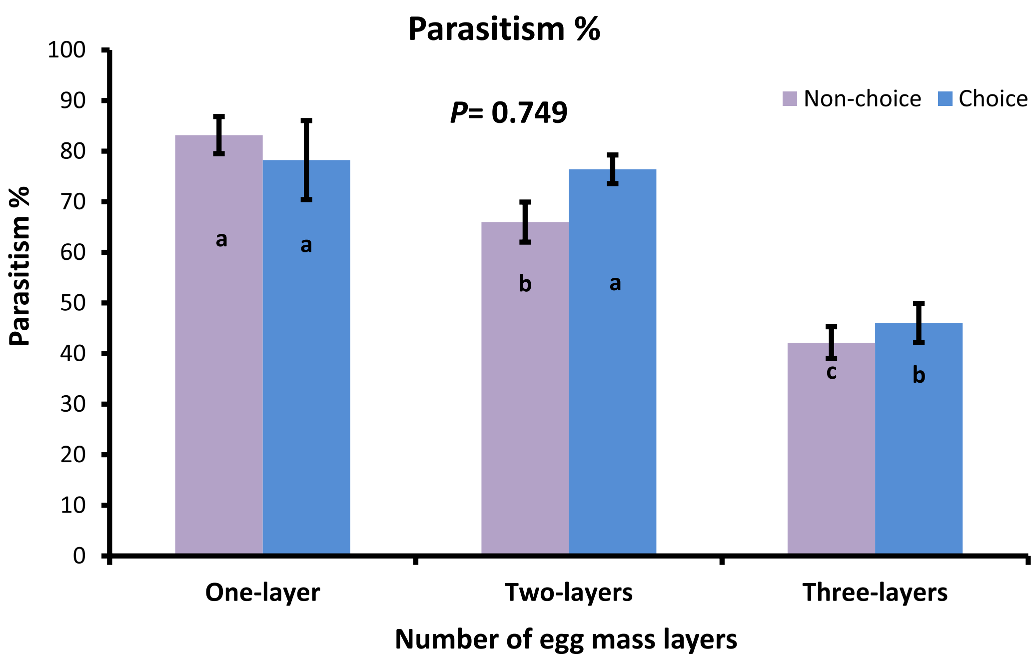

Fig. 3 Proportion of *Trichogrammatoidea bactrae* parasitism on *Spodoptera frugiperda* related to numbers of egg mass layers in non-choice and choice tests. Non-significant differences were recorded in the parasitism rate between both tests ($P > 0.05$). Columns denoted different letters within the same test are statistically significant ($P < 0.05$)

found between non-choice and choice tests in the female % from one- and two-layer egg masses; the only exception was on three layers (Fig. 6).

F_1 progeny

Efficacy of the produced *T. bactrae* parasitoid from *S. frugiperda* egg masses on the next generation (F_1 progeny) was evaluated based on the rates of parasitism, adult emergence, and female (Table 4). The mean percent of parasitism in F_1 progeny was similar somewhat with that recorded in parental generation in terms of one (79.13%) and two layers (66.16%) that had significantly ($P < 0.05$) the highest rate than three-layer (42.53%) egg masses, regardless of the

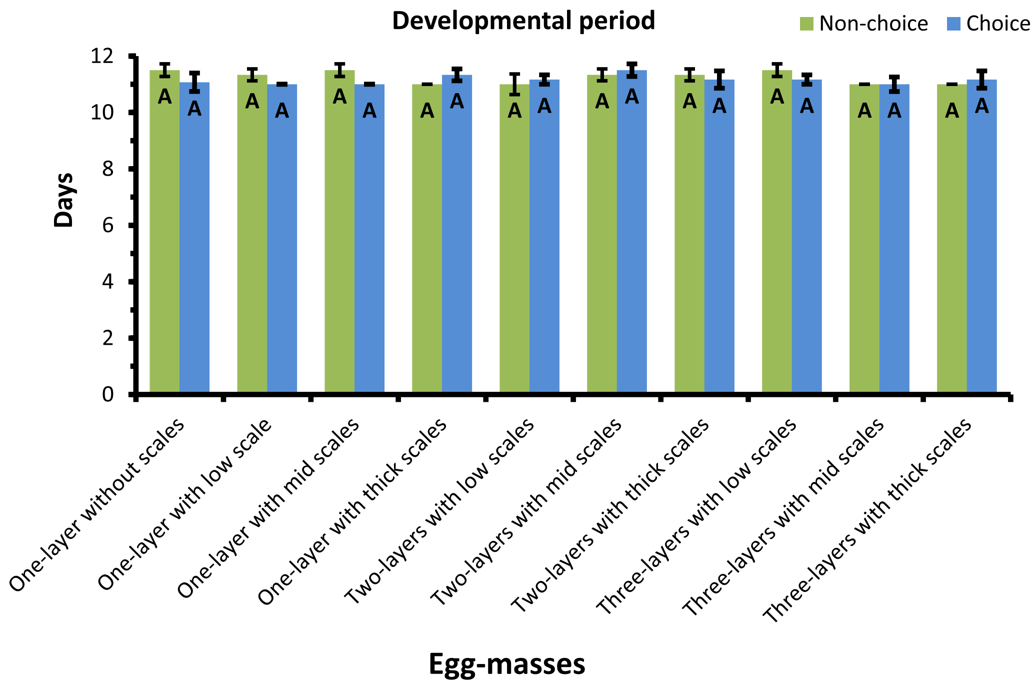

Fig. 4 Developmental duration (days) of *Trichogrammatoidea bactrae* emerged from *Spodoptera frugiperda* with different numbers of egg mass layers and scales' thickness in non-choice and choice tests. The parasitoid durations between both tests were insignificantly different ($P > 0.05$)

Table 2 Percentages of adults' emergences of *Trichogrammatoidea bactrae* from *Spodoptera frugiperda* egg masses on different layers' and scales' thickness in non-choice and choice tests

Egg masses	Adult emergence (%)		*t*-test
	Non-choice test	Choice test	*P*-value
One layer without scales	89.69 ± 0.61^{cdB}	92.49 ± 0.81^{cdA}	0.011
One layer with low scales' density	95.82 ± 0.98^{aA}	91.53 ± 2.81^{bcdA}	0.171
One layer with mid-scales' density	92.23 ± 1.85^{bcA}	90.95 ± 1.54^{cdA}	0.514
One layer with thick scales' density	87.94 ± 2.52^{dA}	88.23 ± 2.31^{dA}	0.933
Two layers with low scales' density	92.2 ± 1.62^{bcdB}	96.08 ± 0.87^{abA}	0.047
Two layers with mid-scales' density	96.36 ± 0.67^{aA}	94.82 ± 1.1^{bcA}	0.247
Two layers with thick scales' density	93.88 ± 1.37^{abA}	96.14 ± 0.37^{bA}	0.132
Three layers with low scales' density	88.71 ± 1.1^{dB}	98.03 ± 0.36^{aA}	< 0.0001
Three layers with mid-scales' density	91.71 ± 1.48^{bcdA}	94.42 ± 0.94^{bcA}	0.137
Three layers with thick scales' density	89.42 ± 1.23^{cdA}	90.13 ± 1.58^{dA}	0.727
$F_{(9,140)}$	4.456	4.409	
P_{ANOVA}	< 0.0001	< 0.0001	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows are statistically insignificant ($P > 0.05$)

scale thicknesses. High scaly egg mass with three layers (37.38%) had the least parasitism rate in all exposed treatments. Variations in egg layers and scales density had a great impact on the parasitism % by *T. bactrae* ($F_{9,90} = 113.714$; $P < 0.0001$). Besides, high parasitoid emergence (~ 87–95%) from all tested egg masses was directly influenced by the degree of scale thicknesses only ($F_{9,90} = 2.618$; $P = 0.014$). Afterward, the overall sex ratio of this adult parasitoid in offspring was strongly female-biased in all exposed egg masses ,and it was similar to that observed from the parents. The general mean of females (> 71%) was not changed related to the numbers of egg mass layers ($P = 0.783$), but it significantly varied by the degree of scales' thickness ($F_{9,90} = 2.054$; $P = 0.048$).

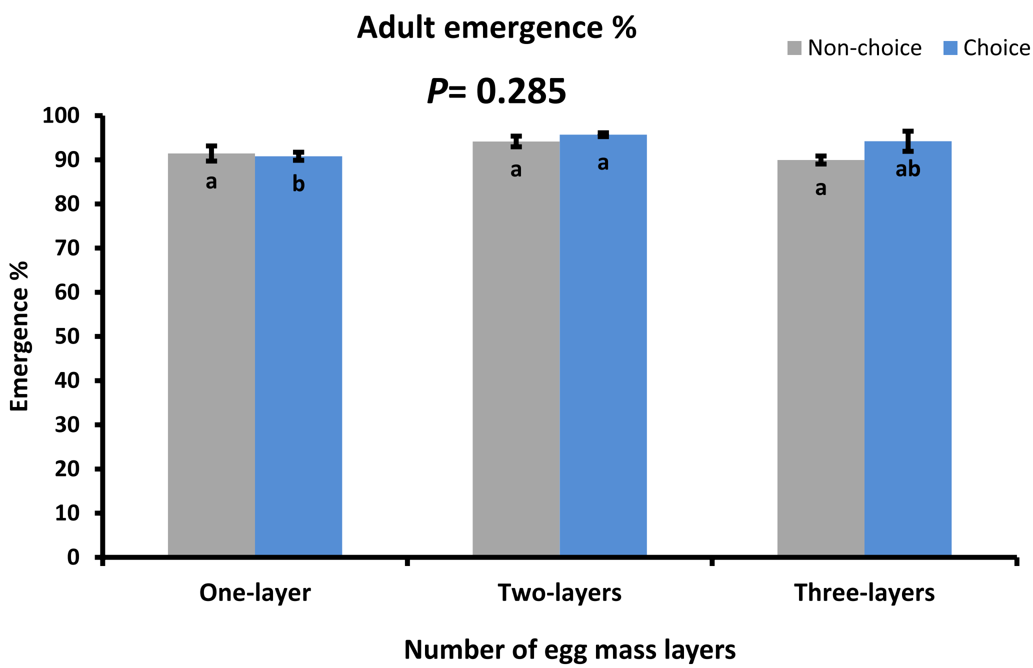

Fig. 5 Adult emergence rate of *Trichogrammatoidea bactrae* emerged from *Spodoptera frugiperda* with different numbers of egg mass layers in non-choice and choice tests. Columns with the same lowercase letters within the same test are insignificantly different ($P > 0.05$)

Table 3 Percentages of *Trichogrammatoidea bactrae* females emerged from *Spodoptera frugiperda* egg masses on different layers' and scales' thickness in non-choice and choice tests

Egg masses	Female (%)		*t*-test
	Non-choice test	Choice test	*P*-value
One layer without scales	74.5 ± 2.02^{aA}	67.75 ± 1.12^{bB}	0.011
One layer with low scales' density	69.8 ± 1.87^{abB}	77.18 ± 1.55^{aA}	0.007
One layer with mid-scales' density	71.00 ± 0.93^{abA}	75.75 ± 3.06^{aA}	0.164
One layer with thick scales' density	62.00 ± 2.11^{cB}	74.00 ± 2.79^{aA}	0.003
Two layers with low scales' density	70.1 ± 1.12^{abA}	72.27 ± 1.72^{aA}	0.304
Two layers with mid-scales' density	70.9 ± 0.98^{abB}	76.57 ± 1.68^{aA}	0.01
Two layers with thick scales' density	69.7 ± 1.53^{abB}	76.75 ± 1.60^{aA}	0.005
Three layers with low scales' density	67.8 ± 2.07^{bcB}	77.00 ± 0.40^{aA}	0.001
Three layers with mid-scales' density	68.4 ± 1.54^{bA}	73.18 ± 1.78^{aA}	0.057
Three layers with thick scales' density	69.2 ± 1.31^{bB}	76.14 ± 1.26^{aA}	0.001
$F_{(9,100)}$	3.865	2.583	
P_{ANOVA}	0.0003	0.01	

Means ± SE sharing the same small letters in the same columns and same capital letters in the rows between tests are statistically insignificant ($P > 0.05$)

Discussion

To overcome the threats caused by *S. frugiperda* on various economically cultivated crops, biological control by using the egg parasitoid could be one of the most effective sustainable management strategies against this pest in its early stage, which is also environmentally safer than harmful chemicals. However, several insect species covered their egg masses with materials for protection against egg parasitoids such as oothecae, scales, setae, feces, or silks. These materials particularly the scales are considered a strong physical barrier to parasitic oviposition behavior (Tian et al. 2020) through decreasing the parasitic potential attempts /or increasing the handling times (Gross 1993). Besides, the layers' number of FAW egg mass is a crucial factor influencing the parasitism potency (Beserra and Parra 2005). Therefore, parasitism

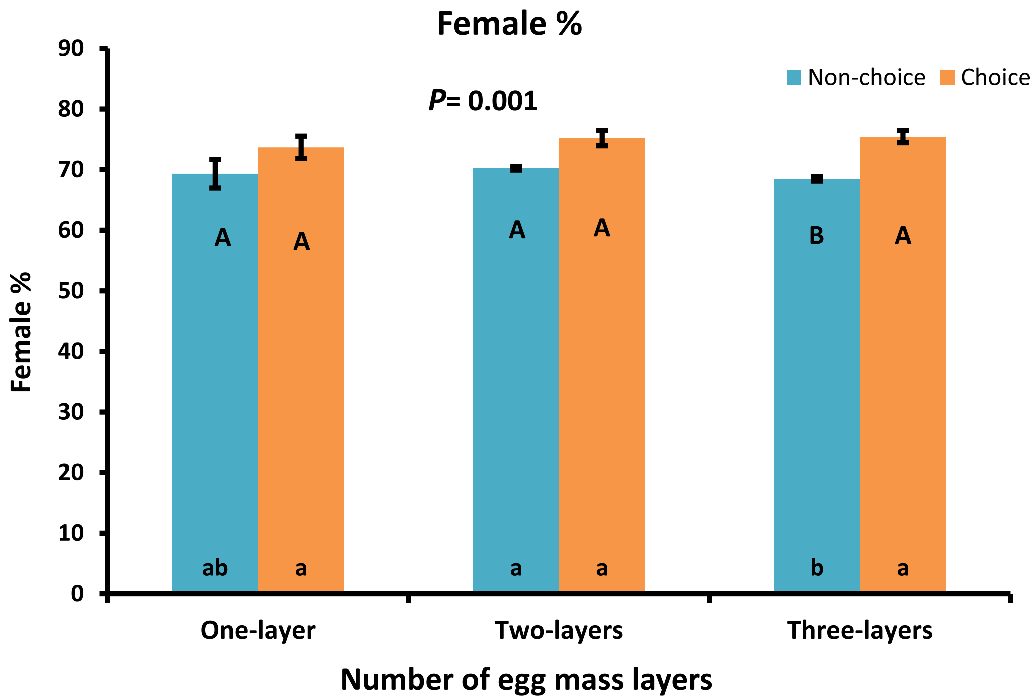

Fig. 6 *Trichogrammatoidea bactrae* females (%) emerged from *Spodoptera frugiperda* with different numbers of egg mass layers in non-choice and choice tests. Different uppercase letters within the same egg mass layer number between both tests, and different lowercase letters within the same test are significantly different ($P < 0.05$)

Table 4 Percentages of parasitism, adult emergence rates and females ratio of *Trichogrammatoidea bactrae* in F_1 progeny emerged from *Spodoptera frugiperda* egg masses on different layers' and scales' thickness

Egg masses	Parasitism (%)	Adult emergence (%)	Female (%)
One layer without scales	87.1 ± 0.49^{a}	90.95 ± 1.65^{bc}	74.00 ± 2.5^{abc}
One layer with low scales' density	85.78 ± 1.11^{a}	90.91 ± 1.50^{bc}	73.4 ± 3.38^{abcd}
One layer with mid-scales' density	76.50 ± 2.09^{b}	85.52 ± 2.53^{c}	68.35 ± 1.57^{d}
One layer with thick scales' density	67.16 ± 1.25^{c}	87.11 ± 2.17^{bc}	75.00 ± 1.96^{ab}
Two layers with low scales' density	73.15 ± 2.69^{bc}	91.97 ± 0.64^{b}	69.54 ± 2.59^{bcd}
Two layers with mid-scales' density	70.82 ± 2.1^{bc}	88.65 ± 2.37^{bc}	74.67 ± 0.82^{ab}
Two layers with thick scales' density	54.5 ± 1.05^{d}	88.94 ± 3.15^{bc}	70.27 ± 1.16^{cd}
Three layers with low scales' density	47.12 ± 0.8^{e}	90.83 ± 0.47^{b}	76.17 ± 0.81^{a}
Three layers with mid-scales' density	43.10 ± 1.67^{f}	95.55 ± 1.03^{a}	73.64 ± 2.00^{abcd}
Three layers with thick scales' density	37.38 ± 2.43^{f}	87.50 ± 0.79^{c}	73.5 ± 0.80^{b}
$F_{(9, 90)}$	113.714	2.618	2.054
P_{ANOVA}	< 0.0001	0.014	0.0489

Means ± SE sharing the same small letters in the same columns are statistically insignificant ($P > 0.05$)

behavior is the main tool for evaluating parasitic efficiency and successful reproduction (Nurindah et al. 1997). Unfortunately, among these species, FAW females laid their eggs with both different layers' and scales' thickness.

Worldwide, several studies investigated the performance of various *Trichogramma* species against *S. frugiperda*, but limited were on *Trichogrammatoidea* species. Hence, this study focused on the impact of FAW egg mass layers and scale thicknesses on the efficacy of *T. bactrae* and their next progeny. Based on the laboratory observations, FAW females laid their eggs irregularly, which make it easier to examine, reach to the exposed eggs and parasitize by the parasitoid, while the majority of un-parasitized lower egg layer failed to complete their development (dead larvae). So, *T. bactrae* wasps parasitized all FAW egg masses deposited either with layers or scales, but with different percentages. Regardless of the presence of scales, single- (83.18, 78.24%) and two-layer (65.99, 76.42%) egg masses had the highest parasitism rates, followed by the three ones (42.15, 46.05%) in both tests (non-choice and choice). These findings are agreeable with those observed previously by Sun et al. (2021) who assessed five different parasitic species (*Trichogrammatoidea lutea* Girault, *Trichogramma mwanzai* Schulten & Feijen, *Trichogramma ostriniae* Pang & Chen, *Trichogramma leucaniae* Pang & Chen and *Trichogramma japonicum* Ashmend) against FAW egg masses with different ages, and they found that all the 5 species accepted and parasitized the egg masses, but *T. lutea* wasps had the highest proportion of parasitism from the 5 examined species, while *T. japonicum* was the worst one. They suggested that *Trichogrammatoidea* species exhibited great potential for directly applied against FAW management based on these previous findings and their better adaptation to different environmental conditions (Tang et al. 2023).

Other supportive studies on various species of *Trichogramma* as reported previously by Beserra and Parra (2005) that the parasitism % of *Trichogramma atopovirilia* Oatman & Platner were (66.24, 45.20, and 40.10%) on *S. frugiperda* egg masses with one, two, and three layers, respectively, regardless of scale's density. Moreover, Jaraleño-Teniente et al. (2020) observed the parasitic potential of *T. atopovirilia* as 70.14% on FAW egg masses under laboratory conditions, while 29.23% only by *Trichogramma pretiosum* (Riley), and several times non-parasitism was recorded. In this regard, Beserra and Parra (2004) confirmed that *T. atopovirilia* had a higher ability for parasitization on overlapping eggs with and without scales than *T. pretiosum* by overcoming their physical barriers. Therefore, this parasitoid species was more effective against FAW and could be used in the augmentative biological control strategy. In contrast to these previous concepts, Figueiredo et al. (2015) noticed the greatest parasitism percent of *T. pretiosum* on FAW egg masses after (1–3 releases) (69.8, 79.2, and 68.75%, respectively) in maize fields (cultivar BR 106), and hence the yield productivity increased with 19.4%. Besides, the rate of this parasitoid on FAW eggs was (> 50%) when released either on indoor or open fields (Zhu et al. 2019). In this context, *T. pretiosum* was the most dominant species, surveying 93.79% of the FAW-parasitized eggs than 2.07% by *T. atopovirilia* on different regions in maize fields, São Paulo, Brazil, as confirmed by Beserra et al. (2002). In India, Bueno et al. (2008) found that *Trichogramma chilonis* Ishii had the greatest potential on FAW egg masses than *T. pretiosum*, reaching the deepest layer and causing maximum parasitism. This parasitoid species was noted naturally in the Indian ecosystems.

In contrast to the present results, the parasitism % of *Trichogrammatoidea* sp. on FAW egg masses was 25% compared with 78% by *Telenomus remus* Nixon (Hymenoptera: Platygastridae), under laboratory conditions (Laminou et al. 2020), 7% by *T. chilonis* (Tian et al. 2020), and 35.4% by the same parasitoid species (Yuan et al. 2022). Another contradictory study by Dong et al. (2021) that reported *Trichogramma dendrolimi* Matsumura parasitized 22% only of egg masses without scales, but 46% by *T. pretiosum*. In addition, the parasitic performance of *T. dendrolimi* was about 40% on naked FAW egg masses either without or with low scales (Hou et al. 2022). However, the rates registered 15.87, 29.98, and 25.73% on FAW eggs parasitized by *T. chilonis*, *T. dendrolimi*, and *T. pretiosum*, respectively, as noticed by Yang et al. (2022). Certainly, these parasitism rates in the previous findings were very low in comparison with our experimental results on FAW egg masses either in non- or choice tests, regardless of the layers' or scales' presence.

The thick coverage of scales on FAW egg masses negatively influenced the parasitism by different *Trichogramma* species, and in some cases, the rate reached 13% with *T. dendrolimi* and 20% with *T. pretiosum* as mentioned previously by Dong et al. (2021) but was 9% with *T. dendrolimi* by Hou et al. (2022) and ≤ 10% with *T. atopovirilia* by Beserra et al. (2005). This hypothesis may be due to the high scales on egg masses hindering the female parasitoid from examining, moving on, and/or depositing their eggs on the host. In this regard, different species of *Trichogramma* females reabsorbed their oocytes under unfavorable conditions or hosts deprivation; leading to decrease the parasitism rate and the fertility period (Hougardy et al. 2005). Contrary, obtained results exhibited the greatest ability of *T. bactrae* to parasitize on thick scaly eggs based on the layers' numbers and the least rate was on three layers with 36% in non-choice test than 40% in choice one. Subsequently, scale presence on FAW egg masses was not the sole factor determining parasitic performance based on the achieved data. Some other factors as suggested by Li et al. (2023) such as the thickness, texture, odor, and even color of the scales may affect the behavior of parasitoids, besides the number of egg layers.

Finally, the variation in the parasitism percentages observed in the previous and current studies may be referred to the *Trichogramma* species /or strains used in the study, experimental conditions, geographical origin, scale thicknesses and the selected layers of FAW egg masses. All these reasons may illustrate the failure in some field reports when using *Trichogramma* against

FAW. For successful biological control program against this invasive pest, the adaptation of a specific and aggressive parasitoid strain from a specific region must be taken into consideration.

On the other hand, the developmental duration of *T. bactrae* in all tested FAW egg masses was in the same line with that noted previously from other *Trichogramma* species on the same target pest by Sun et al. (2021) on *T. lutea*, *T. mwanzai*, *T. ostriniae*, *T. leucaniae* and *T. japonicum*, *T. chilonis* (Li et al. 2023), and *T. pretiosum* at 25 °C (Bueno et al. 2010). All of these previous parasitoid species lasted about 10–12 days until their emergence. However, the period decreased somewhat in *T. dendrolimi* with about (9.2–9.5 days) based on the FAW daily oviposition egg mass (Li et al. 2023). According to the rearing temperature, *T. pretiosum* completed their development in 7 days at 32 °C and in 20 days at 18 °C (Bueno et al. 2010) and was (8.4–8.9 days) in F_1 progeny (Yang et al. 2022).

Afterward, high numbers of adult parasitoid emergence (87–98%) were observed from FAW egg masses with different morphological characteristics. This present finding was consistent with that noticed previously by Bueno et al. (2010) who recorded the emergence % of *T. pretiosum* was (> 88%) from FAW egg masses at different tested temperatures (18-32ºC), about (97–99%) in *T. dendrolimi* and *T. pretiosum* from egg masses without and with scales coverage (Dong et al. 2021), and (95–99.5%) in *T. dendrolimi* and *T. chilonis* from the same pest according to their oviposition days (2, 4, 8 days) (Li et al. 2023).

Obviously, the proportion of female offspring is another important parameter in biological control. Greater numbers of females in their progeny are essential for mass production and direct reduction of the target pest when applied in the field (Bueno et al. 2009). In this regard, the majority of emerged *T. bactrae* adults from all exposed FAW egg masses were female-biased in both tests and F_1 progeny. This performance was in accordance with those registered by Li et al. (2023) that the overall *T. dendrolimi* and *T. chilonis* adults from FAW egg masses were female with about (> 81%) (Li et al. 2023). Besides, the number of females in F_1 progeny of *T. dendrolimi* and *T. pretiosum* species increased to 85.13 and 100%, respectively, as mentioned by Yang et al. (2022) and then decreased somewhat to 76.44% in the next generation (F_2) in *T. dendrolimi*. However, Sun et al. (2021) observed the female rates of five *Trichogramma* species ranged about (63–82%) from FAW egg masses. Contrary to these previous results, Yang et al. (2022) noted that the F_1 female proportion in *T. chilonis* was (55.47%) only.

Based on our laboratory observations, the highest percent of produced females were from highly scaly eggs with different layers when the parasitoid was offered simultaneously to all egg masses (choice test). This hypothesis may be due to the parasitoid decision to deposit high numbers of their fertilized eggs in the egg host for producing females in their progeny, even if the presence of high scales on the egg mass to maintain the survival and sustainability. This finding agrees with the opinion stated previously by Suzuki et al. (1984) that female wasps can determine the sex ratio of their offspring by regulating the entry of sperm stored in the spermatheca through parasitization on the egg host.

Without a doubt, there is a critical need for further field research on the parasitic potential of *T. bactrae* against *S. frugiperda* egg masses for achieving the biological control and suppressing the damage of this pest on maize crops as the main preferable host. This concept was previously tested and applied with different *Trichogramma* species in maize fields, and hence, the damage rate decreased as reported by (Yang et al. 2022) that noted a remarkable suppression in corn's damage rate after releasing three *Trichogramma* species.

Certainly, this laboratory study of the effect of FAW egg masses with different physical barriers (layers and scales) on parasitic performance of *T. bactrae* lays a strong foundation for the biological control of this new invasive pest, paving the way for the field application of this parasitoid species on large scale in the near future.

Conclusions

The egg parasitoid, *T. bactrae*, is an efficient and promising bio-control agent against *S. frugiperda* due to the greatest ability to overcome the layers' number and scales' thickness, parasitizing all types of egg masses, in choice and non-choice tests. Besides, their efficiency on the produced offspring (F_1) was highly recommended based on the obtained results. High rates of adult parasitoid emergence and female-biased were recorded on all tested FAW egg masses. Hence, the selection of an aggressive parasitoid that abled to parasitize effectively all FAW egg masses is urgently needed for the successful management of this destructive pest in the field.

Abbreviations

FAW Fall armyworm
RH Relative humidity
F_1 Offspring (next generation)
h Hour
P Probability

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HOM designed, planned and conducted all the experiments, collected and analyzed the data, drafted and finalized the manuscript. AHE & HFD were responsible for the manuscript revision. All authors read and approved the final manuscript.

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