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RESEARCH

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Host-instar selection, interspecific competition, and reproductive capacity of extant and novel parasitoids (Hymenoptera: Braconidae) on Egyptian cotton leafworm

Esmat Hegazi[1*] and Wedad Khafagi[2]

Abstract

Background *Cotesia marginiventris* (Cresson) and *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) are larval endoparasitoids of *Spodoptera frugiperda* (Smith) and *S. littoralis* (Boisd.), respectively. *Cotesia marginiventris* was introduced in Egypt to control the recent invasive pest, *S. frugiperda* and local one, *S. littoralis*. Therefore, competition between the two parasitoid species may occur.

Results When single females of *C. marginiventris* (Exp. 1), single females of *M. rufiventris* (Exp. 2), and one female each of *C. marginiventris* and *M. rufiventris* (Exp. 3) were offered a choice of 60 1st, 60 2nd, and 60 3rd instars, of the tested host species, *S. littoralis* and different parasitization levels were obtained. In the non-competitive context (Exps. 1, 2), all instars were parasitized, but *C. marginiventris* and *M. rufiventris* parasitized primarily 2nd and 3rd instar host larvae, respectively. In the competitive context (Exp. 3), the same preference pattern emerged but the percentage of parasitism was lower than in each of the two non-competitive contexts.

Conclusions *M. rufiventris* appears to be a superior competitor when simultaneously exploiting the host with the other parasitoid species. The knowledge of this work can be used to predict and/or understand possible outcomes of a biological control program using the two biocontrol agents against one or the two pests species, and to gain insight into possible biotic interference between species at the same trophic level exploiting the same host.

Highlights

- *Cotesia marginiventris* (Cresson) and *Microplitis rufiventris* Kok. parasitize young instars but differ in their degree of host selection instar of *Spodoptera littoralis* (Boisd.).
- Brood sex ratio (proportion of females), realized lifetime fecundity, and clutch size were relative positively to instar preference, 2nd instar for *C. marginiventris*, and 3rd instar for *M. rufiventris*.
- Extrinsic competition occurs between the two species, and *M. rufiventris* is typically the superior of the two species. Some parasitized hosts resulted from parasitization by single wasp species, produced no parasites and showed intrinsic completion

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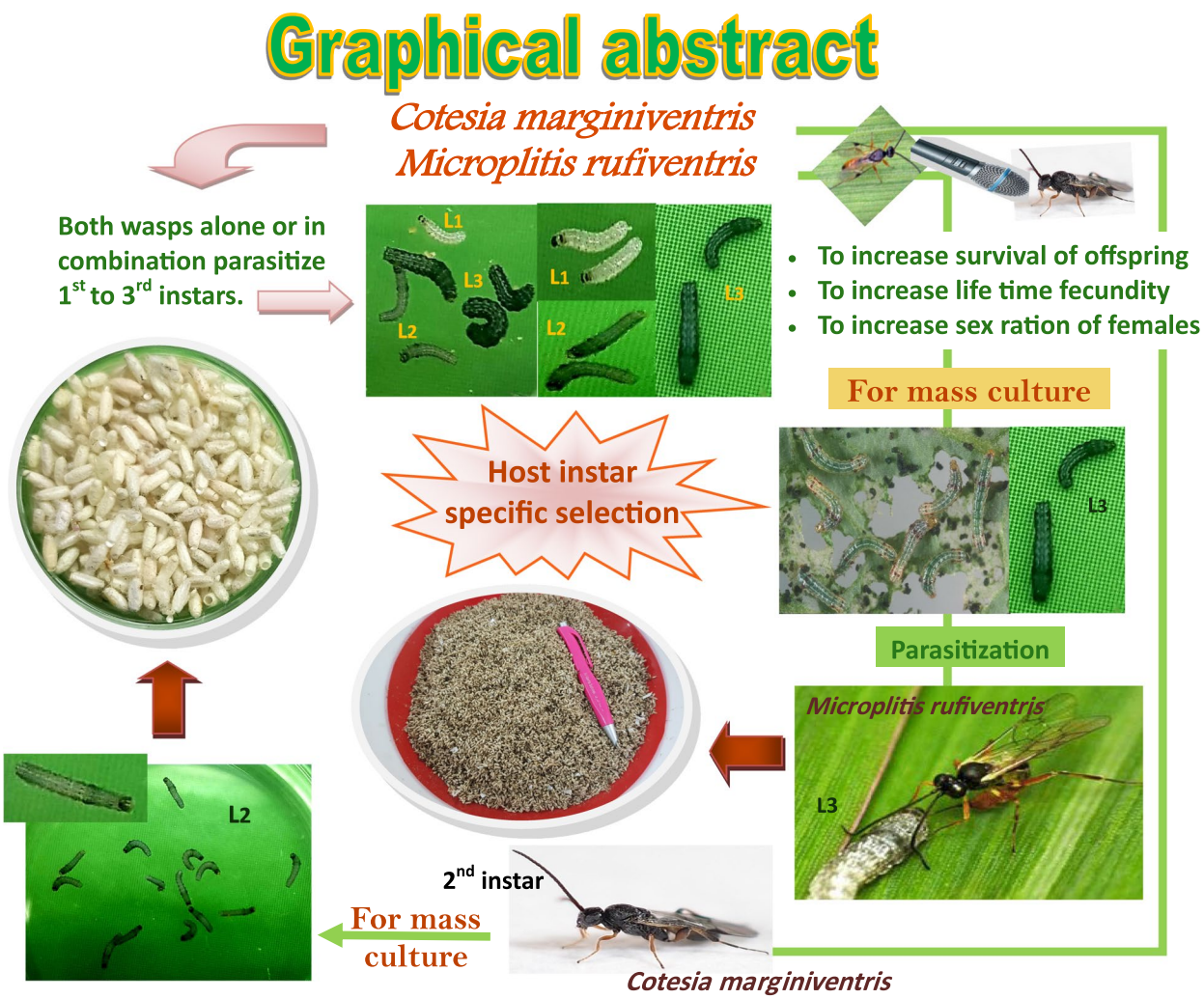
Keywords *Cotesia marginiventris*, *Microplitis rufiventris*, *Spodoptera littoralis*, Instar preference, Interspecific interaction

Graphical abstract



Background

In a healthy ecosystem, diverse and balanced numbers of species exist, constantly interacting. That interaction is a vital part of how insects develop and change over time. When studying species, it is important to analyze the way they interact with their ambient environment. So, it is important to understand the interactions between introduced parasitoid insects and their coexistence with local species that use the same resource.

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae), formerly in the genus *Apanteles* Foerster (Mason 1981), is a larval endoparasitoid that attacks many economically important lepidoptera species such as tobacco cutworm *Spodoptera litura* (Fabricius), fall armyworm *S. frugiperda* (J. E. Smith), beet armyworm *S. exigua* (Hübner) Southern armyworm *S. eridania* (Stoll), soybean looper *Chrysodeixis includens (Walker)*, and *Heliothis* spp. (Lepidoptera: Noctuidae) in agroecosystems (Tamò et al. 2006). The parasitoid frequently causes high mortality among these pests (e.g., Capinera 1999). It has been used as a model organism for studying tri-trophic interactions between crop plants, pests and their natural enemies (Farmer 1997; Fatouros, et al. 2005). *Cotesia marginiventris* was originally described from Cuba and is native to the West Indies. It also occurs in the USA, Mexico

and South America (Mason 1981). It was imported to Egypt in 2016 from Switzerland but not released in Egypt (Dr. Ted Turlings, Institute of Biology, University of Neuchatel, Switzerland) for trials against local pest (*S. littoralis*).

Microplitis was formerly classified under the genus *Microgaster latreille,* 1804 with the type species *Microgaster sordipes* Nees (Telenga 1955). The solitary endoparasitoid *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) oviposits and develops in several noctuid caterpillars in Egypt, including the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Gerling 1969); the lesser cottonworm, *S. exigua* (El-Minshawy 1963); *S. latebrosa* Lederer (Hammad et al. 1965); the cotton bollworm, *Helicoverpa armigera* Hbn. (Ibrahim and Tawfik 1975) and the new invasive pest, *S. frugiperda* in Egypt (Youssef 2021).

Both *C. marginiventris* and *M. rufiventris* are arrhenotokous koinobiont parasitoids whose hosts continue to feed and grow during much of physiological interaction between parasitoid offspring and their hosts (Harvey 2005). During oviposition, female parasitoids inject an egg together with venom and calyx fluid containing a polydnavirus that halts the host development to pupal form (Hegazi et al. 2005).

Many koinobiont larval endoparasitoids attack hosts of various instars that continue feeding and growing during parasitism, and the amount of available resources may change greatly between oviposition and parasitoid offspring completing its larval development (Gu et al. 2003; Elzinga et al. 2003). In the field, both *C. marginiventris* and *M. rufiventris* preferentially attack earlier instars of their hosts (Turlings et al. 1990a, b). There are plans to import *C. marginiventris* from Switzerland into Egypt to control *S. frugiperda* and *S. littoralis*. Thus for, few studies have focused on the interaction of these two parasitoid species (Ruberson and Whitfield 1996), it is not known if competition between the two species may occur. Therefore, before undertaking large-scale release of *C. marginiventris* and *M. rufiventris*, it is important to determine the host instar(s) they preferentially parasitized and whether interspecific competition reduces their combined efficacy as biological control agents. Results derived from this study are relevant and may contribute to make decision about single or multiple parasitoid species to control a single pest species. In this study, the relevance of studying the interactions among the two species of parasitoids that could be used simultaneously as biological control agents for one local and can be on the invasive pest species in future.

Methods

Maintenance of parasitoid and host colonies

Cultures of the parasitoids: *C. marginiventris* and *M. rufiventris* were reared for several generations and kept on young larvae of *S. littoralis* before experiments. The colonies were reared following the methods of Hegazi and El-Minshawy (1979). Both colonies of *S. littoralis* and *M. rufiventris* originated from field crops in Egypt, including cotton *Gossypium hirsutum* L. (Malvales: Malvaceae). Field-collected insects were added to the colonies twice a year to increase genetic diversity. *Cotesia marginiventris* was imported from Switzerland. Early 3rd instar of *S. littoralis* were used as hosts for both parasitoids. The wasp, *C. marginiventris* was maintained on *S. littoralis* larvae according to methods used for *M. rufiventris.*

Mating in both *C. marginiventris* and *M. rufiventris* wasps occurred as soon as both sexes were present, thus male and female wasps held together in glass vials (25 by 100 mm) for 2–4 h were presumed mated. Mated females of 1 day old (hereafter mated females) were held together with accompanying males throughout the test period. The wasps were provided with droplets of honey and distilled water solution at 1:1 daily.

Host-instar preference and interspecific interaction

The study was divided into two parts. Firstly, to determine whether the host instar (host size) of *S. littoralis* larvae "at the time of" parasitism had an influence on parasitization rate, choice tests were carried out. These were *C. marginiventris* + *S. littoralis* (Exp. 1), and *M. rufiventris* + *S. littoralis* (Exp. 2). The wasps were offered 180 larvae of the 1st three *S. littoralis* instars for 3-h exposure period/day. That was to check the preference of female wasp for the smaller (1st), as opposed to the relatively larger (2nd and 3rd) *S. littoralis* larvae, when they are simultaneously offered each parasitoid species in a choice test setting.

Secondly, in part 2, the effect of interspecific competition between *C. marginiventris* and *M. rufiventris* in a choice test setting was investigated (Exp. 3). So, in this experiment one female each of *C. marginiventris* and *M. rufiventris* was offered a choice of similar number of 1st, 2nd, and 3rd instars of *S. littoralis* larvae. For the two trials, the host larvae were grouped into instars: late first (L1; determined by their color and weight) and newly molted second (L2) and third (L3), (determined by the presence of a molted head capsule). For each replicate, *S. littoralis* larvae that had been food-deprived—empty guts—for 3 h were released at the same time inside the ventilated parasitization cage (110 × 110 × 35 mm). So, parasitization cage in each case contained one female

parasitoid for Exp. 1 or Exp. 2 and two wasps (one of each species) for Exp. 3. Each parasitization cage was left for 1.5 h to get natural distribution among used hosts before releasing the wasp(s). In each case 60 larvae/instar was considered as unlimited number/individual wasp (Hegazi and El-Minshawy 1979) exposure for 3-h per day.

The newly emerged adult male and female wasps used for the experiments, were introduced in each ventilated glass vials (70 × 30 mm) and divided into three subgroups for the Exp. 1–Exp. 3, (n = 12–15 mated females/Exp). A sufficient supply of honey solution was maintained daily in each glass vial of adult females and males. Tracking of individual females and their "performance"-parasitism by a stereoscope—on 8 consecutive bioassay days was kept. The date of death of each tested females was recorded.

In all cases, mated females were daily used for a 3-h exposure period/day (to minimize interspecific competition in Exp. 3) and each served as a replicate. After exposure period, the different host instars were re-separated and each placed in a clean transparent plastic box (155 × 155 × 60 mm) covered with organdy cloth and provided daily with fresh standard artificial diet for the host larvae. Each test was replicated 10–12 times.

The host larvae were checked daily until they pupated or died. The host larvae that died prior to pupation were dissected to ascertain if they had been parasitized. Parasitism was confirmed by the presence in the cadaver of an egg or immature parasitoid larva. The date and the numbers of different *S. littoralis* instars parasitized were recorded. The date and the number of parasitoid cocoons and unspun larvae were also recorded. The date of death of each tested females was recorded. All experiments were conducted and cultures maintained at 26 ± 1 °C, 65 ± 5% RH, and a photoperiod of 12:12 (L: D) hrs.

The potential competition between the two parasitoid species was only assessed in a single pest species (*S. littoralis*) and did not on the invasive pest species (*S. frugiperda*) due to quarantine measures. The experiments by *C. marginiventris* were carried out in 2016 and the parasitoid still was under laboratory containment facility. At present, the *S. frugiperda* is already an established species in several regions in Egypt and neighboring countries.

Remaining eggs at death

Microplitis rufiventris females have two ovaries, each with two ovarioles that are in contact with each other throughout their length. The distal portion of each ovariole forms a gourd-shaped structure for storing mature eggs. The commencement of the lateral oviducts is swollen and referred to as a calyx where a water-soluble fluid of bluish hue (calyx fluid) is found. The two lateral oviducts unite medially into the common oviduct, which extends close to the base of the ovipositor. Mature ovarian eggs were found in the calyx and reservoir regions. *Cotesia marginiventris* wasp has almost similar ovaries. The ovaries of both wasps at death were dissected under a binocular dissecting microscope.

To standardize counting the eggs, only mature eggs were counted. To test the importance of host instar interaction on egg load, the ovaries of both female wasps (*M. rufiventris* and *C. marginiventris*) at death at the end of the test were dissected (Khafagi & Hegazi 1998) under a binocular dissecting microscope and mature reservoir, and calyx eggs were counted. To standardize counting the eggs, only mature eggs were counted.

Statistical analysis

Where appropriate, data were subjected to one-way analysis of variance to determine differences between means. Student's t test was used for statistical analyses. Percentages of data were transformed to arcsin square root of proportions before statistical analysis, but the untransformed means ± SEM were presented for comparison (SAS Institute 1989).

Results

Host-instar preference and interspecific competition

The reproductive longevity (mean ± SD) of the wasps was 8.9 ± 1.6 and 8.3 ± 0.3, days (n = 8 females) for *C. marginiventris* and *M. rufiventris*, respectively. The difference was non- significant (t = 1.9, P < 0. 05), so the parasitization activity of each wasp was limited for the 1st 8 ovipositional period. Figure 1 shows the life time of total number of parasitized *S. littoralis* larvae (mean ± SE) by *C. marginiventris* (Exp. 1) and by *M. rufiventris* (Exp. 2) adult females (n = 10 females/Exp.). When a single female of *C. marginiventris* was given a choice among three equally available host instars of *S. littoralis*, the mean total number of parasitized hosts varied significantly (F = 34.5; df = 2, 27; P < 0.05) among the host stages. The total number of parasitized *S. littoralis* larvae/host instars by the female reached up: L1 (76.8 ± 8.9), L2 (109.2 ± 4.5), L3 (59.8 ± 3.4). The wasp significantly parasitized more 2nd instars than larger or smaller instars.

When a single female of *M. rufiventris* (Exp. 2) was given the same choice among the host instars of *S. littoralis*, the total number of parasitized hosts varied significantly among the host instars, but with different figures and trend. The mean total number of *S. littoralis* larvae parasitized by the female increased significantly (F = 126.7; df = 2, 27; P < 0.05) with increasing size of host instar: L1, L2 and L3. The wasp accepted more L3 than L2 or L1.

However, when both *C. marginiventris* and *M. rufiventris* were given the same choice with same instars of *S. littoralis* larvae in one arena (Exp. 3), the same pattern

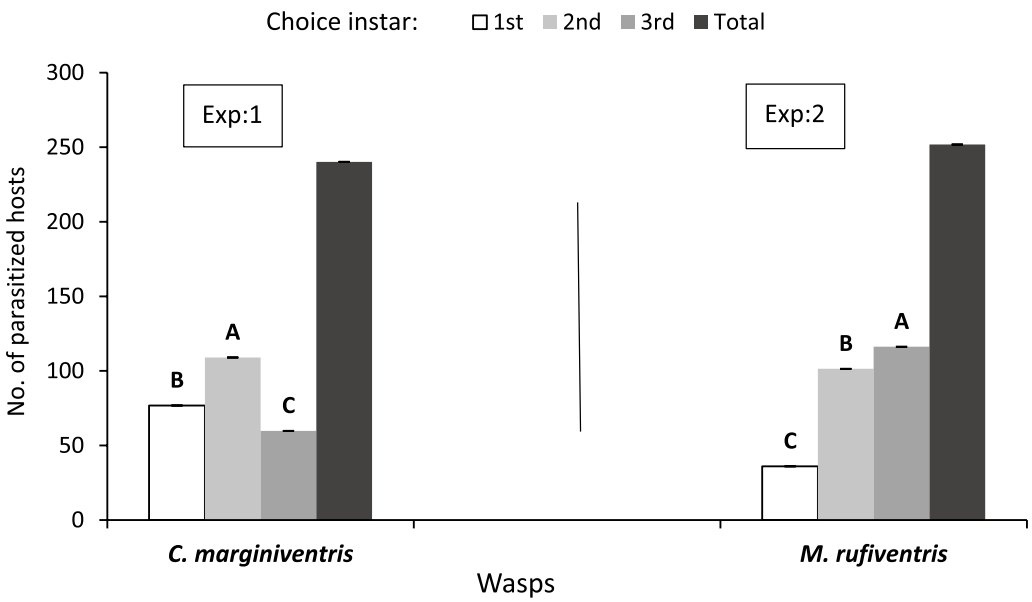


Fig. 1 Lifetime mean number (± SE) of parasitized *S. littoralis* larvae by *C. marginiventris* (Exp. 1) and *M. rufiventris* (Exp. 2) adult females ($n = 10$/Exp.) when each wasp given 180 host larvae of 1st–3rd instars "during 8 consecutive days." Letters above the bars of each set represent significant differences among the different instars (one-way ANOVA, $P < 0.05$)

of parasitism was obtained, but with lower figures. For *C. marginiventris*, the number of *S. littoralis* parasitized by a female wasp did not increase significantly ($F = 11.1$; df = 2, 27; $P < 0.05$) with 3rd host instar: L1 (45.2 ± 3.3), L2 (76.0 ± 7.3), L3 (45.0 ± 3. 7) and with same pattern of parasitism level of Exp. 1 (Fig. 2). However, parasitism by *M. rufiventris* parasitoid varied significantly ($F = 171.7$; df = 2, 27; $P < 0.05$) with the number of *S. littoralis* parasitized increasing significantly with increasing age of host instar: L1 (14.0 ± 1.7), L2 (52.6 ± 5.3), L3 (111.4 ± 3.2).

Figure 3 shows the mean daily parasitization by *C. marginiventris* female when introduced alone and when

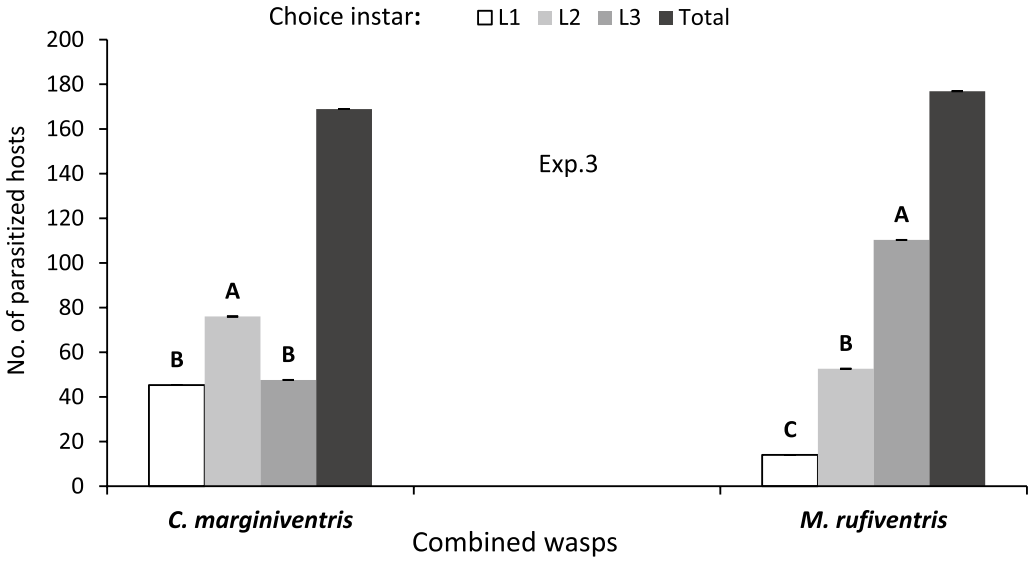


Fig. 2 Lifetime mean number (± SE) of parasitized *S. littoralis* larvae by coexisted wasps of *C. marginiventris* and *M. rufiventris* (Exp. 3) adult females ($n = 10$/Exp.) when wasps given 180 host larvae of 1st–3rd instars "during 8 consecutive days." Letters above the bars of each set represent significant differences among the different instars (one-way ANOVA, $P < 0.05$)

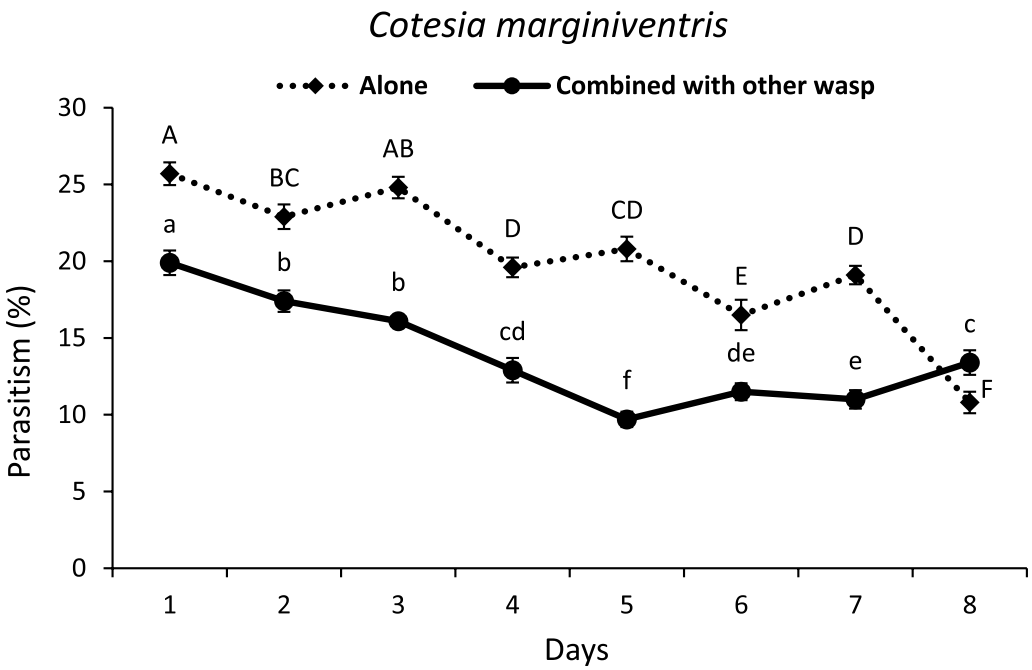


Fig. 3 Daily percentage (Mean ± SE) of parasitism of *S. littoralis* larvae by *C. marginiventris* female alone or when combined with *M. rufiventris* wasp and introduced each time to 180 1st–3rd host larvae (60 larvae/instar) into the parasitization cage. Points with the same uppercase or lowercase letter are not significantly different ($P > 0.05$)

combined with *M. rufiventris* in the same cage. Generally, the percentage of larvae parasitized daily by *C. marginiventris* changed significantly with female age when the wasp introduced alone ($F = 41.7$; df = 7, 56; $P < 0.05$) and when combined with the other species ($F = 28.1$; df = 7, 56; $P < 0.05$). The number of parasitized hosts produced was related the day that the females were exposed to hosts. Peak parasitization activity occurred from day one to day three (58.1% of total parasitism) and then gradually decreased to its minimum on day 8 (age dependent parasitization). Percentage of total parasitism of wasps introduced alone in the parasitization cage was significantly higher ($t = 2.9$, $P < 0.05$) than wasps combined with *M. rufiventris*. This suggests that the interspecific competition between the two wasps' species reduced the total parasitism of *C. marginiventris* by 30.1%.

Figure 4 shows the mean daily percent of parasitism by *M. rufiventris* when introduced alone and when combined with *C. marginiventris* into the parasitization cage to different host instars. Like *C. marginiventris*, the percentage of larvae parasitized daily by *M. rufiventris* changed significantly with female age when the wasp introduced alone ($F = 52.0$; df = 7, 56; $P < 0.05$) and when combined with the other species ($F = 80.1$; df = 7, 56; $P < 0.05$). Peak parasitization activity occurred from day one to day four (57.6% of total parasitism) and then gradually decrease to its minimum on day 8 (age dependent parasitization). However, percentage of total parasitism of the single wasp was significantly higher ($t = 3.1$, $P < 0.05$) than wasps combined with *C. marginiventris* (Table 1). Also, the interspecific competition between the two wasps reduced the total parasitism of *M. rufiventris* by 31.1%.

Table 1 shows the effect of non-competitive context (Exps. 1 and 2) and competitive context (Exp. 3) by *C. marginiventris* and *M. rufiventris* wasps on the female percentage of total progeny and remaining mature eggs in the oviducts of the wasps. In Exp. 1, females of *C. marginiventris* attacked and accepted more the relatively medium (2nd) instars, i.e., this instar preferred over equally available smaller (1st) or larger instars (3rd). However, when *C. marginiventris* wasps were introduced in combination with *M. rufiventris* wasps (Exp. 3), the wasp accepted significantly more median size 2nd instar, than smaller or larger instars. The sex ratio of total progeny was significantly in favor of females for those wasps developed in L2 (for Exp. 1, $F = 15.9$; df = 2, 27; $P < 0.05$ or Exp. 3, $F = 8.4$; df = 2, 27; $P < 0.05$). The highest percentage of females were produced by L2, 45.5 and 40.3%, in Exps.1 and 3, respectively. In case of *M. rufiventris*, the females attacked and laid eggs in all three larval instars of *S. littoralis* and they always accepted more the relatively larger (3rd) instars (Table 1). The preference for L3 was influenced by the relative difference in larval size

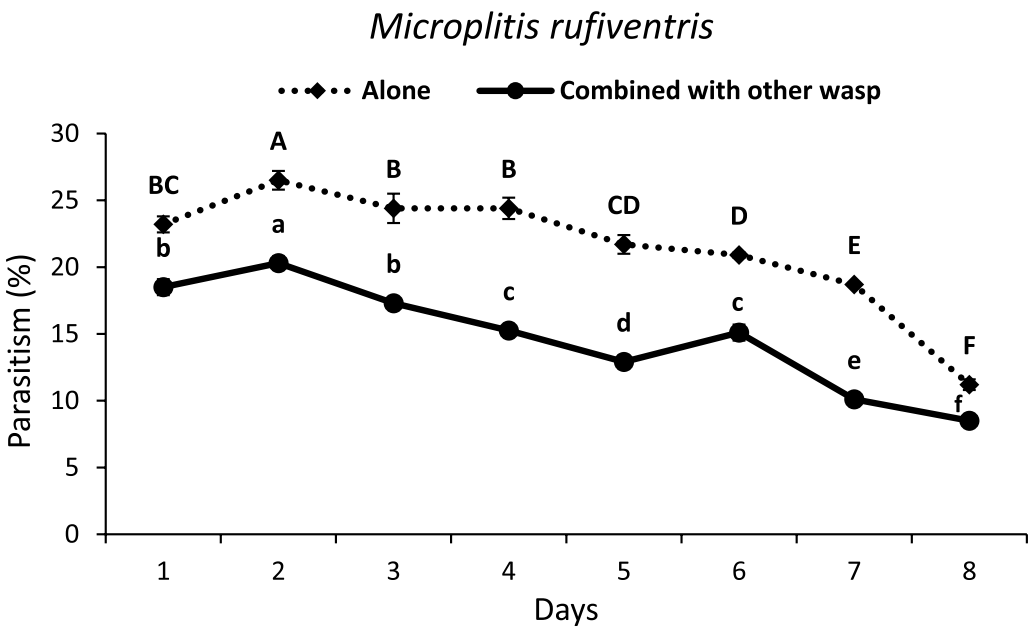


Fig. 4 Daily percentage (Mean ± SE) of parasitism of *S. littoralis* larvae by *M. rufiventris* female alone or when combined with *C. marginiventris* wasp and introduced each time to 180 1st–3rd host larvae (60 larvae/instar) into the parasitization cage. Points with the same uppercase or lowercase letter are not significantly different ($P > 0.05$)

between the available host stages. The same table shows that the life time preference and female % of total progeny of *M. rufiventris* introduced alone (Exp. 2) or in combination with other wasps (Exp. 3) were significantly greater among the 3rd instar (for Exp. 2, $F = 36.7$; df = 2, 27; $P < 0.05$ or for Exp. 3, $F = 12.4$; df = 2, 27; $P < 0.05$). The highest percentage of females were produced by L3, 43.1 and 49.9%, in Exps. 2 and 3, respectively.

Realized and potential fecundity

The parasitization schedule (single species vs combined species) of female parasitoids significantly affected their lifetime fecundity (i.e., the number of eggs laid for an entire lifetime): for both wasps used singly ($t = 13.7$, $P < 0.05$) and combined wasps ($t = 9.1$, $P < 0.05$) (Fig. 5). This consequently affected daily parasitism by wasps used singly (Figs. 3 and 4). The lifetime fecundity of singly parasitization by *C. marginiventris* wasps (Exp. 1) was 240.2 ± 2.0 eggs and reduced into 166.2 ± 9.6 eggs when *M. rufiventris* introduced in the parasitization cage (Exp. 3).

Also, the lifetime fecundity of *M. rufiventris* females when used singly (Exp. 2) was 255.8 ± 7.8 eggs and reduced to 178.0 ± 6.3 eggs in the presence of *C. marginiventris* wasp (Exp. 3). Percentage of female progeny was higher for hosts parasitized during their 2nd instar for *C. marginiventris* and 3rd instar for *M. rufiventris*. Also, potential fecundity (i.e., the number of eggs laid for entire life time + remaining eggs in the oviducts) was parasitization method-dependent, i.e., releasing single wasp species vs releasing both species together (Table 1, Fig. 6). The remaining mature ovarian eggs at death were higher in combined wasps comparing with wasps used singly.

Discussion

Cotesia marginiventris is new to Egypt and has been found to parasitize the larvae of *S. littoralis* in the laboratory *M. rufiventris* is one of the most common and widely distributed solitary endoparasitoid of *S. littoralis* larvae in Egypt. Also, *C. marginiventris* (generalist) and *M. rufiventris* (specialist) are parasitoids of lepidopteran larvae and differ in their degree of host specificity. Both wasp species are very promising candidates in an augmentation program for control of the invasive pest, *S. frugiperda* and local one, *S. littoralis*, in Egypt. However, little is known about the individual or combined effects of intrinsic and extrinsic competition on the effectiveness of both parasitoids as biological control agents.

It is well known that plant and host cues attract parasitic wasps (Hymenoptera) to host plants and host insects. Frass and host larvae are the other two components among complete plant–host complex (Chen and Fadamiro 2007). To determine whether the host instar of *S. littoralis* larvae at parasitism has an influence on instar selection, we had to minimize the effect of chemical information of some of these factors that attract the parasitoids. This was done by fasting host larvae for 1.5 h to

Table 1 Effect of non-competitive context (Exp. 1 and 2) and competitive context (Exp. 3) by *C. marginiventris* and *M. rufiventris* wasps on the mean number (±SE) lifetime parasitization, female % of total progeny and remaining mature eggs in the oviducts, when 180 *S. littoralis* larvae (60/instar) introduced to mated females of each of Exp. 1, Exp. 2 and Exp. 3

	Only *C. marginiventris* Parasitized hosts			**Only *M. rufiventris* Parasitized hosts**			**Combined wasps**					
							Hosts Parasitized by *C. marginiventris*			**Hosts Parasitized by *M. rufiventris***		
	L1	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**	**L1**	**L2**	**L3**
Female % of total progeny	29.4 ± 1.6 b	45.5 ± 2.2 a	24.6 ± 2.3 b	21.3 ± 2.1 c	31.6 ± 2.1 b	43.1 ± 1.2 a	28.1 ± 3.7 b	40.3 ± 2.1 a	24.9 ± 2.4 b	14.4 ± 7.1 b	25.9 ± 3.2 b	49.9 ± 2.3 a
Life time parasitization (No.)	76.8 ± 5.9 b	109.2 ± 3.0 a	54.2 ± 3.4 c	36.0 ± 2.7 c	101.4 ± 2.3 b	118.4 ± 5.5 a	45.2 ± 3.3 b	76.0 ± 7.3 a	45.0 ± 3.7 b	14.0 ± 1.7 c	52.6 ± 5.3 b	111.4 ± 3.2 a
Remaining eggs in the oviducts												
Reservoir	15.4 ± 4.0			11.6 ± 3.9			13.6 ± 1.9			16.3 ± 1.8		
Calyx	4.8 ± 1.6			7.8 ± 1.3			8.1 ± 1.7			11.3 ± 1.1		
Total	20.2 ± 5.6			19.4 ± 4.3			21.7 ± 2.7			27.5 ± 1.7		

For each set, means within a row sharing with the same letter are not significantly different (one-way ANOVA, $P < 0.05$)

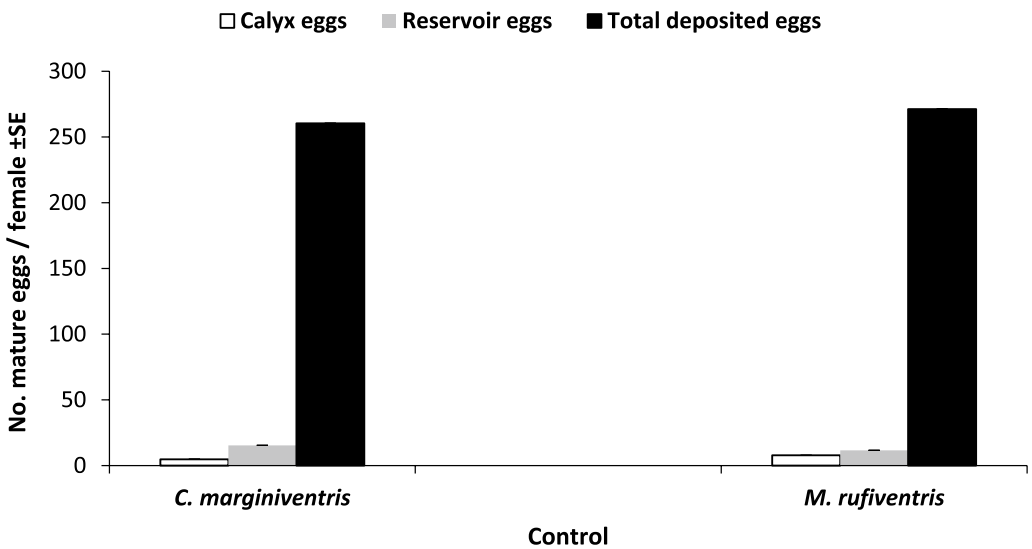


Fig. 5 Remaining eggs at death in the oviducts and potential fecundity ((Mean ± SE, remaining eggs + eggs laid for entire lifetime) of *C. marginiventris* and/or *M. rufiventris* (control) when each introduced alone to 180 of 1st three instars of *S. littoralis* larvae

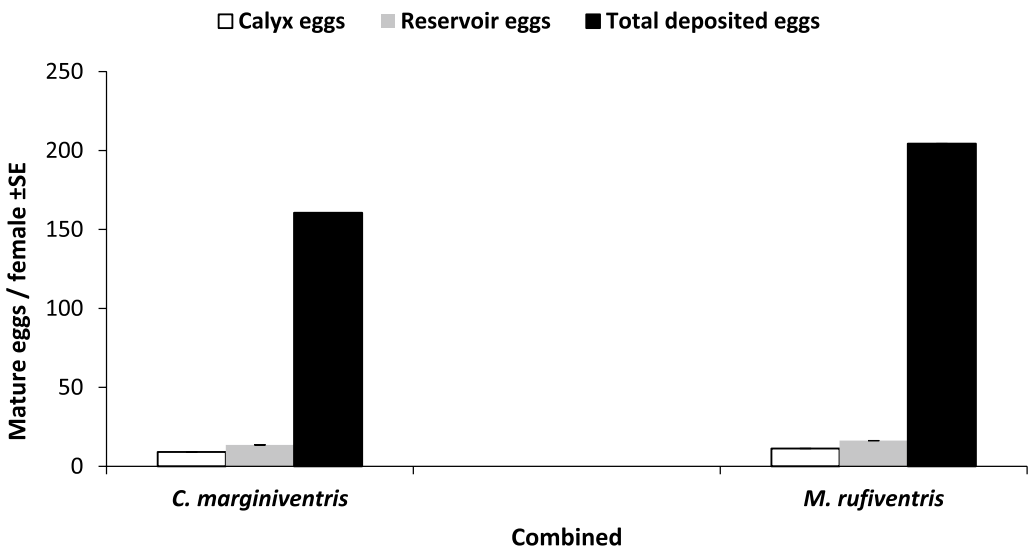


Fig. 6 Remaining eggs at death in the oviducts and potential fecundity (remaining eggs, (Mean ± SE) + eggs laid for entire lifetime) of *C. marginiventris* and *M. rufiventris* when both females coexisted together against 180 of 1st three instars of S. *littoralis* larvae

almost exclude frass during exposure period and by using clean and one-time use of empty parasitization cage to exclude larval food cues and using unlimited hosts for short exposing periods of only 3 h a day.

In this work, we first described daily host instar selection by single newly emerged mated female of *C. marginiventris*, and *M. rufiventris* or both wasps when combined together. We showed that first, second and third instars of *S. littoralis* were parasitized by either *C. marginiventris* or *M. rufiventris*, respectively. Choice tests revealed that there were significant differences in parasitism among the three susceptible instars, with the highest percentage of parasitism found in second instars for *C. marginiventris* (Exp. 1), and third instars for *M. rufiventris* (Exp. 3). Accordingly, in both cases of wasps, parasitism was instar-specific. So, these wasps in the field are important for minimizing damage through biological control. The *C. marginiventris* was introduced to Egypt in containment facility but not released in the field. Introduction was only for laboratory experiments.

Importing this wasp may be good for biological control of both, the new invasive pest, *S. frugiperda* and local

one, *S. littoralis*. However, more evaluations on many native and non-target noctuid species are needed before introducing an exotic biocontrol agent.

Parasitization levels by *C. marginiventris* on the first three instars indicated preference for the 2nd instar hosts avoiding both smaller (1st) and larger (3rd) hosts. First instar may be suboptimal for the immature development and production of less female progeny, and third instar (larger) hosts may be risky to attack and less effective in subduing and handling for the female and production of more male offspring. Time costs for *C. marginiventris* increased with *Spodoptera* large instars. So, host choice is affected by host size. Parasitizing proportionally more L2 than L1 or L3 instars. *Cotesia marginiventris* may need more time for parasitizing L3 than 2nd instars for host handling and oviposition. So, parasitoid preference reflects differences in handling costs, oviposition success and sex ratio among host types (Harvey et al. 1994).

Both of *C. marginiventris* (Mattiacci and Dicke 1995a) and *M. rufiventris* (Hegazi et al. 1977) are both synovigenic and well-studied parasitoids. In non-ovipositing *Microplitis* wasps, females normally reach a peak of egg maturation rate at first day of emergence. Egg maturation slowed down once the maximum oviduct egg load was reached on second post eclosion day (Hegazi et al. 2014). This can impact biocontrol, i.e., before the host larvae become larger, including the short lifespan of the females. Constant releases would be required to keep the pests under control as there are overlapping generations of the pests.

In the present study, *C. marginiventris* accepted significantly more median size (2nd instar) than smaller or larger instars, indicating the following ranked order of preference: L2 > L1 > L3. *M. rufiventris* accepted significantly more 3rd instar than smaller ones, indicating the following ranked order of preference: L3 > L2 > L1. Also, percentage of female progeny was significantly higher when females of *C. marginiventris* were associated with second instars and when females of *M. rufiventris* were associated with third instar. It seems that *C. marginiventris* and *M. rufiventris* laid eggs preferentially on 2nd and 3rd instars, respectively. Larger hosts (L3) may contain more nutritional resources for offspring development and growth. Both female wasps can distinguish between instars and use this information to optimize their progeny and sex allocation.

Two types of competitions can occur between the wasps: intrinsic and extrinsic competition. Intrinsic competition occurs inside the larval host body. By contrast, extrinsic competition occurs between adult parasitoids outside the host body (Ode et al. 2022). Parasitization by single species vs combined species significantly affected their daily parasitism and consequently their lifetime fecundity, indicating that extrinsic competition occurs between the two species, and *M. rufiventris* was typically the superior of the two species. Also, parasitism level was always higher when each wasp was introduced singly into the parasitization cage, compared with parasitization level resulted from combined wasps. On the other hand, in all experiments, some hosts exhibited arrested development and reduced growth and showed no wasp emergence after 10–12 days. When they were dissected in Pringle's (1938) saline solution to verify that parasitization had occurred and to check that teratocytes were present. These hosts contained 2–3 no living parasitoids (nonfunctional wasps) and were considered as "pseudo-parasitized," i.e., these hosts were accidentally parasitized more than once (superparasitism, in Exp. 1 or Exp. 2), showing intrinsic competition (Harvey et al. 2013).

Multiparasitism occurs when *C. marginiventris* attacks a host that has been already attacked by *M. rufiventris* (Exp. 3) (Godfray 1994). This interaction involves strong intrinsic competition, because individual hosts are sufficient for the development of one parasitoid only (Harvey et al. 2013), and the competition was in favor of *M. rufiventris*. Also, in both parasitoids' species, the average daily parasitism of *S. littoralis* by a single wasp was always higher than introducing combined wasp species, due to some factors including extrinsic competition.

The results obtained showed that host instar at parasitism influenced both daily parasitization rate, total fecundity and sex ratio of female progeny. Percentage parasitism by *C. marginiventris* in Exp. 1 (single species) or Exp. 3 (two species) was significantly higher and in female favor for second instar than for the other two host instar classes. Also, percentage parasitism and sex ratio of female progeny by *M. rufiventris* in Exp. 2 or Exp. 3 was significantly higher for third instars than for the other two host instar classes, indicating that host instar influenced both parasitization rate and sex ratio by both wasps. Also, percentage parasitism by *M. rufiventris* in all Exp. 3 was significantly higher than those recorded for *C. marginiventris* wasp which could account for the observed preference pattern and interspecific interaction (extrinsic competition) between the two wasps in presence of the different instars of *Spodoptera* larvae. Highest parasitization by *C. marginiventris* occurred when the adult female introduced alone into the parasitization cage (Exp. 1) comparing with female combined with another female species (Exp. 3) indicating extrinsic competition and intrinsic competition as mentioned above. In short, sex progeny was significantly in favor of females when wasp females of *C. marginiventris* were associated with second instar and when females of *M. rufiventris* were associated with third instar. So, brood sex ratio (proportion of females), realized lifetime fecundity, and

clutch size were relative positively to instar preference, 2nd instar for *C. marginiventris*, and 3rd instar for *M. rufiventris*.

Parasitoid survival is highest when parasitism is initiated in earlier host instars, due to age-related changes in internal (physiological) host defense and their suitability for a parasitoid development (Ode et al. 2022). Most parasitoids have the ability to determine host quality during the parasitism process, and hosts will often be accepted or rejected according to their species, size, and developmental stage (Li et al. 2006). For a specific parasitoid, host stage preference can occur for a range of reasons. Preference may be based on increased survival of offspring or ease of increased survival of offspring (Mattiacci and Dicke 1995a). The present work demonstrates the role of host size regarding in parasitoid's host selection.

There are differences in host quality associated with the age of the host that influence the developmental performance of the larval parasitoid. Also, as the developing host grows in size, it increases its capacity for physical and behavioral defenses, which would potentially increase risks for the parasitoid (Mattiacci and Dicke 1995b). *Cotesia marginiventris* exhibited a marked preference for a specific instar larva; especially second instars followed by the smaller instars, both are generally less mobile (ease of parasitism), take less handling time, and have fewer effective anti-parasitoid defenses than older instars (3rd instar), which are larger and more active. Also, it was observed that when third instar offered to the wasp female, the reproductive capacity of the wasps was reduced, and "pseudoparasitized = unsuccessfully parasitized hosts" risks for the parasitoid' larvae increased the risks for the parasitoid increased. Result of this behavior is to optimize the percentage of parasitism, parasitoid development, females' progeny. Selecting third instar in *M. rufiventris* exhibited a marked preference, where the percentage of successful parasitism was higher and pseudoparasitism was lesser than females attaching the younger instars. Second and third instars of *S. littoralis* were better hosts for *C. marginiventris* and *M. rufiventris*, respectively than other instars. Therefore, it is recommended to use the obtained information in mass cultures of both wasps. Later on a future study on *S. frugiperda* will be extended.

Conclusion

Main results showed that parasitoid wasps exhibited a marked preference for a specific host larval stage and these stages are different for each parasitoid species. Further, host larval stage at parasitism influences parasitization rate and sex ratio of the progeny of both parasitoid species. Percentage of female progeny was higher for hosts parasitized during their 2nd instar for *C. marginiventris* and 3rd instar for *M. rufiventris.* Finally, *M. rufiventris* appears to be a superior competitor when simultaneously exploit the host with the other parasitoid species. The knowledge generated from this work may increase our knowledge on performance of a new biocontrol agent in Egypt if released with the extant parasitoid *M. rufiventris*. If the study was conducted with *S. frugiperda*, probably that *C. marginiventris* would be the winner for that pest as they origin from the same area. We do not know how the 2 parasitoids will interact with *S. frugiperda* and *S. littoralis* in the field as this study is limited to *S. littoralis*, due to strictly quarantine measures for *S. frugiperda*. Competitive and non-competitive studies on the two parasitoid species (*C. marginiventris* and *M. rufiventris*) with the rationale to determine the possibility of classical biological control against *S. frugiperda* will be conducted.

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The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Consent for publication
The manuscript has not been published in completely or in part elsewhere.

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