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Intrinsic Competition between Extant and Novel Parasitoids (Hymenoptera: Braconidae) in the Cotton Leafworm *Spodoptera littoralis* (Boisd.) under Laboratory Conditions

Wedad Khafagi[1], Sania Shwiel[1], Rafiaa A, Rabab[2] and Esmat. Hegazi[3]

1. Plant Protection Research Institute, Alexandria, Egypt
2. Faculty of Agriculture,University of Ezzitona
3. Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Abstract: Parasitoids may compete at the adult stage when foraging hosts (extrinsic competition), or at the larval stage, when supernumerary parasitoids develop in the same host (intrinsic competition).A study including lepidopteran pest *Spodoptera littoralis* and novel (*Cotesia marginiventris*) and extant (*Microplitis rufiventris*) hymenopteran endoparasitoids, was carried out to investigate the effect of single and double parasitism at ovipotion times of 2hr and 48hr on endo-development of the wasps and competitive interactions between the parasitoid larvae. No significant differences in the endo-development time of the wasps were found between single or superparasitised hosts. Multiple parasitism increased the endo developmental time of both wasps. Singly parasitized hosts by *M. rufiventris* produced more wasps than those of *C. marginiventris*. Also, the percentage of emergence success by superparasitized *M. rufiventris*, was, greater than those recorded by superparasitized *C. marginiventris*. Muliparasitized hosts produced more *C. marginiventris* than *M. rufiventris* wasps. However, the outcome of competition, by both wasp larvae principally depended on the wasp species that was first to attack and on the time interval between two oviposition's by wasp females. The intrinsic and competitive interactions between the parasitoid larvae in multiple parasitism experiments was more frequently than in superparasitism experiments. The novel parasitoid, *C. marginiventris* was intrinsically superior competitor comparing with the extant *M. rufiventris*, so the use of the two species simultaneously may lead to competitive exclusion and influence the stability of the extant parasitoid population. The competition was asymmetrical, meaning one species (the exotic) had a competitive advantage as competitor being the victor whatever the sequence of attack, over the other species. Introducing *C. marginiventris* to Egypt, is not a good option for biological control of both, the new invasive pest, *S. frugiperda* and local one, *S. littoralis*.

Keywords: *Cotesia marginiventris, Microplitis rufiventris, Spodoptera littoralis*, single and double parasitism, endo-development, intrinsic competition

INTRODUCTION

Competition involving insect parasitoids falls into two broad categories: extrinsic (among free-living adults searching for host resources) and intrinsic (among immature parasitoids developing on or inside the host) (Price 1972; Bogran et al. 2002; Harvey, et al. 2009). The importance of competition in driving parasitoid community and niche structure has been the subject of many theoretical studies (Comins & Hassell, 1996). Moreover, competitive exclusion in which an introduced parasitoid species displaced a native species has recently been observed in the field.

The solitary endoparasitoid, *Cotesia marginiventris* is a general parasitoid of noctuid moths. It attacks mostly very young larvae (late first to second instar). However, second instars are preferred (Hegazi & khafagi 2024). *C. marginiventris* was originally described from Cuba and is native to the West Indies. It also occurs in the United States, Mexico and South America (Mason, 1981). *C. marginiventris*, was 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)

Microplitis was formerly classified under the genus Microgaster latreille, 1804 (Telenga 1955). *M. rufiventris* occurs in Afghanistan, China, Cyprus, Egypt, Israel, Jordan, Romania, Turkey, Turkmenistan, Uzbekistan and Iran (Yu et al. 2012). The solitary endoparasitoid *M. rufiventris* 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 & Tawfik 1975) and the new invasive pest, *S. frugiperda* in Egypt (Youssef 2021). In the field, this parasitoid prefers to attack earlier instars of *S. littoralis* (late first to third instar larvae) when they still live in clusters near the place of egg deposition. However, third instars are preferred (Hegazi et al. 1977). At oviposition both female wasp species inject along with the parasitoid egg, venom and calyx fluid that contains polydnavirus (Hegazi et al., 2005). Both species preferentially parasitize early-instars and occupy similar ecological niches. The overlap in the host range of these parasitoids indicates that they are competitors, which may be likely influence their abundance in cotton field and in natural habitats.

It is intended to introduce *C. marginiventris* in Egypt to control the recent invasive pest, *S. frugiperda* and local pest *S. littoralis* (Hegazi & Khafagi 2024). Therefore, intrinsic and extrinsic competition between the two species may occur. The present study investigated the effect of single (control) and double parasitism on the endo-development of the wasps and the consequences of intrinsic and competitive interactions between the parasitoid larvae under laboratory conditions, using *S. littoralis* larvae (Lepidoptera, Noctuidae) under laboratory conditions.

MATERIAL AND METHODS

Insect Rearing

Cultures of the *M. rufiventris* parasitoids were reared in the Department of Applied Entomology and Zoology, mass rearing quarantine facility in Alexandria, Egypt following the methods developed by Hegazi et al., 1977 and Hegazi & El-Minshawy, 1979. *C. marginiventris* was imported from Switzerland (Prof. Ted Turling). The wasp was maintained using *S. littoralis* larvae according to methods used for *M. rufiventris*. Adult wasps were kept in cages (40 × 40 × 40 cm) and maintained at 26–27 ◦C, 60–70% RH and a photoperiod of L12: D12. *M. rufiventris* and *S. littoralis* from wild populations in Egypt were periodically added to the mass reared stock wasps at 3-month intervals to maintain genetic variability. Both wasps were reared on late second instar larvae of *S. littoralis*. Parasitoids were provided with fine drops of pure honey streaked on the topside of the cages and water on

moist cotton wool balls (5–6 cm in diameter). Mating in both *M. rufiventris* and *C. marginiventris* wasps occurred as soon as both sexes were present, thus male and female wasps held together for 2hr were presumed mated. Mated females of 1 day old were held together with accompanying males throughout the test period.

Experimental Procedure

The intrinsic competition between the two parasitoids was determined under three scenarios. In the first and second scenarios, early second-instar Larvae (3 days old) were used for oviposition (Table1). Some host larvae were parasitized once by one species of parasitoid female (control). In some other host larvae, two oviposition sequences were performed within a maximum interval of 2hr, namely, sequence A (oviposition by *M. rufiventris*, followed by *M. rufiventris*), sequence B (oviposition by *M. rufiventris*, followed by *C. marginiventris*), sequence C (oviposition by *C. marginiventris*, followed by *C. marginiventris*) and sequence D (oviposition by *C. marginiventris,* followed by *M. rufiventris*). In the second scenario, two oviposition sequences were performed within a maximum interval of 48hr, namely, sequence E (oviposition by *M. rufiventris*, followed by *M. rufiventris*), sequence F (oviposition by *M.rufiventris*, followed by *C. marginiventris*), sequence G (oviposition by C. *marginiventris*, followed by *C. marginiventris*) and sequence H (oviposition by *C. marginiventris*, followed by *M. rufiventris*).

In the third scenario, early third instar *S. littoralis* larvae (5 days old) were used (Table2). Two oviposition sequences were performed only within a maximum interval of 2hr, namely, sequence A (oviposition by *M. rufiventris*, followed by *M. rufiventris*), sequence B (oviposition by *M. rufiventris*, followed by *C. marginiventris*), sequence C (oviposition by *C. marginiventris*, followed by *C. marginiventris*) and sequence D (oviposition by *C. marginiventris*, followed by *M. rufiventris*). Every control host was attacked only once by a parasitoid female (single parasitism). All the females used for the experiments were one day old and able to oviposit more than 35 eggs per day. The host larvae were grouped into instars and ages within the instar, i.e., second instar and third instar. For each group of host larvae, 6–8 1-d-old mated female parasitoids which had no previous contact with host larvae were used singly and each served as a replicate. Oviposition was induced by placing the female wasp in a glass vial (7.0 × 1.8 cm) and tapping the female into contact with the host larvae which was removed immediately after a single oviposition. Larvae that were accidentally parasitized more than once were discarded.

Every control host larva was attacked only once by a parasitoid female. Each experiment involved a total of 50 host larvae/3 rep. For each repetition, ten host larvae (20%) were dissected 2 d after the second oviposition to detect the presence of the two parasitoid species in the same host in double parasitism. Thus, 30 of each multiple (B &D) and superparasitism (A&C) in parasitized Spodoptera larvae were dissected for each of the oviposition sequences. The remaining parasitized hosts (75%) per repetition were transferred individually to small petri dishes containing artificial diet and maintained under the conditions previously described until the emergency of the parasitoid cocoons, which were then identified and counted to determine the number of parasitoids of each species that emerged after the multiple and superparasitism experiments. An ovipositional attack was defined as the female encounter resulting in the insertion of the ovipositor into the host larva.

In each case the developmental time (egg to cocoon) was also recorded. Dissections of hosts produced parasitoid larvae were then carried out. Each host larva was placed in a drop of Ringer solution in a wax tray and opened under a dissecting microscope. Twenty dissections were made at each time interval for each order of oviposition. In each trial, the stung larvae (30–40 larvae/female) were observed daily and the successful emergence of the parasitoid wasps was noted.

The experiments were conducted in incubators (type Hann, Munden, Germany), equipped with eight fluorescent 30-Watt cool white flurescent tube per cabinet. In all experiments, the light onset started at 5 am. The light intensity during photophase was approximately at 1270 foot candles. Light was measured with a Weston light meter (Model) (Weston Electrical Instrument Company, Newark, NJ). All cabinets were located in a controlled room.

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 arcsine square root of proportions before statistical analysis, but the untransformed means ± SEM were presented for comparison (SAS Institute 1989).

RESULTS

Double parasitism, including Superparasitism, results from oviposition by one or more individuals of the same parasitoid species into the same host individual (conspecific female). While multi parasitism results from oviposition by two or more different parasitoid species in the same host individual. The interactions can lead to competition between the parasitoid larvae, impacting their development and survival.

Effect of Parasitism Patterns on the Endo-Developmental History of the Parasitoids

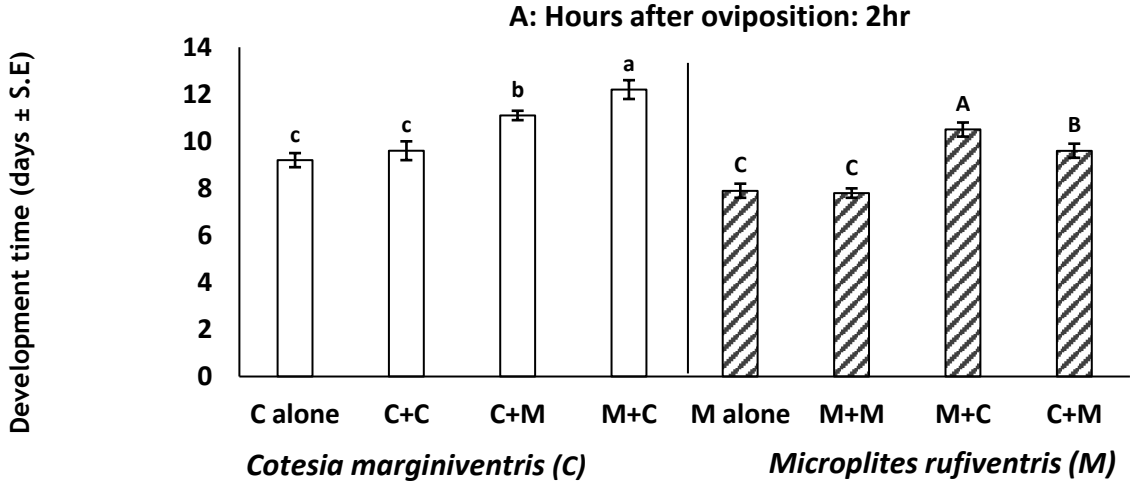
The endo-developmental history (egg to cocoon) for different parasitism patterns at 2hr oviposion or 48h intervals by *C. marginiventris* and *M. rufiventris i*n the 2nd and 3rd instar larvae of *S. littoralis* is shown in Tables 1&2. Fig. 1 showed that the effect of parasitism patterns (single parasitism, superparasitism and multiple parasitism) in the 2nd instar *S. littoralis* larvae at 2hr ovipositon intervals on the endo-development time varied significantly in both *C. marginiventris* ($F=18.75$; $df=3, 36$; $P<0.05$) and *M. rufiventris* ($F=24.0$; $df=3, 36$; $P<0.05$) wasps. The same trend was also observed in the 2nd instar *S. littoralis* larvae at 48h oviposion intervals in both *C. marginiventris* ($F=8.73$; $df=3, 36$; $P<0.05$) and *M. rufiventris* ($F=10.66$; $df=3, 36$; $P<0.05$) wasps (Fig. 1).

Table 1: Effect of double ovipositions by *Cotesia marginiventris* and *Microplitis rufiventris* at two oviposition time intervals in the second instar larvae of *Spodoptera littoralis* on percentage of emergence success and development time of the wasps.

Order of parasitism		Hours after oviposition	Emergence success (%)			Development time (days ± S.E)	
First wasp	Second wasp	/ Oviposition sequence	M.	C.	Total	M.	C.
Microplitis	-	Alone/ control	99.6	-	99.6	7.9 ± 0.1	-
Microplitis	Microplitis	2hr/A	98.8	-	98.8	7.8 ± 0.16	-
Microplitis	Cotesia	2hr/B	33.63	36.44	70.1	10.5 ± 0.6	12.25 ± 0.5
Cotesia	-	Alone/ control	-	92.76	92.76	-	9.2 ± 0.16
Cotesia	Cotesia	2hr/C	-	90.48	90.48	-	9.6 ± 0.4
Cotesia	Microplitis	2hr/D	31.8	54.98	86.8	9.6 ± 0.4	11.1 ± 0.3
Microplitis	Microplitis	48hr/E	91.6	-	91.6	8.0 ± 0.2	-
Microplitis	Cotesia	48hr/F	36.7	42.5	79.2	10.0 ± 0.4	10.7 ± 0.45
Cotesia	Cotesia	48hr/G	-	83.3	83.3	-	10.0 ± 0.3
Cotesia	Microplitis	48hr/H	25.6	63.8	89.4	9.2 ± 0.3	11.4 ± 0.3

Table 2: Effect of double ovipositions by *Cotesia marginiventris* and *Microplitis rufiventris* at 2hr time intervals in the third instar larvae of *Spodoptera littoralis* on percentage of emergence success and development of the wasps.

Order of parasitism		Hours after oviposition	Emergence success (%)			Development time (days ± S.E)	
First wasp	Second wasp	/ Oviposition sequence	M.	C.	Total	M.	C.
Microplitis	-	Alone (control)	97.5	-	97.5	7.4 ± 0.1	-
Microplitis	*Microplitis*	2hr/A	91.8	-	91.8	7.3 ± 0.1	-
Microplitis	*Cotesia*	2hr/B	19.76	67.47	87.23	8.5 ± 0.3	11.1 ± 0.25
Cotesia	-	Alone (control)	-	70	70	-	8.9 ± 0.14
Cotesia	*Cotesia*	2hr/C	-	71.4	71.4	-	9.3 ± 0.13
Cotesia	*Microplitis*	2hr/D	21.2	68	89	10.4 ± 0.2	11.9 ± 0.2



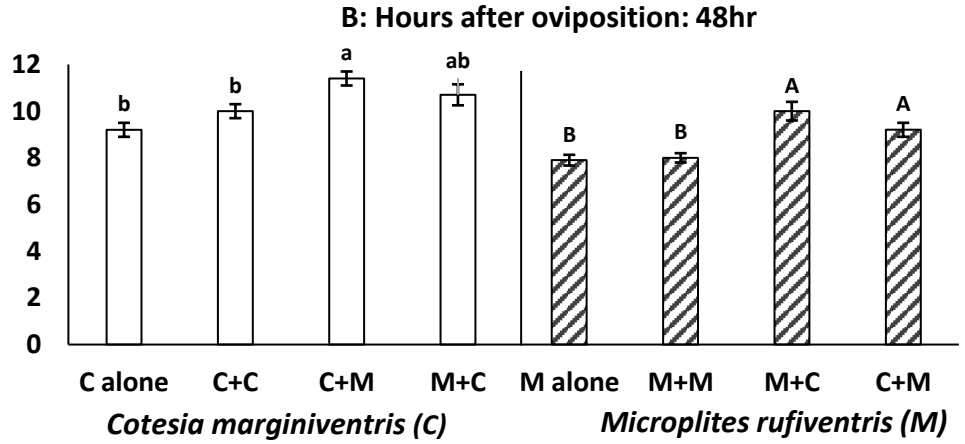


Figure 1: Effect of parasitism pattern, single parasitism (C & M), superparasitism (CC & MM) and multiple parasitism (CM & MC) in the 2nd instar *Spodoptera littoralis* larvae at 2 and 48hr intervals oviposition on endo-development time of the wasps.

The effect of different parasitism patterns on the endo-developmental history of the both parasitoid wasps in the 3rd instar *S. littoralis* larvae at 2hr interval oviposition time was also varied significantly in both *C. marginiventris* ($F=35.5$; df=3, 36; $P<0.05$) and *M. rufiventris* ($F=31.1$; df=3, 36; $P<0.05$) (Fig. 2).

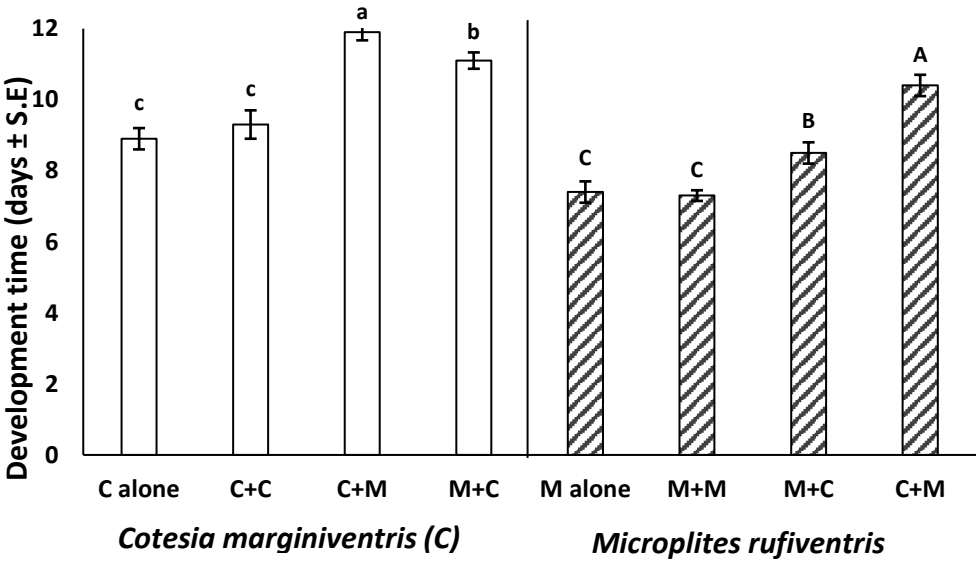


Figure 2: Effect of parasitism pattern, single parasitism (C & M), superparasitism (CC & MM) and multiparasitism (CM & MC, MC and CM) in the 3rd instar *Spodoptra littoralis* larvae at 2hr interval oviposition on development time of the wasps.

Single Parasitism

The endo-development of *C. marginiventris* was longer by more than one days than those of *M. rufiventris*. The effect of single parasitism on egg-to-cocoon development times for *C. marginiventris* did not vary significantly between the 2nd instar hosts (9.2 ± 0.3 days) and 3rd instar hosts (8.9 ± 0.3 days). Also, there were no significant differences in

development times of *M. rufiventris* in 2nd (7.9 ± 0.3 days) and 3rd (7.4± 0.3 days) instar host larvae (Figs. 1 & 2).

Self Superpasitism (Conspecific Superparasitism)

For *C. marginiventris*, no significant differences were found between the development time of *C. marginiventris* in both single parasitizesd (9.2± 0.16days) and superpasitized *S. littoralis* larvae. (9.6± 0.4days). Also, for *M. rufiventris* wasps, No significant differences in the developmental time was found between single (7.9± 0.05 days) or superparasitised (7.8± 0.16 days) host larvae (Table1).

Multiple Parasitism

The scenario of endo-developmental history was different in multiparasitism experiments which involves attacks from different parasitoid species (*C. marginiventris* and *M. rufiventris*) on the same host larvae. In multipararsitized hosts (Figs. 1), the endo-development time of the wasps significantly increased in both wasp species comparing to single or superparasitism experiments. For example, in oviposition sequence B (oviposition by *M. rufiventris*, followed by *C. marginiventris*), the development time lasted 10.5± 0. 6 and 12.25± 0.5days, respectively. In sequence D (oviposition by *C. marginiventris*, followed by *M. rufiventris*), the development time lasted 9.6± 0. 4 and 11.1± 0.3days, respectively (Table1). So, the endo-development times differed among the multiple parasitization patterns (Figs.1). In both wasps, the endo-development time of the wasps was significantly shorter in both wasp species in single and superparasitism, comparing to multipleparasitism.

The same scenario was observed in 48hr oviposition intervals, sequence E, F, G and H (Tables 1, Fig. 1). In both wasps, the endo-development time of the wasps was significantly shorter in single and superparasitism experiments comparing to multiple parasitism one.

The endo-developmental history of different parasitism patterns at 2hr oviposion intervals by *C. marginiventris* and *M. rufiventris* in the third instar larvae of *S. littoralis* larvae is shown in Table 2, Fig. 2. Again, in both wasps, the endo-development time of the wasps was significantly shorter in both wasp species in singly parasitized or superparasitized larvae comparing to multipleparasitism. (For *C. marginiventris*, $F=35.5$; $df=3, 36$; $P<0.05$. and *M. rufiventris*; $F=31.1$; $df=3, 36$; $P<0.05$).

Competitive Interactions in Double Parasitism

The study demonstrates that both parasitoid species were able to oviposit on previously parasitized hosts. Fig. 3A, shows the effect of single (control) and double ovipositions at 2hr intervals by *C. marginiventris* and *M. rufiventris* in the 2nd instar *S. littoralis* larvae on percentage of emergence success (pupae produced=cocoons) of the parasitoid wasps. In singly parasitized hosts by *C. marginiventris* and *M. rufiventris*, the percentage of emergence success reached up to 92.8 ± 0.5 and 99.6 ± 0.3%, respectively. The difference was significant ($t= 11.78$, $P < 0.05$). So, singly parasitized hosts by *M. rufiventris* produced more wasps than those of *C. marginiventris*. The percentage emergence success in superparasitized experiments by the same wasps varied significantly ($t= 10.39$, $P < 0.05$).

The percentage of emergence success by superparasitized *M. rufiventris*, oviposition sequence A, was greater (98.8 ± 0.5) than those recorded by superparasitized *C. marginiventris* (90.8 4± 0.5), oviposition sequence C (Table 1). However, in multiple parasitism experiments, when *C. marginiventris* was the first to oviposit (sequence D), the emergence success of total wasps reached up to 86.8% Of these wasps 54.98% were *C. marginiventris* and 31.85 were *M. rufiventris*. While, when *M. rufiventris* was first to oviposit (sequence B), the percentage of total emerged wasps significantly dropped to 70.1 ± 0.85%. Of these wasps 36.44 ± were *C. marginiventris* and 33.63± % were *M. rufiventris*. Muliparasitized hosts produced more *C. marginiventris* than *M. rufiventris* wasps. The difference between total emergences success in multiple ovipositions was significantly varied (t= 18.13, P < 0. 05). So, the interactions can lead to competition between the parasitoid larvae, impacting the survival and development of *M. rufiventris*.

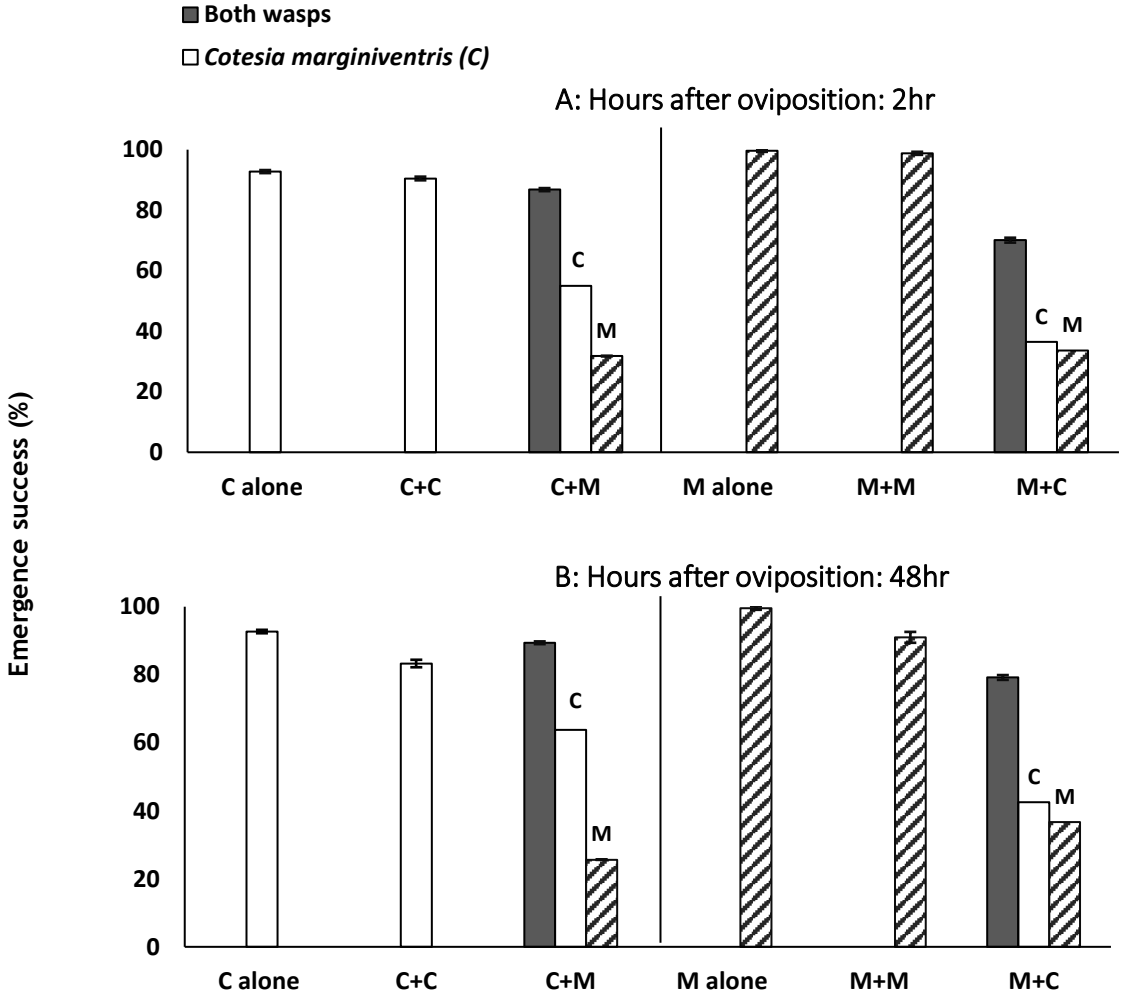


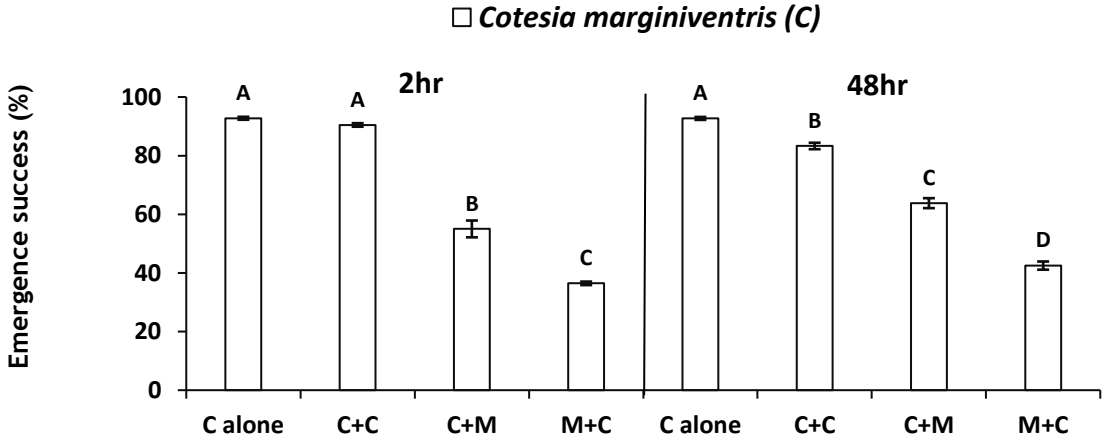
Figure 3: Effect of double parasitism by *Cotesia marginiventris* and *Microplitis rufiventris* in the 2nd instar *Spodoptera littoralis* larvae on percentage of emergence of the parasitic wasps.

Fig. 3, shows the effect of single and double ovipositions at 48hr intervals by *C. marginiventris* and *M. rufiventris* in early 2nd instar *S. littoralis* larvae on percentage of

emergence success of the parasitoid wasps. In singly parasitized hosts by *C. marginiventris* and *M. rufiventris*, the percentage of emergence success reached up to 92.7 ± 0.5 and 99.6 ± 0.5%, respectively. The difference was significant (t= 11.96, P < 0. 05). The percentage emergence success in superparasitized experiments by the same wasps varied significantly (t= 4.93, P < 0. 05). The percentage of emergence success by superparasitized *M. rufiventris*, oviposition sequence E, was greater (91.6± 1.0) than those recorded by superparasitized *C. marginiventris* (83.3 4± 1.1), oviposition sequence G (Table 1). However, in multiple oviposition experiments, when *C. marginiventris* was the first to oviposit (sequence H), the emergence success of total wasps reached up to 89.4% Of these wasps 63.8% were *C. marginiventris* and 25.6 were *M. rufiventris*. While, when *M. rufiventris* was first to oviposit (sequence F), the percentage of total emerged wasps significantly dropped to 79.2 ± 0.7%. Of these wasps 42.5 ± were *C. marginiventris* and 36.7± % were *M. rufiventris*. However, muliparasitized hosts produced more *C. marginiventris* than *M. rufiventris* wasps. The difference between total emergences success in multiple oviposition was significantly varied (t= 10.55, P < 0. 05). So, the interactions can lead to strong competition in sequence H and stronger competition in sequence F, between the parasitoid larvae, impacting the survival and development o *M. rufiventris*.

Fig. 4. shows the effect of parasitism patterns by *C. marginiventris* and *M. rufiventris* at 2 and 48hr. interval oviposition times in early 2nd instar *Spodoptera littoralis* larvae on percentage of emergence success of *C. marginiventris*. The emergence success of *C. marginiventris* varied significantly either after 2hr (F=242.6; df=3, 36; P<0.05) or 48hr (F=329.4; df=3, 36; P<0.05). The detrimental effect depended on the type of species oviposits first in double parasitism.

Fig. 4. Shows also, the effect of parasitism patterns by *C. marginiventris* and *M. rufiventris* at 2 and 48hr oviposition times in early 2nd instar *Spodoptera littoralis* larvae on percentage of emergence success of *M. rufiventris*. The emergence success of *M. rufiventris* varied significantly either after 2hr (F=383.9; df=3, 36; P<0.05) or 48hr (F=1103; df=3, 36; P<0.05). The detrimental effect in the double parasitism depended on the wasp species included first which impacting the survival and development of the wasps. The novel wasp was stronger competitor than the extant wasp impacting the survival and development of the 2nd wasp (Fig. 4).



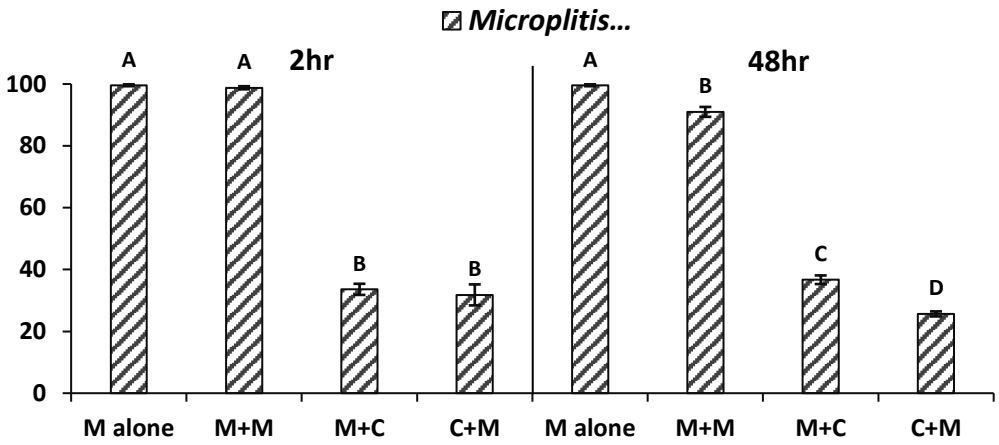


Figure 4: Effect of intervals between double ovipositions (2 or 48 hr) by *Cotesia marginiventris* and *Microplitis rufiventris* on percentage of emergence success of the parasitic wasps.

Fig. 5 shows the effect of single and double oviposition's at 2hr intervals by *C. marginiventris* and *M. rufiventris* in the 3rd instar *S. littoralis* larvae on percentage of emergence success of the parasitoid wasps. In singly parasitized hosts by *C. marginiventris* and *M. rufiventris*, the percentage of emergence success reached up to 70.0 ± 0.5 and 97.5 ± 1.3%, respectively. The difference was significant (t= 19.43, P < 0. 05). The study demonstrates that both parasitoid species were able to oviposit on previously parasitized 3rd instar hosts. In single parasitism experiments, the 3rd host instars significantly produced (t= 19.43, P < 0. 05) more *M. rufiventris* (97.5 ± 1.3%) compared to *C. marginiventris* wasps (70.0 ± 0.5%). The percentage emergence success in superparasitized experiments by the same wasps varied significantly (t= 14.23, P < 0. 05). The percentage of emergence success by superparasitized *M. rufiventris*, oviposition sequence A, was greater (91.8 ± 1.3) than those recorded by superparasitized *C. marginiventris* (71 4± 0.7), oviposition sequence C (Table 2). However in multiple oviposition experiments, when *C. marginiventris* was the first to oviposit (sequence D), the emergence success of total wasps reached up to 89.0%. Of these wasps 68.0% were *C. marginiventris* and 21.0 were *M. rufiventris*. While, when *M. rufiventris* was first to oviposit (sequence B) (Table 2), the perecentage of total emerged wasps significantly dropped to 87.2 ± 0.8%. Of these wasps 67.47 were *C. marginiventris* and 19.76 % were *M. rufiventris*. The difference between total emergences success in multiple oviposition was significantly varied (t= 18.13, P < 0. 05). However, muliparasitized hosts produced more *C. marginiventris* than *M. rufiventris* wasps. So the interactions can lead to competition between the parasitoid larvae, impacting the survival and development of *M. rufiventris*.

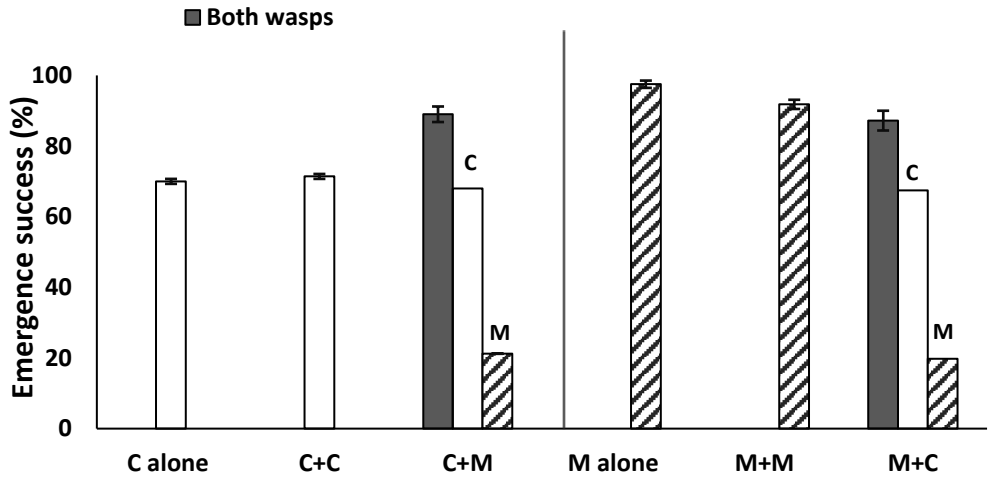


Figure 5: Effect of double ovipositions at 2hr intervals by *Cotesia marginiventris* and *Microplitis rufiventris* in the third instar *Spodoptera littoralis* larvae on percentage of emergence success of parasitic wasps.

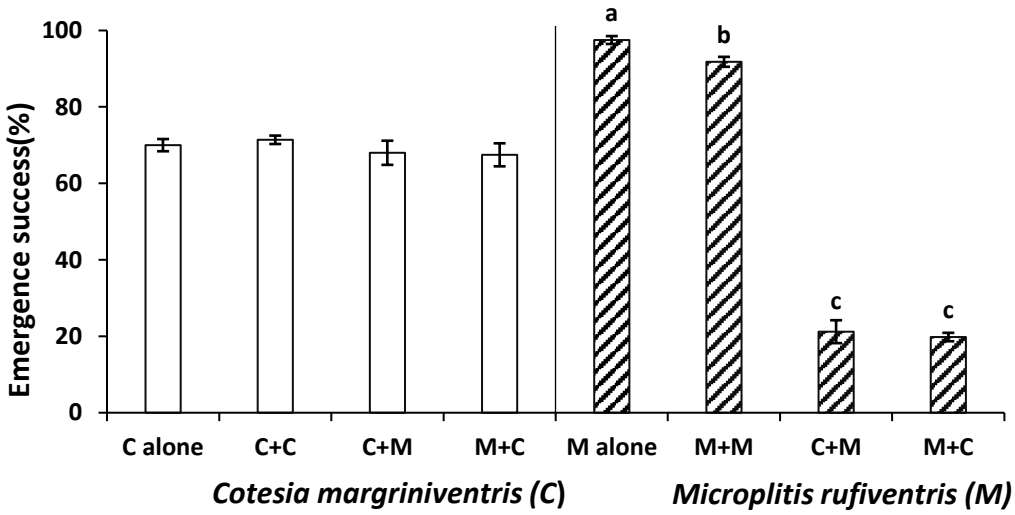


Figure 6: Effect of double ovipositions at 2hr intervals by *Cotesia marginiventris* and *Microplitis rufiventris* in the third instar *Spodoptera littoralis* larvae on emergence success of *C. marginiventris* and *M. rufiventris* wasps.

Fig. 6. shows the effect of parasitism patterns by *C. marginiventris* and *M. rufiventris* at 2hr oviposition internal time in the 3rd instar *Spodoptera littoralis* larvae on percentage of emergence success of *C. marginiventris* and *M. rufiventris*. The emergence success of by both wasps was different. Parasitism patterns significantly affected the percentage of emergence success in *M. rufiventris* ($F=844.4$; $df=3, 36$; $P<0.05$). No detrimental effects were recorded in single or superparasitism. The detrimental effect depended on the wasp species in the double parasitism. The perecentage of emerged wasps significantly dropped when parasitism included both wasp species. However, no significant differences were detected in percentage of emergence success of *C. marginiventris* between parasitism patterns.

Also, significantly greater number of *C. marginiventris* wasp larvae emerged from the 2th (n = 112) host instars containing two wasp eggs (superparasitism) than counterparts (n = 115) containing two wasp eggs in the 3rd of instar larvae Tables 1 &2).

In multiple parasitism the outcome of competition principally depended on the wasp species that was first to oviposit and host instar attacked. In all cases the novel wasp, *C. marginiventris* was the stronger competitor being the victor whatever the sequence of attack impacting the survival and development of the extant wasp. The detrimental effect in the double parasitism depended on the host instar and the wasp species included which impacting the survival and development of the other wasp.

DISCUSSION

The study explores how competition between two species of solitary parasitoid wasps: the indigenous *M. rufiventris* and the exotic *C. marginiventris*, is affected when they parasitized the same host, a phenomenon known as heterospecific superparasitism (=multiparasitism).

The *M. rufiventris* and the *C. marginiventris* can play a significant role in managing *Spodoptra* spp in the Egyptian cotton fields agro-ecosystem. *M. rufiventris* and *C. marginiventris* are both synovigenic, koinobiont larval solitary endoparasitoids of *S. littoralis* and *S. frugiperda*, respectively. (Hegazi and Khafagi 2024). The overlap in the host range of these parasitoids indicates that they are competitors, which may be likely influence their abundance in natural habitats. The study demonstrated that both parasitoid species were able to oviposit on previously parasitized 2nd and 3rd host instars. Superparasitism occurs when a female wasp (e.g. *C. marginivetris*) oviposits in a host previously parasitised by itself (self superparasitism) or by other female(s) of the same species (conspecific superparasitism). No significant differences in the developmental time was found between single or superparasitised host larvae by *C. marginiventris* or *M. rufiventris*. The effect of single and double parasitism at 2hr intervals by *C. marginiventris* and *M. rufiventris* in the 2nd instar *S. littoralis* larvae on percentage of emergence success of the parasitoid wasps was significantly different. Singly parasitized hosts by *M. rufiventris* produced more wasps than those of *C. marginiventris*. The percentage of emergence success by superparasitized *M. rufiventris*, was greater than those recorded by superparasitized *C. marginiventris*. Also, the effect of single and double ovipositions by *C. marginiventris* and *M. rufiventris* in the 3rd instar *S. littoralis* larvae on percentage of emergence success of the parasitoid wasps was different. In single parasitism experiments, the 3rd host instars significantly produced significantly more *M. rufiventris* compared to *C. marginiventris* wasps. The percentage emergence success in superparasitized experiments by the same wasps varied significantly. The percentage of emergence success by superparasitized *M. rufiventris*, was significantly greater than those recorded by superparasitized *C. marginiventris*. The data suggest that superparasitism in *C. marginiventris* or *M. rufiventris* will have no effect on its mass rearing for the biological control of *S. littoralis*. The fitness costs of superparasitism are highly variable. For example, in Dendrocerus carpenter (Curfis) (Megaspilidae), conspecific superparasitism resulted in a female-biased sex ratio, supporting the notion that superparasitism may be beneficial (Mackauer and Chow, 2016). In another study, delayed developmental time of Ooencyrtus pityocampae (Mercet) (Encyrtidae) was observed in a superparasitised host, Samia cynthiaricini (Jones) (Lepidoptera: Saturniidae) (Tunca et al., 2016). In the present study, no significant differences in the developmental time was found

between single or superparasitised host larvae by *C. marginiventris* or *M. rufiventris*. Multiparasitism occurs when a wasp female (e.g. *C. marginiventris*) attacks a host that has been already attacked by other female (e.g. *M. rufiventris*). Multiple parasitism increased the endo developmental time, but superparasitism not. Also, muliparasitized hosts produced more *C. marginiventris* than *M. rufiventris* wasps. When more eggs are deposited in the same host, elimination by physical combat, behavior known as siblicide, or physiological suppression takes place (Salt 1961; Mackauer 1990; Godfray 1994; Vinson & Hegazi 1998).

At oviposition, the wasp female injects a minute amount of venom and calyx fluid (CxF) containing polydnavirus/ ovipositional thrust (Hegazi et al. 2005), that may create a suitable physiological milieu for the growth and development of her eggs and larvae. In superparasitized hosts, the same wasp female or a second wasp female inject the same factors into the same host that have the same physiological or nutritional requirements factors injected by the first parasitoid female. So, in superparasitized hosts, no significant differences in the endo- development time of the wasps were found between single or superparasitised hosts by *C. marginiventris* or *M. rufiventris*.

Physiological mechanisms used to exclude competitors include physical and biochemical factors that originate with the ovipositing female wasp or her progeny. The immature development of solitary parasitoidism is restricted to resources found in a single host larva. When two parasitoids of the same (superparasitism) or different species (multipleparasitism) attack the same host, there is competition for monopolization of host resources. Dissections of parasitized hosts indicated that eggs of *M. rufiventris* hatched between 18 and 24 h after oviposition, while those of *C. marginiventris* took around 28- 35 h to hatch. First-instar larvae of both wasp species have a pair of sharp mandibles (active phase). Mandibles are sickle-shaped and are clearly suitable for brushing off the aggregation of the blood cells and fixing them into the body of a competitor under conditions of double parasitization (Hegazi & Furher,1985).

During competition between first and second instar larvae oviposited at an interval of 48 h, the first instar always killed a mandibulate second instars or other first instar wasp larva for eggs deposited at interval 2hr by physical attacks using the mandibles. Significantly greater number of *C. marginiventris* wasp larvae emerged from the 2th host instars containing two wasp eggs (superparasitism) than counterparts containing two wasp eggs in the 3rd of instar larvae. Hegazi & Khafagi 2024, reported that the first, second and third instars of *S. littoralis* were found to be parasitized by either *C. marginiventris* or *M. rufiventris*, respectively. But, choice tests revealed that the highest percentage of parasitism found in second instars for *C. marginiventris* and third instars for *M. rufiventris*.

The scenario was different in multiparasitism experiments, i.e wasp factors. It seems that the first wasp species parasitoid injects factors that differed from those injected by another wasp species. In all double ovipositions, the competition was in favor of *C. marginiventris* (the winning parasitoid). This indicates that the physical and biochemical factors originated by *C. marginiventris* were more effective than ones originated by *M. rufiventris*. The resulting intraspecific competition between two parasitoid larvae leads to the death of one larva that failed to get enough nutrition and died precociously , but the other larva completes its development(the winning parasitoid). That is to say, the consumption of host resources by (the winning parasitoid) may to some extent be detrimental to the development of the other parasitoid larva. We found that in multiple

parasitism experiments, the competition was asymmetrical, meaning one species (the exotic) had a competitive advantage over the other. Wasp larvae involved in physical combat and those that encapsulated by blood cells that sealing the other wasp larva off from the host environment were common in muliparasitized larvae than in superparasitized host larvae (intrinsic completion).

The current study demonstrated that *C. marginiventris* was a superior larval competitor compared with *M. rufiventris* '. Competitive exclusion in which the novel parasitoid species partially displaced the extant species has been observed in the present test. So, importing *C. marginiventris* may be not a good option for biological control of both, the new invasive pest, *S. frugiperda* and local one, *S. littoralis*.

The eggs of many braconid parasitoids liberate large cells called teratocytes from the serosal membrane into the host hemocoel at hatching. These cells exhibit several functions, including the regulation of host growth and immunosuppression (Dahlman 1990; Firlej et al. 2007; Sasaki & Godfray 1999). Given their large size, it has been suggested that teratocytes may have a trophic function, providing critical additional food to immature parasitoids developing inside the host (Kadono-Okuda et al. 1998). The number of circulating teratocytes is higher in superparasitized hosts than in singly parasitized hosts. So it is suggested that *C. marginiventris*, teratocytes may influence the outcome of competition. However, more evaluations on many native and non-target noctuid species are needed before introducing this exotic biocontrol agent.

The current study demonstrated that the novel parasitoid, *C. marginiventris* was a superior larval competitor compared with local parasitoid, *M. rufiventris*, because the number of cocoons of the former wasp that emerged in the multiple parasitism experiment was much greater than *M. rufiventris*.

The results of the study provides useful information to use suitable host age of *S. littoralis* for mass production and mass rearing programs using *C. marginiventris* and *M. rufiventris* parasitoids. For inundative release of these species, it would appear to be most efficient from a production standpoint to use second instar for *C. marginiventris* and third instar for *M. rufiventris*.

Authors Contributions

Hegazi conceived the idea, wrote the first draft of the manuscript and general supervision of the research and was a major contributor in writing the manuscript. Khafagi collected the data and participated in its design and coordination and aided to draft the manuscript. Showiel and Rabab supervised some parts of the work.

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Data Availability Statment

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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