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Incidence of Superparasitism in Relation to some Biological Aspects of the Egg Parasitoid, *Trichogramma evanescens* West. (Hymenoptera: Trichogrammatidae)

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ABSTRACT

Superparasitism is a widespread phenomenon in insect parasitoids and may be advantageous in certain circumstances. The present study aimed to highlight the effect of superparasitism on some biological aspects of *Trichogramma evanescens* Westwood, the common, native egg parasitoid species in Egypt, when reared on different hosts under laboratory conditions. Eggs of seven lepidopterous hosts i.e. *Helicoverpa armigera* (Hb.), *Pectinophora gossypiella* (Saund.), *Earias insulana* (Boisd.), *Spodoptera littoralis* (Boisd.), *Galleria melonella* L., *Anagasta (Ephestia) kuehniella* (Zeller) and *Sitotroga cerealella* (Oliver) were used in the study. Developmental period, emergence rate, longevity and sex ratio were the considered biological aspects of *Trichogramma,* in no-choice and choice experiments. The superparasitism occurred in both small and large host eggs, as more than one adult emerged from a single host egg with different average numbers of emerged adults, ranged between 1.20 and 2.73 adults in *Sitotroga* (the smallest egg volume) and *Helicoverpa* (the largest), respectively. The volume of the host's egg and incidence of superparasitism had no influence on the developmental period of immature stages of *T. evanescens* in the seven tested hosts, while some other biological aspects of the parasitoid species such as; emergence rate, female percentage and adults' longevity had relatively been affected and significant differences were found.

Key words: *Trichogramma evanescens*, Superparasitism, Host size, Biological aspects.

INTRODUCTION

Superparasitism is a widespread phenomenon in insect parasitoids and may be advantageous in certain circumstances. In solitary parasitoids, superparasitism (the allocation of an egg (or more) to an already parasitized host) has a payoff, measured in offspring produced and costs, measured in eggs and time invested. Solitary parasitoids that are capable of host discrimination must adopt the strategy that ensures the best use of their egg load and available lifetime.

Trichogramma species are the most widely used insect natural enemy in the world (Li-Ying, 1994), due to their wide geographic distribution, high specialization and efficacy against many lepidopteran important crop insect pests and partly because they are easy to mass rear. Physical cues such as size, shape, color and curvature of the host eggs are the most important criteria used by *Trichogramma* species to select hosts (De Jong and Pak, 1984 and Borah and Basit, 1994). Among these physical factors, host size is critical in host acceptance and a major factor regulating clutch size and sex ratio adjustment (Schmidt, 1994).

Superparasitism affects subsequent parasitoid development (Ahmad *et. al.* 2002) and has been suggested as a factor leading to extend emergence periods in several *Trichogramma* species (Parra *et al.* 1988).

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) is the most common, native and widely distributed egg parasitoid species in several fields; vegetable and fruit crops in Egypt. Besides, it has been recorded in different Egyptian habitats (El-Heneidy and Shoeb, 2007).

George (1936) stated that *T. evanescens* is able to distinguish healthy from parasitized hosts, and when few hosts are available can restrain itself for 8 hours to the deposition of 5 %, of its available eggs. Discriminative ability is perfect; but the restraint is limited and, in a longer period of time or when several parasitoids are together, it breaks down and superparasitism occurs. When the superparasitism is slight and only two or three eggs are laid in a host, the competition between the parasitoid progeny leads to the victory and emergence of one; but when it is severe either a dwarfed or imperfectly developed individual or none at all emerges. As the density of parasitoids in a fixed population of hosts is increased, more and more superparasitism occurs. Tuncbilek and Ayvaz (2003) reported that fresh eggs (6- 48 h) and (72- 96h) of the Mediterranean flour moth, *Anagasta (Ephestia) kuehniella* (Zeller) were more accepted to *T. evanescens* than older ones.

Suzuki *et. al.*, (1984) studied the role of sex-controlling behavior at oviposition in generating primary sex ratios, and the effect of larval competition on secondary sex ratios, were studied in the gregarious endoparasitoid, *Trichogramma chilonis*.

Moreira *et. al.*, (2009) studied behavioral traits of *Trichogramma pretiosum* Riley parasitizing

Sitotroga cerealella (Oliver) eggs aiming to a better understanding to the results of parasitism and superparasitism.

The present study aimed to highlight the effect of superparasitism on some biological aspects of *T. evanescens* when reared on some hosts under laboratory conditions.

MATERIALS AND METHODS

Experiments for studying the phenomenon of superparasitism and impact of the size of host eggs on some biological aspects of the egg parasitoid *T. evanescens* were carried out under laboratory conditions. To ensure incidence of superparasitism, individual host eggs were offered to a quite number of mated parasitoid females and number of emerged adults/host egg was estimated.

Eggs of seven lepidopterous host species that have different sizes of eggs and have been recorded as hosts for *Trichogramma*, namely; the American bollworm *Helicoverpa armigera* (Hb.), the pink bollworm *Pectinophora gossypiella* (Saund.), the spiny bollworm *Earias insulana* (Boisd.), the cotton leaf worm *Spodoptera littoralis* (Boisd.), the greater wax moth *Galleria melonella* L., the Mediterranean flour moth *Anagasta (Ephestia) kuehniella* (Zeller) and the grain moth *Sitotroga cerealella* (Oliver) were used in the present study. The eggs used were obtained from the stock cultures at different departments of the Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. As well, the *Trichogramma* was obtained from its mass rearing unit at the Department of Biological Control (DBC), PPRI, ARC at Giza.

T. evanescens was reared for 2-3 generations on each of the seven tested hosts before recording any data. Rearing was maintained at 25±1 °C, 65±5% R.H. and 12L: 12D. The parasitoid individuals used in all experiments were less than 24h old and were fed on droplets of bee honey. As well, fresh laid eggs (less than 24h old) of each host were offered separately in two different types of experiments; (no-choice) by offering only one host eggs for a certain period of time (12 hours) and (choice) by offering eggs of the seven host species together to the parasitoid (Tuncbilek and Ayvaz, 2003). Glass tubes (2x8 cm) were used for exposure of host eggs to the *Trichogramma* adults.

Dimensions of each host egg were measured (30 eggs/host), using a micrometer lens fixed in a stereomicroscope. Comparisons among the eggs of different hosts were dependent upon the egg's volume. The volume (V) was calculated using the formula of Ernest and Richard, (2005) $V = (\pi D^3)/6$ where (π is a constant value ≈ 3.14 and D is diameter), for spherical shaped egg and $V = (\pi W^2 L)/6$ (W = width, L = length), for oval shaped egg. 15 replicates were used for each host (n= 15 males and 15 females). Parasitized eggs were checked daily until emergence of the parasitoid adults.

Biological Aspects

Biological aspects of *Trichogramma* resulted from eggs of each host species were considered in the two types of experiments were:

- **Developmental period** of immature stages (duration was estimated by dividing total developmental period from egg laying to adult emergence into two stages; 1) egg-larval period, from parasitization (egg laying) to pupal stage (color of eggs turns black), and 2) pupal period, from black eggs (pupal formation) to adult emergence.
- **Emergence rate** was estimated by counting and separating black eggs of each host individually and counting number of emerged adults per female in the two types of experiments (no-choice and choice).
- **Longevity** of both females and males was estimated by keeping 50 emerged adults in glass vials, providing them with droplets of honey as food and checking the vials daily to record dead individuals. The dead ones were sexed, recorded and their longevities were calculated.
- **Sex ratio** was estimated as percentages of females among emerged adults. The sex was determined according to the morphological characteristics of antennae.

Statistical analysis

ANOVA with significant difference tests according to Fisher's Least Significant Difference (LSD) at 5% significance was used to analyze obtained data. The analysis was performed by using Statview 4.0 software (Haycock *et al.*, 1992).

RESULTS AND DISCUSSION

Impact of dimensions and volumes of hosts' eggs

Average dimensions [(width and length) or diameter] of hosts' eggs of the seven insect species; (*Sitotroga, Pectinophora, Earais, Anagasta, Galleria, Spodoptera* and *Helicoverpa)* were measured individually using a micrometric lens. As for the eggs of *Earais*, only the (diameter) was measured because of its spherical shape. Volume of each host's egg, the most effective factor influencing the incidence of superparasitism and consequently the number of adults emerge from each host's egg was estimated according to the formula of Ernest and Richard, (2005). Obtained data were summarized in table (1). The dimensions and volumes of the eggs varied among different host species used in the study. The volumes ranged between 0.000350 and 0.000752 u liter in *Sitotroga* (the smallest) and *Helicoverpa* (the largest), respectively. Egg size increased proportionally to the size of moths of each host. The seven hosts could be arranged in an ascending order, according to the volume of each host's egg as follows; *Sitotroga, Pectinophora, Anagasta, Earais, Spodoptera, Galleria* and *Helicoverpa*. Schmidt and Smith, (1987) stated that the exposed surface area may be the cue used by the parasitoid to adjust its progeny allocation to the number of local hosts. Tuncbilek and Ayvaz (2003) reported that fresh eggs (6- 48 h) and (72- 96h) of *A. kuehniella* were more accepted to *T. evanescens* than old ones.

Averages in number of *T. evanescens* adults emerged from a single host egg were also presented in table (1). The superparasitism took place in all hosts' eggs, as an average of more than one adult/ one host's egg was recorded. The average number of emerged adults per one host egg increased as the host egg size/ volume increased. The average number ranged between 1.20 and 2.73 adults in *Sitotroga* (the smallest egg size/ volume) and *Helicoverpa* (the largest), respectively in the no-choice experiments. Respective numbers ranged between 1.13 and 2.80 in case of the choice experiment. Statistical analysis showed that there were significant differences among the numbers of emerged adults/ single host egg in both experiments, except between the largest two hosts *Galleria* and *Helicoverpa* (Table 1).

When superparasitism occurred by oviposition of two or three *Trichogramma* eggs in a single host egg, the competition between the parasitoid progeny leads to the victory and emergence of only one adult. When it is severe, either a dwarfed or imperfectly developed individual or none at all emerge (George, 1936). Klomp and Teerink (1967) stated that progeny with the greatest fecundity and longevity were produced when only one parasitoid egg was allocated to each host, regardless to host's size. Bai *et al.* (1992) and Bjorksten and Hoffmann (1995) reported that *T. evanescens* prefers larger host eggs more than smaller ones. However, chemicals from the offered host eggs might have been involved in the host recognition and acceptance process because natural hosts were used in these experiments. Under laboratory conditions, an average of 2.1 wasps emerged from eggs of the corn earworm, *Helicoverpa zea*, vs 1.0, from the smaller diamondback moth egg (Hoffmann *et al.*, 1995). Several authors have mentioned that *Trichogramma* spp. reared on small host eggs were less fecund or had lower searching ability than individuals reared in larger host eggs (Bigler *et al.*, 1987; Bergeijk *et al.*, 1989; Greenberg *et al.*, 1998, Luck *et al.*, 1999 and Moreira *et. al.*, 2009).

Influence of superparasitism on some biological aspects of *T. evanescens*

Developmental period

Durations of the immature stages of *T. evanescens* in eggs of the seven tested hosts, in the two types of experiments (no-choice and choice) were estimated as two main developmental periods (egg-larval and pupal periods). Consequently, total developmental periods were also estimated. Obtained data were recorded in table (2). Average durations of the egg-larval period ranged between 4.0-4.2 and 3.9-4.2 days, in the seven tested hosts in the no-choice and choice experiments, respectively, without significant difference among the hosts. For the pupal period, respective duration averages ranged between 5.9–6.3 and 6.0 – 6.4 days, with significant difference between *Earais* and *Spodoptera* in the no-choice experiments and among all hosts, except between each of the *Sitotroga, Earais, Anagasta* and *Spodoptera, Galleria* and *Helicoverpa* in the choice experiment. Total developmental periods ranged between 10.1–10.5 and 10.0–10.5 days in the seven tested hosts and in the two types of experiments, respectively, with significant differences among the hosts, except among *Sitotroga, Pectinophora* and *Spodoptera* in the no-choice experiments and among *Sitotroga, Pectinophora, Anagasta* and *Helicoverpa* in the choice experiment (Table 2).

Generally, it could be concluded that the host egg's volume and/or superparasitism had almost no influence on the developmental period of *T. evanescens* in the seven tested hosts under the laboratory conditions; 25±1 °C, 65±5% R.H. and 12L: 12D, as the significant differences recorded were very narrow. Yadav *et al.* (2001) reported that superparasitism, which is of common occurrence in *Trichogramma* spp. in the laboratory, may cause an increase in the developmental time in late females.

Emergence rate

Emergence rates of *T. evanescens* adults from the seven tested hosts, in the two types of experiments were presented in table (3). Average emergence rates ranged between 87.1 – 92.2 and 84.1 – 90.2 %, in the no-choice and choice experiments, respectively, with significant differences among the hosts. The emergence rates were relatively higher in case of the no-choice experiments.

Female's percentage (Sex ratio)

Female percentages among emerged progeny were calculated as an indication of the sex ratio

Table (1): Mean host egg sizes (dimensions/ volumes) and number of adults emerge/single host egg.

Host	Dimensions (μ m) and Volume ($^{\mu}$ liter) Host egg			Number of adults/single host egg	
				No- choice	Choice
Sitotroga	W = 160 ± 9.2	L= 260 ± 7	V= 0.00350	1.2 ± 0.35d	1.13 ± 0.32^{d}
	R = (135 – 170)	(244 – 272)		(0.8 - 1.9)	(0.8 - 1.9)
Pectinophora	W = 180 ± 7.4	L= 270 ± 8.2	V= 0.00458	1.33 ± 0.42^{cd}	1.33 ± 0.38^{cd}
	R = (160 – 190)	(236 – 264)		(0.8 - 2.1)	(0.9 - 2.0)
Anagasta	W = 180 ± 11.2	L= 270 ± 8.2	V= 0.00458	1.47 ± 0.52^{bcd}	$1.4 \pm 0.51b^{cd}$
	R = (164 – 196)	(263 – 284)		(1 - 2)	(1 - 2)
Earais	D= 216 ± 10.2		V= 0.00527	1.67 ± 0.49^{bc}	1.73 ± 0.4^{6b}
	R= (200 - 228)			(1 - 2)	(1 – 2)
Spodoptera	W= 211 ± 9.8	L= 243± 9.8	V= 0.00566	1.73 ± 0.46^{b}	1.67 ± 0.49^{bc}
	R= (206 – 236)	(224 – 462)		(1 - 2)	(1 – 2)
Galleria	W= 200 ± 12.2	L= 305 ± 7.8	V= 0.00647	2.4 ± 0.51^{a}	2.74 ± 0.52^{a}
	R= (184 – 216)	(300 – 322)		(2 – 3)	(2 – 3)
Helicoverpa	W= 220 ± 11.6	L= 297 ±7.6	V= 0.00752	2.73 ± 0.7^{a}	2.8 ± 0.68^{a}
	R= (204 – 238)	(270 – 306)		(1 - 4)	(1 – 4)
LSD				0.3635	0.3549

W= Width L= Length D= Diameter V= Volume R= Range

Means with the same letter are not significantly different.

Table (2): Effect of host's egg size on developmental period of *T. evanescens* emerged adults.

Host	Developmental period Stage (1) (days)		Developmental period Stage (2) (days)		Total developmental period (days)	
	No- choice	Choice	No- choice	Choice	No- choice	Choice
Sitotroga	4.1 ± 0.2	4.1 ± 0.1	6.1 ± 0.2	6.1 ± 0.4	10.2 ± 0.52^{bc} (9.5 – 11)	10.2 ± 0.5^{bc} (9.5 – 11)
Pectinophora	4.2 ± 0.3	4.0 ± 0.2	6.0 ± 0.1	6.2 ± 0.3	10.2 ± 0.51^{bc} (9.4 – 11)	10.2 ± 0.52^{bc} (9.5 – 11)
Anagasta	4.15 ± 0.1	3.9 ± 0.3	6.2 ± 0.2	6.1 ± 0.5	10.33 ± 0.44^{abc} (9.8 – 11)	10.1 ± 0.6^{bc} (9.9 – 10.3)
Earais	4.22 ± 0.2	4.0 ± 0.3	5.9 ± 0.4	6.0 ± 0.3	10.1 ± 0.13^{c} (9.9 – 10.3)	10.0 ± 0.39^{c} (9.3 – 10.7)
Spodoptera	4.1 ± 0.1	4.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.4	10.4 ± 0.24^{ab} (10 – 10.8)	10.5 ± 0.24^{a} (10.1 – 11)
Galleria	4.0 ± 0.2	4.2 ± 0.2	6.2 ± 0.1	6.2 ± 0.5	10.2 ± 0.16^{bc} (9.9 – 10.4)	10.4 ± 0.32^{ab} (10 – 10.8)
Helicoverpa	4.15 ± 0.1	4.1 ± 0.2	6.1 ± 0.2	6.4 ± 0.4	10.53 ± 0.24^{a} (10.1 – 11)	10.2 ± 0.52^{bc} (9.5 – 11)
LSD	0.321	0.246	0.346	0.284	0.258	0.286

Table (3): Effect of host's egg size on emergence rate, females' percentage and adult longevity of *T. evanescens*.

Host	Emergence rate (%)		Percentage of females		Longevity of Females		Longevity of males	
	No-choice	Choice	No- choice	Choice	No- choice	Choice	No- choice	Choice
Sitotroga	88.1 ± 2.2	86.0 ± 3.4	48.0 ± 2.2^{d}	50.0 ± 3.2^{c}	10.4 ± 0.7^{a}	9.85 ± 1.06^{a}	5.051 ± 0.2^{ab}	5.1 ± 0.26^{a}
Pectinophora	92.2 ± 3.1	90.1 ± 2.3	50.4 ± 2.2^{cd}	52.6 ± 3.5^{d}	$9 8 \pm 0.8^{ab}$	9.6 ± 1.06^{a}	4.86 ± 0.4^{bc}	4.73 ± 0.49^{b}
Anagasta	87.1 ± 2.2	88.1 ± 4.5	51.4 ± 2.7^{c}	53.4 ± 2.2^{d}	9.85 ± 1.0^{ab}	9.8 ± 0.86^{a}	4.67 ± 0.52^{c}	4.73 ± 0.48^{b}
Earais	90.1 ± 3.4	88.0 ± 3.3	54.2 ± 2.4^{b}	56.2 ± 2.6^{c}	10.2 ± 1.1^{ab}	9.4 ± 1.06^{a}	5.2 ± 0.49^{a}	$4,5 \pm 0.21^{bc}$
Spodoptera	92.1 ± 3.1	90.2 ± 3.4	55.6 ± 3.8^{b}	$57.6 \pm 3.1b^{c}$	9.6 ± 0.99^{bc}	9.8 ± 0.94^{a}	5.0 ± 0.21^{ab}	4.67 ± 0.44^{b}
Galleria	92.2 ± 4.1	87.1 ± 2.5	58.5 ± 3.2^{a}	59.5 ± 3.2^{b}	9.0 ± 0.85^{cd}	9.4 ± 0.82^{a}	4.8 ± 0.38^{bc}	$4. 2 \pm 0.22^{c}$
Helicoverpa	87.1 ± 3.2	84.1 ± 3.4	60.9 ± 5.1^{a}	62.9 ± 4.2a	8.8 ± 1.08^{d}	8.4 ± 0.74^{b}	4.67 ± 0.4^{c}	4.2 ± 0.24^{c}
LSD	3.342	3.234	2.4152	2.3542	0.6893	0.6903	0.2845	0.2902

(Table 3). Female percentages in the seven host species, ranged between 48.0 – 60.9 and 50.0 – 62.9 %, in the no-choice and choice experiments, respectively, with significant differences among the hosts. Lowest and highest female percentages were recorded in case of the *Sitotroga* (the smallest egg size/ volume) and *Galleria* and *Helicoverpa* (the largest), respectively (Table 3). These results may be attributed to the impact of the size/ volume of the host's egg on the sex ratio of *T. evanescens*, as the female percentage increased when the parasitoid was offered large host egg size (Bai *et al.*, 1992). Suzuki *et al.*, (1984) concluded that immature mortality is not significantly different between the sexes when a host egg is parasitized by a single *Trichogramma* egg, while females suffer higher immature mortality than males when superparasitism occurs. Also, wasps adjust their progeny sex ratios to a wide range of host size.

Female and male longevity

Longevities of females and males of *T. evanescens* adults emerged from the seven tested hosts in the no-choice and choice experiments were summarized in table (3). Female longevity ranged between 8.8–10.4 and 8.4–9.8 days in the seven hosts in the no-choice and choice experiments, respectively without significant difference among the hosts in the no-choice experiments, while the differences were significant in the choice experiments. As for the male longevity, it ranged between 4.7–5.2 and 4.0–5.1 days in the two types of experiments, respectively, with significant differences among the hosts. Also, females' longevity was almost double that of males and both sexes emerged from large host eggs lived shorter than those emerged from small host eggs (Table 3). Offspring longevity, however, was similar for females emerged from hosts from which one or two adults emerged Moreira *et. al.*, (2009)

In conclusion, it could be stated that superparasitism occurred in both small and large host eggs, as more than one adult emerged from a single host egg. The volume of the host's egg and incidence of superparasitism had no influence on the developmental period of immature stages of *T. evanescens* in the seven tested hosts, while some other biological aspects of the parasitoid species such as; emergence rate, female percentage and adults' longevity were relatively affected and significant differences were found. Obtained results agree with those reported by Nandihalli and Lee, (1995).

Superparasitism has a payoff, measured in offspring produced and costs, measured in eggs and time invested. *Sitotroga*, *Anagasta* and *Corcyra* are the most factitious hosts used for mass rearing of the egg parasitoid, *T. evanescens*. Therefore, for successful colonization, it is suggested that when the parasitoid is reared in the laboratory for mass liberation, care should be taken to avoid superparasitism in the host eggs, for which special techniques have to be evolved; and also the initial population of target pest eggs in the field has to be taken into consideration at the time of the release. Superparasitism is a very potent factor that determines the success or failure of the colonization of *Trichogramma* in the field.

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