
The Effect of Host Density and Viability on Superparasitism Behavior of *Trichogramma cacoeciae* and *T. principium* Females

Muhanad Harba*, Imad Idris

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Damascus, Syria

Email address:

ascientific@aec.org.sy (M. Harba)

*Corresponding author

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Abstract: Non-choice laboratory experiments were conducted to estimate the influence of host density and viability of codling moth eggs on the superparasitism behavior of *Trichogramma principium* Sug. et Sor and *T. cacoeciae* Marchal (Hymenoptera: Trichogrammatidae). There was a strong effect of number of emerged offspring of *T. principium* and *T. cacoeciae* and host density on the mean number of parasitized eggs. The tendency of *T. principium* and *T. cacoeciae* females to oviposit was increased when high number of *Cydia pomonella* was available. The results presented that the host density influenced in the percentage of superparasitized eggs. The superparasitism appeared to be more noticeable when sterile *C. pomonella* was offered. Superparasitism behavior was noticed in both parasitoids species, regardless of host density and viability. The study demonstrates the success of *T. principium* and *T. cacoeciae* in the existence of high host density and in an integrated program employing the sterile insect technique for *C. pomonella* management.

Keywords: *Trichogramma*, Superparasitism, Host Density, Host Viability

1. Introduction

The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is considered to be the most important pest of apple in Syria that need to be addressed due to the economic impact of this insect [27]. Insecticides are widely used to control this pest, but this method is costly, nonselective, environmentally unfriendly and only effective for a short period of time in treated areas [2]. Moreover, resistance to insecticides is a recorded frequent phenomenon among Lepidoptera [18]. Other control methods such as biological ones should be used. Therefore, strategies relying on biological control measures are being considered as economic alternatives for managing Lepidopteran species in the field [24]. The egg parasitoid *Trichogramma* species using can be considered as the most common biological control agents [1]. The wide use of *Trichogramma* can be related to their abundant distribution and because they are highly efficient and specialized. The egg parasitoid to parasitize over 200 insect species belonging to 70 families of

8 insect orders, especially Lepidoptera were reported [12]. Previous investigations showed the efficacy of using *T. platneri*, *T. cacoeciae* and *T. principium* (Hymenoptera: Trichogrammatidae), as biological tools against *C. pomonella*. Moreover, the compatibility of use these species in an integrated program employing the sterile insect technique for *C. pomonella* management [5], [28], [29], [31], [43]. However, females of *T. principium* and *T. cacoeciae* were able to oviposit more than one egg per *C. pomonella* egg while not more than one egg per *Ephesia kuehniella* Zeller egg [27]. Such this phenomenon is known as the superparasitism behavior [33]. Several factors including: Host acceptance, age's host egg, [6], [36], emergence rate, adult's longevity [32], [35], sex ratio, offspring female body size and longevity [10], [34], intraspecific intrinsic competition (IIC) and temperature [13], [20], could play an effective role in *Trichogramma* superparasitism. In literature, there is a lack of information on the relationship of superparasitism of *T. cacoeciae* and *T. principium* with viability and host density. Therefore, from practical point of

view, it is very important to investigate if the superparasitism behavior of *T. principium* and *T. cacoeciae* would be influenced by the density and viability of host egg. For this purpose, we investigated the mean numbers of parasitized eggs and emerged F_1 progeny per parasitoid female, fecundity or the mean numbers of eggs (indicated by the number of parasitoid larvae in the host eggs) laid per parasitoid female, and percentages of superparasitized host eggs. Moreover, the relationship between superparasitism behavior and viability of host eggs was assessed for the two tested *Trichogramma* species.

2. Materials and Methods

2.1. Host Culturing and Parasitoid

Parasitoid culturing *T. principium* and *T. cacoeciae* used in this study were reared on eggs of *Ephestia kuehniella* Zeller and the codling moth colony has been maintained in our laboratory for several years. Rearing procedures were fully described by Makee [27].

2.2. Effect of Host Density on Parasitism and Superparasitism

In our present experiment, three groups of 15 newly emerged *T. cacoeciae* females were used. The groups of females 1, 2 and 3 were exposed to 5, 10 and 15 *C. pomonella* eggs (aged <24 h), respectively. A droplet of honey was added to feed parasitoids' females in all tested groups. The parasitoid females, in each group, were left with the host egg for 24 h under $25\pm 1^\circ\text{C}$ and $75\pm 5\%$ R. H. The host eggs kept at constant conditions to allow *Trichogramma* development, after all females were removed. Same procedure was followed when *T. principium* females were tested. After 4 days, host eggs turning black were recorded as parasitized. A daily observation was carried out until emergence of parasitoid progeny. In day 9, the number of *T. principium* and *T. cacoeciae* larvae per host egg was counted to determine the superparasitized eggs. A host egg with more than 1 parasitoid larva was described as superparasitized egg. The number of *T. principium* and *T. cacoeciae* larvae in host eggs represents the fecundity (number of eggs) of *T. principium* and *T. cacoeciae* females. In each tested host egg group, the following parameters were determined:

1-Mean parasitized eggs per *T. principium* and *T. cacoeciae* female = Total numbers of parasitized host eggs/total number of females per group.

2-Mean numbers of emerged F_1 progeny per *T. principium* and *T. cacoeciae* female = Total number of emerged F_1 progeny/total number of females per group

3-Mean fecundity of *T. principium* and *T. cacoeciae* females = Total number of parasitoid larvae in parasitized host eggs/total number of females per group.

4-Percentage of superparasitized eggs = Total number of host eggs with more than 1 parasitoid larva/total number of parasitized host eggs.

2.3. Effect of Host Eggs Viability on Superparasitism

To obtain sterile host eggs newly emerged codling moth females were irradiated with 100 Gy, using a ^{60}Co source (Issledova Gamma Irradiator, Techsnabexport Co. Ltd, Russia) at a dose rate of 60 Gy/min. Irradiated females were paired with unirradiated males and sterile eggs were removed daily. Fertile eggs were obtained by pairing unirradiated males with unirradiated females. Both sterile and normal egg were used in non-choice experiments. To study the effect of host viability on superparasitism of *T. cacoeciae* two groups of newly emerged *T. cacoeciae* females were used ($n=25$ female). In group 1, the females were individually exposed to 20 fertile *C. pomonella* eggs (<24 h old). In group 2, the females were singly exposed to 20 sterile *C. pomonella* eggs (<24 h old). In both groups a droplet of honey was added to feed parasitoid females. The *T. cacoeciae* females, in each group, were held with the host egg for 24 h at $25\pm 1^\circ\text{C}$ and $75\pm 5\%$ R. H. After that, the host egg was removed and kept under the same conditions to allow *Trichogramma* to develop. A daily observation until emergence of parasitoid progeny was carried out. In day 9, the numbers of *T. cacoeciae* larvae per host egg was counted to determine the superparasitized eggs. Same procedure was followed when the effect of host viability on superparasitism of *T. principium* females were tested. Statistical analysis was done using the StatView computer program at the 5% level ($P=0.05$). Analysis variance (ANOVA) was apply to evaluate the differences between means. Differences between means were tested for significance sing Fisher's Least Significant Difference (LSD). The percentages were analysis by applying normal approximation test (analysis of proportion).

3. Results

3.1. Effect of Host Density on Parasitized Egg and F_1 Progeny

Our result illustrates that there was a vigorous effect of the host density on the number of parasitized eggs and emerged F_1 progeny of *T. principium* and *T. cacoeciae* (Table 1). This was shown by a significantly high mean numbers of parasitized eggs and emerged F_1 individuals when a great number of host eggs was offered ($F=71$, $df = 5, 84$, $P<0.05$; $F=116$, $df = 5, 84$, $P<0.05$, respectively). The mean numbers of parasitized eggs started to increase significantly when 10 *C. pomonella* eggs were offered to *T. cacoeciae*. No significant difference was noticed in the mean number of parasitized eggs of *T. cacoeciae* when the numbers host eggs was increased to 15 eggs. The mean numbers of F_1 progeny of *T. cacoeciae* was increased significantly when the numbers of *C. pomonella* eggs was increased to 15 eggs. While in *T. principium*, the mean numbers of parasitized eggs, and F_1 progeny were increased by increasing the number of host eggs. The highest value of parasitized host eggs, and F_1 progeny was obtained when a female was exposed to 15 *C. pomonella* eggs (Table 1). Regardless of host density, significant differences were showed in the mean numbers of

parasitized eggs, and emerged F_1 progeny between *T. principium* and *T. cacoeciae*. The result reveals that the mean numbers of parasitized eggs by *T. principium* was

significantly higher than that of *T. cacoeciae*. Whereas, the mean numbers of F_1 progeny of *T. principium* was significantly lower than that of *T. cacoeciae* (Table 1).

Table 1. The effect of host density on the number of parasitized codling moth eggs and number of emerged F_1 progeny of *T. cacoeciae*, *T. principium*.

Number of host eggs/group ^a	Mean no. of parasitized eggs (\pm SE)		Mean no. of F_1 progeny (\pm SE)	
	<i>T. cacoeciae</i>	<i>T. principium</i>	<i>T. cacoeciae</i>	<i>T. principium</i>
5	4 \pm 0.06b*	4.8 \pm 0.13c*	8 \pm 0.13b*	5.5 \pm 0.13c*
10	6 \pm 0.13a*	8 \pm 0.34b*	8 \pm 0.13b*	7 \pm 0.11b*
15	6.6 \pm 0.11a*	9 \pm 0.30a*	9 \pm 0.00a*	8 \pm 0.13a*

Means within a column followed by the same letters are not statistically different at $P < 0.05$ level (Fisher LSD).

*Means within a row are statistically different at the $P < 0.05$ level (Fisher LSD).

^a There were 15 replicates (parasitoid female) per group.

3.2. Effect of Host Density on *T. principium* and *T. cacoeciae* Fecundity

The results show that there was a significant effect of host density on the fecundity of *T. principium* and *T. cacoeciae* females ($F = 77.5$, $df = 5, 84$, $P < 0.05$). When *T. principium* and *T. cacoeciae* females were exposed to 5 eggs of *C. pomonella*, the mean fecundity was significantly

lower than those exposed to 10 and 15 host eggs (Table 2). There was no significant difference in the fecundity between *T. principium* and *T. cacoeciae* when their females were exposed to 5 and 10 codling moth eggs. However, the mean fecundity of *T. principium* females was significantly higher than that of *T. cacoeciae* when 15 *C. pomonella* eggs were offered (Table 2).

Table 2. The effect of codling moth eggs density on fecundity of *T. cacoeciae* and *T. principium* females.

Number of host eggs/group ^a	Mean fecundity /female (\pm SE)	
	<i>T. cacoeciae</i>	<i>T. principium</i>
5	12 \pm 0.35c	11.5 \pm 0.37c
10	14 \pm 0.38b	16 \pm 0.33b
15	16 \pm 0.35a*	20 \pm 0.38a*

Means within a column followed by the same letters are not statistically different at the $P < 0.05$ level (Fisher LSD).

*Means within a row are statistically different at the $P < 0.05$ level (Fisher LSD).

^a There were 15 replicates (parasitoid female) per group.

3.3. Effect of Host Density on the Superparasitism of *T. principium* and *T. cacoeciae*

The result demonstrates that the percentage of superparasitized eggs by *T. cacoeciae* and *T. principium* was directly influenced by the host density (Table 3). The percentage of superparasitized eggs was significantly reduced, when a high number of host eggs was offered. In contrast, the percentages of unparasitized and once

parasitized eggs (egg with one parasitoid larva) were significantly increased by increasing the host density (Table 3). Regardless of the host density, there were no significant differences between *T. principium* and *T. cacoeciae* in the percentage of superparasitized and once parasitized eggs (Table 3). Significant difference in the percentage of unparasitized eggs was observed between *T. principium* and *T. cacoeciae* (Table 3).

Table 3. Effect of host density on percentages of superparasitized, once parasitized and unparasitized codling moth eggs by *T. cacoeciae* and *T. principium* females.

Number of host eggs/group ^a	Superparasitized eggs%		Once parasitized eggs%		unparasitized eggs%	
	<i>T. cacoeciae</i>	<i>T. principium</i>	<i>T. cacoeciae</i>	<i>T. principium</i>	<i>T. cacoeciae</i>	<i>T. principium</i>
5	97a	96a	3c	4c	19c*	4c*
10	84b	87b	16b	13b	33b*	23b*
15	74c	80c	26a	20a	65a*	39a*

Percentages, in a column, followed by the same letter are not significantly different $P < 0.05$ (Analysis of proportion).

* Percentages, in a row, are significantly different $P < 0.05$ (Analysis of proportion).

^a There were 15 replicates per group.

It should be mentioned that our observation shows that all F_1 progeny of *T. principium* and *T. cacoeciae* were produced from superparasitized eggs whereas none of once parasitized eggs was able to hatch and produce progeny, regardless of the host density. When the number of parasitoid larvae was decreased, the numbers of emerged offspring was reduced. Our observation illustrates that the highest number of *T.*

principium and *T. cacoeciae* larvae per *C. pomonella* egg was 5 larvae (Figures 1 and 2). Figure 1 indicates that the percentage of once and twice parasitized host eggs was significantly increased by increasing the host density. Whereas, the percentage host eggs with 3, 4 and 5 of *T. cacoeciae* larvae was significantly reduced by increasing the host density (Figure 1). Most of *C. pomonella* eggs had 3

parasitoid larvae, when *T. cacoeciae* female was exposed to 5 eggs. Whereas, the percentage host eggs with 2 larvae was the highest, when 10 and 15 host eggs were offered. It should be mentioned that when *T. cacoeciae* female was exposed to 5 *C. pomonella* eggs the percentage of once parasitized eggs was significantly lower than superparasitized eggs (Figure 1). In case of *T. principium*, the percentage host egg with 2

parasitoid larvae was significantly higher than all other types of eggs, regardless of the host density (Figure 2). Percentage host eggs with 3 larvae did not differ significantly by increasing the host density. None of *C. pomonella* eggs had 4 larvae when *T. principium* female was exposed to 10 and 15 codling moth eggs. Percentage host eggs with 5 larval parasitoids did not changed (1%) at various host densities.

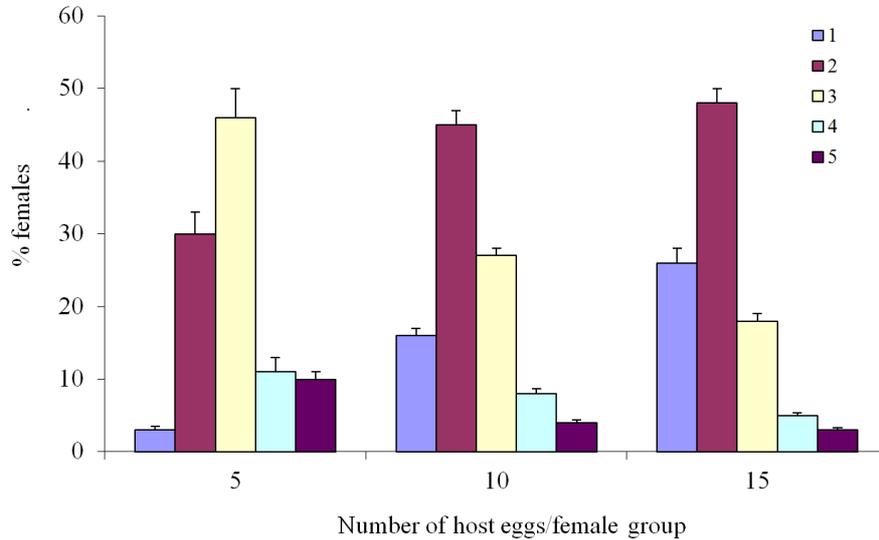


Figure 1. Percentage host eggs with different number of parasitoid larvae when a female of *T. cacoeciae* was exposed to various number of codling moth eggs for 24h.

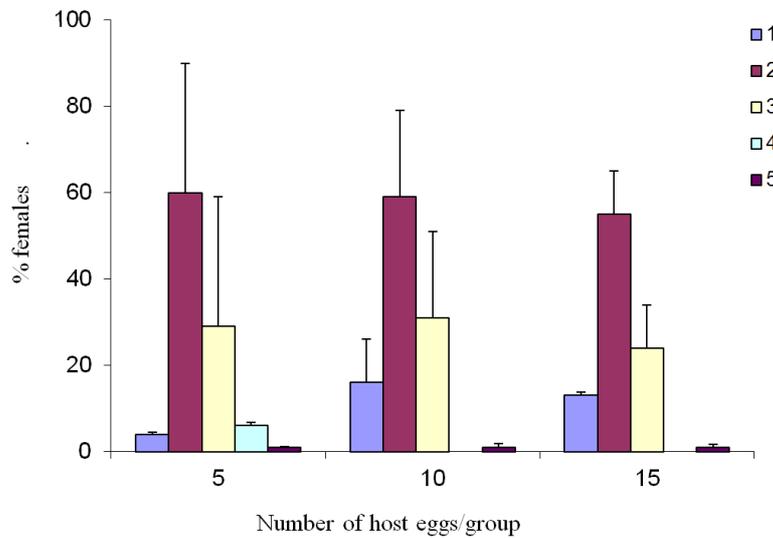


Figure 2. Percentage host eggs with different parasitoid larva when a female of *T. principium* was exposed to various numbers of codling moth eggs for 24h.

3.4. Effect of Host Eggs Viability on Superparasitism

When *T. principium* and *T. cacoeciae* females were exposed to sterile and fertile *C. pomonella* eggs, the percentage of superparasitism of sterile eggs was significantly higher than that of fertile eggs (Figure 3). The results revealed that the percentage of superparasitized eggs by *T. cacoeciae* was significantly lower than of *T. principium*, regardless of the viability of *C. pomonella* eggs (Figure 3). For both species, the percentage sterile host eggs host with 1 parasitoid larva was significantly lower than those with 2 and 3 larvae but it was higher than those with 4

and 5 larvae. The result shows that the highest percentage of sterile eggs was those with 2 larvae of *T. principium* and *T. cacoeciae* (Table 4). When *T. principium* and *T. cacoeciae* females were exposed to sterile and fertile *C. pomonella* eggs, the percentage of sterile eggs with 1 larva was significantly lower than that in fertile eggs. However, the percentage of sterile eggs with 3 parasitoid larvae was significantly higher than that in fertile eggs. There were no significant differences between fertile and sterile host eggs in percentages eggs with 2, 4 and 5 larvae, regardless of parasitoid species (Table 4).

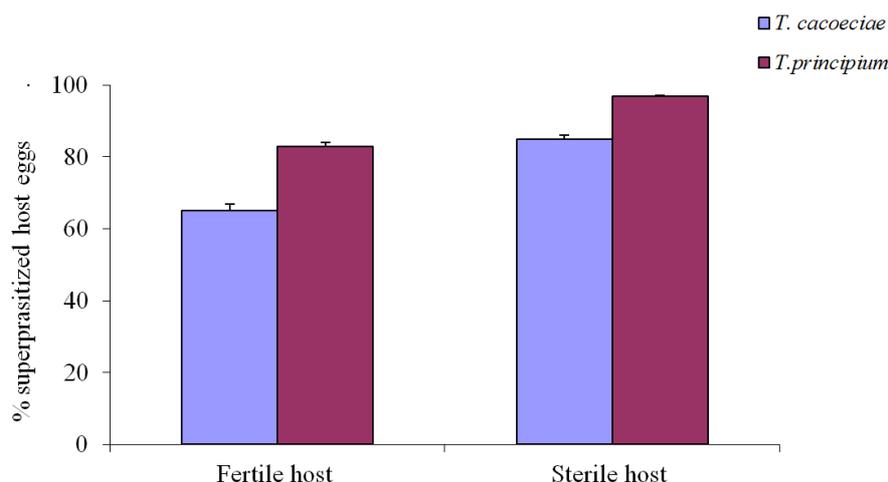


Figure 3. Percentage superparasitized eggs when a female of *T. cacoeciae* and *T. principium* attacked fertile and sterile codling moth eggs.

Table 4. Percentage host eggs with various number of parasitoid larvae when a female of *T. cacoeciae* and *T. principium* was exposed to fertile and sterile eggs of codling moth.

No. parasitoid larvae Per host egg	<i>T. cacoeciae</i>		<i>T. principium</i>	
	Fertile host	Sterile host	Fertile host	Sterile host
1	35a*	15c*	17c*	3c*
2	36a	40a	48a	48a
3	19b*	32b*	29b*	42a*
4	10c	9d	4d	7b
5	0	4e	2d	0

Percentages within a column followed by the same letters are not statistically different at the $P < 0.05$ level (Normal approximation test).

* Percentages within a row, for each species, are statistically different at the $P < 0.05$ level (Normal approximation test).

4. Discussion

Host characteristics including size, chorion thickness, nutritional content, age, egg distribution and viability directly influence the parasitism as well as the number, quality and sex ratio of parasitoids [14], [27], [28], [30]. Previous studies showed that parasitism is often affected by host density [21]. However, an aggregative response of parasitoid adults to host density does not necessarily lead to a density-dependent pattern of parasitism in host population. Parasitism patterns of parasitoids range from inverse density dependence, through density independence, to positive density dependence [8], [21]. Our results demonstrated that when *T. principium* and *T. cacoeciae* were exposed to *C. pomonella* eggs, the number of parasitized host eggs was increased by increasing the number of host eggs (Table 1). Therefore, both parasitoids showed a positive response to host density. Such result was more pronounced in *T. principium*. Correspondingly, Previous investigations reported that parasitoids were searching and ovipositing more frequently on apple trees with higher host density

[25]. Contrary, it was reported that when *Choristoneura Parallela* (Robinson) eggs were attacked by *T. minutum* Riley the parasitized egg masses had fewer number of eggs than unparasitized egg masses [41]. It has been founded that the effectiveness of *T. cacoeciae* against *Lobesia botrana* Denis & Schiffermuller is reduced with increasing host density. However, the effect of host

density was not significant on the effectiveness of *T. cacoeciae* against *E. kuehniella* [34]. Like the number of parasitized eggs, the number of emerged F_1 progeny of *T. principium* and *T. cacoeciae* was positively influenced by host density (Table 1).

It is well known that an important factor of the potential success of a parasitoid is its fecundity and the numbers of progeny a female can produce. Our results indicated that there was a strong relationship between the average numbers of eggs laid by *T. principium* and *T. cacoeciae* and the density of *C. pomonella* eggs. Therefore, these parasitoids were able to deposit more eggs when the host density was increased, especially in *T. principium* (Table 2). The *Trichogramma* adjust their progeny according to host density, laying more eggs when host density was low [39]. The ability of a parasitoid female to attack host eggs is related to the availability of mature eggs in this female. Therefore, the numbers of parasitized host eggs by a parasitoid female is limited by the availability of mature eggs. Previous investigations have been demonstrated that in some *Trichogramma* species females emerge with fully mature eggs. It is known that *T. principium* and *T. cacoeciae* are partially pro-ovigenic and synovigenic [40], [47]. Pro-ovigenic female parasitoid emerges with full lifetime complement of mature eggs. Whereas, synovigenic female parasitoid emerges with a supply of mature eggs [20]. When such female gets suitable nutrients, by feeding on host, further eggs can be matured [7]. In such situation, the egg

maturation in *Trichogramma* females continues throughout their lifetime consequently the final number of eggs produced can be more than the initial one [11]. Our result indicated the average number of eggs laid by *T. principium* and *T. cacoeciae* ranged from 12-16 and from 11.5-20 eggs, respectively, depending on host density provided during the first 24 h (Table 2). It was likely that the exposure to high number of *C. pomonella* eggs stimulated the females of parasitoids to mature more eggs. Therefore, *T. principium* and *T. cacoeciae* matured additional eggs when they were exposed to high number of host eggs. The deposition of a single egg (or a clutch of eggs) by a parasitoid female in a host egg already parasitized, by same female or conspecific females, results in superparasitism behavior. Our study indicated the occurrence of this behavior in both tested parasitoids (Table 3). Therefore, superparasitism behavior is quite dominant in *T. principium* and *T. cacoeciae*. Similar behavior was reported in many species' parasitoids such as: *Eupelmus vuilleti*, *T. atopovirilia*, *T. pretiosum*, *T. evanescens*, *Diachasmimorpha longicaudata*, *T. pretiosum*, *Chelonus sp. nr. curvimaculatus* [12], [15], [22], [32], [33]. Many species are able to distinguish between unparasitized and parasitized hosts [4]. Our results illustrated that both parasitoids tend to show a preference for certain host eggs and parasitized them repeatedly, this led in many instances to a host egg contained multiple parasitoid eggs (Figures 1 and 2). Therefore, *T. principium* and *T. cacoeciae* females were willing to parasitize already parasitized host eggs, by same female, although unparasitized eggs were available (Table 3). Thus, this fact suggests that such superparasitism behavior is an intrinsic characteristic of these species. Corresponding results were obtained in *Cotesia glomerata*, *T. atopovirilia* and *T. pretiosum*, [13], [19].

Moreover, our results indicated that the percentage of *C. pomonella* eggs that were superparasitized was higher than once parasitized eggs (Table 3). Whereas, the percentage of host eggs that were superparasitized by *C. sp. nr. curvimaculatus* was just about equal to that were parasitized once [37].

In *T. evanescens* Westwood and *C. glomerata* the superparasitism usually leads to larger brood sizes with smaller individual offspring [46]. Contrary, in *C. flavipes* superparasitism did not result in larger brood size due to higher immature mortality in superparasitized hosts [16]. Previous studies showed that in superparasitized eggs by *C. sp. nr. curvimaculatus* only one parasitoid complete development in a host egg. Our observations showed that all the emerged F_1 progeny of *T. principium* and *T. cacoeciae* were from superparasitized eggs, whereas once parasitized eggs were unable to produce any progeny. Thus, progeny dead may attributed to presence more of yolk in host egg and parasitoid's larvae has not pupated until all the yolk has been consumed [26]. Therefore, the superparasitism behavior has in some species including *T. principium* and *T. cacoeciae* positive effects in terms of the number of offspring. Generally, superparasitism behavior of *Trichogramma* females is affected by several factors such as the quality and quantity of nutrients available in host, which depend on host

size, host age, and parasitoid density [16], [46]. In fact, studies on superparasitism behavior in relation with host density have been rarely carried out.

In current study, there was a negative relationship between the host density and the percentage of superparasitized host eggs. The superparasitism varied according to host density. So, when host density was increased from 5 to 15 eggs the superparasitism was reduced from 97 to 74% in *T. cacoeciae* and from 96 to 80% in *T. principium* (Table 3). Therefore, the superparasitism was recorded even when a good number of host eggs was present for one *T. principium* and *T. cacoeciae* female. Therefore, providing parasitoids' female with excess host eggs did not prohibit the occurrence of superparasitism. Thus, host density appeared to be a strong factor in governing the superparasitism in *T. principium* and *T. cacoeciae*. Hentz et al. [22] found that as the host density increased the percentage of superparasitized eggs by *C. sp. Nr. curvimaculatus* was slightly decreased. However, Bezemer and Mills [3] found that the number of eggs laid by *Mastrus ridibundus* (Gravenhorst) per host remained constant across various host density. Another essential factor which could affect superparasitism behavior of the parasitoid species is the host viability. It was reported that the percentage parasitized false codling moth eggs that produced more than one *T. cryptophlebiae* Nagaraja adults was significantly influenced by host egg fertility [9]. Our current non-choice laboratory experiments revealed that *T. principium* and *T. cacoeciae* did not discriminate between fertile and sterile eggs, so both types of eggs served as suitable hosts for superparasitism. However, the percentage of superparasitism was higher when sterile *C. pomonella* eggs were offered, especially in case of *T. principium* females (Figure 3). Irradiation can suppress the immune system of F_1 eggs [23]; therefore, they became more suitable for superparasitism like fertile eggs, most of sterile host eggs contained 2-3 parasitoid larvae and only few of them had 4-5 larvae (Table 4). The forgoing study could add significant information to our understanding of the biology of *T. principium* and *T. cacoeciae*, such information is needed for their successful establishment in apple field. Our study demonstrated that these parasitoids performed well in high or low host density. If host density is high the number of parasitized host eggs will be increased, while in low host density the superparasitism will be increased. Consequently, both situations will be good for parasitoid population. Additionally, our results proved that *T. principium* and *T. cacoeciae* can successfully work when they are combined with sterile insect technique for management of codling moth population.

5. Conclusion

The key finding in this work emphasis on the increased correlation between *T. principium* and *T. cacoeciae* parasitizing with the number and density of *C. pomonella* eggs. In addition, the number of *T. principium* and *T. cacoeciae* eggs and its emerged F_1 progeny positively

influenced by *C. pomonella* eggs density. Our work also proved that *T. principium* and *T. cacoeciae* behavior can be described as superparasitism dominant that increased in the host mature eggs and decreased with the host eggs density increase regardless the fertility. Eggs from irradiated host enhanced the superparasitism phenomena that may attribute to immune suppression in the laid eggs.

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