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Preference and performance of *Trichogramma embryophagum* when parasitizing *Cydia pomonella* and two stored-product moths

Fatemeh HAGHI GOLBAGHI[1], Seyed Hosein GOLDANSAZ[2], Shima RAHMANI[3], Mohammad Reza ATTARAN[4]
[1]*Department of Entomology and Plant Pathology, College of Aboureyhan, University of Tehran, Iran*
[2]*Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
[3]*Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[4]*Biological Control Research Department, Iranian Research Institute of Plant Protection, Tehran, Iran*

Abstract

Trichogramma species are polyphagous biocontrol agents most commonly used against the egg stage of various groups of insect pests. Host preference is a key trait in parasitoids and it is important to characterize the main hosts of parasitoids before using them in biocontrol programs. In the present study, the parasitism of *Cydia pomonella* (L.) (Lepidoptera Tortricidae) by *Trichogramma embryophagum* (Hartig) (Hymenoptera Trichogrammatidae) (a parasitoid species commonly distributed in Europe and Central Asia against the pest) was compared with parasitism on *Sitotroga cerealella* Oliver (Lepidoptera Gelechiidae) and *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) using choice and no-choice experiments. In the no-choice test, parasitism on *C. pomonella* was higher than on *S. cerealella* and *E. kuehniella*. In the parasitoid's early life, parasitism on *C. pomonella* was four times greater than on the two other hosts. In addition, parasitoid emergence from *C. pomonella* was also higher than from the two other hosts on the first day of parasitism. In the choice test, *C. pomonella* eggs were preferred as hosts (vs. *S. cerealella* and *E. kuehniella*) during the first two days of the parasitism assay as well as during total parasitism assay overall. Furthermore, the production of females was higher in *C. pomonella* than in the other hosts (early in parasitism and during whole parasitism assay, both in non-choice and choice tests). Female longevity was greater on *C. pomonella* than on *S. cerealella* and *E. kuehniella* during the no-choice test and was even lower during the choice test. Female abnormality and preadult mortality were more frequent in *S. cerealella* and *E. kuehniella* than in *C. pomonella* during the choice test while no significant difference was detected for these traits when hosts were presented in the no-choice test. Overall, the results indicated that *C. pomonella* is a preferable host for *T. embryophagum*; these results may be useful for further development of biocontrol programs relying on *T. embryophagum*.

Key words: parasitism, host preference, parasitoid specialization, no choice test, choice test.

Introduction

The codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae) is one of the most important pests of apple and pear around the world, capable of damaging up to 95% of these products. Quince, peach, walnut, and plum are other fruit crops that are damaged by this insect (Ismail and Albittar, 2015; Basheer *et al*., 2016). Use of natural enemies such as pathogens, nematodes, predatory mites, insect predators and parasitoids is one strategy in managing codling moth populations (Blomefield and Giliomee, 2012; Ismail and Albittar, 2015; Ksentini and Herz, 2019). Among parasitoids, several Hymenopteran families have been reported, with the majority of species belonging to Braconidae and Ichneumonidae (Velcheva and Atanassov, 2016). In the family Trichogrammatidae, one species, *Trichogramma cacoeciae* Marchal (Hymenoptera Trichogrammatidae) has been considered as a biocontrol agent of codling moth in several papers (Botto and Glaz, 2010; Ismail and Albittar, 2015; Basheer *et al*., 2016; Ksentini and Herz, 2019). In Denmark, alongside *T. cacoeciae*, mass release of *Trichogramma evanescens* Westwood was examined for reduction of *C. pomonella* (Sigsgaard *et al*., 2017). However, according to the Sigsgaard *et al.* (2017), further studies are required to assess the suitability of *Trichogramma* species against *C. pomonella* eggs.

For more than 40 years, *Trichogramma* spp. have been used in many countries as biological control agents against various insect pests, mostly lepidopterans, on fruit trees and on crops such as vegetables, cotton, corn, rice, and sugarcane (Smith, 1996; Poorjavad *et al*., 2018). Indeed, *Trichogramma* species are one of the most commonly used groups of natural enemies. They can be cultured relatively easily and are able to manage the host before crop damage occurs (Smith, 1996; Yang *et al*., 2016). However, most parasitoids especially species of the *Trichogramma* genus possess different host preferences and performances when facing their variable hosts (Godfray, 1994; Mansfield and Mills, 2004; Roriz *et al*., 2006; Desneux *et al*., 2009; 2012; Chailleux *et al*., 2012; 2013a; Paraiso *et al*., 2013). Thus, it is essential to evaluate host ranges of parasitoids such as *Trichogramma* for possible use in biological control programs (Paraiso *et al*., 2013). Parasitism preference and fitness of parasitoid offspring such as demographic traits, larval survivorship, growth and development time, emergence rate, adult weight, and fecundity are the factors affected by the host quality (Godfray, 1994; Roriz *et al*., 2006; Iranipour *et al*., 2010; Pizzol *et al*., 2010; 2012; Chailleux *et al*., 2013b; Yang *et al*., 2016; Zhang *et al*., 2017).

It is necessary to find the host range of a parasitoid to evaluate the potential worth of a biological control agent (Mansfield and Mills, 2004). No-choice and choice tests are the most common methodologies utilized for host range evaluation (Van Driesche and Murray, 2004). If the agent is not monophagous according to a no-choice test, then a choice test is necessary to find the host preference. Choice tests are employed to show if one host is preferred over other physiologically acceptable hosts, or whether all acceptable hosts are equally selected (Mansfield and Mills, 2004). Selection of the optimal host occurs through host acceptance (preference) and the appropriateness of the host for growth and development of the parasitoid (performance) (Godfray *et al*., 1994). Under controlled laboratory conditions, host preference is an important step in assessment of biological control agents (Mansfield and Mills, 2004).

In this study, both of the common test types (no-choice and choice tests) were used to more accurately estimate the parasitoid host range (Murray *et al.*, 2010) with emphasis on *C. pomonella* as an important insect pest. However, such methods have limitations. For example, in no-choice tests, host ranges are often misjudged while in choice tests, false negative or false positive results may occur due to the unknown reactions to mixed host cues (Van Driesche and Murray, 2004; Murray *et al.*, 2010).

Trichogramma embryophagum (Hartig) (Hymenoptera Trichogrammatidae), as other *Trichogramma* species, is commonly used for augmentative biological control of many insect pests in agricultural and forestry environment, while also representing an important component of the natural ecological community (Maini and Mosti, 1988; Ivanov and Reznik, 2008). Nevertheless, there is little ecological information about this species (Voinovich *et al.*, 2003, Ivanov and Reznik, 2008; Reznik *et al.*, 2011; Poorjavad *et al.*, 2014) and there have been few data about its host suitability and preference so far (Hassan *et al*., 1988; Hassan, 1989; Schöller *et al*., 1996; Reznik and Umarova, 1990). According to the previous experiments, this wasp can parasitize several insect pests such as *C. pomonella* (Hassan *et al*., 1988), *Ephestia kuehniella* Zeller, *Ephestia elutella* (Hubner) (Schöller *et al*., 1996), *Sitotroga cerealella* Oliver (Reznik and Umarova, 1990), *Adoxophyes orana* Fischer von Roslerstamm (Hassan *et al*., 1988), and *Ectomyelois ceratoniae* Zeller (Poorjavad *et al*., 2014). In this study, using choice and no-choice tests, the efficiency of *T. embryophagum* on *C. pomonella*, as a pest, was evaluated and compared to *S. cerealella* and *E. kuehniella*, as two common storage insect pests, whose eggs are a usual factitious host for rearing of Trichogrammatidae.

Materials and methods

Parasitoid rearing

The original population of *T. embryophagum* was collected from apple orchards of the Damavand region (Tehran province, Iran) and the species was identified by Jamasb Nozari, Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. *T. embryophagum* were transferred to the laboratory and reared on *E. kuehniella* eggs during 15 generations under controlled conditions (25 ± 1 °C, 50 ± 5% RH and 16L:8D photoperiod. The parasitized eggs were transferred into glass tubes (7 cm height, 1 cm diameter) under the above conditions until the adult wasps emerged. Newly emerged parasitoid wasps were fed with a 35% honey solution (McDougall and Mills, 1997) on the inside wall of the tubes.

Hosts rearing

Three lepidopteran species were used in this study as hosts: *C. pomonella*, *S. cerealella* and *E. kuehniella*. *C. pomonella* larvae and pupae were collected from apple orchard of Jaban village near of Damavand region. The codling moths were kept in a growth chamber under conditions of 26 ± 1 °C, 50 ± 5% RH and 16L:8D. Larvae were reared on an artificial diet suggested by Hilker (1989) for several generations before examinations. A piece of cotton soaked into a mixture of water and honey (10%) was also used for feeding the adult moth. *S. cerealella* was reared in the laboratory at 25 ± 1°C, 70 ± 5% RH and 16L:8D. Barley seed was supplied as food according to Hassan (1981). *E. kuehniella* was obtained from Chemical Ecology laboratory in Department of Plant Protection at the University of Tehran. The larvae were reared on an artificial diet (43.5% wheat flour, 3.0% yeast and 10% wheat bran) according to Lima *et al*. (2000) at 27 ± 1 °C, 60 ± 5% RH and 16L:8D.

No-choice test

For evaluating the fitness and longevity, 50 female wasps mated during 24 hours were placed individually into the experimental units (glass tubes: 1 cm diameter, 10 cm length) with 40 host eggs laid within a period of 24 hours. These eggs were glued by a colourless and odourless paste on the surface of cards. The eggs were replaced by new ones every day. The experiment was conducted at 25 ± 1 °C, 50 ± 5% RH and 16L:8D. Providing new eggs for the parasitoids continued until the death of all wasps. The black eggs were counted as preadult mortality, after the fifth day of the wasp's life span. The longevity of the adult parasitoids was measured by counting the live and dead insects every day. In addition, deformity of progeny wings as emerged female abnormality (Poorjavad *et al*., 2014), sex ratio, and emergence rate were also determined (Mansfield and Mills, 2004).

Choice test

In the choice test, 20 eggs from each host species were glued on one card and put into the glass tubes as experimental units. The egg cards in every tube were exposed to one mated female of *T. embryophagum* which had emerged and mated 24 hours previously. The egg cards were replaced every day by new ones, and the experiment was replicated 30 times. After 24 hours, each card was removed and the card sections carrying 20 eggs belonging to each different hosts were cut and placed separately into new tubes. All parameters measured in the no-choice test (parasitism rate, adult female longevity, sex ratio, preadult mortality, abnormality and emergence rate) were also assessed in this experiment (Yokomi and Tang, 1995).

Statistical analysis

The normality of data was tested initially in MATLAB software.

If it was necessary (p value was lower than 0.05), the formula of DEGREES(ASIN(SQRT(Number/100))) in Excel was used to normalize the data.

General linear models (PROC GLM, SAS Institute, 2003) and comparison of means using Duncan's multiple range test (DMRT) were conducted in order to determine the differences in biological parameters in choice and no-choice tests (SAS Institute, 2003). The significant level was P < 0.05.

Results

No-choice test

Table 1 reports the number of *C. pomonella*, *S. cerealella* and *E. kuehniella* eggs parasitized by *T. embryophagum* during the first five days of parasitism in no-choice experiments. The number of eggs parasitized showed significant changes only during the first day ($F_{2, 147}$ = 43.52; P < 0.0001). Also, the number of parasitized eggs of *C. pomonella* during the entire five days of development was greater than that of *S. cerealella* and *E. kuehniella* ($F_{2, 147}$ = 30.00; P < 0.0001) (table 1). Thus, *C. pomonella* was the suitable host followed by *S. cerealella* and *E. kuehniella*, respectively. Finally, there was no significant difference in preadult mortality of the parasitoids on the three host eggs ($F_{2, 147}$ = 0.7; P < 0.49) (table 1).

T. embryophagum female longevity on *C. pomonella* eggs was longer than that of the other hosts (figure 1).

The number of females emerging in the no-choice test showed significant differences between the three host egg species on the first day only ($F_{2, 147}$ = 29.45; P < 0.0001) (table 2). The emergence of females from *C. pomonella* throughout the five days was approximately 1.5 times greater than that of the other two hosts ($F_{2, 147}$ = 15.02; P < 0.0001) (table 2). On the other hand,

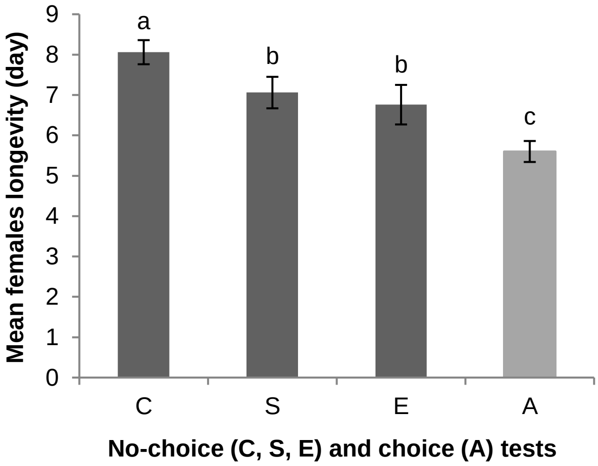

Figure 1. Mean *T. embryophagum* females longevity in days (± SE) in no-choice (C: *C. pomonella*, S: *S. cerealella* and E: *E. kuehniella*) and choice (A) tests. Bars with the same letters are not statistically different by Duncan multiple range test (at *P* < 0.05 level).

Table 1. No-choice test: number of eggs parasitized by *T. embryophagum* on *C. pomonella*, *S. cerealella* and *E. kuehniella* during the first five days of parasitization, total period of parasitization, and percentage of preadult mortality. Means followed by the same letter, across columns, are not significantly different from one another at *P* < 0.05 (GLM, Duncan's multiple range test).

Hosts	Mean number of parasitized eggs (± SEM)						Percentage of preadult mortality (± SEM)
	1st day	2nd day	3rd day	4th day	5th day	Total	
C. pomonella	17.86 ± 0.77 a	8.6 ± 0.71a	5.87 ± 0.44a	3.42 ± 0.25a	2.67 ± 0.24a	38.02 ± 1.1a	5.98 ± 1.18a
S. cerealella	7.82 ± 1.07b	8.53 ± 0.81a	4.14 ± 0.47a	2.88 ± 0.42a	2.73 ± 0.26a	25.0 ± 1.39b	5.61 ± 0.91a
E. kuehniella	5.48 ± 1.11b	7.87 ± 1.1a	4.67 ± 0.65a	2.52 ± 0.32a	2.94 ± 0.44a	22.2 ± 2.04b	7.88 ± 2.01a
P	<0.0001	<0.819	<0.07	<0.164	<0.82	<0.0001	0.49
F	43.52	0.20	37.72	1.83	0.2	30.00	0.70
df	2,147	2,145	2,142	2,133	2,123	2,147	2, 147

Table 2. No-choice test: emergence of *T. embryophagum* females from *C. pomonella*, *S. cerealella* and *E. kuehniella* eggs, during the first five days of parasitization and percentage of female abnormality. Means followed by the same letter, across columns, are not significantly different from one another at *P* < 0.05 (GLM, Duncan's multiple range test).

Hosts	Mean parasitoid female emergence (± SEM)						Percentage of female abnormality (± SEM)
	1st day	2nd day	3rd day	4th day	5th day	Total	
C. pomonella	11.08 ± 0.64a	5.16 ± 0.51a	3.18 ± 0.32a	1.79 ± 0.22a	0.93 ± 0.22a	21.98 ± 1.02a	3.03 ± 0.72a
S. cerealella	5.16 ± 0.75b	5.67 ± 0.59a	2.66 ± 0.33a	1.55 ± 0.17a	1.45 ± 0.16a	15.90 ± 1.09b	3.83 ± 0.61a
E. kuehniella	3.66 ± 0.76b	4.85 ± 0.69a	2.97 ± 0.48a	1.35 ± 0.34a	1.17 ± 0.32a	13.4 ± 1.28b	1.77 ± 0.82a
P	<0.0001	<0.64	<0.64	<0.48	<0.29	<0.0001	0.14
F	29.45	0.44	0.44	0.72	1.22	15.02	1.98
df	2,147	2,145	2,143	2,133	2,124	2,147	2, 147

female abnormality in the emerged females did not showed any significant difference between the three host eggs ($F_{2, 147}$ = 1.98; P < 0.14) (table 2).

There were significant differences in the sex ratio (number of females / total individuals) of the emerged parasitoids among all three hosts from the first to the seventh day (table 3). In *C. pomonella*, more females than males emerged during the first four days. However, in *S. cerealella* the number of females emerged was greater than that of males on the second and third days; in *E. kuehniella*, this ratio was greater on the second, third, and fourth days (table 3).

Choice test

The results of choice test revealed that the parasitism mean of *T. embryophagum* on the three different egg hosts during the first three days differed significantly (P < 0.001). On the first day, *C. pomonella* was parasitized 4.4 times more than the two other hosts (table 4). The number of eggs parasitized during the total of 5 days of development period on *C. pomonella* was also more than that of *S. cerealella* and *E. kuehniella* ($F_{2, 87}$ = 38.55; P < 0.0001) (table 4). Also, preadult mortality on *C. pomonella* eggs was significantly greater than on the other host eggs ($F_{2, 87}$ = 5.74; P < 0.0005) (table 4).

Female longevity of *T. embryophagum* in the choice test was shorter than in the no-choice experiment where the wasps were not permitted to choose their hosts (figure 1).

The number of emerged females in the choice test during the five days showed significant differences between the three host eggs on the first ($F_{2, 87}$ = 15.73; P < 0.0001), second ($F_{2, 87}$ = 11.24; P < 0.0001), and third days ($F_{2, 87}$ = 4.13; P < 0.019) (table 5). Female emergence in *C. pomonella* was greater than in the other treatments. Furthermore, *T. embryophagum* female abnormality on *C. pomonella* eggs was significantly greater than on *S. cerealella* and *E. kuehniella* eggs ($F_{2, 87}$ = 3.50; P < 0.032) (table 5).

The sex ratio (number of females / total individuals) of the parasitoid reared on different hosts is presented in table 6. The percentage of females that emerged during the first three days was greater than that of males in all egg hosts, but the number of emerging females declined during the next days (table 6). There was no significant difference between three egg hosts (table 6).

Discussion

Although *Trichogramma* species were in some instances considered polyphagous, a great differences across the species with regards to biological parameters and the number of eggs parasitized under equal conditions

Table 3. No-choice test: sex ratio (%) *T. embryophagum* females, developed on *C. pomonella*, *S. cerealella* and *E. kuehniella* eggs. Percentage followed by the same letter, within the rows, are not significantly different from one another at *P* < 0.05 (GLM, Duncan's multiple range test).

Day	*C. pomonella*	*S. cerealella*	*E. kuehniella*
1	61.99 ± 2.2a	40.40 ± 5.1b	29.50 ± 5.2b
2	61.40 ± 3.0a	57.82 ± 4.3a	41.55 ± 4.9b
3	57.78 ± 4.5a	69.53 ± 4.8a	64.91 ± 5.1a
4	51.08 ± 5.4a	52.21 ± 6.8a	60.83 ± 6.06a
5	36.71 ± 5.3ab	47.65 ± 6.1a	29.63 ±5.9c
6	29.55 ± 5.5a	41.72 ± 7.3a	36.85 ±7.7a
7	18.12 ± 5.7b	40.57 ± 8.3a	20.53 ± 7.6ab
8	8.33 ± 5.1a	27.88 ± 8.5a	20.83 ± 11.4a
9	14.29 ± 9.7a	27.27 ± 14.1a	5.56 ± 5.5a
10	0a	16.67 ± 16.6a	0a
11	0	0	0
Whole days	30.84 ± 7.3a	38.33 ± 5.8a	31.01 ± 6.3a

Table 4. Choice test: the number of egg parasitized by *T. embryophagum* on *C. pomonella*, *S. cerealella* and *E. kuehniella* during the first five days of parasitization, total period of parasitization, and percentage of preadult mortality. Means followed by the same letter, across columns, are not significantly different from one another at *P* < 0.05 (GLM, Duncan's multiple range test).

Hosts	Mean number of parasitized eggs (± SEM)						Percentage of preadult mortality (± SEM)
	1st day	2nd day	3rd day	4th day	5th day	Total	
C. pomonella	4.26 ± 0.63a	5.4 ± 0.56a	4.66 ± 0.70a	1.42 ± 0.37a	1.34 ± 0.11a	17.83 ± 1.14a	10.09 ± 2.37a
S. cerealella	0.96 ± 0.26b	1.7 ± 0.24b	3.4 ± 0.66a	1 ± 0.22a	0.69 ± 0.40a	7.73 ± 1.02b	3.24 ± 1.60b
E. kuehniella	0.93 ± 0.27b	1.03 ± 0.51b	1.53 ± 0.35b	0.85 ± 0.23a	1.13 ± 0.51a	5.40 ± 1.02b	1.93 ± 1.34b
P	<0.0001	<0.0001	<0.0015	<0.34	<0.62	<0.0001	<0.0045
F	20.08	25.41	7.04	1.09	0.47	38.55	5.74
df	2,87	2,87	2,87	2,81	2,66	2, 87	2, 87

Table 5. Choice test: emergence of *T. embryophagum* females from *C. pomonella*, *S. cerealella* and *E. kuehniella* eggs during the first five days of parasitization and percentage of female abnormality. Means followed by the same letter, across columns, are not significantly different from one another at $P < 0.05$ (GLM, Duncan's multiple range test).

Hosts	Mean parasitoid female emergence (± SEM)						Percentage of female abnormality (± SEM)
	1st day	2nd day	3rd day	4th day	5th day	Total	
C. pomonella	2.9 ± 0.49a	3.16 ± 0.48a	2.5 ± 0.46a	0.71 ± 0.19a	0.39 ± 0.23a	9.67 ± 0.57a	3.80 ± 1.45a
S. cerealella	0.63 ± 0.18b	1.16 ± 0.42b	2.3 ± 0.49a	0.46 ± 0.14a	0.39 ± 0.30a	4.83 ± 0.35b	0.45 ± 0.45b
E. kuehniella	0.7 ± 0.2b	0.7 ± 0.20b	0.966 ± 0.41b	0.42 ± 0.14a	0.60 ± 0.33a	3.40 ± 0.088b	0.74 ± 075b
P	<0.0001	<0.0001	<0.019	<0.401	<0.83	<0.0001	<0.032
F	15.73	11.24	4.13	0.90	0.18	18.63	3.50
df	2, 87	2, 87	2, 87	2, 81	2, 66	2, 87	2, 87

Table 6. Choice test: sex ratio (%) *T. embryophagum* females, developed on *C. pomonella*, *S. cerealella* and *E. kuehniella* eggs. Percentage followed by the same letter, within the rows, are not significantly different from one another at $P < 0.05$ (GLM, Duncan's multiple range test).

Day	*C. pomonella*	*S. cerealella*	*E. kuehniella*
1	79.56 ± 5.4a	71.53 ± 10.8a	81.14 ± 8.3a
2	58.04 ± 5.4a	67.89 ± 9.2a	58.59 ± 11.5a
3	58.49 ± 7.0a	64.75 ± 6.8a	71.72 ± 8.2a
4	49.47 ± 9.3a	44.23 ± 10.1a	53.03 ± 13.1a
5	15.98 ± 6.5a	52.78 ± 19.8a	44.64 ± 13.8a
6	20.0 ± 14.4a	25.0 ± 25a	75.0 ± 25a
7	25 ± 25a	0a	50 ± 50a
8	0	0	0
Whole days	38.32 ± 9.5a	40.77 ± 10.3a	54.27 ± 8.9a

were found (Schöller and Hassan, 2001). Choice and no-choice tests (Yokomi and Tang, 1995; Mansfield and Mills, 2004; Roriz *et al.,* 2006; Yang *et al*., 2016; Mohammadpour *et al*., 2019) were used in this study for assessing host suitability and preferences of *T. embryophagum*, an important polyphagous biological control agent. *C. pomonella* eggs in both choice and no-choice tests were parasitized more often than were the eggs of *E. kuehniella* and *S. cerealella*. Also, *S. cerealella* (Reznik *et al*., 2002; 2011; Ivanov and Reznik, 2008) and *E. kuehniella* (Schöller *et al*., 1996; Pandir *et al*., 2013) eggs have been shown by many authors to be lepidopteran hosts for *T. embryophagum*. On the other hand, Schöller *et al*. (1996) showed that *E. kuehniella* was a less suitable host for *T. embryophagum* compared to *T. evanescens.* In this study, we observed that *C. pomonella* eggs were the most attractive hosts among the other species. There are several *Trichogramma* species with an ability to parasitize codling moth eggs (Hassan *et al*., 1988; Sigsgaard *et al*., 2017; Ksentini and Herz, 2019). Hassan (1989) demonstrated that *T. embryophagum* had a stronger preference to *C. pomonella* in comparison with *S. cerealella*. Elsewhere, Hassan *et al*. (1988) reported 50.06% reduction in *C. pomonella* damages after mass releasing of *T. embryophagum.* On the other hand, *A. orana* seemed a fairly more suitable host for this parasitoid due to reduction of its damage by 50.8%.

Egg parasitoids of phytophagous insects are capable of using a wide range of chemicals such as semiochemicals of host eggs for selecting their host (Fatouros *et al*., 2007). The odour of some host eggs may provide chemical cues with stronger attractants for *Trichogramma* spp. than the other hosts (Brotodjojo and Walter, 2006). An egg parasitoid female must determine where to search and how long to invest in a given microhabitat. Those decisions may have significant effects on foraging gains and parasitism rates (Gingras and Boivin, 2002).

There are several features such as host appearance, size, colour, texture and structure of egg surface, thickness of chorion and structural integrity which affect the host acceptance and parasitization (Vinson, 1976).

Hardness and thickness of the host egg chorion are one of the main restrictive factors for oviposition (Kivan and Kilic, 2002). *Trichogramma* spp. cannot penetrate the ovipositor into the chorion of larger host eggs thicker than 20 µm. In smaller host eggs, also the ratio of chorion width to volume of egg is a limiting factor in parasitization (Mansfield and Mills, 2002).

Consoli *et al.* (1999) found that the presence of hairy structures on egg clusters of *Spodoptera littoralis* (Boisduval) acts as a physical barrier, causing reduction in the parasitism rate of *Trichogramma* under both field and laboratory conditions. *S. cerealella* eggs are fusiform, 0.60 × 0.27 mm. The anterior end is truncate, but the posterior end is rounded. The reticulation consists of major longitudinal ridges connected by lesser cross ridges. On the other hand, *E. kuehniella* eggs are ellipsoid, 0.51 × 0.29 mm. Nearly all eggs show chorion folding at the anterior end and some of them have a nipple like appearance. Chorion surface is marked by a networked pattern of sinuous ridges joining together with tubercles (Arbogast *et al*., 1980). *C. pomonella* eggs are flat shaped, 1.35 × 1.05 mm. The upper part of the eggshell is convex and the surface finely structured. The lower side in contact with the substrate is flat (Fehrenbach *et al*., 1987).

Larger host eggs are distinguished more clearly than smaller host eggs at a greater distance by *Trichogramma* and this is why larger hosts are more likely to be preferred and selected (Bruins *et al*., 1994; Mansfield and Mills, 2004). Karamaouna and Copland (2000) showed that hosts larger than 1 mm were more appropriate for the growth and development of parasitoids due to their higher quality in comparison with other sizes. Heavier eggs of the host species support the development of a higher number of parasitoids per host egg (Roriz *et al*., 2006). In this study, *C. pomonella* eggs were larger and heavier than the other egg hosts (≈ 2 mm in diameter; 0.065 mg vs. ≈ 0.5 mm in width; 0.049 mg in *E. kuehniella* and ≈ 0.6 mm in diameter; 0.023 mg in *S. cerealella* eggs; unpublished data).

Trichogramma species use the egg colour to gather information on the age of a host egg, host characters and conditions, and the probability of previous parasitism (Lobdell *et al*., 2005). According to Lobdell *et al*. (2005) *Trichogramma ostriniae* Pang et Chen showed egg colour preference in the ranking of yellow > white > green > black. In this study, colour did not seem to have a major role in the host preference of *T. embryophagum*. It is because the *C. pomonella* egg was white and the *S. cerealella* and *E. kuehniella* eggs were yellowish white. However, *C. pomonella* eggs were more attractive to the wasp.

Many characteristics discussed above can influence the other traits of parasitoids such as their number, quality, and sex ratio (Hoffmann *et al*., 2001). Sex ratio in many species of parasitoid wasps has been influenced by the host size and quality. In most parasitoids, the number of female offspring coming out from large and high-quality hosts is higher than from smaller ones with a lower quality (King, 1987; Fox *et al*., 1990). In most wasp species, a larger number of female offspring is produced early in the life span. However, older female adults produce more sons due to depletion in their sperm supplies (King, 1987). According to the present results, sex ratio in both of the experiments was male-based after calculating the emergence of all males and females' post-appearance. Nevertheless, inspection on each day showed that throughout the first to third or fourth days, females appeared more than males.

In the no-choice test, females lived longer in *C. pomonella* eggs; also, more females emerged from *C. pomonella* eggs. These results suggest that the eggs of *C. pomonella* have probably a higher nutritional quality and are more suitable host for *T. embryophagum* as was found for another association (Huang and Gordh, 1998). On the other hand, only in the choice test, female abnormality and preadult mortality were greater in the wasps parasitizing *C. pomonella* eggs than in the other hosts with unknown reasons. In addition, preadult mortality and female abnormality in the no-choice test were more than in the choice test, but there was no significant change between hosts when the wasp could not choose their host species. Preadult mortality and abnormality rate were low in the choice test compared with another experiment, possibly because the parasitoids could select more appropriate hosts. Dahlan and Gordh (1998) found a larger number of abnormal wasps emerging from artificial eggs due to its lower quality. The food quality did not seem to have a critical role in this experiment. As shown previously, parasitized eggs, emergence of females, and sex ratio in this study changed during different days. As abnormality and preadult mortality were calculated for the total time, both parameters could be greater on the last days of the parasitoids' life time.

Keeping parasitoids in the laboratory for several generations can lead to changes in the performance of parasitoids in response to genetic variations in the hymenopteran colony or alteration in the laboratory environment (Hoffmann *et al*., 2001). On the other hand, under field conditions, many other factors may affect the parasitism rates. In the main habitat, host plant characters such as plant-derived semiochemicals (Mansfield and Mills, 2004) may have been responsible for parasitism efficiency (Romeis *et al*., 1999). Additionally, phytophagous physiological defences react against parasitoid attack in multifaceted ways (Godfray, 1994). Since *Trichogramma* release in the control of some pests has not always been efficient (Sigsgaard *et al*., 2017), we need to enhance our knowledge about biological characteristics and ecological traits of the tritrophic combinations of the host plant, the pest, and the parasitoid for a successful biological control (Smith, 1996).

In this study, *T. embryophagum* indicated preference to *C. pomonella* eggs as a proper host in comparison with *S. cerealella* and *E. kuehniella*. This feature should be remembered when this natural enemy is used as a powerful agent in pest management programs.

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Authors' addresses: Shima RAHMANI (corresponding author: shima.rahmani351@gmail.com), Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Iran; Fatemeh Haghi GOLBAGHI, Department of Entomology and Plant Pathology, College of Aboureyhan, University of Tehran, Tehran, Iran; Seyed Hosein GOLDANSAZ, Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran; Mohammad Reza ATTARAN, Biological Control Research Department, Iranian Research Institute of Plant Protection, Tehran, Iran.

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