Studying the effect of the insect host on some biological characteristics of the parasitoid *Trichogramma cacocaie* marchal under laboratory conditions

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Abstract

The parasitoid *Trichogramma cacoeciae* Marchal, 1927 was reared in an artificial environment on eggs of three insect hosts of the order Lepidoptera, namely the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), the carob moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) and the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae), in order to select the appropriate host for the quantitative laboratory rearing of this parasitoid. The results showed that the preferred host for rearing the parasitoid in the laboratory was the eggs of the codling moth, because the duration of the different stages and the complete generation on this host was shorter than on the two hosts, the carob moth and the Mediterranean flour moth. The sex ratio was also the highest possible and in favor of females when reared on the eggs of the codling moth, the percentage of parasitism was the highest possible on the eggs of the codling moth. The percentage of parasitism mortality was the lowest possible on the codling moth compared to the other two hosts. Therefore, it can be concluded that the laboratory rearing of the parasitoid in an artificial environment is preferable to be on the eggs of the codling moth, compared to the other two hosts.

Keywords: biological control, *Trichogramma cacoeciae,* parasitoid, egg parasitoids.

Introduction

The expansion and growth in the cultivation of field crops and fruit trees has led to the emergence and spread of many agricultural pests that affect these plants at various stages. The problem of protecting these crops from pest damage has also occupied a large part of human interest and thinking, which requires the use of chemical pesticides. In general, the use of pesticides to combat agricultural pests has produced many problems, the most important of which are: environmental pollution, the emergence of resistant strains of pests, the transformation of some secondary pests into primary pests that cause damage to cultivated plants, in addition to disrupting the natural balance between agricultural pests and their biological enemies [1]. Therefore, biological control was employed by utilizing natural enemies to eliminate harmful pests and insects or reduce the pest numbers to a level that causes economic damage.

The reproduction of biological enemies (parasitoids and predators) in the laboratory to produce large numbers of them with the aim of releasing them against specific pests at specific times is one of the main goals in this field. The Trichogramma parasitoid is one of the biological enemies that parasitizes the eggs of many insects. It attacks more than 400 species of insects and works to reduce their numbers in nature, as the parasitoid completes its entire life cycle inside the host egg and emerges as a complete insect. Parasitism only succeeds in newly formed eggs. Wasps of the genus Trichogramma are also very important in biological control, especially against the eggs of the order Lepidoptera that attack crops and cause great economic damage, such as the eggs of the codling moth and almond worm, the European corn stalk borer, the tomato leaf miner *Tuta absuluta*, the carob moth and others. The parasitoid *T. cacocaie* is used to control many agricultural pests including the grape worm *L. botrana*, the codling moth *C. pomonella*(L.), the peach fruit worm *C. funebrana*, the peach fruit worm *G. molesta*, the olive moth *P. oleae* and on cotton crops [2]. The species of the genus *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are among the most widespread egg parasitoids in the control of many types of insects [3], and they are found in many countries in Europe and North Africa. The strains of this parasitoid are distinguished by their ability to adapt to climatic conditions, especially high temperatures at the release site [4].

Trichogrammatids represent a large group of small parasitic wasps that attack the eggs of various economically important insects. These wasps lay their eggs inside the eggs of pests and grow on them, eliminating them even before they begin to cause damage, which gives them importance in biological control[5]. Therefore, work was done to find methods that are part of the integrated pest management program, including the quantitative rearing of the parasitoid and its dissemination in farms to reduce the harmful insects it attacks. Due to the presence of centers for breeding biological enemies in the country, it was necessary to conduct this research to determine the appropriate host of insect pests that the parasitoid attacks to be able to quantitatively rear it, by knowing the biological characteristics of the parasitoid on the different hosts that it will be reared on, and choosing the most appropriate host for quantitative rearing of this parasitoid. The study aimed to rear the parasitoid *T. cacoeciae* in the laboratory on the eggs of three hosts that were reared in an artificial environment, namely the Mediterranean flour moth *E. kuehniella*, the carob moth *E. ceratoniae*, and the codling moth *C. pomonella* L. to choose the appropriate host for quantitative rearing of this parasitoid.

Materials and Methods

Source of the parasitoid *T. cacoeciae*

A quantity of apple fruits infected with the codling moth was collected, then each fruit was placed in a suitable glass beaker (15x20cm), covered with a piece of cloth for ventilation and to prevent insects from escaping, then a wavy piece of cardboard was placed in each package (2x2cm) to prevent the larvae. The beakers were placed in the

laboratory at a temperature of (25±1°C), relative humidity (60±10%) and lighting duration (16L: 8D). The beakers were monitored daily and the emerging insect parasitoids were collected. The insect parasitoids were classified using insect classification keys[6].

Rearing of *T. cacoeciae*

Eggs of the fig moth *E. cautella* Walker (Lepidoptera: Pyralidae) exposed to UV light at 254nm wavelength for 12min were used to rear and maintain the parasitoid *T. cacoeciae* in the laboratory [7]. Eggs (24h old) were spread on pieces of pale-yellow cardboard (1.4x5cm) previously glued with gum Arabic on one side. These pieces were stored in glass tubes (2.5x20 cm) containing adult Trichogramma insects, and the tube openings were closed with cotton wool containing a 5% sugar solution with a drop of natural honey (1:1ratio) to feed the adult parasitoids. When the host eggs turn black, indicating that they are infected with the parasitoid, the cardboard is removed and divided into four parts, each of which is placed in a new tube prepared with cardboard containing the host eggs and closed with a piece of cotton that also contains the sugar solution to feed the emerging wasps. The glass tubes were placed in the laboratory under the same previous storage conditions [8].

Laboratory treatment of the parasitoid *T. cacoeciae* on eggs of the Mediterranean flour moth

The Mediterranean flour moth *E. kuehniella* was reared on an artificial diet consisting of 81% wheat grits, 12% glycerin, 6% molasses and 1% dry yeast[9]. To obtain the eggs of the Mediterranean flour moth, each pair (male+ female) of newly emerged adults was transferred into a medium-sized lantern bottle. The bottle was placed on a plastic Petri dish containing a black filter paper moistened with distilled water to place the eggs on and to facilitate their viewing and counting. The upper opening of the lantern was covered with a gauze cloth containing a central opening with a piece of cotton moistened with a sugar solution (5%) to feed the adults[10]. The eggs laid by the females were collected daily at the age of 24hours. The eggs were placed on black circular filter papers (8.5-9cm in diameter) inside a Petri dish at a rate of 50eggs per paper. The eggs were sprayed with distilled water to prevent drying. Each dish was placed inside a 1000ml glass bottle, open at both ends, with the germination plate containing the eggs placed on one end and the other closed with a piece of gauze containing in the middle a piece of cotton soaked in a 5% sugar solution with a drop of natural honey (1:1 ratio) to feed the entire parasitoid. A pair of newly emerged parasitoids (male + female) were released inside. Five replicates were made, in addition to the control treatment in which the parasitoid was not released. All replicates were placed inside the rearing room under the same previous storage conditions.

Laboratory treatment of the parasitoid *T. cacoeciae* on the eggs of the carob moth *E. ceratoniae*

Carob moth was reared using 5 plastic bottles with a diameter of (20x30cm), each bottle containing 150g of Zahidi dates. A pair of newly emerged adult insects (male+ female) were released inside each bottle. The bottles were placed in the laboratory at a temperature of 25±1°C, relative humidity (60±10%), and lighting duration of 16L: 8D[11]. After emergence, adults were collected using test tubes and then placed inside glass mating bottles with a diameter of (20x30cm), each bottle contained a Petri dish containing (date flour, wheat bran and distilled water), and the eggs were removed through a fine mesh tulle in the feeding place [12]. After collecting the eggs laid by the females daily at the age of 24hours, the eggs were placed on black filter papers and divided into four pieces at a rate of 50eggs per piece. The eggs were sprayed with distilled water to prevent drying. The filter papers were placed inside 500ml plastic bottles with a piece of cotton soaked in a 5% sugar solution with a drop of natural honey (1:1ratio) to feed the entire parasitoid. A pair of newly emerging parasitoids (male+ female) were released inside them. Five replicates were made of them in addition to the control treatment in which the parasitoid was not released. All replicates were placed inside the rearing room under the same previous storage conditions.

Laboratory treatment of the parasitoid *T. cacoeciae* on the eggs of the codling moth *C. pomonella*

The codling moth was reared on an artificial medium consisting of 20g agar powder, 50g corn flour, 50g pollen, 50g yeast, 4.5g vitamin C, 1.8g sodium benzoate, 1.8g methyl propane, and 780ml distilled water [13]. A set of 4 volumetric flasks with a capacity of 1000ml were made. 100g of local codling moth were placed inside each flask with a piece of cotton soaked in a 5% sugar solution to feed the adults. Two pairs of newly emerged codling moths (2males + 2females) were released inside each flask. The flasks were closed from the top with a cloth for ventilation and to prevent the adults from escaping. The eggs laid by the females were collected daily at the age of 24hours. The eggs were placed on black circular filter papers (8.5-9cm in diameter) inside a Petri dish at a rate of 50eggs per paper. The eggs were sprayed with distilled water to prevent drying. Each dish was placed under a lantern bottle open at both ends. The upper end was closed with a piece of gauze containing in the middle a piece of cotton soaked in a 5% sugar solution with a drop of natural honey (1:1ratio) to feed the entire parasitoid. A pair of newly emerged parasitoids (male+ female) were released inside it. Five replicates were made of them in addition to the control treatment in which the parasitoid was not released. All replicates were placed inside the rearing room under the same previous storage conditions.

Statistical analysis

The effect of the insect host on some biological characteristics of the parasitoid *T. cacoeciae* was studied, with calculation of the sex ratio (number of females: number

of males), the life span of males and females and the parasitism rates. Each insect host was considered a treatment and five replicates were used for each treatment. The results were analyzed using ANOVA, at a probability level of 1%. Excel 2013 was used to analyze the results statistically.

Results and Discussion

The effect of the insect host on some aspects of the life of the parasitoid *T. cacoeciae*

The results (Table1) indicated that the shortest development duration of the parasitoid stages was 7.92, 3.8 and 11.72days for the egg-pupa, pupa-adult and egg-adult stages, respectively, when the parasitoid was reared on codling moth eggs. The longest durations were 18.6, 10.65 and 29.25days for the mentioned stages, respectively, when reared on Mediterranean flour moth eggs. The differences between rearing on Mediterranean flour moth eggs and carob moth and codling moth eggs were significant. There was no significant difference between the codling moth and the carob moth at a probability level of 1%, as well as for the average mortality rates, as the highest value was 75.33% when reared on Mediterranean flour moth eggs, then 16.56% when reared on carob moth eggs, and the lowest was 12.57% when reared on codling moth eggs. The difference was significant between the host, the Mediterranean flour moth, and the hosts, the carob moth and the codling moth. There was no significant difference between the codling moth and the carob moth at a probability level of 1%.

Table (1): Average life cycle durations of the parasitoid *Trichogramma cacoeciae* reared on three different hosts.

life cycle stages	development duration/day (mean ± standard error)			
	LSD 0.01	codling moth	carob moth	Mediterranean flour moth
egg - pupa	5.8	7.92±0.07b (6-8)	9.35±0.22b (7-11)	18.6±0.13a (16-20)
pupa - adult insect	3.58	3.8±0.01b (3-5)	5.45±0.05b (4-7)	10.65±0.1 (9-13)
egg - adult insect	9.57	11.72±0.13b (10-13)	14.8±0.1b (12-17)	29.25±0.14a (27-33)
parasitism percentages%	44.02	82.56±5.05a (69-100)	3.21±79.81a (60-95)	4.3±0.009b (3-5)
mortality rate %	35.14	12.57±1.15b (7.08-20)	16.56±2.21b (9.9-26)	75.33±6.09a (55.12-100)

Means followed by similar letters on the same line indicate no significant difference at the 1% probability level.

The lowest average parasitism percentage (4.3%) was recorded when reared on Mediterranean flour moth eggs, followed by 79.81% when reared on carob moth eggs, and the highest value was 82.56% when reared on codling moth eggs. The difference was significant between the host, Mediterranean flour moth eggs, and the eggs of the two

hosts, carob moth and codling moth. There was also no significant difference between the codling moth and carob moth at a probability level of 1%. The codling moth and the carob moth are the hosts that the parasitoid parasitizes to a greater extent in the wild in apple and pomegranate orchards, compared to the Mediterranean flour moth, so the success of the parasitoid's rearing on the hosts is attributed to the eggs of the codling moth and the eggs of the carob moth, and to a lesser extent to the rearing of the parasitoid on the eggs of the Mediterranean flour moth[14].

Effect of insect host on emergence percentages and sex ratios of the parasitoid *T. cacocaie*

The results showed a difference in the sex ratios of the parasitoid *T. cacoeciae* when reared on eggs of different hosts (Table2). The highest sex ratio of the parasitoid was (1female: 3.66male) when reared on eggs of the codling moth, followed by (1female: 1.76male) when reared on eggs of the carob moth, and the lowest was (1female: 0.42male) when reared on eggs of the Mediterranean flour moth. The sex ratios of parasitoids vary in laboratory conditions according to the percentages of female and male emergence. When the percentage of female emergence was 19.5%, the sex ratio was (1female: 0.42male), when the percentages of female emergence increased, the sex ratios of the parasitoid also increased in both hosts, the carob moth and the codling moth. They were highest when reared on codling moth eggs (1female: 3.66male). These results are consistent with [15], which stated that the sex ratios are in favor of males when parasitizing an unsuitable host, and increase when parasitizing a host preferred by the parasitoid, as the codling moth is one of the preferred hosts for the parasitoid [16]. The average lifespan of the female and the male did not differ when reared on the two hosts, the carob moth and the codling moth, with an average of (46.30days for the female and 52.20days for the male) and (48.34 for the female and 54.30 for the male) days, respectively as for the Mediterranean flour moth, the average lifespan of the female was 9.20days and the male was 12.87days.

The insect host that is reared in the laboratory on artificial food affects the duration of the different stages of the parasitoid, so that it is as short as possible on the preferred host of the parasitoid, It also affects the sex ratio, and this ratio is as high as possible when reared on the preferred host of the parasitoid. It also affects the percentage of parasitism, and therefore the laboratory rearing of the parasitoid in an artificial environment is preferable to the eggs of the codling moth, because it is the preferred host of the parasitoid. In a laboratory and field study [17], he pointed out some of the characteristics and features of the parasitoid *T. cacocaie*, that the females lived for more than 27days on the host *C. pomonella*, which is the longest compared to the rest of the studied species, with a fertility rate of 115.3eggs/female. It was also shown that the efficiency of the parasitoid depends on several factors, including high fertility, the rate of offspring produced, the acceptance of the host by the parasitoid, the ability to spread in the environment, the length of survival, and tolerance to high temperatures.

Table (2): Effect of the insect host on the emergence percentages and sex ratios of the parasitoid *Trichogramma cacoeciae*

The host	LSD 0.01	parasitoid lifespan/day (Mean ± standard error)		sex ratio		percentage of % emergence		No. males	No. females	total No. individuals
		♂	♀	♂	♀	♂	♀			
Mediterranean flour moth	6.08	12.87 ±1.15b	9.20 ±0.13b	0.42 : 1		80.50	19.50	33	8	41
carob moth	42.02	52.20 ± 0.09a	46.30 ±0.07a	1.76 : 1		43.08	65.92	100	176	267
codling moth	46.04	59.54 ±0.10a	48.34 ±0.08a	3.66 : 1		21.42	78.57	63	231	294

Means followed by similar letters on the same line indicate no significant difference at the 1% probability level.

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