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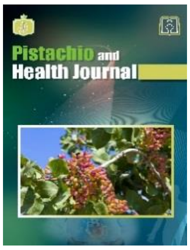

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ORIGINAL ARTICLE

Some biological characteristics of *Ooencyrtus pityocampae* Mercet parasitoid of *Brachynema signatum* Jakovlev under laboratory conditions

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Background: Biological control is an important component of an eco-friendly approach in integrated pest management to prevent insecticide-resistant pests and minimize using pesticides. *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) is an egg parasitoid of *Brachynemasignatum* (Hem.: Pentatomidae) in the pistachio orchards of Iran.

Materials and Methods: In this study, biological characteristics, such as immature developmental time, adult longevity, parasitism percentage, and sex ratio were investigated.

Results: Mean egg, larval, pupal, and total development time of *O. pityocampae* were 1.94±0.01, 3.42±0.03, 7.11±0.03, and 11.84±0.06 days, respectively. On five different food regimes (honey, water,10% honey-water, no food diet, honey, and *B. signatum* egg), adult longevity of females and males were 39.29±1.45, 38.5±3.33, 9.3±0.56, 5.05±0.91, and 19.81±0.63 days and 40.05±1.56, 37.5±1.05, 7.8±0.29, 3.89±0.13, and 9.09±0.74 days, respectively. Female wasps were laying eggs during the first two weeks after adult emergence, and maximum parasitism (39.27±0.01 Eggs) was observed on day 3 of adult life span. Interestingly, sex ratio was positively correlated with *B. Signatum* egg mass size increased from 0.11 to 0.26 when the egg mass size increased from 10 to 40.

Conclusions: These laboratory findings indicate that *O. pityocampae* may show good potential as a biocontrol agent against *B. signatum* under IPM programs.

Keywords: Biological control; egg parasitoid; Encyrtidae; green pistachio stink bug

1. Introduction

The pistachio green stink bug, *Brachynema signatum* Jakovlev (Hemiptera: Pentatomidae), has great importance in pistachio production due to the quantitative and qualitative damages it causes. Serious infestation of pistachio nut by *B. signatum* makes it unpleasant or unmarketable. Piercing of the soft-shelled pistachios by the stylets of the stink bug and sucking the nutrients causes epicarp lesion on the hull in the early season [1] and kernel necrosis in the midseason as well as transmission of a fungal pathogen, *Nematospora coryli*, in pistachio nuts [2]. Due to the stink bug wide host-range, adult dispersal habits, and diverse habitats this group of pistachio pests are generally difficult to control. Injudicious use of pesticides can damage human health and the environment. Given the concerns associated with pesticide use, using natural enemies for biological pest control is a preferred approach to *B. signatum* management [1]. The egg parasitoids are considerable biocontrol agents because they attack the host before it develops and inflicts feeding damage [1].

Field surveys have revealed the existence of egg parasitoids of the green pistachio bug in Iran [3]. *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) is a polyphagous egg parasitoid that commonly attacks the pine processionary caterpillar, *Thaumetopoea pityocampa* (Denis and Schiffermfiller) (Lepidoptera: Thaumetopoeidae), in southern Europe [4]. It is also a parasitoid of *B. signatum* eggs in Iran. This species is a synovigenic endoparasitoid that benefits from a supply of carbohydrates to produce more eggs for a longer period of time [4-7].

Tiberi *et al.* [8] reported that the augmentation of *O. pityocampae* has beneficial in controlling processionary caterpillar, *Thaumetopoea pityocampa* (Denis and Schiffermfiller) (Lepidoptera: Thaumetopoeidae) in young pine trees. Masutti *et al.* [4] studied the rearing of *O. pityocampae* on artificial eggs. Dulaurent *et al.* [9] assessed the response of *O. pityocampae* and *Baryscapus servadeii* Domenichinito different trophic resources, i.e. two types of honeydew produced by two aphid species feeding on oak or pine trees. Tunca *et al.* [10] investigated the biologicalvariables that influence the parasitism of *O. pityocampae* on the new laboratory host *Philosamia ricini* Danovan (Lepidoptera: Saturniidae).

So far, egg parasitoids have proven to be successful biological control agents against several insect pests. However, the biology of the majority of these effective parasitoids is not fully understood. Therefore, in this study, we investigated several biological parameters of *O. pityocampae* on the green pistachio stink bug *B. signatum*,

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including the development time, longevity, parasitism percentage, and sex ratio.

2. Materials and Methods

2.1. Insect rearing

Adult pistachio green stink bugs *B. signatum*, were collected in a pistachio orchard in Rafsanjan, Kerman province, southeastern Iran (30°42′2″ N and 55°53′51″ E) during the summer season. The collected bugs were returned to the laboratory and were reared in plastic boxes (20×30×10 cm), provided with mesh-covered holes (0.5 mm aperture), in a climatic chamber (25±1°C, 70±5% RH and L16:D8 photoperiod), and fed with a diet of weeds (*Salsola kali* Linnaeus and *Zygophyllum fabago* Linnaeus) and water. Food was changed every 1–2 days, and separate boxes were used for nymphs and adults. Egg masses were collected daily and used partly to maintain the colony and partly to raise parasitoid. The *O. pityocampae* colony was originally collected with the egg traps method. For this purpose, the egg masses laid on *Salsola kali*o, which is obtained from the stock laboratory colony, and stapled to yellow cards (7.0 × 7.0 cm) installed in different directions within the non-commercial pistachio orchards and on *Salsola kali* plant at a height of 50.0 cm. Cards were collected every three days, and parasitized eggs were held in an incubator until wasps emerged. Adult parasitoids were isolated in glass vials (1.5 × 5.0 cm) covered with tissue paper held in place with a rubber band, provided with a drop of diluted honey on the side of the vial and held in a climate-controlled growth chamber (27±1°C, 65±5% RH, L16:D8 photoperiod). Egg masses of *B. signatum* were collected daily, and each mass was exposed to a single parasitoid for 24 h, then transferred to a clean glass tube for incubation. Male and female parasitoids were paired for mating within 48 h of adult emergence.

2.2. Development time

The development time, defined as the time needed to complete development from egg-laying to adult emergence, was determined using 10 mated adult females of *O. pityocampae* (first generation parasitoid adults). Each 3-day-old female parasitoid was placed inside a semitransparent plastic box (10×12 cm) and exposed to around 100 *B. signatum* eggs (which were collected after 24 h). Female parasitoids were allowed to lay eggs in the host eggs for 6 h under controlled conditions (27±1°C, 65±5% RH, L16:D8 photoperiod). Parasitized eggs were maintained under the same conditions. The development of the parasitoid was observed every 6 hours until the adult emerged.

2.3. Effect of the feeding regime on longevity

Newly emerged one *O. pityocampae* male-female pair (second-generation parasitoid adults) were introduced into a plastic cup (5×6 cm) and provided with the following feeding regimes: (1) 10% honey-water; (2) honey; (3) water; (4) no food (control); and (5) the *B. signatum* egg and honey. To assess longevity, a daily check was performed to determine whether wasps were alive or dead. Accidental death (e.g. insects glued on honey) was excluded from the results. At least 10 replicates were carried out per treatment, kept at 27±1°C, 65±5% RH and 16:8 L: D photoperiod.

2.4. Parasitism percentage

In each of the 10 replicates, one *O. pityocampae* male-female pair (first generation parasitoid adults), which emerged on the same day, was put into plastic boxes (10×12 cm), which contained droplets of honey and were provided with 100 fresh host eggs (which were collected after 24 h) per day until the death of the parasitoids. The number of host eggs exposed was proportional to the number of live females (100 egg/1 female). The host eggs were taken out after 24h exposure to allow the parasitized eggs to develop. Until all the *O. pityocampae* females were dead, this protocol was repeated every day, and the eggs were offered to the parasitoids. Upon emergence, the *O. pityocampae* adults were counted and sexed.

2.5. Sex ratio

To test the influence of the egg patch size on the sex ratio of the females, host egg densities containing 1, 10, 15, 20, 25, 30, 35, and 40 eggs per adult female (second-generation parasitoid adults) were used. These patch sizes were selected on the basis that the parasitoid is likely to encounter small to very big host patches in the field. They were counted under a light microscope before they were offered to the test insects. One individual female per vial represented a replication, and a total of ten replications were made for each of the patch treatment. Upon emergence, *O. pityocampae* females were kept with males for 24 h to allow mating. Females were then isolated, provided with a drop of honey solution, and used in experiments when they were 2-3 days old (the age at which a female can produce the maximum number of progeny). The parasitized egg masses obtained from the experiments were labeled and incubated at 27±1°C, 65±5% RH, and L16:D8 conditions until emergence. The emerged adult progeny along with the number of females out of the total progeny (number of adults) were counted and recorded. The males and females of *O. pityocampae* were easily distinguished based on differences in antennae, which are longer and setiform in males in comparison with females.

2.6. Data analysis

Data are presented as means (±SE) per treatment. A t-test was used to compare the development time between wasp sexes. The development time and the effect of feeding regime on the longevity of *O. pityocampae* were analyzed using a one-way ANOVA.

3. Results

3.1. Development time

The development time of *O. pityocampae* did not significantly differ by gender (t=-1.78; df=54; *p*=0.8), and mean egg-to-adult (total) development time of *O. pityocampae* was 11.68±0.07 and 11.84±0.06 days for male and female parasitoids, respectively. Mean egg, larval, and pupal development time of *O. pityocampae* were 1.94±0.01, 3.42±0.03, and 7.11±0.03, respectively.

3.2. Longevity of *O. pityocampae*

The effect of food on longevity of female *O. pityocampae* (F=56.32, df=3, *p*<0.001) and male *O. pityocampae* (F=276.46, df=3, *p*<0.001) was meaningful in terms of the longevity of wasps fed with honey and water diet, and only honey diet was significantly higher than control for both males and females (*p*<0.001). There was no significant difference in the female wasps longevity fed with honey and water and just honey (T=0.94, df=56, *p*>0.05 Table 1). When exposed to the honey and *B. signatum* egg, the mean longevity of *O. pityocampae* females was estimated to be 19.81 days (Table 1).The longevity of both male and female *O. pityocampae* was significantly affected by the feeding regime (F=4.41, df=1, *p*<0.053, Table 1). The longevity of water-fed wasps was significantly lower than that of the wasps fed with honey for both males and females (F=91.56, df=3, *p*<0.001, Table 1). The longevity of female *O. pityocampae* whose feeding regime was based on host dependent resources such as host feeding was significantly higher than that of female wasps fed with water and significantly lower than that of wasps fed with honey (F=90.87, df=2, *p*<0.001, Table 1).
The adult parasitoids disappeared shortly after emerging in the non-food environment, which shows the important role of nutrition in increasing adult parasitoids longevity. Other than this diet, there is no difference between genders of *O. pityocampae* (T=-1.60, df=40, *p*>0.05, Table 1).

Table 1. Effect of the feeding regime on the longevity of *O. pityocampae adults*

Treatment	Longevity (mean±SE)	
	Male	Female
Honey	40.05±1.56 [a]	39.29±1.45 [a]
Water	7.8±0.29 [c]	9.3±0.56 [c]
Honey and water	37.5±1.05 [a]	38.5±3.33 [a]
Non-food	3.89±0.13 [c]	5.05±0.91 [c]
Honey and *B. signatum* egg	9.09±0.74 [c]	19.81±0.63 [b]

Within the columns means followed by the same letters are not significantly different (P < 0.05)

3.3. Parasitism percentage of *O. pityocampae*

Parasitism is very low in the first 24 hours of parasitoid life span and parasitizing *B. signatumeggs* (7.27±2.54 eggs) at low levels. Understanding the efficient age of parasitoids is very important to obtain effective and successful parasitism in field release programs as well as for mass production. The maximum number of eggs deposited by one *O. pityocampae* female was observed on the 3rd (39.27±0.01eggs) days of its adult life span. The egg laying period of *O. pityocampae* lasted 14.45±0.68 days (Fig. 1). A female *O. pityocampae* lays 122.45±4.30 eggs in its whole life time. The average adult longevity was 9.09±0.74 and 19.81±0.62 days for females and males, respectively.

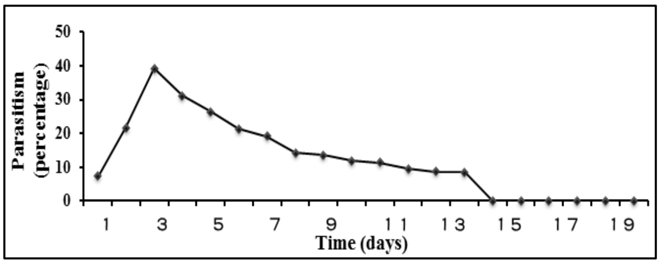

Fig. 1. Parasitism percentage of *Ooencyrtus pityocampae* on *Brachynema signatum* eggs

3.4. Sex ratio of *O. pityocampae*

Sex ratio of *O. pityocampaeon B. signatum* eggs in the laboratory is shown in Fig. 2. The sex ratio (female: male) of *O. pityocampae* is 4.2:1.

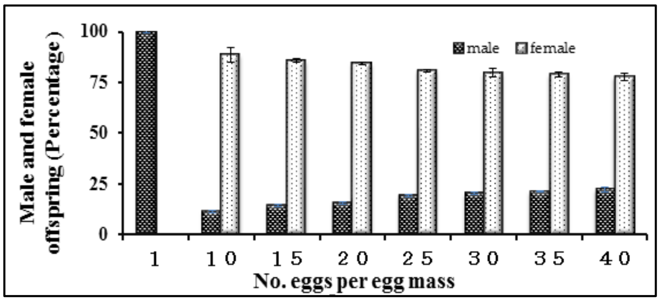

Fig. 2. Effects of the host density on percentage male and female offspring of the parasitoid

4. Discussion

4.1. Development time

According to many researchers, there are different types of development times in *Ooencyrtus* species at different temperatures and in different hosts. For example, Rahim *et al*. [11] studied the effect of temperature on the development time of *O. papilionis*. Ashmead showed that in this parasitoid no *development* of immatures occurs if the temperature is over 32.5°C. According to the survey by Muesebeck and Dohanian [12] in both *O. kuvanae* and *O. anasae* development from egg to adult takes about 18-35 days, depending on temperature. Viggiani [13], during investigations on *O. gonoceri* Viggiani which is the parasitoid of *Gonocerus acuteangulatus*. Goeze, showed that the preimmaginal development takes place in 8-12 days at 23-26°C and at 50-70% of R.H. Maple [14] has also reviewed the biology of *O. johnsoni* Howard on *Murgantia histrionica* Hahn eggs. Based on his results, the development of immatures is approximately 17 days. Masutti [15] reported that in *O. pityocampae* on *Thaumetopoea pityocampa Denis* & Schiffermuller eggs the development from egg to adult takes about 22-25 days. These apparent contrasting results could be due to using different hosts. Our results showed that short *development* period of *O. pityocampae* on *B. signatum* eggs can be an important feature for mass rearing in the biological control of the pest.

4.2. Longevity of *O. pityocampae*

The longevity of wasps fed with honey and water and just honey was significantly higher than water-fed wasps for both males and females (p<0.001), which shows that honey, due to having sugars such as Sucrose, Glucose, Fructose, and vitamins, plays a role in increasing longevity toward water [16]. The longevity of *Ooencyrtus* was influenced by temperature and feeding regime. Rahim *et al.* [11] reported that in *O. manii* longevity of both sexes decreases with an increase in temperature; males live 4 to 1.2 days at temperatures of 15-36°C whilst females live from 10-1.7 days at the same temperatures. The lower longevity we observed in female wasps exposed to the honey and *B. signatum* egg in comparison with that observed for those fed with honey (Table 1) could be due to a less favorable feeding regime and/or the occurrence of oviposition activity. These results emphasize the importance of providing food sources to the adult wasps through habitat management (*e.g.* flowering plants) to enhance conservation or augmentation of biological control.

4.3. Parasitism percentageof *O. pityocampae*

Egg-laying period, number of eggs, and longevity of egg parasitoids of *in vitro* depend on many factors, including temperature and relative humidity. According to many researchers, a change in temperature and relative humidity can also change longevity, egg-laying period, and number of eggs laid by wasps [17]. In addition, in the different generations of the egg-laying period, number of eggs and longevity is changed. Also, the nature of the substrate at the oviposition site may affect rates of parasitism. For instance, in *O. kuvanae*, the eggs of the host on red maple (smooth bark) have a higher rate of parasitism than those on oak (rough bark) [18]. Aung *et al*. [19] stated that the reproductive potential of 4–day–old females of *O. nezarae* is higher than 20–day–old and 1–day–old females. Also, Rajapakse *et al.* [20] stated that the optimum age of *Cotesia marginiventris* for successful parasitism of *Spodoptera frugiperda* ranged from 48 h to 96 h [20] and that *C. marginiventris* which were younger or older than the above age class were not able to parasitize a host. The results of the present study are consistent with the results of this researcher.

4.4. Sex ratio of *O. pityocampae*

Sex ratio may be influenced by several factors including temperature, seasonal fluctuations, and microorganisms like *Wolbachia*. For example, in *O. fecundus*, all progeny are female if the ovipositing female is subjected to temperatures of 30°C, but all progeny are male if the ovipositing female is subjected to a temperature of 35°C [21]. Battisti *et al.* [5] reported that males are very rare in natural populations (0.033%), whereas Tiberi *et al.* [22] observed male emergence under laboratory conditions only when temperature at oviposition exceeded 30°C. Sex ratio of *O. pityocampae* parasitoid on the different eggs of host showed that females start egg-laying sequences by laying male eggs early in an oviposition bout in accordance with the males Ist strategy. These results suggest that changes in the sex ratio in each batch depend on the number of host eggs. According to the predictions of the local mate competition model, a decrease in the number of hosts offered to the females should result in an increased proportion of male eggs laid [23]. When parasitoid wasps lay eggs in a patch, unfertilized eggs may be laid during the early duration of oviposition [24, 25]. Such a 'male-first strategy' facilitates the production of optimal sex ratios by female parasitoids. If multi foundresses oviposit in a patch, there may be a reduction in clutch size per foundress because of limited oviposition sites in the patch. Higher proportion of male offspring means higher fitness to foundresses, because male offspring have the potential to mate with the female offspring of the other foundresses, and the value of males increases [26-28]. Therefore, laying more unfertilized eggs at the beginning of oviposition should also bring a potential benefit to foundresses. When mating takes place in small patches and involves mostly relatives, theories predict that females are selected to maximize their fitness by producing few males, just the number necessary to guarantee that all their daughters will be inseminated, and a female biased sex ratio will also bring less local mate competition (LMC) among the sibling male offspring [26-28]. But reduction of males also increase the probability of virginity in offspring. Foundresses may reduce the risk by ensuring sufficient male offspring. In addition, in parasitic Hymenoptera, mated females store sperm in the spermatheca and can manipulate offspring sex ratio (males/total offspring) by controlling fertilization during oviposition. The haplodiploid sex-determination system provides the mothers with a mechanism to control progeny sex ratio, because males develop from unfertilized eggs (haploid) and females from fertilized eggs (diploid) [24, 29]. Tracy and Nechols [30] also reported that in *O. anasae* the proportion of females increases with the number of hosts parasitized.

5. Conclusions

According to the results of the present study, it seems that this parasitoid can be expressed as a good candidate for mass rearing and releasing in nature, because the development time is rather short, and the proportion of high female has appropriate rate parasitism, and longtime longevity such as single characteristics in terms of considered characteristics have appropriate species for rearing and releasing clump for probability doing besides easy rearing. The obtained results in this paper reported ease in mass rearing on this parasitoid and effective control of pistachio green stink bug.

Conflicts of interest

The authors declare no conflicts of interest.

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