Reproductive biology of *Ooencyrtus egeria* (Hymenoptera: Encyrtidae) reared on eggs of two economically important pistachio stink bugs: *Brachynema germari* and *Acrosternum arabicum* (Hemiptera: Pentatomidae)

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Information

Article Type:
Original Article

Article History:

Received: 26.05.2022
Accepted: 15.09.2022

Doi: 10.22123/PHJ.2023.365944.1141

Keywords:
Biological control
Egg parasitoid
Parasitism
Sex ratio

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Abstract

Background and Objectives: *Ooencyrtus egeria* Huang and Noyes is an effective solitary egg parasitoid of *Brachynema germari* Kolenati and *Acrosternum arabicum* Wagner, the most common stink bugs of pistachios in Rafsanjan, Iran. Basic biological information on parasitoid and host interaction is essential for increased efficiency and successful biological control programs.

Materials and Methods: In the present study, some important biological characteristics of *O. egeria* on two pistachio green stink bugs, *A. arabicum* and *B. germari*, were evaluated to define the best laboratory host for the mass rearing of this wasp.

Results: The eggs of *B. germari* had better nutritional quality for *O. egeria* females, thus, having the highest parasitism rate compared to the eggs of *A. arabicum*. The number of female offspring that emerged from *B. germari* eggs was higher than that of *A. arabicum*; however, the highest survival rate and the lowest mortality rate were recorded for the *A. arabicum.*

Conclusion: Although the laboratory results show that the survival rate is higher and the mortality rate is lower for *A. arabicum, B. germari* is a more suitable host for the rearing of *O. egeria* in laboratory conditions due to the higher parasitism rate and sex ratio. The present research findings could be used in pistachio orchards to provide necessary data enabling the best use of this egg parasitoid as a biocontrol agent.

►Please cite this article as follows:
Sedigh H, Ranjbar F, Jalali MA, Ziaaddini M. Reproductive biology of *Ooencyrtus egeria* (Hymenoptera: Encyrtidae) reared on eggs of two economically important pistachio stink bugs: *Brachynema germari* and *Acrosternum arabicum* (Hemiptera: Pentatomidae). Pistachio and Health Journal. 2022;5(3):62-69.

1. Introduction

Pistacia vera Linnaeus (Anacardiaceae) is one of the most important agricultural products of Iran. Mites and insects are among the significant factors causing damage to the product, thus a slight decrease in its production and export. Among the key pests of pistachio are green stink bugs, invading pistachio orchards during the growing season and causing considerable quantitative and qualitative damage to the crop [1,2]. The green stink bugs of pistachio orchards in Iran mainly belong to the Pentatomidae and Lygaeidae families. *Brachynema germari* Kolenati and *Acrosternum arabicum* Wagner have been reported as the most abundant and important pentatomid species of pistachio pest [1, 3, 2]. Using chemical pesticides incompatible with natural enemies leads to the resurgence of pests; thus, their effect on the environment and natural enemies is negative. Egg parasitoids are highly valued since they attack the first stage of life before causing any damage and feeding on the plant, and they have a high potential for biological combat against the pest [4]. Different species of two families, Scelionidae and Encyrtidae, which are the egg parasitoids of pistachio stink bugs, have been reported in Iran. The genus *Ooencyrtus* from the Encyrtidae family was first named by Ashmid (1900). One of the distinctive features of this genus is its egg stalk, which remains as a protrusion from the host eggshell after laying, and the larvae can use atmospheric air directly from the protruding part of the egg [5]; this feature can be used to count the number of parasitized eggs of the host. Various species from the Encyrtidae family, including *Ooencyrtus egeria* Huang and Noyes, with a high population and activity period in pistachio regions of Rafsanjan, have been collected and identified from the crop pentatomid population [6, 7]. In order to determine the suitability of an egg parasitoid wasp as an acceptable agent for biological control of the pest population, characteristics such as reproductive parasitism capacity, developmental time, mortality rate, and sex ratio should be investigated [8]. Due to the high importance of using egg parasitoid as one of the effective methods in biological control programs against pentatomid bugs, some important biological characteristics of *Ooencyrtus egeria* on two pistachio green stink bugs, *A. arabicum* and *B. germari*, were investigated in this research to define the best laboratory host for the mass rearing of this egg parasitoid.

2. Materials and Methods

2.1. Insects

In Rafsanjan, Adults of *A. arabicum* and *B. germari* were collected from orchards pistachio trees under the weeds of prickly salt, *Kali turgidum* (Dumort) Guterm (Amaranthaceae) plants; they were identified using the keys of Safavi [9] and Ribes and Schmitz [10]. Collected stink bugs were kept and reared on an alternative food diet, green beans, *Phaseolus vulgaris* in plastic containers (12 x 30 x 20 cm) with ventilated lids in a climate-controlled room under conditions: 27 ± 1 C°, 65 ± 5% RH, and 16:8 (L:D) photoperiod. The egg trap method was used to collect parasitoid wasps. The one-day-old egg masses obtained from stink bugs rearing in the laboratory were glued on yellow cards with dimensions of 7 x 7 cm. They were installed in the pistachio orchards on *Kali turgidum* at a height of 50 cm in different directions of the pistachio trees. Cards were collected after 48 h, and the parasitized eggs were placed in the incubator with the standard

conditions mentioned above until the emergence of adults. *O. egeria* species were identified and isolated using the identification key; then, they were compared with the identified samples kept in the laboratory of the Vali-e-Asr University of Rafsanjan.

In order to access parasitoids with a specific lifespan, males and females collected from egg masses were exposed to one-day-old eggs in falcon tubes (15 ml); then, the eggs were collected on the next day and kept in the growth chamber until the emergence of adults. A 10% honey and water mixture was used to feed the parasitoids.

2.2. Experiments

To determine the biological characteristics of parasitoids, a number of eggs (<24 h, 30 eggs) of *A. arabicum* and *B. germari* (separately) were glued together on paper and given to one-day-old male and female *Ooencyrtous egeria* (10 replicates) in the plastic centrifuge tubes. After 24 h, they were removed and replaced with 30 new ones; this process continued daily until the end of the life of the females. The parasitism rate was recorded based on the observation and counting of eggs with respiratory siphons; then, the survival and mortality rates were calculated by counting the number of emerged adults and recording the number of unhatched eggs. Also, the sex ratio was determined by counting the number of males and females who emerged.

3. Results

The trend of parasitism and daily oviposition of *O. egeria* on both pistachio green stink bug species is shown by a line graph (Fig. 1).

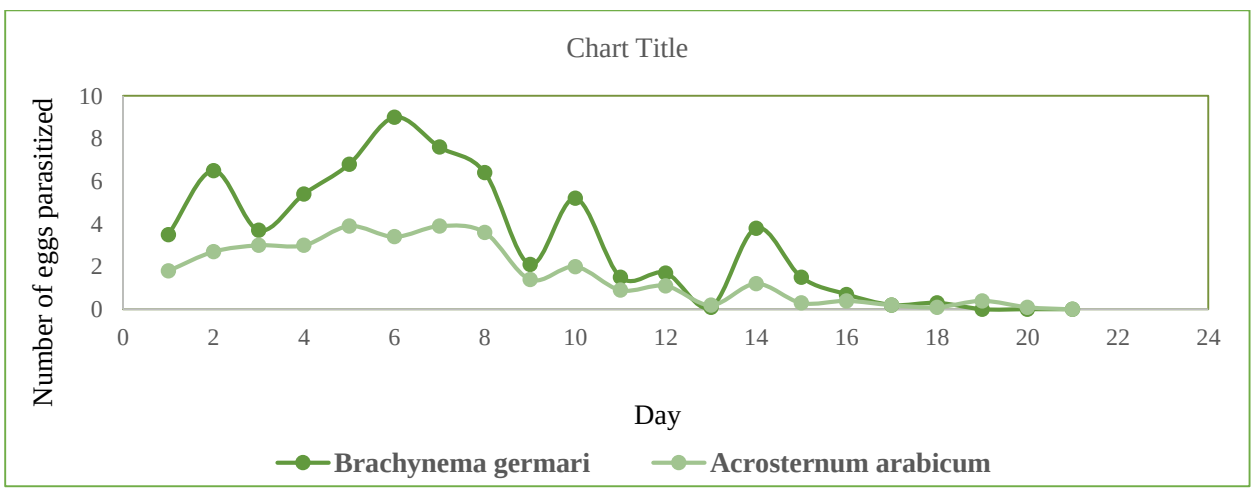

Fig. 1. Daily Oviposition process of *O. egeria* female on eggs of *B. germarii* and *A. arabicum.*

Oviposition started with small amounts on the first day and increased with the age of the females. The highest amount of egg laying on *B. germari* was recorded on the sixth and, on average, on the fifth to seventh days for *A. arabicum*.

The data analysis of the survival and mortality rates has shown a significant difference in these two parameters; accordingly, the highest survival rate and the lowest mortality rate were recorded for the *A. arabicum*. Although the amount of sex ratio in both species of green bugs did not show any significant difference, the highest number of released

females was recorded for *B. germari* (Table 1). The developmental time of *O. egeria* females was similar in both hosts (Fig. 2). The parasitism rate of *O. egeria* was significantly higher on the eggs of *B. germari* (Fig.3).

Table 1. Mortality rate, survival rate and sex ratio of *Ooencyrtous. egeria* on eggs of *Brachynema germari* and *Acrosternum arabicum*

Species	Mortality Rate (%)	Survival Ratea (%)	Sex Ratiob (%)
Brachynema germari	1.26	98.7	55.1
Acrosternum arabicum	0.04	99.6	32.8
df	18	18	18
Tc	2.500	-2.500	2.022
Sig (2-tailed)	0.022	0.022	0.058

[a]Number of eggs with a hole/total number of eggs with stalk.
[b]Number of females/(number of females+males).
[c] Data were arcsin transformed when necessary after running normality test

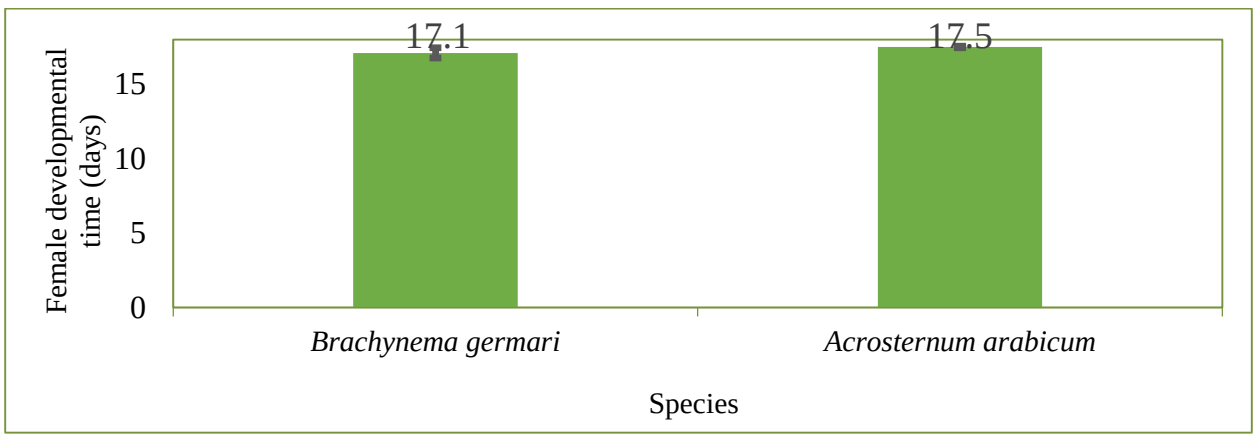

Fig. 2. Developmental time of *O. egeria* female on eggs of *B. germarii* and *A. arabicum* (*t*= 164.56, *df*= 19, *p*= 0.203)

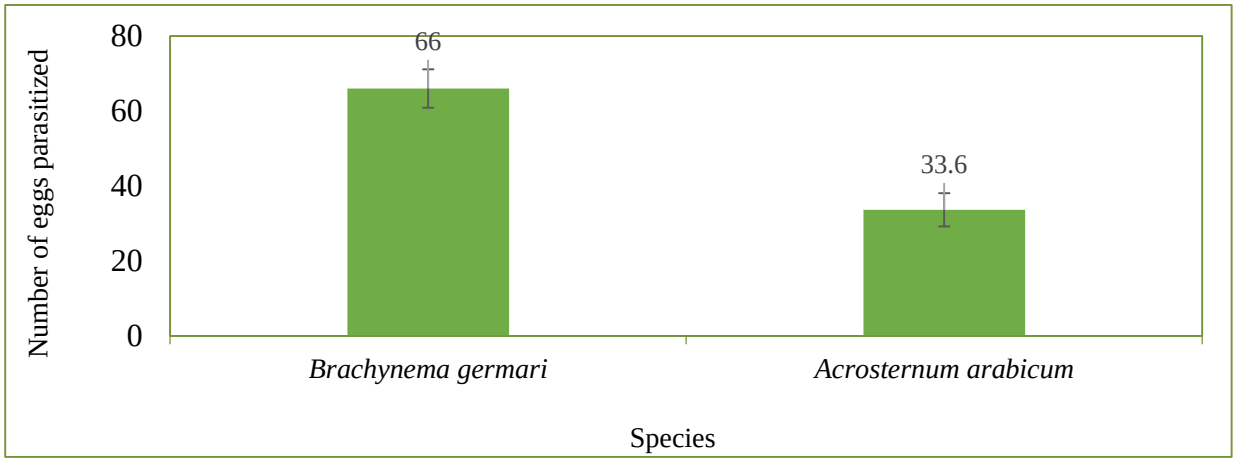

Fig. 3. Total parasitism of *O. egeria* female on eggs of *B. germarii* and *A. arabicum* (*t*= 11.579, *df*= 19, *p*< 0.001).

4. Discussion

Biological information of natural enemies on various hosts seems essential to have a reliable and effective protocol in mass rearing and biocontrol agent releasing.

Parasitism is influenced by various factors of the internal physiology of the wasps, host conditions, and even environmental conditions. When parasitoids approach the host area, the quality of the hosts found must be evaluated before oviposition. The quality of the host is determined by its diagnosis and size, physiological conditions, and the parasitism state. This evaluation increases the efficiency of egg allocation and better offspring production. After the diagnosis of the host species and the stage of life, parasitoids must determine the number of their eggs and sex. The size and age of the host are two important traits related to its quality [11]. Features such as the shape, size, texture, color, and chemical composition of the host play a role in its recognition and acceptance; the most important factor that varies these features can be the host species [12]. The eggs of *B. germari* had better nutritional quality for *O. egeria* females, with the highest parasitism rate compared to those of *A. arabicum*.

In several pieces of research, the effect of different environmental factors and host species on biological and demographic characteristics, diversity, and abundance of species of parasitoid wasps of the genus *Ooencyrtous* has been studied [13-18].

In addition to the effects of the host species, the size of the host egg can affect the body size of the egg parasitoid and its lifespan [19]. The quality of the host egg in terms of longevity is very important in the oviposition behavior of parasitoids. The growth rate of the embryonic stages can be the most crucial factor for the acceptance or non-acceptance of the host egg for parasitism [11, 20].

The number and quality of the host (the size and age), as well as the species and the state of parasitism, are effective factors in determining the sex of the progeny of egg parasitoids. The ability of parasitoid males in the number of matings and transfer of sperm to females can also affect the total number of female offspring [11, 21, 22]. In this study, the number of female

offspring that emerged from *B. germari* eggs was higher than that of *A. arabicum*.

The sex of the progeny and the degree of parasitism are also influenced by the host species and are variable according to the parasitoid species [8, 21]. Various factors, such as temperature or host species, affect the sex ratio. For instance, in the species *O. fecundus*, if the female lays eggs at a temperature of 30 degrees Celsius, all the offspring will be female, but if the egg is laid at a temperature of 35 degrees Celsius, all the offspring will be male. Given the inherent defense capabilities that insect eggs can have against parasitism, for parasitoids, the early stage of the embryonic development of the host is appropriate for parasitism [23]. In the present study, the egg-laying started at a very low level on the first day and gradually increased and reached its maximum on the 5th to 7th days of the life of the female; then, it decreased again and reached zero with age. It seems that *O. egeria* has only a few full eggs, and the rest mature gradually over time and renewed energy. Therefore, feeding on the host's egg contents is one of the key factors during the maturation stage [24].

It is very important to know the most appropriate oviposition age of parasitoids to use in a mass release of biological control agents. For *B.germari*, the maximum amount of egg was recorded on the 6th day, while for *A. arabicum*, it took place between 4 and 7 days of female age.

The oviposition period, the number of eggs, and the life span of the parasitoid in laboratory conditions depend on various factors, such as temperature and relative humidity, with changes in which, for example, the length of the oviposition period and the number of eggs laid by the females change quickly.

The developmental time of a parasitoid is among the most important quality indicators [25]. On the other hand, in situations where the host density is low in nature, the parasitoid with a longer life span can regulate its reproduction; it has a higher power compared to the species with a shorter life span [26].

5. Conclusion

In this study, there was no significant difference between the development of *O. egeria* on eggs of both stink bug species. In general, although the laboratory results show that the survival rate is higher and the mortality rate is lower for *A. arabicum*, *B. germari* is a more suitable host for the rearing of *O. egeria* in laboratory conditions due to the higher parasitism rate and sex ratio.

Acknowledgments

We are grateful to the Vali-e-Asr University of Rafsanjan, Iran, for financial support to HS. (Ph.D. student No. 98368002). This study was funded in part by Biotechnology development council, Tehran, IRAN (grant agreement No biodc-2002585-2002254.1*)*.

Conflict of Interest

The authors of the present research declare that there is no conflict of interest.

Code of Ethics

In this research, no living thing has been used, and all research stages have been conducted in a laboratory.

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