

Research Article

Laboratory evaluation of *Pauesia antennata* (Hymenoptera: Braconidae), specific parasitoid of *Pterochloroides persicae* (Hemiptera: Aphididae)

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Abstract: *Pauesia antennata* Mukerji (Hymenoptera, Braconidae) as the unique parasitoid of the giant brown peach aphid, *Pterochloroides persicae* Cholodkovsky (Hemiptera, Aphididae) is considered to be the most effective biological control agent of this pest. In this study, the assessment of selected biological parameters of *P. antennata* demonstrated that the longevity of mature parasitoid was 3.90 ± 0.22 , the developmental time was 14.48 ± 1.05 and the life span was 19.46 ± 0.68 days. The maximum flight activity and oviposition were observed at the second and third days of the parasitoid lifespan. The parasitism, emergence rates and the sex ratio were affected by the variations in the number of *P. persicae* relative to each population of the parasitoid.

Keywords: Adult longevity, developmental time, sex ratio, host density, parasitoid density, Tunisia, biological control

Introduction

In 1987, the Tunisian stone fruit sector was confronted with an outbreak of an accidentally introduced insect pest, the brown peach aphid, *Pterochloroides persicae* Cholodkovsky (Hemiptera, Aphididae), causing severe crop loss (El Trigui and El-Shérif, 1989; Mdellel *et al.*, 2011; Mdellel and Ben Halima Kamel, 2012). This pest is thought to originated from China and has slowly dispersed to new areas in Asia, North Africa, Europe and North America (Cross and Poswal, 1996; Khan *et al.*, 1998; Stoetzel and Miller, 1998; Ateyyat and Abu-Darwich, 2009). It is an aggregative aphid species, forming huge colonies, mainly on the underside of large branches as well as trunks of young peach trees. The aphids severely

consume the plant sap and also induce the development of sooty moulds, due to the production of copious amounts of honeydew, which reduce the photosynthetic capacity of leaves and fruits causing abnormal size and color (Cross and Poswal, 1996). Worldwide, the control of aphid pests depends mainly on the frequent applications of synthetic insecticides, which often have negative impact on the aphid natural enemies (Sun *et al.*, 1994). Beside the insufficient efficacy of chemical control, it has many negative effects, such as the development of resistance (Gibson *et al.*, 1982) and associated environmental problems, such as pollution of water, presence of toxic residues in food and the effects of pesticides on human health (Laher *et al.*, 2000; Akol *et al.*, 2002; Pavela *et al.*, 2004). Biological control as a major component of integrated pest management, an active alternative method, is not only ecologically, but also economically sound. For a successful biological control of aphids, it is necessary to search for effective natural enemies, particularly

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those that can be used to control aphids on crops (Rabasse and van Steens, 1987). Indeed, some natural enemies have been identified attacking the brown peach aphid, such as a Coccinellidae: *Coccinella algerica* Kovar (Mdellel and Ben Halima Kamel, 2012), entomopathogenic fungi: *Thaxterosporium turbnatum* Kenneth (Entomophthorales, Neozygitaceae) and *Entomophthora turbinata* Kenneth (Entomophthorales, Entomophthoraceae) (Kenneth, 1977; Ben-Zev *et al.*, 1988) and a parasitoid wasp *Pauesia antennata* Mukerji (Hymenoptera, Braconidae) (Cross and Poswal, 1996; Rakhshani *et al.*, 2005). This parasitoid is commonly recorded as predominant natural enemy of aphids in Central Asia (Rakhshani *et al.*, 2005). It is the unique specific parasitoid of the brown peach aphid (Kairo and Poswal, 1995). Kairo and Poswal (1995) and Cross and Poswal (1996) revealed that the lifespan of adult of *P. antennata* is influenced by many factors such as temperature, humidity, food and availability of the host. Little data has been recorded on the fecundity, sex ratio, parasitism emergence rate and its functional responses. These biological parameters should be studied before any biological control program. Indeed, the introduction of *P. antennata* from its area of origin can be very promising for the control of the brown peach aphid. However, the introduction of this parasitoid needs the mass rearing and requires some knowledge on biological parameters in order to have a general idea about the biology of this parasitoid before its release in the field.

In this research, some biological parameters of *P. antennata* reared on *P. persicae* were studied and the effect of host and parasitoid density on parasitism, emergence rate and sex ratio were determined.

Materials and Methods

Stock colony of *Pterochloroides persicae*

Samples of the brown peach aphid, *Pterochloroides persicae* were collected from peach trees in March 2011 and were maintained in the laboratory in the High Agronomic Institute of Chott Mariem in Tunisia. The aphids were reared on peach shoots in KNOP

solution (Knop, 1965) in Plexiglas cages (60 cm × 60 cm), at 21 ± 1°C, a photoperiod 16: 8 (L: D) hours and a relative humidity of 60 ± 10% (Adabi *et al.*, 2010; Mdellel *et al.*, 2011). These aphid colonies were used for rearing of the parasitoid, *Pauesia antennata*.

Stock of *Pauesia antennata*

Fifty mummified individuals of *P. persicae* collected in May 2011 on almond trunks from Iran (Taftan: 28°36'00" N, 61°07'57" E) were reared in laboratory quarantine conditions in Tunisia. After emergence, the parasitoids were released into the cages containing the colony of *P. persicae*. Three droplets of honey were offered on the internal sides of the cage in order to improve the survival and fertility the adult parasitoids (Mondedji *et al.*, 2002).

Flight and oviposition activities

In order to assess the parasitic activity of *P. antennata* during its life period, a newly emerged couple of the parasitoid (aged less than twelve hours) was exposed to the colony of *P. persicae* mostly consisting of fifty to one hundred individuals. In all experiments, final nymph stage and early adult of *P. persicae* were used. These two developmental stages are reported to be the most attacked by *P. antennata* (Abdel Hak, 2001). Two droplets of honey were offered in the cages as complementary food. Parasitoid flight and oviposition activity were followed daily during the lifespan of the females from 6 AM to 10 PM. The parasitoid flight was determined after visual monitoring. The numbers of mummies corresponding to each day were recorded. In total, three colonies of *P. persicae* were treated and the total number of aphids tested was between 150 and 300 individuals.

Bionomics of *P. antennata*

To determine the total developmental time, the longevity, the cumulative fecundity, the parasitism, emergence rates and the sex ratio of *P. antennata*, one couple of the parasitoid was exposed to a colony of about 100 individuals of *P. persicae*. The number of mummified aphids was recorded and then incubated until emergence of the adult parasitoids. The duration of the post embryonic development,

the longevity of females and males and the fecundity were recorded. The parasitism and emergence rates were also determined according to Mondedji *et al.* (2002). At the end of each experiment, the sex ratio of the offspring was also calculated. This experiment was repeated for six couples of the parasitoids, separately.

Impact of aphid density on parasitism

To determine the effect of various host densities on the biological parameters of *P. antennata*, individual wasps were exposed to three different densities of *P. persicae* (D1 = 50, D2 = 100, D3 = 150 aphids per cage). Two droplets of honey were added in the cages as food for adult parasitoids. For each host density, one newly emerged parasitoid couple (aged less than twelve hours) was released into each one of the cages. Aphids were checked daily until mummification. In total, eighteen colonies were treated (six colonies for each aphids density: D1, D2 and D3). The parasitism rate, the emergence rate and the sex ratio were recorded.

Impact of parasitoid density on parasitism

To determine the effect of the parasitoid density on parasitism rate, emergence rate and sex ratio of *P. antennata*, 1, 2 and 3 pair adult parasitoids (male and female) were introduced separately to each *P. persicae* density (D1, D2 and D3) (Table 1). The number of mummified aphids and mummies from which parasitoids had emerged were counted. Also, the sex ratio of the emerged parasitoid was recorded, separately. This procedure was replicated 6 times for each *P. persicae* density, with all series of parasitoid density.

Statistical analysis

For the laboratory assays, the experimental data were statistically analyzed using the SPSS ver. 17 software by one-way analysis of variance followed by SNK test, with statistical significance set at $\alpha = 0.05$. The number of mummified aphids, emerged parasitoid and sex of each emerged aphid for different host densities and parasitoid numbers were also compared.

Results

Flight and oviposition activity

Results revealed that the longevity of adult parasitoids male and female ranged from three to four days. We found that *P. antennata* parasitizes its host mainly in the morning. Indeed, $94 \pm 2.6\%$ of ovipositions were observed between 8.00 AM and 12.00 AM and more than $83.6 \pm 3.4\%$ of ovipositions were made precisely between 10.00 AM and 12.00 AM. A significant difference ($F = 30.18$, $df = 3.00$, $P = 0.011$) was found in the number of eggs laid per hour, during oviposition period of the female parasitoids (Table 2). In the second and the third days, the number of oviposition per hour was respectively 3.66 ± 2.80 and 4.16 ± 1.72 . However, it was 0.16 ± 0.04 and 0.33 ± 0.08 in the first and the fourth day, respectively.

Table 1 The number of *Pterochloroides persicae* and parasitoids, *Pauesia antennata*, used in each treatment.

Number of aphids / cage	Number of couples of parasitoid		
50 (D1)	1	2	3
100 (D2)	1	2	3
150 (D3)	1	2	3

Table 2 Fecundity of *Pauesia antennata* over time during life.

Mean \pm SE (number of eggs / h) ¹			
1 st Day	2 nd Day	3 rd Day	4 th Day
0.16 ± 0.04^b	3.66 ± 2.8^a	4.16 ± 1.72^a	0.33 ± 0.08^b

¹ Values followed by the same letters are not significantly different (SNK test, $P < 0.05$).

Behavioral parameters

The assessed biological parameters of *P. antennata* are shown in Table 2. Mean developmental time of *P. antennata* reared on the last nymphal stage and young adults of *P. persicae* was 14.48 ± 1.05 days, the longevity of adult parasitoids male and female mixed was 3.90 ± 0.22 and the mean generation time was

19.46 ± 0.64 days. The parasitism and the emergence rates were 40.5 ± 12.4% and 36.4 ± 17.2%, respectively. The sex ratio was slightly female biased (0.51 ± 0.04).

Impact of aphid density on parasitism

Impact of the host aphid density on the rate of parasitism, emergence rates and sex ratio of *P. antennata* was assessed. The results are shown in Table 3. The parasitism and the emergence rates decreased with increasing rate of the aphid densities. The parasitism rates were counted as 45 ± 16.1, 36.4 ± 9.9 and 27.5 ± 8.1, for the three densities of *P. persicae*, D1, D2 and D3, respectively. There was a significant difference ($F = 6.18$, $df = 2$, $P = 0.01$) between parasitism rate on aphids densities D1 and D1 (Table 4). The emergence rates were 40.8 ± 1.6, 31.2 ± 11.2 and 27.3 ± 12.2 on D1, D2 and D3 densities, respectively. There was also a significant difference ($F = 11.47$, $df = 2$, $P = 0.02$) of emergence rates in the case of D1, D2 and D3. Our results indicated that the increase of population size has an impact on sex ratio of the emerged parasitoids (Table 4). While, in D1, the sex ratio was slightly male biased (45 ± 3% of emergences were female) it was slightly female biased in the two other aphid densities (53 ± 6% and 54 ± 4% for D2 and D3 respectively). The density of the host had also a significant impact on sex ratio ($F = 8.99$, $df = 2$, $P = 0.03$) between the emergence rate in D1 versus D2 and D3.

Table 3 Biological parameters of *Pauesia antennata* reared on *Pterochloroides persicae*.

Parameters ¹	Mean ± SE
Developmental time (day)	14.48 ± 1.05
Longevity (day)	03.90 ± 0.22
Generation time (day)	19.46 ± 0.68
Parasitism rate (%)	40.50 ± 12.40
Emergence rate (%)	36.40 ± 17.20
Sex ratio (Female: male)	0.510 ± 0.40

¹ 100 individuals of *P. persicae* with one pair of parasitoids was used.

Impact of parasitoid density on parasitism

The assessment of the impact of the density of *P. antennata* on parasitism rate, emergence rate and sex ratio are shown in table 5. The parasitism rates were assessed as 45 ± 16.1, 35.8 ± 5.1 and 31.3 ± 5.3 at 1, 2 and 3 couples of *P. antennata* for D1. A significant difference ($F = 26.33$, $df = 2$, $P < 0.001$) was observed between parasitism rates on D1 using 2 and 3 couples of parasitoids versus one couple. These results prove that the parasitism rate decreases when the parasitoids density increases on D1. Nevertheless, in higher aphid densities (D2 and D3), the parasitism rate was also increased (Table 5). A significant difference of parasitism rates ($F = 15.31$, $df = 2$, $P = 0.001$) was observed on D3 comparing D2 and D1. The emergence rates were 40.8 ± 21.6, 44.1 ± 16 and 47.5 ± 4.5 when a single couple of parasitoid was introduced to D1, D2 and D3 respectively. We detected significant differences between emergence rates ($F = 5.27$, $df = 2$, $P = 0.018$), when 1, 2 and 3 couples of parasitoids were released into D2. A significant difference ($F = 21.47$, $df = 2$, $P = 0.002$) was also observed in emergence rate of the parasitoids when the same densities of female parasitoids were released into D3 of the host aphid. As for sex ratio of emerged parasitoids, no significant difference was observed when we introduced 1, 2 or 3 couples of parasitoids respectively with D1, D2 and D3. Nevertheless, the sex ratio was slightly female biased when aphid's densities increased for different parasitoids densities. Indeed, a significant difference of sex ratio ($F = 8.23$, $df = 2$, $P = 0.04$) was observed when introducing one couple of parasitoids on D1 in comparison with D2 and D3. A significant difference of sex ratio was observed also with 2 and 3 couples of *P. antennata* on D1 in comparison to D2 and D3 ($F = 18.14$, $df = 2$, $P < 0.001$; $F = 54.64$, $df = 2$, $P = 0.002$).

Table 4 Parasitism rate, emergence rate and sex ratio of *Pauesia antennata* reared on *Pterochloroides persicae*.

Parameters ¹	Mean ± SE ²		
	50 (aphids/cage)	100 (aphids/cage)	150 (aphids/cage)
Parasitism rate (%)	45.0 ± 16.1 ^b	36.4 ± 9.90 ^{ab}	27.5 ± 8.10 ^a
Emergence rate (%)	40.8 ± 1.60 ^a	31.2 ± 11.2 ^b	27.3 ± 12.2 ^b
Sex ratio	0.47 ± 0.03 ^b	0.53 ± 0.06 ^a	0.54 ± 0.04 ^a

¹ One pair of parasitoids was used.

² Values followed by the same letter within a row are not significantly different (SNK test, P < 0.05).

Table 5 Impact of parasitoid and aphid host density on parasitism rate, emergence rate and sex ratio of *Pauesia antennata*.

Parameters	Number of aphids/cage	Mean ± SE		
		1 CP	2 CP	3 CP
Parasitism rate (%)	50	45.0 ± 16.1	35.8 ± 5.1	31.3 ± 5.30
	100	36.4 ± 9.90	45.6 ± 4.3	53.0 ± 11.2
	150	27.5 ± 20.7	46.2 ± 3.1	56.6 ± 9.30
Emergence rate (%)	50	40.8 ± 21.6	31.2 ± 1.80	47.5 ± 4.50
	100	44.1 ± 16.0	52.4 ± 6.30	55.2 ± 7.54
	150	47.5 ± 4.50	58.3 ± 2.60	61.2 ± 6.30
Sex Ratio	50	0.48 ± 0.03	0.45 ± 0.02	0.43 ± 0.06
	100	0.53 ± 0.06	0.55 ± 0.04	0.58 ± 0.03
	150	0.54 ± 0.04	0.56 ± 0.09	0.57 ± 0.02

CP: Number of couple of parasitoids.

Discussion

It was evidenced that female individuals of *P. antennata* start oviposition from the first day after emergence with a maximum activity recorded at the second and the third days. According to Cross and Powel (1996) and like other aphidiinae species (Starý, 1970) *P. antennata* is a pro-ovigenic species, since the oviducts contain some mature eggs when emerging. Further eggs are produced throughout their reproductive life and usually females continue parasiting throughout their adult life with maximum activity occurring a couple of days after emergence. We demonstrated also that the longevity of *P. antennata* ranged from 3 to 4 days. Cross and Poswal (1996) showed that *P. antennata* has very short lifespan (5-6 days). The same authors proved that adult parasitoids of *P. antennata* feed on host honeydew which is an essential food source. The longevity of *P. antennata* seems much shorter than that of *Aphidius ervi*

Haliday, which was 12.29 ± 0.43 days at 20°C (Malina and Praslicka, 2008). Longevity also depends on many other factors such as temperature, water availability and the presence of hosts. Similarly, Baghery-Matin *et al.*, (2009) indicated that adult female of *Aphidius sonchi* Marshall, a parasitoid of the sowthistle aphid *Hyromyzus lactucae* (L.) can live longer in the absence of hosts than in their presence. The same authors proved also that females supplied with water and honey were able to live longer than those without honey.

The development time was 14.48 ± 1.05 days. It was longer than of *Aphidius colemani* Viereck which was 13.9 days at 20°C reared on *Aphis gossypii* Glover and shorter than that of *Aphidius ervi* Haliday on *Aphis pomi* deGeer (16.5 days) (Harizanova and Ekbon, 1997). The post embryonic development period can be influenced by many factors such as temperature, humidity and host (Starý, 1988). The impact of temperature on developmental time of *P. antennata* was demonstrated by

Abdel Hak (2001) who proved that this period is 16.4 days at 15°C and 10.5 days at 30°C. Welling *et al.* (1986) assessed also the impact of temperature in *Lysiphlebus testaceipes* (Cresson), the developmental time ranged between 53.53 ± 0.48 at 10 °C to 8.86 ± 0.06 days at 26°C. We demonstrated that, upon introduction of one couple of *P. antennata*, the parasitism and emergence rates decreased when the aphid population densities were high (D2 and D 3). The behavior of *P. antennata* in this experiment is consistent with the classic Holling's type II functional response (Holling, 1959). Indeed, the number of parasitized aphids increased with the increase in aphid density but with a progressively decreasing rate. A type II functional response has also been found in some other parasitoids species such as *Ephedrus cerasicola* Starý (Hymenoptera, Braconidae) released with different densities of *Myzus persicae* (ranging from 1 to 120 individuals) after 24 hours. Similarly, He *et al.*, (2006) demonstrated that the mean number of parasitized aphids and laid eggs during *Aphidius ervi* female's life time increased with the increase of host density and the daily parasitism rate decreased when the host density increased to 50/cylinder. These results indicate that the parasitoid can adjust the oviposition strategy in response to host density. We demonstrated that the release of 2 or 3 couples of *P. antennata* will cause a high parasitism and emergence rates. Similarly, Ben Halima Kamel (2011) demonstrated that the higher parasitism rate was recorded when releasing 4 couples of *L. testaceipes* for a density of 80 individuals of *A. gossypii*. We demonstrated also that sex ratio of *P. antennata* is slightly female biased when *P. persicae* population densities increase. This suggests that the female parasitoid can adjust the sex allocation strategy in response to the host density. A similar case was recorded for *Aphelinus mali* Haliday, which attacks the woolly apple aphid (Mueller *et al.*, 1992). Similarly, He *et al.* (2006) demonstrated that the proportion of female progeny was higher with host densities ranging from 25 to 100 aphids / cylinder. Such information could be

important when considering host parasitoid density ratio in biological control programs.

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ارزیابی آزمایشگاهی زنبور *Pauesia antennata* (Hymenoptera: Braconidae) پارازیتوئید اختصاصی شته *Pterochloroides persicae* (Hemiptera: Aphididae)

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چکیده: زنبور *Pauesia antennata* Mukerji (Hymenoptera: Braconidae) تنها گونه پارازیتوئید شته خالدار هلو، *Pterochloroides persicae* Cholodkovsky (Hemiptera, Aphididae) بوده و به‌عنوان مؤثرترین دشمن طبیعی این آفت شناخته می‌شود. با بررسی خصوصیات بیولوژیک زنبور *P. antennata* مشخص شد که طول عمر حشرات کامل 0.22 ± 0.39 روز، دوره پیش از بلوغ 1.05 ± 1.48 روز و کل دوره زندگی 0.68 ± 1.94 روز می‌باشد. بیش‌ترین فعالیت پرواز و تخم‌گذاری حشره ماده در روزهای دوم و سوم دوره زندگی آن مشاهده شد. درصد پارازیتیسیم، خروج حشرات کامل و نسبت جنسی پارازیتوئید در جمعیت‌های مختلف رهاسازی شده متأثر از تراکم‌های مختلف شته میزبان بود.

واژگان کلیدی: عمر حشرات کامل، دوره پیش از بلوغ، نسبت جنسی، تراکم میزبان، تراکم پارازیتوئید. تونس، مهار زیستی