



# Biological Parameters of *Aphidius smithi* Sharma and Subba Rao (Hymenoptera: Aphidiinae), A Parasite of the Pea Aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae) Under Laboratory Conditions

Tasleem Akhtar<sup>1\*</sup>, Muhammad Farooq Nasir<sup>2</sup>, Imran Bodlah<sup>2</sup> and Muhammad Adnan Bodlah<sup>3</sup>

<sup>1</sup>Department of Plant Protection, Pest Warning and Quality Control of Pesticides Lahore, Pakistan

<sup>2</sup>Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Murree Road, Rawalpindi, Pakistan

<sup>3</sup>Fareed Biodiversity and Conservation Centre, Department of Agriculture Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Punjab, Pakistan

## ABSTRACT

*Aphidius smithi* (Aphidiinae: Hymenoptera) is an important endoparasitoid of pea aphid (*Acyrtosiphon pisum*) which has been utilized to determine the effectiveness of this agent in reducing pest damage. Biology of the *A. smithi* reared on *A. pisum* in the laboratory at 23±1°C has been studied. The development cycle of *A. smithi* from larvae to adult was completed in about 11 days. The pre-mating period of males (n=10) varied between 5 and 8 min (mean: 4 min). Copulation time (n = 10 pairs) was between 33 and 55 sec (mean: 45.2 sec). Oviposition time (n = 10 females) was between 1 and 2 sec (mean: 1.5 sec). Female parasitoids lived longer (5.75 days) than male parasitoids (4 days) when offered honey and water as a food diet. Lifespan of adult male and females was shorter i.e. 2.25 and 2.75 days respectively when fed upon dissected aphid. When adult parasitoids were released on pea plant provided with pea aphid, mean period of life for male and female was 4.75 and 6 days respectively. Sex ratios of field collected mummies were female biased (60%). Two species of hyperparasitoids viz. *Asaphes suspensus* (68%) and *Pachyneuron aphidis* (54%) were involved. Examination for phenotypic polymorphism showed that field population of *A. smithi* contained both dark and light pigmentation pattern of abdominal segments while laboratory reared samples were only dark pigmented in both sexes. The findings of this study can help in defining strategies for the rearing to release this parasitoid in biological control programs against *A. pisum* in Pakistan.

## Article Information

Received 28 March 2022

Revised 25 April 2022

Accepted 17 April 2022

Available online 11 August 2022 (early access)

## Authors' Contribution

TA and MFN planned and performed the experiment. IB identified the hyper parasitoids, helped in the data analysis and write up of the paper.

## Key words

*Aphidius smithi*, *Acyrtosiphon pisum*, Aphid parasitoid, *Aphidius* biology, Phenotypic polymorphism

## INTRODUCTION

Aphids are important pests of cultivated crops in Pakistan. They not only reduce the yield of crops but also serve as vectors of disease. Irshad (2001) mentioned

that about 92 species of aphids are in Pakistan. Naumann-Etienne and Rемаудиере (1995) listed 300 different species of aphids in various ecological zones of Pakistan from different host plants. Additionally, many studies (Hassan *et al.*, 2010; Bodlah *et al.*, 2011, 2017; Amin *et al.*, 2017a, b; Kanturski *et al.*, 2017; Maryam *et al.*, 2019) have added many new records from Pakistan but still no formal updated list of aphid fauna is available. Among different aphid species, *Acyrtosiphon pisum*, commonly known as the pea aphid, is a sap-sucking insect in the Aphididae family. It is considered as the model aphid species as its reproductive cycle, including the sexual phase and the overwintering of eggs can be easily completed on host plants under laboratory conditions (Aqueel *et al.*, 2014). Considering their economic importance towards agricultural crops, it is

\* Corresponding author: [taslstar@yahoo.com](mailto:taslstar@yahoo.com)  
0030-9923/2022/0001-0001 \$ 9.00/0



Copyright 2022 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

necessary to adopt timely control measures in order to avoid losses caused by these tiny creatures. In Pakistan, aphids have mostly been controlled by insecticidal applications. However due to adverse effects of insecticides, total reliance on them cannot be made (Irshad, 2001). So, there is a need to design a complete integrated pest management (IPM) strategy to overcome the side effects of pesticides. In IPM programme, the role of parasitoids and predators are very important (Snyder and Ives, 2003). Predators and parasitoids both are playing an important role in successful control of aphids but aphid parasitoids have achieved more success than predator's *viz.* 21.8% and 4.1%, respectively (Hirose, 2006) and commonly used in biological control programs in greenhouses and field situations (Boivin *et al.*, 2012). In Pakistan, the importance of biological control has been discussed by Irshad (1987) and Mohyuddin (1981). Biological control work on aphids in Pakistan has also been reported (Alam *et al.*, 1969; CIBC, 1977; Habib, 1973; Hamid, 1983; Hamid *et al.*, 1974; Khalil *et al.*, 1990; Mughal and Munshi, 1985; Mustafa *et al.*, 1996; Suhail *et al.*, 1999). *A. smithi* is native to Pakistan and India (Sharma and Subbarao, 1958) but still has not been reared for controlling pea aphid in Pakistan. Current studies would provide basis for its possible utilization as a bio-control agent for the management of pea aphid in Pakistan. In Pakistan, both pea aphid and its parasitoid have been reported (Starý *et al.*, 1998; Naeem *et al.*, 2005). Several studies have been done on mass rearing of *A. smithi* and utilizing of these natural enemies in the field in parts of the world but poor studies has been done in Pakistan.

To serve as a baseline of information in our efforts to determine the effectiveness of *A. smithi* as a biological control agent of *A. pisum*, we studied various developmental stages of *A. smithi* on its host, adult longevity, host age preference for parasitism, sex ratio, pre-oviposition time and oviposition behavior, pre mating time and mating time, sex ratio of field collected mummies at laboratory conditions, mummy coloration and position of emergence hole and phenotypic differences of its field population. The present study will help the future workers of Pakistan to utilize this parasitoid in integrated pest management programs as a source of effective biological control agents for pea aphid management under the field conditions as well as in green houses.

## MATERIALS AND METHODS

### *Study site*

Present investigations were carried out at biological control laboratory of Entomology Department, PMAS-Arid Agriculture University, Rawalpindi during 2012-13. Pea crop was sown with double rows spaced at least 12

to 18 inches apart over an area of 1 acre for collection of aphids, parasitoids and hyperparasitoid. All recommended agronomic practices were applied to the crop.

### *Rearing of Acyrthosiphon pisum*

Adult females of *A. pisum* were collected from the field of pea. In laboratory, females aphids (n=50) were released on the leaves of pea plants (n=6) in order to maintain aphid culture. Aphids of the same age (1<sup>st</sup> instars to 4<sup>th</sup> instars) were maintained and used for further experiments.

### *Rearing of Aphidius smithi*

Both male and female individuals of *A. smithi* were collected from field of pea plants and reared on *A. pisum* in biological control laboratory of Entomology Department, at 23±1°C. Male and female individuals of *A. smithi* were released in separate glass jars and kept there for mating. Hundred aphids (3<sup>rd</sup> instars obtained from aphid culture) were placed in 4 glass jars, containing pea plant leaves, covered with muslin cloth. Three mated females were taken and released in glass jar with aphids for 48 h. They were provided with 10% honey solution soaked in cotton. Mummified aphid on the pea plant leaves were collected from glass jars after 4-5 days and put in small gelatinized capsules (2×0.5cm) until emergence. Emerged individuals were again used to maintain culture of *A. smithi*.

### *Life cycle of Aphidius smithi*

In order to study the life cycle of *A. smithi*, 500 aphids (3<sup>rd</sup> instars of *A. pisum* from aphid culture) were nourished on pea plant leaves in four glass jars. Each jar was provided with fresh pea plant leaves after two days. Five mated females of *A. smithi* (from maintained culture) were released in jars for a period of 48 h. After removal of females from the jar, mummified aphids (at least 10 from each jar) were also removed after every 24 h for dissection. Ten mummified aphid from each jar were taken on daily basis and dissected daily under the microscope (Swift SM-80 with magnification 2xs and 4xs). Color photographs of each developmental stage were also taken. Morphological variations in each stage from larval instars to adult stage were noted and snapped under Nikon microscope. Time taken by each stage was noted in days.

### *Host age preference for parasitism*

To check the effect of parasitism on aphid age, 1-day old, 2 days old, 3 days old and 4 days old were secured from maintained culture of aphids. Each age group was replicated three times in Petri plates supplied with 20 aphids reared on pea plant leaves. Three mated females of *A. smithi* from maintained culture were released in each replicate and remained for their whole life. The parasitoid

was given with honey and moistens cotton. The mummies were counted at each second day after ten days of release in Petri dishes and then removed from leaf.

#### *Effect of different diets on parasitoids longevity*

In the first treatment, four replications of five *A. smithi* individuals both males and females were released in Petri plates supplied with artificial diet (honey + water on cotton wool). Adults were allowed to feed on artificial diet until death. Numbers of days of insect life were counted from 1<sup>st</sup> day of release till death. In the 2<sup>nd</sup> treatment, five individuals of *A. smithi* were released for their whole life time (until death) on potted plants of pea with 3<sup>rd</sup> instars nymphs of *A. pisum* in laboratory. Potted plants were wrapped with polythene bags. Polythene bags were provided with a piece of muslin cloth for ventilation. Number of days of life was counted from 1<sup>st</sup> day of release of adult until death. In the 3<sup>rd</sup> treatment, five females and males were allowed to feed on dissected *A. pisum* in Petri plates with four replications. Number of days of life was counted from 1<sup>st</sup> day of release of adult until death.

#### *Determination of pre-mating time, copulation time, pre-oviposition time*

In order to observe pre-mating time and copulation time, ten observations were made by releasing 10 males and 10 females of *A. smithi* in Petri dishes plates. Pre-mating and mating time were noted using stop watch in seconds. Similarly, pre oviposition time was also noted for ten mated females when released in Petri dishes containing 3<sup>rd</sup> instars aphid (*A. pisum*) with three replicates.

#### *Male and female population of field collected parasitoids and hyperparasitoid*

For determination of male and female population of field collected mummies, about 200-500 aphid mummies were collected after every 5 days and reared under laboratory conditions till the emergence of parasitoids and hyperparasitoids. Emerged parasitoids and hyperparasitoids were separated into males and females to determine the effect of hyperparasitism on sex ratio.

#### *Mummy coloration and position of emergence hole*

Mummies were observed under binocular microscope for their colouration and position of emergence hole made by parasitoids and hyperparasitoids and coloured photographs were snapped.

#### *Determination of phenotypic differences of field population of Aphidius smithi*

Phenotypic differences of field collected population as well as laboratory collected population of *A. smithi*

were observed under microscope and snapped.

## RESULTS

#### *Life stages of Aphidius smithi*

Various life stages of *A. smithi* were studied on *A. pisum* in the laboratory at 23±1°C. During oviposition, mated female of *A. smithi* laid eggs singly in the adipose tissues of aphid. Parasitized aphid continued feeding and remains attached to the leaves of host plant after oviposition (Fig. 1A). Mummified aphid changed into golden yellow (Fig. 1B). After oviposition period, hatching started after 72±1h, blackish larva was observed on feeding of soft adipose tissue in the aphid's metasoma (Fig. 1C). After 96±1h, dissections showed that blackish larva changed into yellow colour (Fig. 1D). On 5<sup>th</sup> day of development, larva was still yellowish in colour but it was entirely changed from the 4<sup>th</sup> day larva, head, mesosoma and metasoma were clearly differentiated (Fig. 1E). Other body parts like antennae, legs and wings started to develop but transparent in colour (Fig. 1F). There were four larval instars completed inside the mummified aphid. The metasoma was cleaned out then mesosoma and head. On 6<sup>th</sup> day, larva started segmentations. Head and mesosoma changed into dark colour; antennae and wings were not completely developed at that time (Fig. 1G). On 7<sup>th</sup> day, no considerable changes occurred in the larva. On 8<sup>th</sup> day of development, larva changes into pre pupae stage (Fig. 1H). On 9<sup>th</sup> day, larva turned into pupa (Fig. 1I). On the 11<sup>th</sup> day, pupa changed into mummy (Fig. 1J). At the same day, adult emerged out by making an opening in the metasoma of the host (Fig. 1K). Total life cycle of *A. smithi* inside the host (*A. pisum*) from oviposition to emergence was completed in 11<sup>th</sup> day (Table I).

**Table I. Development duration of *Aphidius smithi* when reared on *Acyrtosiphon pisum*.**

Biological parameters	n	Mean ± SE
Hatching period (h)	3	81±1.45
Ovi-position to mummification time (h)	3	80±8
Pupal period (h)	3	177±5.10
Ovi-position to emergence time (h)	3	245±18.17

#### *Host age preference for parasitism*

Percent parasitism was extensively dependent on aphid age (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars) being used. Maximum parasitism was observed in 3<sup>rd</sup> instars of *A. pisum* (83.44%) followed by 4<sup>th</sup> instars who had 65.22% parasitism. Aphids of 1<sup>st</sup> and 2<sup>nd</sup> instars showed 24.88-40% parasitism (Fig. 2).

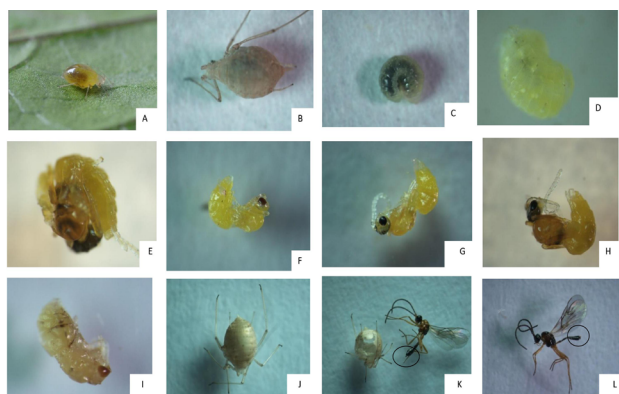


Fig. 1. Life cycle of *Aphidius smithi* on *Acyrthosiphon pisum*; parasitized aphid (A), mummified aphid (B), newly hatched (C), later stage (D), 4<sup>th</sup> days old larvae (E), 5<sup>th</sup> days old larvae (F), 6<sup>th</sup> days old larvae (G), 8<sup>th</sup> day pre pupae stage (H) and on 9<sup>th</sup> changed into pupae (I). fully developed mummy (J), pupae changed into adult female at 11 days and emerged from host on the same day (K), male adult parasitoids (L).

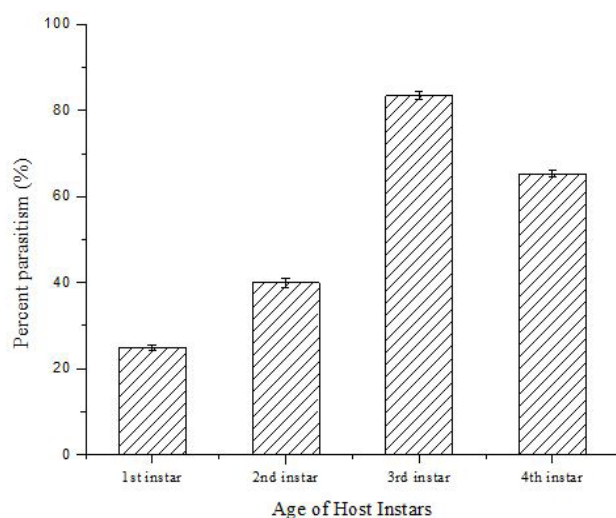


Fig. 2. Comparison of different aphid parasitized by *A. smithi* female in relation to host age (mean values with SD, n=20).

#### Adult longevity

Data analysis showed that diet had a significant effect on adult parasitoids longevity. In first treatment when honey solution was used as food, the average life of male and female was 4 and 5.75 days, respectively. In second treatment, mean life of male and female was 2.25 and 2.75 days, respectively when fed upon dissected aphid. While in the 3<sup>rd</sup> treatment, when adult parasitoids were released on pea plants provided with pea aphid as a host to feed on

honey dew, their longevity was relatively longer than other artificial food therefore mean life of male and female was 4.75 and 6 days, respectively (Fig. 3).

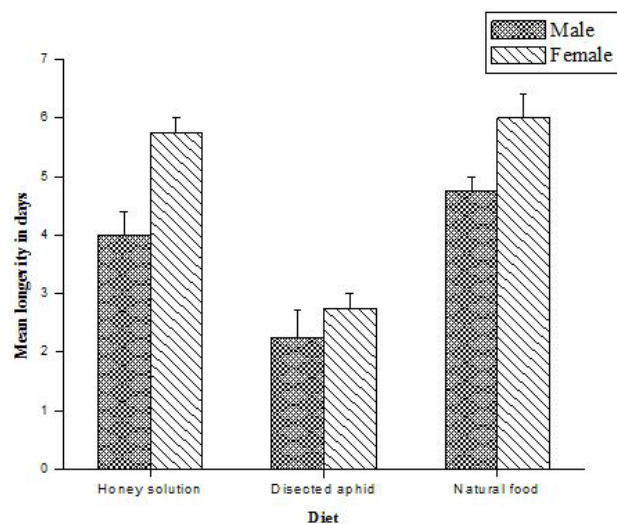


Fig. 3. Effect of different diet sources on adult longevity of *Aphidius smithi* (mean values with SD, n= 5).

#### Pre-mating time, copulation time, pre-oviposition time and oviposition time

One or two males copulated with virgin females within few minutes of emergence while others showed no attraction towards the virgin females until 2 to 3 h after emergence. Mostly pre-mating time of males (n= 10) was between 5 and 8 min with average time of  $4 \pm 0.53$  min. however, it was longer in females. The majority of females rejected all copulatory attempts at least two h after emergence. When newly emerged females were confined with males for a period of 12 h, all were started to be mated i.e., they produced offspring of both sexes. Copulation time (n= 10 pairs) was between 33 and 55 sec with mean value of  $45.2 \pm 2.63$  sec. Pre-oviposition period was between 1 and 8 min with mean value of  $2.8 \pm 0.65$  min while its oviposition time was between 1 and 2 sec with mean value of  $1.7 \pm 0.21$  sec (Table II).

Table II. Various biological parameters of *Aphidius smithi* at  $23^\circ\text{C} \pm 1$ .

Biological Parameter	n	Mean $\pm$ SE
Pre- mating time of male (Min)	10	$4 \pm 0.53$
Mating time of male (Sec)	10	$45.2 \pm 2.63$
Pre-oviposition time (Min)	10	$2.8 \pm 0.65$
Oviposition time (Sec)	10	$1.7 \pm 0.21$



#### Male and female population of aphid parasitoids and hyperparasitoids in field conditions

A total of 1855 mummies were collected from the field throughout experiment. Out of them 744 were the males (40%) and 1111 were the females (60%) (Table III). A total of 417 aphid mummies were found to have hyperparasitism. Two species of hyperparasitoids viz. *Asaphes suspensus* and *Pachyneuron aphidis* were involved. Sex ratio of these two species was female biased. Out of 417 hyperparasitoids, 257 (62%) were females and 160 (38%) were males. *A. suspensus* was the most abundant hyperparasitoid (68%) which parasitized *A. smithi* while *P. aphidis* was the least numbers (54%) (Table IV).

**Table III.** Sex ratio of field collected parasitoids during 2012-13.

Sex ratio	Collected parasitoid	Percentage	Average±S.E
Male	744	40	37.2±4.30
Female	1111	60	55.55±7.13

**Table IV.** Species wise sex ratio of field collected aphid hyper parasitoids during 2012-13.

Name of species	Female (%)	Male (%)	Total
<i>Asaphes suspensus</i>	155 (68%)	72 (32%)	227
<i>Pachyneuron aphidis</i>	102 (54%)	88 (46%)	190

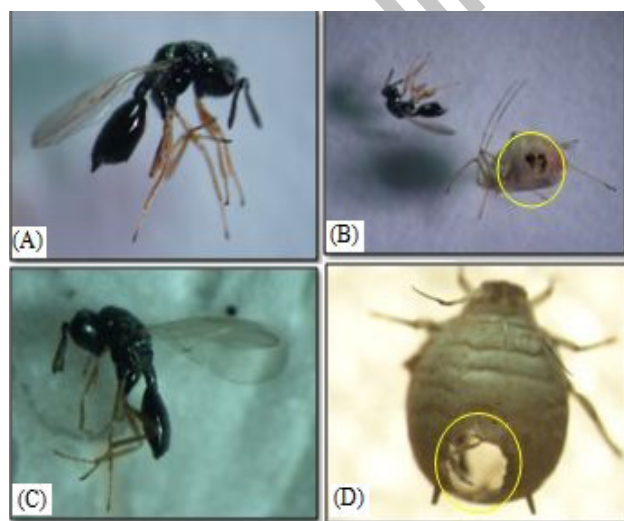


Fig. 4. Mummy coloration and position of emergence hole of hyperparasitoids with its respective species. *Pachyneuron aphidis* (A, B) and *Asaphes suspensus* (C, D).

#### Mummy colouration and position of emergence hole of hyperparasitoids

Colour photographs of hyperparasitoids mummies with emerging hole were taken under Nikon microscope. From light yellowish mummy of *A. suspensus*, adult hyperparasitoid produced an apical irregular hole at the middle end (Fig. 4A, B) while from the whitish yellow mummy of *P. aphidis*, adult hyperparasitoid made an irregular hole on the left side above cornicle (Fig. 4C, D).

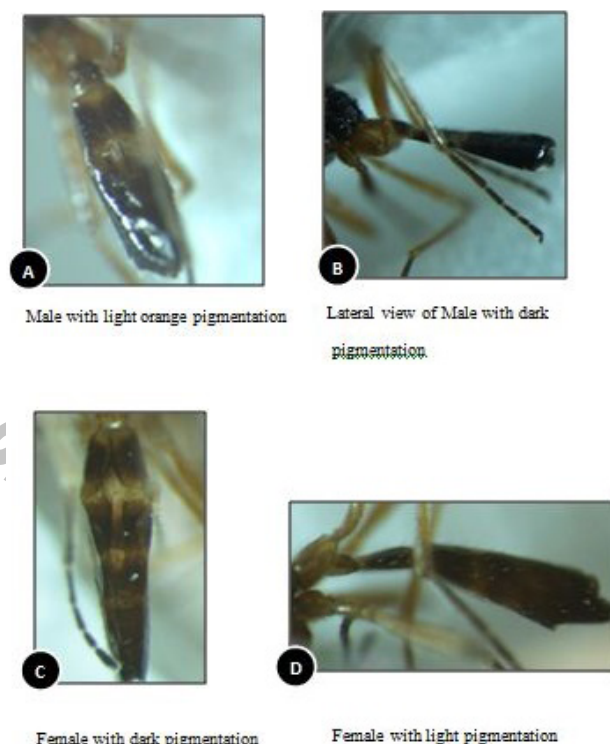


Fig. 5. Phenotypic polymorphism in field population of *Aphidius smithi*.

#### Phenotypic polymorphism

Field population of *A. smithi* contained both dark and light pigmentation pattern of abdominal segments while laboratory reared samples were only dark pigmented in both sexes. Field reared dark phenotype contained all abdominal segments pigmented with regular bands while light phenotype with bright orange pigmentation (Fig. 5A-D). Phenotypic polymorphism in laboratory reared population of *A. smithi* resulted in a single pigmentation pattern of both genders (Fig. 6A-B).

## DISCUSSION

Biological control agents are often first tested under laboratory condition to evaluate their potential for success

(Kalyebi *et al.*, 2015). Parasitic natural enemies of aphids such as *Aphidius* spp., have been successfully used in the suppression of aphid populations (Van, 2012). Our study constitutes the developmental time, adult longevity, coloration and position of emergence hole of mummified aphid, phenotypic differences, parasitism rate, and sex ratio of *A. smithi*.



Fig. 6. Phenotypic polymorphism in laboratory reared population of *Aphidius smithi*.

Successful introduction of a biological control agent such as parasitoids requires knowledge of its lifecycle and interactions with the host (Mutitu *et al.*, 2013). In the present research, various life stages of *A. smithi* on *A. pisum* were studied in the laboratory at  $23\pm 1^{\circ}\text{C}$ . Total life cycle of *A. smithi* inside the host (*A. pisum*) from oviposition to adult emergence was completed in 11 days (Table I) similarly to other *Aphidius* species at  $20\text{--}22^{\circ}\text{C}$  (Kalule and Wright, 2004; Dhiman, 2006; Colinet *et al.*, 2005). The development behavior of *A. smithi* was also similar to the results given by Starý (1970) for *Aphidius* species. Adult female laid eggs singly in the host. The hatching larva lived inside the body cavity of host and used all material leaving just exoskeleton of aphids. Larva made a clean circular hole with one of its mandibles in the Venter of aphid body and fixed the later to the plant part with a silk-like secretion. The larva pupated within the aphid body and dead aphid changed into a mummy. The adult parasitoid cut with its mandibles a circular hole in abdominal part of aphid, usually between the siphunculi. The colour and form of the mummies and the form of the emergence hole may be used to some extent for identification of the most abundant species of Aphidiinae attacking known hosts on a certain crop (Powell, 1982). Aphidiinae parasitoids exhibit a well integrated symbiotic

relation with their host during larval development of *A. smithi* in pea aphid feeding on holidic diets (Cloutier, 1978, 1986; Cloutier and Mackauer, 1979).

In biological control, host instars selection by a parasitoid is among the most important factors that affect its potential to reduce an aphid population (Hågvar and Hofsvang, 1978). In the present investigation, maximum parasitism was observed in 3<sup>rd</sup> instars of *A. pisum* (83.44%) followed by 4<sup>th</sup> instars (65.22%) than 1<sup>st</sup> and 2<sup>nd</sup> instars (Fig. 2) As the 1<sup>st</sup> instars (1-day) showed higher mortality because they are more fragile than 3<sup>rd</sup> and 4<sup>th</sup> instars aphids. Reason interrelated with the mortality of 1<sup>st</sup> instars (1-day aphid) might be more susceptible to injuries during oviposition as an effect of venom or sting injection. As compare to the adult aphids that did not have such type of issues from parasitoids. *A. smithi* generally preferred older aphids which were larger in size and age than other Aphidiinae species which are usually prefer small or medium sized aphid nymphs (Perdikis *et al.*, 2004). All our observations also agreed with the results of (He *et al.*, 2005; Lin and Anthony, 2003). Therefore, the present study indicates that host preference could be an important factor for effectiveness of *A. smithi* against *A. pisum*.

Successful biological control is to some extent dependent on the longevity of beneficial insects. Availability of carbohydrates in adipose tissue of host can improve the nutrition of parasitic insects, and thereby increase their longevity. Evidence suggests that individual fitness benefits afforded by food sources are important for a time-limited parasitoid (Williams and Roane, 2007). Providing parasitoids with food will result in increased longevity and subsequent parasitism rates (Irvin *et al.*, 2007). Longevity is generally influenced by searching activity, mating, oviposition, body size, temperature, humidity, photoperiod and diet (Jervis and Copland, 1996). In present study, food provision of female parasitoids affected their longevity significantly. The adult parasitoid of *A. smithi* lived shorter when fed only on water and honey solution compared to those kept with pea aphid as a host to feed on honey dew, their longevity was relatively longer than other artificial food therefore mean life of male and female was 4.75 and 6 days, respectively. Development time of females was greater than of males on all host diet. Present study revealed that nutritional needs of parasitoids on artificial diets are not always efficient because of low nutritional quality. On the contrary, the importance of host feeding and natural food supplied to parasitoids by host species that is related to the long lasting host parasitoid interactions still maintain its validity and reliability. These results are in conformity with the already recorded observations of Bodlah *et al.* (2012) on *Diaeretiella rapae* who stated that female lived longer ( $11.1\pm 0.16$  days) than males ( $9.4\pm 0.18$  days). Adult

longevity of parasitoids was even shorter when they were kept with dissected aphid (Rakhshani, 2001). Female longevity of *Aphidius gifuensis* is also greater than male (Chi and Su, 2006).

In our study, average pre-mating time of males was 4 min; however, it was longer in females at least two h after emergence. Average copulation time was 45.2 sec. Mean pre-oviposition period was 2.8 min while its oviposition time was between 1 and 2 sec (Table II). All our observations agreed with the observations of Sheng and Carver (1985).

Male and female population of field collected mummified aphid were female biased. Females were more in number as compared to the males because they consisted of 59.89% of the total collected mummies from the field (Table III). It has been determined in many studies that hymenopterous females regulate their sex ratio in response to variety of ecological (availability of food, temperature etc.) and biological factors (parasitoid age, host size, host age, host density etc.) (Medeiros *et al.*, 2006; Bogdanovic *et al.*, 2009; Yu *et al.*, 2003; Fuester *et al.*, 2003; Garcia-Medel *et al.*, 2007). Similar results were found by Stary (1988) who reported that population of parasitoids under field condition was female biased. It was also confirmed by King (1987) that the sex ratio of adult wasp under field conditions was female biased. Our results are also correlated with the study of Kos *et al.* (2008) who documented that population of several species of aphid parasitoid was female biased. *Aphidius* parasitoids frequently produce female biased sex ratios early in adult life (He and Wang, 2006). Two species of hyperparasitoids *viz.* *Asaphes suspensus* and *Pachyneuron aphidis* were identified. *A. suspensus* was the most abundant hyperparasitoid species (54%) which parasitized the *A. smithi* while *P. aphidis* was in least numbers (46%) (Table IV).

Phenotypic polymorphism involving coloration or pigmentation pattern was examined in field population of *Aphidius smithi* by Mackauer (1968). Our results are in line with Mackauer (1968) with reference to field population with both dark and light pigmentation pattern of abdominal segments. Phenotypic differences were more distinct in female gender. The difference of pigmentation in field population may be due to continuous fluctuation in temperature and humidity of the field environment which were constant under laboratory condition and resulted in a single pigmentation pattern in laboratory reared samples of both genders.

As pea aphid is a serious pest in Pakistan and many other countries of the world. So our standardized parameters will not only helpful for only the mass rearing of *A. smithi* in Pakistan but other countries too. After

mass rearing in bio-control laboratories, this parasitoid like other bio-control agents, would be the part of IPM programs under the field conditions as well as in green houses in Pakistan.

## ACKNOWLEDGEMENT

We are grateful to Department of Entomology, PMAS Arid Agriculture University Rawalpindi for providing Biological control laboratory facility, research area and technical facilities.

### Statement of conflict of interest

The authors have declared no conflict of interest.

## REFERENCES

- Alam, M.M., Beg, M.N., Syed, R.A., and Shah, S., 1969. *Survey of parasites of insect pests of cultivated and useful plants and survey of insect destroying weeds and their parasites*. Final Report, Pakistan Station. Commonwealth Institute of Biological Control, pp. 243.
- Amin, M., Mahmood, K., and Bodlah, I., 2017a. Aphids (Homoptera: Aphididae) infesting medicinal and aromatic plants in the Poonch Division of Azad Jammu and Kashmir, Pakistan. *J. Anim. Pl. Sci.*, **27**: 1377-1385.
- Amin, M., Mahmood, K., Bodlah, I., and Khan, M.R., 2017b. New additions to Pakistan's Aphididae (Hemiptera: Aphidoidea) damaging *rosa* species. *Sarhad J. Agric.*, **33**: 511-518. <https://doi.org/10.17582/journal.sja/2017/33.4.511.518>
- Aqueel, M.A., Abu-Bakar, M.R., Rashad, M.B., Muhammad, A.S., Irfan, M., Muhammad, M.J., and Simon, R.L., 2014. Tritrophic interactions between parasitoids and cereal aphids are mediated by nitrogen fertilizer. *Insect. Sci.*, **6**: 813-820. <https://doi.org/10.1111/1744-7917.12123>
- Bodlah, I., Bodlah, M.A., and Hussain, M., 2017. New distributional records of aphid, *Melanaphisdonacis* (Passerini) in Osia and surrounding areas, Punjab. *Pak. J. appl. Agric. Biotechnol.*, **2**: 48-51.
- Bodlah, I., Naeem, M., and Mohsin, A.U., 2011. Checklist distribution host range and ecology of Aphidoidea (Homoptera) from the rainfed region of Punjab province of Pakistan. *Sarhad J. Agric.*, **27**: 93-101.
- Bodlah, I., Naeem, M., and Mohsin, A.U., 2012. Distribution, hosts and biology of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae:



- Aphidiinae) in Punjab, Pakistan. *Pakistan J. Zool.*, **44**: 1307-1315.
- Bogdanovic, A.I., Ivanovic, A., Tomanovic Z., Zikic, V., and Stary, P., and Kavallieratos, G., 2009. Sexual dimorphism in *Ephedra persicae* (Hymenoptera: Braconidae: Aphidiinae): Intra specific variation in size and shape. *Can. Entomol.*, **141**: 550–560. <https://doi.org/10.4039/n09-029>
- Boivin, G., Hance, T., and Brodeur, J., 2012. Aphid parasitoids in biological control. *Can. J. Pl. Sci.*, **92**: 1-12. <https://doi.org/10.4141/cjps2011-045>
- Chi, H., and Su, H.Y., 2006. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. *Environ. Ent.*, **35**: 10–21. <https://doi.org/10.1603/0046-225X-35.1.10>
- CIBC, 1977. *Natural enemies of graminaceous aphids*. Final Report Pakistan Station, Common wealth Institue of Viological Conrol, pp. 17.
- Cloutier, C., 1978. *A study of the host-parasite association between the pea aphid, Acyrthosiphon pisum (Hemiptera: Aphididae), and some of its hymenopteran parasites: Growth and food utilization during parasitism*. Ph. D. thesis, Simon Fraser University.
- Cloutier, C., 1986. Amino acid utilization in the pea aphid *Acyrthosiphon pisum* infected by the parasitoid *Aphidius smithi*. *J. Insect Physiol.*, **32**: 263–267. [https://doi.org/10.1016/0022-1910\(86\)90037-5](https://doi.org/10.1016/0022-1910(86)90037-5)
- Cloutier, C., and Mackauer, M., 1979. The effect of parasitism by *Aphidius smithi* (Hymenoptera: Aphidiidae) on the food budget of the pea aphid, *Acyrthosiphon pisum*. *Can. J. Zool.*, **57**: 1605–1611. <https://doi.org/10.1139/z79-210>
- Colinet, H., Salin, C., Boivin, G., and Hance, T., 2005. Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecol. Ent.*, **30**: 473-479. <https://doi.org/10.1111/j.0307-6946.2005.00716.x>
- Dhiman, S.C., 2006. *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) a potential biocontrol agent of mustard aphid, *Lipaphis erysimi* (Kalt.). *Ins. Environ.*, **3**: 101-109. **(Please check name of journal)**.
- Dolphin, K., and Quicke, B.L.J., 2001. Estimating the global species richness of an incompletely described taxon: An example using parasitoid wasps (Hymenoptera: Braconidae). *Biol. J. Linn. Soc.*, **73**: 279-286. <https://doi.org/10.1111/j.1095-8312.2001.tb01363.x>
- Fuester, R.W., Dunning, K.S., Taylor, P., and Ramaseshiah, G., 2003. Male biased sex ratio in *Glyptapanteles flavicoxis* (Hymenoptera: Braconidae), a parasitoid of gypsy moth (Lepidoptera: Lymantriidae). *Annls entomol. Soc. Am.*, **96**: 553–559. [https://doi.org/10.1603/0013-8746\(2003\)096\[0553:MSRIGF\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2003)096[0553:MSRIGF]2.0.CO;2)
- Garcia-Medel, D., Sivinski, J., Diaz-Fleischfr, F., Ramirez-Romeror, M., and Aluja, M., 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single or multiple-species cohorts in field cages: Influence of fruit location and host density. *Biol. Cont.*, **43**: 12–22. <https://doi.org/10.1016/j.biocontrol.2007.06.008>
- Habib, R., 1973. Natural enemies and their role in limitation and control of *Brevicorne brassicae* L. in Pakistan. *Tech. Bull. Commonw. Inst. biol. Contr.*, **16**: 69-78.
- Hagen, K.S., and Schlinger, E.I., 1960. Imported Indian parasite of pea aphid established in California. *Calif. Agric.*, **14**: 5-6.
- Hagvar, E.B., and Hofsvang, T., 1978. Aphid parasitoids (Hymenoptera, Aphidiidae): Biology, host selection and use in biological control. *Biocont. News Inf.*, **12**: 13-41.
- Hamid, S., 1983. Natural balance of *graminicolous* aphids in Pakistan. *Surv. Popul. Angron.*, **3**: 665-673. <https://doi.org/10.1051/agro:19830708>
- Hamid, S., Shah, M.A., and Anwar, A.M., 1974. Investigations on *Acyrthosiphon pisum* Harris with special reference to its parasite *Aphidius smithi* Sharma and Subba Rao. *Tech. Bull. Commonw. Inst. biol. Contr.*, **17**: 69-85.
- Hassan, A.S., Rafi, M.A., Javed, H., Zia, A., Naeem, M., Khan, I.A., and Bilal, H., 2010. Aphidoidea (Homoptera) from the northern areas of Pakistan. *Sarhad J. Agric.*, **26**: 609-611.
- He, X.Z., and Wang, Q., 2006. Asymmetric size effect of sexes on reproductive fitness in an aphid parasitoid *Aphidius ervi* (Hymenoptera: Aphidiidae). *Biol. Contr.*, **36**: 293-298. <https://doi.org/10.1016/j.biocontrol.2005.09.014>
- He, Z.X., Wang, Q., and Teulon, D.A.J., 2005. The effect of parasitism by *Aphidius ervi* on development and reproduction of the pea aphid, *Acyrthosiphon pisum*. *N. Z. Pl. Prot.*, **58**: 202-207. <https://doi.org/10.30843/nzpp.2005.58.4273>
- Hirose, Y., 2006. Biological control of aphids and coccids: A comparative analysis. *Popul. Ecol.*, **48**: 307-315. <https://doi.org/10.1007/s10144-006-0005-5>
- Hofsvang, T., and Hågvar E.B., 1978. Larval morphology and development of *Aphidius colemani*



- and *Ephedrus cerasicola* Stary (Hym., Apidiidae). *Norw. J. Ent.*, **25**: 1-8.
- Irshad, M., 1987. Biological control of pests and its scope in Pakistan. *Sci. Indust.*, **6**: 40-49.
- Irshad, M., 2001. Aphids and their biological control in Pakistan. *Pak. J. Biol. Sci.*, **4**: 537-541. <https://doi.org/10.3923/pjbs.2001.537.541>
- Irvin, N.A., Hoddle, M.S. and Castle, S.J., 2007. The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*. *Biol. Contr.*, **40**: 69-79. <https://doi.org/10.1016/j.biocontrol.2006.09.005>
- Jervis, M.A., and Copland, M.J.W., 1996. The life cycle. In: *Insect natural enemies, Practical approaches to their study and evaluation* (eds. M.A. Jervis and N. Kidd). Chapman and Hall, pp. 63-162. [https://doi.org/10.1007/978-94-011-0013-7\\_2](https://doi.org/10.1007/978-94-011-0013-7_2)
- Kalule, T., and Wright, D.J., 2004. The influence of cultivar and cultivar-aphid odors on the olfactory response of the parasitoid *Aphidius colemani*. *J. appl. Ent.*, **128**: 120-125. <https://doi.org/10.1111/j.1439-0418.2004.00821.x>
- Kalyebi, A., Overholt, W.A., Schulthess, F., Mueke, J.M., Hassan, S.A., and Sithanathan, S., 2015. Functional response of six indigenous trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) in Kenya: Influence of temperature and relative humidity. *Biol. Contr.*, **32**: 164-171. <https://doi.org/10.1016/j.biocontrol.2004.09.006>
- Kanturski, M., Zia, A., and Raf, M.A., 2017. The lachnus of Pakistan with description of a new species (Hemiptera: Aphididae: Lachninae). *J. Asia Pac. Ent.*, **20**: 1219-1227. <https://doi.org/10.1016/j.aspen.2017.09.001>
- Khalil, S.K., Khan, M., Farmanullah, and Naeem, M., 1990. Studies on the entomopathogenic fungus *Verticillium lecanii* (Zimm) for the control of green peach aphid *Myzus persicae* Sulz. *Sarhad J. Agric.*, **6**: 597-600.
- King, B.H., 1987. Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol.*, **62**: 367-396. <https://doi.org/10.1086/415618>
- Kos, K., Tomanovic, Z., Obradovic, O.P., Laznik, Z., Vidrih, M., and Trdan, S., 2008. Aphids (Aphididae) and their parasitoids in selected vegetable ecosystems in Slovenia. *Acta Agric. Slov.*, **91**: 15-22. <https://doi.org/10.2478/v10014-008-0002-9>
- Lin, L., and Anthony, A.R., 2003. The effect of parasitoid host-size preference on population growth rates: an example of *Aphidius colemani* and *Aphis glycines*. *Ecol. Ent.*, **28**: 542-550. <https://doi.org/10.1046/j.1365-2311.2003.00536.x>
- Mackauer, M., 1968. Phenotypic polymorphism in *Aphidius Smithi* Sharma and Subba Rao (Hymenoptera, Aphidiidae). *Entomophaga*, **13**: 281-287. <https://doi.org/10.1007/BF02371911>
- Maryam, S., Sandhu, A.A., Bodlah, I., Aziz, M.A. and Aihetasham, A., 2019. Contribution to aphid's fauna of Gujranwala (Punjab), Pakistan. *Punjab Univ. J. Zool.*, **34**: 09-16. <https://doi.org/10.17582/journal.pujz/2019.34.1.9.16>
- Medeiros, R.S., Ramalho, F.S., Lemos, W.P., and Zanuncio, J.C., 2006. Age-dependent fecundity and life-fertility tables for *Podisus nigrispinus* (Dallas) (Het., Pentatomidae). *Anns. entomol. Soc. Am.*, **99**: 401-40.
- Mohyuddin, A.I., 1981. *A review of biological control in Pakistan*. Proc. 2<sup>nd</sup> Cong. Zool. Tandojamm, pp. 21-79. [https://doi.org/10.1016/0006-3207\(81\)90073-2](https://doi.org/10.1016/0006-3207(81)90073-2)
- Mughal, S.H., Munshi, G.H., And Rizvi, N.H., 1985. A survey of *Aphidivorous coccinellids*. *Pak. Agric.*, **7**: 35-36.
- Mustafa, G., Saeed, M., Ali, A., and Latif, M., 1996. Population dynamics of aphids and predatory coccinellid beetles of sorghum crop at Bahawalpur. *Secod. Int. Cong. entomol. Sci.*, **19**: 21-22.
- Mutitu, K., Garnas, J.R., Hurley, B.P., Wingfield, M.J., Harney, M., Bush, S.J., and Slippers, B., 2013. Biology and rearing of *Cleruchoides noackae* (Hymenoptera: Mymaridae), an egg parasitoid for the biological control of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae). *J. econ. Ent.*, **106**: 1979-1985. <https://doi.org/10.1603/EC13135>
- Naeem, M., Shehzad, F., and Khan, M.R., 2005. Biosystematics of aphid parasitoids (Hymen: Aphidiidae: Aphelinidae) from Potohar region of the Punjab. *Ent. Mon. Maga.* Human Press, U.S.A., **141**: 219-226.
- Naumann-Etienne, K., and Remaudière, G., 1995. A commented preliminary checklist of the aphids (Homoptera: Aphididae) of Pakistan and their host plants. *Parasitica*, **51**: 1-61.
- Perdikis, D.C., Lykouressis, D.P., Garantonakis, N.G., and Iatrou, S.A., 2004. Instar preference and parasitization of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by the parasitoid *Aphidius colemani* (Hymenoptera: Aphidiidae). *Eur. J. Ent.*, **101**: 333-336. <https://doi.org/10.14411/eje.2004.044>
- Powell, W., 1982. The identification of hymenopterous parasitoids attacking cereal aphids in Britain. *Syst. Ent.*, **7**: 465-473. [https://doi.org/10.1016/0013-7495\(82\)90011-1](https://doi.org/10.1016/0013-7495(82)90011-1)

- [org/10.1111/j.1365-3113.1982.tb00457.x](https://doi.org/10.1111/j.1365-3113.1982.tb00457.x)
- Rakhshani, E., 2001. *Identification of natural enemies of Chromaphis juglandicola (Hom.: Aphididae) and biology of its parasitoid wasp, Trioxys pallidus (Hym.: Aphidiidae)*. Msc. thesis, College of Agriculture, Univ. of Tarbiat Modares, Tehran, Iran.
- Rakhshani, E., Talebi, A.A., Manzari, S., Tomanović, Ž., Stary, P., and Rezwani, A., 2007. Preliminary taxonomic study of the genus *Praon* (Hymenoptera: Braconidae: Aphidiinae) and its host associations in Iran. *J. Entomol. Soc. Iran*, **26**: 19-34.
- Sharma, A.K., and Subbarao, B.R., 1958. Description of two new parasites of an aphid from North India (Aphididae: Ichneumonidae and Pteromalidae: Chalcidoidea). *Indian J. Ent.*, **20**: 181-188.
- Sheng, S.L., and Carver, M., 1985. Studies on the biology of *Aphidius Sonchi* Marshall (Hymenoptera: Aphidiidae), a parasite of the sow thistle aphid, *Hyperomyzus lactucae* (L.) (Hymenoptera: Aphididae). *Bull. entomol. Res.*, **75**: 199-208. <https://doi.org/10.1017/S0007485300014280>
- Snyder, W.E., and Ives, A.R., 2003. Interactions between specialist and generalist natural enemies: Parasitoids, predators, and pea aphid. *Biol. Contr. Ecol.*, **84**: 91-107. [https://doi.org/10.1890/0012-9658\(2003\)084\[0091:IBSAGN\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[0091:IBSAGN]2.0.CO;2)
- Stary, P., 1970. Migration and parasitization of the pea aphid, *Acyrtosiphon pisum* (Harris). *Biology*, **25**: 787-796.
- Stary, P., 1988. Aphidiidae. In: *Aphids, their biology, natural enemies and control 2b* (eds. A.K. Minks and P. Harrewiegn). Elsevier, pp. 177-184.
- Stary, P., Naumann-Etienne, K., and Remaudiere, G., 1998. A review and tritrophic associations of aphid parasitoids (Hymenoptera; Braconidae, Aphidiidae) of Pakistan. *Parasitica*, **54**: 3-21.
- Suhail, A., Sabir, A.M., Hussain, A., and Saeed, A., 1999. Predatory efficacy of *Coccinella septempunctata* L. on cotton aphids, *Aphis gossypii* Glov. *Pak. J. biol. Sci.*, **2**: 603-605. <https://doi.org/10.3923/pjbs.1999.603.605>
- Talebi, A.A., Rakhshani, E., Fathipour, Y., Stary, P., Tomanović, Ž., and Mazhar, N.R., 2009. Aphids and their parasitoids (Hym., Braconidae: Aphidiinae) associated with medicinal plants in Iran. *Am. Eur. J. Sustain. Agric.*, **3**: 205-219.
- Van Den Bosch, R., Schlinger, E.I., Lagace, C.F., and Hall, J.C., 2012. Parasitization of *Acyrtosiphon pisum* by *Aphidius smithi*, a density-dependent process in nature. (Homoptera: Aphididae) (Hymenoptera: Aphidiidae). *Ecology*, **47**: 1049-1055. <https://doi.org/10.2307/1935655>
- Wiackowski, S.K., 1960. Laboratory studies on the biology and ecology of *Aphidius smithi* Sharma and Subba Rao. *Bull. Acad. Polon. Sci.*, **8**: 503-506.
- Wiackowski, S.K., 1962. Laboratory studies on biology and ecology of *Aphidius smithi* Sharma and Subba Rao (Hymenoptera, Braconidae) introduced to California for biological control of the pea aphid, *Acyrtosiphon pisum* (Harris). (In Polish.) *Zeszyty Probl. Postepow Nauk Roln.*, **35**: 137-41.
- Williams-Iii, L., and Roane, T.M., 2007. Nutritional ecology of a parasitic wasp: Food source affects gustatory response, metabolic utilization, and survivorship. *J. Insect Physiol.*, **53**: 1262-1275. <https://doi.org/10.1016/j.jinsphys.2007.06.017>
- Yu, S.H., Ryoo, M.I., Na, J.H., and Choi, W.I., 2003. Effect of host density on egg dispersion and the sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). *J. Stored Prod. Res.*, **39**: 385-393. [https://doi.org/10.1016/S0022-474X\(02\)00032-2](https://doi.org/10.1016/S0022-474X(02)00032-2)