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Aqsa Arshad
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Muhammad Ishaque Mastoi
Pakistan Agricultural Research Council PARC Islamabad, Pakistan

Waqas Wakil
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Asim Munawar
1. Department of Entomology, University of Agriculture, Faisalabad, Pakistan
2. Institute of Insect Science, College of Agriculture and Biotechnology, Zhejiang University, China

Fazlullah
Centre for Agriculture and Biosciences International CABI, Rawalpindi, Pakistan

Aamer Sohail
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Abid Ali Soomro
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Correspondence
Asim Munawar
1. Department of Entomology, University of Agriculture, Faisalabad, Pakistan
2. Institute of Insect Science, College of Agriculture and Biotechnology, Zhejiang University, China

Parasitism behavior of parasitoid and host relationship of *Acerophagus papayae* on *Paracoccus marginatus* under laboratory conditions

Aqsa Arshad, Muhammad Ishaque Mastoi, Waqas Wakil, Asim Munawar, Fazlullah, Aamer Sohail and Abid Ali Soomro

Abstract
Acerophagus papaya, a solitary endoparasitoid, is widely used in classical biological control of papaya mealybug, *Paracoccus marginatus*. The parastism behaviour of *A. papayae* on two host stages of *P. marginatus* at ambient environment was examined. The results revealed that there were more percent parasitism when *A. papayae* offered adult female and 2nd instar nymph of *P. marginatus* in choice situations (44±5.47%) as compared to adult female only (36±5.47%). The higher number of female parasitoids, were emerged from adult female hosts while the developmental time for male parasitoids was shorter than female in both no choice and choice situations. This information may lead that parasitoid efficiency will be increased with diverse stages of papaya mealybug in the field.

Keywords: Parasitoid, parasitism, *A. papayae, P. marginatus*, papaya mealybug

Introduction
The Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, is a small polyphagous sucking insect pest which causes massive damage to a large number of economically important vegetables, ornamentals and fruits crops [18, 21, 5]. Papaya mealybug caused serious injuries on papaya plantation when it was first time appeared in Cuba 1999 [4]. This pest is invaded in India in 2008 attacking many economically important agricultural crops [10]. According to Tanwar *et al*. [23] papaya mealybug generally active in warm temperatures. A decline in papaya production in Bangladesh was reported. The invasion of this non-indigenous pest is responsible and causing a huge economic losses to farmers [8, 18]. Papaya mealybug potentially poses a danger to several agricultural products in the globe, especially in Florida and other parts of the world such as California and Hawaii. Classical biological control of this noxious pest has been recognized as an significant component in management [24, 18] and at this time, a parasitoid *Acerophagus papayae* (Noyes and Schauff) being widely used in classical biological control of papaya mealybug in Dominican Republic, India, Palau, Puerto Rico, Guam and Sri Lanka [17, 7, 22].
Parasitoid *A. papayae* Noyes and Schauff can successfully control papaya mealybugs [19] and the biology of this solitary endoparasitoid varies according to the development of papaya mealybug on different host plants [20]. The researcher's first time report *P. marginatus* from Indonesia and India, causing huge damage to papaya fruit productions and cautioned about its potential occurrence and spreading in the neighboring countries [17, 16, 18] for the first time confirmed the presence of *P. marginatus* along with its parasitoids especially *A. papayae* in the Papaya orchards in Malaysia. During the last decades, *A. papayae* has been widely introduced in many countries such as Sri Lanka, Palau, Guam and India to manage the population of *P. marginatus* [7, 18]. *Acerophagus papayae* introduced as the dominant species in controlling the papaya mealybugs. An amount of Rs.122 crores have been saved by farmers for not advocating pesticide application for the past six months in cassava, papaya and mulberry due to release of this endoparasitoid [11].
For successful and continuous augmentative biological control programme of *P. marginatus* involved mass rearing of *A. papayae* in large sufficient populations to suppress mealybug's outbreak. Earlier researches carried out their experiments on parasitism of *A. papayae* on *P.*

marginatus without differentiating the male and female mealybugs instar nymphs [3]. Better understanding of host stages of prey for parasitism sex ratio and developmental time of *A. papayae* will help to understand in effective augmentative biocontrol.

2. Materials and Methods

2.1 Study site

The research was carried out in the laboratory of the Insect Pest Management Program (IPMP) National Agricultural Research Center, Islamabad Pakistan during January 2018 to April 2018 to fulfill the objectives of research work.

2.2 Collection of Papaya mealybug, *P. marginatus* and its parasitoid, *A. papayae*

Multiple visits were made during this experimental period to collect initial individuals of *P. marginatus* and its parasitoid, *A. papayae* from different horticultural and field crops in National Agriculture Research Centre (NARC), Islamabad Pakistan for experiment. Identification of both the pest and parasitoid was made in National Insect Museum with help of available literature under the guidance of experts of the museum.

2.3 Rearing of Papaya mealybug, *P. marginatus*

The laboratory culture of *P. marginatus* was maintained on sprouted potatoes and pumpkin. Clean potato sprouts and pumpkin were used as hosts for rearing of *P.marginatus*. Potato tubers and pumpkin was thoroughly washed under tap water and air dried. Air dried potatoes were transferred to the trays containing sterilized sand, and covered with black cloth to promote rapid sprouting and kept in dark place. Every day the trays were sprinkled with water to maintain the moisture. After some days when sprouting reached 3.0 to 4.0 cm in length, the potatoes were cleaned and transferred into rearing boxes. Depending upon the size of potato tubers and pumpkin each was infested with five to six ovisacs of *P. marginatus*, under the temperature (25±2℃) and humidity (60±5%). To maintain the colony of *P. marginatus*, for each week 10 potato sprouts and pumpkin were infested with ovisacs of papaya mealybug (Fig.1).

Fig 1: Rearing *P. marginatus* on pumpkin and potatoes

2.4 Rearing of endoparasitoid, *Acerophagus Papayae* (Noyes and Schauff)

Fresh mummies of papaya mealybug, *P. marginatus* were collected from a different sources mainly from Guava and Brinjal at NARC. Highly infested leaves with parasitoid, *A. papayae* were collected. Mummies were separated and closed in a glass jars covered with muslin cloth for emergence of newly emerged parasitoids. The main purpose of rearing of *A. papayae* was to maintain a culture for further experimentation. Unripen fruits infested with 2nd and 3rd instar of *P. marginatus* were offered to *A. papayae* for further rearing of pure culture in the laboratory. After one week of introduction of *A. papayae*, mummified mealybugs were collected from culture with the help of camel hairbrush and placed individually for adult parasitoid emergence in glass vials. Mummies were separated under microscope and were placed in separate capsules and kept in controlled conditions [18]. Depending on suitability streak of honey solution was given to each parasitoid better adult life and egg laying capacity (Fig 2).

Fig 2: Endo Parasitoids culture rearing

2.5 Experimental setup

2.6 Selection of host plant

Brinjal were selected as a host plant along with petiole placed in each petri dish. A hole was made in the bottom of the each petri dish. The cover of petri dish was cut in the center in square shape to attach muslin cloth with glue to allow the air circulation inside the petri dish. The petiole inserted through the hole at the bottom of the Petri dish. Each petri dish with a Brinjal leaf was placed on a cup of water to prevent the desiccation of leaf and leaf remain fresh [18].

2.7 Treatment structure

Ten adult female mealybug individuals (T1) without choice situation and five adult female mealybug + five 2nd instar nymph stage mealybugs in choice situation (T2) were placed on Brinjal leaf in petri dishes with 5 replications. A couple of endoparasitoid was released for 48 hours to each petri dish. A streak of 80% honey solution was also offered to a parasitoid. The experiment was conducted at an ambient environment of 18-26°C, 50-60% relative humidity and 10:14 (L: D) h photoperiod. The parasitoid was removed after 48 hours and the mealybug cohort retained in same petri dish to continue its development. The mealybug cohort was examined daily upon mummification, the mummies were collected and isolated in separate vials until eclosion of adult endo parasitoids. The percent parasitism, sex ratio and developmental time of *A. papayae* on each host stage of *P. marginatus* were recorded (Fig 3).

Fig 3: Showing experimental structure

2.8 Data Analysis
Data was analyzed by standard deviation and find out their mean.

3. Results and Discussions
3.1 Parasitism
The parasitism rate is more important to check the efficiency of *A. papayae* (Fig. 4). Our results revealed more parasitism (44±5.47) when endoparasitoid, *A. papayae* offered adult female and 2nd instar nymph *P. marginatus* in choice situations (T2) as compared to adult female *P. marginatus* only (T1) (36±5.47). Under choice treatment (T2), *A. papayae* preferred 2nd Instar *P. marginatus* nymphs (34±5.47) while 10±00 parasitism rate was recorded in adult female *P. marginatus* (Table 1). Mastoi *et al.* [15] in their studies found highest percent parasitism in 2nd instar female *P. marginatus* while lowest was in adults. Amarasekare *et al.* [3], Amarasekare *et al.* [1], Shylesha *et al.* [22] also noted highest percent parasitism in 2nd instar *P. marginatus* without sex differentiation. They further reported less parasitism in adult female *P. marginatus* than 3rd Instar female. Kalyanasundaram *et al.* [11] Lyla *et al.* [13], Shylesha *et al.* [22] reported that less parasitism rate in adult female *P. marginatus* may be due to bigger size of adults with strong defensive behavior and require more time to handle bigger host.

Table 1: Percent parasitism of *P. marginatus* by endoparasitoid, *A. papaya*

Replications	Adult Female *P. marginatus* (Without choice)	Adult Female v/s 2nd instar *P. marginatus* (With choice)		
		Overall parasitism	Adult Female	2nd instar
R1	30	50	10	40
R2	40	40	10	30
R3	30	40	10	30
R4	40	50	10	40
R5	40	40	10	30
Mean ± STDEV	36±5.47	44±5.47	10±0	34±5.47

3.2 Sex Ratio
The higher number of female endoparasitoids, *A. papayae* were emerged from adult female *P. marginatus* in without choice and choice situations. In no choice situations the sex ratio was 38.3:61.6±37.08 (Male: Female) while in choice situations (adult female v/s 2nd instar *P. marginatus* nymphs) the sex ratio was 0.00:100±0.0 in adult females and 69.99:29.99±04.56 in 2nd instar *P. marginatus* in nymphs (Table 2, Fig 4). According to the previous findings of Amarasekare *et al.* [3], Amarasekare *et al.* [2] 2nd instar host stage of *P. marginatus* yielded more male parasitoids as compared to other stages. King. [12] has also revealed that female endoparasitoids emerged from larger size of hosts and male parasitoid emerged from smaller sized hosts.

Fig 4: *Acerophagus papayae* parasitizing papaya mealybug, *Paracoccus marginatus*

Fish *et al.* [6], Hemerik. [9] have also conducted same type of experiment but was not on papaya mealybug, they reported higher number of female parasitoid *Anaesius bambawalei* emerged from third instar cotton mealybug, *P. solenopsis* nymphs, whereas, the higher proportion of male parasitoids emerged from second instar nymphs.

Table 2: Sex ratio of endoparasitoid, *A. papayae* emerged from host *P. marginatus*

Replications	Adult Female *P. marginatus* (Without choice)	Adult Female v/s 2nd instar *P. marginatus* (With choice)	
	Adult female	Adult female	2nd Instar nymph
R1	33.33:66.66	0.00:100	75.00:25.00
R2	00.00:100	0.00:100	66.6:33.3
R3	33.33:66.66	0.00:100	66.6:33.3
R4	25.00:75.00	0.00:100	75.0:25.0
R5	100:00.00	0.00:100	66.6:33.3
Mean±STDEV	38.3:61.6±37.08	0.00:100±0.0	69.99:29.99±4.56

3.3 Developmental time of endoparasitoid, *A. papayae*

The developmental time for male *A. papayae* is shorter than female in no choice and choice situations. The developmental time for male *A. papayae* recorded was 16.75±3.3 and 16.0±1.0 in adult female and 2nd instar *P. marginatus* nymphs respectively in no choice and choice situations. The developmental time for female *A. papayae* was 19.5±2.6 days in adult female *P. marginatus* in no choice treatment while in choice treatment developmental time for female parasitoid was 17.2±0.8 in adult female and 17.0±0.7 in 2nd instar *P. marginatus* (Table 3). Fish *et al.* [6], Mastoi *et al.* [14] have also observed same type of results that the developmental time of female parasitoids (i.e. *A. bambawalei* and *A. papayae*) is always longer than males.

Table 3: Developmental Time of male and female *A. papaya*

Replications	Adult female *P. marginatus* (Without choice Treatment)		Adult female v/s 2nd Instar *P. marginatus* (Choice Treatment)			
			Adult female *P. marginatus*		2nd Instar nymph *P. marginatus*	
	Male Parasitoid	Female Parasitoid	Male Parasitoid	Female Parasitoid	Male Parasitoid	Female Parasitoid
R1	15	18	--	18	15	17
R2	--	17	--	17	15	17
R3	20	20	--	17	17	17
R4	19	23	--	18	17	18
R5	13	--	--	16	16	16
Mean ± STDEV	16.75±3.3	19.5±2.6	--	17.2±0.8	16±1	17±0.7

4. Conclusion

In recent decades due to multiple applications of pesticides on Agricultural crops as well as on fruit orchards have imposed a huge burdens on sustainable produce. Pesticides not only control target insect pests but also eliminate the natural enemies. There are also many growing concerns over the excessive applications of pesticides, like resistance to pests, environmental effects and human health, chemical residues in fruits and vegetables and high cost production [22]. Mealybugs especially papaya mealybug, *P. marginatus* causes huge infestations to papaya cultivations in the worldwide. Due to waxy filaments on their body its becomes very difficult to manage this pest with frequently applications of pesticides. There is a need to develop a tactics which should be eco-friendly and provide sustainable pest control [19]. Biological control have attained more attentions from recent years, and could provide an effective and sustainable Insect Pest Management. Parasitoid, *A. papayae* have been approved a potential biocontrol agent for the management of papaya mealybug, *P. marginuts* [15]. Researcher now a days are in effort to found suitable ways for the augmentative release of Parasitoid, *A. papayae* against papaya mealybug, P. marginatus under field conditions. We hope this study will provide an effective knowledge to overcome upcoming challenges in field of biological control.

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6. Authors Contributions

Authors conducted all the research trials, wrote the manuscript and analysis of data. All the authors of this manuscript have read and approved this article.

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