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Host instars preference, density-dependent parasitism and behavioral perspective of parasitoids (*Aphidius colemani, Aphidius matricariae* and *Aphelinus abdominalis*) in *Aphis glycines* and *Aphis gossypii*

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ABSTRACT

Three parasitoid species *Aphidius colemani, Aphidius matricariae* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) were evaluated concerning their parasitism potential in two aphid species, *Aphis glycines* and *Aphis gossypii* (Hemiptera: Aphididae). The feeding of these two aphid species, even at low sums, can significantly damage photosynthesis and is found to transmit many kinds of plant viruses, which impact potential adverse effects on the plants. The overall parasitization on all nymphal ages in *As. glycines* was accomplished by *Ad. colemani* (60.50%), *Ad. matricariae* (49.16%) and *Al. abdominalis* (40%), while in *As. gossypii* parasitism exhibited by *Ad. colemani* (79.48%), *Ad. matricariae* (65.33%) and *Al. abdominalis* (58.83%). *Aphelinus abdominalis* exhibited the lowest parasitism in both given species as hosts. Significant differences in parasitism of different parasitoids and host species were observed. Concerning the preference of nymphal instars, we found that parasitoids species prefer to parasitize 1st- 4th instars in *As. gossypii* while in *As. glycines* 2nd, 1st, 3rd and 4th. Our results showed that the parasitism increases with the increase of parasitoid numbers and hosts densities.

Introduction

Biological control is considered a potentially effective and economical strategy in the integrated pest management of devastating insects (Naranjo et al., 2015). Insecticides are generally toxic to the environment and the reliance on biological control is desirable to avoid their extensive use and deleterious effects (Van Lenteren et al., 2018). Despite extensive precautions, biological control is often cheap, safest and the most cost-effective practical approach in the long-term management of a variety of insect pests. Therefore, agriculture professionals are concentrating on biological control-based integrated pest management to limit the use of chemicals and to protect the natural beneficial fauna.

Aphids (Hemiptera: Aphididae) are important insect pests of agricultural crops, which cause huge economic losses (Wickremasinghe and Van-Emden, 1992; Van Emden and Harrington, 2007; Gripenberg et al., 2010; Wieczorek et al., 2019). Aphids are phloem feeding insects and are vectors of a diversity of plant viruses (Ng and Perry, 2004; Wang et al., 2006; Vilcinskas, 2016). They defecate sticky honeydew on plants that provide the nourishment for the growth of sooty mold fungus and lead to the plummeting of the photosynthesis (Chomnunti et al., 2014). Due to their invasive ability, dispersal behavior and nature of parthenogenetic reproduction, aphids have become global insect pests (Messing et al., 2007; Wieczorek et al., 2019). Many species of aphid are reported over the globe (Blackman and Eastop, 2017). Among them, *Sitobion avenae, Rhopalosiphum padi, Myzus persicae, Myzus obtusirostris, Rhopalosiphum maidis, Schizaphis graminum, Melanaphis sacchari, Aphis gossypii* and *Aphis glycines* are reported in Pakistan (Hamid, 1983; Mustafa et al., 1996; Stray et al., 1998; Amin et al., 2017).

Aphis gossypii Glover, 1877 and *As. glycines* Matsumura, 1917 are infesting a variety of crops including cotton and soybean, respectively (Alam and Hafiz, 1963; Wu et al., 2004). *Aphis gossypii* (cotton aphid) is an important insect pest of cotton and many other host crops (Leclant and Deguine, 1994). It is a sap-sucking insect and causes the deformation of leaves and buds, stunting growth and plant development (Leite et al.,

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2006) and can cause significant reduction in the yield (Ramalho, 1994; Furtado et al., 2009).

Aphis glycines (soybean aphid) is a pest of soybean native to Asia (Wu et al., 2004), and is well recognized as invasive species in other part of the world (Ragsdale et al., 2011; Natukunda and MacIntosh, 2020). It causes impairment by slurping plant, leaves and stem fluids, and leads to yield reduction to the extreme level with the rapid increase in its population densities (Sun et al., 2000; Wu et al., 2004).

The parasitoid wasps belonging to Aphidiinae (Hymenoptera, Braconidae) encompasses over 50 genera (Smith and Kambhampati, 2000) with relatively close dispersal to their hosts (Stary, 1975). The parasitoids of the genera *Aphidius* and *Aphelinus* are the most used in the biological control programs of aphids (Völkl et al., 2007; Boivin et al., 2012). The wasp species *Ad. colemani* Viereck, 1912, *Ad. matricariae* Haliday, 1834 as well as *Al. abdominalis* Dalman, 1820 (Hymenoptera: Aphelinidae) are tropical aphid parasitoids with an origin in the South Asia and most likely spread worldwide through central Asia (Schlinger and Mackauer, 1963; Stary, 1975; Van Steenis, 1995; Blumel and Hausdorf, 1996; Stary et al., 1998; Molck and Wyss, 2001).

These species are potentially effective in biological control management in glasshouses and commercially used for plant louse management in different parts of the world such as Europe, North America (Fernandez and Nentwig, 1997) and Australia (Wilson et al., 2004; Khatri, 2017). Although few studies have reported the bio-control of aphids in Pakistan, the majority of them explored the prevalence, ecology, biology and host range of natural enemies (Hamid, 1983; Mustafa et al., 1996; Stray et al., 1998). Nevertheless, little is known regarding the feeding and parasitizing potential of parasitoid wasps in the agro-climatic conditions of Pakistan.

Host-parasitoids interaction is an important component of the biological control program, which may affect the proficiency of parasitoids (Berhow et al., 2013). It is worth to acquire the knowledge of vital behavioral trait such as host-parasitoid interactions, host selection behavior, host suitability, host density, parasitoid's selectivity among different host species and host nymphal stages. The understanding of these attributes is essential for the successes of biological control and pest management programs (Keller and Tenhumberg, 2000; Sidney et al., 2010; Yazdani et al., 2015). The information regarding parasitoid's preference for specific developmental stages, foraging, host-selection approaches and population dynamics diaspora is important for successful mass-rearing facilities and parasitoids release to implement the pest control (Lin and Ives, 2003; He and Wang, 2006; Henry et al., 2009; Shrestha et al., 2015). Therefore, it is quite logical to investigate the in-situ effectiveness of parasitoid wasps against their aphids hosts. The present study investigated the parasitism potential of three parasitoid wasps (*Ad. colemani, Ad. matricariae,* and *Al. abdominalis*) at different larval stages of host and host density-dependent parasitism against two aphid species (*As. glycines* and *As. gossypii*).

Material and methods

The parasitism responses in the nymphal instars and host choices (density-dependent parasitism) of three hymenopteran parasitoid species (*Ad. colemani*, *Ad. matricariae* and *Al. abdominalis*) were investigated over the host population of two aphid species (*As. glycines* and *As. gossypii*).

Collection of samples, culturing and handling of aphid-parasitoids experiments

The winged and wingless aphids, *As. glycines* (soybean) and *As. gossypii* (cotton), were collected from the pesticide-free agricultural fields located in different cities (Faisalabad, Sahiwal, Bahawalnagar, Tandojam and Peshawar) of Pakistan during 2017-2020. The hand-picking, aspirators and net methods after visual inspection were used for insect collection. The collected insect samples were brought in the Entomology Laboratory, Department of Zoology, Government College University, Faisalabad, Pakistan and identified based on their morphological characters under the microscope following the methods and keys provided by Lagos-Kutz et al. (2014) and Voegtlin et al. (2004).

The aphid colonies were maintained separately on soybeans and cotton plants in a greenhouse (25 ± 7 °C, RH: 70 ± 10%) for four years. First to fourth-instar aphids were transferred to clipped soybean and cotton leaves for experiments in the laboratory. The host instars were distinguished based on their size and age calculation (Inaizumi and Takahashi, 1989; Wool and Hales, 1996; Tilmon et al., 2011). The laboratory conditions were maintained (24 ± 2 °C, 65 ± 5% RH, and Light 16h: Dark 08h) for the rearing of the aphid and parasitoid species. The rearing experiments were conducted separately in specially designed rearing chambers (Length x Height x Width: 38 x 34 x 30 cm). These rearing chambers were made up of perpex sheets and were purchased from the local market.

For parasitoids collection, the adult parasitoids and the mummies of parasitized aphids were collected from the soybean and cotton field crops during 2017-2020. The mummies were placed in petri dishes until the completion of the parasitoid's life cycle. The adult aphid parasitoids that emerged out from the mummies were sorted and identified as *Ad. colemani, Al. abdominalis* and *Ad. matricariae* (Japoshvili and Karaca, 2009; Rakhshani et al., 2012). Adult parasitoids were nourished with a diet consisting of honey, sugar solution, and water in the ratio of 25:25:50, respectively. The cotton soaked in diet were put in the petri dishes and placed in the rearing chambers. Separate petri dishes of water were also provided in the rearing chambers. The datasets regarding parasitism, progenies and hosts-parasitoid behavioral interactions for each experiment were recorded daily until the completion of the parasitoid's life cycle.

Effect of parasitoids on the age (nymphal instars) of aphid species (non-choice experiments)

The continued existence and parasitism perspective of aphid parasitoids were determined by releasing a mated female (>12h old) of each parasitoid in the individual petri dishes (95 x 15 mm). The mating of aphids was ensured by pairing newly emerged virgin female with a one day old virgin male in a 3 ml glass vial for 30 min. The mated female was transferred to another 3 ml glass vial and nourished with diet (honey, sugar solution and water in the ratio of 25:25:50, respectively) soaked in cotton until their use in subsequent experiments. The separate petri dishes containing thirty nymphs of first instar *As. glycines* or *As. gossypii* were provided with fresh leaves of soybean and cotton, respectively. The parasitoids were left for 24h to parasitize the aphids. Likewise, thirty nymphs of 2nd, 3rd and 4th instar aphids were also parasitized with each parasitoid for 24h in individual petri dishes containing fresh leaves. The moistened filter papers were also placed in the petri dishes with the fresh leaves to maintain the moisture level. The mummified aphids of each instar were counted once per day in the morning, and transferred separately in 3 ml micro-centrifuge tubes to count the subsequent parasitism data. The parasitized aphids were then transferred to the plastic containers (L x H x W: 42 x 36 x 32 cm) containing fresh leaves of soybean and cotton for maintaining the culture. The experiment was conducted in three replicates for each treatment at maintained conditions (24 ± 2 °C, 65 ± 5% RH, and Light 16h: Dark 08h) in the laboratory.

Effect of parasitoids on the age (nymphal instars) of aphid species (choice experiments)

The experiment was conducted to determine the host preference of parasitoids towards any particular age of aphid nymphs (1st, 2nd, 3rd and 4th instar). The host nymphal instars differentiation was calculated based on their size and age (Inaizumi and Takahashi, 1989; Wool and Hales, 1996; Tilmon et al., 2011). A mated parasitoid female (>12h old) of *Ad. colemani, Al. abdominalis,* and *Ad. matricariae* was released into a separate petri dish containing 120 aphids (thirty of each 1st, 2nd, 3rd, and 4th instar) on the fresh leaf surface. After 24h of parasitization, the parasitized and non-parasitized aphids were isolated according to their ages (nymphal instars) and the datasets were recorded separately in three replicates for each host and parasitoid species at standardized handling conditions as mentioned earlier. The parasitism percentage was determined by the formula as follows.

$$Parasitism\ percentage = \frac{Number\ of\ \ parasitized\ individuals}{30} x100 \qquad (1)$$

Density-dependent parasitism (host: parasitoid ratio)

These experiments were performed to find out if the population density (number) of the host (aphid nymphs) and parasitoid have any potential effect on the parasitization. The specialized perspex cages were prepared for the introduction of both host and parasitoid in controlled conditions. The parameters i.e. duration of host exposure, temperature conditions during experiment, and size of containers are those mentioned in the previous experiments. Adequate quantity of food was provided in each experiment to avoid any mortality due to food shortage. The mated parasitoid females (>12h old) of *Ad. colemani, Al. abdominalis,* and *Ad. matricariae* were released in different ratios of parasitoid: host with the fresh plant leaf. The different ratios of host and parasitoids numbers were 1:1, 10:1, 50:5, 100:10 and 200:20. The age of *As. glycines* was 3-6 days old and *As. gossypii* was 1-3 days old. Five separate cages (C1, C2, C3 C4 and C5) were utilized to access the parasitism by releasing a specific host: parasitoid ratio. The host-parasitoid ratio (C1= 1:1) was treated as a positive control. The parasitoids were permitted for 36h to parasitize the aphids. The experiments steered separately in three replicates for each host and parasitoid species (Table 1) under standardized laboratory conditions as mentioned earlier.

Statistical analysis

The assumptions of normality and homogeneity of variance were tested during the statistical analysis. The data of parasitism were subjected to analysis of variance (ANOVA) and the significant results at $p < 0.05$ were estimated by the Post Hoc Tukey's HSD multiple comparison range tests. The data is presented as mean ± standard deviation (SD) and standard error (SE), which was calculated using Microsoft Excel 2013®. We performed assumptions of each statistical test and steered analyses in R version (R Core Team, 2020).

Results

Host-parasitoid interaction revealed the preferential parasitism of parasitoids concerning host age (nymphal instars), and diversity in parasitism with the change in ratio of host-parasitoid numbers.

Effect of parasitoids on the age (nymphal instars) of soybean aphid (*As. glycines*)

The data indicated a significant difference among parasitism percentage of three parasitoids confronting on different instars (1st, 2nd, 3rd and 4th) of *As. glycines* (soybean aphid). The parasitoid *As. colemani* exhibited the higher parasitism percentage followed by that of *Ad. matricariae,* (moderate) and *Al. abdominalis* (least) on each tested instars (1st, 2nd, 3rd and 4th) of *As. glycines* (Fig. 1 A). The parasitism percentages of *Ad. colemani* (68.66%, 82.00%, 59.33% and 32.00%), *Ad. matricariae* (53.33%, 74.66%, 40.66% and 28.00%) and *Al. abdominalis* (42.66%, 56%, 36.66%, and 24.66%) were recorded on first, second, third and fourth instar aphids, respectively (Fig. 1 A). The mean parasitism of *Ad. colemani*, *Ad. matricariae* and *Al. abdominalis* on all tested ages (nymphal instars) was 60.50%, 49.16% and 40.00%, respectively. The parasitism of three tested parasitoids were significantly different among each other [$F(2, 12) = 14.290$, $p = 0.005$] (Table 2). *Aphidius colemani* showed the highest parasitism followed by *Ad. matricariae* and *Al. abdominalis* (Fig. 2 A and Table 2).

The parasitism was significantly affected by the age of *As. glycines*. A significant difference in parasitism [$F(3, 12) = 32.326$, $p = 0.000$)] was recorded at different ages (nymphal instar) of *As. glycines* (Table 2). 2nd instar was the highly preferred host age for parasitism by all three tested parasitoids, followed by 1st instar as the second preferred host age (Fig. 2 A). Host age has a significant influence on the fraction of female progeny of parasitoids that remained highest in 2nd instar, followed by 1st and 3rd instar and least in the 4th instar of *As. glycines*.

Density-dependent parasitism (different ratios of host, *A. glycines*: parasitoids)

The density of parasitoid and host population revealed significant effect on parasitism [$F(3, 12) = 14.891$, $p = 0.000$)] that was tested with different number ratios of host (*As. glycines*) and parasitoids females (host: parasitoid = 1:1, 10:1, 50:5, 100:10 and 200:20) in the separate cages (C1, C2, C3, C4 and C5), respectively (Table 3).

The parasitism percentage of *Ad. colemani* (parasitoid females) in tested (host: parasitoid) ratios were 100% (C1= 1:1), 64% (C2 = 10:1), 70% (C3 = 50:5), 78% (C4 = 100:10) and 82% (C5= 200:20). The ratio (C5= 200:20) exhibited higher aphid parasitization percentage compared to other tested ratios, except for the positive control (C1= 1:1) that showed 100% parasitism. This pattern of parasitoids revealed that parasitization increases with the increase of parasitoid and host numbers (Fig. 3 A).

Table 1
Different ratios of host parasitoids in different cages.

Cage No.	Ratio (Host: Parasitoid)	Details (*As. glycines* 3-6 days / *As. gossypii* 1-3 days)
C1	1:1	One aphid (*As. glycines* / *As. gossypii*): One parasitoid female (*Ad. colemani* / *Ad. matricariae* /*Al. abdominalis*)
C2	10:1	Ten aphid (*As. glycines* / *As. gossypii*): One parasitoid female (*Ad. colemani* / *Ad. matricariae* /*Al. abdominalis*)
C3	50:5	Fifty aphids (*As. glycines* / *As. gossypii*): Five parasitoid females (*Ad. colemani* / *Ad. matricariae* / *Al. abdominalis*)
C4	100:10	100 aphids (*As. glycines* / *As. gossypii*): Ten parasitoid females (*Ad. colemani* / *Ad. matricariae* /*Al. abdominalis*)
C5	200:20	200 aphids (*As. glycines* / *As. gossypii*): Twenty parasitoid females (*Ad. colemani* / *Ad. matricariae* / *Al. abdominalis*)

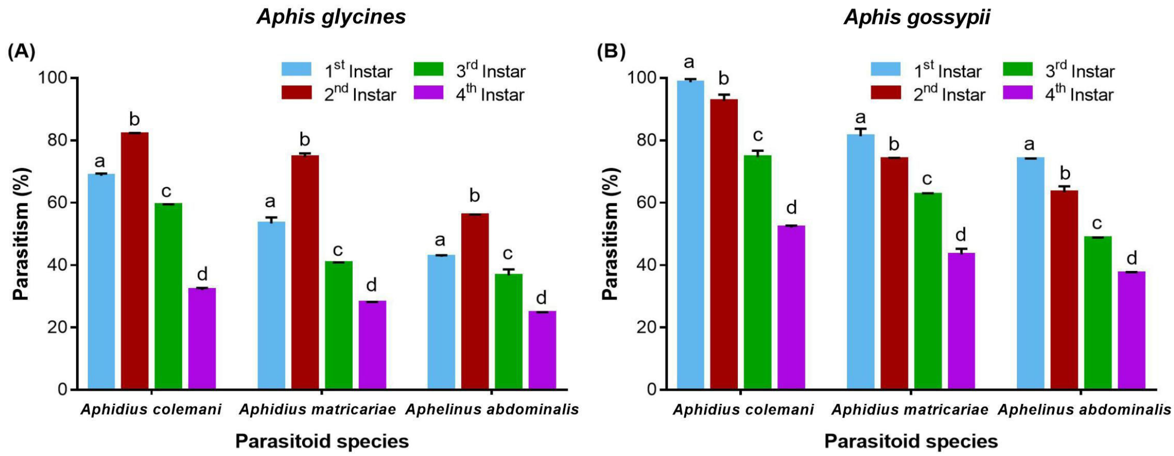

Figure 1: Comparison of parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) on different ages (nymphal instars) of the **(A)** *As. glycines* (n= 30) and **(B)** *As. gossypii* (n= 30).

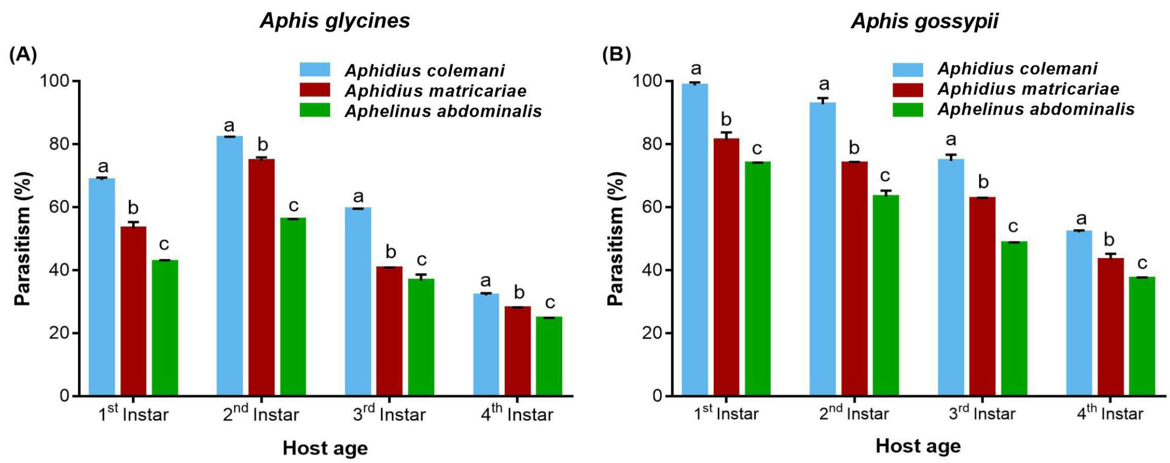

Figure 2: Parasitism percentage of parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) on different host instars of **(A)** *As. glycines* (n= 30) and **(B)** *As. gossypii* (n= 30).

Table 2
Host-parasitoid (nymphal instars): Replicated mean parasitism efficacy in numbers ± standard deviation. Mean values marked with the same letter are not significantly different at P < 0.05, by Tukey's test.

Host species	Parasitoid – Parasitism (mean ± SD)			Among parasitism of parasitoid		Larval stage (instars)				Between larval instars	
	Ad. colemani	*Ad. matricariae*	*Al. abdominalis*	(df) F-value	P-value	First instar	Second instar	Third instar	Fourth instar	(df) F-value	P-value
As. glycines	18.15 b ± 6.35	14.75 cd ± 5.97	12.00 d ± 3.91	(2,12) = 14.290	0.005*	16.47 bc ± 3.92	21.27 ab ± 4.02	13.67 c ± 3.63	8.47 d ± 1.10	(3,12)= 32.326	0.000**
As. gossypii	23.85 a ± 6.30	19.60 b ± 4.97	16.75 c ± 4.84	(2,12) = 45.728	0.000**	25.40 a ± 3.80	23.00 ab ± 4.45	18.60 bc ± 3.90	13.27 c ± 2.21	(3,12)= 76.513	0.000**

*(** Highly significant and *significant).*

Table 3
Host-parasitoid ratios: Replicated mean density-dependent parasitism in numbers ± standard deviation. Mean values marked with the same letter are not significantly different at P < 0.05, by Tukey's test.

Host species	Parasitoid – Parasitism (mean ± SD)			Among parasitism of parasitoid		Parasitoid: Aphid density ratio					Parasitism among density ratio	
	Ad. colemani	*Ad. matricariae*	*Al. abdominalis*	(df) F-value	P-value	C 1 (1:1)	C 2 (10:1)	C 3 (50:5)	C 4 (100:10)	C 5 (200:20)	(df) F-value	P-value
As. glycines	7.50 a ± 0.69	5.18 b ± 0.40	3.8 c ± 0.70	(2, 12) = 256.701	0.000**	1 ± 0.00	5.05 cd ± 1.59	5.13 c ± 1.80	5.67 bc ± 1.94	6.13 ab ± 1.70	(3, 12)= 14.891	0.000**
As. gossypii	8.03 a ± 0.58	5.99 b ± 0.57	4.68 c ± 0.63	(2, 12) = 298.956	0.000**	1 ± 0.00	5.73 bc ± 1.51	5.83 b ± 1.69	6.33 ab ± 1.43	7.02 a ± 1.47	(3, 12)= 27.109	0.000**

*(** Highly significant), C1 (Cage 1 Control), C2 (Cage 2), C3 (Cage 3), C4 (Cage 4), C5 (Cage 5).*

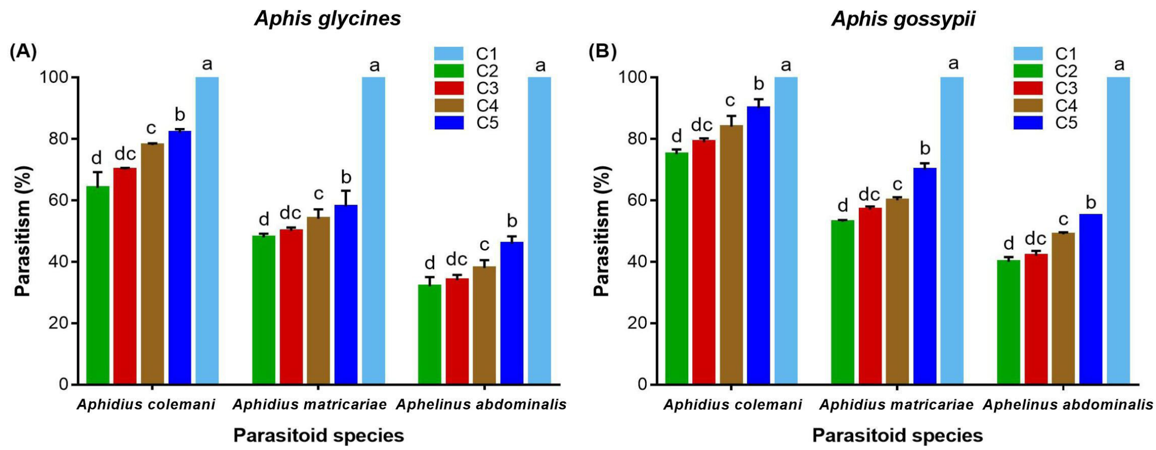

Figure 3: Comparison of parasitism of parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) on different host density levels of **(A)** *As. glycines* and **(B)** *As. gossypii.* C1 (1:1) Control, C2 (10:1), C3 (50:5), C4 (100:10), C5 (200:20).

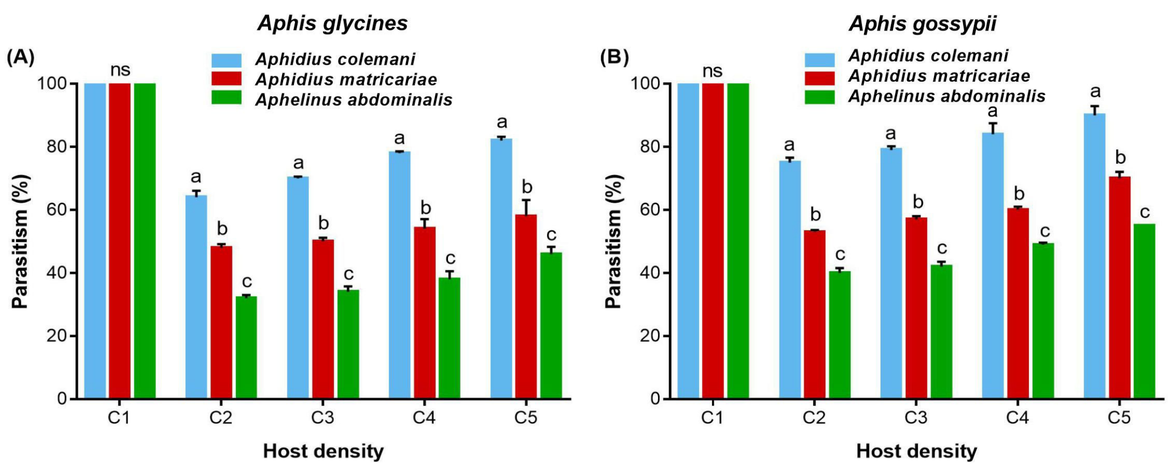

Figure 4: Parasitism percentage of parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) at different host density levels of **(A)** *As. glycines* and **(B)** *As. gossypii.* C1 (1:1) Control, C2 (10:1), C3 (50:5), C4 (100:10), C5 (200:20).

The percentage parasitism of *Ad. matricariae* female in different tested ratios (host: parasitoid) were 100% (C1= 1:1), 48% (C2= 10:1), 50% (C3= 50:5), 54% (C4= 100:10) and 58% (C5= 200:20). The ratio (C5= 200:20) resulted in higher aphid parasitization percentage compared to other tested ratios except for the positive control (C1= 1:1) that showed 100% parasitism. Thus, parasitization of *Ad. matricariae* increases with the increase of parasitoid and host numbers (Fig. 3 A).

Aphelinus abdominalis female parasitoids exhibited the parasitism percentage in different tested ratios (host: parasitoid) with values 100% (C1= 1:1), 32% (C2= 10:1), 34% (C3= 50:5), 38% (C4= 100:10) and 46% (C5= 200:20). The ratio (C5= 200:20) resulted in higher aphid parasitization percentage compared to other tested ratios except positive control (C1= 1:1) that showed 100% parasitism. Thus, parasitization of *Al. abdominalis* increases with the increase of parasitoid and host numbers (Fig. 3 A).

The parasitism percentage of *Ad. colemani*, *Ad. matricariae* and *Al. abdominalis* females in tested host: parasitoid ratios were C1 (100%, 100% and 100%), C2 (64%, 48% and 32%), C3 (70%, 50% and 34%), C4 (78%, 54% and 38%) and C5 (82%, 58% and 46%), respectively (Fig. 4 A).

Among parasitoids, the density-dependent parasitism of *Ad. colemani, Ad. matricariae* and *Al. abdominalis* were also significantly different from each other [$F (2, 12) = 256.701$, $p = 0.000$] (Table 3). *Ad. colemani* showed significantly higher parasitism, followed by that of *Ad. matricariae,* and lower parasitism in *Al. abdominalis* for four tested ratios C2 (10:1), C3 (50:5), C4 (100:10) and C5 (200:20), respectively (Fig. 4 A). The parasitism increases with the increase in the number of parasitoids and hosts (Fig. 4 A).

Effect of parasitoids on the age (nymphal instars) of cotton aphid (*A. gossypii*)

The data indicated a significant differential parasitism percentage among three parasitoids against different nymphal instars (1st, 2nd, 3rd and 4th) of cotton aphid, *As. gossypii* (Fig. 1 B). The parasitoid *Ad. colemani* exhibited the higher parasitism percentage followed by that of *Ad. matricariae,* (moderate) and *Al. abdominalis* (least) on each tested nymphal instars (1st, 2nd, 3rd and 4th) of *As. gossypii* (Fig. 1 B). *Ad. colemani* parasitism percentages were 98.60%, 92.66%, 74.66%, and 52.00% on 1st, 2nd, 3rd and 4th host instar, respectively. *Ad. matricariae* parasitism values were 81.33%, 74.0%, 62.66%, and 43.33% on 1st, 2nd, 3rd and 4th instar aphids, respectively. *Aphelinus abdominalis* parasitization percentage values were 74.0%, 63.33%, 48.66% and 37.33% on 1st, 2nd, 3rd and 4th instar aphids, respectively (Fig. 1 B). The mean parasitization of *Ad. colemani, Ad. matricariae* and *Al. abdominalis* on all ages (instars) of aphid was 79.48%, 65.33% and 55.83%, respectively. The parasitism of three tested parasitoids was significantly different among each other [$F (2, 12) = 45.728$, $P= 0.000$] (Table 2). *Aphidius colemani* has the highest parasitization percentage in all four nymphal instars followed by the parasitization of *Ad. matricariae* and *Al. abdominalis* (Fig. 2 B and Table 2).

The parasitism was significantly affected by the age of *As. gossypii.* We observed that 1st instar was the highly preferred host age for parasitism by all three tested parasitoids followed by 2nd, 3rd and 4th instar of *As. gossypii,* [F (3, 12) = 76.513, p = 0.000] (Table 2). Host age also showed a significant effect on the proportion of female progeny of parasitoid, which were maximum in 1st instar, followed by 2nd and 3rd instar (descendent tendency), and the least parasitization in the 4th instar aphids of *As. gossypii*.

Density-dependent parasitism (different ratios of host, *As. gossypii*: parasitoids)

The density of parasitoid and host population revealed significant effect on parasitism [F (3, 12) = 27.109, p = 0.000] that was tested with different number ratios of host (*As. gossypii*) and parasitoids females (host: parasitoid = 1:1, 10:1, 50:5, 100:10 and 200:20) in the separate cages (C1, C2, C3, C4 and C5), respectively (Table 3).

The parasitism exhibited by *Ad. colemani* (parasitoid females) in different tested ratios (host: parasitoid) were 100% (1:1), 75% (10:1), 79% (50:5), 84% (100:10) and 90% (200:20). The ratio (C5= 200:20) resulted in a higher aphid parasitization percentage compared to other tested ratios, except for the positive control (C1= 1:1) that showed 100% parasitism. The behavioral pattern of parasitoids revealed that parasitization increases with the increase of parasitoid and hosts numbers (Fig. 3 B).

Aphidius matricariae females parasitism in different tested ratios (host: parasitoid) were 100% (1:1), 53% (10:1), 57% (50:5), 60% (100:10) and 70% (200:20) (Fig. 3 B). The ratio (C5= 200:20) resulted in a higher aphid parasitization percentage compared to other tested ratios, except for the positive control (C1= 1:1) that showed 100% parasitism (Fig. 3 B).

Aphelinus abdominalis ffemales parasitism values were 100% (1:1), 40% (10:1), 42% (50:5), 49% (100:10) and 55% (200:20). The C5 ratio (200:20) resulted in higher aphid parasitization percentage compared to other tested ratios, except for the positive control C1 (1:1) that exhibited 100% parasitism (Fig. 3 B).

Among parasitoids, the density-dependent parasitism of *Ad. colemani, Ad. matricariae* and *Al. abdominalis* were also significantly different from each other [F (2, 12) = 298.956, p = 0.000] (Table 3). *Aphidius colemani* females showed significantly higher parasitism followed by that of *Ad. matricariae* for all tested ratios C2 (10:1), C3 (50:5), C4 (100:10) and C5 (200:20), respectively (Fig. 4 B). *Aphelinus abdominalis* parasitoids exhibited the lowest parasitization as compared to the other two parasitoid species (Fig. 4 B and Table 3).

Aphidius colemani, Ad. matricariae and *Al. abdominalis* exhibited parasitism for C1 (100%), C2 (75%, 53%, 40%), C3 (79%, 57%, 42%), C4 (84%, 60%, 49%) and C5 (90%, 70%, 55%) respectively (Fig. 4 B). The parasitism percentage increases with the increase in the number of parasitoids and hosts.

Discussion

Biological control is considered a good alternative approach for the management of aphid species (Rasool et al., 2020) and the practice of using parasitoids against aphids is successful (Boivin et al., 2012). The knowledge about host-parasitoid interactions to gauge their whereabouts and responses is necessary for the successful implementation of any biological control program. The research is particularly necessary where crop varieties with low-yields are being swapped with high-yield and new pest problems have emerged. Studies aiming to explore the parasitism potential of parasitoids concerning the host age and host-parasitoid ratio are obligatory for the effective implementation of biological control in aphid management programs.

The present study investigated the parasitism potential of three parasitoid wasps against two aphid species (*As. glycines* and *As. gossypii*). Host-parasitoid interaction revealed the preferential parasitism of parasitoids about host age (nymphal instars) and the changes in parasitism concerning the ratio of host-parasitoid numbers. Our findings indicated a significant difference among parasitism percentage of three parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) on two aphid host species (*As. glycines* and *As. gossypii*). The parasitoid *Ad. colemani* exhibited a higher parasitism percentage compared to *Ad. matricariae,* (moderate parasitism) and *Al. abdominalis* (least parasitism). The parasitoids can attack, reproduce, and develop diversely in diverse host species. However, they prefer to choose the ideal host species for their development process (Ghimire and Phillips, 2014) and due to availability of high-quality nutrition (Bueno et al., 1993; Sidney et al., 2010).

The preference for parasitizing some aphid species over others has also been reported in *Aphidius* (Prinsloo, 2000). Some hosts are assumed to be more adequate for the parasitoid; we found high parasitization of three parasitoids on the cotton aphid as compared to the soybean aphid considering the host conferring substantial adaptive advantages of species assessed. Parasitoid's potential may vary when associated with different host species due to variation in adult size, survival, food preference and precision phenomenon (Birch, 1948). The parasitoid species have the ability to adapt and feed on alternative hosts if their favorite hosts are not available in the field (Cameron and Walker, 1984; Zepeda-Paulo et al., 2013). *Aphidius colemani* readily accepted several recognized alternate hosts (Stary, 1975). The alternative host imposes different selection pressures on parasitoid populations. Responsively inhabitants may follow different evolutionary trajectories. The progress of local-host adaptation in populations during divergent natural selection processes could increase the effectiveness of biological control (Zepeda-Paulo et al., 2013).

The presence of less preferred species persuades a resilient disruption consequence and reason in the decrease of parasitism by the preferred species (De-Rijk et al., 2013). A performance like this might influence the parasitoid potential of biological control worth on the availability of multiple host species (Ferrari et al., 2008). It is evident from the datasets that 2nd instar of *As. glycines* was the highly preferred host age followed by 1st, 3rd and 4th nymphal instars, whereas in *As. gossypii,* 1st, 2nd, 3rd and 4th instars in descending order were preferred for parasitism by three tested parasitoids. Certain transformations may be pragmatic for host proclivity and cause alterations in aphid defense reactions and unpredictable behaviors among aphid nymph stages and even at species level (Hagvar and Hofsvang, 1991). Veteran parasitoid females were more productive in confronting smaller than bigger aphids and presented higher oviposition in small aphids (Kouamé and Mackauer, 1991). Host quality is one of several important aspects that regulate female decisions of oviposition in insects (Courtney and Kibota, 1990). Therefore, a female can raise her progeny in total number by focusing on aphids that may spurt parasitization with a minimum capacity (Gerling et al., 1990). The smaller (1st - 2nd) nymphal instars are generally more common than larger (3rd - 4th) in aphid field population and therefore are more predictable to be confronted (Chau and Mackauer, 2001; He et al., 2005; Kouamé and Mackauer, 1991). These parasitoids may choose hosts conferring age preference and the optimization between food supply and ovipositional costs. It seemed that the quantity and quality of host-parasitoid interaction influence efficiency and parasitization perspective. Previous studies confirmed that parasitoids are capable of parasitizing all the stages of their different aphid hosts with emphasis on preference (Lopez et al., 2009; Jokar et al., 2012; Velasco-Hernandez et al., 2017). During our studies, we found that all the three parasitoids more frequently parasitized the 2nd instar, followed by 1st, 3rd and laterally 4th nymphal instars in *As. glycines.* In *As. gossypii* results are partially in association with earlier findings. The preference of parasitoids (*A. ervi* and *A. colemani*) for advanced nymphal instars of aphids (*M. persicae* and *A. gossypii*) has significant effect on the quality of parasitism, which is also depicted from other host-parasitoid interactions (Perdikis et al., 2004; Colinet et al., 2005; He and Wang, 2006; Rehman and Powell, 2010; Farhad et al., 2011; He et al., 2011; Jokar et al., 2012; Hopkinson et al., 2013; Yang et al., 2015; Yazdani et al., 2015). *Aphelinus abdominalis* and *Ad. matricariae* prefer to oviposit the firsts developmental stages (Gerling et al., 1990; Shrestha et al., 2015) due to the capacity to avoid the defensive strategy of 3rd and 4th instar (Chau and Mackauer, 2001; Wyckhuys et al., 2008; Farhad et al., 2011; Pasandideh et al., 2015). These studied parasitoids are more likely preferring earlier stages than intermediate stages of the nymph. However, these effects might be due to absence of defensive, aggressive behavior (Gerling et al., 1990) and less developed immune system of the host (Stoepler et al., 2013).

The density-dependent parasitism (%) of parasitoids (*Ad. colemani, Ad. matricariae* and *Al. abdominalis*) were found significantly different in different number ratios of hosts (*As. glycines* and *As. gossypii*) and parasitoids females (10:1, 50:5, 100:10 and 200:20), respectively. All three investigated parasitoids gave maximum parasitism in a ratio (200:20), and displayed the gradual increase of the parasitization of aphids per parasitoid in other ratios. The mean parasitization steadily increases with the increase of parasitoids and hosts in quantity which further impacts the effectual increase in qualitative parasitism. The tested assumptions of host-parasitoid ratios were not discussed in earlier literature. The result differences may be due to the parasitoids fitting to diverse biotypes, geographic isolation, sympatric speciation and their idiosyncrasy to specific hosts (Atanassova et al., 1998; Takada and Tada, 2000).

Aphelinus abdominalis diverges to the other *Aphidius* species in better behavioral responses at high temperatures (Molck and Wyss, 2001). These traits may be explored more intensely in combination with semi-field and field experiments in the future (Bilal et al., unpublished). The comparison of three parasitoids for the evaluation of two aphid species exhibited the host preference and diverse configurations of the capacity to parasitize among themselves. During the parasitism process, mostly parasitoids can determine host quality conferring to their species, developmental stage and size where the hosts will often be accepted or rejected. Thus, both host preference and host density seems to have a significant role in biological control management programs.

Conclusions

The introspective host preference and parasitoid parasitism depend on the readiness of resources and female choice. Present results are of eminence for bio-control programs and principal thinking whether in the absence and presence of multiple hosts, veteran parasitoid usage may be anticipated or not. *Aphidius colemani* exhibited high, *Ad. matricariae,* moderate and *Al. abdominalis* low parasitism potential for *As. gossypii* (79.48%, 65.33%. 58.83%) and *As. glycines* (60.50%, 49.16%, 40%), respectively. *Aphidius colemani* developed better on *As. gossypii* than on *As. glycines. Aphidius matricariae* showed better and *Al. abdominalis* less performance as compared to *Ad. colemani* in both host species. However, *Al. abdominalis* parasitized both species and poor parasitism in *As. glycines.* Parasitoids showed specific preferences to early nymphal instars of both aphid species. Host: parasitoid ratios showed that parasitism increases with the increase in number of parasitoids and hosts. Transformations between host-parasitoid interactions and such behaviors are obligatory to study due to their potential for host manipulation as these features may influence the efficiency in aphid field populations. The outcomes of the present study may have an effect on the population growth of two aphid species of cotton and soybean.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution statement

BR conceived and designed the study; BR, ZM and MFA; data curation; BR and RM, formal analysis, BR, ZM and MFA conducted investigation; BR and TY methodology; BR and JI, project administration,; BR and RM Software- equal,; BR and JI, supervision,; JI and BR, validation; BR, writing – original draft-lead; BR and JI, writing – review and editing-equal; All authors participated in writings of the manuscript and approved the final version.

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