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Biological Parameters and Feeding Potential of *Micromus timidus* Hagen (Neuroptera: *Hemerobiidae*) on Selected Aphid Species

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Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

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ABSTRACT

In the present study, we investigated on the developmental biology, adult longevity and fecundity of *Micromus timidus* Hagen on *Aphis craccivora* (Koch), *Aphis gossypii* Glover, *Rhopalosiphum maidis* (Fitch) and *Uroleucon compositae* (Theobald) at room temperature (28±2^{0}C) and relative humidity of 70%. The study design followed was completely randomized design (CRD). The study was conducted with three replications with 10 larvae in each replication for biology studies and for adult parameters 3 replications with 10 pairs in each replication used. Shortest incubation period, II instar and III instar duration, total larval period, pre-pupal period, and egg to adult period of *M. timidus* were recorded on *A. craccivora*, shortest I instar duration was recorded on *U. compositae* and least pupal period on *A. gossypii*. The developmental periods for all trait were extended when fed on *R. maidis*. The reproductive traits *viz.,* oviposition period and adult longevity (male and female) were maximum when *M. timidus* adults fed on *A. gossypii*, and the highest fecundity was recorded when fed on *A. craccivora.* Feeding potential in *M. timidus* increases progressively across successive instars, with III instar larvae exhibiting the highest aphid predation rate, and first instar larvae displaying the lowest feeding activity. The maximum feeding potential of the larva was noted on *A. craccivora* (142.47 ± 31.66), while, the adult feeding potential increased when it was given with *A. gossypii* (1335.10 ± 192.74).

Keywords: Micromus timidus; Hemerobiidae; biology and feeding potential.

1. INTRODUCTION

Brown lacewings are small medium-sized insects belonging to the family, *Hemerobiidae*, known to be the third largest family in the order Neuroptera. *Hemerobiidae* is represented by approximately 600 species worldwide (Oswald, 2004, Farahi et al. 2009). The brown lacewings feed on small soft bodied arthropods (aphids, scale insects, spider mites etc.) (New, 2002). Most brown lacewings are aphidophagous predators, play a role in biological control, being more effective at low aphid densities than green lacewings (Chrysopidae) (Pekas et al.2023). The low-temperature requirement of brown lacewings also gives them a survival advantage during cold spells and frosts in temperate climates (Neuenschwander et al. 1975, Kovanci et al. 2014). *Micromus timidus* Hagen was introduced from Australia to the Hawaiian Islands in 1919 for the control of various aphids, particularly on corn and sugarcane plants (Williams, 1927). *Hemerobius nitidulus* F. and *H. stigma* Stephens were introduced from Europe to Canada as biological control agents against the balsam woolly aphid, *Adelges piceae* (Ratzeburg), during the 1930s (Smith et al.1957). However, neither species was recovered (Garland, 1978) possibly because they did not adapt to the climate of Canada.

In India, *Micromus igorotus* Banks was used for biological control of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner in southern parts of the country during the aphid outbreak, 2003 to 2006. A standardized mass production technology has been developed for the *M. igorotus* in the laboratory using *C. lanigera* as a laboratory host and the field release dosage has been standardized (1000 to 1500 cocoons per acre area) (Vidya, 2007, Vidya et al. 2010). Presently the pest is under control by the predators (*M. igorotus* and *Dipha aphidivora* (Meyrick)) (Srikanth et al. 2015) and a parasitod (*Encarsia flavoscutellum* Zehntner) (Srikanth et al.2012) and farmers are not taking any interventions for the pest.

Micromus timidus has been recorded as a predator of many aphid species *viz*., *Aphis gossypii* G., *A. spiraecola* Pagenstecher, *Myzus persicae* (Sulzer) (Rao et al. 1990, Rao, 1969, Devi et al. 2002, Navi et al. 2011), *Longiunguis sacchari* (Zehntner) (Patnaik et al. 1977), *Lipaphis erysimi* (Kalt) (Raychaudhuri et al. 1981), *Toxoptera aurantii* (Boyer de Fonscolombe) (Radhakrishnan et al. 1989, Agarwala et al. 1958), *Pentalonia nigronervosa* Conquerel (Agarwala et al. 1958), *Aphis craccivora* Koch [Navi et al. 2011, Radhakrishnan et al. 1989), *C. lanigera* (Vidya et al. 2010, Navi et al. 2011, Arakaki, 1992), *Myzus nicotianae* Blackman (Rao et al. 1990, Navi et al. 2011, Singh et al. 1994), *Cervahis quercus* Takahashi (Shantibala et al. 1994) and *Aphis nerii* Boyer de Fonscolombe (Chao et al. 2021).

In the present study, we investigated the biology (developmental and reproductive traits) and host specificity of *M. timidus* on four aphid species *viz*., *A. craccivora*, *A. gossypii*, *R. maidis* and *U. compositae* under 28±2^{0}C and 70% RH.

2. MATERIALS AND METHODS

2.1 Rearing of *M. timidus*

The investigation of the current study was carried out at the Department of Agricultural Entomology, Gandhi Krishi Vignana Kendra (GKVK), University of Agricultural Sciences (UAS), Bangalore, Karnataka India from November 2022 to September 2023. The adults of *M. timidus* were collected from the maize field infested with *R. maidis* from K block, Department of Genetics and Plant Breeding, UAS, Bangalore.

The adults of *M. timidus* were reared in a round plastic box (22 x 10 cm) by providing cowpea twigs infested with *A*. *craccivora* under laboratory conditions at 28±2^{0}C temperature and 70% RH. The freshness of the twig was maintained by inserting the lower end of the twig in a glass vial containing water and plugged with a cotton wad. The mouth of the rearing container was covered with black cloth held in position by a rubber band to avoid the escape of adults. The white cotton thread strings were given as the oviposition substrate and the oviposition substrate was selected based on the early works on *Micromus igorotus* Vidya et al. 2008.

The eggs laid on cotton thread string were collected and placed in the rearing container (17 x 5.5 cm) for hatching. After hatching, larvae were provided with aphids-infested cowpea twigs and at the time of pupation, a folded paper sheet was provided for pupation. Folded paper sheets with pupae were kept for adult emergence in the round plastic container (22 x 10 cm).

2.2 Biology and Feeding Potential of *M. timidus* on the Selected Species of Aphids

The biology and feeding potential of *M. timidus* were studied on 4 different aphid species *viz., A. craccivora, A. gossypii, R. maidis* and *U. compositae.* The basis for the selection of the four-aphid species is mainly based on the availability and ease of maintenance in the field as well as in the polyhouse condition. For maintaining the above aphid cultures, the host plants like cowpea (*Vigna unguiculata* (L.) Walp.), bhendi (*Abelmoschus esculentus* L.), maize (*Zea mays* L.), safflower (*Carthamus tinctorius* L.) and niger (*Guizotia abyssinica* (L. f.) Cass.) were maintained both in the field and in the polyhouse of AINP on Agricultural Acarology, Department of Entomology, University of Agricultural Sciences, Bangalore. Each crop was grown in the field in an area of 2.5 x 4.5m^2 and allowed for natural infestation and if not, artificial aphid infestation was done. The same crops were also maintained in the pots in the polyhouse and artificial infestation was done. The field/polyhouse-maintained aphid culture was used for the study.

Freshly a laid hundred eggs of *M. timidus* were placed in a clean plastic box with a lid (5.5 x 17cm) and incubated under laboratory conditions (28±2^{0}C and 70% RH) and examined at 24-hour intervals to record the incubation period. Upon hatching, 30 neonate larvae were reared individually in clean glass vials (5.0 x 2.5 cm) and were provided with respective aphid species as food till pupation. The study was conducted with three replications with 10 larvae in each replication. Larval instar duration was recorded based on the presence of cast skin and size. The observation on the prepupal (the time larva stops feeding and starts constructing the cocoon till completion) and pupal period (from the time of completion of cocoon construction till adult emergence) was recorded. A known number of respective aphid species were given to each larva and the number of aphids consumed was recorded at 24-hour intervals. The total number of aphids consumed by each instar was calculated separately and recorded. Aphids that fed were recognized by their shriveled appearance compared to the live aphids.

The reproductive traits *viz.,* pre-oviposition, oviposition, post-oviposition period, male and female longevity and fecundity were studied in the laboratory. Hundred freshly emerged adults were released into the ovipositional container (22 x 10cm) by providing respective aphids as adult food. Sexing was done (Joshi et al. 2011) and one pair *viz.,* one male and one female were paired and released in a separate container (9 x 9cm) containing respective aphid species. The mouth of the container was covered with muslin cloth and fastened with a rubber band after providing cotton white thread strings as an oviposition substrate. After 24-hour intervals, both oviposition substrate and aphids were replaced with fresh ones. From 3 replications (10 pairs in each replication), pre-ovipositional period, ovipositional period, and post-ovipositional period, the longevity of both males and females and fecundity were recorded. The number of eggs laid by the female every day was recorded to find out the fecundity. Since the adults are also predators, they were provided with a known number of aphids every day and the total number of aphids consumed was recorded.

2.3 Statistical Analysis

The mean and the standard deviation are calculated for different larval and adult parameters. Test for significance is done for life stages and feeding potential for the different species of aphids fed by the predator using Duncans Multiple Range Test (DMRT) in Web-Agri Stat Package (WASP 2.0).

3. RESULTS

3.1 Biological Parameters of *Micromus timidus* on Four Aphid Species

The comparative account of different biological parameters (developmental traits and developmental periods) of *M. timidus* on four aphid species *viz*., *A. gossypii, A. craccivora, R. maidis* and *U. compositae* are presented in the Table 1 and Fig. 1. The incubation period of *M. timidus* on four aphid species varied significantly from 3.30 days to 3.90 days. The significantly shortest incubation period was recorded on *A. craccivora* (3.30 days), was on par with *U. compositae* (3.46 days) and was significantly higher on *R. maidis* (3.90 days). The total larval duration was significant and varied from 5.20 to 6.15 days. The larval duration was significantly shorter on *A. craccivora* (5.20 days) and was extended on *R. maidis* (6.15 days). The larval period on *A. craccivora* was on par with *U. compositae* (5.53 days) and differed statistically from *A. gossypii* (6.00 days) and *R. maidis* (6.15 days). The pupal period on four aphid species did not vary significantly. Pupal period was extended on *R. maidis* (7.10 days) followed by *U. compositae* (6.23 days), *A. gossypii* (5.56 days) and a shorter pupal period was recorded on *A. craccivora* (5.53 days).

The total developmental period from egg to adult varied significantly among the given hosts. The mean shortest developmental period was recorded on *A. craccivora* (14.03 days) and conversely longest duration on *R. maidis* (17.15 days). All the biological parameters like incubation period, total larval duration, pupal duration and developmental period were completed in the shortest time on *A. craccivora* as the host, and conversely, they took the longest duration when *R. maidis* was used as the host*.*

3.2 Reproductive Traits of *Micromus timidus* on Four Aphid Species

The comparative account of different reproductive traits of *M. timidus* on four aphid species is presented in Table 2. The pre-oviposition period varied significantly from 5.08 to 8.00 days. The least pre-oviposition period was recorded on *A. craccivora* (5.08 days) which was followed by *R. maidis* (6.10 days), *A. gossypii* (7.00 days) and *U. compositae* (8.00 days) (Fig. 2). Oviposition period *viz.*, egg-laying duration was 23.50, 23.84, 23.90 and 30.60 days on *R. maidis, A. craccivora, U. compositae* and *A. gossypii,* respectively and differed non-significantly (Fig. 2). The shortest post-oviposition period was recorded on *A. gossypii* (2.10 days) followed by *A. craccivora* (2.24 days) and *R. maidis* (3.20 days) and the longest duration was on *U. compositae* (4.30 days), there was no significant difference between the aphid species (Table 2). Fecundity is a crucial trait for natural enemies. There is a significant difference in the fecundity of *M. timidus* across different aphid species. Maximum fecundity was on *A. craccivora* (591.67 eggs/female) over a period of 23.84 days followed by *A. gossypii* (390.50 eggs/female) and *R. maidis* (362.10 eggs/female). In contrast, significantly lower number of eggs was recorded on *U. compositae* (176.90 eggs/female). The peak egg-laying by the *M. timidus* female was between the 10^{th} and 11^{th} day. On *A. craccivora* peak egg laying was on 11^{th} day *i.e.,* 36.78 eggs/female and 25 eggs/female on the 10^{th} day were recorded on *A. gossypii.* The maximum egg laying was seen on the 10^{th} day of the oviposition period on both *R. maidis* (33.80 eggs/female) and *U. compositae* (14.10 eggs/female) (Fig. 3). Adult longevity (male, female and mean duration) on four aphid species differed non-significantly. Survival time of *M. timidus* male was 36.70, 27.30 and 30.40 days on *A. gossypii*, *R. maidis* and *U. compositae* respectively. The female duration was maximum on *A. gossypii* (39.70 days) followed by *U. compositae* (36.20 days), *R. maidis* (32.80 days) and *A. craccivora* (31.16 days). The mean longevity of adults ranged from 30.05 to 38.20 days, with the maximum longevity on *A. gossypii* and the lowest on *R. maidis*.

Table 1. Comparative biology of *Micromus timidus* on different aphid species

Aphid species	Duration (days)								
	Incubation period	I instar	II instar	III instar	Total Larval period	Pre-pupal period	Pupal period	Pre-pupal + pupal period	Total life cycle
A. craccivora	3.30 ± 0.46[a]	2.00 ± 0.00	1.80 ± 0.70	1.40 ± 0.61[a]	5.20 ± 0.87[a]	2.40 ± 0.95[a]	3.13 ± 1.20	5.53 ± 1.94	14.03 ± 2.42[a]
A. gossypii	3.63 ± 0.55[b]	2.03 ± 0.48	1.90 ± 0.30	2.06 ± 0.93[b]	6.00 ± 0.82[b]	2.56 ± 1.33[a]	3.00 ± 1.55	5.56 ± 2.84	15.20 ± 3.48[a]
R. maidis	3.90 ± 0.54[c]	2.05 ± 0.50	2.00 ± 0.00	2.10 ± 0.30[b]	6.15 ± 0.48[b]	3.25 ± 0.43[b]	3.85 ± 0.36	7.10 ± 0.62	17.15 ± 0.85[b]
U. compositae	3.46 ± 0.49[ab]	1.90 ± 0.30	2.00 ± 0.45	1.63 ± 0.88[a]	5.53 ± 0.99[a]	2.86 ± 1.20[ab]	3.37 ± 1.35	6.23 ± 2.49	15.23 ± 2.96[a]
CD value at 0.05	0.263	NS	NS	0.39	0.43	0.56	NS	NS	1.40

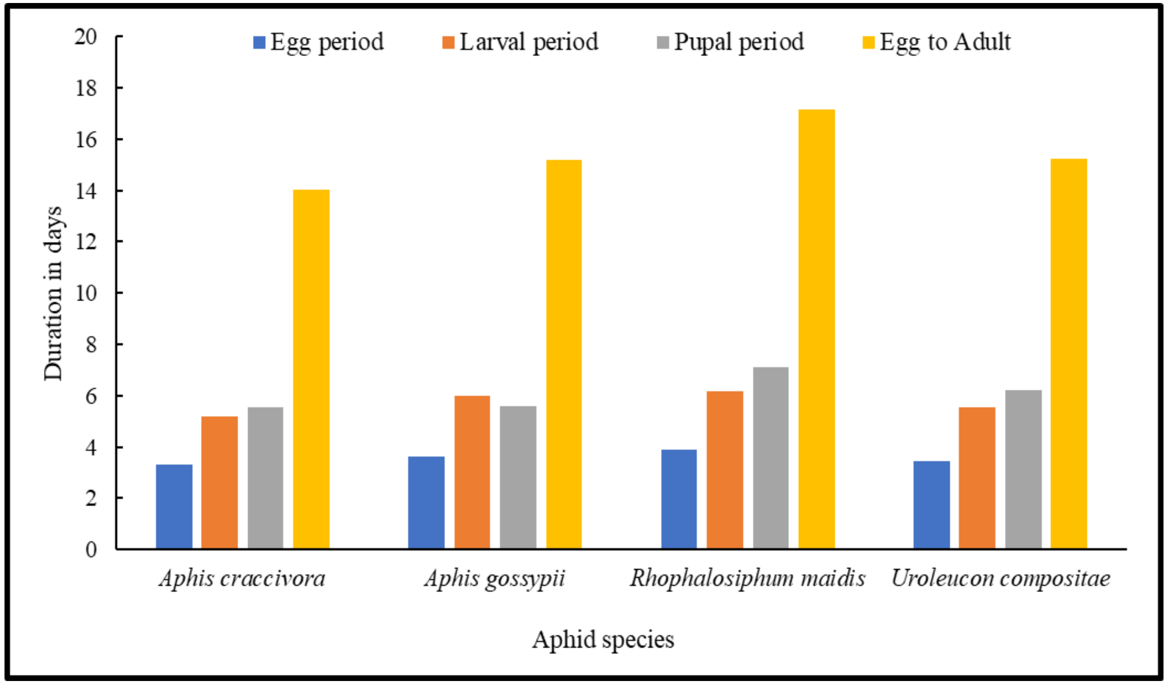

Fig. 1. Developmental biology of *Micromus timidus* on different aphid species

Table 2. Reproductive traits of *Micromus timidus* adult on different aphid species

Aphid species	Duration (days)			Fecundity	Longevity (days)		
	Pre-oviposition period	Oviposition period	Post-oviposition period		Male	Female	Mean
A. craccivora	5.08 ± 1.09[a]	23.84 ± 14.19	2.24 ± 2.44	591.67 ±306.58[a]	34.52 ± 19.55	31.16 ± 15.19	32.84 ± 12.54
A. gossypii	7.00 ± 1.41[bc]	30.60 ± 11.73	2.10 ± 1.38	390.50 ± 168.66[ab]	36.70 ± 13.89	39.70 ± 12.49	38.20 ± 5.40
R. maidis	6.10 ± 1.30[ab]	23.50 ± 10.89	3.20 ± 3.22	362.1 ± 237.50[b]	27.30 ± 6.96	32.80 ± 9.55	30.05 ± 7.16
U. compositae	8.00 ± 1.00[c]	23.90 ± 7.91	4.30 ± 2.49	176.90 ± 102.17[c]	30.40 ± 9.07	36.20 ± 9.29	33.30 ± 7.06
CD value at 0.05	1.10	NS	NS	228.11	NS	NS	NS

Mean values followed by the same letter do not differ significantly by DMRT (P= 0.05), NS indicates non-significant

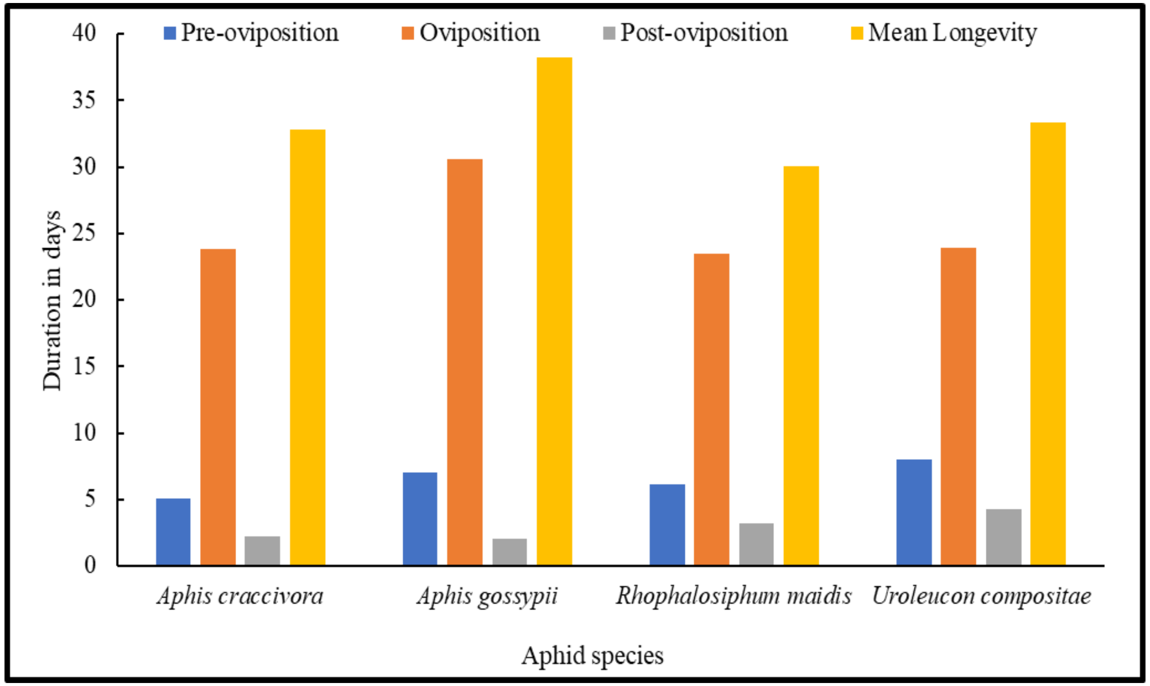

Fig. 2. Reproductive traits of *Micromus timidus* on different aphid species

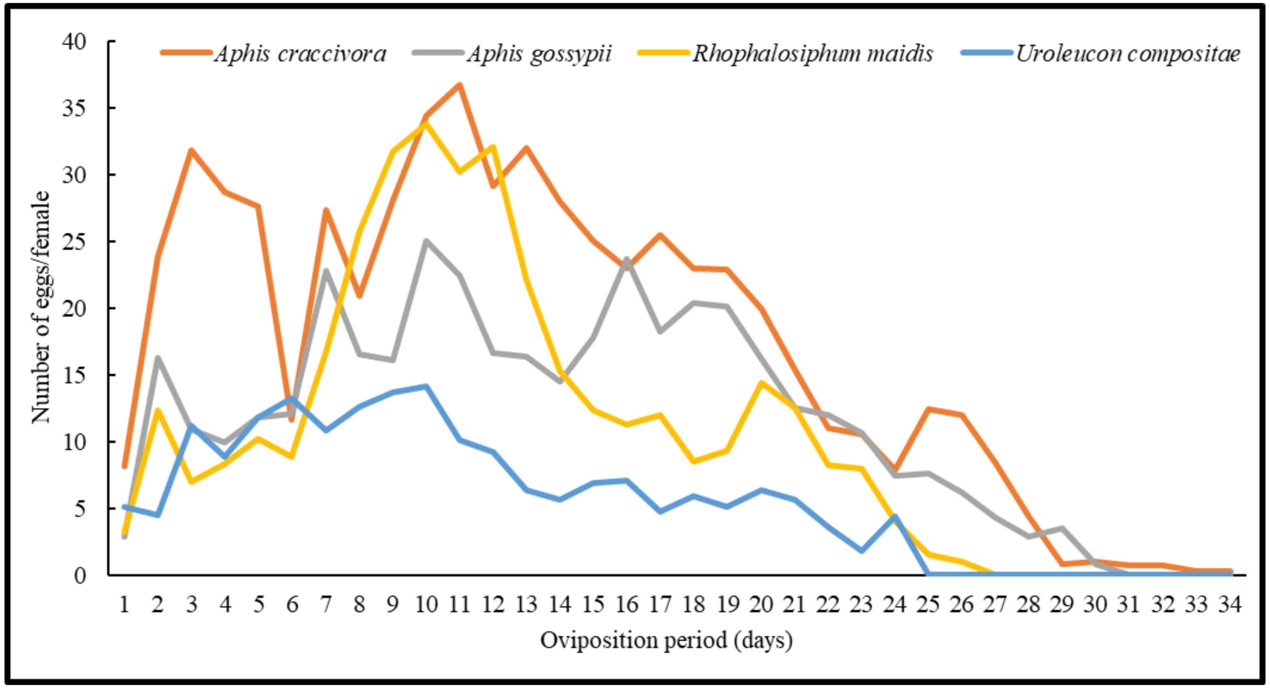

Fig. 3. Fecundity of *Micromus timidus* on various aphid species at daily intervals

Table 3. Comparative feeding potential of *Micromus timidus* on different aphid species

Aphid species	Total number of aphids consumed					
	I instar	II instar	III instar	Larval stage	Adult stage	Total
A. craccivora	46.66 ± 5.83[a]	52.33 ± 22.83[a]	43.47 ± 20.95[b]	142.47 ± 31.66[a]	1305.92 ± 544.55[a]	671.31 ± 686.26[a]
A. gossypii	12.06 ± 1.43[d]	40.23 ± 8.64[b]	72.26 ± 35.10[a]	124.57 ± 41.38[bc]	1335.10 ± 192.74[a]	427.20 ± 534.16[b]
R. maidis	16.75 ± 2.36[b]	46.95 ± 3.71[ab]	72.40 ± 20.31[a]	136.10 ± 21.91[ab]	1041.80 ± 284.29[a]	438.00 ± 457.77[ab]
U. compositae	14.90 ± 1.58[c]	44.53 ± 9.43[b]	52.63 ± 21.41[b]	112.07 ± 24.72[c]	630.7 ± 141.11[b]	241.73 ± 236.37[b]
CD value at 0.05	1.750	7.092	13.202	16.256	372.858	233.848

Mean values followed by the same letter do not differ significantly by DMRT (P= 0.05)

3.3 Feeding Potential of *Micromus timidus* on Four Aphid Species

The results recorded for the feeding potential of *M. timidus* on four aphid species are presented in Table 3. A significant difference in the total feeding by larval stage was observed. A significantly higher number of *A. craccivora* (142.47aphids/larva) were consumed by the larva of *M. timidus*, followed by *R. maidis* (136.10 aphids/larva) and *A. gossypii* (124.57 aphids/larva). Significantly least number of *U. compositae* aphids were consumed by the larva (112.07 aphids/larva). The instar-wise feeding potential also differed significantly in all three instars. Irrespective of aphid species, a greater number of aphids were consumed by III instar compared to I and II instars, except on *A. craccivora* due to shorter duration (1.40 days). All three instars consumed a significantly lower number of *U. compositae*. In comparison to larval feeding potential, the total adult feeding on aphids was higher. Adults consumed significantly lower number of safflower aphids, *U. compositae* (630.7 aphids). The feeding by the adults on the other three aphid species did not differ significantly. The total adult feeding of *A. craccivora, A. gossypii* and *R. maidis* was 1305.92, 1335.10 and 1041.80 aphids respectively.

4. DISCUSSION

In biology studies, the incubation period is slightly higher on *R. maidis* compared to other three aphid species. The values recorded did not differ much from Vidya et al. 2011, Vidya et al. 2007 on *C. lanizera* and *M. sacchari* and Navi et al. 2010 on *A. gossypi* who reported incubation period of 3 to 4 days for *M. timidus*. However, the larval duration of *M. timidus* does not vary significantly among *A. craccivora* and *U. compositae* and completes the larval period faster compared to *A. gossypii* and *R. maidis* may be because of the host likeliness by *M. timidus.* The present findings with respect to larval instars and duration, corroborate with the findings of Radhakrishnan and Muraleedharan 1989, Raychaudhuri *et al*. 1981, Vidya *et al*. 2010 and Navi *et al*. 2010 that *M. timidus* has three larval instars, completed within a period of 7 to 10 days on different species of aphids. Confirming that brown lacewing has short larval duration with three instars. In the present study pre-pupal period *viz*., time taken to construct cocoons (inner and outer cocoon) and pupal period were considered separately. The present values are similar to the findings of Raychaudhuri *et al*. 1981 and Chao *et al*. 2021 on *L. erysimi*, *A. craccivora* and different aphid species respectively. The pupal period does not vary significantly between the hosts. The values reported by Navi et al. 2010 and Radhakrishnan and Muraleedharan 1989 on *A. gossypi* (9 to 10 days) and *T. aurantii* (8 to 10 days) are slightly higher than present study. The values recorded in the present study did not differ much from Vidya *et al*. 2007 and Navi *et al.* 2011 for *M. timidus*. Earlier studies by Vidya, 2007 and Joshi *et al*. 2011 on *M. igorotus* on different aphid species have reported the similar kind of variations in the developmental period. The total life cycle from egg to adult recorded less on *A. craccivora i.e.,* of 14. 30 days showed us host acceptance of *M. timidus* towards *A. craccivora* which can be used as host for mass multiplication of *M. timidus.*

Neuenschwander, 1976 reported longer adult duration of 72.20 days for *Hemerobius pacificus* Banks. Similarly, Sato and Takada 2004 reported higher longevity of three *Micromus* spp. on *A. craccivora*. Vidya, 2007 and Joshi *et al*. 2011 have reported longer adult duration and higher fecundity by *M. igorotus* on different aphid species. The reason for variation in the adult longevity or higher fecundity of *M. timidus* may be due to the species itself. The life cycle and adult parameters greatly vary with species of brown lace wing and the nutritional quality of the aphid species, which is influenced by host plants on which the host insect has fed. Only pre-oviposition period and fecundity varied significantly among the host selected it may be due to that hosts does not play an important role in oviposition and post oviposition period but it play major role in terms of fecundity because the significantly higher number of eggs were laid when adult is given with *A. craccivora i.e.,* 592 eggs in its lifetime. It may be because of the nutrients present in *A. craccivora* compared to other three hosts.

From our feeding potential studies of *M. timidus*, the number of aphids consumed was less on *U. compositae* and it might be because of their larger body biomass. In contrast to this, the adult consumption of *A. gossypii* was higher because of its smaller body biomass. Irrespective of the aphid species, adults consumed a greater number of aphids compared to larvae as they survived for long duration (30-38 days) compared to larval stage (5 to 7 days). Our studies on feeding potential are in corroborate with the findings of Patnaik *et al.* 1977; Raychaudhuri *et al*. 1981 and Radhakrishnan and Muraleedharan 1989. On the contrary, Vidya *et al*. 2009 and Navi *et al*. 2010 have reported higher feeding potential of *M. timidus*.

5. CONCLUSION

The present study investigated on the biology and feeding potential of *M. timidus* on four aphid species. The total life cycle from egg to adult was shortest on *A. craccivora* (14.03±2.42 days) and the adult longevity was 32.84±12.54 days which did not differed significantly from other aphid species. Apart from these parameters, fecundity of *M. timidus* was highest when fed on *A. craccivora* (591.67± 306.58). Hence, by considering the life cycle, reproductive parameters, and feeding potential, it can be concluded that *A. craccivora* is the most suitable laboratory host for the laboratory rearing of *M. timidus,* followed by *A. gossypii*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text to image generators have been used during the writing or editing of manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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