Research Article

Biology and Feeding Efficiency of Zigzag Beetle, *Menochilus sexmaculatus* (Fab.) (Coleoptera: Coccinellidae) Fed on Mustard Aphid, *Lipaphis erysimi* (kalt.) (Hemiptera: Aphididae) Under Controlled Conditions

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Abstract | Research was conducted to evaluate the biological attributes and feeding efficiency of *Menochilus sexmaculata* fed on *Lipaphis erysimi* under laboratory conditions. The mean developmental time from egg till adult emergence was 41.60±0.70 days under laboratory conditions of 24±1c° with 55±5 percent relative humidity (R.H.). The incubation period was 4.00±0.25 days. The pre-oviposition, oviposition, and post-oviposition periods were observed at 8.6, 24.1 and 6.0 days, respectively. The total laid eggs/female were 288.4 eggs. The pre-pupae and pupae developmental time was 1.00±0.00 and 6.00±0.08 days, respectively. The overall mean male longevity was 33.40 days, while the mean female longevity was 38.70 days. In larval feeding efficiency, the maximum mean number of aphids (479.6) were consumed by larvae while the minimum mean number of aphids (36.8) were consumed by 1st larva instar. Female beetle was recorded with a maximum mean number of aphids (2561) consumption as compared to male beetles (1921). Furthermore, the apparent mortality was highest at egg stage, making it the most vulnerable stage in the beetle's life cycle, followed by 1st instar larval stage, which also showed higher mortality. The maximum survival fraction was observed for pre-pual and fourth instar larvae, while the minimum was found for the egg stage. Mortality survival ratio (MSR) was found to be maximum at the egg stage and minimum at the 4th instar and pre-pupal stages, while the Indispensable mortality (I.M.) was found to be maximum at the egg stage and minimum at 4th instar and pre-pupal stages. Life expectancy (ex) was found to be maximum in the egg phase (4.67) and minimum in the pupal phase (1.5). The total K value for the whole generation was 0.33.

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Introduction

Aphids are economically important pests of cultivated crops in Pakistan. There are 92 aphid species that have been reported in Pakistan. Some of these are serious pests of stone fruits, vegetables and oil seed crops, Aphids injury soft shoots straight by sucking cell saps and indirectly transmit viruses that influence both quantity and quality of the crops (Irshad, 2001).

In Pakistan, the aphids *Lipaphis erysimi* (Kalt.), *Bervicoryne brassica* (L.) and *Myzuspersicae* (Sulz.) are mostly found attacking oilseed crops in the winter and spring season (Amer, 2009). *L. erysimi* is a cosmopolitan specie and considered the main constraint to South Asia's brassica crop (Landin, 1982). The colonies of *L. erysmi*, *B. brassica* and *M. persicae*are present on both (upper and lower) surfaces of the leaves, leaf axles, leaf folds developing heads, and leaf stalk and are rarely found at lower plant portion near the soil. Aphids infested seedling/plant becomes stunted, and due to continuous feeding, yellowing and wilting may cause necrosis in mature plants.

IPM is an integrated approach for pest management in which biological control is an important component. In biological control, natural enemies (pathogen, predator and parasitoid) are used for pest control (Bonnett and Gordon, 1991). Among predators, the Lady bird beetle (Coccillinidae) is the main predator of the aphids. The larvae and adult stages of the ladybird beetle are predacious. They mostly feed mites, aleyrodids and aphids and psyllids. These insects exercises as a natural control for damaging insect pests in the field (George, 1999).

Among the coccinellid beetle, *Menuchilus sexmaculata* (Fab.) normally known as zigzag ladybeetle, is one of the best predators/ natural enemies of aphids (Gilkeson and Kelin, 2001). Rajeshwari and Singh (2022) indicated that three coccinellid beetles comprising *Coccinella septempunctata, Hippodamia variegate* and *Menochilus sexmaculatus* (*Cheilomenes sexmaculata*) along with two chrysopids viz. *Chrysoperla carnea* and *Anisochrysa boninensis*; the syrphids, *Episyrphus balteatus* and chamaemyiid, *Leucopis* sp. were observed feeding on colonies of aphids. *M. sexmaculatus* is predaceous and distributed worldwide in Philippines, South Africa, Southern western Asia, Indonesia, India and Pakistan (Rahman *et al*., 1993). This predator beetle feed on a spacious range soft body insect pest, consisting of plant hopper, jassids, thrips, mealy bugs, white fly and aphids that cause damage to economically important vegetables, fruits and other cultivated crops (Solangi *et al*., 2007).

Due to the predacious importance of *M. sexmaculatus* on various agricultural pests, study was conducted on the biological attributes of life parameter and feeding efficiency of *M. sexmaculatus* on *L. erysimi.* The research study will be advantageous for pest control managers in employing bio-control agents for the IPM program in future.

Materials and Methods

This research was carried out on predatory efficiency and biological attributes of zigzag beetle (*Menochilussexmaculata*) (Coleoptera: Coccinellidae) reared on mustard aphids (*Lipaphiserysimi*) (Aphididae: Homoptera) under laboratory conditions of 24±1°C with 55±5 percent relative humidity (R.H.) in Insectary Biological control lab, at Integrated Pest Management Programme, NARC, Islamabad, during 2019. Cabbage plants were sown in screen house in Insectary on which the aphids were reared. All the agronomic practices were kept constant.

Aphids culture maintenance

For maintenance of the *L. erysimi* culture, aphids infested leaves were collected from cultivated fields of NARC. The aphids were removed by soft camel hair brush and released on the culture host plant in a glass house at the 3rd -4th leaf stage. The aphids culture was left undisturbed to induce patheogenesis for the entire study period.

Rearing of M. sexmaculatus

Initially, the *M. sexmaculatus* beetles and pupae were collected in field and shifted to the lab for rearing. The emerged adults were shifted to small plastic containers covered with muslin cloth and kept under controlled environmental conditions. Aphids infested leaves of cabbage were provided as feed to the adults daily. Tissue paper and fresh leaves were placed into the container for egg laying, which were then collected and shifted to small plastic pots. Upon hatching all the larvae were shifted to small vials separately and were given fresh infested leaves daily till pupation. Adults, upon emergence, were again shifted to the main plastic container.

Experimental study

Two hours 100 fresh-eggs of *M. sexmaculatus* were gathered from stock culture and counted under a binocular microscope. Ten eggs were reserved in each vial (6 x 3) cm replicated 10 times. Percent hatching and incubation period were calculated. Subsequent to hatching, the 1st instar larvae of *M. sexmaculatus* were shifted into vials separately, and provided with aphid-infested cabbage leaves in each vial. Vials were covered with a muslin cloth. After 24 hours, the old-infested leaves were regularly replenished with fresh leaves. Data were collected on molting, survival/ mortality during specific ages and stage of immature stages daily.

The following parameters were recorded.

- The egg incubation period (days) and hatching percentage
- The larval instars developmental period
- Pre-pupal and pupal durations
- Developmental time from egg to adult emergence
- Age and stage specific mortality/survival rate from egg to adult emergence
- A number of males and females emerged

Statistical analysis

Data on developmental time, age, and stage-specific survival/ mortality were subjected to variance analysis with one-way ANOVA via Statistix 8.1. Means were compared using Tukey HSD test at 5% level of significance.

Age and stage-specific life table parameters of immature stages

Age-specific life table parameters: To draw life table parameters, the following assumptions were used (Ali and Rizvi, 2009).

x = age of insect in days

d_x = each interval mortality rate

l_x = survival of the insect

e_x = life probability for the age of each individual x, by

$e_X = t_x / l_x$

$100q_x$ during age mortality x and totalled by equation

$100\times(d_x/l_X)$

l_x and t_x calculated as;

lx = active individual between age x+1and X, and determined as $t_x = l_x + (l_x+1) + (l_x+2)\ (l_x+3) \ldots .. + l_w)$, l_w = final age

Stage-specific life table: The following standard heads were used for stage specific tables.

x=Insect age (days).

l_X=Survived rate of age (X).

d_x=Rate of Mortality ($_X$)

100qx (Apparent Mortality)

$100q_x$ shows the percent death;

Apparent Mortality= $100\times d_x/l_x$

Sx (Survival Fraction)

Data of $100Q_x$ was used for approximation of Sx with the following formula;

$$S_x(\text{each phase}) = L_x(\text{successive phase})/L_x\ (\text{specific phase})$$

MSR (Mortality Survival Ratio)

MSR is the increase in individual numbers, which was calculated as:

$$(\text{Mortality in specificphase})/(L_X \text{ of successive phase}) = MSR$$

I.M. (Indispensable Mortality)

No. of emerged adult x MSR of specificphase) = I.M.

K value

$K = k_E + k_G1 + k_G2 + k_G3 + k_G{}^4 + k_{PP} + k_P$, where k_E, k_g1, k_g2, k_g3, k_g4, k_{PP} and k_P. These are the k-values at the oviposition stage, first instars to fourth instar, pre-pupae and pupal stage.

Biological attributes of adult female *M. Sexmaculatus*

To find out the biological attributes of adult female *M. sexmaculatus*, ten newly emerged pairs from stock culture were shifted in adult rearing jars and provided with cabbage leaves infested with *L. erysimi* aphids daily. The laid eggs by each female were gathered on daily basis in each replicate. The process was continued throughout their life span, and data was calculated on the following parameters.

- Pre-oviposition period
- Oviposition period
- Post oviposition period
- Number of eggs/female
- No. of eggs/Female/day

The predatory potential of various larval instars fed on L. erysimi aphids

Twenty (20) freshly emerged 1st instar larvae were collected and transferred into transparent vials separately. These larvae were provided with 20 aphids in each vial and the number increased with each larval instar. Data was documented by counting the numbers of consumed, dead and unconsumed aphids in each vial on daily basis under binocular microscope. The same was carried on till pupation.

Feeding efficiency of adult males and females fed on L. erysimi aphids

Five males and five females adult beetles were kept in adult rearingjars separately and provided with a known number of aphids (250-300) on a daily basis. The consumed and unconsumed aphids were counted after every 24 hours in each replication till the mortality of adults.

Data analysis

The data was analyzed by statistical software (Statistix 8.1) and mean values were compared using Least Significant Difference test.

Results and Discussion

Developmental time (days) ± S.E. of immature stages of M. Sexmaculata fed on L. Erysimi aphids (1st to 3rd) nymphal instars

The mean developmental time of immature stages of *M. sexmaculata* fed on *L. erysimi* was studied. The developmental time from egg to adult emergence was 41.60±0.70 days. The incubation period was 4.00± 0.25 days. Non-significant difference (2.44±0.03 and 2.11±0.03days) was recorded in the developmental time of 1st and 2nd larval instars, respectively. Statistical significance was recorded for the developmental time of 3rd and 4thinstar larvae and larvae with 3.60±0.03, 5.20±0.07 and 13.35±0.22 days, respectively. The pre-pupae and pupae developmental time was 1.00±0.00 and 6.00±0.08 days, respectively (Table 1). These findings were in agreement with those of Sharma and Joshi (2013), who found incubation duration as (3.5 ± 0.53) days, 1st instar (3 ± 0.11) days, 2nd instar (1) day, 3rd instar (2 ± 0.14) days, 4th instar (12) days, and pupal duration of (4.5±0.47) days. The same conclusions are also in line with Solangi *et al.* (2007) where it was accounted for the incubation period of *M. sexmaculata* eggs as 7.5, 7.1 and 7.2 days, with a larval duration of 1st, 2nd, 3rd and 4thinstar as (6.5-7.0, 4.4-5.5, 3.9-6.5 and 6.6-8.5) days, respspctively, and pupal duration of 3.1-5.5 days, while reared on various three species of aphids (*R. Maidis, A. gossypii*and *T. trifolli*). Similarly, Esbijerg (1980) also recorded the incubation period as (5-6) days. Rajput (1990) revealed pupal period of *M. sexmaculatus* in the range of 4 to 6 days averaging of 4.7 days.

Table 1: *Developmental time (days) ± SE of immature stages of M. Sexmaculata fed on L. Erysimi aphids (1st to 3rd) nymphal instars.*

Developmental stages	Developmental time ± SE	Minimum duration in days	Maximum duration in days
Incubation	4.00± 0.25 d	3	5
1st instar	2.44±0.03 e	2.00	3.00
2nd instar	2.11±0.03 e	1.50	2.80
3rd instar	3.60±0.03 d	3.00	4.00
4th instar	5.20±0.07 c	4.00	6.00
Larvae	13.35±0.22 b	10.00	16.00
Pre pupae	1.00±0.00 f	1.00	1.00
Pupae	6.00±0.08 c	5.00	7.00
Egg to adult emergence	41.60±0.70a	33.00	49.60
LSD value	4.385		

Means followed by the same lower case letter against different larval instars column wise are non-significantly different at 5 ≤ 0.05 using Tukey HSD test.

Mean pre-oviposition, oviposition, post oviposition period, number of eggs female^{-1} and number of eggs female^{-1} day^{-1} of M. sexmaculata fed on L. erysimi under lab conditions

The pre-oviposition, oviposition, and post oviposition periods were (8.6, 24.10 and 6.0 days), respectively. The total laid eggs/femalewas 288.4 eggs. The lowest number of eggs (2) was laid on day 9 while the highest number of eggs (25) was laid on day 20 (Figure 1). These results coincide with that of Solangi *et al.* (2007), who reported theseperiods as 3.1-3.7, 23.4-27.7 and 3.5-4.5 days, respectively, for female M. *sexmculata*. Priyadarshani *et al.* (2016) determined the pre-oviposition period as 3.0 ± 0.1 days and the period for oviposition as 43.3 ± 0.9 days when reared on *A. craccivora*. Tank and Korat (2007) investigated the period for oviposition of *M. sexmaculatus* as 16.1 ± 2.5 days cultured on *A. craccivora*. Sharma and Joshi (reported the oviposition period of 11 days and fecundity of *M. sexmaculata* per female as 700 eggs, when reared on *A. gossypii*. Similarly, Saha (1987) accounted for 1391 eggs female^{-1} when *A. gossypii* was offered to *M. sexmaculatus*. The variation may be due to the types of prey provided to the beetles (Moya-Larano, 2011).

Male/Female longevity of M. sexmaculata feed on L. erysimi

The results regarding the male/female longevity of *M. sexmaculata* adults revealed that the mean male longevity was 33.40 days while the mean female longevity was 38.70 days (Figure 2). The results are similar to those of Sharma and Joshi (2013). They reported that female life duration was long as compared to males.

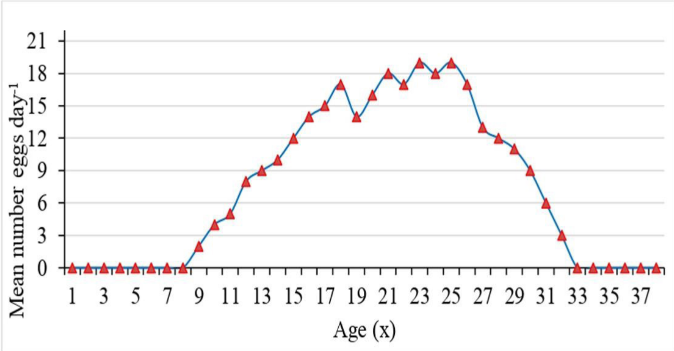

Figure 1: *Pre-Oviposition, oviposition, post oviposition period, mean no. of eggs/female and mean no. of eggs/female/day of M. sexaculata feed on L. erysimi.*

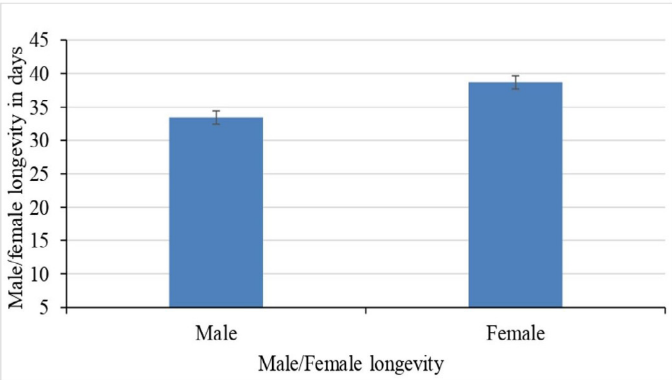

Figure 2: *Male/female longevity of M. sexaculata feed on L. erysimi.*

Feeding efficiency of M. sexmaculata (Larval stages)

The feeding efficiency results indicated that all beetle's instars are voracious feeders of the aphid species (Figure 3). The 5th instar larvae consumed the maximum mean number of aphids (479.6) followed by fourth, third, and second instar larva with a mean consumption of 221.3, 147.6 and 64.9 aphids, respectively. While, the first instar larvae consumed the minimum mean number of aphids (36.8). Solangi *et al.* (2007) also reported that prey consumption difference was highly significant between the third and fourth instar but statistically not significant between the 1st and 2nd instars. More aphids were consumed by the 4th instar and adult *M. sexmaculatus.* Least preference to mustard aphid by *M. sexmaculatus* grubs was reported by Verma *et al.* (1983). He determined that mean daily consumption of aphids by 1st to 4th instar larvae was 49.47, 25.04 and 57.11 on *R. maidis, A. gossypii* and *T. trifolii,* respectively. Patel and Vyas (1984) reported that the highest mean consumption of *A. craccivora* by *M. sexmaculata* grubs was 79.0 per day.

Feeding efficiency of M. sexmaculata (Male/ Female) adults

The feeding efficiency of *M. sexmaculata* (male/ female) Adults showed that the highest mean number of aphids were consumed by female adults (2561), while male beetles consumed the lowest mean number of aphids (1921) (Figure 4). The investigations are in line with that of Priyadarshan *et al.* (2016) as adult *M. sexmaculata* females consumed significantly more aphids (1624) than males (1300). Similarly, Bukero et al. (2019) also found that male adults consumed 79.1 ± 0.10 aphids/day/beetle as compared to female adults 89.1 ± 0.16 aphids/day/beetle.

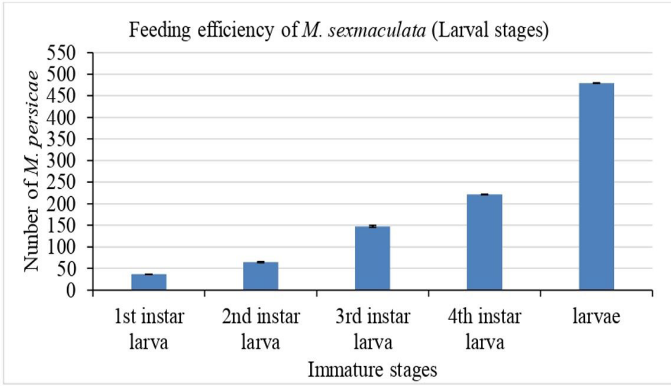

Figure 3: *Feeding efficiency of M. sexaculata (Larval stages) feed on L. erysimi.*

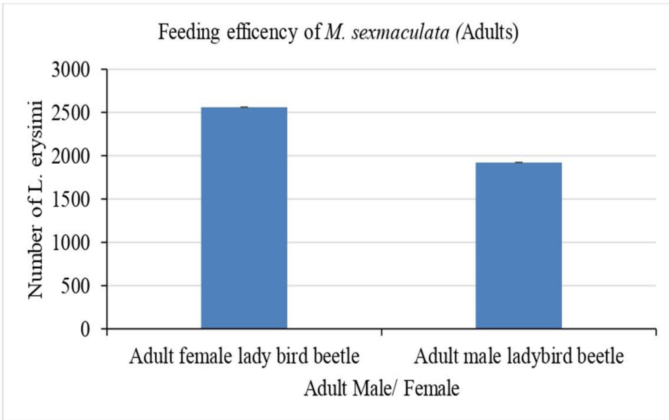

Figure 4: *Feeding efficiency of M. sexaculata (Male/female) adults.*

Stage specific life table of M. sexmaculata fed on M. persicae

Apparent mortality: The apparent mortality in the egg phase, first, second, third and fourth larval instars was (19.0, 16.0, 10.2, 8.1 and 5.35), respectively. The apparent mortality of pre-pupa and pupal phases were 3.77 and 7.84, respectively. These observations revealed that the egg stage was the most vulnerable stage in the life cycle. Among the larval instars,

Table 2: *Stage Specific Life table of M. sexmaculata feed on L. erysimi.*

Stage X	lx	Dx	Lx	100qx	SX	TX	MSR	IM	Log lx	ex	k-values
Egg	100	19	90.5	19	.81	467	0.25	11.75	2	4.67	0.1
1st instar	81	13	74.5	16	.83	376.5	0.20	9.4	1.90	4.64	0.07
2nd instar	68	7	64.5	10.2	.89	302	0.11	5.17	1.83	4.44	0.05
3rd instar	61	5	58.5	8.1	.91	237.5	0.09	4.23	1.78	3.89	0.04
4th instar	56	3	54.5	5.35	.94	183	0.05	2.35	1.74	3.26	0.02
Pre-pupa	53	2	52	3.77	.96	128.5	0.04	1.88	1.72	2.42	0.02
Pupa	51	4	49	7.84	.92	76.5	0.17	7.99	1.70	1.5	0.03
Adult	47	47	23.5						1.67		K=0.33
Male/Female	18/29		9/14.5								

Where lx= No. surviving at the beginning of the stage; dx = No. dying In each stage; Lx= No. alive b/w age x and x+1; 100qx = Apparent mortality; Sx = Survival fraction; MSR= Mortality/ survivor ratio; IM= Indispensable mortality; Ex= Life expectancy; K= killing power.

1st instar larval stage was the most fragile than the other instars and hence indicated higher mortality at this stage.

Survival fraction

The survival fraction (Sx) of egg phase, first, second, third and fourth instar larvae was 0.81, 0.83, 0.89, 0.91 and 0.94, respectively. While the survival fraction of pre-pupa and pupal phases was 0.96 and 0.92, respectively. The maximum survival fraction was observed for pre-pupal and 4th instar larvae, while the minimum was found for the egg stage.

Mortality survivor ratio

Mortality survival ratio (MSR) during egg phase, 1st, 2nd, 3rd and 4th instar larvae was found to be 0.25, 0.20, 0.11, 0.09 and 0.05, respectively, while mortality survival ratio of pre pupal and pupal phases was 0.04 and 0.17, respectively (Table 2). Thus, maximum MSR was found at the egg stage and minimum at the 4th instar and pre-pupal stages.

Indispensable mortality

Indispensable mortality (I.M.) during egg phase, 1st, 2nd,3rdand 4th larval instars was 11.75, 9.4, 5.17, 4.23 and 2.35, respectively. While, the indispensable mortality for pre-pupa and pupae was 1.88 and 7.99, respectively (Table 2). I.M. was found maximum at the egg stage and minimum at 4th instar and pre-pupal stages.

Life expectancy (ex)

The life expectancy (ex) was maximum at the egg phase (4.67) and minimum at the pupal phase (1.5). The life expectancy was found to be 4.64, 4.44, 3.89 and 3.26 for 1st, 2nd, 3rd, and 4th larval instars, respectively (Table 2).

K-values

The k-value in egg and pupal phases was maximum (0.1) and minimum (0.03) at the fourth larval instar and pre-pupal stages, respectively. The total k-value for the whole generation was 0.33 (Table 2).

Conclusions and Recommendations

It is important to determine the characteristic biological attributes in order to successfully mass rear the predators in biological control programs. Life table data also helps evaluate future progeny and estimate the total number to bereleased in successful biological control programmes. The current research established the life table parameters of *M. sexmaculatus,* suggesting its beneficial use in biological control programmes, due to its greater egg output, faster development, higher longevity and significant feeding efficiency.

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Novelty Statement

This study demonstrates the potentiality of Zigzag beetle for the control of Mustard aphid as a biological control mechanism and highlights the use of environmentally safe technique for the control of crop pests.

Author's Contribution

Ashfaq Hussain: Conducted experimental study, collected data, wrote paper abstract, methodology, results and discussion.

Javed Khan: Conceived the idea, Design experiment, analyzed data, facilitated and supervised overall study at Insectary Biological control lab.

Ammara Blouch: Article drafting, introduction and references.

Ahmad-Ur-Rahman Saljoqi: Overall supervision, technical proof reading.

Ashraf Khan: Assisted in experimental design, Statistical data analysis, and manuscript proof reading.

Zaheer Sikandar: Provided technical assistance in experimentation and manuscript writing.

Conflict of interest

The authors have declared no conflict of interest.

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