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Biology of vegetable leaf miner (*Liriomyza sativa* Blanchard) on yard long bean

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Abstract

Vegetable leaf miner (*Liriomyza sativa*) being a dipteran fly possesses polyphagous feeding nature. Among the insect pests attacking yard long bean, vegetable leaf miner once considered as a secondary pest is now appearing as a primary pest due to disruption of associated natural enemies. With a view to study the biology, investigation was carried out in entomology laboratory, Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka during January to April, 2021. Results revealed that at laboratory condition, incubation period was 2.12 ± 0.31 days, larval stage lasted for 4.57 ± 0.49 days and pupal stage remained for 7.85 ± 0.67 days. Irrespective of sex, adult leaf miner lived for 1.5 ± 0.46 days without food source whereas 4.9 ± 2.20 days with supplementary food source. Higher pupal mortality (54.08%) was observed than larval mortality (21.07%).

Keywords: biology, leaf miner, yard long bean

Introduction

The Dipteran vegetable leaf miner (*Liriomyza sativae*) belongs to the family Agromyzidae. Among the economically important Agromyzid flies *Liriomyza. sativae* Blanchard described by Blanchard (1938) [4] from Argentina is highly polyphagous in nature infesting diverse host plants in the families' Cucurbitaceae, Leguminosae, Solanaceae, Brassicae, Asteraceae, Compositae and others (EPPO, 2009) [8]. Previous literature states that in Bangladesh so far four leaf miner species infesting on seventeen (17) host plants have been identified (Mazumdar and Bhuiya, 2014) [14]. It is found that one of the economically important *Liriomyza* species (*Liriomyza trifolii)* was accidently introduced in India from American sub-continent along with chrysanthemum cuttings (Anonymous, 1991) [1] but in case of Bangladesh no such information is available.

Bangladesh preponderantly possesses an agriculture based economy as agriculture contributes to about 12.09% of the country's GDP and generates employment opportunity for around 37.75% of the total labour force (BBS, 2020) [3]. Despite rice domination in cropping sector, vegetable production in the country is currently booming at a fast rate and Bangladesh is the third largest producer of vegetables in the world (FAO, 2019) [9]. Currently, sixty various kinds of vegetables are being produced under different seasons in the country (DAE, 2019) [7]. Among them yard long bean is popular and high valued vegetable cultivated in summer season when there is a shortage of vegetable supply in the market. At present, after meeting local needs yard long bean is also exported to many countries in the world along with other vegetables. It is considered to be a fascinating source of vitamins and minerals along with enough protein.

These days, one of the notable hindrances of yard long bean production faced by farmers in the country is the high occurrence of insects and pests. Among the insect pests attacking yard long bean, vegetable leaf miner once considered as a secondary pest is now appearing as a primary pest due to disruption of associated natural enemies. This happens as a consequence of overuse of chemical pesticides along with the development of pesticide resistance as a pivotal part of the biology of vegetable leaf miner is its capability to develop resistance to insecticides (Parrella, 1987) [16]. As a holometabolic insect, leaf miner passes through four life stages viz. egg, larva, pupa and adult having the egg and larval stages inside the leaf, the pupal stage into the soil and the adult is free. When the larva is ready to pupate, it cuts a slit and come out of the mine on the leaf surface. Adults emerge through the dorsal anterior end of the puparium, the whole process taking between 5 minutes to more than 1 hour (Perrella, 1987) [16]. At optimal temperatures (30^0C), the vegetable leaf miner completes development from egg to adult stage in about 15 days (Capinera, 2005) [5]. The adult female flies oviposit just below the leaf surface, from where after hatching the newly formed larvae starts to feed the mesophyll contents and proceeds forwards forming mines which turns parchment white and affects photosynthesis. Feeding and oviposition punctures along with mines can affect crops in six different ways such as exterminating young seedlings, reducing crop yields, vectoring diseases, instigating 'sunburning' of the fruit, declining the aesthetic value of ornamental plants, and creating problems for plant quarantine (Perrella, 1987) [16]. *Liriomyza* infestation can causes up to 70 % yield losses (Zoebisch *et al.* 1984) [20]. Due to uncontrolled population increase up to 90% of tomato foliage may be lost (Schuster, 1978) [19]. Besides direct damage producers also have to lose export markets confronting quarantine bans from importing countries due to presence of leaf miner. Even after withdrawal of complete bans, phytosanitary measures such as fumigation and irradiation are needed to be performed to fulfill the requirements of importing countries resulting in raised export costs (Reitz *et al.* 2013) [17]. Wherefore, it is

essential to have a thorough knowledge on biology of concerned pest for developing a successful management program as pesticide selection changes according to nature of life stages.

In Bangladesh very few researches have been done on biology of vegetable leaf miner. So it is justifiable to do the basic research on different biological aspects of leaf miner. Hence, this study was undertaken to generate information on the biology of *Liriomyza sativae* species on yard long bean.

Materials and Methods

Present investigation on biology of vegetable leaf miner, *Liriomyza sativae* (Diptera: Agromyzidae) was carried out in entomology laboratory, Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka during January to April, 2021.

Collection of adult vegetable leaf miner

Infested yard long bean leaves were collected from the yardlong bean field of central farm, Sher-e-Bangla Agricultural University and reared in rearing boxes at laboratory for obtaining adult male and female vegetable leaf miner flies. The emerged adult vegetable leaf miner flies were collected meticulously with the help of small vial. After identification of male and female adults with the help of morphological characteristics, they were paired for further investigation.

Preparation of oviposition boxes

Four rearing boxes containing two healthy infestation free yard long bean seedlings each were maintained for oviposition following CRD method. Ten pairs of adult vegetable leaf miner flies were introduced in cage for oviposition. Eggs laid by female vegetable leaf miner on the leaves of yard long bean were observed every day to know the further development. The leaf containing pupa was transferred in glass petridish (70 cm×1.5 cm) having moist tissue paper at normal room laboratory condition for further development. As a food supplement five percent honey solution was provided to adult flies. During investigation the observations were also continued on different larval instars and subsequent developmental stages till the death of adult flies

Egg period

Yard long bean leaves having eggs were collected for examining with microscope and after examining leaves were placed in a petridish for further development. Leaves were observed at every 6 hours till hatching to record the incubation period.

Larval period

Upon hatching the vegetable leaf miner larvae were allowed to feed inside the mine and observed two times daily. By critically examining the mine diameter with microscope, data on different larval instars period were recorded. The duration from egg hatching to pupation was considered as larval period.

Pre puparium period

Full grown larvae came out of the mine and crawled to find suitable places for pupation. This time was recorded as Pre puparium period.

Pupal period

For studying the pupal period of the concerned insect the same larvae was observed daily upto adult emergence and pupation inside or outside the leaf mine was noted. The duration from pupal formation to adult emergence was considered as pupal period.

Adult longevity

To study the adult longevity emerged adults were introduced individually in to another petridishes. In case of this study two types of observation were made viz. with food supplement and without food supplement. As a food supplement fresh yard long bean leaves were provided. Observations were continued to death of the individual adult. In both cases period between adult emergence and adult death was recorded as adult longevity.

Length of mine

Length of mine made by vegetable leaf miner larvae on leaf of dahlia, cabbage, cauliflower, tomato and squash was measured. Due to zigzag pattern of the tunnel a fine thread was used to measure the length. Hence a piece of fine thread was placed carefully on the mine and the thread length was calculated by placing it on a scale. The found thread length was recorded as the length of mine.

Pre adult mortality percentage

Leaves containing 86 larvae were transferred into five petridishes and placed on a moist tissue paper to protect leaves from desiccation while each petridish was considered as a replication. Petridisishes were covered with lid and monitored daily up to adult emergence. Number of formed pupa and number of emerged adult were counted and recorded. Larval mortality percentage and pupal mortality percentage were calculated by using the following formula.

$$\text{Larval mortality percentage} = \frac{\text{Initial no of larvae- no. of formed pupa}}{\text{Initial number of larvae}} \times 100$$

$$\text{Pupal mortality percentage} = \frac{\text{Initial no of pupa- no. of emerged adult}}{\text{Initial number of pupa}} \times 100$$

Data analysis

Range and mean of different parameters were analyzed using Microsoft Excel Program.

Result and Discussion

Development time

Gathered data on development time of leaf miner were collected according to different life stages namely egg stage, larval stage, pupal stage and adult stage (Table 1).

Incubation period

Observations during study revealed that white, elliptical eggs were intercalated into plant tissue just underneath the leaf surface at leaf base or leaf tip or haphazardly on entire leaf surface. Considering previous findings (Ghelani *et al*., 2020) [10] and aforementioned observation here, it can be stated that egg laying behavior of leaf miner is still ambiguous. During this incubation period eggs hatched into larvae and the presented data in Table 1 exhibited that incubation period of leaf miner lasted for an average 2.12 ± 0.31 days ranging from 1.67 days to 2.75 days at normal room temperature (25-30^{0}C). This egg day's period was

almost close to the periods 2.7 ± 0.01 days on melon plants and 2.5 days on *Vigna unguiculata* for *L sativa* at 25^{0}C described by Araujo *et al.* (2013) [2] and Costa- Lima *et al.* (2009) [6] respectively. This obtained result is also similar to the result found by Lanzoni *et al.* (2002) [12] who recorded 2.1 days as egg period for *L. trifolii* on *V. unguiculata*.

Larval period

It was observed that the apodous newly hatched larvae were colourless while as it progressed towards maturity turned yellow in colour. It was also marked that the larva passed through three distinct instars prior to pupation and all the phenomenon regarding larval changes were happened inside the mine made by larva itself. The observed three larval instar stages and total larval period have been described below.

First larval instar

First instar larva was found colourless and due to its transparent colour it was arduous to detect first instar larva inside mine. Data presented in Table 1 showed that the first larval instar period ranged from 1.00 day to 1.50 days with an average 1.04 ± 0.12 days and this duration was similar to the duration verified by Ghelani *et al.* (2020) [10] who found 1.10 ± 0.18 days for first instar larval period. Parrella and Bake (1988) [15] also found 0.85 days of this period which was also in close proximity with this study.

Second larval instar

During this period the larva was supposed to develop colour and became pale greenish in colour and later on it turned pale yellow. Findings illustrated in Table 1 exhibited that the period of second larval instar ranged from 1.00 day to 2.00 days with an average 1.23 ± 0.38 days which was in the conformity with the findings of Parella and Bake (1988) [15] who reported 1.23 days for second instar larval period. Given result was also almost close to the result obtained by Ghelani *et al.* (2020) [10] who found an average 1.69 ± 0.28 days for this stage ranging from 1.45 to 2.18 days.

Third larval instar

Third instar larva was found dark yellow in colour and bigger in size than preceded instars. Due to these features larva at this stage could be easily visible inside mine from a distant with naked eyes. Data presented in Table 1 revealed that the third instar larval period varied from 2.00 days to 3.00 days with an average 2.23 ± 0.41days which almost agreed with the findings obtained by Ghelani *et al.* (2020) [10] indicating that the third instar larval period lasted for an average 1.97 ± 0.33 days ranging from 1.45 to 2.18 days. Again in another study Parrella and Bake (1988) [15] found 1.42 days for third larval instar period which was a little bit less than given value. Actually the variation in present study could be accepted as previous findings obtained by Fagoonee and Torry (1983) [11] had already proved that larval period was dependent not only on temperature but was also affected by antibiosis factors.

Total larval period

From Table 1 it was observed that the total larval period ranged from 4.00 days to 5.00 days with an average 4.57 ± 0.49 days which was almost similar to the duration confirmed by Costa – Lima *et al.* (2009) [6] who found 4.9 days as larval period for *L. sativae* on Vigna unguiculata (Fabaceae) at 25^{0}C. Moreover, the present finding was in close agreement with the findings obtained by Ghelani *et al.* (2020) [10] who reported an average larval period 4.76 ± 0.79 ranging from 3.99 to 6.14 days.

Close observations during study revealed that full grown larva cut a semi- circular slit at or near the mine and came out of the mine. This intermediate condition between puparium formation and pupation was rarely described by most researchers. Some merged this duration with larval period while other merged with pupal period. Gathered information regarding this intermediate condition has been discussed below:-

Pre puparium period

It was observed that after getting outside of the mine the fully matured yellow coloured, cylindrical shaped larva stopped feeding and remained inactive. It was also found that during this period the larva showed wriggling movement which was supposed to find suitable place for pupation. Data illustrated in Table 1 revealed that this condition continued for 2-4 hours with an average 2.83 ± 0.81 hours. This obtained result is in accordance with Leibee's (1984) [13] work who recorded 2.67 ± 0.18 hours for the duration of pre puparium condition at 35^{0}C. Saradhi and Patnaik (2006) [18] also reported that this condition lasted for 2 -5.50 hours which are in close proximity with this current research finding.

Pupal period

During investigation larva was found to pupate at leaf margin or on leaf in field condition while in laboratory condition on sides and bottom of the rearing petridishes. The previous literature stated that after getting out of the mine larva generally drops from the leaf and burrows into the soil to a few cm depths to form a puparium (Parrella, 1987) [16]. Initially the pupa was yellow in colour and coarctate and it turned gradually from light brown to dark brown or black. Data presented in Table 1 Showed that pupal period ranged from 7.00 to 9.00 days with an average 7.85 ± 0.67 days at room temperature (25-30^{0}C). The almost similar period for pupal stage was recorded by Costa Lima *et al.* (2009) [6] and Lanzoni *et al.* (2002) [12] which was 8.0 days for *L. sativa* and 8.2 days for *L. trifolii* on *V. unguiculata* respectively.

Total development period (Egg to adult)

It was noticed that the emergence of adult from puparium generally occurred during early morning hours and just after emergence it climbed upon the wall of rearing petridishes. The newly emerged adults were found yellow in colour and upon maturity original colour was developed. The matured adult was black coloured with a bright yellow spot on the mat grey mesonotum.

It was shown in Table 1 that the total development period ranged from 16.12 to 22.37 days with an average 19.56 ± 2.83 days which was in close proximity with the findings obtained by Costa Lima *et al.* (2009) [6] and Lanzoni *et al.* (2002) [12] indicating 16.5 days egg to adult period for *L. sativae*, 18.7 days for L. trifolii on *V. unguiculata* respectively. They are also almost similar to the duration recorded by Araujo *et al.* (2013) [2] indicating 15.9 ± 0.04 days for *L. sativa* on melon plants.

Table 1: The length of developmental periods of *Liriomyza sativae* on yard long bean

Developmental time in days			
Life stages	**Mean ± SD**	**Range**	**No. of observation**
Egg	2.12 ± 0.31	1.67- 2.75	40
First Instar larva	1.04 ± 0.12	1.00 -1.50	40
2nd Instar larva	1.23 ± 0.38	1.00 -2.00	40
3rd Instar larva	2.23 ± 0.41	2.00 -3.00	40
Total larval period	4.57 ± 0.49	4.00 -5.00	40
Pre puparium period	0.12 ±.04 (2.83 ± 0.81 hours)	0.08 -0.17 (2-4 hours)	20
Pupa	7.85 ± 0.67	7.00 -9.00	40
Egg to Adult	19.56 ± 2.83	16.12 -22.37	40

Adult longevity

Collected information related to adult longevity of leaf miner (disregarding sex) was furnished in Table 2. The given table data showed that with supplied food source (50% honey) the adult remained alive for an average 4.9 ± 2.20 days ranging from 2.00 days to 8.00 days whereas without food source the adult leaf miner survived for an average 1.5 ± 0.46 days varied from 1.00 to 2.00 days. This result was similar to the result obtained by Saradhi and Patnaik (2006) [18] which was 3-9 days adult survival for with food condition along with an average 5.35 ±2.08 days and 1-2 days for without food condition along with an average 1.90 ±0.30 days.

Table 2: The adult longevity of *L. sativae* on yard long bean

Condition	Mean ± SD (Days)	Range (Days)	No. of observation
Without food source	1.5 ± 0.46	1.00 -2.00	20
With food source	4.9 ± 2.20	2.00 -8.00	20

Total life duration of *L. sativa* in laboratory

Data displayed in Table 3 revealed that in without food condition total life duration of *L. saivae* was an average 19.56 ± 2.83 days ranging from 17.12 -22.37 days whereas in with food condition this duration ranged from 18.25 - 28.58 days with an average 23.62 ± 5.40 days.

Table 3: Total life duration of *L. sativae* in Laboratory

Condition	Mean ± SD	Range	No. of Observation
Without food source	19.56 ± 2.83	17.12 -22.37	20
With food source	23.62 ± 5.40	18.25 -28.58	20

Pre – adult mortality percentage of *L. sativae* at different life stages in laboratory condition

The observed pre-adult mortality percentage (%) of *L. Sativae* at two development stages namely larval and pupal stage has been presented in Table 4. The table data showed that during the investigation period 21.07% larval mortality and 54.08% pupal mortality were observed respectively. Previous findings revealed that Araujo *et al.* (2013) [2] found 5% larval mortality and 21.8% pupal mortality on melon whereas Costa Lima *et al*. (2009) [6] observed 15.2% larval mortality and 21.20% pupal mortality on *V. unguiculata* respectively which were lower than the present findings. Other literature also varied on survival and mortality percentage of larva and pupa of *Liriomyza* (Leibee, 1984; Lanzoni *et al*., 2002) [13, 12]. However, it is pertinent to note that copious biotic and abiotic factors can affect the survival as well as mortality rates of larva and pupa (Parrella, 1987) [16] such as host plant, parasitoid abundance, climatic conditions etc. Hence, it is hard to mention exact possible reasons for this variation.

Table 4: The pre- adult mortality percentage (%) of *L. sativae* at different life stages in Laboratoy

Different life stages	Mortality percentage (%)	No. of observation
Larva	21.07	86
Pupa	54.08	50

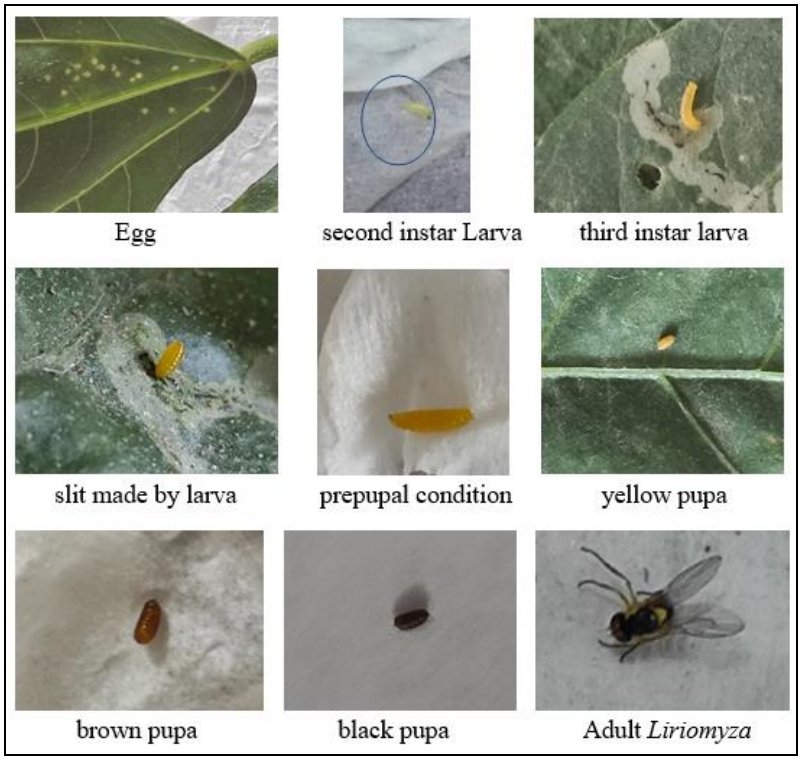

Plate 1: Biology of *Liriomyza sativa* on yard long bean

Mine length of *L. sativae* on different host plants

It was noticed that immediately after hatching, the newly formed larva started to construct mine and moved forward by feeding the mesophyll content and with the larval development the length and breadth of mine was also increased. Obtained data related to mine length on different host plants have been displayed in Table 5. Given data in the table exhibited that the highest average mine length (6.47 ± 1.11 cm) was observed on Yard long bean ranging from 4.9 -7.9 cm followed by Dahlia with average 5.60 ± 1.38 cm ranging from 4.4-5.6 cm and Tomato with average 5.05 ± 1.21 cm ranging from 2.8 -6.0 cm. respectively. On the other hand, the lowest mine length (3.48 ± 1.61 cm) was recorded on cabbage ranging from 2.0 -5.9 cm followed by Squash with average mine length 4.93 ± 0.92 cm ranging from 3.5 -5.9 cm. Actually findings related to mine length on various host plant are scarce. Saradhi and Patnaik (2006) [18] found an average 8.26 ± 0.13 cm length of mine created by *L trifolii* on French bean which was close to present findings. However, it is worth pointing out that the feeding behavior of *Liriomyza* can be influenced by thickness of leaf, presence of plant trichomes, phenolic content and nutritional value of host (Parrella, 1987) [16].

Table 5: Mine length of *L. Sativae* on different host plants

Host plant	Mean ± SD (cm)	Range (cm)	No. of observation
Tomato	5.05 ± 1.21	2.8 -6.0	6
Yard long bean	6.47 ± 1.11	4.9 -7.9	6
Cabbage	3.48 ± 1.61	2.0 -5.9	6
Squash	4.93 ± 0.92	3.5 -5.9	10
Dahlia	5.60 ± 1.38	4.4-5.6	6

Summary and Conclusion

Overall information on Biological aspects revealed that *Liriomyza sativae* passed egg period under leaf surface, larval period inside mine and pupal period outside the leaf whereas adult leaf miner fly is free living. At laboratory condition, total egg to adult development period was 19.56 ± 2.83 days as well as irrespective of sex, adult leaf miner lived for 1.5 ± 0.46 days without food source whereas 4.9 ± 2.20 days with supplementary food source. As *Liriomyza* passes different life stages at different habitats, selection of pesticides will be divergent based on life stages to manage them successfully. Obtained findings from the biology study will be helpful to choose appropriate pesticides for controlling leaf miner. Being a polyphagous insect vegetable leaf miner has the ability to infest new host plants in adverse situation. Further research on various biological aspects as well as nature of damage will facilitate the researchers to devise worthy management options of vegetable leaf miner.

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