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BIOECOLOGY OF PIGEONPEA POD FLY, *MELANAGROMYZA OBTUSA* MALLOCH IN KARNATAKA, SOUTH INDIA

S. M. A. S. REHMAN[1], S. ONKARA NAIK[2], K. P. KUMAR[1], A. K. CHAKRAVARTHY[2]* AND B. HANUMANTHE GOWDA[2]
[1]Department of Agricultural Entomology, UAS, GKVK, Bengaluru - 560 065, Karnataka
[2]Division of Entomology and Nematology,
Indian Institute of Horticultural Research (ICAR-IIHR), Hessaraghatta Lake post, Bengaluru - 560 089, Karnataka
e-mail: chakravarthyakshay@gmail.com

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***Corresponding author**

ABSTRACT
Studies on bioecology of pod fly *Melanagromyza obtusa* Malloch on pigeonpea (*Cajanus cajani* L.) in field and greenhouse were undertaken. There were no significant differences in the time taken to complete the life cycle of the pod fly under caged (20.30 ± 0.42 days) and field conditions (20.00 ± 0.17 days). Longevity of mated as well as unmated females was significantly higher than the respective males under field conditions. The sex ratio (male: female) was female biased most of the times during the study period. On an average, 37.1 ± 9.42 eggs were laid by each female during ovipositional period. Ten to fifteen days old pods were the most preferred for oviposition by the pod fly. Pods occurring on pigeon pea at the top canopy received significantly higher number of eggs 20.8 ± 4.99 per 10 pods compared to middle (11.67 ± 2.47 eggs/10 pods) and bottom portions 2.52 ± 1.67 eggs/ pods, nevertheless this depends on plant height.

INTRODUCTION

The pigeon pea pod fly *Melanagromyza obtusa* Malloch (Diptera: Agromyzidae) is a serious pest of pigeonpea reported throughout south and south-east Asia (Shanower *et al*., 1998). Females deposit eggs on the pigeonpea green pods and other host plants, and the developing larva first feeds just under the epidermis of the seed like a leaf miner. Pigeon pea is an important crop in semi-arid tropical and subtropical areas. Especially in Asia it is a major source of protein for humans (Shanower *et al*., 1999). As many as 250 insect species have been recorded to attack pigeon pea (Upadhyay *et al*., 1998) among which the pod-borers are the most damaging pests, inflicting considerable damage to the reproductive parts of the plant.

Pod yield losses ranging between 5-30% (Talekar, 1990) due to *M. obtusa* is reported during winter and spring from several countries and the main country suffering from its pestilence is India because of its wide spread cultivation (>90% of the world production) (Talekar, 1990; Akhauri *et al.,* 1994 and Shanower *et al*., 1998). Therefore, comprehensive study on the bioecology of the pod fly, that is lacking was initiated.

MATERIALS AND METHODS

Experiments were conducted at the University of Agricultural Sciences, Gandhi Krishi Vignan Kendra (GKVK), Bangalore, during *kharif and summer* (2011 - 2013). Geographically, the experimental site was located at 12°58′ N and 77°35′ E and 930 MSL.

Biology

The biology of the pest was conducted under field and laboratory cage conditions. Observations commenced from the pod initiation stage that followed first bloom (mid-October, 2010) (Plate 1). One hundred tender pods (10-15 days old) were randomly labelled from the field (bulk plots were used for the study) everyday at 10.30 to 11.00 h [as adult emergence and copulation is known to occur during early morning hours) (Ipe, 1974; Singh and Lallan Rai, 1984). Pods were opened in the field and observed for eggs. Number of eggs that were freshly laid (freshly laid eggs are glistening white and possess a tapering and transparent anterior process which gradually becomes milky white and rounded as the days progress) and that had freshly hatched (freshly hatched eggs appear similar to a collapsible sack which dries up as the days progress) on each pod was counted. The total egg and larval periods were recorded. One hundred larvae that freshly came out of the seeds for pupation were collected and transferred to individual glass vials (5.5 x 1.3 cm) to determine the pre-pupal and total pupal periods. Date records on each labelled pod and larva were maintained.

Seventy-five potted plants @ 1 plant/earthen pot of size were used for observations under caged conditions using nylon net of 200 mesh size. Five mated female flies (24 h old) were released into the cage during early morning hours (7.00 hours). Prior to release, pods were provided to the females and mating was confirmed through egg laying. All the females were excluded from their respective cages 24 h after release.

Later, five plants were stripped-off all the pods everyday to examine egg hatching. All the other plants remained in their respective cages to prevent further oviposition.

The difference between egg laying and hatching gave the total incubation period for the eggs. Similarly, five plants were observed everyday to note the days taken for larval instars. The pods were cut opened carefully and the larva inside was taken out with great care as they are delicate. The instars were confirmed based on the size and structure of the posterior spiracle on abdomen. Size of the larva was also measured. To apply Dyar's law (1890) for segregating larvae into instars later, the full-grown larva, which emerged from the seeds, were collected and its size measured. Days taken for the larva to emerge from the seeds gave the total larval period. Once the larvae emerged, they were transferred individually to marked glass vials (5.5 x 1.3 cm) (with date of emergence) and shifted to the laboratory to determine the total larval duration. Subsequently, pre-pupal period and total pupal periods were recorded.

Adult longevity

Fifteen adult males and females each were individually placed in glass vials (5.5 x 1.3 cm) covered with muslin cloth and tightly secured using rubber band. Food was not provided to one batch consisting of 15 males and 15 females and pure honey was provided as food to the other batch. A drop of honey was placed along the sides of the vials and 15 pairs of adults were placed pair-wise (a male and a female) and food was withheld. In another batch of 15 pairs of adults, few drops of pure honey were provided as food. The entire set up was maintained under room conditions (23 ± 3^0c t, 75 to 98 % RH). Observation regarding adult longivity was noted after every twelve hours.

Sex ratio

Sex ratio of adult flies was determined in the field during *Kharif* and *Rabi* 2010, 2011 and 2012. The first observation was taken 20 days after pod initiation stage while the second observation was taken 30 days after the first, every season. The following two methods were followed to determine the sex ratio. Two hundred pupae were collected randomly from the pods during each observation. The collected pupae were left for adult emergence in the laboratory under room conditions (23 ± 3^0c t, 75 to 98 % RH). Among those that had survived and emerged, sexes were identified based on the presence or absence of ovipositor (ovipositor is prominent in females). The number of males and females were counted and the sex ratio was determined.

Sex of the adults was determined using sweep net method during field observations. Two successive sweeps to and fro were made at one place over the canopy of the plants. The sweeps were made at an angle of approximately 30°. The material collected in the net was transferred into a glass container (750 ml vol) before making the next pair of sweeps. Sweeps were done at 9.00 to 9.30 hours, as adults are known to emerge during 6.00 hours to 9.00 hours. Twenty such pairs of sweeps were made in the field.

Fecundity

Fecundity of the pod fly was investigated under laboratory conditions. Ten pairs of freshly emerged adults were used. Each pair was released into a plastic container (0.3 m dia) containing ten, approximately fifteen day old, pods. The pods were placed in a container with water in such a way that the proximal end of the pod touched the water. A drop of honey was provided as food for the adults. The experiment was set up at 25ºC and 60 % RH. After 24 h of release, the container with pods was removed from the cage and replaced by another one containing fresh pods. The pods exposed to the flies were dissected and the number of eggs laid was noted. This continued till the female survived. Total number of eggs laid by a female during its life cycle and daily pattern of egg-laying were recorded.

RESULTS AND DISCUSSION

Pod fly biology

The time taken to complete the life cycle of the pod fly under greenhouse as well as field conditions is given in Tables 1 and 2. Under caged conditions, the egg stage lasted for 2.6 ± 0.07 days, larval stage for 8.9 ±0.08 days and pre-pupa for 0.5 ± 0.02 days. The total developmental period of the fly extended up to 20.30 ± 0.42 days (Table 1). Under the field situation, egg stage remained for 3.00 ±0.13 days, larval stage for 7.17 ±0.29 days, pre-pupa for 0.23 ± 0.01 days and pupa for 9.1 ± 1.27 days. The total developmental period (from egg to adult stage excluding adult longevity) extended to 20.00 ±0.17 days (Table 2). There were no significant differences in the time taken to complete the life cycle of the pod fly under caged and field conditions during October-November, 2010

Table 1: Duration of developmental stages of pod fly in caged conditions (Mean of 20 replications)

	Duration (days)							
	Egg	Larval instars				Pre-pupa	Pupa	Total developmental period
		1st	2nd	3rd	Total			
Mean	2.6	2.4	3.3	3.2	8.9	0.5	10.5	20.30
SEm ±	0.07	0.02	0.04	0.03	0.08	0.02	0.37	0.42

Table 2: Duration of developmental stages of pod fly in field conditions (mean of 20 replications)

	Duration (days)				
	Egg	Larva	Pre-pupa	Pupa	Total developmental period
Mean	3.0	7.17	0.23	9.1	20.00
SEm ±	0.13	0.29	0.01	0.27	0.17

Table 3: Influence of food and mating on longevity (in days) of adult pod fly (Mean of 25 adults)

	With food (honey) Without mate		With mate		Without food Without mate		With mate	
	Male	Female	Male	Female	Male	Female	Male	Female
Mean	7.27	12.06	5.6	10.6	3.37	5.17	3.97	4.27
SD ±	2.98	5.86	1.06	3.12	0.85	1.58	0.92	1.03

Table 4: Morphometrics of developmental stages of pod fly (Mean of 20 eggs)

Developmental stages	Size of the stage (in mm) Length	Width
Egg	1.01 ± 0.07	0.6 ± 0.2
I instar larva	0.7 ± 0.03	0.17 ± 0.2
Full grown larva	4.1 ± 0.3	1.26 ± 0.09
Pupa	3.02 ± 0.4	1.2 ± 0.2
Adult male	2.82 ± 0.18	0.09±0.02
Adult female	3.1 ± 0.34	0.53±0.04

(Fig. 1).

Comparisons were also made with respect to time taken to complete each of the developmental stages between field and caged conditions. Results revealed that all the developmental stages varied but not significantly between the two conditions. Egg and larval durations were significantly lower while pre-pupal and pupal periods were higher under greenhouse conditions.

There was no significant difference in the time taken to complete pod fly life cycle under field and laboratory conditions 20.00 ±0.17and 20.30 ± 0.42 respectively. The results are in conformity with Ahmad (1982) who made comprehensive studies on several aspects of the pod fly. Subharani and Singh (2009) also reported that *M. obtusa* completed total life cycle in 41 days. Khokhar and Sucheta (2003) conducted biological studies on different stages of pigeonpea pod fly. Dahiya *et al.* (1999) reported that 20-28°C and 51-53 per cent relative humidity are conducive for *M. obtusa* larval development. Singh and Rai (1984) observed three larval instars that lasted each for 1.45, 2.10 and 2.39 days, respectively. The larval duration of this agromyzid fly is split among three instars (Ipe, 1974). Singh and Rai (1984) mentioned that the larval duration lasted for 5 to 6 days.

Influence of food on adult longevity

Experiments conducted on the influence of food on adult longevity showed that honey aids in extending adult longevity. When provided with 20 per cent honey solution adult longevity of both males and females were significantly higher under mated as well as unmated conditions. Longevity of Adults reared under starvation with food timed for 7.27 ± 2.98 days and 5.6 ± 1.06 days in unmated and mated males respectively. Under starvation, unmated males timed for 3.37 ± 0.85 days and mated males timed for 3.97 ± 0.92 days. With food, unmated females timed for 12.06 ± 5.86 days and mated females timed for 10.6 ± 3.12 days whereas under starvation unmated females timed for 5.17 ± 1.58 days and mated females timed for 4.27 ± 1.03 days (Table 3).

Longevity of mated as well as unmated females were significantly higher than the respective males under conditions when food was provided. Longevity did not differ significantly between sexes when starved and allowed for mating. However, females timed longer than males when starved and kept without male. Female longevity was significantly higher when left without mating irrespective of whether provided with food or not. However, unmated males timed significantly longer than the mated males only when food was provided. Under starvation, the differences were non-significant ($P < .0.05$ at 1 df). Adult longevity showed that both males and females had significantly ($P > 0.05$ at 1df) higher longevity when fed with 20 per cent honey solution. Female longevity was significantly higher when left without mating irrespective of whether the adults were provided with food or not.

Morphometrics

The egg length was 1.01±0.07 mm and width 0.6±0.2 mm. The early instar larval length and width were 0.7±0.03 and0.17±0.2 mm, whereas fully grown up larva measured4.1±0.3 and 1.26 ±0.09 mm,. Similarly pupal length and width were 3.02±0.4 and 1.2±0.2 mm, respectively (Table 4). Adult male and female were 2.82±0.01 and 0.09±0.02, 3.1±0.34 and 0.53±0.04 mm in length and width, respectively.

Sex ratio

The sex ratio of the adult pod flies was assessed using two methods *viz.,* laboratory assessment and sweep net method under field conditions (Tables 5 and 6). In laboratory, where a fixed number of healthy pupae were observed, the sex ratio (male: female) appeared female biased most of the times (1: 2.13) during *kharif,* 2010; 1:2.33 during *kharif,* 2011; 1:1.86 during *rabi,* 2011 and 1: 1.63 during *kharif,* 2012. Sex ratio was male biased (l: 0.79) only during *rabi,* 2010. During *rabi* and *kharif* of 2011, the sex ratio appeared female biased (1:1.86 and 1: 2.33, respectively) (Table 5). The numbers of adults caught in the sweep net also varied across seasons in different years. The maximum female population was observed in *kharif* compared to *rabi* in general. In 2010 male to female ratio was 1:1.36, 1:0.41 in *kharif* and *rabi*, respectively. Similarly, in 2011 it was 1:0.67 (kharif) and 1: 1.43 (rabi) and in 2012 kharif, it was 1:2.14 (Table 6).

The sex ratio of the adult pod fly under laboratory conditions appeared female biased during kharif and *rabi* seasons. Ipe (1974) observed under laboratory conditions the adult sex ratio that indicated 49.85 males for every 50.15 females. Similarly, the sex ratio of pod fly under field conditions revealed female biased only during *kharif* 2010 and 2012. But during *Rabi* 2010 and 2012 no such variations were observed (Tables 5 and 6).

Fecundity

Maximum oviposition by pod fly was recorded during the

Table 5: Number of adult male and female pod flies and the sex ratio in laboratory (N = 100 pupae)

Sowing season	Male	Female	Total adults observed	Ratio(male to female)
Kharif, 2011	64	136	200	1:2.13
Rabi, 2011	112	88	200	1:0.79
Kharif, 2012	60	140	200	1:2.33
Rabi, 20112	70	130	200	1:1.86
Kharif, 2013	76	124	200	1:1.63

Table 6: Number of adult male and female pod flies in sweep net method (N = 100 adults)

Sowing season	Males	Females	Total adults	Ratio (male : females)
Kharif, 2011	151	205	356	1:1.36
Rabi, 2011	92	38	130	1:0.41
Kharif, 2012	170	114	284	1:0.67
Rabi, 2012	150	215	365	1:1.43
Kharif, 2013	49	105	154	1:2.14

Table 7: Fecundity of the pod fly

Pair	No. of eggs laid by each female						Total No. of eggs laid
	1st day	2nd day	3rd day	4th day	5th day	6th day	
I	2	15	24	10	2	0	53
II	3	5	25	7	0	0	40
III	1	8	17	10	0	1	37
IV	1	9	16	11	4	0	41
V	5	12	9	18	1	0	45
VI	2	8	12	7	0	0	29
VII	0	26	7	0	0	0	33
VIII	4	14	15	7	0	0	40
IX	2	15	15	3	0	0	35
X	1	4	13	0	0	0	18
Mean	2.1	11.6	15.3	7.3	0.7	0.1	37.1

Table 8: Influence of age of pod on the oviposition behavior of pod fly

Pod age (days)	Number of eggs	
	Field conditions*	Laboratory conditions**
5	12.18	08.87
10	30.38	16.09
15	32.68	15.18
20	06.69	05.42
25	01.63	01.17
SEm±	02.39	01.62
CD (α = 0.05)	07.23	04.56

second and third day after mating (11.6 ±6.41 and 15.3 ±5.76 eggs per female, respectively). A few eggs (2.1 ±1.52 eggs/female) were laid on the first day. Beyond third day, the fecundity declined and most females ceased oviposition by sixth day. On an average, 37.1 ± 9.42 eggs were laid by each female during ovipositional period (Table 7).

Maximum oviposition by pod fly was recorded during the 2nd and 3rd day after mating. Beyond 3rd day the fecundity declined and most females stopped ovipositing by 6th day. On an average, each female during ovipositional period laid on an average 37.00 and 9.00 egg. Jose (1986) reported *M. obtusa* laying about 79-80 eggs per female, incubation period 2.5 to 3 days and 70 per cent of the eggs hatched successfully. Ipe (1974) reported that single egg was laid at a time and the maximum number of eggs were found on single pod was as high as 22 with the average number of 4 eggs per pod in a heavily infested field. Reed and Lateef (1990) pointed that under favorable conditions the lifecycle of the pod fly was completed in less than 3 weeks. Jose (1986) recorded 2.5 - 3.0 days incubation period under laboratory conditions and 2.5 - 3.5 days under field conditions.

Influence of pod age on oviposition

Data under laboratory and field conditions showed maximum oviposition on pods aged 10 and 15 days (30.38 and 32.68 eggs per 40 pods, respectively) under field conditions and 16.09 and 15.18 eggs per 25 pods, respectively under laboratory conditions (Table 8). The differences between oviposition on 10 and 15 days old pod was statistically non-significant. Pods of 25 days old were the least preferred recording 1.63 eggs/40 pods and 1.17 eggs /25 pods, respectively. Ten to fifteen days-old pods were the most preferred for oviposition by the pod fly compared to 15 days older pods.

Pod age influenced ovipositional behavior of pod fly under field and laboratory conditions. Results of the experiments under laboratory conditions showed maximum oviposition on pods aged between 10 to 15 days. Under field conditions the pod fly maximally oviposited between 10 to 15 days-old pods (Table 8). Pods of 25 days old were the least preferred for the oviposition. Sithanantham *et al*. (1987) studied the distribution of eggs at different age of the pod indicated that

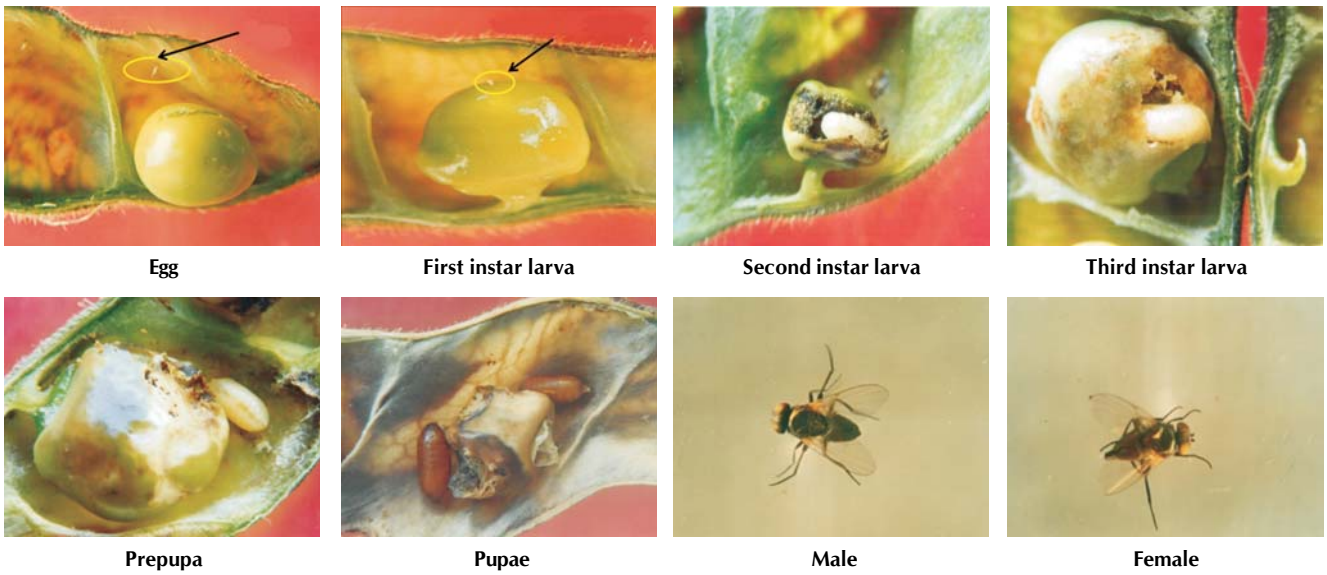

Figure 1: Life stages of podfly

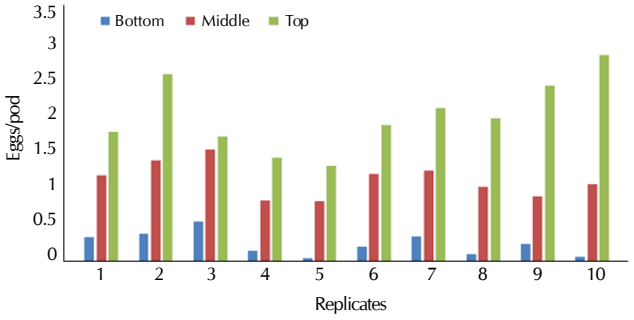

Figure 2: Distribution of eggs in different canopy portions of the pigeon pea plant

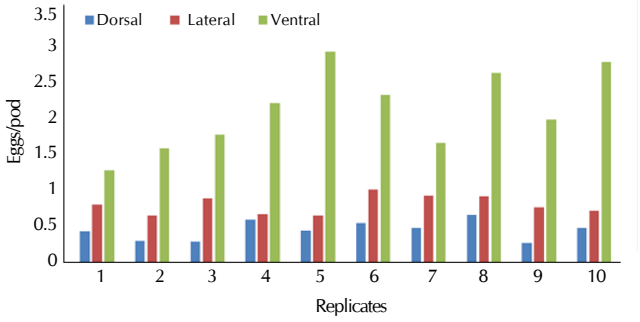

Figure 3: Distribution of eggs in different positions of the pod

partly matured pods carried more number of eggs than tender, mature and dry pods. Reddy *et al*. (1981) observed two flushes of podding in pigeonpea and second flushes yielded more compared to the first. The pod borer, *H. armigera* damage more of second flush while pod fly attack both the first and second flushes of pods. Cotyledon infestation by *M. abtusa* resulted in a higher number of dead seeds or abnormal seedling. Such seeds are difficult to separate during the cleaning and grading process (Kashyap and Purnia, 1995). *M. obtusa* lays eggs on young pods (Reed and Lateef, 1990) preferably on the ventral surface (Singh *et al*., 1982).

Egg distribution on pods disposed at different positions

Distribution of eggs on pigeonpea pods at select canopy positions varied significantly. Pods occurring on pigeonpea at the top canopy received significantly higher number of eggs of pod fly (20.8 ± 4.99 eggs /10 pods) compared to middle (11.67 ± 2.47 eggs/10 pod) and bottom (2.52 ± 1.67 eggs/pod) position. Oviposition per ten pods was significantly higher in middle canopy than bottom canopy. Higher the elevation, higher the number of eggs deposited up to plant height (Fig.2). Spatial distribution of eggs on pigeon pea pod varied significantly. Pigeon pea pods at top canopy received significantly higher number of eggs of pod fly compared to middle and bottom canopy positions. Higher the elevation, greater was the number of eggs deposited on pigeon pea pods. There was statistical significance among the 3 canopy positions by ANOVA at 1 % significance (Fig. 2). Sithanantham *et al*. (1987) assessed *M. obtusa* egg distribution spatially and reported that top portion of plant canopy was the most preferred for oviposition.

Ovipositional preference for pod side

It was found that oviposition occurred on dorsal, lateral and ventral sides of a pod (Fig. 3). However, the ventral side was the most preferred. Oviposition on the ventral side was significantly higher (21.98 ± 5.73 eggs/ 10 pods) compared to lateral (8.34 ± 1.33 eggs/ 10 pods) and dorsal (4.68 ± 1.39 eggs/ 10 pods) sides of the pod. Lateral sides of the pods were significantly more preferred than the dorsal side of the pod. On an average, a total of 35 ± 6.55 eggs/l0 pods were laid under the existing experimental conditions (Fig. 3). It was found that oviposition by gravid pod fly occurred on all sides of the pod. However, the ventral side was the most preferred.

M. obtusa laid eggs on egg pods (Reed and Lateef, 1990) and on ventral surface of the leaf (Singh *et al*., 1982). Eggs were

inserted in such a way that they were not visible from outside the pod (Ahmad, 1938; Singh and Rai, 1984). Oviposition on the ventral side was statistically significant compared to other sides of the pod (Fig. 3). Singh *et al*. (1982) reported that the inside dorsal suture of the green pods received more eggs compared to developing pods. Singh *et al*. (1982) also reported that more number of eggs was laid at the middle locule followed by basal apical locule.

Pigeon pea podfly biology revealed that under caged and field conditions, the insect complete life cycle on an average in 20 days. Ten to fifteen days old pigeon pea pods need maximum protection as they are maximally preferred for oviposition. Pods borne at about a meter from ground were the most preferred for oviposition. So these pods need protection on priority.

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