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The Behavior and Feeding Preference of the 12-Spotted Beetle *Epilachna indica* MULSTANT (Coleoptera: Coccinellidae: Subfamily Epilachninae) Towards the Black Nightshade *Solanum nigrum* (Family: Solanacea)

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Abstract: The behavior of fifty one 12-spotted ladybird beetles *Epilachna indica* (Coleoptera: Coccinellidae: subfamily: Epilachninae) on ten black nightshade *Solanum nigrum* (Solanaceae) plants was observed from 08:00 to 19:00 h in the field at Ulu Kelang, Selangor, Malaysia. 29.26% time was spent feeding compared to 7.22% mating, 51.05% resting and walking and 12.45% flying. Optimal feeding time in the field was from 08:00 h to 10:00 h with peak feeding occurred at 09:30 h. Leaf disc choice bioassay showed that *E. indica* prefers to feed on both *Solanum melongena* and *S. nigrum* leaf discs. In the laboratory, 72 h continuous observation on ten beetles showed that the leaf area consumption of *S. nigrum* was 1.202±0.085 mm^2 h^{-1} for one beetle. This study indicates that *S. nigrum* is a potential trap plant for pest management of the economically important egg plant. *Solanum melongena*. Future study on feeding stimulant on *S. nigrum* will enlighten the understanding of host selection in *E. indica*.

Key words: *Epilachna indica*, *Solanum nigrum*, feeding behavior, feeding preference

INTRODUCTION

Epilachninae are economically important beetle pests. In Malaysia, Yusof and Khoo (1989) reported that *Epilachna indica* are found attacking *Phaseolus vulgaris* L., sweet potato *Impomoea batatas* (L.) Lam, bitter gourt *Momordica charantia* L., eggplant *Solanum melongena* L., cucumber *Curcumis sativus* L. and watermelon fruit *Citrullus vulgaris*. In Taiwan, both larvae and adults of some species of Epilachninae feed on the leaves of Solanaceae, Cucurbitaceae and Urticaceae (Richards and Filewood, 1990).

Epilachna indica can be differentiated from *Epilachna vigintioctopunctata* from the twelve spots found from adult beetles (Fig. 1). The *E. vigintioctopunctata* has twenty-eight round spot on its elytra whereas the body is red similar to *Epilacha indica*. The only difference in the larvae is that those of *E. indica* is often more yellowish in color and armed with numerous branched yellowish spines (Fig. 2).

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Fig. 1: An adult *E. indica* has twelve black spot differentiating it from *E. vigintioctopunctata.* The leaf area eaten are shown

Fig. 2: Larva of *E. indica* is yellowish in colour and armed with numerous branched yellowish spines

Adult *E. vigintioctomaculata* and *E. vigintioctopunctata* attacks cucubitaceous plants when they cannot find solanaceous plant to feed (Abe and Matsuda, 2000). Abe and Matsuda (2000) reported both *E. vigintioctomaculata* and *E. vigintioctopunctata* were not stimulated to feed by solanine found in *S. tuberosum* and *S. nigrum* and tomatine contained in *Lycopaersicon esculentum* the solanaceous host plants. *Solanum nigrum* contain alkaloid, solanine. Alkaloids have never been reported to act as feeding stimulant. Both species are possibly stimulated to feed by some other substances contained in solanaceous host plants such as potato and tomato (Abe and Matsuda, 2000). In *E. vigintioctomaculata* methyl linolenate maximized the feeding activity of sugars at the concentration contained in the potato leaves suggesting that methyl linolenate plays an important role in the host selection of *E. vigintioctomaculata* (Endo *et al*., 2004).

Food preference is influenced by odor, taste, vision, age of plant and thickness of leaves and proportion of crude fibre, parenchymatous tissue and water content present (Katakura *et al*., 1989). The feeding preference was influenced by chemical factors as in three

Aulocophora leaf beetle species (Abe and Matsuda, 2005). Insect plant interaction may be evaluated by quantitative measurement of leave area consumed by herbivorous insect. Katakura *et al.* (1989) accessed leaf tissue removal by scoring and approximating percentange visually. Escoubas *et al.* (1993) measured leaf eaten areas using video-image analysis software when screening Hokkaido plants for antifeedant activity against *Spodoptera litura.* Imura and Ninomiya (1998) reported on the quantitative measurement of leaf area consumption by *Epilachna vigintocpuntata* towards solanaceous weed *Solanum carolinense* using image processing. Imura and Ninomiya (1998) showed that leaf area removal is a better measure for herbivore impact on the host plants than fresh or dry weight measure in terms of photosynthesis reduction.

Fauziah and Subramaniam (2008) reported that 100 ppm of metholic extract of Azadirachtin caused optimal antifeeding behavior of *E. indica*.

Solanum nigrum is a widely distributed tropical plant. The leaves of the plant have been used in some parts of Plateau State, Nigeria for food and medicinal purposes (Wannang *et al*., 2008). Unlike the egg plant *S. melongena,* the fruits of *S. nigrum* does not have economic importance, since it is not consumed by human.

Katakura *et al.* (1989) reported that adult beetles selected their natural hosts when given choices. This study investigated the potential use of *Solanum nigrum* as a trap plant in pest management of egg plant *Solanum melongena* by studying the feeding behavior and optimal feeding time of *E. indica* on the black nightshde plant *Solanum nigrum.*

MATERIALS AND METHODS

Study Venue

This study was conducted from 1st April until 3rd December 2002. The laboratory experiments were conducted at the Institute of Post Graduate Study, University Malaya, Kuala Lumpur, Malaysia. The field studies were conducted in the fields of Malaysia Agricultural Research Institute Jalan Kebun, Ulu Kelang, Malaysia.

Beetles and Plants

The 12-spotted ladybird beetles *Epilachna indica* (Coleoptera: Coccinellidae: Sub family Epilachninae), used in the study were collected from *Solanum nigrum* L. Black nightshade plants (Fig. 3, 4) grown in the field station of Malaysian Agricultural Research Development

Fig. 3: *Solanum nigrum* plants used in the observation

Fig. 4: Fruit of the blacknight shade a *S. nigrum* plant at Jalan Kebun, Ulu Kelang, Selangor, Malaysia

Institute (MARDI) at Jalan Kebun, Ulu Kelang, Selangor, Malaysia. The ladybirds were freshly collected prior to each experiment. The plants used for the study were about 0.4 m in height. Beetle of the same size were used for all experiments.

Experiment 1. Field Observation of *Epilachna indica* Behavior on Ten *S. nigrum* Plants

A field observation was conducted in farm at MARDI, Jalan Kebun on Oct 2nd 2002 from 08:00 until 19:00 h on 51 beetles on 10 plants of *S. nigrum* randomly chosen. The behavioral activities under conditions were observed for 12 h. The number of beetles feeding, resting, walking, mating and flying on all ten plants was recorded every 30 min throughout the observation period. The aim of this experiment was to determine the behavioral pattern of *E. indica* on *S. nigrum* in the field.

Experiment 2. Twenty Four Hours Observation on Feeding Duration of *E. indica* in a Control Condition in a Rearing Cage

Ten beetles were placed in a rearing cage together with a wild Solanaceous plant, *Solanum nigrum* (Fig. 5). Observation was undertaken every hour for 24 h from 06:00 on 3rd October 2002 until 06:00 h the following day. The number of beetles feeding and duration of each feeding was recorded. The method was modified from Fauziah *et al*. (2003), who observed feeding behavior of *E. indica* on *Solanum melongena* for 12 h then repeated the observation twice on every alternate days.

Experiment 3. Measurement of Leaf Area Consumption by *S. nigrum* and Optimal Feeding Time of *E. indica*

Ten ladybird beetles, *Epilachna indica* were individually placed in the cylindrical plastic container of diameter 4.0 cm and 5.5 cm in height together with a leaf of *S. nigrum*. The feeding activity of the beetles was recorded every half an hour for 3 days continuously from 06:00 h on Oct 3rd until 06:00 h on 6th Oct 2002. In this experiment, leaf consumption was determined by measuring leaf removal by area eaten by the beetles. Each leaf was traced onto a graph paper after 24 h and after 72 h. The rate of consumption of the beetles per day in square millimeter square (mm^2) was determined by counting the areas eaten. The optimal feeding time on each of the 3 days was also assessed.

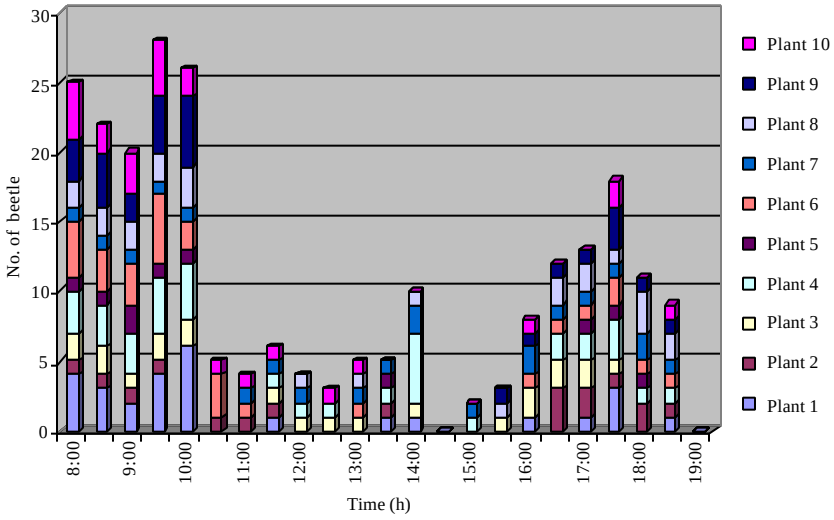

Fig. 5: Population of feeding *E. indica* on ten *S. nigrum* plants in the field

Experiment 4. Feeding Preference of *E. indica* between Leaf Discs of *S. melongena* and *S. nigrum* and Between Leaf Discs of *S. nigrum* and the Leaves of a Wild Solanaceous Plant

Beetles were given a choice whether to feed on leaf disc from *S. melongena* or *S. nigrum* following the method used by Wada and Munakata (1986). Two pieces of *S. melongena* leaf disc of 2.0 cm diameter and two pieces *S. nigrum* leaf discs 2.0 cm diameter were placed opposite each other in a petri dish of 9.0 cm in diameter. Five beetles were released in the center of the petri dish and their feeding preference was recorded. Two days prior to this experiment, beetles to be used in this experiment were not given food but only water. The experiments began at 10:00 h and ten replicate petri dishes were observed. The number of beetle found on each leaf disc was recorded at 10:30 h. This experiment was conducted during the peak feeding time for *E. indica* on *S. nigrum* plants after result obtained from earlier experiments. Beetle preferences were calculated by comparing the mean numbers recorded on each leaf type. Three treatment was given in this experiment:

- *S. nigrum* compared with *S. melongena*
- *S. nigrum* compared with a wild solanaceous plant and
- *S. melongena* compared with a wild solanaceous plant

RESULTS

The Behavior of Fifty One *E. indica* on ten *S. nigrum* Plants in the Field

The behavioral pattern of *E. indica* in the field can be obtained from the results summarized in Fig. 5, 6, 7 and 8. Table 1 shows the number of beetles showing different behaviors at each half hour observation from 08:00 h to 18:30 h on ten *S. nigrum* plants in the field. *Epilachna indica* fed and mated (Fig. 9) on the leaves or branches of the *S. nigrum* plants. Some beetles were motionless and some were walking on the leaves whilst other beetles were flying in between the leaves and branches or from one *S. nigrum* plant to another. The mean frequency of each behavior throughout the study was recorded. The

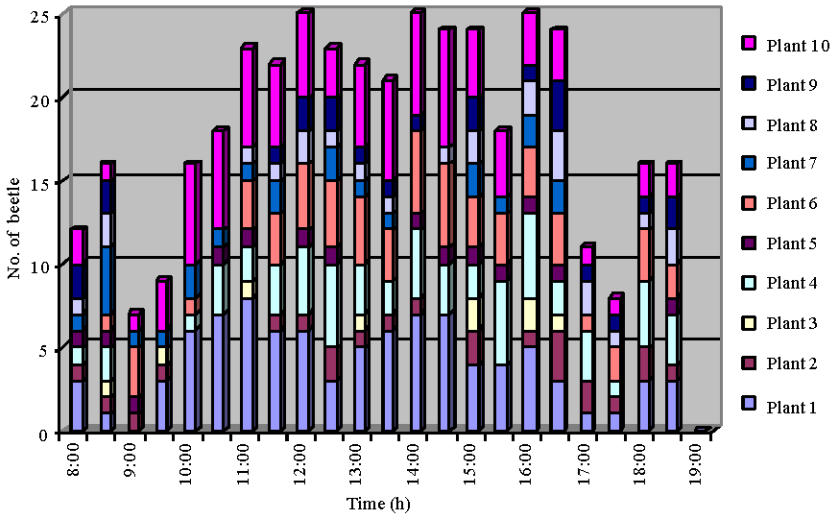

Fig. 6: No. of motionless and walking *E. indica* on ten *S. nigrum* plants in the field

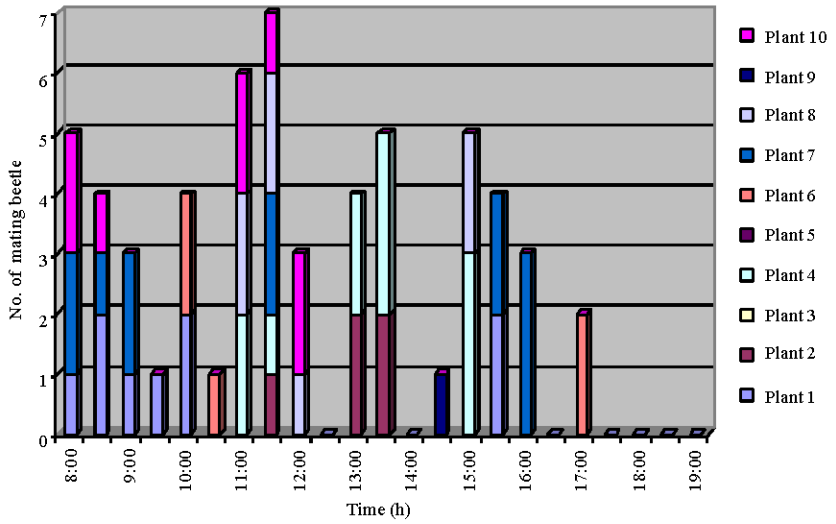

Fig. 7: Population of mating pairs of *E. indica* on ten *S. nigrum* plants in the field

frequency of beetles showing the behavior of feeding, mating, walking or motionless and flying on all ten plants were 235, 58, 401 and 100 times, respectively. This study shows *E. indica* beetles spent most time motionless or walking followed by feeding, flying with the least activity mating during the period of 08:00 to 18:30 h.

Feeding Behavior

Figure 5 shows two cycles of feeding periods for *E. indica* on *S. nigrum* plants in the field. The first cycle of feeding was from 08:00 to 10:00 h with the first peak of feeding with a maximum number of beetles feeding at 09:30 h (N = 28). Fewer beetles were feeding between

Table 1: The population of *E. indica* on ten *S. nigrum* plants in the field from 08:00 h until 18:30 h observed every half an hour on 2nd October 2002

Time (h)	Total no. of *E. Indica* on 10 *S. nigrum* plants in the field	No. of beetles			
		Feeding	Motionless or walking	Mating	Flying
08:00	48	25	12	5	6
08:30	51	22	16	4	9
09:00	29	19	7	3	0
09:30	40	23	9	1	7
10:00	50	26	16	4	4
10:30	32	5	18	1	9
11:00	18	4	23	6	5
11:30	38	6	22	7	3
12:00	35	4	24	3	4
12:30	30	3	23	0	4
13:00	38	5	22	4	7
13:30	33	5	21	5	2
14:00	35	10	24	0	1
14:30	29	0	24	1	4
15:00	31	2	24	5	0
15:30	31	3	18	4	6
16:00	36	8	24	3	1
16:30	39	14	23	0	2
17:00	33	13	11	2	7
17:30	35	18	8	0	9
18:00	35	11	16	0	6
18:30	29	9	16	0	4

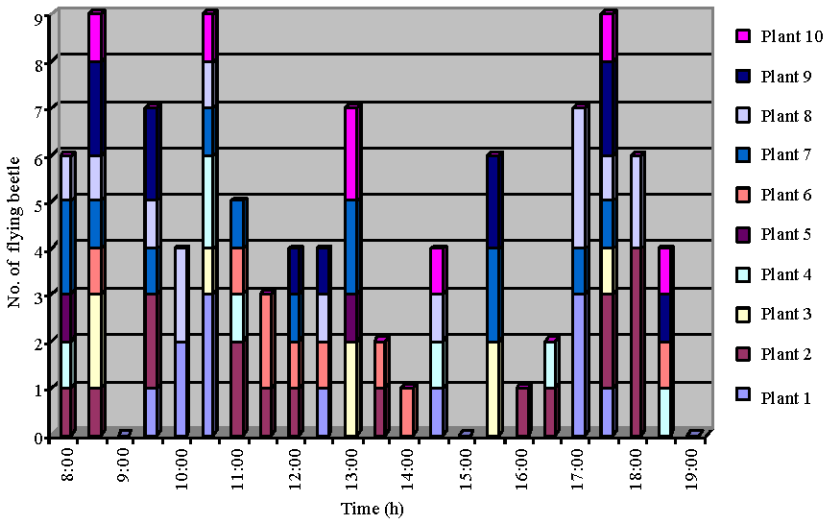

Fig. 8: Population of flying beetles on ten *S. nigrum* plants in the field

10:30 to 14:00 h, with a mean of 4.5±1.05. The second cycle of feeding was from 16:00 to 18:30 h and has a lower peak. The second peak of beetles feeding was at 17:30 h (N = 18). The mean number of beetles feeding throughout the first cycle was 24.0±3.54 while the mean number of feeding beetles during second cycle was lower at 20.285±3.55. The minimum number of feeding beetles on *S. nigrum* plant throughout the observation was at 15:00 h (N = 2). No beetle was feeding at 14:30 and 19:00 h.

Walking and Motionless Behavior

Figure 6 shows the number of beetles motionless or walking on ten *S. nigrum* plants in the field from 08:00 to 17:00 h. There were always some beetles motionless or walking throughout the observations. The highest number of beetle motionless or walking was from 11:00 to 16:30 h (N = 24 to N = 25). The least number of beetles walking or motionless was at 09:00 h (N = 7), 09:30 h (N = 9), 17:00 h (N = 11) and 17:30 h (N = 8). The mean number of beetle walking or motionless throughout the observation period was 18.23±6.68.

Mating Behavior

Figure 7 shows that mating activity of *E. indica* occurs in daytime on *S. nigrum* plants in the field at Jalan Kebun, Ulu Kelang Selangor, Malaysia. The adult size of *E. indica* in this study was between 1.3 to 1.5 cm (Fig. 1). The males and females can be identified by looking at the size and observing the end of the ventral side of the abdominal segments. The abdomen showed differences between the sexes of the beetle. Mounting pairs began mating from 08:00 h (N = 5) and occurred throughout the day until 17:00 h (Fig. 7). The highest number of pairs mating was at 11:30 h (N = 7). The mean number of mating pairs throughout the observation period was 3.5±1.79 which was rather low compared to other behaviors.

Flight Behavior

The number of *E. indica* flying every half an hour on ten *S. nigrum* plants in the field between 08:00 to 18:30 h is shown in Fig. 8. The beetles flew sporadically throughout the day moving in between the leaves or from leaf to branch or onto another *S. nigrum* plant nearby. The mean number of flying beetles on *S. nigrum* plant in the field was 4.70±2.67. The maximum number of flying beetles (N = 9) was at 08:30, 10:30, 13:00 and 17:30 h.

Feeding Duration of Ten *E. indica* on a *S. nigrum* Plant in the Rearing Cage in Control Condition

Over a 24 h period, the activity of ten beetles on a single *S. nigrum* plant in a cage in control condition was recorded. Figure 10 shows that 34% of time was spent on feeding, 18% walking, only 1% flying and most of the time, 47%, the beetles were motionless. This is due to the resting time during the night which was included in the 24 h continuous recording period. The total time spent by ten *E. indica* B1 to B10 on feeding, walking, flying and motionless was 8.31±0.99 h, 4.20±1.23 h, 1.30±0.48 h, 11.30±1.25 h, respectively. Figure 9 shows the feeding activity within 24 h of ten beetles on a single *S. nigrum* plant in the cage. The optimal feeding time was between 09:00 to 14:00 h whereby all ten beetles were feeding

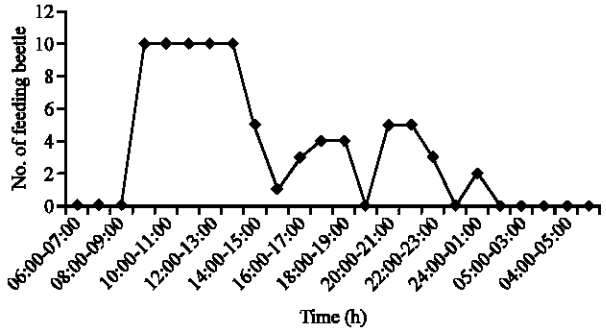

Fig. 9: Feeding pattern of *E. indica* for 24 h on a *S. nigrum* plants in a rearing cage

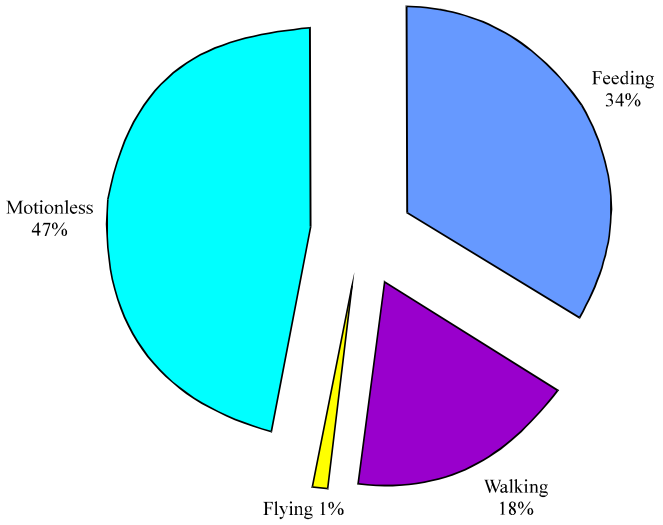

Fig. 10: Percentage time spent by *E. indica* on different behavior during a 24 h study

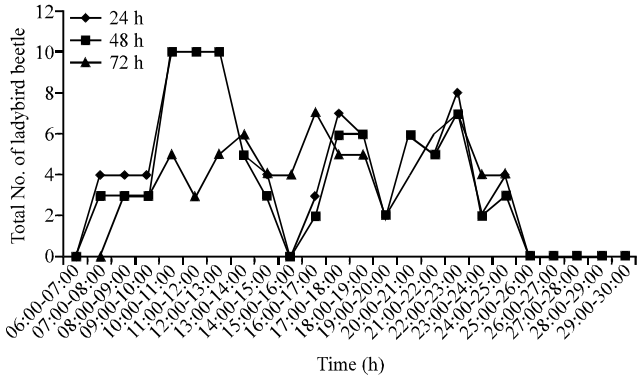

Fig. 11: Feeding pattern of ten *E. indica* on 3 successive days 4th, 5th and 6th Oct 2002 in caged *S. nigrum* plant

throughout. No beetle was feeding at 19:00 to 20:00 h and 23:00 to 24:00 h. Feeding began to stop completely from 02:00 to 08:00 in the cage (Fig. 9).

Optimal Feeding Time of *E. indica* on *S. nigrum* in Control Condition Observed for 72 h Observation

Figure 11 shows the feeding pattern of *E. indica* over 3 successive days from 3rd Oct to 6th Oct 2002 on a *S. nigrum* plant in a rearing cage. On the first two days the optimal feeding time was from 10:00 to 13:00 h with all ten ladybird beetles feeding every hour for duration of 3 h. Then, the numbers of beetles feeding decrease with only one feeding at 15:30. There was further feeding activity throughout the evening between 17:00 until 01:00 when all feeding ceased until 06:00 h. However, on the third day, feeding occurred sporadically throughout the day with no obvious peak period.

Table 2: *Solanum nigrum* leaf consumption by *E. indica* ladybird

Time	Measurement of leaf area removal through feeding by beetle (mm^2) (N = 10) 1	2	3	4	5	6	7	8	9	10	Mean per beetle±SD (mm^2)	Feeding rate per beetle (mm^2/nr)
After 24 h	28	24	30	26	34	38	32	24	26	30	29.2±4.308	1.217
After 48 h	56	60	62	50	68	58	62	64	68	66	61.4±5.660	1.279
After 72 h	72	80	78	84	90	76	80	74	88	78	80±5.812	1.111
Mean±SD											1.202±0.085	

Table 3: No. of beetles found feeding on leaf discs of *S. melongena*, *S. nigrum* and a wild solanaceous plant in feeding preference experiment

Petri dish replicate	No. of beetles on leaf discs: Petri dish A: *S. melongena* disc 1 and 2	Petri dish A: Wild leaf disc 3 and 4	Petri dish B: *S. nigrum* disc 1 and 2	Petri dish B: Wild leaf disc 3 and 4	Petri dish C: *S. melongena* disc 1 and 2	Petri dish C: *S. nigrum* disc 3 and 4
1	4	1	4	1	2	2
2	3	2	3	2	3	2
3	3	1	3	2	3	2
4	5	0	4	1	1	3
5	4	0	5	0	3	2
6	5	0	5	0	2	2
7	4	1	4	1	2	3
8	5	0	5	0	3	2
9	3	2	3	2	2	2
10	4	1	4	1	3	2
Total	40	8	40	10	24	22
Mean	4	1.455	4	1	2.4	2.2
SD	0.816	0.189	0.816	0.816	0.699	0.422

Leaf Consumption of *S. nigrum* by *E. indica*

Table 2 shows the measurement of *S. nigrum* leaf area removal after being eaten by beetles in pill bottles after 24, 48 and 72 h. The mean leaf area of *S. nigrum* eaten per bottle after 24, 48 and 72 h was 29.2±4.308 mm^2, 61.4±5.660 mm^2 and 80.0±5.812 mm^2. The feeding rate was 1.217 mm^2 h^{-1} per beetles after 24 h. 1.279 mm^2 h^{-1} per beetles after 48 h and 1.111 mm^2 h^{-1} per beetle after 72 h. The mean feeding rate over 3 days was 1.202±0.085 mm^2 h^{-1} per beetle.

Feeding Preference of *E. indica* on Leaf Disc Choice Test

Table 3 summaries the result of the leaf disc choice test. The mean number of beetles found feeding on *S. melongena* leaf disc was 4.0±0.816 compared to 1.454±0.788 on control wild solanaceous plant leaf disc. Similarly, the beetles exhibited preference to feeding on *S. nigrum* plant with the mean number of beetle 4.0±0.816 compared to 2.4±0.699 on control the wild solanaceous plant. Beetles not found on any leaf disc were not scored in the experiment. However, there was no significant difference between the mean numbers of beetles found feeding on the leaf disc of *S. melongena* which was 2.4±0.6999 compared to on *S. nigrum* leaf disc which was 2.2±0.421. Results of this experiment shows that *E. indica* have equal preference to eat *S. melongena* and *S. nigrum* leaf over the wild plant (control).

DISCUSSION

This study is the first study that reports a continuous observation of all the behavioural activity of *Epilachna indica* on *Solanum nigrum* plants in the field. Results of this study

confirms that *E. indica* feeds on *Solanum nigrum* plants in the field, cage and in the laboratory. The optimal feeding time of fifty one *E. indica* on ten *S. nigrum* plants under the sun in the field at Ulu Kelang Selangor was from 08:00 to 10:00 h whereas the optimal feeding time for ten *E. indica* on a *S. nigrum* plant in the cage was from 09:00 to 14:00 h. In natural conditions in the field physical factors such as the heat of the sun, wind speed, relative humidity, air temperature and other environmental factors will have the effect on beetle activity. In the cage the plant is sheltered from the heat of direct sunlight. There was two feeding cycle exhibited by *E. indica* on *S.nigrum* in the field. The periods of the first feeding cycle was 08:00 to 10:00 h and with the second cycle from 16:00 to 18:30 h. The period between 10:00 to 15:30 h are the hottest time in the field. At these time the beetles were mostly motionless or walking on the plant.

Similarly, the optimal feeding period of *E. indica* on egg plant S. *melongena* in the field at Somme Estate, Kedah was from 08:00 to 10:00 h (Fauziah *et al*., 2003). The second cycle of feeding by *E. indica* on *S. melongena* was from 17:00 to 20:00 h (Fauziah *et al*., 2003) whereas on the black nightshade *S nigrum* plant the second cycle of feeding period starts earlier from 16:00 to 18:30 h. These long feeding periods contrast with that of *E. tredecimnotata* which lasts for only 1 or 2 h each morning (Carroll and Hoffmann, 1980).

Bioassay using leaf disks in this study shows that *E. indica* prefers to eat *S. melongena* over control leaf disk of an unknown wild Solanacea plant. Similarly *E. indica* prefers *S. nigrum* over the control. However when given choices between leaf disks of *S. melongena* and *S. nigrum* equal number of beetles were found feeding on both *S. melongena* and *S. nigrum* confirming that *E. indica* like to eat both *S. melongena* and *S. nigrum*.

Food preferences in Epilachnines are influenced by odor, taste and age of the food plant (Katakura *et al*., 1989). The basis for discrimination is chemical with scent being detected by olfactory and gustatory sensilla on maxillary palps and antenna (Fischer and Kogan, 1986). Restriction to a particular plant family and species selection within the family is dependent on both odor and taste. However Srivasta (1957) contended that the age of food plant was not important in food selection trials with *E. vigintitioctopunctata*. Endo *et al*. (2004) suggested that methyl linoleate plays an important role in host selection of *E. vigintioctomaculata*. Cucurbitacins, contained specifically in cucurbitaceous plants, acted as feeding stimulants for *E. admirabilis*, *E. boisduvali*, *E. vigintioctopunctata* and *E. vigintioctomaculata* (Abe and Matsuda, 2000). Abe and Matsuda (2005) reported that chemical factors influences the feeding preferences of three *Aulocophora* leaf beetle species. This study shows that *E. indica* likes to eat both *S. melongena* and *S. nigrum* suggesting that *S. nigrum* contain chemical compound that stimulates *E. indica* to feed on it. Fauziah *et al*. (2003) reported 2-furancarboxaldehyde was the feeding stimulant which caused feeding behavior by *Epilachna indica* towards *Solanum melongena*. Behavioral bioassay towards chemical components extracted from the leave of *S. nigrum* would enlighten our understanding on host plant selection in the ladybird beetle *E. indica*.

This study confirms that *S. nigrum* can be used in pest management strategy of egg plants against *E. indica*. Results of this study is important and of significance providing a new strategy for Malaysian vegetable farmers. *Solanum nigrum* maybe grown in margins surrounding egg plants *S. melongena*. This would lure *E. indica* to stay feeding on *S. nigrum* protecting *S. melongena* from *E. indica* attack thus increasing the quality and quantity of egg plant production in Malaysia without the need to use pesticide ensuring safe product for Malaysian consumer and safer environment for all.

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