Feeding stimulant for *Epilacha indica* (Coleoptera: Coccinellidae) from the leaf surface of *Solanum melongena*

Fauziah Abdullah, Subramanian Partiban and Faizah Abdullah

Institute of Biological Sciences, Faculty of Science, University Malaya 50603 Kuala Lumpur, Malaysia

ABSTRACT A study was conducted to observe the 12 spotted lady bird beetle, *Epilacha indica* (Coleoptera:Coccinellidae) feeding on the eggplant *Solanum melongena* (Family: Solanaceae) in a farm in Somme Estate, Serdang, Kedah. The study was conducted for 24 hours on 3 different days and subsequently followed by a 12 hour observation on feeding behavior conducted in the laboratory. The optimal feeding time was found to be between 0800h to 1000h and between 1700h to 2000h in the laboratory and 1700h to 2000h in the field. The leaves of *Solanum melongena* were collected and an ethanol extract obtained. *Epilacha indica* exhibited strong feeding behavior to two fractions as shown by column chromatography. Analysis of the fractions using Gas Chromatography-Mass Spectrometry gave 2-furanencarboxaldehyde as the compound found in the purified extract which causes feeding behavior in *Epilacha indica*.

INTRODUCTION

The egg plant *Solanum melongena* belongs to the family Solanaceum. It is also known by its common name as brinjal. The eggplant has many leaves and is about 0.5 to 1.0 m in height. The leaves are green to purplish in colour. The fruit of the egg plant is a popular vegetable. The fruits are round or long and can be white green or purple in colour. The feeding preference of phytophagous insects is largely mediated by the presence of secondary chemicals found in the plant. Plant epicuticular waxes affects feeding behaviour differently in various insects [1, 2, 3, 4]. Insects have the capacity to detect non-polar substances on the leaf surface [2]. Phytophagous insects usually make sensory exploration of the leaf surface before biting to check whether the plant is a suitable diet or not.

The ladybird beetle, *Epilacha indica* is one of the pests of the eggplant where both larva and adult beetle feed on the upper surface of the leaves and flowers of the egg plant of all stages. *Epilacha indica* is a yellow-headed ladybird beetle with 12 small black rounded spots on the surface of its red elytra and its size is between 10 mm to 12 mm. The prothorax and legs are yellow in colour.

The objectives of this study are to determine firstly the optimal feeding time for *Epilacha indica* and to elucidate the compounds found in the leaves of the egg plant which makes the leaf palatable for the beetles.
MATERIALS AND METHODS

Plant material and beetles
Leaves of the eggplant, *Solanum melongena* (Family: Solanaceae) were taken from Sowme Estate, Serdang, Kedah. Forty beetles, *Epilachna indica* (Coleoptera: Coccinellidae) (Figure 1) were collected in December 2001 from two-month-old eggplant trees. The beetles were kept together in a plastic container and fed with fresh eggplant leaves everyday.

Extraction of leaves
*Solanum melongena* leaves were rinsed with tap water for 1 to 2 minutes and followed by distilled water after which the leaves were blotted with tissue paper. The clean leaves were cut with razor into small pieces and blended with 100 ml of 70% ethanol. After 24 hours the mixture was evaporated utilising a rotary evaporator.

Isolation and Purification
20 g of silica gel 60 was weighed and allowed for swelling for 24 hours in 100 ml of 70% ethanol. The slurry was then poured into a column filled with glass wool at the end. The reservoir was then filled with 70% ethanol while the column tap was opened to allow ethanol to flow through the column for about 30 minutes in order to clean the column from air bubbles and contamination. The outlet trap can be used to adjust the flow to about 2 ml/min. 2.0 ml of the leaf extract was introduced into the column. Then 50 ml of 70% of ethanol was slowly poured into the column. The mobile phase was let to flow and the eluant fractions were collected at 2 minute intervals. A total of 25 fractions of 2ml each were collected and named fraction 1 to 25 respectively.

24 hour observations on feeding behavior in the field
Five two-month old *Solanum melongena* trees were chosen for the 24 hour observation. Every hour the number of beetles that were feeding on each of the five eggplants were recorded from 0900h to 0800h on 11 December 2001. The 24 hour observation was repeated on the third (13 December 2001) and fifth day (15 December 2003).

12 hour observations on feeding behavior in laboratory
20 *Epilachna indica* were taken from the rearing chamber and their feeding time was observed in the laboratory for 12 hours from 0800h to 2000h 20 December 2001. Five beetles were put in a petri dish together with a *Solanum melongena* leaf. The number of beetles feeding were recorded. The observation was repeated twice using different beetles on 21 December 2001 and 22 December 2001.

Figure 1. The 12-spotted ladybird beetle *Epilachna indica*
Feeding Bioassay using extract
A 10 μl eluant fraction (E) was placed at one side of a petri dish lined with a 10 cm diameter filter paper. 10 μl of 70% ethanol was placed on the other side as control (C) (Figure 2). Three *Epilachna indica* B1, B2 and B3 were released at the center of the filter paper (Figure 2). The response of the beetle towards the fraction was observed for 5 minutes. The observation was repeated using the other 24 fractions. The feeding bioassay was conducted during the optimal feeding time which was observed from the 24 hour and 12 hour studies on feeding behavior in the field and in the laboratory respectively.

Thin Layer Chromatography Analysis (TLC)
The fraction which gave positive response in the feeding bioassay was analysed using TLC plate silica gel F254. Diethyl ether and ethanol of 70:30 solvent mixture was used. The separation that occurred on the TLC plate was observed under UVGL-58 UV light of short wave 254nm / long wave 366nm. The spots which appeared were removed and dissolved in solvent and again bioassayed using another set of beetles.

Feeding Assay on TLC Spot
The components which appeared as spots on the TLC plate were removed and dissolved in 2.0 ml solvent mixture. Response of *Epilachna indica* towards each compound was observed in a feeding assay as conducted earlier.

Analysis using Gas Chromatography-Mass Spectrometry (GCMS)
The spots that appeared on TLC plate which indicated positive response during feeding assay were analysed in Gas Chromatography Mass-Spectrometry (GCMS). The temperature of oven was kept at 40°C for 5 minutes then increased to 280°C at 10°C/min, then maintained at 280°C for 10 minutes. The flow rate of the helium gas was 1.3 ml per minute. A BP5 capillary column of 29.0 m in length of diameter 0.25 mm was used.

Figure 2. Arena for feeding bioassay
RESULTS

Figure 3, 4 and 5 showed the number of beetles feeding on five Solanum melongena plants observed in Somme Estate every hour for 24 hours on three different days. The result obtained indicated that the optimal feeding time of Epilachna indica was 1700 h to 2000 h.

![Graph showing the number of beetles feeding on Solanum melongena leaves.](image)

**Figure 3.** Number of Epilachna indica feeding on Solanum melongena leaves for the first 24 hour study on 11 December 2001.

![Graph showing the number of beetles feeding on Solanum melongena leaves.](image)

**Figure 4.** Number of Epilachna indica feeding on Solanum melongena leaves for the second 24 hour study on 13 December 2001.
Figure 5. Number of *Epilachna indica* feeding on *Solanum melongena* leaves for the third 24 hour study on 15 December 2001.

However, for the 12 hour observation on feeding in the laboratory the optimal feeding time of *Epilachna indica* was observed twice, from 0800 h to 0900 h and from 1700 h to 1900 h as shown in Figure 6.

Figure 6. Number of *Epilachna indica* feeding on *Solanum melongena* leaves for three days of 12 hour study in laboratory on 20, 21, and 22 December 2001.
Positive feeding is shown in Figure 7(a) while negative feeding is shown in Figure 7(b). Positive response is indicated when *Epilachna indica* move and reach the extract spot at E and try to feed on it. Negative response is indicated when *Epilachna indica* does not approach the spot. These bioassays were repeated five times for every fraction using different beetles in each bioassay.

**Figure 7(a).** Movement of ladybird beetle when response is positive

**Figure 7(b).** Movement of ladybird beetle when response is negative
Table 1 Summarizes the feeding assay using the 25 fractions of the extract. The most positive response was obtained with fraction 4 and fraction 18.

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+: when only 1 beetle approached or feed on extract

+++: when only 2 beetles approached or feed on extract

++++: when only all 3 beetles approached or feed on extract

-: when beetle did not approach or if approached did not feed the extract spot and moved to other place

Table 1. Feeding Response of *Epilachna indica* towards fractions

Figure 8 shows the chemical components obtained after fraction 4 and 18 had undergone Thin Layer Chromatography. One spot was observed for fraction 4 and for fraction 18. The retention time, RF of the spot from fraction 4 was 0.44 and the RF of fraction 18 was 0.39.
Figure 8. Thin Layer Chromatography showing spots from fraction No.4 and No.18

The chromatogram obtained after GCMS gave a peak at retention time of 7.84 for fraction 4 and another peak at retention time of 8.15 for fraction 18 (Figure 9 and Figure 10). Both fractions contain the same compound, 2-Furancarboxaldehyde present in the leaves of eggplant, *Solanum melongena* which caused positive feeding response by the twelve-spotted ladybird beetle *Euphyra edonis*. The chemical structure of 2-Furancarboxaldehyde is given in Figure 11.

Figure 9. The chromatogram profile of spot no. 4 obtained from TLC
DISCUSSION

The ladybird beetle from the subfamily Epilachninae are often destructive to plants in the Cucurbitaceae and Solanaceae families. In this study, Epilachna indica were observed to feed on the green leaves and in between the veins of Solanum melongena leaving behind leaf skeletons. This may cause death to young plants and stunt older Solanum melongena plants [5]. Some species of ladybirds are specialists requiring specific food for their reproduction [6]. Phytophagous insects that feed on plants cultivated by man such as the eggplants often cause economic loss. In the Somme Estate, Kedah Epilachna indica did not feed between 1100h to 1400h. This could be because the leaf surface is hottest during this period since the sun is directly overhead. Whereas in the laboratory at least one beetle was found feeding in the petri dish during this period.
Chrysomelid beetles are stimulated to feed by n-alkanes and fatty alcohols [7]. The feeding stimulant for the leaf surface of Populus were n-behenyl alcohol (C25), n-lignoceryl alcohol (C24), n-hexacosanol (C26), n-octacosanol (C28), n-triacontanol (C30) and α-tocopherolquinone was isolated and identified by [8]. A mixture of alcohol and α-TQ synergistically stimulates beetle feeding [8], while glucosinolates initiate and prolong feeding in many insect species which feed specifically on crucifers [9]. This was also reported to be true in the cabbage flea beetle, Psylliodes chrysocata [10]. Sugars, amino acids, proteins, vitamins, sterol and phosholipids have all been reported to be insect phagostimulants [11].

Methanolic extracts of leaves of Solanum dulcamara was positive in the “Chilo dipping test” for ecysts steroids [12]. Solanaceae is a large family with at least 2300 species [13] surveyed 128 species of Solanaceae species and found that Solanum nigrum contain 20-hydroxyecdysone and polydione B (5f), 20-hydroxyecdysone as major compounds.

The presence of 2-furanacarbonylaldehyde appeared to contribute to a defensive mechanism for protecting the developing maize kernel from fungal attack [14].

In summary, the feeding stimulant in the eggplant Solanum melongena is probably 2-furanacarbonylaldehyde since Epilachna indica showed positive feeding response to the compound. Thus in this study it is suggested that Epilachna indica feed on 2-furanacarbonylaldehyde for its defence system.

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REFERENCES