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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (19): 991-997, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.991.997

Effect of Hadda Beetle, *Epilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae) Infestation on Eggplant Leaf (*Solanum melongena* L.) and Bio-control Potential of Essential Oil Formulations

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Abstract: The present study evaluates the nature of feeding damage of *Epilachna vigintioctopunctata* on leaves of *Solanum melongena* L. and antifeedant activities of essential oil formulations against *E. vigintioctopunctata*. Investigations were made on the morphological and anatomical damage caused by the feeding activity of the adults and grubs of *E. vigintioctopunctata*. Adult *E. vigintioctopunctata* scraped the green matter of the upper and lower sides of the leaves of *Solanum melongena* leaving behind only a network of veins. This characteristic scraping made the leaves papery and the infested eggplants exhibited inter venal damage or holes on the leaves. The reduction percentage of leaf fresh weight and leaf dry weight in infested leaves after one generation were 11.4 and 15.2%, respectively. In the grub infested leaves of eggplant, fully damaged epidermis, parenchyma cells and phloem cells of vascular bundles were observed. The total mean percentage of infested leaf damage was 12.75±0.43%. Maximum antifeedant activities of 80.06, 61.92% were observed in oil formulation III and formulation I, respectively at 100 ppm concentration against the fourth instars grub of *E. vigintioctopunctata*. This would be a good alternative for the chemical pesticides.

Key words: *E. vigintioctopunctata*, eggplant, feeding damage, antifeedant activity, oil formulation

INTRODUCTION

Eggplant, *Solanum melongena* L. is a common and popular vegetable crop grown in the subtropics and tropics (Sarker *et al*., 2006) and one of the major vegetable crops in India. The eggplant is commonly known as brinjal in India and aubergine in Europe. Eggplant is a perennial but grown commercially as an annual crop by small and marginal farmers in India. Eggplant is infested by a dozen of insect pest species, among which the most serious and destructive one is the *Epilachna vigintioctopunctata* Fab.

The spotted leaf beetle or Hadda beetle, *Epilachna vigintioctopunctata* Fab. (syn. *Henosepilachna vigintioctopunctata* Fab.), is the key-pest of the solanaceous and cucurbitaceous plants (Islam *et al*., 2011). This pest is widely distributed in Southeast Asian countries, Korea, Australia and it is common in South India, also occurs in other parts of India (Kapur, 1950). Due to its infestation, considerable economic loss is occurring during every crop season, adversely affecting both quality and quantity of crop output. The grub and adult feeds on the leaves, retarding the plant growth, which leads to loss of fruit production. Fruit reduction in yield up to 60% has been reported (Mall *et al*., 1992). The damage was greater during April-October and 80% of leaves were injured (Rajagopal and Trivedi, 1989) and the high incidence of the pest has been reported during temperature range of 24-31°C and relative humidity 58-75% RH in the field (Ramzan *et al*., 1990; Ghosh and Senapati, 2001). The pest has been noticed since many decades in different parts of India (Krishnamurti, 1932; Mathur and Srivastava, 1964; Rajagopal and Trivedi, 1989). The outbreak of *E. vigintioctopunctata* was noticed during 2004-2005 on a medicinal plant, *Withania somnifera* (Venkatesha, 2006) in Bangalore, India. The success of this pest species in Asian countries indicates its wide geographical range, high polyphagy, mobility, high fecundity and propensity to develop resistance to insecticides.

Different varieties of eggplant are available in India on the basis of their size, shape and colour (Nisha *et al*., 2009). Earlier reports explain eggplant varieties, biology of *E. vigintioctopunctata* and importance of eggplant fruits

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in antioxidant activity (Nisha *et al*., 2009). Literature scan also reveals that the *E. vigintioctopunctata* feeds exclusively on Solanaceae and the feeding activity were varied in daylight and darkness (Richards and Filewood, 1990). Abdullah and Abdullah (2009) have been reported that in one generation about 29.26% time was spent by *Epilachna* beetle for feeding on *S. nigrum* and the optimal total feeding time in field was from 8:00 to 10:00 h. Sinha and Krishna (1969) have been recorded that Epilachna vigintioctopunctata Fab. on *Luffa aegyptiaca* show that these insects prefer flowers in light and leaves and flowers in dark. However, there is a lack of data on the nature of feeding damage caused by *E. vigintioctopunctata* on eggplant leaves, especially with respect to morphological and anatomical damage and its potential control measures. Considering this fact and the importance of eggplant fruits in cuisines, the present study was undertaken to evaluate and compare feeding damage of *E. vigintioctopunctata* on eggplant leaves and antifeedant activity of prepared essential oil formulations were tested against *E. vigintioctopunctata* grub.

MATERIALS AND METHODS

Plant culture and insect rearing: Eggplants were grown individually in 20 cm mud pots at the four to six leaf stages in Department of Zoology, Madras Christian College (12°55'N Latitude and 80°06'E Longitude, India) and every single plant was placed into an individual rearing cage (60×60×60 cm) covered with nylon net. Then 2-day-old *E. vigintioctopunctata* (20 numbers) were released into this cage for 72 h to allow adult beetle's for oviposition. After then the eggs were introduced into new eggplants that were kept in rearing cages till to complete one generation for morphological and anatomical study. Control plants were kept in another rearing cage with similar environment.

Morphological investigation: Five leaves were collected from each of infested and control plants (one leaf from one plant) after completing one generation of *E. vigintioctopunctata* for examination of leaf morphology. The damaged area of each leaf was observed and the morphological changes were recorded. Data on fresh and dry leaf weights (g) were measured by a digital weighing machine (Model AB54-S, Japan). After measuring leaf fresh weight, leaves were placed in an oven (Model STD-115, India) at 80°C temperature for 48 h and dry weight was recorded.

Anatomical investigation: The anatomical study was performed as previously established method of Touhidul and Shunxiang (2009) with slight modifications. The infested and control leaf samples were fixed in FAA (50% Ethyl alcohol [95%], 10% formalin [37%] Formaldehyde, 5% Acetic Acid, 37% dH_2O) after making small pieces of about 5 mm in length. Then the samples were dehydrated using Tertiary Butyl Alcohol (TBA) series (65, 75, 85 and 95%). The leaf samples were embedded in wax and hardened by keeping under laboratory conditions (27±2°C, relative humidity 68±4 and photoperiod 16 h light: 8 h dark) for 24 h. Serial cross sections of the hardened materials were obtained at the thickness of 8-12 μm using a hand rotating microtome (WESWOX Optic Model MT-1090 A, India) and the sections were stained with Rose Bengal stain for two minutes. Finally, stained sections were transferred to a microscope slide, cover slipped and immediately viewed through a stereo zooming microscope (Wild M7S TYP 308700, Switzerland. Thereafter, the stained infested leaves were randomly selected under microscope; cell damage was observed including vascular bundles, photographed and compared with control.

Study on feeding damage: Adults of *E. vigintioctopunctata* were maintained separately in a container (8×10×10 cm). The beetles were fed with fresh, undamaged eggplant leaves. Ten replications were maintained. The total area of the leaf before feeding was recorded using the portable Leaf Area Meter AM-300, USA. After 24 h the leaf fed upon by the beetle was removed and the damaged area was measured (cm^2) using the established method of Fauziah *et al*. (2003) with slight modification. Thus the mean percent damage caused by the beetle on the leaves was recorded and tabulated.

Preparation of oil formulation: Neem oil, Ginger oil, Karanj oil were purchased from local market and Lime oil, Lemon oil, Orange oil, Basil oil were extracted in laboratory using a Clevenger-type apparatus (Model MI 1000, Sunbim, India) where the peel and plant material were subjected to hydro distillation. Conditions of extraction were: 50 g of air-dried sample and water (1:10 ratio) in 70°C for 4-6 h distillation. Outer peels were used for lime, lemon, orange oil extraction and leaves of *Ocimum basilicum* L. were used for basil oil extraction. Details of chosen oils are presented in Table 1. Four different essential oil formulations were prepared by mixing above mentioned oils, making a total of 10 mL including emulsifier and the details of prepared oil formulations are given in Table 2. Stock solutions (1% in Tween 20) of all the four formulations were prepared and stored in 4°C for further bioassay screening.

Table 1: Details of plant oils taken for preparation of oil formulation

Plant Name	Family	Parts used for oil extraction	Name of the oil
Azadirachta indica A.Juss.	Meliaceae	Seed	Neem oil**
Zingiber officinale Willd.	Zingiberaceae	Rhizome	Ginger oil**
Ocimum basilicum L.	Lamiaceae	Leaf	Basil oil*
Citrus limonia L.	Rutaceae	Peel	Lemon oil*
Citrus sinensis L.	Rutaceae	Peel	Orange oil*
Pongamia pinnata L.	Fabaceae	Seed	Karanj oil**
Citrus aurantifolia Christm.	Rutaceae	Peel	Lime oil*

*Oils extracted in laboratory, **Market purchased oils

Table 2: Details of different oil formulations subjected for antifeedant activity

Formulation I (mL)	Formulation II (mL)	Formulation III (mL)	Formulation IV (mL)	Control (mL)
Neem oil 3	Karanj oil 5	Neem oil 4	Lemon oil 3	Emulsifier 1
Lime oil 4	Zinger oil 1	Karanj oil 4	Orange oil 4	Distilled water 9
Basil oil 2	Lemon oil 3	Zinger oil 1	Basil oil mL 2	
Emulsifier 1	Emulsifier 1	Emulsifier 1	Emulsifier 1	
Total = 10	Total = 10	Total = 10	Total = 10	Total = 10

Within the column, Emulsifier used was Tween 20

Antifeedant activity: The antifeedant activity of essential oil formulations were tested in *S. melongena* leaves using leaf disk no-choice method (Isman *et al*., 1990). The prepared stock solution of oil formulations were taken in two concentrations, viz 100 and 50 ppm. The activity was tested against the fourth instar grub of *E. vigintioctopunctata*. The fresh eggplant leaf discs of 5 cm diameter were punched using iron cork borer, test concentration (100, 50 ppm) of formulations were applied in leaf discs and air dried for 5 min. Four hour pre-starved fourth instar grubs of *E. vigintioctopunctata* were introduced into treated leaf discs. The leaf discs treated with emulsifier (Tween 20) were considered as control. Five replicates were maintained for each treatment and with five grubs per replicate (n = 25). The total area of the leaf before feeding was recorded using the portable Leaf Area Meter AM-300, USA. After 24 h the leaf fed upon by the beetle was removed and the consumption rate was measured (cm^2) using the established method of Fauziah *et al*. (2003). The percentage of antifeedant activity was calculated using the following formula and the calculated values were analysed in Graph Pad Prism version 3.0.

$$\text{Antifeedent activity} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treatment}} \times 100$$

Statistical analysis: The results were presented as mean±SE for morphological study. Statistical analyses of all the data obtained in morphological studies were evaluated using One-Way ANOVA (SPSS Program; Version 11.5). The differences were considered as significant at p = 0.05. The calculated percent antifeedant activity was analysed in Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, USA.

RESULTS

Effect of *E. vigintioctopunctata* on leaf morphology: The morphological parameters of control and infested leaves are summarized in Table 3. Significant differences were observed on two morphological parameters i.e., leaf fresh weight (F = 221.7, P = 0.05 and df = 1) and leaf dry weight (F = 409.6, p = 0.05 and df = 1) after completing one generation of *E. vigintioctopunctata*. The reduction percentage of these two parameters in infested leaves was 11.4 and 15.2%, respectively. The infested leaves became papery and withered in 24 h.

Effect of *E. vigintioctopunctata* on leaf anatomy: In the *E. vigintioctopunctata* grub infested leaf, there were no damage observed in the xylem cells, but damaged cells were observed in the phloem, epidermis and parenchyma cells of infested leaf (Fig. 2b). There were no damaged cells observed in control leaves during present investigation (Fig. 2a). The non-damaged vascular bundles contained both xylem and phloem in control; while some damaged vascular bundles contained fully damaged phloem in infested leaf. But adults of *E. vigintioctopunctata*, scraped the whole green matter of the upper and lower epidermis, parenchyma cells and vascular bundles of the leaves of *S. melongena* leaving behind only a network of veins. The leaves were fully damaged leaving only holes on the leaves. There were no possibilities of anatomical study in the adult infested leaf since the whole green matter was eaten up by the insect.

Feeding damage of *E. vigintioctopunctata* on leaf: The morphological parameters of control leaf area and infested leaf damaged area are summarized in Table 4. The mean damaged area of infested leaves (n = 10) was

Table 3: Mean±SE values of two morphological parameters-leaf fresh weight and leaf dry weight of eggplant leaf

		Confidence level (95%)			Confidence level (95%)	
Treatment	Fresh leaf weight (g)	Lower	Upper	Dry leaf weight (g)	Lower	Upper
Control	1.25±0.09[a]	1.23	1.28	0.82±0.37[a]	0.72	0.92
Infested	0.68±0.37[b]	0.58	0.78	0.06±0.003[b]	0.05	0.07

Values for each parameter followed by a different letter indicate a significant difference at $p<0.05$ (One-way ANOVA); n = 5

Table 4: Mean±SE values of two morphological parameters-total leaf area and leaf damaged area of eggplant leaf

		Confidence level (95%)			Confidence level (95%)	
Treatment	Leaf area $(mm)^2$	Lower	Upper	Damaged area $(mm)^2$	Lower	Upper
Control	3261.80±19.11[a]	3218.56	3305.04	00±00[a]	-	-
Infested	2980.30±28.06[b]	2916.80	3043.80	439.50±15.88[b]	403.58	475.42

Values for each parameter followed by a different letter indicate a significant difference at $p<0.05$ (One-way ANOVA), n = 10

Fig. 1(a-c): (a) Control leaf, (b) *E. vigintioctopunctata* grub infested leaf and (c) *E. vigintioctopunctata* adult infested leaf of *S. melongena*

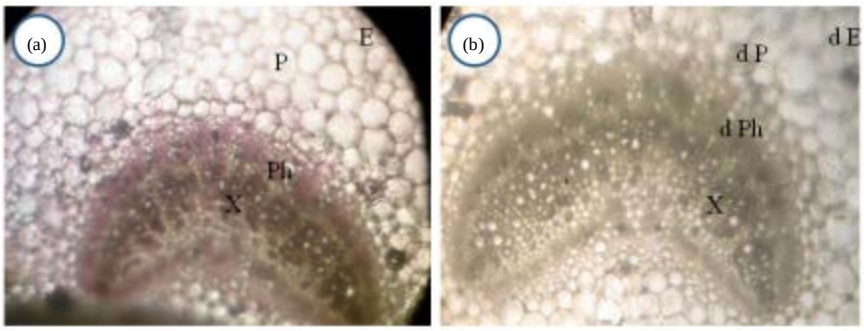

Fig. 2(a-b): Cross section of grub infested leaf of eggplant (b) showing damaged epidermis (d E), damaged Parenchyma cells (d P), damaged phloem cells (d Ph) and non-damaged xylem (X) compared with (a) normal control

439.50±15.88 mm^2. The total mean percentage of infested leaf damage was 12.75±0.43%. The morphological damage of adults and grubs on eggplant leaves were compared with control (Fig. 1).

Antifeedant activity of oil formulations: The results of antifeedant activity of different essential oil formulations against *E. vigintioctopunctata* grubs are given in Fig. 3. Maximum antifeedant activities of 80.06, 61.92% were observed in formulation III and formulation I, respectively at 100 ppm concentration against the fourth instars grub of *E. vigintioctopunctata*. Least antifeedant activity was noticed in formulation IV with 13.34, 33.42% at 50 and 100 ppm concentration, respectively. Likewise formulation

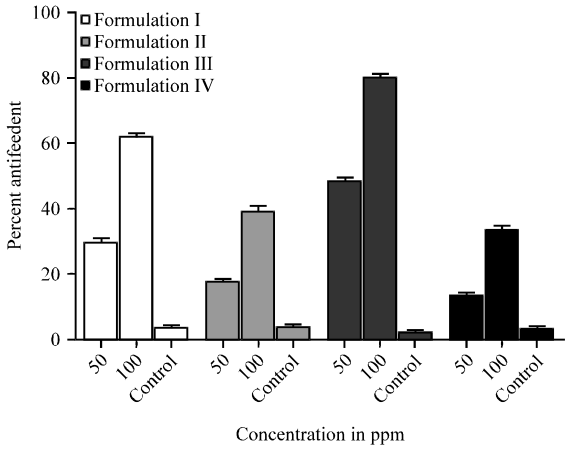

Fig. 3: Percent antifeedant activity of different oil formulations against fourth instars grub of *E. vigintioctopunctata*. Results are given in mean±SD of five replicates each

II also recorded less antifeedant activity with 39.08% at 100 ppm concentration. In formulation III treated leaves it was noted that some grubs were died at 100 ppm concentration. In control the introduced grubs were very active and normal feeding activity was observed.

DISCUSSION

Eggplant, *S. melongena* is a common and very popular vegetable crop grown in the sub-tropics and tropics. *E. vigintioctopunctata* is known as a specialist herbivore of solanaceous plants (Shinogi *et al*., 2005) and it is common pest of Asian region (Sharma *et al*., 2012). There are many plant species have been reported as its host plant so far include *S. melongena*, *S. tuberosum*, *S. photeinocarpum*, *S. torvum* and *Lycopersicon esculentum* (Shirai and Katakura, 1999).

The present study indicates that the *E. vigintioctopunctata* infestation has showed significant impacts on morphology and anatomy of eggplant leaves. The total reduction percentage on leaf morphology of two parameters (leaf fresh weight and leaf dry weight) in infested leaves was 11.4 and 15.2%, respectively. In the anatomical study of grub infested leaves, the epidermal cells were fully eaten up by grub, damaged parenchyma cells and damaged phloems were observed; but xylem cells were not damaged. Phloem plays an important role of transporting organic substances of photosynthesis to other parts of the plant. Thus, after one generation, the stunted growth was observed, fruit setting were delayed and the fruit yield was drastically reduced in both grub and adult infested plants. This is because; the insect infestation on leaves completely decrease chlorophyll content and which directly affects the photosynthesis. A similar result was observed in rice plant by whitefly (Watanabe and Kitagawa, 2000) and whitefly on eggplant leaves (Touhidul and Shunxiang, 2009). This results in significant economic loss every year.

E. vigintioctopunctata causes high feeding damage on eggplant (Solanaceae); but it also feeds on other plants like Chinese cabbage, *Arabidopsis*, kidney bean, cucumber and its feeding efficacy were varied (Shinogi *et al*., 2005). Another report says that *E. vigintioctopunctata* attacks cucurbitaceous plants if its primary host plants (Solanaceae) were not available (Abe and Matsuda, 2000). Bernys and Chapman (1994) documented that *E. vigintioctopunctata* recognizes the host plants via preformed compounds as has been reported in other phytophagous insects. *E. vigintioctopunctata* is known to be one of the devastating pests, continually since from many decades (Mathur and Srivastava, 1964; Mohansundaram and Uthamaswamy, 1973; Rajagopal and Trivedi, 1989; Verma, 2006). Earlier studies explains, Odors, taste, vision, age of the plant, thickness of the leaves and phytochemicals stimuli are the main factors which influence food preference of *E. vigintioctopunctata* (Katakura *et al*., 1989; Endo *et al*., 2004; Abe and Matsuda, 2005).

Natural insecticides are generally pest specific, biodegradable, usually non-allergic to human as well as non-target organisms (Bowers, 1992; Pavunraj *et al*., 2012), and oil formulations possess novel compounds with a wide range of activities (Hussien *et al*., 2011). Plant derived essential oils are richly available in Indian market. Considering this, the antifeedant activity was evaluated and the present study showed that formulation III was potent against *E. vigintioctopunctata* grubs. Some of the grubs were died at 100 ppm concentration of formulation III. This result was comparable with earlier report of Diaz Napal *et al*. (2010) that they have screened a flavonoid Pinocembrin, which strongly affected the survival of *E. paenulata*. In another experiment the seed oil from *P. glabra* exhibited maximum antifeedant activity and no feeding was observed after 24 and 48 h of treatment (Swaminathan *et al*., 2010). Earlier, the efficacy of neem oil on the mortality, growth and feeding responses of *E. dodecastigma* (Wied.) showed that all the larval instars were susceptible to this oil (Anam *et al*., 2006). The feeding of *E. indica* towards extracts of *Azadirachta indica* were also evaluated (Abdullah and Subramaniam, 2008). Recently, the interaction between the insect pest, *H. vigintioctopunctata* and the fungal pathogen, *Alternaria alternata* was studied on *Withania somnifera* leaves (Sharma *et al*., 2012).

In conclusion, present results helps one to know about the feeding damage caused by *E. vigintioctopunctata* on leaves of *S. melongena* and the

antifeedant activity explains that the oil formulation III could be used for bio-control of *E. vigintioctopunctata*. This would serve as a good eco-friendly bio pesticide, which is biodegradable and cost-effective than chemical pesticides.

ACKNOWLEDGMENTS

The authors are thankful to Dr. R.W. Alexander Jesudasan, Principal, Madras Christian College, Chennai, India for providing lab facilities. We thank Dr. Mohan Daniel, Department of Zoology, Madras Christian College, Chennai, India for his guidance and valuable comments in writing manuscript. We also wish to extend thanks to Mr. Vetri Selvan for help in insect culture maintenance.

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