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Methyl linolenate as a feeding stimulant for the 28-spotted potato ladybird, *Henosepilachna vigintioctopunctata*? A molecular docking approach

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ABSTRACT

The 28-spotted potato ladybird, *Henosepilachna vigintioctopunctata* is a key pest of *Solanum melongena* in Asian countries. In order to develop reliable tools to control this pest, it is important to explore the feeding activity and understand the influence of potential feeding stimulants present in the host plants for the pest development. Here we focused on a modern approach integrating feeding assays, GC-MS analysis and molecular docking, in order to shed light on potential feeding stimulants from young, mature and senescent eggplant leaves, routing the development of *H. vigintioctopunctata* larvae. As a general trend, food utilization indices showed best results when beetles fed on mature leaves, if compared to pests fed on young or senescent leaves. High feeding index and larval survivability were noted when beetles fed on mature leaves, followed by marked total lipid level deterioration. GC-MS analysis identified the main presence of methyl linolenate in *S. melongena* leaf extract. Therefore, we evaluated this compound using a novel approach based on a combination of bioinformatics and feeding assays, to identify potential compounds affecting the metamorphosis of *H. vigintioctopunctata*. A correlation between the deprival of lipids and larval growth was argued considering the marked interaction between methyl linolenate and ecdysone protein receptor. Overall, this research provides useful knowledge to understand the importance of feeding stimulants routing larval growth and development in *H. vigintioctopunctata.*

1. Introduction

The Coccinellidae family comprises a number of important biological control agents, which feed on aphids, scales, moth larvae and other pest eggs. On the other hand, some ladybirds are pests of economic importance. A good example is represented by the subfamily Epilachninae, which is composed by phytophagous species [59]. *Henosepilachna vigintioctopunctata* (Fabricius) (Coleoptera: Coccinellidae), known in Asia as hadda beetle, is one of the most important pests attacking Solanaceae crops in Asian countries [6,25,52]. India loses about 30% of its crops every year due to pests and diseases [61]. The *H. vigintioctopunctata* destructive potential is high at both the adult and larval stages, since both of them feed on epidermal tissues of leaves, flowers and fruits, causing high yield loss [21]. The affected leaves of the plant become skeletonized, gradually dry and drop down. The larvae focus their feeding activity

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on the leaf lower surface while adult beetles usually feed on the leaf upper surface [32], delaying plant growth, leading to loss of fruit production up to 60% [38].

Plant metabolites play a vital role during the host plant selection process by a given insect pest. Earlier studies focused on the relative importance of odor, taste, vision, age of the plant, thickness of the leaves and phytochemicals stimuli influencing food preference of *H. vigintioctopunctata* [1,18,30]. There are several reports regarding the control measures of this insect pest of *S. melongena* [42,62,66]. Nowadays, novel interdisciplinary approaches are used in prevention and control of this pest, such as formulation of artificial diet Insecta LFS for its control [31], as well as pneumatic control technology [13]. However, to the best of our knowledge, there are no studies shedding light on the eggplant foliage chemistry and the eggplant-borne VOC-mediated interactions with the pest *H. vigintioctopunctata*. The cause of the strong attraction to the specific insect towards eggplant foliage is not well explored.

In order to develop reliable tools to control this pest, it is crucial to investigate the feeding activity and understand the influence of potential feeding stimulants present in the eggplant host for pest development. Here, we focused on a modern approach integrating feeding assays, GC-MS analysis and molecular docking, in order to shed light on potential feeding stimulants from young, mature and senescent eggplant leaves, routing the development of *H. vigintioctopunctata* larvae. GC-MS analysis identified the main presence of methyl linolenate (methyl all-cis-9,12,15-octadecatrienoat; $C_{19}H_{32}O_2$) in *S. melongena* leaf extract. Therefore, we evaluated this compound using a novel approach based on a combination of bioinformatics [40] and feeding assays, to identify candidate molecules which potentially affect the metamorphosis of *H. vigintioctopunctata*. A correlation between the deprival of lipids and larval growth was argued considering the marked interaction between methyl linolenate and ecdysone protein receptors.

2. Materials and methods

2.1. Collection of plant material and insect rearing

S. melongena leaves of different ages were collected from plantations in Karamadai (11°13′55.6″N 76°58′00.3″E), South India. Leaves were classified based on the growth period of leaf emergence through continuous monitoring in the field. Size, color, and texture of the leaves were noted, and leaves were classified as fresh young (≥1 week old), mature (2–4 weeks old), and senescent (5–7 weeks old). *H. vigintioctopunctata* beetles were collected from the same geographical location and reared at the Department of Zoology, Avinashilingam University, Coimbatore, India, following the standard method by Refs. [39,58].

2.2. Food utilization efficiency measures (FUEM) on young, mature, and senescent S. melongena leaves

First to fourth generation instar larvae of similar size were designated, weighed and grown separately into sterilized glass jars, fed with three types of eggplant leaves for 24 h and then weighed again. Estimation of fresh weight gain during the period of study was determined by the differences in weight of instars (i.e. by subtracting initial and final weight during the period of study). Instars that fed on each type of leaf were weighed and dried in a hot air oven, and weighed again to determine the percentage dry conversion value, which was used to estimate dry weight. After 24 h feeding by the insect, eggplant leaves (young, mature, and senescent) were oven dried and weighed to determine dry weight gain of the diet given to the instar. Sample leaves from the each type of leaf were weighed, oven dried, and reweighed to estimate percent dry weight conversion values to allow estimation of the dry weight of the diet supplied to the larvae. The quantity of the food consumed was estimated by determining the difference between the dry weight of diet remaining at the end of each experiment and total dry weight of diet initially provided. For each tested larval instar, 20 individuals were evaluated on each type of eggplant leaf tested as food source. Food utilization indices, all based on dry weight, were GR = growth rate; CR = consumption rate; RGR = relative growth rate; CI = consumption index; AD = approximate digestibility; ECI = efficiency of conversion of ingested food; ECD = efficiency of conversion of digested food; LS = larval survivability, calculated with the formula by Waldbauer [67] with slight modifications [60,64,55,68].

2.3. Determination of moisture content

One gram of each type of eggplant leaf was placed in a hot-air oven at 50 ± 1 °C temperature for 72 h, and materials that showed constant dry weight were removed from the oven and weighed in a monopan balance (±0.01 mg). The percentage water content depended on the difference in the fresh and dry weight of each type of leaf. The moisture content determination was repeated for five times for each type of tested leaves [57].

2.4. Determination of ash free weight

A portion (25 ± 0.5 mg dry weight/replicate) of each type of the oven-dried samples was burned in a 25 ml heat-resistant porcelain crucible with a lid on the top in a muffle furnace (SUNVIC, UK). The temperature of the furnace was raised slowly to 450 ± 10 °C, for 15 min. Formation of carbonates and hydrated salts was attained by weighing the ash with 5% solution of ammonium carbonate [$(NH_4)_2CO_3$], and drying to constant weight. The ash content of the sample was determined by weighing the ash produced during burning of a particular leaf sample, and the percent of ash in the sample was calculated from the equation: weight of ash produced/dry weight of the sample) × 100 [4,7,35]. Then, the ash free weight was calculated from the equation: (Dry weight − Ash content) as mg/g fresh weight of leaf tissue. All the above measurements were carried with five replicates.

2.5. Determination of primary and secondary biochemical constituents

The freshly harvested young, mature, and senescent leaves of *S. melongena* were used for the estimation of primary (i.e. total carbohydrates, total proteins and total lipids) and secondary biochemical (i.e. total phenols and total amino acids) constituents. Each measurement was replicated five times.

2.6. Total carbohydrates

One g of each type of leaf was cut into pieces (0.5 ± 0.2 cm) plunged and boiled in 5 ml of 80% ethanol for 5 min in a hot water bath. The leaf tissue in the solvent was cooled and crushed in a mortar with pestle. Soluble carbohydrates suspended in the supernatant were extracted and residual leaf bits were treated with the former process for complete extraction. The extract of respective leaf tissue was filtered through Whatman No. 41 filter paper. The soluble sum of carbohydrates was estimated with the anthrone method [16]. The pellet of each type of leaf extract was kept separately for further biochemical analysis.

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