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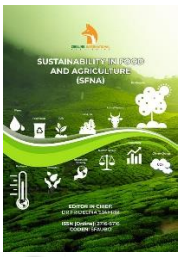

RESEARCH ARTICLE

ANTIFEEDANT ACTIVITY OF LEAF EXTRACTS AGAINST THE ADULTS OF RED PUMPKIN BEETLE *RAPHIDOPALPA FOVEICOLLIS* LUCAS 1849 (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Red pumpkin beetle is the most destructive polyphagous pest of cucurbit vegetables. Antifeedants from plant extracts and their phytochemical constituents can be used as a safer alternative to harmful chemical pesticides to control agricultural pests and have emphasized their potential as ecologically safe pesticides. In the present study, the crude solvent leaf extracts of selected plants were screened and tested at 0.1% concentration after 24 hours for their antifeedant activity against the adults of *Raphidopalpa foveicollis* by leaf disc no choice bioassay method. Amongst them, more than 50% antifeedant activity was noted in the ethyl acetate of *Cassia fistula* (83.43); hexane and diethyl ether of *Leucas aspera* (67.28 and 69.89) and *Streblus asper* (61.34 and 72.43), and dichloromethane extract of *Plumbago zeylanica* (60.15) while rest of the leaf extracts exhibited varying degree of moderate or lesser antifeedant activity. This indicated that the active principles present in the leaf extracts inhibited the feeding behaviour of *Raphidopalpa foveicollis* by making the food unpalatable resulting in feeding deterrence. The results obtained in the present study suggests that further studies should concentrate on the activity of the phytochemical compounds present in each promising extracts of the plant tested.

KEYWORDS

Raphidopalpa foveicollis, antifeedant activity, solvent leaf extracts.

1. INTRODUCTION

The red pumpkin beetle, *Raphidopalpa foveicollis* is widely distributed all over South-East Asia, Africa, Europe, Mediterranean region, Australia, Greece, Sri Lanka, Nepal, Burma, Iraq and in almost all states of India (As, 1983; Butani and Jotwani, 1984, Mckinlay et al., 1992; Atwal and Dhaliwal, 2005). The adult beetle is red in colour, 6-8 mm in length, having glistened yellowish red to yellowish brown elytra. The life cycle of this beetle is 32-65 days. The female lays around 150-300 yellowish pink spherical eggs in the soil which turns orange after a couple of days and the egg period is 5-8 days. The grubs become fully grown in 13-25 days and pupate in the soil, and the prepupal and the pupal period ranges from 2-5 and 7-17 days respectively. Red pumpkin beetle is the most destructive pest of cucurbit vegetables which occurs throughout the year and causes severe damage to the crops especially at seedling stage (Rajak, 2001; Rahaman and Prodhan, 2007). This pest is reported to cause 80.63% damage on musk melon, 71.69% on long melon, 13.88% on ash gourd and 7.63% on snake gourd especially in Indo-Pakistan subcontinent (Latif and Khan, 1952; Butani and Jotwani, 1984). Originally red pumpkin beetle is a pest of pumpkin, bottle gourd, and musk melon, but later on it was reported to be polyphagous as it started to feed on all cucurbitaceous vegetables causing damage to more than 81 species (Nath and Thankur, 1965; Doharey, 1983; Bhutani and Jotwani, 1984; Rahman and Annadurai, 1985; Raman, 1985; Saljoqi and Khan, 2007). Beetles attack right after the germination and slow down the growth of plants due to serious attack. Vines are damaged by feeding on cotyledon or leaves from seedling stage to maturity. The grubs feed on root tissue and cause direct damage to the newly developed seedlings besides stem and fruits (Narayanan and Batra, 1960). Adults prefer young seedlings, flowers and young tender leaves, and cause irregular holes on leaves leading to severe attack on the young seedlings of cucurbitaceous crops resulting in the death of plants (Bogawat and Pandey, 1967; Allam, 1969; Bhutani and Jotwani, 1984; Khan and Hazela, 1987; Waterhouse and Norris, 1987; Johri and Johri, 2003).

Antifeedants offer first line of crop protection against notorious insects. A substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent and it is reported that antifeedants have profound adverse effects on insect feeding behavior (Isman, 1994, 2002; Hummelbruner and Isman, 2001). Discovery of antifeedants from plant extracts has emphasized a potential method for the development of "ecologically safe pesticides" (Weires and Riedl, 1991). Insect antifeedants are chemical substances that are naturally present in a plant. Botanical antifeedants inhibit feeding or disrupt insect feeding and eventually insects starve to death owing to phytochemical constituents which possesses obvious antifeedant property against insects (Talukder, 2006; Rajashekar et al., 2012).

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Phytochemicals with insect antifeedant potential can be used as a safer alternative to harmful chemical pesticides to control agricultural pests. Antifeedants attracted considerable attention ever since the striking antifeedant effects of azadirachtin which was first documented against the desert locust (*Schistocerca gregraria*) (Butterworth and Morgan, 1968). Crude extracts from leaf, stem, root and seed of various plant species consisting of complex mixtures of active principles have been reported to possess antifeedant properties (Leatemia and Isman, 2004). Hummelbruner and Isman reported that synergistic effects of complex mixtures (crude extracts) of phytochemicals are important in plant defenses against insect herbivores (Hummelbruner and Isman, 2001). Keeping in view the above-mentioned aspects, the present study was attempted to screen and test the antifeedant activity of leaf extracts against the adults of red pumpkin beetle.

2. Materials and Methods

2.1 Plant collection and preparation of phytoextracts

Plants belonging to diverse families and genera were collected from Siruvani Hills (10°56′17″N 76°41′14″E), 37Km from Coimbatore, Tamil Nadu, India and utilized for the present study based on available literature, abundant availability, medicinal and insecticidal properties (Table 1). Taxonomical identity of the plants was confirmed at the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India. The leaves of the collected plants from the field were then brought to the laboratory, washed with dechlorinated water, shade dried under room temperature and were powdered individually using an electric blender. Each powdered plant leaf material was sieved using a kitchen strainer. One kilogram of each powdered leaf material was sequentially extracted with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol for a period of seventy-two hours and then filtered. The filtered content was then subjected to rotary vacuum evaporator until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained were stored in sterilized amber coloured bottles maintained at 4^0C in a refrigerator. Standard one per cent stock solution for each leaf extract was prepared by dissolving 100mg of each crude solvent extract in 100mL of acetone.

Table 1. List of plants utilized for the present study

Plant species	Family	Common Name	Vernacular name (Tamil)
Alstonia scholaris (L.) R.Br.	Apocynaceae	Blackboard tree	Palai
Cassia fistula L.	Fabaceae	Golden shower	Sarakkonnai
Eclipta prostrata (L.)	Asteraceae	False daisy	Karisalankanni
Evolvulus alsinoides (L.)	Convolvulaceae	Dwarf morning glory	Vishnukranthi
Leucas aspera (Willd.) Link	Lamiaceae	Common leucas	Thumbai
Plumbago zeylanica L.	Plumbaginaceae	Chitrak	Chittiramoolam karimai
Rhynchosia minima (L.) DC.	Fabaceae	Burn mouth vine	Kaliyantuvarai
Sapindus emarginatus Vahl	Sapindaceae	Soapnut tree	Poovan kotti
Sida rhombifolia L.	Malvaceae	Cuban jute	Kurundotti
Streblus asper Lour.	Moraceae	Sand paper tree	Kurripila
Strychnos nux-vomica L.	Loganiaceae	Strychnine tree	Yettimaram

2.2 Rearing of *Raphidopalpa foveicollis*

The adults of *Raphidopalpa foveicollis* collected from cucurbits plants near agricultural fields at Padappai, Kancheepuram, district, Tamil Nadu, India were brought to the laboratory at the Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India and taxonomical identity was confirmed prior to rearing and mass culturing of the beetles. The collected beetles were released into the glass jars containing fresh pumpkin leaves which were provided as food and maintained at room temperature of 27 ±2°C and relative humidity of 70-80%. The mouth of the glass jars was closed with muslin cloth held by rubber bands. Fresh pumpkin leaves were replenished on a daily basis. Cyclic generation of beetles on emergence were transferred to new rearing glass jars provided with fresh tender pumpkin leaves which were replaced on a daily basis. This process of culture method was repeated, and the culture was maintained throughout the study period.

2.3 Antifeedant bioassay

The experiment was conducted using leaf disc no choice bioassay method. For the bioassay, the F_1 generation of *Raphidopalpa foveicollis* adults from the culture was used. Fresh pumpkin leaf disc (1350sq.mm) were dipped in 0.1% concentration of each solvent leaf extract. After solvent evaporation at room temperature, the treated leaf disc was kept inside an individual transparent cage (45 x 45 x 45cm) made of acrylic sheet. Leaf discs sprayed with acetone and water served as negative and positive control respectively. A single unsexed pre starved (24 hours) adult was then introduced into the cage, and thereafter the cage was closed with muslin cloth. The adult was allowed to feed on the treated leaf discs for a period of twenty-four hours. A total of three trials with five replicates per trial were carried. At the end of the experiment, unconsumed area of leaf disc was measured with the aid of a leaf area meter and per cent antifeedant activity was calculated based on the formula (Singh and Pant, 1980).

$$\text{Antifeedant activity (\%)} = \frac{\text{Leaf disc area consumed in control - Leaf disc area consumed in treated}}{\text{Leaf disc area consumed in control + Leaf disc area consumed in treated}} \times 100$$

2.4 Statistical analysis

Data was subjected to ANOVA and Tukey HSD post-hoc tests to determine differences in response between the treated bioassays and controls, and the response between extracts of each plant. The differences were considered as significant at P=0.05 level. All statistics were conducted in IBM SPSS Statistics v22 with significance set at 95% confidence (SPSS, 2010).

3. Results

All leaf extracts screened and tested exhibited varying levels of antifeedant activity. Amongst them, the extracts which exhibited antifeedant activity above 80% was the ethyl acetate extract of *Cassia fistula* (83.43); between 60 and 80%, it was hexane and diethyl ether of *Leucas aspera* (67.28 and 69.89) and *Streblus asper* (61.34 and 72.43), and dichloromethane of *Plumbago zeylanica* (60.15); between 40 and 60%, it was hexane, diethyl ether and methanol of *Cassia fistula* (51.20, 52.35 and 43.47), hexane and diethyl ether of *Eclipta prostrata* (55.11 and 43.54), ethyl acetate of *Leucas aspera* (47.60), hexane of *Plumbago zeylanica* (42.35), diethyl ether and ethyl acetate of *Rhynchosia minima* (45.73 and 50.00), *Sapindus emarginatus* (55.25 and 41.73), *Sida rhombifolia* (46.66 and 58.99), methanol of *Sapindus emarginatus* (48.53), ethyl acetate of *Streblus asper* (41.77) and diethyl ether of *Strychnos nux-vomica* (41.48). All the remaining solvent extracts exhibited antifeedant activity below 40%. Data was found to be significant between the control and treated bioassays and also between extracts of each plant species and the values were significant at P=0.05 level by one way ANOVA followed by Tukey test performed. The green coloured bars represent more than 50% antifeedant activity while the black ones below 50% among the leaf extracts of each plant species tested (Figure1; Table 2).

Table 2. Statistical analysis for antifeedant activity of leaf extracts against *Raphidopalpa foveicollis*

Source of variation	SS	df	MS	F	*P*-value	F crit
Plants (including controls)	14645.53	12	1220.461	7.39707	1.87E-07**	1.960121
Solvent extracts	1746.639	4	436.6598	2.646544	0.044666*	2.565241

**Highly significant @ P value = 0.001; *Significant @ P value = 0.05

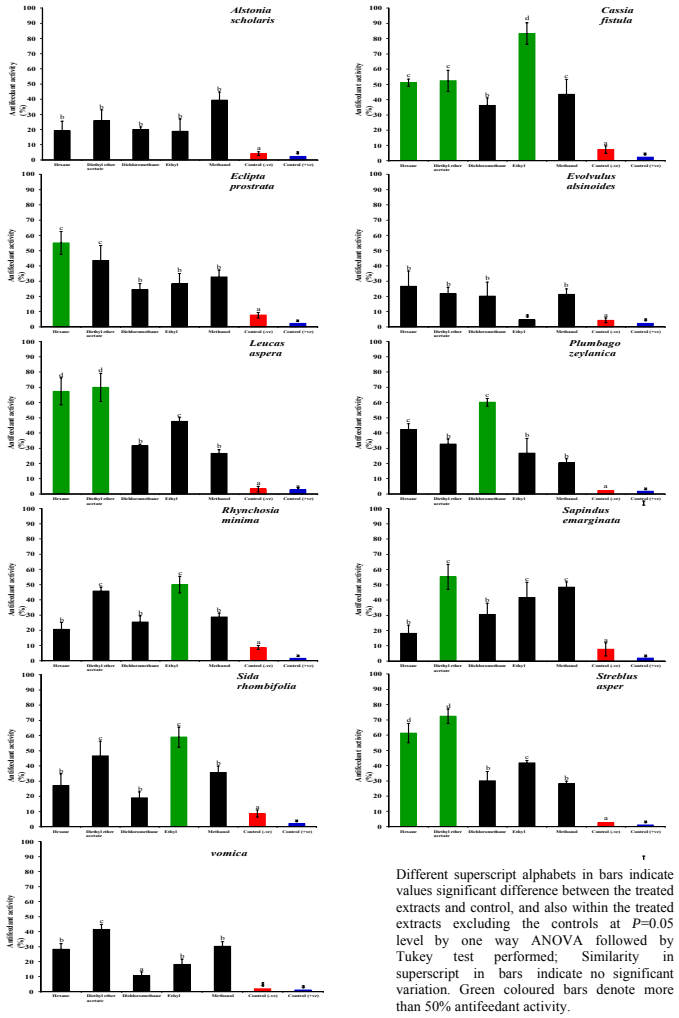

Figure 1. Antifeedant activity of leaf extracts against *Raphidopalpa foveicollis*

4. Discussion

Red pumpkin beetle has been a limiting factor in cucurbit production as it injures almost all cucurbits and the extent of damage by this pest is severe. Bioassays against insects have been used for decades as a means of elucidating the activity of many chemical components or extracts. The major goals achieved by employing bioassay techniques are to determine the roles of naturally occurring chemicals, identify the mechanism of resistance in crop plants and to find various insect control agents. The basic design to study antifeedants is to present to an insect a substrate with the candidate chemical and to measure the response of the insect. Therefore, substrate choice and presentation are important factors for a successful bioassay (Schoonhoven, 1982). Natural substrates could be whole plant, leaves, leaf discs, or specialized substrates such as twigs. Koul has reported on the individual types of biological assays used for evaluating antifeedant efficiency. In general, bioassays can be divided into two groups according to the mode of the experiment: a choice or a no-choice bioassay (Koul, 2005). The principle is that insects can choose either control or treated discs (choice) or insects may be exposed to the test substance only (no choice). The no-choice situation often is more representative but at the same time is very sensitive and this was used in the present study. Leaf discs are commonly used in preference or consumption bioassays with chewing insects. These assays are important in estimating the biological potential of the antifeedant effect of plant extracts in screening studies, and they correspond as much as possible to the conditions of the practical application. However, it must be emphasized that these assays are short term. For the purpose of practical use, further bioassays must be performed in crude extracts or phytochemical compounds which exhibits the best biological activity. These bioassays are irreplaceable and therefore was used in the present study to screen leaf extracts for antifeedant activity against the red pumpkin beetle.

Antifeedant activity of plant extracts have been reported against insects (Arivoli and Samuel, 2012, 2013). In the present investigation, the rate of feeding by the adult beetles was highly reduced by the leaf extracts of *Cassia fistula*, *Leucas aspera*, *Streblus asper* and *Plumbago zeylanica* as they exhibited more than 60% antifeedant activity. Botanicals for the management of this beetle as feeding inhibitor are reported (Rathod et al., 2009). Leaf extract of *Ageratum conyzoides, Momordica charantia*, whole plant extracts of *Mentha longifolia*, *Mentha piperita*, *Mentha spicata*, *Ocimum basilicum*, *Ocimum canum*, *Pogostemon heyneaus*, *Salvia officinalis* and seed extracts of *Pachyrhizus erosus* and *Strychnos nuxvomica* were considered effective in the management of red pumpkin beetle as feeding deterrents (Chandravadana and Pal, 1983; Pande *et al*., 1987; Chandel et al., 2009; Vishwakarma et al., 2011). In the present study, the antifeedant activity varied significantly based on the solvents used for extraction. Maximum antifeedant activity was recorded in hexane and diethyl ether whereas minimum in methanol solvent extracts. The ethyl acetate extract of *Cassia fistula* exhibited the highest antifeedant activity by reducing the feeding rate of *Raphidopalpa foveicollis* in the present study. The difference in the antifeedant indices is caused by the different chemical compounds contained in each extract, which affects the eating behavior of insects. This indicated that the active principles present in the leaf extracts inhibit feeding behaviour by making the food unpalatable resulting in feeding deterrence. The present study results corroborated with a previous report which also stated that it was the ethyl acetate extract of *Cassia fistula* and a quinone compound by name rhein which showed antifeedant activity against *Helicoverpa armigera* (76.13%) and *Spodoptera litura* (56.79%) (Duraipandiyan et al., 2011).

Antifeedant property of phytoextracts and their compounds against adult coleopteran pests have been reported. Crude extracts of plants showed activity against cucumber beetles, particularly the spotted beetle (Reed et al., 1981). Frazier signified that antifeedants can be found amongst all the major classes of secondary metabolites as they are toxic substances against insects (Frazier, 1986). Various secondary metabolites including alkaloids, chromenes, coumarins, cucurbitacins, phenolics, phenols, polyacetylenes, quassinoids, saponins, tannins, terpenes and terpenoids were reported as potent insect antifeedants (Schoonhoven, 1982; Adeyemi, 2010; Lingathurai et al., 2011; Kumar et al., 2013; Adeyemi and Mohammed, 2014; Jose and Sujatha, 2017). Effective natural antifeedants are triterpenes, sesquiterpene lactones and alkaloids, cucurbitacines, quinines and phenols and the potent antifeedants belong to the terpenoid group, which has the greatest number and diversity of known antifeedants compounds (Nawrot et al., 1986; Norris, 1986; Van beek and Groot, 1986; Adeyemi and Mohammed, 2014). Azadirachtin and salannin, from the ethanolic extract of neem seeds showed high antifeedant activity against striped beetle (Redfern et al., 1978; Uebel et al., 1978). Quinones and compounds with aldehyde groups have been reported to be insect antifeedants (Morimoto et al., 1999). Anthraquinones and naphthoquinones possessed antifeedant activity against the striped beetle (Reed et al., 1981). Anthraquinones significantly inhibited feeding against carpet beetles (Morimoto et al., 2002). Azadirachtin, a complex tetranortriterpenoid limonoid from the neem seeds, is the main component responsible for antifeedant (Mordue et al., 2000). A group researchers pointed that azadirachtin which is prominent constituent of *Azadirachta indica* is established as a pivotal antifeedant ingredient (Chaudhary et al., 2017; Ghoniem and Hamadah, 2017). Methanolic leaf extract of *Momordica charantia* was found to have strong feeding deterrent property against the adults of red pumpkin beetle and it was found that the phytochemical triterpenoid was responsible for the antifeedant property as it inhibited the feeding of the beetle (Chandravadana and Pal, 1983; York, 1992; Abe and Matsuda, 2000). Among triterpenoids the deterrents identified were momordicine I and II which caused significant reduction of feeding by red pumpkin beetles (Chandravadana, 1987; Abe and Matsuda, 2000).

Antifeedants deter phytophagous insects against food consumption. Food selection among insect herbivores is a highly specialized phenomenon.

While olfactory and physical aspects of plants or their organs can be important in insect host finding and acceptance, the choice of food is based primarily upon contact chemoreception of various allelochemicals (Miller and Strickler, 1984; Frazier, 1986; Stadler, 1992). Antifeedants work by producing feeding inhibition stimulant such that it disturbs the stimulation perception for eating. Yet, the mechanism of antifeedant action is still unfamiliar, and it can be only supposed that antifeedants depress or terminate insect feeding through mechanisms that involve chemosensory-based food rejection. The acceptance by a phytophagous insect of a plant species as a suitable host depends on the capability of the insect's chemosensory system to detect plant tissues with favourable levels of feeding stimulants and feeding deterrents (Munakata, 1975; Schoonhoven, 1982; Bentley et al., 1990; Bernays and Chapman, 2000). Antifeedant effects are highly correlated with the sensory response of chemoreceptors on the insect mouthparts (Mordue et al., 1998). Feeding behavior depend upon both neural input from the insects chemical senses in the taste receptors on mouthparts and oral cavity, and central nervous integration. In particular, dietary experience has influenced the ability of insects to taste plant chemicals that may have served as signals of suitability or unsuitability. Certain dietary constituents appeared to suppress the development of taste sensitivity to deterrents in an insect (Renwick, 2001). Avoidance of allelochemicals, when looked at from a behavioral point of view, is the outcome of interactions with chemoreceptors characterized by broad sensitivity to a spectrum of deterrents (Mullin et al., 1994). Although research of chemoreceptors is important for a general understanding of efficiency of individual antifeedant substances, from the practical point of view, experiments based on simple biological tests are those mostly used in antifeedancy studies. Nevertheless, it should be emphasized that most of the antifeedant research is in the preliminary trial stage, although the activity of many phytocompounds and plant extracts are known (Koul, 2005). The results obtained in the present study suggests that further studies should concentrate on the activity of the phytochemical compounds present in each promising extracts of the plant tested. Consequently, it is expected that in the near future efficacious plant-based compounds will be formulated as antifeedants, from the huge biodiversity provided by nature.

5. Conclusion

Screening plant extracts for antifeedant effects on insects is one of the approaches used in the search for current botanical insecticides as secondary plant compounds deter insects from feeding. These phytochemical antifeedants play a major role in the unsuitability of non-host plants as food for insects. Isolation and structure elucidation of these chemicals is important not only for understanding the ecological aspects of insect pests relationship, but also for their potential in control of them.

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