Anti-feedant activity of some biopesticides on *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae)

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ABSTRACT

The antifeedant activity of *Azadirachta indica* (L.) A. Juss (neem) leaf extract, seed kernel extract, and seed oil; *Pongamia glabra* Vent seed oil; *Madhuca latifolia* (Roxb.) Macbeth oil and two fungal origin biopesticides, *i.e*., conidia of *Metarhizium anisopliae* (Metchnioff) Sorokin and the enzyme preparation of the fungus, *Myrothecium verrucaria* (Albertini & Schwein) were evaluated against the adult *H. vigintioctopunctata* under laboratory conditions during 2005-06. Among the botanicals evaluated, *P. glabra* oil showed the maximum anti-feedant activity. No feeding was observed up to 48 hours after treatment. Mortality was noticed 72 hours after treatment and cent per cent mortality was recorded 7 days after treatment at all the concentrations. Neem oil showed 60 per cent mortality at 5 per cent concentration. The leaf extract and seed kernel extract of *A. indica* had less anti-feedant activity as compared to the oil formulations of *A. indica* and *M. latifolia* (based on the per cent leaf area consumed). A decrease in feeding was evidenced after treatment with *M. verrucaria*, *M. verrucaria* and *M.anisopliae*.

Keywords: *Henosepilachna vigintioctopunctata*, crop pest, antifeedant activity, botanicals, entomopathogens

INTRODUCTION

Henosepilachna vigintioctopunctata (F.) is one among the most injurious pests of solanaceous crops in India, especially *Solanum melongena* and *S. tuberosum*. Mathur and Srivastava (1964) recorded the beetle to feed on *Datura stramonium* L., *D. metel* L., *D. innoxia* Mill., *Solanum nigrum* L. and *Withania somnifera* (L.) Dunal. Sachan and Rathore (1979) observed the beetle to feed on six wild solanaceous plant species however, young larvae were unable to survive on *Solanum pubescens*. Biopesticides, consisting beneficial microorganisms, nematodes or other safe biologically based active ingredients can effectively control insect pests, plant diseases and weeds, besides maintaining human and environmental safety and limit the use of chemical pesticide. Of the various plant extracts evaluated for their antifeedant activity against the phytophagous coccinellid, *H. vigintioctopunctata*, extracts from the neem tree have been widely studied. The limonoid, azadirachtin, has been reported to have adverse effects on the endocrine system of the bean beetle, *Epilachna varivestis*, and to cause sterility in the female insects (Schmutterer *et al*., 1981). Schluter and Schulz (1983) reported that this compound causes degradation in larval epidermis preventing the larvae from molting. Insect larvae treated with meliantriol with concentration as low as 0.2 µg/larva were observed to cease feeding. Similarly, many workers have evaluated the efficacy of microbial pesticides against the beetle. In the present work, impact of *A. indica* oil, neem seed kernal extracts, neem leaf extracts, *Madhuca latifolia* seed oil and *Pongamia glabra* seed oil and three fungal pathogens on antifeedant activity of *H.vigintioc topunctata* was recorded under laboratory conditions.

MATERIAL S AND METHODS

Antifeeding activity of botanicals

Antifeedant activity of fresh *Azadirachta indica* A. Juss leaf extract, seed kernel extract, seed oil; *Pongamia glabra* Vent seed oil and *Madhuca latifolia* (Roxb.) Macbeth seed oil evaluated under laboratory conditions by slight modification of the disc dipping method suggested by Butterworth and Morgan (1971) using fresh leaf discs of *Withania somnifera* (L.) Dunal. Four different concentrations of the botanicals were made in distilled water, *A. indica* leaf extract (10, 5, 2.5 and 1.25 per cent) and *A. indica* seed kernel extract; concentrations for *P. glabra*, *A. indica* (5, 2.5, 1.25 and 0.625 per cent) and *M. latifolia* oils with an addition of Tween 80 for proper miscibility.

To prepare *A. indica* seed kernel extract, the mature kernels of neem were collected and shade dried. The kernels were crushed with the help of domestic mixer. The powder was

passed through 60 mesh sieve and mixed with distilled water @ 50 g powder in 150 ml of distilled water. This extract was considered to be of 100 per cent concentration from which the desired concentrations were made.

In case of *A. indica* leaf extract, one kilogram of fresh, tender leaves were plucked and put in a glass container and one litre of lukewarm water was added. The leaves were allowed to stand soaked in water for 12 hours and then macerated in a mixer-grinder. The extract was filtered through a muslin cloth and collected in a glass jar. This extract was considered as 100 per cent solution from which the desired concentrations were made. Fresh *W. somnifera* leaves were cut into uniform discs with an area of 962.5-mm^2 and were impregnated with different concentrations of the botanicals to be evaluated. The leaf discs were drip-dried at room temperature and provided as food to the adults of Epilachna beetle which were reared in petridishes (10 cm diameter). Ten healthy beetles were taken in each replicate. For the control, the leaf disc was dipped in distilled water and drip-dried before being given to the beetles.

A parallel experiment was conducted to evaluate the antifeedant activity of the botanicals under field conditions was laid out, for which, *W. somnifera* dunal plants were grown in pots and sprayed with different concentrations of the botanicals. To observe the antifeeding activity, one hour after treatment, treated leaf discs were drip-dried and provided to the adult beetles; whereas, to observe the residual effect of antifeedant activity, the leaf discs were provided to the adult beetles 24 and 48 hours after treatment from the treated pot plants. Thereafter, only untreated leaf discs were provided as food to the adult beetles and observations were recorded until 7 days after treatment.

Antifeeding activity of entomopathogens

The two bio-pesticides, conidia of *Metarhizium anisopliae* (Metchnioff) Sorokin and the enzyme preparation of the fungus, *Myrothecium verrucaria* (Albertini & Schwein) were evaluated for their anti-feedant activity against the adult *H. vigintioctopunctata*. These formulations were obtained from NCL (National Chemical Laboratory), Pune and, as per Protocol, the treatments contained – *M. anisopliae* aqueous formulation @ 5 x 10^{12} conidia and *Myrothecium verrucaria* (Albertini & Schwein) enzyme @ 2 U/ml. In order to get the desired concentrations, 0.3 g powder of either formulation was dissolved in 1.25 litres of water with 1.25 ml of Tween 80. Evaluation was done using topical application and leaf dip method.

Observations were made on 3, 5 and 7 days after treatments. In case of leaf dip method, the treated leaf discs were provided only once for 24 hours. Thereafter, untreated fresh leaf discs were given every day. In case of topical application, fresh untreated leaf discs were provided one hour after treatment. The leaf area consumed was calculated by using a graph paper expressing the anti-feedant activity in per cent area fed by the test insect. The percentage feeding was computed using the graphical method, wherein the leaf discs were plotted on a graph and the area fed by the test insect marked. The number of squares in the area consumed was counted and expressed as a percentage: Area fed (mm^2) / Total area (mm^2) X 100

RESULTS

Antifeeding activity of botanicals

The seed oil from *P. glabra* had the maximum antifeedant activity and no feeding was observed after 24 and 48 hours of treatment (Table 1). *Madhuca latifolia* oil had relatively greater anti-feedant activity than *A. indica* oil for all the concentrations tested. The antifeedant activity

Table 1. Antifeedant activity of *W. somnifera* leaves on *H. vigintioctopunctata* adults

Botanicals	Leaf area feeding (%)		Mortality in (%) 7th day
	After 24 hrs.	After 48 hrs.	
A. indica oil			
5.0	13.09	6.86	60
2.5	5.60	5.20	40
1.25	17.25	5.50	20
0.625	14.54	8.73	No mortality
A. indica kernel			
10.0	13.19	19.64	40
5.0	12.99	14.13	40
2.5	10.50	19.10	No mortality
1.25	14.12	17.04	No mortality
A. indica leaf			
10.0	12.09	6.13	20
5.0	21.19	31.17	No mortality
2.5	23.53	17.97	No mortality
1.25	11.94	4.26	No mortality
M. latifolia oil			
5.0	0.31	7.06	20
2.5	2.59	7.38	20
1.25	13.51	11.95	No mortality
0.625	9.56	12.26	No mortality
P. glabra oil			
5.0	No feeding	No feeding	100
2.5	No feeding	No feeding	100
1.25	No feeding	No feeding	100
0.625	No feeding	No feeding	100

Note: In untreated control the leaf area fed ranged from 42 to 95 per cent.

Table 2. Feeding response of adult *H. vigintiocto-punctata* on botanical treated *W. somnifera*

Treatments Concentrations (%)	Leaf area feeding (%)				
	10	5	2.5	1.25	0.625
A. indica oil	-	1.09	0.307	0.10	17.14
A. indica seed kernel extract	12.36	13.60	13.60	6.075	-
A. indica leaf extract	12.36	15.70	14.025	10.07	-
M. latifolia oil	-	7.01	12.10	9.60	12.10

of *A. indica* seed kernel extract was better than that of *A. indica* leaf extract. *P. glabra* oil treatment showed 100 per cent mortality at all the concentrations 7 days after treatment; whereas, *A. indica* oil showed 60, 40 and 20 per cent mortality at 5, 2.5 and 1.25 per cent concentrations, respectively; *A. indica* seed kernel extract showed 40 per cent mortality at 10 and 5 per cent; *M. latifolia* oil showed 20 per cent mortality at 5 and 2.5 per cent and *A. indica* leaf extract registered 20 per cent mortality at 10 per cent concentration.

The leaf and seed kernel extracts of *A. indica* had less anti-feedant activity as compared to the seed oil treatments of *A. indica* and *M. latifolia* based on the per cent leaf area consumed (Table 2). The lowest concentration of oil formulation (0.625 %) had similar effect of anti-feeding as the higher concentrations (2.5, 5.0 and 10.0 %) of leaf and seed kernel extracts of *A. indica*. Besides, *A. indica* oil had relatively stronger anti-feedant activity than *M. latifolia* oil at 5, 2.5 and 1.25 per cent concentrations.

Antifeeding acitivity of fungal pathogens

Evaluation of the other biopesticides for feeding deterrence showed that there was a subsequent decrease in the percentage feeding after treatment with both the fungal preparations (Table 3). The respective feeding was 15.84, 16.88 and 15.22 per cent 3 days after treatment for *M. verrucaria* when provided as food after leaf dip, *M. verrucaria* and *M. anisopliae* when applied topically. Five days after treatment, the respective figures were 11.01, 14.39 and 14.96 per cent; whereas, 7 days after treatment the anti-feedant activity happened to enhance further and the respective feeding was 3.63, 4.57 and 1.81 per cent only.

Table 3. Effect of entomopathogenic fungi on the feeding behaviour of *H. vigintioctopunctata* adults

Fungal Preparations	Leaf area consumed (%)			Per cent mortality (7^{th} day)
	3 DAT	5 DAT	7 DAT	
Myrothecium verrucaria enzyme (leaf dip)	15.84	11.01	3.63	No mortality
Myrothecium verrucaria enzyme (topical)	16.88	14.39	4.57	No mortality
Metarhizium anisopliae (topical)	15.22	14.96	1.81	No mortality

DISCUSSION

During the present investigation it was notable that among seed oils *P. glabra* had the maximum anti-feedant activity and no feeding was observed 24 and 48 hours after treatment followed by *M. latifolia* and *A. indica* oil for all the concentrations tested 24 hours after treatment. The anti-feedant activity of *A. indica* seed kernel extract was better than that of *A. indica* leaf extract 24 hours after treatment; however, 48 hours later the effect was *vice versa* and the leaf extract was more effective as an anti-feedant. The botanical treatments also showed mortality as observed 72 hours after treatment until 7 days. The leaf and seed kernel extracts of *A. indica* had relatively less anti-feedant activity than seed oil treatments of *A. indica* and *M. latifolia* based on the per cent leaf area consumed; besides, the lowest concentrations of oils had similar effect of anti-feeding as the higher concentrations of leaf and kernel extracts of *A. indica*. This indicates that the seed kernel and leaf extracts have to be used at higher concentrations than the oils.

Earlier, Reddy *et al*. (1990) reported that petroleum ether (1 %) extracts of *Azadirachta indica* A. Juss and *Annona squamosa* L., reduced the number of *H. vigintiocto punctata* larvae infesting brinjal by 88.0 and 92.99; 85.98 and 91.02 per cent 24 hours and 3 days after spraying, respectively. Similarly, Rao *et al*. (1990) recorded that extracts of *Annona squamosa* L., *Argemone mexicana* L., *Calotropis gigantea* Ait., *Datura stramonium* L., *Ecalyptus globulus* Labill, *Pongamia glabra* Vent and *Ricinus communis* L. (0.5%) gave cent per cent protection against second instar larvae of *H. vigintioctopunctata* indicating high anti-feedant effect.

In the present investigation, the other bio-pesticides showed an increasingly lower percentage of feeding, which is indicative of the fact that the fungal preparations take relatively more time to bring about the desired effect; however, no mortality was recorded. Beevi and Jacob (1982) reported that 3^{rd} and 4^{th} instar larvae of *H. vigintiocto punctata* were most susceptible to the fungus, *Fusarium moniliforme* Shel., variety *subglutinans* (100 % mortality after 5 days), followed by 1^{st} and 2^{nd} instar larvae (96.7 and

90.7 per cent mortality, respectively). Pupae were less susceptible than larvae (80 per cent mortality) and adults were least susceptible (76.7 % mortality). Under field conditions, the fungus caused 96.7 per cent mortality of the pest after 7 days when applied to bitter gourd at the concentration of 7.5 x 10^5 conidia/ ml; whereas, at lower concentrations, it caused between 50 and 83.3 per cent mortality. According to Rajendran and Gopalan (1999), *Bacillus thuringiensis* Berliner used as a leaf dip caused a maximum mortality of 68.9 per cent to the first instar larvae and a minimum of 13 per cent to the pre-pupal stage of *H. vigintioctopunctata*. The adults were less susceptible to *B. thuringiensis*. The respective percent egg hatchability was 12.2, 11.7, 17.9, 12.9 and 18.7 when freshly laid, 1, 2, 3 and 4 days old eggs were treated with 0.25 per cent B. *thuringiensis*. Direct spraying of *Beauveria bassiana* B. killed 58.1 per cent first instar larvae and 35.2 per cent pre-pupal stage larvae. The adults were not susceptible to *B. bassiana*, though the maximum mortality being 10.3 per cent in the case of newly emerged adults. The fungus caused 54.6 per cent hatchability of one-day-old eggs of the spotted beetle. Markandeya *et al*. (2001) studying the anti-feedant activity and mortality of *H. vigintiocto punctata* on aubergine leaves treated with biotox (*B. thuringiensis*) observed that the average leaf area consumed after 48 hours by each grub was only 5.05 cm^2, while each adult fed on only 1.5 cm^2, thus the leaf area protection due to *B. thuringiensis* was 56.39 per cent from grubs and 80.62 per cent from adult beetles. They also recorded 39.03 per cent mortality of grubs and 5.22 per cent mortality of adults.

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