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Pesticidal activity of Indigenous plant extracts against brinjal hadda beetle, *Henosepilachna vigintioctopunctata* (Fab.) (Coccinellidae; Coleoptera)

Thulasi S, S Arivudainambi
Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India

Abstract
An experiment conducted to assess the pesticidal properties of twenty plants recommended by the aboriginal people of Western Ghats of Tamil Nadu were studied by conducting laboratory bioassay against third instar grubs of *Henosepilachna vigintioctopunctata* (Fab.) (Coccinellidae; Coleoptera). Among the various treatments, increased antifeedant index was noted in *Wrightia tinctoria, Andrographis paniculata, Cymbopogon citrates, Glorisa superba,* and *Justicia gendarussa* at five percent concentration. The antifeedancy and repellency indexes were recorded at maximum range of 83.33 to 100 percent respectively. The insect growth regulatory activity was more pronounced in *W. tinctoria* and *A. paniculata* at two percent concentration where 83.33 and 91.04 per cent was noted respectively.

Keywords: *Henosepilachna vigintioctopunctata,* antifeedant, repellent, insect growth regulatory activity and insecticidal action

Introduction
The western ghats mountain range running down the south- west side of India is a treasure trove of wildlife. In Tamil Nadu, it extends from the Niligris in the north to marunthuvazhmalai in Kannyakumari district in the south. Its altitude ranges from 2,000 to 3,000 metres. It covers an area of 2,500 sq. Km. Kodaikanal is also called as the princess of the hill stations with an elevation of 2100 m and to be an authentic store house of plant diversity. Nearly 1300 species of plants are recorded in this area. The western ghats in Tamilnadu are residence to paliyar and irulas tribes. The people living in the biodiversity affluent areas are knowledgeable about the flora and their medicinal values (Azaizeh, 2003) [2]. The present study aims to explore the potent pesticidal plants of western ghats in tamilnadu by conducting intensive survey among the tribes, local healers and resource persons. Then shortlisted plants were bio-assayed against Brinjal hadda beetle to validate their pesticidal properties.

Materials and Methods
A survey was conducted among the aboriginal people, local curators and farmers of study area about the poisonous and insecticidal plants from October 2019 to March 2020. In total 30 plants were suggested by the folklore. By literature survey and in discussion with the scientists of Botanical survey of India, Coimbatore, botanists of Annamalai University and local healers 20 plants were shortlisted for studying their anti insect properties. The plants intended to experiment were collected with the help of local tribes and processed then brought to the phyto- insecticide research laboratory, Department of Entomology, Annamalai University.

Extraction of Selected Plants
The plants parts intended to study were collected and shade dried for two weeks. Then the dried plants parts were ground using electric blender each separately. Powders of the plants were made into 10 g thimbles individually using Whatmann no.40 filter paper placed in 500 ml conical flasks and extracted with double distilled water at room temperature. After 72h, the thimbles were removed carefully, and the extracts were collected separately and consider as 10% stock solution. From the stock, 5 per cent concentrations were made by diluting with double distilled water and used in the preliminary screening.

Rearing of *Henosepilachna vigintioctopuncta* Fabricius (Coccinellidae: Coleoptera)
Twenty days old seedling of brinjal (AU 1 variety) raised in the nursery were planted in plastic container of 10 cm diameter containing the mixture of red soil, sand and compost at the ratio of 1:2:1, @ two seedling per pot and watered. After three weeks, the plants raised in pots were kept in a nylon mesh netted insect cages (60x60x120cm) @ 10-12 pots in a cage and infested with field collected adults of *H. vigintioctopuncta*. There were 50-60 adults released in to the cage. Then from third day onwards eggs were collected from the cage and incubated under laboratory condition of 27±2^{0}C temperature and 70±5% relative humidity with a 12:12 (L:D) photoperiod. The emerged grubs were reared by supplying fresh leaves at two times a day. Thus, a continuous laboratory culture was maintained and used in experiments. There were four instars with the total larval duration of 9-18 days. Pupae took 3-7 days to emerge. Adult longevity lasts for 2-4 days.

Antifeedant and repellent assay (No- choice poison food bioassay)

Antifeedant and repellent activities of the extracts were studied by following leaf disc bioassay no choice method against grubs and adults. A leaf disc of 4.5 cm diameter was punched using cork borer and was applied with 200 µl of respective concentration of each extract separately. After air drying, leaf discs were placed in petri dishes and allowed 3 h pre starved third instar grubs and 1 day old adults @ 6/dish were released separately. Progressive consumption of leaf area by the grubs and adults after 24 h of feeding was recorded in control and treated discs using leaf area meter. Leaf area protected over control was obtained and antifeedancy grading was done based on the below scale.

Table 1

Percent leaf area protection	Antifeedancy and Repellency rating	Grade
>81	Strong inhibition	A
51-81	Medium inhibition	B
21-50	Weak inhibition	C
<20	Insignificant inhibition	D

The percent repellency over control was worked out by recording the data related to the settling of grubs and adults on treated and control discs after 6h.

Insecticidal and Insect growth regulatory activity (No- choice poison food bioassay)

The method described in antifeedant assay was followed. But, the concentrations considered as antifeedant or repellent in the above experiments were avoided and lower concentrations were used. After the exposure, untreated fresh brinjal leaves were supplied to the grubs and adults and reared continuously. The exposed grubs were reared up to adult emergence and the data on mortality and malformations of grub, pupa and adults were recorded. All the experiments were replicated thrice.

Statistical Analysis

The data recorded in the experiments subjected to analysis of variance (ANOVA) under CRD by adopting the procedures described by Gomez and Gomez (1984) [3]. Necessary data transformation made before analysis and the computer based Wasp Agristat package used for the calculation.

Results and Discussion

In the antifeedant bioassay, out of twenty plants tested at 5% concentration, the maximum of 92.25% larval and 89.93% adult antifeedancy over control was recorded repectively in *W. tinctoria*. This was followed by 90.52% larval and 89.44% adult antifeedancy over control was recorded in *A. paniculata.* Insects exposed to treatment showed low respiration rates, reduced locomotor activity and avoidance responses to treated surfaces. This might be due to the presence of various biochemical constituents such as wrightial, triaconatanol, tryptanthrin, cycloeucalenol, indigotin and campetosterol as reported by Srivastava (2014) [8].

In the present study, other than the above mentioned two plants, three other plants such as *C. citrates, G. superba* and *J. gendarussa* recorded medium inhibition of 85.91, 80.91 and 77.25% larval respectively; 81.25, 73.43 and 70.08% adult antifeedancy over control respectively. Our results are in accordance with the reports of Angelica *et al.* (2020) [1] reported that *C. citrates* essential oil caused 89.88% adult antifeedancy against *Ulomoides dermestoides* adults due to presence of citral, geraniol, nerylacetal and geranyl acetate. Nebapure *et al.* (2017) [6] reported that repellency potential of glory lily, whereas seed methanol extract exhibited highest repellency (66±9.91%) against *Tribolium castaneum* adults. The minimum range of 63.20 to 5.63% larval and 59.54 to 3.01% adult antifeedancy over control was recorded in other than above mentioned plants.

The highest repellency of grubs and adults over control was noted in *W. tinctoria* and *A. paniculata* of (100, 83.33; 83.33, 78.33%). The repellent activity might be due to the presence of alkaloids and flavonoids that are able to irritate the olfactory senses of the grubs and adults. Sakthivadivel (2019) [7] reported that *W. tinctoria* fruit extract showed repellency against mosquitoes for period of 192 min in aqueous extract and 176 min in petroleum ether extract.

The high percent of pupal malformation (83.33%) and mortality (16.67%) was seen at *W. tinctoria.* The reduction in fecundity of adults in *W. tinctoria* (91.04%) and *A. paniculata* (88.65%) are statistically on par. The moderate pupal malformation and adult fecundity were showed by *C. citrates, G. superba, J. gendarussa* and positive control (Azadirachtin-300 ppm).

The malformed pupae exhibited dark brownish colour, disappearance of blackspots, damaged portions, size reduction and abnormal shapes. Fecundity reduction was seen in adults. Comparatively the results revealed that in control females lay 30-35 eggs/cluster and 6-7 clusters/adult. But, the treated insects lay only 2-3 clusters. In addition, it was observed that treated insects laid pale coloured eggs.

Many sterols in the *W. tinctoria* may disrupt ecdysteroid metabolism in insects resulting in inhibition of emergence behavior. These results are consistent with the earlier report on hexane, methanol and ethyl acetate extract of *W. tinctoria* fruit at different concentrations showed that the highest larval mortality at methanol extract and 100% malformed adults in ethyl acetate extracts at 5% concentration (Babu *et al.*, 2017) [3]. The plants belonging to the family Apocynaceae, Acanthaceae, Poaceae and Colchicaceae have pronounced pesticidal value. Further characterization may end with newer, more effectual and eco-friendly compounds.

Table 2: Antifeedant and repellent activities of certain botanicals against grubs and adults of *H. vigintioctopunctata*

Treatment No.	Treatments (5% concentration)	Percent leaf area fed		Percent Antifeedancy over control*		Percent Repellency over control*		Antifeedancy and Repellency rating
		Grub	Adult	Grub	Adult	Grub	Adult	
1	*Actiniopteris radiata*	94.37±1.84^{l}	98.99±2.75^{l}	5.63 (13.49)^{k}	3.01 (10.78)^{k}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
2	*Aglaomorpha quercifolia*	70.00±0.90^{h}	87.77±1.51^{j}	30.00 (33.20)^{gh}	21.23 (27.39)^{gh}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
3	*Alstonia scholaris*	50.72±0.83^{g}	75.77±0.96^{i}	49.28 (44.59)^{f}	45.23 (42.22)^{f}	16.67 (24.06)^{f}	16.67 (24.06)^{f}	D
4	*Andrographis paniculata*	9.48±1.42^{a}	15.56±1.21^{a}	90.52 (72.17)^{ab}	89.44 (71.09)^{ab}	83.33 (65.94)^{b}	78.33 (65.94)^{ab}	A
5	*Bridelia retusa*	96.05±2.26^{lm}	98.99±2.75^{l}	3.95 (11.03)^{k}	3.01 (10.78)^{k}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
6	*Chromolaena odorata*	83.27±1.11^{j}	98.97±2.72^{l}	16.73 (24.11)^{j}	14.03 (20.07)^{j}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
7	*Cipadessa baccifera*	69.90±0.90^{h}	76.46±0.94^{h}	30.10 (33.26)^{g}	23.54 (28.99)^{g}	16.67 (24.06)^{f}	0.00 (2.03)^{f}	D
8	*Clerodendrum trichotomum*	80.65±1.05^{ij}	97.94±2.66^{l}	19.35 (26.07)^{ij}	12.06 (21.77)^{ij}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
9	*Cymbopogon citratus*	14.09±1.19^{b}	18.75±1.04^{b}	85.91 (68.00)^{b}	81.25 (66.34)^{b}	83.33 (65.94)^{b}	66.66 (54.74)^{b}	B
10	*Euphorbia neriifolia*	75.89±0.97^{i}	98.46±2.61^{l}	24.11 (29.39)^{hi}	15.54 (21.56)^{hi}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
11	*Gloriosa superba*	22.75±0.99^{cd}	26.57±1.07^{d}	77.25 (61.54)^{cd}	73.43 (59.67)^{cd}	66.66 (54.74)^{c}	50.00 (45.00)^{c}	C
12	*Holoptelea integrifolia*	53.17±0.83^{g}	97.37±2.66^{l}	46.83 (12.39)^{k}	29.63 (33.01)^{k}	16.67 (24.06)^{f}	0.00 (2.03)^{f}	D
13	*Ipomoea obscura*	75.51±0.96^{hi}	98.24±2.51^{l}	24.49 (29.64)^{ghi}	19.76 (25.76)^{ghi}	16.67 (24.06)^{f}	16.67 (24.06)^{f}	D
14	*Justicia gendarussa*	19.09±1.05^{c}	29.92±1.76^{c}	80.91 (64.12)^{c}	70.08 (54.34)^{c}	66.66 (54.74)^{c}	66.66 (54.74)^{c}	B
15	*Mallotus Philippensis*	36.20±0.86^{e}	96.55±2.13^{e}	63.20 (52.59)^{e}	59.45 (50.09)^{e}	16.67 (24.06)^{f}	0.00 (2.03)^{f}	D
16	*Melia dubia*	43.13±0.84^{f}	58.99±0.99^{f}	56.87 (48.95)^{e}	48.01 (40.01)^{e}	33.33 (35.25)^{e}	16.66 (24.06)^{e}	D
17	*Ocimum basilicum*	52.06±0.83^{f}	65.6±0.75^{h}	47.94 (43.82)^{f}	44.40 (41.11)^{f}	33.33 (35.25)^{e}	16.66 (24.06)^{e}	D
18	*Polygala arvensis*	43.10±0.83^{f}	58.99±0.98^{f}	56.90 (48.97)^{e}	51.01 (47.23)^{e}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
19	*Rauvolfia tetraphylla*	83.45±1.12^{d}	98.22±1.84^{l}	16.55 (23.97)^{j}	15.78 (22.99)^{j}	0.00 (2.03)^{g}	0.00 (2.03)^{g}	D
20	*Wrightia tinctoria*	7.75±1.57^{a}	10.07±1.44^{a}	92.25 (73.97)^{a}	89.93 (72.78)^{a}	100 (87.97)^{a}	83.33 (65.94)^{a}	A
21	Positive control (Azadirachtin 300 ppm-0.03% EC)	25.31±0.95^{f}	30.31±1.14^{f}	-	-	-	-	C
22	Control	97±2.71^{l}	97±2.71	-	-	-	-	D
	C.D. (0.05%)	3.66	4.13	3.82	3.76	2.14	2.07	

*Values are mean (%) of the three replicates ± standard error
Values in parenthesis are arc sine transformed
Values with various alphabets differ significantly

Table 3: IGR and insecticidal activities of certain botanical extracts against grubs and adults of *H. vigintioctopunctata*

Treatment No.	Treatments (2% concentration)	Plant parts used	Percent IGR activity over control*		Percent mortality over control*	
			Grub**	Adult#	Grub	Adult

1	*Actiniopteris radiata*	Leaves	16.67 (24.06)[e]	10.34 (18.67) [i]	33.33 (35.25) [b]	0.00 (2.03) [e]
2	*Aglaomorpha quercifolia*	Roots	0.00 (2.03)[f]	7.89 (16.18) [i]	0.00 (2.03) [d]	0.00 (2.03) [e]
3	*Alstonia scholaris*	Leaves	16.67 (24.06)[e]	23.45 (28.94) [f]	16.67 (24.06) [c]	16.67 (24.06) [d]
4	*Andrographis paniculata*	Leaves	66.67 (54.75)[b]	88.65 (70.39) [a]	33.33 (35.25) [b]	66.66 (54.74) [b]
5	*Bridelia retusa*	Leaves	0.00 (2.03)[f]	0.00 (2.03) [l]	0.00 (2.03) [d]	0.00 (2.03) [e]
6	*Chromolaena odorata*	Whole plant	0.00 (2.03)[f]	3.45 (10.14) [j]	0.00 (2.03) [d]	0.00 (2.03) [e]
7	*Cipadessa baccifera*	Leaves	16.67 (24.06)[e]	15.68 (23.28) [h]	16.67 (24.06) [c]	0.00 (2.03) [e]
8	*Clerodendrum trichotomum*	Whole plant	16.67 (24.06)[e]	16.89 (2.23) [gh]	0.00 (2.03) [d]	0.00 (2.03) [e]
9	*Cymbopogon citratus*	Leaves	50.00 (45.00)[c]	78.23 (62.21) [bc]	50.00 (45.00) [a]	66.66 (54.74) [b]
10	*Euphorbia neriifolia*	Leaves	0.00 (2.03)[f]	1.04 (5.85) [k]	33.33 (35.25) [b]	0.00 (2.03) [e]
11	*Gloriosa superba*	Tubers	50.00 (45.00)[c]	74.23 (59.51) [c]	0.00 (2.03) [d]	50.00 (45.00) [c]
12	*Holoptelea integrifolia*	Leaves	16.67 (24.06)[e]	23.31 (28.84) [f]	0.00 (2.03) [d]	0.00 (2.03) [e]
13	*Ipomoea obscura*	Leaves	0.00 (2.03)[f]	21.54 (27.63) [f]	16.67 (24.06) [c]	16.67 (24.06) [d]
14	*Justicia gendarussa*	Leaves	50.00 (45.00)[c]	75.98 (60.67) [b]	16.67 (24.06) [c]	66.66 (54.74) [b]
15	*Mallotus Philippensis*	Fruits	16.67 (24.06)[e]	0.00 (2.03) [l]	0.00 (2.03) [d]	0.00 (2.03) [e]
16	*Melia dubia*	Leaves	33.33 (35.25)[d]	21.09 (27.31) [fg]	16.67(24.06) [c]	16.66 (24.06) [d]
17	*Ocimum basilicum*	Leaves	16.67 (24.06)[e]	40.65 (39.60) [e]	16.67 (24.06) [c]	16.66 (24.06) [d]
18	*Polygala arvensis*	Leaves	0.00 (2.03)[f]	21.21 (27.31) [f]	0.00 (2.03) [d]	0.00 (2.03) [e]
19	*Rauvolfia tetraphylla*	Roots, Fruits	16.67 (24.06)[e]	54.72 (47.71) [d]	0.00 (2.03) [d]	0.00 (2.03) [e]
20	*Wrightia tinctoria*	Immature pods	83.33 (65.94)[a]	91.04 (72.69) [a]	16.67 (24.06) [c]	83.33 (65.94) [a]
21	Positive control (Azadirachtin 300 ppm- 0.03% EC)	-	66.67 (54.75)[b]	79.63 (63.19) [b]	0.00 (2.03) [d]	50.00 (45.00) [c]
22	Control	-	-	-	-	-
	C.D. (0.05%)	-	2.39	3.16	3.01	2.11

** percent pupal malformation over control
# Percent reduction in fecundity over control
* Mean of three replications
Values in parenthesis are arc sine transformed
Values with various alphabets differ significantly

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