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INSECTICIDAL AND ANTIFEEDANT ACTIVITIES OF AQUEOUS LEAF EXTRACTS AGAINST *Epilachna vigintioctopunctata* (COCCINELLIDAE: COLEOPTERA) UNDER LABORATORY CONDITIONS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The aqueous leaf extracts of different plant such as *Cryptostegia grandiflora, Mansoa alliacea, Allamanda cathartica, Allamanda blanchetti* and *Xanthium strumarium* and leaves of tree species such as *Kigelia pinnata, Bauhinia Purpurea, Drypetes roxburghi, Conocarpus lancifolius* and *Polyalthia longifolia* were studied against the grubs of *Epilachna vigintioctopunctata* at various concentrations under laboratory conditions. Among the plant tested, the maximum insecticidal activity and leaf area protection was noticed in *Xanthium strumarium* 93.33% and 91.62% at 7% concentration with an estimated LC50 of 1.11% followed by *D. roxburghii* (80.00% & 85.55%) and *C. lancifolius* (73.33% and 82.90%) respectively. The minimum per cent mortality was noticed in the treatment, *Mansoa alliacea* 7% with 40.00% and its antifeedancy were 50.97%. Results revealed that both the antifeedancy and mortality was increased with increasing in concentration of the plant extracts.

Keywords: Aqueous leaf extracts; *Epilachna vigintioctopunctata*; leaf area protection and natural insecticide.

1. INTRODUCTION

Brinjal or Egg plant (*Solanum melongena* Linn.) is one of the most important vegetable crops grown all over in India. Brinjal is heavily infested by a number of insects/pests among *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae), which is one of the most destructive pests extensively found all over in tropics. It is one of the polyphagous pests present on solanaceous and cucurbitaceous crops.

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E. *vigintioctopunctata* beetle causes considerable economic losses to brinjal depending on place and season for variations of prevailing environmental conditions and its yield loss upto 80% [1]. It is highly destructive at both, adult and grub stages which feed on the epidermal tissues of leaves, flowers, and fruits by scrapping the chlorophyll content.

Frequent and indiscriminate use of pesticides in the vegetable fields for insect pest management results in various environmental and ecological problems such as widespread development of pest resistance, undesirable effects on non-target organisms, presence of toxic residues in food, outbreak of secondary pests [2-4]. Evidences of pesticide threats to human health and economic effects have been documented in several studies [5]. These problems have highlighted the need for development of new, safer and ecofriendly pest control measures. Naturally occurring plant products play an important role to replace or minimize the excessive use of pesticides as they constitute a rich source of bioactive components [6]

Plant extracts contain botanical insecticides or phytochemicals that could be used to repel, deter or limiting their fecundity and survival of various insect pest species [1,7]. Using various plant extracts, attempts have been made to save brinjal crops [8,9], against the attacks of the beetle. Ahmed (2007) reported that aqueous extract sprays of the castor oil plant *Ricinus communis* L. (*F. Euphorbiaceae*) suppress the beetle incidence and its attack on foliage. Subsequently, methanol extracts of *R. communis* were found to have larvicidal properties against the mosquitoes *Aedes aegypti* [10]. Anam et al. [11] reported that neem oil significantly prolonged larval and pupal periods and some of the treated larvae never reached to the pupae. Pupal recovery and adult emergence were greatly reduced in treated larvae. The aqueous extract of Calotropis procera showed strong repellent activity against the *Henosepilachna elaterii* [11]. In the present investigation, screening and evaluation of different plant aqueous extracts were tested against the grubs of *Epilachna* beetle.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The leaves of *Cryptostegia grandiflora, Mansoa alliacea, Allamanda cathartica, Allamanda blanchetii, Xanthium strumarium, Kigelia pinnata, Bauhinia purpurea, Drypetes roxburghi, Conocarpus lancifolius and Polyalthia longifolia* were collected from Annamalainagar (11.3921° N, 79.7147° E), Chidambaram and properly authentied. The leaves were shade dried and powdered in an electric blender and stored in air tight container in refrigerator till further use. From the stock 100 g of powdered leaves was extracted with 500 mL (1:5) of HPLC water and continuously shaking the bottle for 4 hrs by magnetic stirrer and kept it undisturbed for overnight separately.

2.2 Mass Culturing of *E. vigintioctopunctata*

Adults of *E. Vigintioctopunctata* were collected from the brinjal fields in and around Chidambaram. Grubs were reared in potted brinjal, karappadi (local) covered with wire mesh cages (45 × 45 ×45 cm) under laboratory conditions at 27 ± 2°c, 85%. Damaged plants were replaced by fresh plants regularly till pupation. Then emerged adult beetles, properly sexed were released in potted plants in cages covered with cylindrical nylon mesh (60cm height and 20 cm diameter). After oviposition, the eggs on the plants were left undisturbed for hatching. Immediately after hatching, the grubs were transferred on to fresh and healthy host plant leaves by using a fine brush. The cut end of each leaf petiole was wrapped with wet cotton to avoid dessication and then placed in a circular basin and covered with a *Kada* cloth. Once the leaf was consumed by the grub, it was replaced by a fresh leaf. The grub was reared up to adult emergence [12].
Insecticidal assay

Fresh brinjal leaves were treated with different concentrations (1%, 3%, 5% and 7%) of aqueous extracts. Brinjal leaves treated with water are kept as control. To avoid early drying of the leaves the petioles were tied with cotton plug and placed in a plastic container (30 cm × 15 cm). In each container10 pre-starved (3 h) third instar were released and covered with muslin cloth. Three replications were maintained for all concentrations and the number of grubs died was noted after 24 h. Percentage of larval mortality was calculated and corrected by using Abbot's formula

> Percent mortaliy (%) = [(% grubs mortality in treatment -% grubs mortality in control × 100) / 100 -% grubs mortality in control]

2.3 Antifeedant Assay

Feeding deterrent of certain plant extracts was studied using leaf disc with no choice method. Fresh brinjal leaf discs (5cm dia.) were dipped in concentration of test material. After air drying, each leaf disc was placed in petridish containing wet filter paper to avoid early drying of the leaf disc and 4 hours prestarved grubs were introduced into the petridishes. Three replications were maintained for each concentration and each replication had five grubs in each experiment. Progressive leaf area consumed by the grub after 24 hours feeding was recorded both in treated and control discs using leaf area meter. Leaf area consumed in plant extracts treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Jannet et al., [13].

AFI = C-T/C+T × 100

Where

AFI = Antifeedant Index;
C= Area protected in control leaf disc;
T=Area protected in treated leaf disc.

3. RESULTS AND DISCUSSION

The insecticidal activities of aqueous leaf extract was tested against the grubs of *Epilachna vigintioctopunctata* and the Lethal Concentration (LC_{50}) along with Upper and Lower Confidence Limit (UCL and LCL) were obtained and the results are presented in Tables 1 and 2. Among the treatments tested against *E. vigintioctopunctata*, *Xanthium strumarium* showed mortality of 93.33% at 7% concentration followed by *Dryptes roxburghii* (7%) were 80.00% and their LC_{50} values were 1.11% and 1.90% respectively. The LC_{50} values of leaf extract *Conocarpus lancifolius* showed 2.68%. The least insecticidal activity was noticed with *Mansoa alliacea*, were 40% at 7% and its LC_{50} values were 10.21%. The LC50 values of *Cryptostegia grandiflora* 6.65% and *Bauhinia purpurea* 6.59% were found almost similar. Roy *et al*. 2012 was investigated *that Xanthium strumarium* for its insecticidal effect against *Callosobrucus chinensis* and reported that 4% cocklebur fruit extract showed the highest mortality of 26% and their per cent repellency were 53.3% at 2 days after treatment and 3 hours after treatment, respectively. And also they revealed the lowest fecundity (113.7 female-1), highest percentage of adult emergence inhibition (37.0%) and lowest percentage (42.3%) of seed damage when they were reared on pulse grains mixed with 4% extract.

Table 1. Insecticidal assay of selected plant extracts against the grubs of *Epilachna vigintioctopunctata*

Treatments no.	Treatments	Cumulative percent mortality (24 HAT)			
		1%	**3%**	**5%**	**7%**
1.	*Cryptostegia grandiflora*	13.33 $(21.40)^{cg}$	20.00 $(26.55)^{gh}$	46.66 $(43.07)^{e}$	53.33 $(46.89)^{f}$
2.	*Mansoa alliacea*	6.66 $(14.95)^{hi}$	20.00 $(26.55)^{gh}$	33.33 $(35.24)^{hi}$	40.00 $(39.21)^{g}$
3.	*Xanthium strumarium*	53.33 $(46.89)^{a}$	53.33 $(46.89)^{a}$	80.00 $(63.51)^{a}$	93.33 $(75.94)^{a}$
4.	*Allamanda Cathartica*	20.00 $(26.55)^{de}$	26.66 $(31.07)^{f}$	40.00 $(39.21)^{g}$	53.33 $(46.89)^{ef}$
5.	*Allamanda blanchetti*	20.00 $(26.55)^{de}$	26.66 $(31.07)^{ef}$	40.00 $(39.21)^{g}$	46.66 $(43.06)^{g}$
6.	*Kigelia pinnata*	13.33 $(21.40)^{g}$	33.33 $(35.24)^{cd}$	40.00 $(39.21)^{fg}$	46.66 $(43.07)^{fg}$
7.	*Bauhinia purpurea*	6.66 $(14.95)^{i}$	26.66 $(31.07)^{f}$	33.33 $(35.24)^{i}$	60.00 $(50.75)^{de}$
8.	*Dryptes roxburghii*	40.00 $(39.21)^{b}$	53.33 $(46.89)^{a}$	66.66 $(54.72)^{b}$	80.00 $(63.44)^{b}$
9.	*Conocarpus lancifolius*	33.33 $(35.24)^{c}$	46.66 $(43.07)^{b}$	60.00 $(50.75)^{c}$	73.33 $(58.91)^{c}$
10.	*Polyathia longifolia*	20.00 $(26.55)^{de}$	33.33 $(35.24)^{d}$	53.33 $(46.89)^{d}$	60.00 $(50.75)^{e}$
11.	Control	0.00 $(0.00)^{j}$	0.00 $(0.00)^{i}$	0.00 $(0.00)^{j}$	0.00 $(0.00)^{h}$
	C.D (0.05)	1.278	1.519	2.373	4.038
	SE(m)	0.433	0.515	0.804	1.368
	SE(d)	0.612	0.728	1.137	1.935

**Mean of three replications*
**HAT- hours after treatment*
**Values in paranthesis are arc sine transferred*
**Values with different alphabets differ significantly according to LSD*

Table 2. LC_{50} of selected plant extracts against larvae of *Epilachna vigintioctopunctata*

Treatments no.	Treatments	LC_{50}[a]	UCL[b]	LCL[b]
1.	*Cryptostegia grandiflora*	6.65	3.33	13.28
2.	*Mansoa alliacea*	10.21	3.67	28.44
3.	*Xanthium strumarium*	1.11	0.42	2.90
4.	*Allamanda Cathartica*	7.76	2.58	23.32
5.	*Allamanda blanchetti*	10.03	2.14	46.90
6.	*Kigelia pinnata*	7.85	2.89	21.33
7.	*Bauhinia purpurea*	6.59	3.94	11.01
8.	*Dryptes roxburghii*	1.90	0.87	4.12
9.	*Conocarpus lancifolius*	2.68	1.37	5.25
10.	*Polyathia longifolia*	4.78	2.56	8.91
11.	Control	134.12	130.16	143.79

a LC_{50} represents lethal concentrations that cause 50% mortality
b UCL and LCL represents upper and lower confidence levels

Table 3. Antifeedant assay of selected plant extracts against the grubs of *Epilachna vigintioctopunctata*

Treatments no.	Treatments	Cumulative percent anifeedant (24 HAT)			
		1%	3%	5%	7%
1.	*Cryptostegia grandiflora*	37.92 $(37.99)^{hi}$	42.63 $(40.74)^{hi}$	47.99 $(43.83)^{i}$	51.12 $(45.62)^{gh}$
2.	*Mansoa alliacea*	36.13 $(36.93)^{i}$	38.57 $(38.37)^{i}$	48.83 $(44.31)^{hi}$	50.97 $(45.54)^{h}$
3.	*Xanthium strumarium*	80.75 $(63.95)^{a}$	89.76 $(71.66)^{a}$	92.05 $(74.11)^{a}$	91.62 $(73.75)^{a}$
4.	*Allamanda Cathartica*	38.53 $(38.35)^{ghi}$	46.07 $(42.73)^{gh}$	64.36 $(53.33)^{f}$	68.20 $(55.66)^{e}$
5.	*Allamanda blanchetti*	56.95 $(48.97)^{d}$	65.04 $(53.73)^{d}$	70.57 $(57.12)^{def}$	74.63 $(59.75)^{d}$
6.	*Kigelia pinnata*	39.08 $(38.67)^{fghi}$	49.29 $(44.57)^{fg}$	52.39 $(46.35)^{ghi}$	61.20 $(51.45)^{f}$
7.	*Bauhinia purpurea*	53.77 $(47.14)^{e}$	57.30 $(49.18)^{e}$	67.35 $(55.16)^{ef}$	64.22 $(53.24)^{ef}$
8.	*Dryptes roxburghii*	74.75 $(59.83)^{b}$	80.27 $(63.67)^{b}$	84.82 $(67.10)^{b}$	85.55 $(67.64)^{b}$
9.	*Conocarpus lancifolius*	75.36 $(60.25)^{b}$	79.06 $(62.80)^{bc}$	84.46 $(66.79)^{b}$	82.90 $(65.56)^{bc}$
10.	*Polyathia longifolia*	67.36 $(55.13)^{c}$	75.10 $(60.04)^{c}$	77.67 $(61.79)^{c}$	78.60 $(62.43)^{cd}$
11.	Control	0.00 $(0.00)^{j}$	0.00 $(0.00)^{j}$	0.00 $(0.00)^{j}$	0.00 $(0.00)^{i}$
	C.D (0.05)	1.799	3.463	3.813	3.487
	SE(m)	0.61	1.173	1.292	1.181
	SE(d)	0.862	1.659	1.827	1.67

**Mean of three replications*
**HAT- hours after treatment*
**Values in paranthesis are arc sine transferred*
**Values with different alphabets differ significantly according to LSD*

The maximum per cent leaf protection against *E. vigintioctopunctata* was recorded in the leaves of *Xanthium strumarium,* followed by *Dryptes roxburghii* and *Conocarpus lancifolius at* 7% were 91.62%, 85.55% and 82.90% respectively. This was followed by *Polyalthia longifolia* and *Allamanda blanchetti* at 7% with percent antifeedancy were 78.60% and 74.63% respectively. The minimum per cent antifeedanv was evidenced in the treatment, *Mansoa alliacea 7%* (50.97%) (Table 3). All the treatments showed significantly higher antifeedant compared to control, both mortaliy and antifeedancy were increased with increasing concentration (Fig. 1).

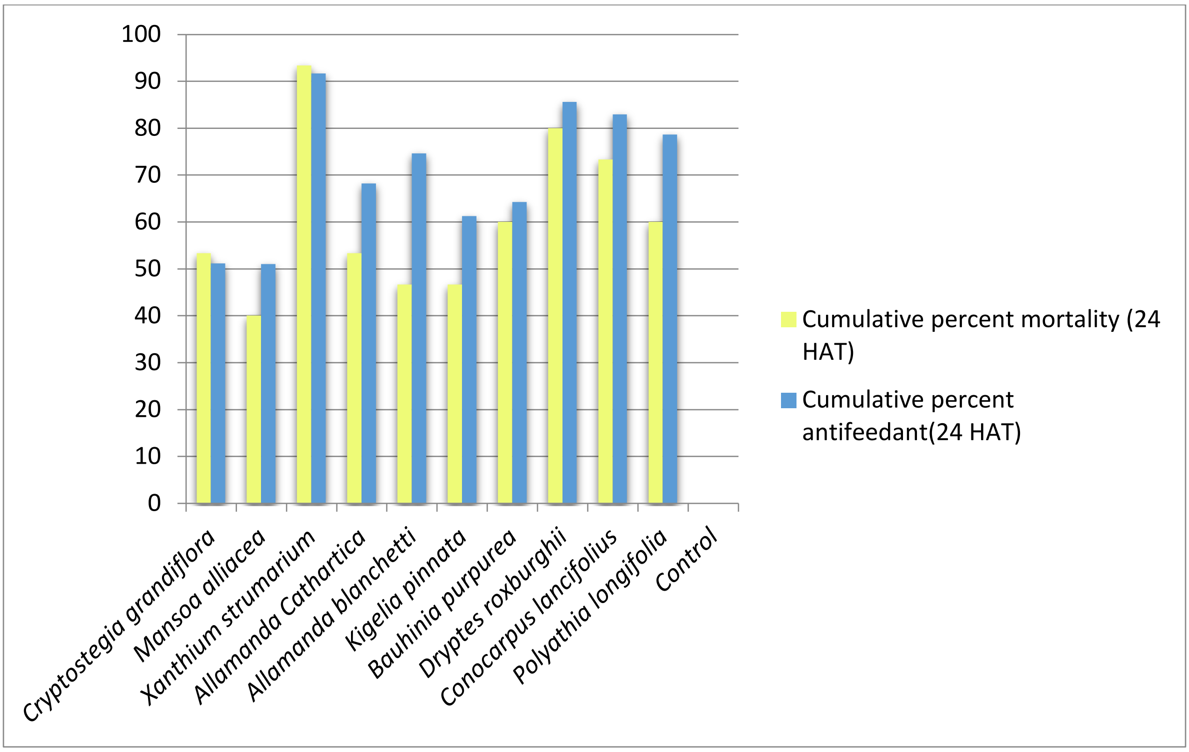

Fig. 1. Comparison of cumulative percent mortality and antifeedant of certain aqueous plant extracts against *Epilachna vigintioctopunctata*

In comparison of standard drug Permethrin (100% activity at 235.7µg cm^{-1}), the crude methanol extract of whole plant against *Callosbruchus analis* exhibited 100% mortality. Hence, it proved *X. Strumarium* as a natural insecticide [14]. The bioactive potential is due to biologically active xanthatin, Xanthumin, sesquiterpene xanthanol, terpenes, caffeic acids,carboxyatractyloside, xanthinin, caffeoylquinic acid, xanthanolides, 8-epi-tomentosin, 3,4-dihydroxy benzaldehyde, ascorbic acid and phytosterols (Kamboj, 2013). The novel bioactive triterpenoid saponion, 3-O [α-L-rhamnopyranosyl-(1→3)-O-β-D-xylopyranosyl] maniladiol (I), along with known compound ursolic acid (II) was reported from the leaves of *Xanthium strumarium* [15]. When taken in sufficient quantity it can cause hypoglycemia and hepatic damage in animals. The mechanism of action has been proposed to be an uncoupling of oxidative phosphorylation which is very essential process for the production of energy and normal metabolic process of a cell. Pollen grains of the plant are reported with allergic components which can cause contact dermatitis [16,17].

4. CONCLUSION

It is evidenced that these plant extracts employed in this study possess toxicity to the insect pests. A through chemical analysis of the active plants was studied in near future and hope it will reveal some interesting facts about the bioactive principle present in the plant, against the target pets. The insecticidal activity of these extracts also suggests a future exploitation of the materials in to potential insect management chemicals with a minimum environmental impact [18-20].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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