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Insect Growth Regulatory Activity of *Adiantum Capillus-veneris* Against *Plutella Xylostella* and *Aphis Craccivora* in Ethanol and Methanol

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Abstract: Insect growth regulatory activity of compounds derived from *Adiantum Capillus-veneris* was studied with Ethanol and Methanol fractions against *P. xylostella* and *A. craccivora. Adiantum capillus-veneris* was collected from the natural habitats in Palampur (H.P.).The larvicidal activity of Ethanol fraction of *Adiantum* also showed significant results .In *P. xylostella,* it showed highest larvicidal activity at higher concentration of 70-85% at 30,000 ppm and at 5,000 ppm conc. the activity was found to be 30-40% between 24-120 h. *A.craccivora* showed 70-75% activity at 30,000 ppm and 50-70% activity at 5,000 ppm between 24-48 h. The larvicidal activity of Methanol extract also showed the same trend as in Ethanol. It showed highest mortality rate against *A. craccivora* at higher conc. followed by *P. xylostella*. In *P. xylostella*, the activity was 65-85% at 30,000 ppm and 30-40% at 5,000 ppm after 24-120 h. LC_{50} values were also found to be highest in *A. craccivora* (9142.2 and 1227.9)in Ethanol, extract after 24 and 48 h as compared to *P. xylostella*, (8093. 6,778.2, 6777.6, 6367.7 and 5376.9) at 24-120 h respectively. The value of Chi–square tests showed significant results in *P. xylostella* and non-significant in *A. craccivora.*The Methanol extract of *A.craccivora* also showed highest LC_{50} values i.e, 11135.5, 557.1 and 4257.0 (24, 48 and 72 h).In *P. xylostella*, LC_{50} value was recorded to be 10094.8, 9761.2, 7683.8, 6967.2 and 6264.8 respectively. The chi–square tests were found to be non-significant in *A.craccivora* and significant results in *P. xylostella* at 24, 48, and 72,120 h and non-significant after 96 h.

Key words: *Adiantum capillus-veneris; Plutella xylostella; Aphis craccivora;* Ethanol; Methanol, larvicidal

INTRODUCTION

Adiantum is a genus of about 200 species of ferns in the family *Pteridaceae*, though some researchers place it in its own family, *Adiantaceae.* The genus name comes from the Greek, meaning "not wetting", referring to the fronds' ability to shed water without becoming wet. They are distinctive in appearance, with dark, often black stripes and rachises, and bright green, often delicately-cut leaf tissue. *Adiantum capillus-veneris* is used as expectorant, diuretic, febrifuge, as hair tonic, in chest diseases, in catarrhal infections, to treat hard tumors in spleen and it is anticancer us [19,25,11,12]. The extracts of *Adiantum capillus-veneris* showed significant inhibitory effect against cucumber green mosaic virus[26].

Chemical composition and antimicrobial activity of the volatile oil and extracts of fronds of *Adiantum capillus-veneris* was studied. Two pure compounds, filicene and filicenal were isolated from the non-polar fraction of the fern and it has exhibited potent analgesic activity [14]. In different studies various constituents of the ferns were isolated and identified [15,23,9]. But there is no study found in open literature describing its insecticidal properties against various pests.

Helicoverpa armigera,H.(Gram pod borer),*Spodoptera litura* F.(Asian armyworm) and *Aphis craccivora* K. Aphids are the four major pests of cultivated crops primarily in tropical and sub-tropical regions [5]. Due to high cost of protecting crops from these pests with chemical pesticides and then increasing resistance and resurgence to many chemical pesticides [2,3,26] there is growing interest in the use of biological products such as bacterial and viral based insecticides and parasitoids [16] and botanical pesticides [21]. The objective of the study was to evaluate commercially available biological and botanical pesticides both individually and partly in combination against *A. craccivora* and *P. xylostella* species on *Adiantum capillus-veneris* to determine their effects under laboratory conditions.

Botanical pesticides are biodegradable [6] and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators [8] and reduces environmental contamination and human

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health hazards. Research on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides are a prerequisite [4] for sustainable agriculture. Botanical pesticides are unique because they can be produced easily by farmers and small industries [21].

MATERIALS AND METHODS

Plant Material: The samples (*Adiantum capillus-veneris*) were collected from their natural habitats in Palampur, Himachal Pradesh, India (32°6¢N latitude and 76°3¢E longitude) in July, 2007 from the damp areas. The samples (entire plant) was washed and kept over the sheets of ordinary filter paper to remove the water. The aerial parts of the plants were separated, shade dried and crushed.

Extract Preparation: The powdered mass (3 replicates of 100 g each) was placed in 1000 ml separatory funnel with cotton plug at the bottom. 250 ml of Ethanol was added to the powdered mass and it was kept undisturbed for 24 h with cotton lid at the top to avoid the loss of solvent. The filtrate was collected after 24 h and the same process was repeated for three days. The crude extract was evaporated under vacuum to give a semi-solid extract. The remaining powdered mass in the separatory funnel was further extracted with other solvent i.e. Methanol and then it was dried and weighed. The weight of two extracts was found to be, 4.2 g- Ethanol and 2.6 g - Methanol).

Insect Rearing: The heavily infested leaves of the larvae of test insects were collected from the natural field conditions and reared in the laboratory controlled conditions. *P. xylostella* was reared on cabbage leaves while *A. craccivora* on cow pea leaves and french bean leaves. Whole twig of the plant was selected for this purpose. The cultures were kept at 25±5°C and 68±5% R.H. under a L16:8 D photoperiod. Second instar larvae were usually preferred during the experiment.

Laboratory Bioassay: Bioassay was conducted by leaf dip method. Petriplates (9.0 cm diameter) were lined with a layer of moist filter paper to maintain humidity in case of castor leaves and bean leaves. First 10 ml of acetone: tritone mixture was prepared using acetone: triton X-100 LR spreader (9:1v/v) (S.D. Fine-Chem. Ltd., India).The *Adiantum* extract (0.1g) was dissolved in the above mixture (1ml) and diluted to 10 ml using distilled water. Four concentrations of 30,000, 20,000, 10,000 and 5000 ppm were prepared . Fresh cabbage leaf disks used in case of *P.xyllostella*(3.5 cm) were dipped in the extracts of these concentrations and air-dried. Leaflets were placed individually with adaxial side up on a filter paper (Whatmann No.1) moistened with distilled water. Controls were treated with distilled water and above tritone:acetone mixture. Second instar larvae were transferred from the culture by a fine hair brush and pre-starved for 2 h. Ten larvae were placed in each petriplate containing the leaf. For *A. craccivora,* bean or cow-pea leaf disks were used and same procedure was followed as mentioned above. The petriplates were sealed properly to avoid desiccation and escape of any larvae .Each concentration was taken in triplicates. Along with treated samples, a control was also prepared in order to check the bioactivity of each insect.

Data Collection and Analysis: The leaf disks were analyzed at 24, 48, 72, 96 and 120 h after treatment. Statistical analysis was conducted till the survival of larvae in both the two extracts.

Probit Analysis: Data for each insecticide / population was analyzed by probit analysis [7] and Abbot's formula [1] was used to correct for control mortality. The ratio of the LC_{50} of populations from the field to the LC_{50} of the susceptible laboratory population was calculated for each insecticide and population in order to determine the resistance factor (RF).The levels of resistance of the populations were considered significantly different if the 95% confidence limits of the LC_{50} values did not overlap.

Calculations: The percent mortality was calculated by using the formula:

$$\text{Percent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

$$\text{Corrected percent mortality} = \frac{\text{T-C}}{\text{100-C}} \times 100$$

Where T= treated, C= control

Regression equation and chi-square values were calculated by using probit analysis [7]. Mortality data were corrected using Abbott's formula [1] Larval mortality was recorded 24 h after treatment and corrected using Abbott's formula [1]. The percent of mortality obtained is analyzed with probit analysis [7] using probit analysis option in the SPSS 10.0 windows:Puss In C.

RESULTS AND DISCUSSION

Larvicidal Activity of Test Compounds: The larvicidal activity of *Adiantum capillus-veneris* in

various extracts made with different compounds was conducted in Government Degree College, Baijnath. This activity was tested against *P.xylostella* and *A.craccivora.*The collection of larvae was made from CSK HPKV, fields and IHBT institute, Palampur.

Ethanol Extract: The larvicidal activity of Ethanol extract of *Adiantum* showed significant results. The extract obtained was viscous in nature. It showed highest mortality rate against *P.xylostella* at high concentration of 30,000 ppm (70 -85%) after time intervals from 24 to 120 h (table 1). At 20,000 and 10,000 ppm concentration, almost similar activity was recorded (70-80%) during the respective time intervals from 24 h to 120 h. When the same extract was tested at lower concentration of 5,000 ppm, it showed (30-40%) activity between 24 h to 120 h.

A.craccivora showed 70-75% activity at higher concentration of 30,000 ppm between 24 and 48 h and 65-70% activity was observed at 20,000 ppm between 24 and 48 h. However, at low concentration of 10,000 ppm, 50-70% activity was recorded between 24 and 48 h. The larval mortality was found to be 100% after 72 h at all the above three concentrations (10,000, 20,000 and 30,000 ppm).When the same extract was tested at 5,000 ppm, it showed 40-60% activity after 24 and 48 h, 80% activity after 72 h and 100% activity after 96 h.

Methanol Extract: The Methanol extract of *Adiantum* was also viscous in nature. The larvicidal activity of this extract also showed the same trend as Ethanol. The extract showed highest mortality rate against *A.*craccivora at higher concentration followed by *P. xylostella*.

In *P. xylostella*, the activity was 65-85% and 65-75% at high conc. of 30,000 and 20,000 ppm respectively from 24-120 h time duration (table 1). However**,** at lower concentration the activity varies from 30-40% at 5,000 ppm (between 24-120 h) and 60-70% activity was observed at 10,000 ppm concentration during the respective time intervals.

A. craccivora showed significant larvicidal activity (70-75%) at higher concentration of 30,000 ppm between 24 and 48 h respectively. Whereas at 20,000 ppm 65-70% activity was recorded. At lower concentration of 10,000 ppm, it showed 50-70% activity between 24 and 48 h respectively and it decreases further at 5,000 ppm (30-50%).However, after 72 h, 100% larval mortality was recorded in all the high concentrations (10,000, 20,000 and 30,000 ppm) except at 5,000 ppm concentration where it was found to be 70%.The activity was recorded to be 100% after 96 h (table 1).

Lethal Concenteration:

Ethanol extract: The LC_{50} values of Ethanol extracts for *P. xylostella* were 8093.6, 7778.2, 6777.6, 6367.7 and 5376.9 after 24, 48, 72, 96 and 120 h at the respective time intervals. When Chi-square analysis was conducted for same pest, the results were found to be significant during all the time periods from 24-120 h. They were recorded to be 15.68, 13.15, 8.97, 12.6 and 11.87 (table 3).

In *A.craccivora*, the LC_{50} values between 24 h and 48 h were recorded to be 9142.2 and 1227.9 (fig.1).However, after 72 h, the LC_{50} value was observed to be 2762.3%. When Chi- square analysis was conducted with same extract, it showed non-significant results in all the time intervals from 24 h to 72 h (0.196, 0.724 and 0.780) (table 2).

Methanol Extract: The LC_{50} values of Methanol extract for *P. xylostella* were 10094.8 and 9761.2 between 24 and 48 h respectively. After 72, 96 and 120 h, LC_{50} values were recorded to be 7683.8, 6967.2 and 6264.8 respectively. Chi-square tests of Methanol extract showed significant activity from 24, 48, 72 h and 120 h whereas after 96 h, it showed non-significant activity (7.066). After 24, 48 and 72 h, the Chi-square values were recorded to be 7.49, 5.49 and 5.07. However, after 120 h, it was found to be 3.76 (table 2).

In *A. craccivora*, LC_{50} values were recorded to be 11135.5, 5557.1 and 4257.0 between 24, 48 and 72 h (fig.2). The Chi-square tests were found to be non-significant in all the time periods after 24, 48 and 72 h (0.718, 12.52 and 0.265).

Comparative study of the results indicated that *A. craccivora* showed the highest LC_{50} values (9142.27) with Ethanol extract .Whereas in *P. xylostell*a, the value was found to be 8093.60 after 24 hrs .Methanol extract of *Adiantum capillus-veneris* showed the highest LC_{50} value (1135.50) in *A. craccivora* after 24 h and *P. xylostella* showed LC_{50} value of 10094.84 after the same duration (table 2,3).

The Chi-square tests showed significant activity with Ethanol extract. The results were found to be significant during 24-120 h. Where as, Methanol extract showed significant activity at 24 ,48 ,72 and 120 h while after 96 h, it showed non-significant activity (table 3).In *A. craccivora*, the Chi-square tests were found to be non-significant in Ethanol and Methanol extract after 24, 48 and 72 h (table 2).

Discussion:

Larvicidal Activity: There are about 2000 plants, which have shown insecticidal properties [13]. It is only in recent years that interest in plant-based products has been revived because of the rising cost of insecticides

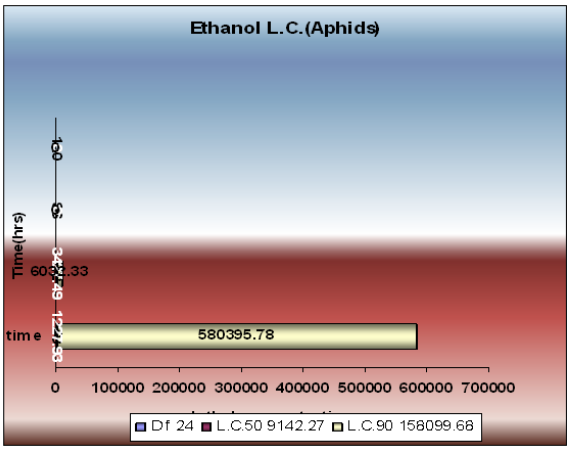

Fig. 1: LC50 of Aphids in Ethanol extract

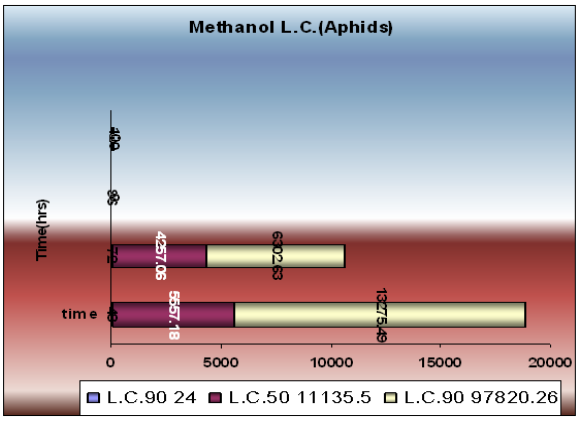

Fig. 2: L.C.50 of Aphids in Methanol extract

Table 1: Percentage mortality of *P.xylostella* and *A.craccivora* against different concentrations of *Adiantum capillus-veneris*

Plant name and part used	*Adiantum* (whole plant)	Extract	Test insect	Percent mortality ± SD																			
				T1 5,000 ppm					T2 10,000 ppm					T3 20,000 ppm					T4 30,000 ppm				
				24	48	72	96	120	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
		Ethanol (C_2H_5OH)	*Plutella Xylostella*	30	30	35	35	40	70	70	72	75	80	70	75	75	80	80	70	75	80	80	85
			Aphis craccivora	40	60	80	100	-	50	70	100	-	-	65	70	100	-	-	70	75	100	-	-
		Methanol (CH_3OH)	*Plutella Xylostella*	30	30	35	35	40	60	60	65	70	70	65	65	70	75	75	65	70	75	80	85
			Aphis Craccivora	30	50	70	100	-	50	70	100	-	-	65	70	100	-	-	70	75	100	-	-

-indicates no activity

Table 2: Pirobt Analysis of *Adiantum capillus-veneris* against *A.craccivora* at different treatments (5,000, 10,000, 20,000 and 30,000 ppm conc.)

Extract	Time	Regression Coefficient	Intercept	Chi –Square	Probability	LC_{50}	LC_{90}	Degrees of Freedom
Ethanol	24	0.44962	-4.10081	.196(Non- Significant)	.907	9142.27970	158099.68128	2
	48	0.20810	-1.48023	.724(Non- Significant)	.696	1227.93919	580395.78925	2
	72	2.26701	-18.45256	.780(Non- Significant)	.677	3427.49633	6032.33253	2
	96	all doses same	-	-	-	-	-	2
	120	-	-	-	-	-	-	2

Table 2: Continue

Methanol	24	0.58976	-5.49535	.718(Non- Significant)		.698	11135.50225	97820.26732	2
	48	1.47165	-12.68979	12.520(Non- Significant)	.002	5557.18645	13275.49147	2	
	72	3.26604	-27.29212	.265(Non- Significant)	.876	4257.06743	6302.63397	2	
	96	all doses same	-	-	-	-	-	2	
	120	-	-	-	-	-	-	2	

-indicates no activity

Table 3: Probit Analysis of *Adiantum capillus-veneris* against *P.xylostella* at different treatments (5,000, 10,000, 20,000 and 30,000 ppm conc.)

Extract	Time	Regression Coefficient	Intercept	Chi –square	Probability	LC_{50}	LC_{90}	Degrees of Freedom
Ethanol	24	0.54743	-4.92621	15.683(Significant)	.000	8093.60	84109.32	2
	48	0.65324	-5.85244	13.155(Significant)	.001	7778.25270	55321.83195	2
	72	0.65257	-5.75654	8.976(Significant)	.011	6777.66776	48303.18867	2
	96	.68569	-6.00693	12.602(Significant)	.002	6367.70608	41332.36135	2
	120	0.68965	-5.92400	11.873(Significant)	.003	5376.96498	34480.42453	2
Methanol	24	0..48813	-4.50048	7.495(Significant)	.024	10094.84	139412.43	2
	48	0.55123	-5.06368	5.490(Significant)	.064	9761.22562	99815.26437	2
	72	0.56391	-5.04527	5.072(Significant)	.079	7683.89958	74569.23790	2
	96	0.65890	-5.83063	7.066(Non- Significant)	.029	6967.29722	48725.42942	2
	120	0.67033	-5.86047	3.767(Significant)	.152	6264.83294	42384.48833	2

-indicates no activity

with development of resistance, cross resistance and toxicity hazards associated with them. In this study an attempt was made to explore the larvicidal potential of *Adiantum*. The data, shown in table 1 and 2 demonstrates the efficacy of extracts of *Adiantum capillus-veneris* against various test insects showing promising activities. The findings of the present investigation indicated promising larvicidal properties against *P. xylostella* and *A. craccivora*.Ethanol and Methanol extracts also showed highest LC_{50} values in *A.craccivora.* A positive correlation was observed between the extract concentration and the percent mortality, the rate of mortality being directly proportional to concentration as observed from the data. Lower dosages (0.4–0.8 ml/m^2) did not produce more than 50% mortality even after 120 h of exposure, except in the case of which showed 65% mortality after 72 h. In some experimental sets, χ^2 was higher and, according to Preisler, 1988 and Preisler et al., 1990, this may be due to unaccountable variations amongst the repetitions such as individuals competing for the same resources. Thus, the mortality values can change without the risk of invalidating the results. Chi-square values were significant at $P<0.05$ level for the extract of Ethanol.

Conclusion: The screening results suggested that *Adiantum* extract have shown larvicidal activity against different test insects like *P.xylostella* and *A.craccivora* and is considered promising. Further studies are needed for active compound isolation, formulations development, their efficacy against pest and cost effectiveness. To the best of our knowledge the results obtained here in with respect to the lethality of Ethanol extracts of *Adiantum* for larvae of *P.xylostella* and *A.craccivo*a have not been previously reported.

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