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Antifeedant effect of *Achyranthes aspera* Linn on Cauliflower Borer *(Hellula undalis),* Fruit and Leaf Borer of Cauliflower (*Spodoptera litura*) and Brinjal Fruit Borer (*Leucinodes arbonalis*)

S. GIRIJA and N. VALARMATHY

Department of Plant Biology and Biotechnology, Ethiraj college for women, Chennai - 08 (India).

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ABSTRACT

Medicinal plants are the local heritage with global importance. Pest problem is one of the major constraints for achieving higher production in agriculture crops. *Achyranthes aspera* was used in the present study to check the larvicidal and antifeedant activity on some insect larvae. The crude ethanolic extract of the plant sample was tested on cauliflower borer, brinjal borer.The mortality rate , the initial and final weight of the larvae was recorded. There was a marked decrease in the food consumption and faecal matter in excreta rate. The overall body weight of the worm increased abruptly in 600µg and 800µg. The larvae attained abnormal size in 800µg. The larvae of 1000µg concentration was not alive after the 3rd day. The faecal matter excreted also showed considerable reduction in 1000µg. Therefore the plant extract showed high antifeedant and less Larvicidal activity on Spodopter litura. It showed that the plant extract showed distinct anti-feedant and growth inhibitory action on all the insect larvae tested.

Key words: Antifeedent activity, *Achyranthes aspera, Hellula undalis, Spodoptera litura* & *Leucinodes arbonalis.*

INTRODUCTION

Medicinal plants are the local heritage with global importance. World is now endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicine that work in concert with the body's own defence. People in Europe, North America and Australia are consulting trained herbal professionals and are using the plant medicines. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities. (Purohit & Vyas, 2004).

Medicinal plants help in alleviating human sufferings. These plants are being integrated to the field of foods as additives, beverages and cosmetics. They are widely used as sweetners, as bitters, as spices, as natural colouring agents and as insecticides. (Purohit & Vyas, 2004).

Pest problem is one of the major constraints for achieving higher production in agriculture crops. India loses about 30% of its crops due to pests and diseases every year. The damage due to these is estimated to be Rs 60,000 crores annually. The use of pesticides in crop protection has certainly contributed for minimizing yield losses. The pesticides, which are needed to be applied carefully, only when the threshold units of pest population is exceeded.

However, quite often the indiscriminate and unscientific use of pesticides has led to many problem, such as pests developing resistance, resurgence of once minor pests into a major problem besides environmental and food safety hazards.

Defoliation caused due to the larvae remains a challenging socio-economic problem in many of the tropical countries. (Udonsi et al., 1986). Search for natural insecticides, which do not have ill effects on the non-target population and are easily degradable, remains to be one of the top priority issues for the tropical countries. (Redwane et al., 2005).

Achyranthes aspera, Linn Amaranthaceae called Devil's horsewhip, prickly chaff flower, Chirchira is an annual weed distributed throughout India in locations upto 915m high.

The plant is much valued in indigenous medicine. It is reported to be pungent, astringent, pectoral and diuretic. It is used as an emmenogogue and in piles and also in skin eruptions. It is useful in cough, asthma, bronchitis, dyspepsia, flatulence, colic, painful inflammations, dropsy, ophthalmopathy, vomiting, leprosy. Traditional healers claim that addition of *Achyranthes aspera* would enhance the efficacy of any drug of plant origin.

Folk medicinal uses of Achyranthes aspera enumerates the medicinal uses of Achyranthes aspera in the treatment of tooth diseases, by the rural and common people of Meerut district. (Tomar, 2007).

Effect of Achyranthes aspera extract on phagocytosis by human neutrophils was carried out by Mali et al., 2007. This paper reveals that the extract has stimulated chemotactic, phagocytic and intracellular killing potency of human neutrophils at the concentration range of 25-100mg/ml.

Ethnobotanical observations of Achyranthes aspera and Aerva with special reference to the people of Nara Desert. This study aims to further explore and document the potential medicinal values of these plants. (Quershi et al., 2007).

The anti-microbial activity of the crude extract of Achyranthes aspera and all fractions were tested. The crude methanolic extract was active against all micro organisms except Escherichia coli, Bacillus subtilis, Y. enterocolitica and Candida albicans. (Chakraborty et al., 2002).

All the plant extracts were found to be antifeedant and growth inhibitory in nature. (Sahayaraj, 1998).

The plant contains triterpenoid, saponins, betaine, achyranthine, hentriacontane, ecdysterone and two glycosides of oleanlic acid. The dried dehusked seeds contain amino acids. Ecdysteroids are polar steroids, almost sugar like in their solubility properties. (Gilbert et al., 2002).

MATERIAL AND METHODS

Achyranthes aspera Linn (Amaranthaceae) is an erect, much branched, suffruticose, 0.3-1m high with ascending branches. In India, the plant is distributed along the road sides, foot paths, rail roads, sand dunes as well as hills upto 900m. (Smith, 1981). It often infests fence rows, open woodland and the borders of forests and coffee fields. It has adapted to a wide range of environments. (Holm et al., 1997).

Collection of the plant material

Leaves of *Achyranthes aspera* were collected from the college premises. The leaves were thoroughly washed and shade dried. The dried leaves were chopped into small pieces and the stem were cut into small bits and shade dried. Both these samples were powdered and stored separately in air-tight containers.

Preparation of the crude extract

The plant sample powders were soaked in the organic solvent (ethanol) in 1:3 ratio and the extract was collected and fresh solvent was decanted each time. The decanted extract was refluxed in a Soxhlet apparatus (Saxena et al). The refluxed crude extract was collected and weighed. (stem 2.4gm and leaf 2.9gm).

Antifeedant activity

The crude extract collected was used for further work. The crude extract (1mg/1ml) was taken from the plant sample. The different concentrations were prepared from the crude extract. (200µg, 400µg, 600µg, 800µg & 1000µg) using ethanol. Pure ethanol was taken as control. The known weight of cauliflower (3g) was taken in three different sterilized petriplates uniformly. The cauliflower was treated with various different concentrations of the plant extracts and then transferred into the respective petriplates. One was maintained as a control without the plant extract. In all the three petriplates 1st larval instar stage of *Hellula undlais* (identified by Mr. Sezhian, (Ent.), Biotech. International Ltd., Chennai) was allowed to grow. The initial weight of the worms, their average reduction/increased weight per day, % of mortality rate were observed. The entire set up was conducted in the room temperature. The similar type of work was carried in brinjal fruit borer (*Leucinodes arbonalis*) (identified by Mr. Sezhian, (Ent.), Biotech. International Ltd., Chennai) on brinjal fruit. The plant extract concentration (200g, 600g and 1000g) was taken. As usual without the plant extract one control was maintained. Antifeedant effect was tested by checking the initial weight and the final weight of the worms.

Another set of work was carried out on Cauliflower leaf using Cauliflower leaf and fruit borer *Spodoptera litura* (identified by Mr. Sezhian, (Ent.), Biotech. International Ltd., Chennai) which is the most serious insect pest, particularly in Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra. It causes significant loss of vegetable yield every year (current science, vol-74, No-6, 25 March 1998). The antifeedant effect was calculated by the initial leaf area with that of the gradual consumed area (in cm) by the larvae, weight of the larvae, abnormality, their mortality rate were observed. To check the results one control was maintained.

The above three sets of observations were also carried out using the stem extract of the plant sample.

Thin layer chromatography

A crude ethanolic extract was spotted on a precoated silica gel plate. The spots were allowed to dry. The silica gel plate was placed in a chromatogram developing tank with toluene, hexane and ethanol in 3:3:1 ratio as solvent. The RF values of the different coloured spots were calculated.

The RF value was calculated using the formula,

$$\text{Rf value} = \frac{\text{Solute Front}}{\text{Solvent Front}}$$

Phytochemical test

1mg of the ethanolic extract was tested for the metabolites by the following phytochemical tests.

Tannin

To 1mg of the extract, few drops of 1% ferric chloride was added and observed for brownish green or blue black colour.

Saponin

2ml of water was added to 1mg of the ethanolic extract and shaken vigorously till persistent froth was observed.

Phlobatannin

To 1mg of the extract, 1% HCl was added and observed for red precipitate.

Flavanoid

To 1mg of the extract, 5ml of dil. NH_3 solution and conc. H_2SO_4 was added and observed for yellow colour.

Steroid

2ml of acetic anhydride and 2ml of conc. H_2SO_4 was added to 1mg extract and observed for violet or blue or green colour.

Cardiac glycosides

To 1mg of the extract, 2ml of glacial acetic acid, 1 drop of $FeCl_2$ and 1ml of conc. H_2SO_4 was added and observed for brown colour which was formed between the two layers. Violet or green ring may appear.

Terpenoid

2ml of chloroform and conc. H_2SO_4 was added to 1mg of the extract and observed for reddish brown colour inference.

Alkaloid

Potassium iodide and Iodine (Wagner's reagent) was added to the extract and observed for red precipitate or pink colour at the top.

TLC analysis

The various bands were eluted separately and tested for their toxic effect. These samples were centrifuged and the supernatant was collected for further studies. The supernatant of the respective bands were tested for their phytochemical analysis and also checked for their antifeedant activity on the respective worms.

RESULTS AND DISCUSSION

The initial weight of the cauliflower borer (Hellula undalis) was given in the tabular column (Table 1). The mortality rate was 100% in 0.8mg/ml and 1mg/ml concentration. In 0.6mg/ml and 0.2mg/ml it was 80% and 20% respectively. Whereas in the control 0% mortality was observed. (Table 2). The weight of the worms was reduced to 50% with that of their initial weight in lower concentration (200µg) where the worms were alive for few days. Both leaf and stem ethanolic extracts showed similar effects. More than 0.6mg/ml concentration seems to be larvicidal to the tested worms.(Fig. 1).

Therefore the worms showed distinct larvicidal effect on higher concentration (800µg and 1000µg) and antifeedant effect in 200µg and 600µg as reported earlier. (*Current Science*, 1998).

The zone of inhibition was recorded and calculated on the leaf and is tabulated. The percentage of zone of inhibition was calculated for about 4 to 5 days. It was calculated using the formula,

The area of feeding in each concentration was recorded and the % of average feeding in various concentration was calculated using the area of feeding in various concentrations and the area of feeding in the control. Then the percentage of inhibition was calculated using the formula,

% of inhibition = (100 - % of average feeding in various concentration)

Table 1: Initial and Final weight of the cauliflower and the worms used

S. No.	Concentration of the leaf & stem extract (µg)	Weight of Cauliflower (g)	Initial weight of the worm (mg)	Final weight of the worm
1.	Control	5	113	171
2.	200	5	128	80
3.	600	5	120	62
4.	800	5	121	120
5.	1000	5	120	118

Table 2: The effect of Hellula undalis larvae on cauliflower

S. No.	Concentration of the extract (µg)	Mortality Rate				
		Day 1	Day 2	Day 3	Day 4	Day 5
1.	Control	+	+	+	+	+
2.	200	+	+	+	+	-
3.	600	+	+	-	-	-
4.	800	-	-	-	-	-
5.	1000	-	-	-	-	-

The average feeding % in various concentration and % of inhibition is calculated for about 4 to 5 days depending on their mortality. It is calculated and tabulated each day.

There was a marked decrease in the food consumption and faecal matter in excreta rate. (Table 4) The overall body weight of the worm increased abruptly in 600µg and 800µg. The larvae attained abnormal size in 800µg. The larvae of 1000µg concentration was not alive after the 3rd day. The faecal matter excreted also showed considerable reduction in 1000µg.

The larvae of 200 µg to 800 µg was allowed to notice for their pupal stage to attain. But the larvae of 600 µg and 800 µg was alive till the 5th day and 4th day respectively. In the two concentrations the larvae showed distinct abnormality and showed irregular growth. (Table 4) After the 6th day even in lower concentrations (200 µg, 400 µg) the larvae were not alive. The control was alive and had attained pupal stage.

Table 3: Effect of cauliflower leaf worm (spodoptera litura) on the various concentrations of the plant extract

S. No	Concentration of the extract (µg)	Days	% of feeding (%)	% of inhibition (%)	Mortality rate (%)
1.	Control	1	100	0	0
		2			
		3			
		4			
		5			
2	200	1	82.25	17.75	0
		2	98.67	1.3	
		3	65.43	34.57	
		4	63.71	36.29	
		5	67.13	32.87	
3.	400	1	67.74	32.26	0
		2	82	18	
		3	48.56	51.44	
		4	56.93	43.07	
		5	57.74	42.26	
4.	600	1	64.52	35.48	0
		2	59.33	40.67	
		3	42.38	57.62	
		4	52.80	47.2	
		5	52.76	47.24	
5.	800	1	40.32	59.68	20
		2	48	52	
		3	40.74	59.26	
		4	44.24	55.76	
		5	-	-	
6.	1000	1	16.13	83.87	20
		2	38.67	61.33	
		3	37.03	62.97	
		4	38.93	61.07	
		5	-	-	

The larvae of Brinjal fruit borer Leucinodes arbonalis was left on the fruits which were sprayed with the different concentrations of the crude extract. The different concentrations taken were 200 µg, 600 µg and 1000 µg. Their effect was observed and their % of mortality rate was tabulated. The observations were done for about 5 days only. In 600 µg and 1000 µg, it showed mortality on the 4th and 3rd day respectively, whereas 200 µg was as similat as the control and was alive till the 5th day.

Therefore the plant extract showed high antifeedant and less Larvicidal activity on Spodopter litura. It showed that the plant extract showed distinct anti-feedant and growth inhibitory in nature. Since Spodoptera litura is a polyphagous pest on some

Table 4: Effect of different concentrations on Spodoptera litura larvae

S.No	Parameter	Control	200 µg	400µg	600µg	800µg	1000µg
1.	Animal Initial Weight (mg)	250	248	252	240	252	251
2.	Animal Final Weight (mg)	293	288	290	340	403	182
3.	Faecal matter (mg)	41.3	36.8	30.3	48.1	70.2	20.8
4.	Mortality rate	Alive	6th day dead	6th day dead	5th day dead	4th day dead	3rd day dead

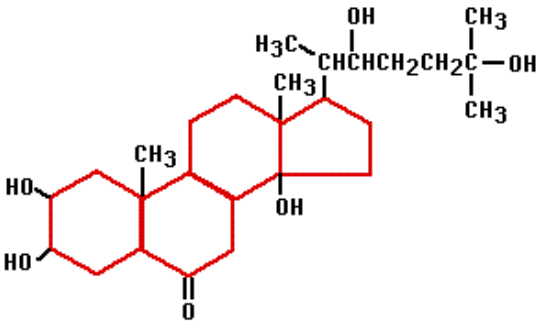
Table 5 : Effect of plant extract on Leucinodes arbonalis, fruit borer of Brinjal

S. No.	Concentration of the extract (µg)	Mortality Rate					% of mortality
		Day 1	Day 2	Day 3	Day 4	Day 5	
1.	Control	+	+	+	+	+	0
2.	200	+	+	+	+	+	0
3.	600	+	+	+	–	–	40
4.	1000	+	+	–	–	–	60

Table 6 : Qualitative analysis of plant extract

S. no	Secondary Metabolites	Result
1.	Tannins	+
2.	Phlobatannins	–
3.	Saponins	+
4.	Flavonoids	–
5.	Steroid	–
6.	Terpenoids	+
7.	Anthraquinone	–
8.	Cardiac glycosides	–
9.	Phytosterols	–
10.	Carbohydrates	+
11.	Phenol	–

Ecdysone



Scheme 1

Table 8: Results of Phytochemical tests for TLC Analysis

S. No	Secondary Metabolites	Bands B1	B2	B3	B4	B5
1.	Tannin	+	-	-	-	-
2.	Steroid	-	+	-	-	-
3.	Alkaloid	-	-	-	-	-
4.	Flavonoid	-	-	-	-	-
5.	Terpenoid	-	+	+	+	-
6.	Saponin	-	-	-	-	+

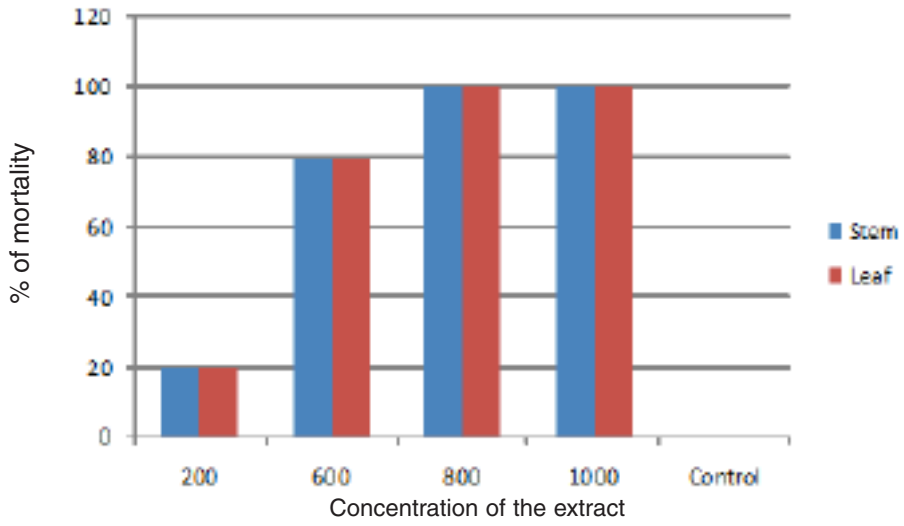


Fig. 1: Larvicidal and antifeedent effect of *Hellula undalis* larvae on different concentrations of the plant extract

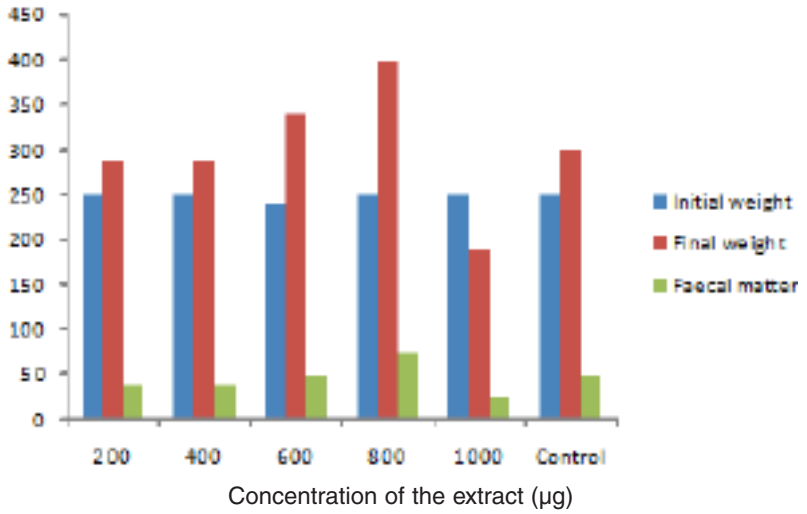


Fig. 2: Effect of different concentration on *Spodoptera litural* larvae

species of plants like cotton, tobacco, chilli, castor, pulse crops, groundnut, tomato and minor spice crops, these extracts can be exploited for the control of this pest. (Sahayaraj, 1998).

The plant extract showed only antifeedant effect on the Brinjal fruit borer (Leucinodes arbonalis). Even in higher concentration (1000 µg) no mortality was reported.

Phytochemical analysis

Phytochemical tests showed positive for Saponin, Carbohydrates, Tannins and Terpenoids, whereas Anthraquinone, Cardiac glycosides, Phytosterols, Phlobatannin, Flavonoids and Phenol were absent or present in minute quantities in both leaf and stem extracts. (Table 6).

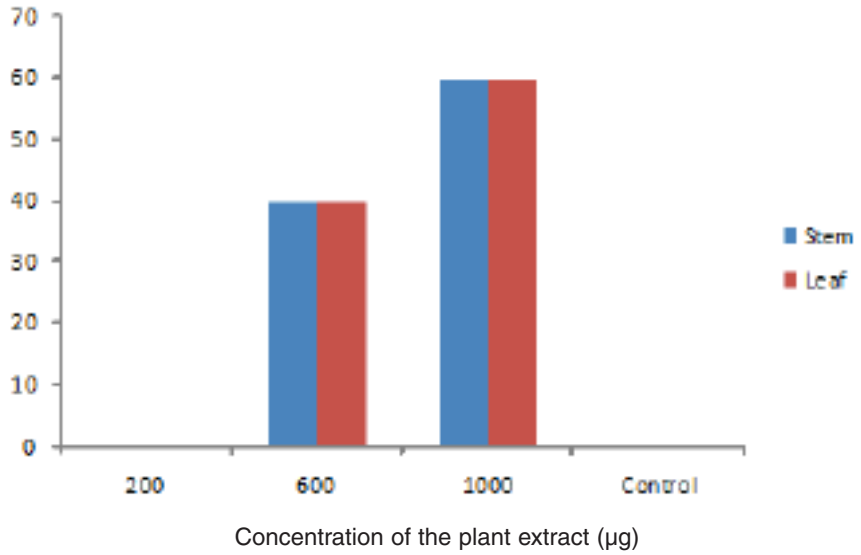


Fig. 3: Effect of plant extract on *Leucinodes arbonalis,* fruit borer of Brinjal

TLC

The developed chromatogram showed five different bands in the used solvent system. The Rf values calculated ranges from 0.25 cm to 0.97 cm.

TLC analysis

The separated bands when tested by phytochemical tests, the secondary metabolites like tannin, steroid, terpenoid and saponins answered positive and the other secondary metabolites were found to be negative. The terpenoid compounds reported maximum % because three bands answered positive for terpenoid compounds.

Therefore the overall results observed showed definite antifeedant activity of plant extract and for some worms this has also showed Larvicidal activity as the earlier investigations studied. (Gopiesh Khana and Kannabiran, 2007)

However, the utilization of these plant extracts for the control of these insects can only be determined through actual field trials.

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