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RESEARCH ARTICLE

BIOEFFICACY OF *HYPTIS SUAVEOLENS* AND *MELOCHIA CHORCORIFOLIA* AGAINST THE ARMYWORM, *SPODOPTERA LITURA* (FAB.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The objective of the present study was to evaluate antifeedant, insecticidal, oviposition deterrent and ovicidal activity of different fractions obtained from the crude extracts of *H. suaveolens* (Lamiaceae) and *M. corchorifolia* (Sterculiaceae) against *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae), four fractions obtained from *H. suaveolens*, fraction III was found to inhibit the feeding ratio of the *S. litura* and it is apparent from the table. While in *M. corchorifolia* only three fractions have been obtained, among them fraction II was found to induced more feeding deterrent activity at 2000 ppm concentration. Ethylacetate extract of *H. suaveolens* yielded four fractions and diethylether extract of *M. corchorifolia* yielded five fractions. As per the data fraction III showed statistically significant ovicidal activity. It is also interesting to note that the same fraction was exerted strong ovipositional deterrent activity. Whereas, ethylacetate extract of *M. corchorifolia* yielded five fractions of which, fraction III showed increased ovicidal activity over the other fractions at higher concentration. Larvicidal activity obtained from the fraction II was significant than the other two fractions of the same extract. The larvicidal activity exhibited by the five fractions of diethyl ether extract of *M. corchorifolia* is shown in table 5. It is clear that fraction III at 1000 and 2000-ppm concentration was found to have increased activity than the other fractions.

INTRODUCTION

The development of integrated pest control programs in controlling the economically important pest, *Spodoptera litura* (Fab.) has gained increased attention in many parts of the world (Zalucki *et al.,* 1986; 1994; Fitt, 1989). *S. litura* is highly polyphagous pest, and this is reflected in the wide taxonomic range of wild and cultivated plants acceptable for oviposition by adults and feeding by larvae (King, 1994). This notorious pest initially feeds on vegetative parts and subsequently on immature pods and ultimately causes severe loss of production. However, many chemicals available for treatment of insect pest are also toxic to natural enemies and gradually the pest will develop resistance to it (Ravikumar *et al*. 1999). Furthermore, indiscriminate use of chemical pesticides results in biomagnifications through food chains subsequently causing various hazardous effects. Use of pesticides that are least harmful to beneficial organisms can potentially ameliorate this problem (Jepson, 1989; croft, 1990; Abdul Kareem 1999). Plants (botanicals) have synthesized a wide array of chemicals to prevent herbivore attack. Botanicals have the advantage of being biodegradable without causing pollution and are safer to the environment (Cunat *et al*. 1990). The objective of the present study was to evaluate antifeedant, insecticidal, oviposition deterrent and ovicidal activity of different fractions obtained from the crude extracts of *H. suaveolens* (Lamiaceae) and *M. corchorifolia* (Sterculiaceae) against the armyworm, *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae).

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MATERIALS AND METHODS

Collection and Solvent Extraction of selected plants

Hyptis suaveolens leaves were collected in September 2005 at Vellore District, and the whole plant of *Melochia corchorifolia* was collected at Salem and Namakkal districts of Tamil Nadu, India. The plant materials were shade dried, powdered using electric blender and sieved through kitchen strainer. 500g of the plant powder was sequentially extracted with organic solvents such as hexane, diethylether, dichloromethane, ethylacetate, methanol and water. Based on their performance against *S. litura* (Elumalai *et al.,* 2002) diethyl ether, ethyl acetate and methanol were identified as potential crude extracts and they were further fractionated with different solvent system using TLC (Table 1). As per TLC known amount of crude extract was loaded in glass column chromatography and were tested for their antifeedant, ovipositional deterrent, ovicidal and larvicidal activity

Table 1. Number of fractions identified and isolated from the selected plants using TLC and Column Chromatography

Solvents	*H. suaveolens*		*M. corchorifolia*	
	NF	Solvents ratio	NF	Solvents ratio
Diethyl ether	4	(Ben: Chl - 1:1)	3	(Ben: Chl - 1:1)
Ethyl acetate	4	(Chl: Met – 9.5:0.5)	5	(Chl: Met – 9.5:1)
Methanol	3	(Ben: Chl – 1:1)	4	(Ben: Chl.: Met – 1:1:1)

NF – Number of Fractions obtained; Ben – Benzene; Chl – Chloroform
Met - Methanol

Table 2. Antifeedant activity of *Hyptis suaveolens* diethyl ether extract fractions against IV instar larvae of *S. litura*

Fractions	Concentrations (ppm) *Hyptis suaveolens*		*Melochia chorcorifolia*	
	1000	2000	1000	2000
Fraction I	54.69^{bc} (47.64)	76.77^{a} (61.14)	37.15^{c} (37.52)	52.5^{b} (46.43)
Fraction II	47.35^{d} (43.45)	65.64^{d} (54.09)	51.6^{b} (49.52)	70.85^{a} (57.29)
Fraction III	63.74^{b} (52.95)	83.43^{c} (65.96)	40.2^{c} (39.35)	55.5^{b} (48.16)
Fraction IV	57.63^{b} (49.37)	61.8^{d} (51.83)	--	--
Azadirachtin 1% (2ml/l)	74.64^{a} (59.74)	74.64^{a} (59.74)	74.64^{a} (59.74)	74.64^{a} (59.74)

- fraction not obtained
Values are mean of five replications and parentheses hold angular transformation. Within the column different alphabets are statistically significant as per LSD test (p=0.005).

Table 3. Ovipositional deterrent activity of *Hyptis suaveolens* ethyl acetate extract and *Melochia chorcorifolia* diethyl ether fractions against *S. litura*

Fractions	Concentrations (ppm) **Hyptis suaveolens**		**Melochia chorcorifolia**	
	1000	**2000**	**1000**	**2000**
Fraction I	36.44^{c} (37.11)	42.66^{d} (40.74)	29.3^{c} (32.77)	42.55^{d} (40.69)
Fraction II	37.6^{c} (37.82)	47.75^{d} (43.68)	42.7^{b} (40.51)	55.6^{b} (48.22)
Fraction III	49.5^{b} (44.71)	64.73^{d} (53.55)	27.7^{cd} (31.76)	38.85^{d} (38.23)
Fraction IV	32.2^{c} (34.57)	40.61^{de} (39.58)	32.47^{c} (34.7)	47.25^{c} (43.39)
Fraction V	--	--	25.95^{cd} (30.59)	35.77^{de} (36.69)
Azadirachtin 1% (2ml/l)	65.88^{a} (54.21)	65.88^{a} (54.21)	63.75^{a} (52.95)	63.75^{a} (52.95)

- fraction not obtained
Values are mean of five replications and parentheses hold angular transformation. Within the column different alphabets are statistically significant as per LSD test (p=0.005).

against *S. litura* larvae and adults used for the experiments were collected from the stock culture.

Antifeedant activity

Antifeedant activity of the different fractions isolated from plant extracts were studied using leaf disc (no – choice) bioassay method. Fresh cotton leaf discs of 3-cm diameter were dipped in 250, 500, 1000 and 2000 ppm concentration of different fractions. Cotton leaf discs treated with neem product. Azadirachtin and without treatment were considered as negative and positive controls. In each petridish, single fourth instar larva of *S. litura* was introduced individually for antifeedant activity. Progress consumption of leaf area by insect after 24 hrs was recorded in control and treated discs. Leaf areas consumed in treated leaf discs were corrected from the control leaf (Blaney *et al.,* 1984).

Oviposition deterrent activity

Oviposition deterrent activities were studied at 250, 500, 1000 and 2000 ppm concentration of the fractions. The different fractions with respective concentrations were

Table 4. Ovicidal activity of *Hyptis suaveolens* and *Melochia corchorifolia* ethyl acetate extract fractions against *S. litura*

Fractions tested	Concentrations (ppm)			
	Hyptis suaveolens		Melochia chorcorifoli	
	1000	2000	1000	2000
Fraction I	15.45^{c} (23.11)	20.83^{cd} (27.13)	20.5^{cd} (26.92)	24.45^{d} (29.6)
Fraction II	18.35^{c} (25.33)	23.71^{c} (29.13)	28.88^{b} (32.46)	30.44^{c} (33.46)
Fraction III	23.64^{b} (29.06)	42.53^{b} (40.69)	24.44^{c} (29.6)	37.55^{b} (37.76)
Fraction IV	16.77^{c} (24.12)	25.44^{c} (30.26)	21.25^{c} (27.42)	27.9^{c} (31.88)
Fraction V	--	--	18.6^{cd} (25.55)	23.88^{d} (29.2)
Azadirachtin 1% (2ml/l)	74.88^{a} (59.87)	74.88^{a} (59.87)	73.82^{a} (59.21)	73.82^{a} (59.21)

- fraction not obtained Values are mean of five replications and parentheses hold angular transformation. Within the column different alphabets are statistically significant as per LSD test (p=0.005).

Table 5. Larvicidal activity of *Hyptis suaveolens* methanol extract and *Melochia corchorifolia* diethyl ether extract fractions against IV instar larvae of *S. litura*

Fractions	Concentrations (ppm)			
	Hyptis suaveolens		*Melochia chorcorifolia*	
	1000	2000	1000	2000
Fraction I	30.19^{bc} (33.27)	63.82^{b} (53.01)	30.09^{b} (33.21)	53.48^{bc} (46.95)
Fraction II	40.26^{b} (39.35)	77.2^{a} (61.48)	33.41^{b} (35.3)	60.2^{b} (50.89)
Fraction III	50.33^{b} (45.17)	53.75^{b} (47.12)	36.82^{b} (37.35)	63.51^{b} (52.83)
Fraction IV	_	_	30.1^{b} (33.27)	53.47^{bc} (46.95)
Fraction V	_	_	15.89^{c} (23.4)	33.41^{d} (35.3)
Azadirachtin 1% (2ml/l)	79.62^{a} (63.15)	79.62^{a} (63.15)	78.94^{a} (62.65)	78.94^{a} (62.65)

- fraction not obtain Values are mean of five replications and parentheses hold angular transformation. Within the column different alphabets are statistically significant as per LSD test (p=0.005).

sprayed on pot cultured cotton plant (leaves) along with selected controls as mentioned in the earlier experiments. Ten pairs of *S. litura* were introduced in a cage containing cotton leaves. Honey solution with multivitamin was provided for adult feeding. After 48 hrs the number of eggs laid by *S. litura* were recorded in both treated and control leaves and the percentage of oviposition deterrence was calculated (Williams *et al*. 1986).

Ovicidal activity

100 freshly laid and 4 hrs old eggs of *H. armigera* were dipped in different fractions of the selected concentrations as mentioned earlier. Eggs with black spot stage were considered as unhatched and then number of hatched and unhatched eggs in control and treated eggs were recorded. Percentage of ovicidal activity was calculated by the formula of Abbott (1925).

Larvicidal activity

Fresh cotton leaves were treated with 250, 500, 1000 and 2000-ppm concentration of different fractions and the controls were provided as that of antifeedant studies. Treated leaves were placed in plastic petriplates; in each petriplates, single fourth instar larva of *H. armigera* was introduced. The number of dead larvae was recorded after 48 hrs and percentages of larval mortality was calculated and corrected by Abbott (1925) formula. Five replicates were maintained for all the concentrations in each experiment. In all the experiments fractions at 1000 and 2000 ppm concentration showed marked activities and hence only those two concentrations were emphasized. The percentage values were angular transformed before two-way analysis of variance and Least Significance Difference (LSD) value was calculated and presented in

the table to separate the mean difference within the different fractions tested.

RESULTS AND DISCUSSION

Antifeedant activity of diethylether extract fractions of *H. suaveolens* and *M. corchorifolia* against *S. litura* are presented in table 2. Among the four fractions obtained from *H. suaveolens*, fraction III was found to inhibit the feeding ratio of the *S. litura* and it is apparent from the table. While in *M. corchorifolia* only three fractions have been obtained, among them fraction II was found to induced more feeding deterrent activity at 2000 ppm concentration. Besides, the antifeedant activity of fractions 1 and 2 are less significant as evidenced from the table (LSD; p=0.005). In the recent past environmental degradation due to consecutive application of chemicals to control the various insect pest, threatened the scientific community worldwide. The use of botanicals is one of the components of Integrated Pest Management (IPM) proved to be the promising tool in controlling the storage as well as the field pests. This indicates that the active principles present in the plant dissolved in this solvent may inhibit the larval feeding behavior or makes the food unpalatable. Earlier Hermawan *et al.,* (1994) reported that the plant extract of *Andrographis paniculata* effectively reduced the feeding of fourth stadium diamond black moth larvae (*Plutella xylostella).* Further antifeedant agent from the neem seed kernel extract (NSKE) reduced feeding rate of *H. armigera* resulted in lowest pod and grain damage (Sarode *et al.,*1995; Shanower *et al*., 1997).

The ovipositional deterrent activity of *H. suaveolens* and *M. corchorifolia* against *S. litura* are presented in table 3. Ethylacetate extract of *H. suaveolens* yielded four fractions and diethylether extract of *M. corchorifolia* yielded five fractions. In *H. suaveolens,* fraction III showed increased ovipositional deterrent activity against the gravid moths of *S. litura* at 2000 ppm concentration, when compared to the control the activity was less but in comparison with other fraction it showed significant increase as evidenced from the data. The ovipositional deterrent activity of different fractions of *M. corchorifolia* revealed that, fraction II was found to have more deterrent activity against the gravid moths of *S. litura* and their significance are apparent. It may due to the consequence volatile present in the solvent extract which makes malfunctioning of the ovariole in female moths. Earlier, Hermawan *et al*. (1998) reported that the leaf extract of *Andrographis paniculata* significantly reduced the egg laying performance of the diamond back moth *Plutella xylostella.* Our findings are also corroborating with the reports of Jeyabalan and Murugan (1997), Patel *et al.,* (1994) and Mehta *et al.,* (1994).

Ovicidal activity against the eggs of *S. litura* by the four fractions obtained from the ethylacetate extract of *H. suaveolens* is given in table 4. As per the data fraction III showed statistically significant ovicidal activity. It is also interesting to note that the same fraction was exerted strong ovipositional deterrent activity. Whereas, ethylacetate extract of *M. corchorifolia* yielded five fractions of which, fraction III showed increased ovicidal activity over the other fractions at higher concentration (Table 4). This might be due to interference with the egg contour and cytoplasmic disintegration. The disturbance of these extracts with egg morphology may plug the micropyles of the chorion thereby preventing the airflow in and out vice versa. The disturbances with egg cytoplasm was reflected in the form of dead eggs with black spot stage and it seems to be arresting of further development of embryo inside the egg. Bhatnagar and Sharma (1994) noticed similar anatomical and physiological disturbances of plant extracts on maize stem borer, *Chilo partellus*.

Methanol extract of *H. suaveolens* yielded three fractions and their larvicidal activity against the fourth instar larvae of *S. litura* is presented in table 5. Larvicidal activity obtained from the fraction II was significant than the other two fractions of the same extract. The larvicidal activity exhibited by the five fractions of diethyl ether extract of *M. corchorifolia* is shown in table 5. It is clear that fraction III at 1000 and 2000-ppm concentration was found to have increased activity than the other fractions. Larvicidal activity was mainly depends on the presence of toxic materials in the plant extracts. Both the plants selected in the present study is non toxic and the plant materials are used for cattle feed in many Indian villages. The present study on insecticidal activity did not show any remarkable findings. Further synergistic studies may enhance the insecticidal activity of the plant extracts and reduced the concentration of pesticides. Manoharan and Uthamasamy (1993) reported that addition of *A. indica* oil to endosulfan and phosalone increased the mortality of larvae compared with the insecticides alone.

Rajasekhar *et al.* (1996) reported that *Parthenium* leaf extract in combination with endosulfan and cypermethrin used for the control of *H. armigera*. In addition Durairaj and Venugopal (1995) were also reported that neem oil 2% + Diflubenzuron 0.03%was found to have significant antifeedant effect and early larval mortality in *H. armigera*. Jaglan *et al.,* (1997) reported that the neem seed and leaf extracts of chloroform:methanol (9:1) extract was the most promising in causing larval mortality with adverse morphogenic effects on various biological parameters of *H. armigera.* In our studies we used only one solvent at time for extraction. Further combination of more than solvents in certain ratio may enhance the larvicidal activity of the selected plants.

Present study methanol extract showed some insecticidal activity. It may due to reduction in the total protein content which is a major component for the metamorphosis of the larval instars, this was clear that the dead larvae showed the symptom of improper metamorphosis from one instar to another instar. This result is also coinciding with the findings of Krishnayya and Rao (1995) who had been reported that the application of plumbagin greatly reduced the protein concentration of *H. armigera*. In the present study *H. suaveolens* and *M. chorcorifolia* exhibited one or more statistically significant activity against *S. litura*. It may include IPM programme for controlling the *S. litura* . The present findings paves the way for purification and identification of the effective principle / compound to control this important polyphagous pest. Further suggest the value of exploring other Indian plants in search for new, environmentally acceptable pest control agents for *S. litura.*

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