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RESEARCH ARTICLE

BIOEFFICACY OF NEEM OIL FORMULATION WITH *HYDNOCARPUS ALPINA* LEAF EXTRACT AGAINST *SPODOPTERA LITURA*

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ABSTRACT

In a laboratory experiment, pesticidal effect of hexane, chloroform and ethyl acetate extracts of *Hydnocarpus alpina* Wt. (Flacourtiaceae) leaves was evaluated against Asian army worm *Spodoptera litura* Fab. at different concentrations viz., 0.625, 1.25, 2.50 and 5.00 per cent. Feeding inhibitory and insecticidal activities were determined by leaf disc method. Among the tested extracts, ethyl acetate extract of *H. alpina* showed maximum antifeedant (72.2%) and larvicidal (66.6%) activities at 5 per cent concentration. Ethyl acetate extract was subjected to fractionation using hexane, ethyl acetate, acetone and methanol solvents by column chromatography and thin layer chromatography. Ten fractions were obtained and were further screened at four different concentrations viz., 125, 250, 500 and 1000 ppm. Among the tested fractions, the eighth fraction showed maximum antifeedant (68.5%) and insecticidal (62.5%) activities at 1000 ppm concentration. Phytochemical analysis of this fraction showed that it contained flavonoids, phenols and triterpenoids. An oil formulation of the eighth fraction with neem oil recorded 78.6 per cent antifeedant and 82.4 per cent larvicidal activities at 100 ppm concentration, which was superior over individual fraction and neem oil treatments.

INTRODUCTION

Asian armyworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is a highly polyphagous, economically important pest in Southeast Asia, India, China, and Japan (Hummelbrunner and Isman, 2001). It infests nearly 150 host plant species including cotton, bendhi, tomato, soybean and sunflower (Rao et al., 1993). In India, 40 species of cultivated crops, wild plants and 11 flowering plants are affected by this pest (Ali et al., 1999). *S. litura* has developed resistance against many synthetic insecticides (Kranthi et al., 2002). The absence of resistance to *S. litura* in host plants and the lack of adequate control measures make it difficult to manage this pest in the fields. Several plant products have been tested against *S. litura* and some promising plants have been reported by many investigators. However the screening of plant extracts against this pest is still continuing throughout the world to find out different kinds of effects of botanicals on this pest and to obtain an ecofriendly and economic biopesticide. The present study was carried out to check the efficacy of *Hydnocarpus alpina* leaf extracts against *S. litura* larva. *H. alpina* is a member of the Flacourtiaceae family, commonly known as 'torathi'. It is traditionally used in the treatment of leprosy (Lima *et al*., 2005).

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MATERIALS AND METHODS

Insects

Newly moulted third instar *S. litura* larvae obtained from a stock culture that was maintained in the laboratory on castor leaves at laboratory conditions (28 ± 2^0C; 57-65% RH; 11 ± 1 hr photoperiod) were used for the study.

Plant collection and extraction

Fresh leaves of *H. alpina* were collected from Vellangiri hills, Coimbatore district, Tamil Nadu. The collected leaves were washed in water, shade dried at room temperature and ground into powder using an electric blender. About 2 kg powder was sequentially extracted with 6 L of hexane, chloroform and ethyl acetate after 72 h soaking. The extract was filtered through filter paper and the resultant extract was concentrated under reduced pressure using rotary vacuum evaporator.

Fractionation

The effective extract, (ethyl acetate extract) was subjected to column chromatography in a silica gel (200 g-acme's 100–200 mesh) glass column. About 24.67 gm crude extract was loaded on the silica gel in the column and eluted with hexane followed by the combination of hexane: ethyl acetate ranging from 95:5 to 0:100. This was followed by the elution with acetone and methanol solvents. The fractions were collected in 200 ml conical flask and concentrated using rotary vacuum evaporator. All the collected fractions (257 fractions) were subjected to thin layer chromatography (TLC) using precoated silica

gel (Merk-60 F254, 0.25mm thick) plate and similar fractions with the Rf values were pooled and finally 10 fractions were obtained.

Phytochemical analysis tests

Phytochemical analysis of the isolated fractions from ethyl acetate extract of *Hydnocarpus alpina* leaves for secondary metabolites such as flavonoids, alkaloids, triterpenoids, tannins, phenols, saponins, quinines, anthraquiones, coumarins and steroids was done using standard methods as described by Horborne (1984).

Treatments and concentrations

The crude extract and fractions were dissolved in acetone to prepare different concentrations viz., 0.625, 1.25, 2.5 and 5 per cent (for crude extract) and 125, 250, 500 and 1000 ppm (for fractions and azadirachtin). Azadirachtin (EID Parry) was included as a reference control. A solvent control (acetone) was also maintained separately.

Oil formulation

Neem oil based formulation was prepared by mixing the effective fraction with neem oil, emulsifier and stabilizer. In a second formulation, neem oil, emulsifier and stabilizer alone were mixed and used for comparison. The method of preparation of oil formulation was the same that of Mariapackiam *et al.,* (2007). The combinations of the oil formulations were as follows:

Formulation 1 (Fn1): Neem oil (89%) + active fraction (1000 ppm) + emulsifier (DMA-NE, Unitop) (10%) +Stabilizer (1%)

Formulation 2 (Fn2): Neem oil (89%) + emulsifier (10%) + Stabilizer (1%)

A commercial neem based pesticide Vijay neem (0.03% EC) was used to compare with the oil formulation of *H. alpina* fraction. To check the effect of stabilizer and emulsifier, which were added in the oil formulation, they alone were used as control. Four different concentrations viz., 12.5, 25, 50 and 100 ppm of oil formulations were prepared in water for bioassay experiments.

Antifeedant bioassay

The antifeedant activity of extracts, fractions and oil formulations was determined by using leaf disc no-choice method of Isman *et al.* (1990). Castor (*Ricinus communis*; Euphorbiaceae) leaf discs (4cm diameter) were cut by a cork borer. The leaf discs were dipped separately in different concentrations of different treatments for 2min and air dried for 10min. The treated and control discs were separately put inside a petridish and one newly moulted third instar, 4 h pre-starved *S. litura* larva was introduced on one leaf disc taken in the petridish. Progressive consumption of leaf area by the larva after 24 h feeding was recorded in control and treated discs using leaf area meter (Delta-T Devices, Serial No. 15736 F 96, UK). The experiments were replicated 15 times and feeding deterrence index for all concentrations of crude, fractions and oil formulations were calculated using the formula of Bentley *et al.,* (1984): (C – T) / (C) x 100, where C is leaf consumption in control and T is consumption in treated discs.

Larvicidal bioassay

In a separate set of experiments, third instar *S. litura* larvae were orally treated with different concentrations of crude extract, fractions and oil formulations through castor leaf discs as mentioned above in the antifeedant experiment. After 24 h treatment, the larvae were fed daily with untreated fresh leaves and larval mortality was recorded for 96h. Fifteen replicates were maintained for each control and treatments. The mortality was adjusted by Abbott's correction factor (Abbott, 1925).

Statistical analysis

The significance of treatments was found out by one way Analysis of Variance (ANOVA) and effective treatment was separated by Tukey's multiple range test. Differences between means were considered significant at $P< 0.05$.

RESULTS

Antifeedant and larvicidal effects

The antifeedant activity of crude extracts of *H. alpina* is presented in Table 1. Among the crude extracts ethyl acetate extract was identified as the most effective with 72.24 percent antifeedant activity at 5 percent concentration. The antifeedant activity of all the three extracts was concentration dependent. The extracts were also toxic to the test insect. Ethyl acetate extract showed maximum larvicidal activity (66.67%) at 5 % concentration (Figure 1).

The ethyl acetate extract was subjected to column chromatography and 10 fractions were obtained. The antifeedant bioassay tests using the fractions on *S. litura* clearly showed that the eighth fraction was very effective (Table 2). At 1000ppm concentration, eighth fraction showed 68.53 per cent antifeedant activity and at 125 ppm concentration it showed 40.59% antifeedant activity. Figure 2 shows the larvicidal activities of isolated fractions. Among the tested fractions at different concentrations eighth fraction registered maximum larvicidal activity (62.51%) at 1000 ppm concentration. The antifeedant and larvicidal activities of all tested fractions were significantly less than that of reference control azadirachtin. Eighth fraction had promising antifeedant and larvicidal activity among the tested fractions.

Neem oil based formulations inhibited the feeding activities of *S. litura* larvae (Table 3). As the concentration increased, the antifeedant activity also increased. Among the tested formulations, formulation 1 showed the highest feeding deterrent activity (78.61%) at 100 ppm concentration. Formulation 2 and commercial neem-based pesticide recorded 64.35 and 69.52 percent antifeedant activity respectively at 100 ppm concentration. Figure 3 shows the larval mortality produced by the oil formulation and commercial pesticide. Larval mortality was maximum in formulation 1. At 100 ppm concentration 82.5 per cent mortality was recorded in formulation 1.

Phytochemical analysis of the fractions

The results of preliminary phytochemical screening of the isolated fractions from the ethyl acetate extract of *H. alpina* leaves are presented in Table 4. Fraction 1 to 4 showed the presence of coumarins, quinones, steroids and tannins; fraction 5 to 7 showed the presence of anthraquinones, phenols, quinones and tannins and fraction 8 to 10 showed the presence of flavonoids, phenolics and tannins.

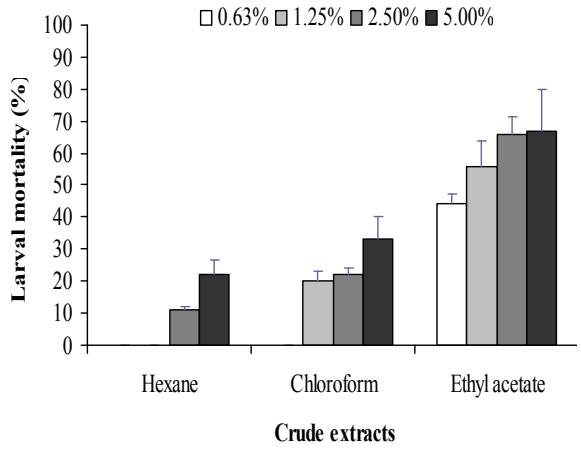

Figure 1. Larvicidal activity of crude extracts of Hydnocarpus alpina leaves against Spodoptera litur.

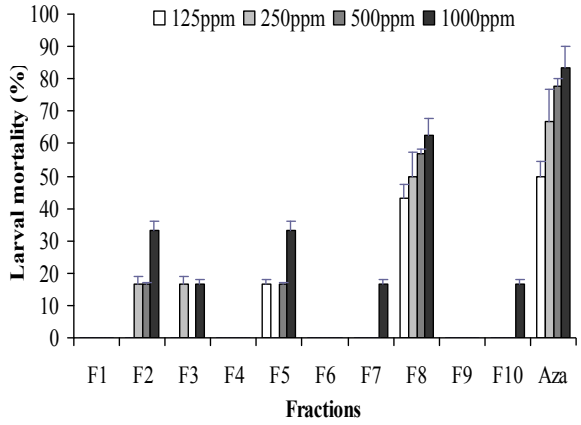

Figure 2. Larvicidal activity of ethyl acetate extract fractions of Hydnocarpus alpina leaves against Spodoptera litura.

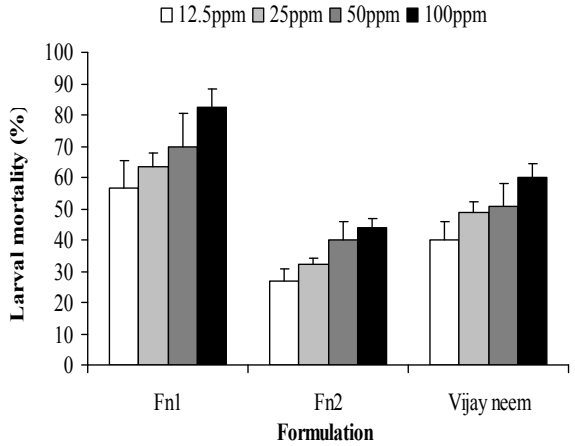

Figure 3. Larvicidal activity of neem oil based formulation of active fraction of ethyl acetate extract of Hydnocarpus alpina leaves against Spodoptera litura

DISCUSSION

In insect-plant interactions, insects often have unique adaptation to their host plants in locating and selecting the plants by the use of chemical, visual and mechanical cues (Schoonhoven *et al.*, 1998). According to Mustaparta (2002), unsuitable plants are avoided by detection of other chemical cues; such chemical substances may have repellent or toxic properties against insects. Based on that principle; botanical pesticides are invented and utilized for control of insect pests. Accordingly, crude extracts of *H. alpina* (i.e., hexane, chloroform and ethyl acetate) were initially screened for the identification of pesticidal potentiality of the plant. Antifeedants offer first line of crop protection against notorious insects. According to Isman (2002) any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent. In general antifeedants have profound adverse effects on insect feeding behavior (Hummelbrunner and Isman, 2001).

In the present study it was observed that ethyl acetate extract of *H. alpina* highly deterred the feeding activity of *S. litura* larvae. Eventhough the feeding was inhibited by the active fraction and the larvae consumed less food on plant extract treated leaves, the botanical exerted lethal effect on the larvae. This result indicates the presence of larval toxin in the ethyl acetate extract of the *H. alpina*. Similarly Raja *et al*. (2004), Susuruk *et al.* (2007), Malarvannan *et al.* (2008) and Samarasinghe *et al.* (2008) have shown that ethyl acetate extract was an effective treatment with maximum feeding deterrent activity against lepidopteran pests. Crude extracts from the leaf, stem, root and seeds of various plant species have been reported to possess antifeedant, insecticidal, and/or growth inhibitory properties (Ekesi, 2000). Bioactivity guided fractionation of complex mixture of phytochemicals leads to separation of simple mixtures of phytochemicals (Hostettmann and Wolfender, 1999). Crude extracts of plants often consist of complex mixtures of active principles (Leatemia and Isman, 2004). Hummelbruner and Isman (2001) reported that synergistic effects of complex mixtures of phytochemicals are also thought to be important in plant defenses against insect herbivory.

Neem oil formulation with eighth fraction of ethyl acetate extract of *H. alpina* (Fn1) showed synergistic activity. Inhibitory effects were increased with increasing concentration of treatments on *S. litura*. Russell and Lane (1993) reported that antifeedants are being successfully used in commercial insect pest management strategies. Hummelbrunner and Isman (2001) have reported that the exposure of several plant chemicals to the insects caused delayed larval development through decreased growth rates. Telang *et al*. (2003) stated that, malformed adult insects were produced as a result of plant toxin treatments and the insects were also short-lived and infertile. These effects could be considered important in the pest population reduction.

In conclusion, the present study has shown the antifeedant and larvicidal potential of *H. alpina* plant. The neem oil based formulation that contained active fraction of *H. alpina* could be considered an eco friendly botanical pesticide.

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Table 1. Inhibitory effect of crude extracts of *Hydnocarpus alpina* leaves on feeding activity of *Spodoptera litura*. (Mean ± SE) (n=15)

Extract	*Concentration (%)*			
	0.625	**1.25**	**2.50**	**5.0**
Hexane	11.34 ± 3.15^{b}	18.74 ± 2.62^{c}	29.78 ± 2.18^{c}	38.57 ± 4.62^{c}
Chloroform	6.44 ± 2.03^{ab}	10.29 ± 3.39^{b}	18.71 ± 4.58^{b}	26.22 ± 4.12^{b}
Ethylacetate	49.15 ± 4.07^{c}	56.77 ± 5.59^{d}	65.67 ± 7.19^{d}	72.24 ± 6.25^{d}
Control	3.34 ± 2.28^{a}			

Values in columns followed by the same letters are not significantly different (Tukey's test; $P \leq 0.05$).

Table 2. Inhibitory effect of ethyl acetate extract fractions of *Hydnocarpus alpina* leaves on the feeding activity of *Spodoptera litura*. (Mean ± SE) (n=15)

Fractions	*Concentration (ppm)*			
	125	**250**	**500**	**1000**
F1	6.84 ± 2.45^{ab}	8.66 ± 1.71^{abc}	9.59 ± 2.31^{bc}	12.57 ± 2.36^{bcd}
F2	5.38 ± 4.33^{ab}	6.02 ± 3.38^{ab}	7.82 ± 2.16^{bc}	9.86 ± 2.70^{bc}
F3	9.39 ± 3.79^{bc}	10.95 ± 5.72^{bc}	12.37 ± 3.06^{cd}	16.04 ± 2.19^{cde}
F4	4.78 ± 2.17^{ab}	7.43 ± 4.29^{ab}	7.57 ± 2.47^{abc}	12.45 ± 5.76^{bcd}
F5	19.94 ± 2.83^{d}	24.54 ± 3.91^{d}	29.35 ± 4.71^{e}	34.88 ± 3.33^{f}
F6	4.37 ± 2.16^{ab}	5.80 ± 4.15^{ab}	5.65 ± 2.42^{ab}	7.28 ± 1.63^{ab}
F7	9.63 ± 2.50^{bc}	10.63 ± 2.11^{bc}	12.37 ± 5.52^{cd}	19.39 ± 6.55^{de}
F8	40.59 ± 4.64^{e}	44.02 ± 3.44^{e}	52.27 ± 2.47^{f}	68.53 ± 4.85^{g}
F9	8.17 ± 2.47^{abc}	8.55 ± 4.27^{abc}	11.17 ± 2.79^{bcd}	12.34 ± 3.86^{bcd}
F10	13.91 ± 3.20^{cd}	15.50 ± 2.27^{c}	16.64 ± 2.50^{d}	21.47 ± 3.77^{e}
Azadirachtin (40.86%)	58.17 ± 6.43^{f}	68.16 ± 4.83^{f}	76.81 ± 3.19^{g}	84.69 ± 4.18^{h}
Control	1.81 ± 0.90^{a}			

Values in columns followed by the same letters are not significantly different (Tukey's test; $P \leq 0.05$).

Table 3. Inhibitory effect of neem oil based formulation of active fraction of ethyl acetate extract of *Hydnocarpus alpina* leaves on the feeding activity of *Spodoptera litura* larvae. (Mean ± SE) (n=15)

Formulation	*Concentration (ppm)*			
	12.5	**25**	**50**	**100**
Formulation 1	51.37 ± 2.50^{c}	59.15 ± 4.62^{c}	66.90 ± 3.44^{c}	78.61 ± 6.53^{c}
Formulation 2	35.15 ± 4.62^{b}	41.25 ± 2.65^{b}	50.47 ± 7.37^{b}	64.35 ± 4.25^{b}
Vijay neem (commercial neem-based pesticide)	42.73 ± 3.73^{bc}	48.63 ± 3.37^{bc}	56.76 ± 2.55^{bc}	69.52 ± 2.36^{bc}
Control (emulsifier+stabilizer +water)	9.34 ± 2.46^{a}			

Values in columns followed by the same letters are not significantly different (Tukey's test; $P \leq 0.05$).

Table 4. Phytochemical tests for ethyl acetate extract fractions of *Hydnocarpus alpina.*

Phytochemicals	**Fractions**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Alkaloids	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	+	+	-	-	-	-
Coumarins	+	+	+	+	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	+	+	+
Phenols	-	-	-	-	+	+	+	+	+	-
Quinones	-	-	-	+	+	+	+	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	-	-	-	-	-	-
Tannin	-	-	+	+	+	-	-	-	+	+
Triterpenoids	-	-	-	-	-	-	+	+	+	+

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