Efficacy of *Cipadessa baccifera* leaf extracts against gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT

***Cipadessa baccifera*, a common medicinal plant of Western Ghats was evakyated fir insecticidal property against *Helicoverpa armigera*. The different extracts of leaves differed significantly in their efficacy with the hexane extract being the most effective in curtailing the fecundity and egg hatchability in the first generation adults. The insecticidal effect against the resultant progeny proved that petroleum ether extract could cause significant reduction in pupation and pupal weight and higher percentage of malformed adults.**

Cipadessa baccifera Miq., belonging to Meliaceae, is a small tree or bushy shrub with pinnate leaves and small flowers. The plant is used traditionally for fuel, as tooth brush, leaf and fruit as cattle fodder and fish poison (personal communication with tribals of Kolli Hills). Medicinally, it is reported to cure piles, diabetes, diarrhea, food poison and head ache (Amit and Shailendra, 2006). It is distributed in North Circas, common on laterite hills, near villages and in dry forests; Deccan, chiefly in hilly country, up to 4,000 ft; Western Ghats, in forest undergrowth up to 5,000 ft (Gamble, 1987).

Four compounds mainly limonoids, *viz*., cipadesin, 17a, 20R-dihydroxypregnan-3, 16-dione, 1,4-epoxy-16-hydroxyheneicos- 1,3,12,14,18-pentaene and 1,4-epoxy-16-hydroxyheneicos - 1,3,12,14-tetraene have been isolated from *C. baccifera* earlier (Luo *et al*., 2000). Limonoids have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Amit and Shailendra, 2006). In particular, cipadesin showed insecticidal activity against the leaf cutting ants, *Atta sexdens rubropilosa* (Leite *et al*., 2005).

Insect pests pose serious threat to crop production and cause 5-15% loss in yield world over (Banerjee, Das and Hui, 2000). Among them, American bollworm, *Helicoverpa armigera,* a polyphagous noctuid is a major pest in India from late seventies (Reed and Pawar, 1982; Guo, 1997). Devastating 181 plant species (of 39 families) (Manjunath *et al.,* 1989), the pest causes an annual loss of US $300 million in pigeon pea and chick pea alone in India (Jayaraj *et al.,* 1990). The miticulous usage of chemicals resulted in insect resurgence, insecticide resistance, erosion of existing natural enemies and environmental pollution (Gunning, Balfe and Easton, 1992).

The plants with medicinal / pesticidal properties are known to possess nearly 30,000 secondary metabolites, which function as insecticidal (Bowers and Nishida, 1980; Schoonhoven, 1993; Tsao and Coats, 1995) and antimicrobial (Kemp and Burden, 1986). These plants/compounds cause deterrence in oviposition and feeding, repellency, growth disruption, reduced fitness and sterility in number of insect species (Ascher and Meisner, 1989; Schmutterer, 1990). They are biodegradable and less toxic to non-target organisms (Wink and Guo, 1995), and hence highly preferable.

Crude methanolic extract of 31 species in 20 genera of Meliaceae including *Cipadessa baccifera* showed growth-inhibiting properties against noctuids, particularly, variegated cutworm, *Peridroma saucia* (Champagne *et al.,* 1993).

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A critical literature survey reveals that *Cipadessa* has not been studied for its pesticidal character. The present study aimed to explore the above-mentioned potential medicinal plant to combat the devastating pest, *H. armigera* under laboratory conditions.

MATERIALS AND METHODS

Plant materials: The leaves of *Cipadessa baccifera* collected from Kolli Hills, Tamil Nadu.

Extraction method: Collected leaves were shade dried and powdered, extracted successively using non-polar to polar solvents *viz.,* hexane, petroleum ether, chloroform and acetone. Aqueous extraction was also done. In each solvent the plant material was soaked for 24h at 30 ± 2°C, filtered and to the residue the same solvent was added. This procedure was repeated thrice to obtain maximum extractable. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator (Harborne, 1998).

Insect culture: *H. armigera* larvae collected from lady's finger field were maintained in the laboratory at 22 ± 2°C and 70 - 75% RH. The larvae were reared on semi-synthetic diet (Shorey and Hale, 1965) in individual containers to prevent cannibalism and contamination.

Insecticidal bioassays

Adult longevity, fecundity and egg hatchability of *H. armigera:* Ten per cent solution of hexane, petroleum ether, chloroform, acetone and water extracts of *C. baccifera* was made in the sugar solution with the respective solvents, which was fed to the adult moths, and the longevity, fecundity and hatchability were checked. Solvent control (10%) and 10% sugar solution (normal control) were also maintained. Five pairs of treated adults were released into the mud pot and maintained as mentioned earlier. Triplicates were maintained for each treatment and the data were subjected to ANOVA (SPSS, 1999) and the means were compared to determine significant differences using Duncan's multiple range tests ($p < 0.05$).

Growth inhibition of larvae: The larvae hatched from the previous treatment on adults (*vide* Table 1) were maintained on semi-synthetic diet till fourth instar. Those survived till fourth instar were tested with the crude extracts of *Cipadessa baccifera* under three conditions *viz.,* (1) diet + extract (2) diet + respective solvent and (3) diet alone. The growth of larvae [pupation (%), pupal weight (mg)] and percent moth emergence (healthy) of the 1st generation were observed. The resultant adults (2nd generation), were also observed for their longevity, fecundity and egg hatchability recorded.

Fractionation

Efficacy of primary fractions of *C. baccifera* on *H. armigera:* 40g of petroleum ether crude extract was dissolved in their respective solvent and fractionated on a silica gel column, using petroleum ether/acetone. The eluted fractions were tested against fourth instar larvae. The pupation and moth emergence details were recorded. Respective solvent and normal control were maintained.

RESULTS AND DISCUSSION

Screening of test plants on adult longevity, fecundity and egg hatchability (first generation): Among the various crude extracts tested, hexane and acetone extracts significantly reduced the adult longevity to 5.6- 6.3 days. However, in water extract, it was up to 12 days (Table 1). This was similar to the results obtained by Subashini, Malarvannan and Renjith Pillai (2004), where the adult longevity of *H. armigera* treated with *Dodonaea angustifolia* crude petroleum ether and chloroform extracts was just three days. In general, there was a wide variation in the fecundity among the treatments. Hexane extract suppressed the fecundity to 87 eggs, while it was induced in water and acetone extracts (1597 and 1235 eggs) (Table 1). Neem seed kernel extract (2.5% azadirachtin) fed against *H. armigera* caused egg mortality and moulting disruption (Hassan, 1999). Similarly, reduction in oviposition was the highest (60.9%) with 10% NSKE and the ovicidal effect decreased with increase in age of eggs (Jeyakumar and Gupta, 1999).

The egg hatchability was in the range of 0-55%. Zero hatchability was observed in chloroform and hexane extracts. The egg hatchability was suppressed (19 %) in acetone extract, despite the induction in egg laying (Table 1). Similar results have been reported with (+)-(E)-endo-beta-bergamoten-12-oic acid, a sesquiterpene acid from wild tomato (Coates *et al*., 1988) and oleandrin, a cardiac glycoside from *Dodonaea angustifolia* (Subashini *et al*., 2004) against *H. armigera*.

Table 1. Bioefficacy of *C. baccifera* in combating *H. armigera* adult (1st generation)

Treatment		Parameters*		
		Adult longevity (days)	Egg (numbers)	Hatchability (%)
Cipadessa extract with	water*	12.0^{def} (10^{cd})	1597^{f} (901^{ef})	35.7^{c} (86.6^{e})
	hexane	5.6^{ab} (5^{a})	87^{a} (18^{a})	0.0^{a} (0.0^{a})
	petroleum ether	9.6^{c} (14^{f})	640^{b} (1247^{h})	54.6^{d} (0.0^{a})
	chloroform	10.3^{cde} (13^{f})	926^{d} (760^{bc})	0.0^{a} (0.0^{a})
	acetone	6.3^{bc} (13^{f})	1235^{e} (1540^{f})	19.1^{b} (0.0^{a})
C.D. at 5%	2.04	170.88	92.67	

Mean of triplicate with five pairs each; Values in parentheses indicate its respective solvent control; *Control - honey Different letters in each column differ significantly (5%) by DMRT

Effect of crude extracts on larval growth and moth emergence of *H. armigera* (1st generation) and adult (2nd generation)

a) Larval growth (1st generation): Among the acetone extract (AE), petroleum ether (PE) extract and water extract (WE) treated larvae (that survived from the previous experiment), the least pupation was noticed in PE extract (26.8%) followed by AE (27.3%). Similarly, petroleum ether extract of *Tribulus terrestris* caused juvenilizing effects on *H. armigera* and *Spodoptera litura* (Gunasekaran and Chelliah, 1985). Petroleum ether extracts of the leaves of *Gnidia glauca* recorded 100% larval mortality in *H. armigera* (Sundararajan and Kumuthakalavalli, 2000). Significant juvenomimetic activity in cotton stainer, *Dysdercus cingulatus* was observed due to petroleum ether extracts of angiosperms belonging to Cannabinaceae, Cactaceae, Cucurbitaceae, Sapindaceae, Solanaceae and Boraginaceae (Neraliya and Srivastava, 1997). Except for acetone extract treated larvae fed with normal diet (AN) (52%) the pupation rate remained more or less equal. In control it was 80%. The variation between treatments was significant.

Similarly, petroleum ether extract treated larvae fed both with extract (PE) and solvent (PS) showed reduction in pupal weight (177.5 and 177.9mg), compared to control 380 mg. There was no difference between AE treated larvae, however, it varied among water extract treated larvae (182.6 mg and 232.1 mg) (Table 2). Reduced pupal weight were observed in *H. armigera* fed with chick pea leaves treated with

Table 2. Effect of *Cipadessa baccifera* extract on growth of *H. armigera* larvae

Treatment		Parameters*		
		Larval growth		Moth emergence (%)
		Pupation (%)	Pupal weight (mg)	Healthy
petroleumether extract treated with	extract	26.8^{a}	177.5^{a}	0.6^{a}
	solvent	28.8^{ab}	177.9^{a}	7.8^{ab}
	normal diet	28.8^{ab}	188.3^{a}	32.4^{c}
acetone extract treated with	extract	27.3^{ab}	229.2^{b}	33.5^{c}
	solvent	34.6^{b}	229.4^{b}	42.5^{c}
	normal diet	51.5^{c}	264.9^{b}	65.6^{d}
water extract treated with	extract	28.7^{ab}	182.6^{a}	26.5^{bc}
	normal diet	31.1^{ab}	232.1^{b}	32.1^{c}
control (normal diet)	80.0^{d}	380.0^{c}	91.3^{e}	
C.D. at 5%	62.9	29.6	18.0	

Each value mean of triplicate. Different letters in each column differ significantly (5%) by DMRT

neem leaf extracts (Sharma and Sheiker, 1997). Semi-synthetic diet containing methanolic extracts of stems and roots of silver fern, *Cheilanthes farinosa* (Josephrajkumar, Subrahmanyam and Devakumar, 2000) and 5% aqueous extracts of *Cynanchum auriculatum* (Ju *et al*., 2000) prolonged the larval period, larval mortality and reduced pupal weight in *H. armigera* The growth inhibitory activity and deterrency to *H. armigera* was witnessed in methanolic extracts of *Melia dubia* in a dose-dependent manner (Koul, Jain and Sharma, 2000).

b) Adult emergence: Similarly, maximum (99%) malformed moths were emerged from petroleum ether extract fed larvae, followed by its solvent control (92%). The least healthy moth emergence (0.6%) was noticed in petroleum ether extract further fed with extract, as against the control (91%) (Table 2 and Fig. 1b). Ethyl acetate extract of *Delphinium cultorum* (Miles, Ramsewak and Nair, 2000), *Artemisia annua*, and *Ageratum conyzoides* displayed antifeedant and larval mortality and failure of normal adult emergence in *H. armigera* (Padmaja and Rao, 2000).

c) Adult (second generation): The adults emerged from previous experiment (*vide* Table 2) lived for more than 10 days in all the treatments except for petroleum ether extract treated fed with extract alone. The less longevity was observed with moths emerged from acetone and petroleum ether solvents, and the highest (14 days) was with water extract.

Fecundity: The fecundity was least (340 eggs) in moths emerged from petroleum ether solvent (PS), followed by acetone extract (AE) with 386 eggs. The moths emerged from petroleum ether extract further fed with normal diet (PN) had the highest fecundity (1561 eggs) followed by water extract fed with normal diet (1425 eggs), acetone extract fed with normal diet (1416 eggs), and in control it was 1493 eggs.

Egg hatchability: Least hatchability (0.57%) was observed in adults emerged from AE, PS and WE, followed by adults emerged from PN (8.8%), AS (12.6%) and AN (20.0%), compared to the control adults (81.6%).

Table 3. Efficacy of petroleum ether fractions of *Cipadessa baccifera* against third instar larvae of *Helicoverpa armigera*

Treatment	Parameters*		
	Larval growth		Moth emergence (%)
	Pupation (%)	Pupal weight (mg)	Healthy
Fraction 1	0.0^{a}	0.00^{a}	0.00^{a}
Fraction 2	20.0^{ab}	150.00^{b}	16.66^{abc}
Fraction 3	80.0^{ed}	233.33^{bc}	12.50^{abc}
Fraction 4	26.6^{bc}	251.66^{c}	11.10^{ab}
Fraction 5	46.6^{cd}	238.33^{bc}	11.10^{ab}
Petroleum ether solvent	66.6^{de}	234.16^{bc}	79.16^{d}
Normal diet	100.0^{d}	306.66^{c}	100.00^{d}
CD (P=0.05)	21.96**	91.42**	30.24**

*Each value mean of triplicate

Different letters in each column differ significantly (5%) by DMRT

Effect of potential fractions on larval growth and moth emergence of *H. armigera*

Of all the fractions of petroleum ether extract of *C. baccifera*, inhibition of larval growth was noticed in the first fraction. Except fraction 3 (80%), all other treatments resulted in less than 50% pupation. The normal control showed 100% pupation. Further, a drastic reduction in pupal weight from 150 mg to 251 mg (normal control- 306 mg) was recorded in all the treatments, which subsequently resulted as malformed moths (83-100%). Vismiones and ferruginins, (lypophilic anthranoids) from *Vismia* inhibited feeding in *Spodoptera* and *H. armigera* larvae and nymphs of *Locusta migratoria* (Simmonds *et al*., 1985). Triterpene, oleanolic acid, in leaves of *Baccharis linearis* at 2.3mg/g diet prevented *H. armigera* pupation (Argandona and Faini, 1993). Glucosinolates, alkaloids and saponins present in *Ajuga reptans* caused feeding deterrence while furanditerpenes and flavonoids caused repellence in chrysomelids (Camps and Coll, 1993). Similarly, two steroidal glycoside esters from the dried leaves of *Physalis peruviana* inhibited the growth of *H. armigera* larvae (Waiss *et al*., 1993; Elliger *et al*., 1994). Azadirachtin and plumbagin led to considerable growth inhibition, larval mortality and reduced pupation in *H. armigera* (Gujar, 1997). Capsicin content of *Capsicum frutescens* extract resulted in significant larval mortality of *H. armigera* (Rethinaraja and Narayanasamy, 1999).

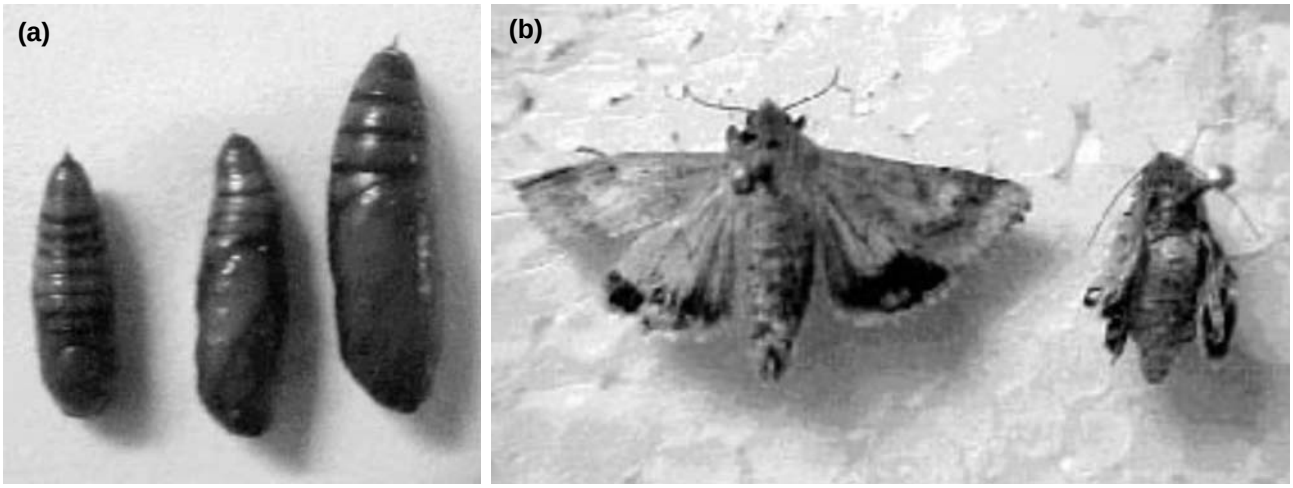

Fig. 1. Effect of *Cipadessa baccifera* extract on growth of *H. armigera*

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