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Bioefficacy studies on insecticidal activities of *Canavalia cathartica* thouar against *Spodoptera litura* fab. (Lepidoptera: Noctuidae)

Arulkodi Pattusamy*, Selvamuthukumaran Thirunavukkarasu
Department of Entomology, Phyto Insecticide Research Laboratory, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract
Bioefficacy studies of *Canavalia cathartica* Thouar seed methanol, ethyl acetate and hexane extracts against *Spodoptera litura* Fab. carried out to find out the impact on feeding, mortality, and duration showed cent per cent feeding deterrence activity in all the three extracts tested and methanol and ethyl acetate extracts showed 40 per cent mortality at cent per cent concentration. Among the reduced concentrations tested, *Canavalia* methanol extract imparted maximum feeding deterrence activity and larval mortality at 70% concentration. In *Canavalia* ethyl acetate extract the maximum feeding deterrence was recorded in 70% concentration (96.76%). The maximum larval mortality was recorded in 70%, 30% and 1% concentrations (66.66%) and highest pupation percentage was recorded in 5% concentration (66.66%). The maximum pupal malformation was recorded in 5% concentration (33.33%). In *Canavalia* hexane extract the maximum feeding deterrence was recorded in 70% concentration. The maximum larval mortality was recorded in 30% concentration and maximum pupation percentage was recorded in 1%. The results revealed that *C. cathartica* extract has the potential to be developed as an effective phyto insecticide.

Keywords: *Canavalia cathartica*, feeding deterrency, larval mortality, pupal malformation

Introduction
Use of insecticides is credited for protecting crops from insect damage and enhancing crop productivity. It was estimated that without the use of pesticides, global food production loss would be 35–45% (Oerke, 2006) [13]. However, "Silent Spring" written by Rachel Carson in the year 1962, made people more aware about the ill effects of synthetic insecticides. Their indiscriminate use has led to resurgence and resistance of insect pests (Bernardes *et al.* 2015, Campos *et al.* 2019) [7, 8]. Botanical insecticides have long been touted as attractive alternatives to synthetic insecticides since they pose little threat to the environment or to human health (Isman, 2006 & 2008) [10, 11]. Yet, only a handful of botanicals are currently used in agriculture and there are few prospects for commercial development of new botanicals. Several factors appear to limit the success of botanicals, most notably regulatory barriers and the availability of competing products (newer synthetics, fermentation products, microbials) that are cost-effective and relatively safe. The need for effective botanicals and newer secondary metabolites with novel modes of action is ever increasing forcing the scientific community for concerted screening and selection. Hence, the present study aimed at testing the anti-insect properties of *Canavalia cathartica* against *Spodoptera litura* Fab.

Materials and Methods
Mass culturing of test insect *Spodoptera litura* Fab
S. litura egg mass collected from the goundnut crop at Kodukkanpalayam (11.71ºN Lat. and 79.66º E Long.) was used to initiate the culture. The egg mass was surface sterilized using 0.05 per cent sodium hypochlorite by dipping. They were then air dried and kept inside incubation chamber till hatching. Hatched neonates were maintained in Bengal gram flour based semi synthetic diet till their pupation. Bengal gram flour, other ingredients mentioned as fraction A and formaldehyde (fraction B) were added to 150ml of distilled water and thoroughly mixed in an electric blender for two minutes. To this mixture, Agar agar boiled in 225 ml of distilled water was added and again blended for a minute. The semi synthetic diet was poured into each cell of sterilized plastic multi cavity tray (26cm x 10cm) containing 32 cells (3cm x 2.5cm). After solidification of the diet, larvae were released @ one per cell. The tray was closed with the lid held in place by a rubber band and kept upside down to avoid faecal contaminants. Faecal pellets were removed daily and the larvae reared till pupation. The pupae were collected, cleaned, sexed, surface sterilized with 0.05 per cent sodium hypochlorite, and transferred to an adult emergence cum oviposition cage (26 x 20 cm) @ five pairs per cage. A clean *Nerium oleander* Linn. twig kept inside the cage acted as oviposition substrate. Ten per cent honey solution fortified with vitamin E (Bayer Ltd.,) socked in absorbent cotton served as adult food. Eggs laid were collected daily and a continuous culture of *S. litura* was maintained in the laboratory (PDBC, 1998) [15].

Table 1: Composition of Bengal gram flour based semi synthetic diet

S. No	Ingredient	Quantity
	Fraction A	
1	Bengal gram flour	60.0g
2	Yeast tablets	10.0g
3	Casein	5.0g
4	Ascorbic acid	1.5g
5	Methyl-P-hydroxybenzoate	1.0g
6	Sorbic acid	0.3g
7	Cholesterol	0.5g
8	Streptomycin sulphate	0.1g
9	Multivitamin tables	6 tablets
10	Vitamin B	1 capsule
11	Distilled water	150ml
	Fraction B	
12	Agar agar	6.0g
13	Formaldehyde	0.3ml
14	Distilled water	225ml

Collection and preparation of Plant materials

Collection of *C. cathartica* seeds

The seeds of *C. cathartica* were collected from the wild plants growing in Kodukkanpalayam village, Cuddalore district. The seeds were shade dried and powered in an electric blender. They were then stored separately in air tight pouches (Ziplock cover 5 in. x 7 in.) at room temperature and used for further extraction.

Preparation of extract

50 g of seed powder was immersed in 250 ml of various analytical grade solvents at room temperature through sequential extraction using three organic solvents (From non-polar to polar: n-hexane (64ºC), ethyl acetate (77.1ºC) & methanol (64.7ºC)) at room temperature for three days and successive extracts were filtered. The filtrate was then evaporated to dryness in a rotary flash vaccum evaporator (Lab-Sil instruments®). The semisolid extractive thus obtained was stored in small glass vials, closed with aluminium foil to prevent the entry of light, and kept in -20 ˚C deep freezer (Blue star ®) (Arivudainambi, 2001) [2].

Preliminary screening of anti-insect properties of extractives

Preliminary screening of anti-insect activities of all the extractives was carried out through a leaf disc no choice bioassay using third instar *S. litura* larvae (Bentley *et al*., 1984) [6]. Fresh castor leaf disc (3 cm^2) was dipped in each extractive (1g dissolved in 1 ml of emulsified acetone), shade dried for few minutes, placed inside a Petri plate (85mm dia.) lined internally with moist filter paper to avoid drying of leaf disc (Ramanan, 2017) [17]. Five pre-starved (for four hours) third instar larvae of *S. litura* were released and allowed to feed for six hours. After six hours, fed leaf area was measured using leaf area meter (Systronics®). The larvae were reared with untreated castor leaves till adult emergence and mortality and malformations were recorded periodically. Respective solvent and absolute controls were also maintained. Each treatment was replicated three times (Selvamuthukumaran, 2008) [18]. The leaf area fed was measured and per cent leaf area protection over absolute control was computed as indicated below

Percent leaf area protection over absolute control

$$= \frac{Per\ cent\ leaf\ area\ protection\ in\ treatment - Per\ cent\ leaf\ area\ protection\ in\ abosulte\ control}{100 - Per\ cent\ leaf\ area\ protection\ in\ absolute\ control} 100$$

Similarly, per cent mortality and malformation was also worked out.

Table 2

S.No.	Per cent anti-insect activity	Rating
1.	> 80	Strong
2.	50 – 80	Medium
3.	20 – 50	Weak
4.	< 20	Insignificant

Extractive showing either strong or medium feeding deterrent or insecticidal or insect growth regulatory activity were further bio-assayed at 1, 5, 10, 30, 70 per cent concentrations.

Evaluation of anti-insect activity of promising extractives at reduced concentrations

The desired concentrations were obtained by dissolving 1, 5, 10, 30, and 70 mg of respective promising extractive in 10ml of acetone each and emulsified with 85 ml of double distilled water and 5ml of Tween 80. A no choice leaf disc bioassay as described earlier was done. The leaf area fed was measured, per cent leaf area protected over absolute control, per cent mortality and malformation was worked out.

The experiments were designed in Completely Randomized Block method; means were transformed accordingly and ranked using Duncan's Multiple Range Test (DMRT).

Result and Discussion

The results revealed that the *Canavalia* Methanol, ethyl acetate and hexane extracts showed cent per cent feeding deterrent activity. Among the solvent extracts tested methanol and ethyl acetate extracts imparted 40 per cent mortality and hexane extract imparted 26.67% mortality (Table 1).

Canavalia methanol extract recorded dose dependent feeding deterrent activity. As the concentration increased, the feeding deterrence and larval mortality were found increasing. The maximum feeding deterrence activity was recorded in 70% (97.05%) followed by 30 % (89.71%), and 10% (85%) concentrations. The larval mortality was also influenced by dose. Cent per cent mortality was recorded in 70% concentration and followed by 30% (93.33%) and 10% concentrations (60 %). Among the concentrations tested, 40 % pupation was recorded in 10% followed by 33.33 % in 5% concentration and 13.33% in 1% concentration. Various kinds of deformities were observed. During pupal molting, the treated larvae failed to detach completely from the *exuvia*. Some pupae did not have fully formed cuticle. The maximum pupal malformation was recorded in 5% concentration (33.33%) followed by 10% concentration (20.00%). In 70% concentration pupation was not recorded due to the cent percent larval mortality. In 30% and 5% concentration no adult emergence was recorded (Table 2).

The maximum feeding deterrence was recorded in 70% concentration (96.76%) followed by 30 % concentration (82.64%), and 10% concentration (76.76%). The larval mortality is dose dependent. The maximum larval mortality was recorded in 70%, 30% and 1% concentrations (66.66%) followed by 10% concentration (53.33%) and 5% concentration (33.33 %). The highest pupation percentage was recorded in 5% concentration (66.66%) followed by 10% concentration (46.66%). In 70%, 30% and 1% concentrations 33.33% pupation was recorded. The maximum pupal malformation was recorded in 5% concentration (33.33%) followed by 10% and 1% concentration (13.33%). However in 70% and 30% concentrations, no pupal malformation was recorded. The least adult emergence was recorded in 30% and 1% concentrations (Table 3).

The larval mortality imparted by *Canavalia* hexane extract was dose dependent. The maximum larval mortality was recorded in 30% concentration followed by 70%, 30%, 10% and 1% concentrations. Pupation percentage recorded was in the following order; 1% > 10% > 70% > 30% > 5%. The

maximum pupal malformation was recorded in 30% concentration (20.00%) followed by 10% and 5% concentrations (13.33%). No adult emergence was recorded in 30% and 5 % concentrations. The maximum feeding deterrence was recorded in 70% concentration followed by 30 %, and 10% (Table 4).

Bioefficacy studies of various botanicals carried out by many authors on *S. litura* supported the present findings. Hexane, chloroform and ethyl acetate leaf extracts of *Blumea mollis* and *Hygrophila auriculata* against *S. litura* revealed increase in feeding deterrence, larvicidal, and pupicidal activities when there was increase in the concentration (Baskar *et al.*, 2011a) [4]. Similarly findings of Ashokaraj and Mahadev (2013) [3] reported that the larvicidal activity of *E. triplinerve* leaf extracts against *S. litura* demonstrated progressively increasing mortality rate. Pavela (2004) [14] obtained 50% feeding deterrence at 3.74% concentration with *Melissa officinalis* leaf extract against *S. littoralis*. The adult emergence inhibition activity of *C.inerme* is also comparable to the present findings and as well with different species of plant extract in different families (Muthukrishnan *et al.*,1999; Pushpalatha and Muthukrishnan, 1999) [12, 16]. Higher feeding deterrent activity, larvicidal, pupicidal and prolonged larval–pupal duration were observed in ethyl acetate leaf extract of *Aristolochia tagala* Cham. by Baskar *et al.*, (2011b) [5]. Arivoli and Tennyson (2013) reported similar maximum feeding deterrence in *Strychnos nuxvomica* ethyl acetate extract, *Vitex negundo* and *Murraya koeingii* hexane extracts.

Table 3: Influence of *C.cathartica* with various solvent extract on the developmental stages of *S. litura*.

Treatment	Feeding deterrent (%)	Larval Mortality 24h (%)	Pupation (%)	Pupal Malformation (%)	Adult emergence (%)
Canavalia Methanol extract	100 (89.26)^{a}	40 (39.23)^{a}	60 (50.77)^{d}	13.33 (21.41)^{a}	46.67 (43.09)^{d}
Canavalia Ethyl acetate extract	100 (89.26)^{a}	40 (39.23)^{a}	60 (50.77)^{d}	13.33 (21.41)^{a}	46.67 (43.09)^{d}
Canavalia Hexane extract	100 (89.26)^{a}	26.67 (31.08)^{b}	73.33 (58.94)^{c}	13.33 (21.41)^{a}	60 (50.78)^{c}
Solvent control	16.81 (24.20)^{b}	6.7 (15.00)^{c}	93.33 (75.94)^{b}	0 (0.74)^{b}	93.33 (75.58)^{b}
Absolute control	0 (0.74)^{c}	0 (0.74)^{d}	100 (89.26)^{a}	0 (0.74)^{b}	100 (89.26)^{a}

Table 4: Influence of Canavalia methanol extract on the developmental stages of *S. litura*.

Treatment	Feeding deterrent (%)	Larval Mortality 24h (%)	Pupation (%)	Pupal Malformation (%)	Adult emergence (%)
1% Conc.	59.12 (50.26)^{cd}	86.66 (68.66)^{c}	13.33 (21.41)^{d}	6.66 (14.95)^{c}	6.66 (14.95)^{d}
5% Conc.	65.29 (53.91)^{c}	66.66 (54.74)^{d}	33.33 (35.26)^{c}	33.33 (35.26)^{a}	0 (0.63)^{e}
10% Conc.	85.00 (67. 41)^{b}	60.00 (50.77)^{d}	40.00 (39.23)^{c}	20.00 (26.56)^{b}	20.00 (26.56)^{c}
30% Conc.	89.71 (71. 52)^{b}	93.33 (75.53)^{b}	6.66 (14.95)^{e}	6.66 (14.95)^{c}	0 (.63)^{e}
70% Conc.	97.05 (81.97)^{a}	100 (89.38)^{a}	0 (0.63)^{f}	0 (0.63)^{d}	0 (0.63)^{e}
Solvent control	48.23 (43.98)^{d}	6.6 (14.88)^{e}	93.33 (75.97)^{b}	0 (0.63)^{d}	93.33 (75.03)^{b}
Absolute control	16.47 (23.94)^{e}	0.0 (0.63)^{f}	100 (89.38)^{a}	0 (0.63)^{d}	100 (90)^{a}
SE(d)	2.838	2.564	2.449	0.548	2.322
CD (P=0.05)	6.146	5.553	5.304	1.187	5.027

Table 5: Influence of Canavalia ethyl acetate extract on the developmental stages of *S. litura*.

Treatment	Feeding deterrent (%)	Larval Mortality 24h (%)	Pupation (%)	Pupal Malformation (%)	Adult emergence (%)
1% Conc.	67.35 (55.16)^{d}	66.66 (54.74)^{a}	33.33 (35.26)^{e}	13.33 (21.41)^{b}	20.00 (26.56)^{d}
5% Conc.	66.47 (54.63)^{d}	33.33 (35.26)^{c}	66.66 (54.74)^{c}	33.33 (35.26)^{a}	33.33 (35.26)^{c}
10% Conc.	76.76 (61.22)^{c}	53.33 (46.91)^{b}	46.66 (43.08)^{d}	13.33 (21.41)^{b}	40.00 (39.23)^{c}
30% Conc.	82.64 (65.46)^{b}	66.66 (54.74)^{a}	33.33 (35.26)^{e}	0 (0.63)^{c}	33.33 (35.26)^{c}
70% Conc.	96.76 (78.46)^{a}	66.66 (54.74)^{a}	33.33 (35.26)^{e}	0 (0.63)^{c}	33.33 (35.26)^{c}
Solvent control	48.23 0(43.98)^{e}	6.6 (14.88)^{d}	93.33 (75.97)^{b}	0 (0.63)^{c}	93.33 (75.97)^{b}
Absolute control	16.47 (23.94)^{f}	0 (0.63)^{e}	100 (89.38)^{a}	0 (0.63)^{c}	100 (89.38)^{a}
SE(d)	2.837	1.881	2.679	0.475	2.542
CD (P=0.05)	6.144	4.073	5.801	1.029	5.505

Table 6: Influence of Canavalia n-hexane extract on the developmental stages of *S. litura*.

Treatment	Feeding deterrent (%)	Larval Mortality 24h (%)	Pupation (%)	Pupal Malformation (%)	Adult emergence (%)
1% Conc.	55.88 (48.38)^{d}	60.00 (50.77)^{d}	40.00 (39.23)^{c}	0 (0.63)^{d}	40.00 (39.23)^{c}
5% Conc.	66.47 (54.63)^{c}	86.66 (68.66)^{a}	13.33 (21.41)^{e}	13.33 (21.41)^{b}	0 (0.63)^{f}
10% Conc.	70.59 (57.20)^{c}	66.66 (54.76)^{c}	33.33 (35.26)^{c}	13.33 (21.41)^{b}	20.00 (26.56)^{d}
30% Conc.	81.18 (64.36)^{b}	80.00 (63.49)^{b}	20.00 (26.56)^{d}	20.00 (26.56)^{a}	0 (0.63)^{f}
70% Conc.	94.11 (76.59)^{a}	80.00 (63.49)^{b}	20.00 (26.56)^{d}	6.66 (14.95)^{c}	13.33 (21.41)^{e}
Solvent control	48.23 (43.98)^{d}	6.6 (14.88)^{e}	93.33 (75.97)^{b}	0 (0.63)^{d}	93.33 (75.97)^{b}
Absolute control	16.47 (23.94)^{e}	0 (0.63)^{f}	100 (89.38)^{a}	0 (0.63)^{d}	100 (89.38)^{a}
SE(d)	2.606	2.367	2.455	0.421	2.370
CD (P=0.05)	5.644	5.126	5.317	0.911	5.133

Table 7: Stages of *S. litura*.

SE(d)	2.957	0.968	3.047	0.392	2.805
CD (P=0.05)	6.674	2.185	6.877	0.886	6.330

Conclusion

The study reveals that the all the solvent extracts of *C. canvalia* tested were found to be having the feeding deteancy, insecticidal activity and growth inhibitory in nature.

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