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Studies on anti-insecticidal properties of *Randia spinosa* poir. against *Spodoptera litura* Fab.

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Abstract
Bioefficacy evaluation of *Randia spinosa* Poir. fruit petroleum ether, chloroform and methanol extractive against *Spodoptera litura* Fab. was carried to find the effect on feeding, development activity and mortality. All the extractives were highly effective at 100% concentration. When tested at reduced concentrations, petroleum ether extractive imparted maximum feeding deterrence at 70% concentration. The highest larval mortality was documented in 70%, 30% and 10% concentrations. Similarly, chloroform and methanol extractives recorded maximum feeding deterrence at 70% concentration (95.88%). However, chloroform extractive was highly effective in imparting larval mortality and it registered 100% larval mortality at all the concentrations tested. The results revealed that *R. spinosa* has insecticidal properties and that can be utilized for development of an effective phyto-insecticide.

Keywords: *Randia spinosa,* feeding deterrent, larval mortality

Introduction
Phyto-insecticides, one of the ancient weapons of pest management as old as crop protection itself, lost their commercial significance after the development of synthetic insecticides. Synthetic insecticides with their efficacy, ease of use, and economical nature became prominent arsenals of pest management in intensive crop production. However, issues of extensive environmental pollution, toxic to non-target organisms, and hazard to human health led to a renewed attention in Phyto-insecticides research (Isman, 2008) [8]. As they coevolved with insects, plant secondary metabolites are very effective natural ingredients that can be relied upon. The secondary metabolites *viz.*,alkaloids, phenolics, terpenoids, *etc*., found widespread among plants (Castro *et al*., 2005; Dietrich *et al*., 2013) possess varied anti-insect properties like repellence, feeding deterrence, insecticidal, and growth regulatory (Isman, 2000) [8]. One such plant, *Randia spinosa* Poir. found to possess wide range of secondary metabolites and locally used as a piscicide. Its ability to stupefy and kill fish after minutes of administration made it a viable piscicide for centuries together. Therefore, the present study was aimed at studying the effects of *R. spinosa* extract on feeding deterrence, larval mortality, development indices, and morphogenetic variations in *S. litura.*

Materials and Methods
Rearing of test insect
S. litura egg mass collected from groundnut crop at Kodukkanpalayam (11.71˚C N Lat. and 79.66˚C E Long.) was used to initiate the culture. Hatched neonates were fed with clean castor leaves in a plastic tray (30 cm dia.). Faecal pellets were cleaned daily and the larvae were reared up to pupation and transferred to an adult ovipositioncage (26 x 20 cm) @ five pairs per cage. A *Nerium oleander* Linn. twig kept in the cage acted as an oviposition substrate. Egg masses were collected and a continuous culture was maintained (Selvamuthukumaran, 2008) [14].

Collection of *R. spinosa berries* and extract preparation
R. spinosa fruits were collected from Kodukkanpalayam village, Cuddalore district. The fruits were shade dried for 30 days and were ground to a fine powder using a mixer grinder (Philips, Chennai, India). The powder was stored in air tight ziplock pouches and used for further extraction. 50 gram of powder was soaked in various analytical grade solvents for three days at room temperature (petroleum ether (55˚C), chloroform (61.2 ˚C), and methanol (64.70 ˚C); non-polar to polar) sequentially and the subsequent extracts were filtered separately. The filterate was evaporated in a rotary flash vacuum evaporator (Lab-Sil instruments, Chennai, India), semisolid extractive obtained was stored in glass vials covered with aluminum foil and kept in a deep freezer (Blue Star, Thane, India) at -20 ˚C. (Arivudainambi, 2001) [2].

Screening of anti-insect properties of extractives
A leaf disc no-choice bioassay was conducted to evaluate anti insecticide properties of all the extractives against third instar *S. litura* larvae (Bentley *et al*., 1984). Fresh castor leaf discs (3 cm^2) were dipped in each extractive (1g extractive dissolved in 1 ml of emulsified acetone), allowed to air dry for few minutes and then placed inside a Petri plate (85mm dia.) lined internally with moist filter paper to prevent drying of leaf discs (Ramanan, 2017) [12]. 3 Four-hour pre starved third instar larvae were relaesed inside the petriplate and allowed to feed the treated leaf disc. Six hours after the treatment, leaf area left unfed was measured using a leaf area meter (Systronics, Ahmedabad, India). The larvae were fed with untreated castor leaves and reared till adult. Observations on mortality and malformations were made periodically. 0.5% neem oil treated leaf disc served as positive control. Absolute and solvent controls were also maintained and each treatment, malformation, and leaf area protection over absolute control were calculated and rated as shown below;

$$\text{Percent leaf area protection over absolute control} = \frac{\text{Per cent leaf area protection in treatment} - \text{Per cent leaf area protection in abosulte control}}{100 - \text{Per cent leaf area protection in absolute control}} \text{X100}$$

Table 1

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

Extractives showing either strong or medium feeding deterrent or insecticidal or insect growth regulatory activity were further bio-assayed at 1, 5, 10, 30, and 70 percent concentrations. Desired concentrations were prepared by diluting 1, 5, 10, 30, and 70 mg of the respective extractive in 100 ml emulsified water. A no-choice leaf disc bioassay was carried out as detailed earlier. Percent leaf area protected, mortality, and malformation were worked out.

The experiments were conducted in a Completely Randomized Design (CRD) and means were transformed accordingly and ranked using Duncan's Multiple Range Test (DMRT).

Result and Discussion

The results of the preliminary screening revealed that all three extractives *viz.*, petroleumether, chloroform, and methanol were found to possess any one or combination of anti-insect properties and were selected for further evaluation at reduced concentrations. All the extractives tested imparted 100%feeding deterrence and 46.66% larval mortality. Petroleum ether extract alone imparted 6.66% pupal malformation. The other two extractives failed to impart any malformation (Table 1).

In confirmatory evaluation at reduced concentrations, petroleum ether extractive imparted medium to strong feeding deterrence. Even at low concentrations of 1% and 5%, it imparted medium feeding deterrence. The larval mortality recorded ranged from 100% (at 10%, 30%, & 70% concentrations) to 93.33% (at 1% & 5% concentrations). The larval mortality was found to decline with declining concentrations. Pupal malformation was noticed only at the lowest concentration tested (1%) and the extractive completely inhibited adult emergence at all the concentrations tested (Table 2).

Such a similar trend was also noticed in chloroform extract which imparted medium feeding deterrence at 1% concentration alone and strong feeding deterrence at all the remaining concentrations. However, all the tested concentrations were highly effective in imparting larval mortality which was recorded as 100% at all the concentrations tested (Table 3).

The toxicity effect of methanol extractive was confirmed at reduced concentrations. The results revealed that the methanol extractive was imparting medium to strong feeding deterrence as other extractives. However, it failed to impart significant larval mortality even at the highest concentration tested (70%). Further, it exhibited medium-level malformation at 5% and 10% concentrations and completely inhibited adult emergence. It also clearly exhibited dose-dependent anti-insect properties (Table 4).

The *Ceasalpinea bonduc* (L.) Roxb. chloroform seed extract when treated against *S. litura* larvae antifeedant, larval mortality and pupal malformation was observed (Kathirvelu *et al*. 2011) [10]. Deepthy *et al*., (2010) [5] recorded that *V. negundo* methanol extract recorded maximum larval mortality (96.3%) against *S. litura*. Kandagal and Khetagoudar (2013) [9] revealed 92 % larvicidal activity was observed at 15 % concentration of aqueous extract of *E.triplinerve* against *S. litura. Adhatoda vasica* showed a high degree of antifeedant activity against *S. litura* (Anuradha *et al*. 2010) [1]. Rathi and Gopalakrishnan, (2005) [13] also reported the toxic effects of *Synedrella nodiflora* methanol extractive counter to *S. litura*. Pavunraj *et al*. 2011 also stated that the *Pergularia daemia* leaf ethyl acetate extract possessed strong feeding deterent against *S. litura*.

Table 1: Anti insect properties of *Randia spinosa* fruit solvent extractives on *S.litura*

Treatment	Percent anti-insect property*			Percent adult emergence
	Feeding deterrence	24h Larval mortality	Pupal malformation	
Petroleum ether extract	100 (89.26)^a	46.66 (43.08)^a	6.66 (14.88)^a	46.66(43.08)^c
Chloroform extract	100 (89.26)^a	46.66 (43.08)^a	0 (0.74)^b	53.33 (46.91)^c
Methanol extract	100 (89.26)^a	46.66 (43.08)^a	0 (0.74)^b	53.33 (46.91)^c
Solvent control	16.81 (24.20)^b	6.6 (14.88)^b	0 (0.74)^b	93.33(75.53)^b
Absolute control	0 (0.74)^c	0(0.74)^c	0(0.74)^b	100(89.26)^a
SE(d)	0.214	1.378	0.088	2.188
CD (P=0.05)	0.483	3.110	0.198	4.939

*Mean of three replications
Values within parentheses are arc-sine transformed
In a column, means followed by same letter(s) are on par by DMRT (p= 0.05)

Table 2: Anti insect properties of *Randia spinosa* fruit petroleum ether extractive on *S.litura*

Treatment	Percent anti-insect property*			Percent adult emergence
	Feeding deterrent	24h Larval mortality	Pupal malformation	
1%	55.29 (48.04)^d	93.33 (75.97)^b	6.66 (14.95)^a	0 (0.63)^d
5%	74.11 (59.43)^c	93.33 (75.33)^b	0 (0.63)^b	6.66 (14.95)^c
10%	87.65 (69.59)^b	100 (89.38)^a	0 (0.63)^b	0 (0.63)^d
30%	92.94 (75.42)^{ab}	100 (89.38)^a	0 (0.63)^b	0 (0.63)^d
70%	95.88 (79.78)^a	100 (89.38)^a	0 (0.63)^b	0 (0.63)^d
Solvent control	48.23 (43.98)^d	6.6 (14.88)^c	0 (0.63)^b	93.33 (75.97)^b
Absolute control	16.47 (23.94)^e	0.0 (0.63)^d	0 (0.63)^b	100 (89.38)^a
SE(d)	2.926	2.081	0.128	1.800
CD (P=0.05)	6.336	4.506	0.278	3.899

*Mean of three replications
Values within parentheses are arc-sine transformed
In a column, means followed by same letter(s) are on par by DMRT (p= 0.05)

Table 3: Anti insect properties of *Randia spinosa* fruit chloroform extract on *S. litura*

Treatment	Percent anti-insect property*			Percent adult emergence
	Feeding deterrence	24h Larval mortality	Pupal malformation	
1%	74.71 (59.83)[d]	100 (89.38)[a]	0 (0.63)	0 (0.63)[c]
5%	83.23 (65.88)[cd]	100 (89.38)[a]	0 (0.63)	0 (0.63)[c]
10%	87.94 (69.98)[bc]	100 (89.38)[a]	0 (0.63)	0 (0.63)[c]
30%	93.82 (76.18)[ab]	100 (89.38)[a]	0 (0.63)	0 (0.63)[c]
70%	95.88 (79.78)[a]	100 (89.38)[a]	0 (0.63)	0 (0.63)[c]
Solvent control	48.23 (43.98)[e]	6.6 (14.88)[b]	0 (0.63)	93.33 (75.97)[b]
Absolute control	16.47 (23.94)[f]	0.0 (0.63)[c]	0 (0.63)	100 (89.38)[a]
SE(d)	2.919	0.127	-	2.288
CD (P=0.05)	6.321	0.275	-	4.955

*Mean of three replications
Values within parentheses are arc-sine transformed
In a column, means followed by same letter(s) are on par by DMRT (p= 0.05)

Table 4: Anti insect properties of *Randia spinosa* fruit methanol extract on *S. litura*

Treatment	Percent anti-insect property*			Percent adult emergence
	Feeding deterrence	24h Larval mortality	Pupal malformation	
1%	69.41 (56.43)[c]	6.6 (14.89)[c]	33.33 (35.26)[c]	60 (50.77)[c]
5%	80.29 (63.68)[b]	33.33 (35.26)[b]	66.66 (54.74)[a]	0 (0.63)[e]
10%	83.23 (65.98)[b]	40 (39.23)[a]	60 (50.78)[b]	0 (0.63)[e]
30%	86.17 (68.30)[ab]	33.33 (35.26)[b]	33.33 (35.26)[c]	33.33 (35.26)[d]
70%	89.41 (71.22)[a]	40 (39.23)[a]	33.33 (35.26)[c]	26.66 (31.08)[d]
Solvent control	48.23 (43.98)[d]	6.6 (14.88)[c]	0 (0.63)[d]	93.33 (75.97)[b]
Absolute control	16.47 (23.94)[e]	0.0 (0.63)[d]	0 (0.63)[d]	100 (89.38)[a]
SE(d)	2.823	1.138	1.571	2.459
CD (P=0.05)	6.114	2.465	3.403	5.325

*Mean of three replications
Values within parentheses are arc-sine transformed
In a column, means followed by same letter(s) are on par by DMRT (p= 0.05)

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