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Evaluation of toxicity of *Cleistanthus collinus* leaf extract against *Spodoptera litura*

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Abstract
The development of resistance to synthetic insecticides among insects and their impact on the environment and humans forced research toward Phyto-insecticides. Although many plants have been explored and their secondary metabolites screened for anti-insect properties, it is still worthier to continue the search as only a minuscule of the secondary metabolite repertoire has been exploited till now. Leaves of *Cleistanthus collinus* (Roxb.) Benth. ex. Hook. f., (Fam.: Euphorbiaceae) was powdered, extracted using acetone and chloroform and their insecticidal effect evaluated against *Spodoptera litra* Fab. The results revealed that both the extractives were effective in imparting insecticidal effect. Among them, chloroform extractive was found to be more effective than acetone. Chloroform extractive imparted maximum mortality of 66.67% at 5 % itself whereas the acetone extractive reached the maximum of 66.67% at 50 % concentration only. Hence, further development of emulsion formulation may be attempted with chloroform extractive.

Keywords: *C. collinus,* larval mortality, *S. litura*

Introduction

Currently, synthetic insecticides such as organophosphates, carbamates, synthetic pyrethroids, etc., are commonly used to manage crop pests. However, the development of resistance to these chemicals among insects and their impact on the environment and humans forced research toward Phyto-insecticides (Charinely *et al*., 1999; Han *et al*., 2006; Kiran *et al*., 2006 and Ciccia *et al*., 2009) [7, 5]. Secondary metabolites of plant origin play an essential role in the defense mechanisms of plants against insects as both coevolved (Broussalis *et al*., 1999). Although many plants have been explored and their secondary metabolites screened for anti-insect properties, it is still worthier to continue the search as only a minuscule of the secondary metabolite repertoire has been exploited till now.

With this background, one of the most promising plants, *Cleistanthus collinus* (Roxb.) Benth. ex. Hook. f., (Fam.: Euphorbiaceae) found in the dry forests of southern and central India, Malaysia, and Africa was selected and evaluated. Traditionally, the aqueous leaf extract is employed as a fish poison, an abortifacient, a suicidal agent, and as traditional medicine (Saratchandra *et al*., 1997; Damodaram *et al*., 2008) [6]. The alcoholic extract of *C. collinus* leaves, roots, and fruits is used to treat gastrointestinal problems and was also found to possess anticancer properties. It possess insecticidal activity against *Tribolium castaneum*, and used as insecticide in rice fields (Harwansh *et al.,* 2010).

Furthermore, farmers use this extract to manage ticks in animal production systems. Panigrahi *et al.* (2013) reported that both aqueous and alcoholic extract treatment of *C. collinus* resulted in 100% tick mortality. Selvamuthukumaran and Arivudainambi (2010) [13] identified that the active ingredient lactone glycoside possesses various anti-insect properties *viz.*, insecticidal, antifeedant, and insect growth regulatory activities. Hence, the present research was undertaken to evaluate effect of *C. collinus* extract on *Spodoptera litura* larval mortality as a preliminary step towards development of suitable formulation.

Materials and methods

Mass culturing of test insect

S. litura egg mass collected from fields in and around Annamalainagar (11.3921˚N Lat. and 79.7147˚E Long.) was used to initiate the culture. The egg mass was surface sterilized using 0.05% sodium hypochlorite by dipping. They were then air-dried and kept inside the incubation chamber till hatching. Hatched neonates were maintained in Bengal-gram flour-basedsemi-synthetic diet poured into a sterilized plastic multi-cavity tray (26cm x 10cm) containing 32 cells (3cm x 2.5cm). Faecal pellets were removed daily and the larvae were reared till pupation. The pupae were collected, cleaned, sexed, surface sterilized with 0.05% 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 an oviposition substrate. 10% honey solution fortified with vitamin E 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) [11].

Plant collection and extraction

C. collinus leaves were obtained from Nagalkuzhi (11.27042˚N and 79.30637˚E), Ariyalur district, Tamilnadu, India and were shade dried for a week. The dried leaves were then powdered for further extraction under cold solvent extraction method. 50 g of leaf powder was soaked separately in 250 ml of acetone (BP: 56˚C) and chloroform (BP: 61.2˚C), agitated intermittently in a magnetic stirrer (Remi make). After three days the extract was filtered and evaporated in a rotary flash vacuum evaporator (Lab-Sil instruments) to obtain crude dried extractive. It was preserved in glass vials covered with aluminum foil and placed in a deep freezer at -20 °C.

Evaluation of anti-insect property

The *C. collinus* leaf extractives were examined for their insecticidal property at various concentrations *viz.*,1, 5, 10, 30, 70, 90, and 100 percent prepared by dissolving respective quantities of extractives in 100 ml emulsified water. 2 ml of respective concentrations were applied on the adaxial and abaxial surfaces of the leaf disc (3 cm dia.) and allowed to air dry for 10 minutes and kept inside a Petri dish (9 cm dia.). 5 four-hour prestarved third-instar larvae were released and allowed to feed for six hours. After six hours, alive larvae were fed with untreated leaf discs. Both solvent and absolute control were maintained. The treatments were replicated three times. The larvae were then fed with untreated castor leaves till pupation. Number of dead larvae was counted and the percent mortality calculated as detailed.

$$\text{Percent Mortality} = \frac{\text{Number of dead larva}}{\text{Total number of larva released}} \times 100$$

Results

The results revealed that both the extractives were effective in imparting insecticidal effect. Both the extractives imparted a maximum of 66.67% larval mortality. Among the two extractives, chloroform extractive was found to be more effective than acetone. Chloroform extractive imparted maximum mortality of 66.67% at 5 % itself whereas the acetone extractive reached the maximum of 66.67% at 50 % concentration only. Both the extractives performed similar in terms of their dose dependent nature in imparting insecticidal effect. The effect started raising from the lowest concentration, reached maximum and got reduced there after. This indicated presence of feeding deterrence property at higher concentrations. Presence of this insecticidal effect was supported by Arivudainambi and Baskaran (2004) [2] who reported that hyper excitation, ataxia, tremors and unbalanced walking in *C. collinus* extract treated larvae of *S. litura* as in organophosphorus and organochlorine poisoned insects. Ahirawar *et al.* (2011) reported *C. collinus* leaf extract as a powerful natural insecticide and recorded that a single application of 50% leaf decoction (50%) was most successful in killing all the treated insects notably rice caseworm, *Nymphula depunctalis* within 12 hours.

Conclusion

Among the extractives, *C. collinus* leaf chloroform extractive was found to be the best which showed 66.67 per cent mortality at a low concentration of 5 %. Hence, further development of emulsion formulation may be attempted with chloroform extractive.

Table 1: Effect of solvent extract of *C. collinus* leaf against *S. litura*

Concentration	Percent larval mortality*	
	Acetone extract	Chloroform extract
1 %	33.33 (35.26)^{e}	46.67 (43.09)^{d}
5%	40.00 (39.23)^{d}	66.67 (54.74)^{a}
10 %	53.33 (46.91)^{c}	60.00 (50.77)^{b}
30 %	60.00 (50.77)^{b}	60.00 (50.77)^{b}
50 %	66.67 (54.74)^{a}	53.33 (46.91)^{c}
70 %	26.67 (31.09)^{f}	40.00 (39.23)^{e}
90 %	13.33 (21.42)^{g}	20.00 (26.57)^{f}
100 %	6.67 (14.96)^{h}	6.67 (14.96)^{g}
Solvent control	0.00 (0.00)^{i}	0.00 (0.00)^{h}
Absolute control	0.00 (0.00)^{i}	0.00 (0.00)^{h}
SE(d)	0.73	0.83
CD(p=0.05)	1.5	1.73

*Mean of three replications

Values within parenthesis are arcsine transformed

In a column, means followed by same letter (s) are on parby DMRT (p=0.05)

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