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Insect Growth Regulatory Activity of *Thevetia nerifolia* Juss. against *Spodoptera litura* (Fab.)

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Summary

Screening for insect growth regulatory activity (IGR) of *Thevetia nerifolia* leaf extracts were evaluated against *Spodoptera litura* (Fab.). Methanol extract of leaves provided 53.8 % larval mortality, 29.6 % pupation and 22.3 % adult emergence at 2.5 % concentration level. The extract was further subfractioned with solvents of different polarity in search of better IGR activity and chloroform extract was found to be most active in terms of larval mortality (27.5-61.5 %), pupation (28.4-60.2 %) and adult emergence (19.8-52.8 %). GI_{50} of the extract was recorded to be 3.02 %. Activity was attributed to the glycosides present in the extract.

Introduction

Out of several agriculturally important insects, *Spodoptera litura* is an economically important polyphagous pest in India, China, and Japan. It is causing considerable economic loss to many vegetable and field crops. It is a polyphagous insect present in high numbers in tropical countries even during rainy seasons with at least 48 families (Ulrichs and Mewis, 2004). It is the first lepidopteron pest to develop resistance in India (Kumar and Reghupathy, 2001; Srivastava and Joshi, 1965). Therefore, an alternative to chemical pesticide is imminent to control the pest. Botanical pesticides are projected as the key plant protection in the new millennium. They are relatively safe and viable alternative to synthetic pesticides as they reduce or prevent insect feeding. Numerous plant species have been reported to possess pest control properties but only a few seem to be ideally suited for practical utilization.

Yellow oleander (*Thevetia* sp.) is a small tree belongs to Apocynaceae family. It is one of the unexplored plant species for insecticidal or insect growth regulatory activity, though other biological activities were studied (Ray et al., 2009; Oji and Okafor, 2000; Ambang et al., 2007; Gata Gongalves et al., 2003). Satpathi and Ghatak (1990) found that methanolic extracts of seeds of *Thevetia nerifolia* Merr, at 1.0 per cent resulted in 100 per cent mortality of fourth instar larvae of *P. xylostella*, 12-24 h after treatment when applied topically. Pesticidal property of *Thevetia* sp. was reported against diamond back moth and other agriculturally important insect pests (Lingappa et al., 2004; Freedman et al., 1979; Mclaughlin et al., 1980; Reed et al., 1981 and 1982). IGR and larvicidal activity of *Thevetia* sp. was evaluated against two species of mosquito and found good larvicidal but very little IGR activity (Lapcharoen et al., 2005. *Thevetia neriifolia* leaf extract was evaluated against *Tribolium confusum* adults and it was found that acetone extract was found to be the most effective toxicant followed by ethyl acetate, petroleum ether and methanol extracts (Khanam et al., 1995). Antifertility potential of *Thevetia peruviana* in male albino rats was also reported (Gupta et al., 2011).

The present study was therefore undertaken to evaluate the insect growth regulatory and larvicidal action of the *Thevetia nerifolia* leaf extracts against *Spodoptera litura* (Fab.).

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Materials and methods

Plant material

Fresh leaves of *Thevetia nerifolia* were collected from Paschim Medinipur District of West Bengal. The botanical identification of the plant was done at Department of Botany, Vidyasagar University, Paschim Medinipur, West Bengal, India.

Insect rearing

Insect cultures of *Spodoptera litura* (F.) (Noctuidae: Lepidoptera) were maintained in the laboratory on castor leaves (*Ricinus communis* L.). Rearing conditions were a 12 h photo regime at 28±1 °C and 60 % relative humidity. Insect cultures were continuously refreshed with new one, found in the vicinity of the research farm of the Indian Agricultural Research Institute, New Delhi, India.

Preparation of extracts

Leaves (2 kg) were chopped and extracted by soaking with hexane (3 × 3 lit) for overnight at room temperature in order to remove fatty materials. Hexane extract was collected and evaporated under vacuum at < 40 °C using rotary evaporator (Heidolph, Germany). Hexane extracted plant materials were then extracted with methanol at 50 °C. Extract was filtered and concentrated under vacuum to obtain a viscous residue. Further fractionation of methanol extract was done using hexane, chloroform and ethyl acetate. Each extract was collected and concentrated separately under vacuum at <45 °C using rotary evaporator.

Contact toxicity

Dry film method was adopted for bioassay of extracts. One milliliter of extract and fraction solution was poured in each rimless glass test tube (25 x 200 mm). The tubes were rotated gently at 100 rpm using hand rotating wheel. Angle of the tubes was adjusted in such a way that the solution covered three-fourth of the inner surface of the tube. The process was continued till the solution dried up leaving behind a uniform film of extract on the inner glass surface. Dry film with acetone was prepared in a similar manner as a control. First instar larvae (12 h old) of *Spodoptera litura* were released using a hair brush, the tubes were plugged with cotton and wrapped in muslin cloth. The larvae were allowed to remain in contact with dry films or 4 h and mortality was recorded thereafter. Three replications per treatment were used for the experiment. Moribund larvae were also counted as dead one.

Growth regulatory activity

The test compounds (500 mg) were weighed accurately in a 5 mL volumetric flask and dissolved into 0.5 mL of suitable solvent. The volume was then made to 5 mL to obtain 10 % stock solution. From these stock solutions, different concentrations (0.5, 1.0, 2.0, 3.0 and 5.0 %) were prepared separately by serial dilution with 0.5 % emulsified water, which in turn was prepared by dissolving 5mL of Tween 80 emulsifier in 1 L of distilled water.

For insect growth regulatory activity, third-instar larvae of *Spodoptera litura* weighing between 30 and 60 mg were treated with various concentrations of the test emulsions under Potter's direct spray tower at a pressure of 340 g cm^{-2}. The sprayed dishes were dried for five min under a fan after which the larvae were transferred to separate rearing bottles. The larvae, similarly sprayed with emulsified water, served as a control. Larval weight was taken at 3 and 7 days after treatment. Ten replications were used per dose for the test. The raw data on different parameters were subjected to angular transformation (arc sine percentage)$^{1/2}$ and analyzed statistically by complete randomized design. Analysis of variance was done, and means were separated by square difference, i.e., critical difference. Inhibition concentration (IC_{50}) was determined based on probit analysis. Insect growth regulatory activity (IGR) was calculated from percent reduction in larval weight gain over control and calculated as

IGR (%) = [(weight gain in control-weight gain in treatment)/ weight gain in control] × 100

Observations on the biological parameters *viz*, larval survival, weight of full grown larvae, larval duration, pupation and adult emergence were also recorded.

Data Analysis

Growth inhibition (GI_{50}) values were calculated by using a Basic LD_{50} program version 1.1 as described by TREVORS, 1986. The raw data on different parameters were subjected to angular transformation (arc sine percentage) and analyzed by complete randomized design. Analysis of variance was done, and means were separated by critical difference (CD) using the statistical package for social sciences (SPSS, version 10).

Results

Larval mortality by contact toxicity was presented in Tab. 1. As extraction concentration increased, toxicity indices also increased, in a dose-dependent manner. Perusal of the data revealed that with increase in concentrations, there was increased larval mortality. LC_{50} of methanol extract was recorded as 2.54 %, wheras hexane extract did not show any contact toxicity. Among subfractions of methanol extracts, LC_{50} ranged between 1.14 to 2.54 %. Among the sub fractions, chloroform extract showed maximum activity and water extract provided maximum survival of the larvae.

Thevetia leaf extracts lowered food consumption and reduced larval growth rate, which was measured in larval weight reduction and calculated as growth inhibition percentage (Tab. 2). Hexane extract provided no growth regulatory activity upto 1.5 % concentration, wheras methanol extract showed promising IGR activity against *Spodoptera litra.* IGR activity ranged between 9.9 to 31.4 % in 0.5-2.5 % concentration. Among the four sub fractions of methanol extract, chloroform provided best and hexane showed least IGR activity. IGR activity ranged between 9.4 to 46.8 % in the concentration range between 0.5-2.5 %. GI_{50} of methanol extract was 7.20 % and among its subfractions, chloroform extract recorded GI_{50} of 3.02 % (Tab. 3).

Tab. 2: Insect growth regulatory (%) activity of *Thevetia nerifolia* against *S. litura.*

Conc [%]	Hexane	Methanol	Methanol fractions			
			Hexane	Chloro-form	Ethyl-acetate	Water
0.5	-	9.9	4.2	9.4	5.8	9.8
1.0	-	13.4	5.8	20.3	13.4	12.7
1.5	-	18.2	15.9	26.1	17.7	22.3
2.0	6.5	26.1	24.3	39.0	29.4	35.2
2.5	10.6	31.4	27.4	46.8	31.6	38.9

Perusal of the data revealed that the methanloic extract showed the mean larval mortality varied from 19.8 to 53.8 % (Tab. 4). Among subfractions, all the extracts showed similar range of bioactivity. Larval mortality ranged between 24.5 to 71.2 % across the solvent polarity range. Water extract comparatively provided least survival of the first instar larvae. The extracts were also influenced the pupation process also (Tab. 4). Pupation varied between 29.6-78.2 % in methanol extract and hexane extract provided no effect on pupation. Pupation percentage ranged between 20.4 to 60.2 % across subfractions. In terms of adult emergence, ethyl acetate subfraction of methanol provided least adult emergence. It ranged between 14.8-46.5 % upon application of 0.5-2.5 % of the extract. Hexane and chloroform fractions of methanol extract recorded comparatively lesser impact on adult emergence of *Spodoptera litura.*

Discussion

Contact toxicity of the leaf extracts were found not so promising. Chloroform and hexane subfractions of methanol extract showed comparatively better activity than other extracts. Compounds responsible for the activity seem to be of medium polarity range. EL-SHAZLY, 2000 also used fractionation of ethanolic extract of *Nerium oleander* for screening of insecticidal activity. Neriifolin was also isolated from the fractionation of the extract. *Thevetia* sp. also reported to have the same cardiotonic glycoside, neriifolin (MCLAUGHLIN et al., 1980). Ethanol extract of *Thevetia peruviana* provided moderately good mosquito larvicidal activity and the LC_{50} ranged between 211.4-346.4 mg L^{-1} (LAPCHAROEN et al., 2005). These findings are supported by EVANS and KALEYSA RAJ (1988). Though the investigation studied the effect using aqueous extract of the plant.

These studies except LAPCHAROEN et al. (2005) did not mention any effects on molting and metamorphosis of the test insect. *T.*

Tab. 1: Contact toxicity of different extracts of *Thevetia nerifolia* leaf against first instar larvae of *Spodoptera litura.*

Extracts	LC_{50} (%)	χ2exp (3df, 95%)	Fiducial limit	Regression equation
Methanol	2.54	7.09	2.11-3.05	Y = 4.36+1.58x
Methanol-Hexane	1.16	5.15	1.04-1.31	Y = 4.84+2.45x
Methanol-Chloroform	1.14	0.69	0.99-1.31	Y = 4.16+1.75x
Methanol-Ethylacetate	1.27	7.22	1.15-1.40	Y = 3.85+1.68x
Methanol-Water	1.79	1.92	1.53-2.08	Y = 4.51+1.93x

Tab. 3: GI_{50} of different solvent extracts against *Spodoptera litura.*

Extracts	GI_{50} (%)	χ2exp (3df, 95%)	Fiducial limit	Regression equation
Methanol	7.20	1.78	3.28-15.8	Y = 3.96+1.22x
Methanol-Hexane	5.67	3.37	3.39-9.46	Y = 3.64+1.80x
Methanol-Chloroform	3.02	1.12	2.29-3.97	Y = 4.16+1.75x
Methanol-Ethylacetate	4.82	1.31	3.01-7.71	Y = 3.85+1.68x
Methanol-Water	4.10	3.26	2.74-6.11	Y = 4.02+1.60x

Tab. 4: IGR activity of *Thevetia nerifolia* leaf extracts against *Spodoptera litura*.

Treatments	Concentration (%)	Larval mortality (%)	Pupation (%)	Adult emergence (%)
Methanol extract	0.5	19.8	78.2^{a} (62.2)	61.3^{a} (51.3)
	1.0	22.6	72.2^{a} (58.2)	50.1^{b} (45.1)
	1.5	27.5	58.3^{b} (49.8)	49.2^{b} (44.5)
	2.0	36.7	42.5^{b} (40.7)	38.1^{c} (38.1)
	2.5	53.8	29.6^{c} (32.9)	22.3^{d} (22.2)
Methanol-Hexane fr.	0.5	24.5	56.5^{a} (48.7)	45.3^{a} (42.3)
	1.0	39.5	53.3^{a} (46.8)	42.3^{a} (40.7)
	1.5	48.4	42.7^{b} (40.8)	35.3^{b} (36.5)
	2.0	55.8	39.2^{b} (38.7)	28.2^{c} (32.1)
	2.5	59.8	33.4^{b} (35.3)	21.4^{d} (27.6)
Methanol-Chloroform fr.	0.5	27.5	60.2^{a} (50.9)	52.8^{a} (46.6)
	1.0	30.4	48.8^{b} (44.3)	40.6^{b} (39.5)
	1.5	41.8	39.1^{b} (38.7)	30.2^{c} (33.3)
	2.0	57.1	30.6^{c} (33.5)	22.6^{d} (28.4)
	2.5	61.5	28.4^{c} (32.2)	19.8^{d} (26.4)
Methanol-Ethylacetate fr.	0.5	27.0	57.5^{a} (49.3)	46.5^{a} (43.0)
	1.0	38.4	48.5^{b} (44.1)	39.5^{b} (38.9)
	1.5	47.7	36.2^{c} (36.7)	30.2^{c} (33.3)
	2.0	61.8	26.5^{d} (30.9)	18.5^{d} (25.4)
	2.5	66.4	20.4^{d} (26.9)	14.8^{d} (22.6)
Methanol-Water fr.	0.5	29.5	56.8^{a} (48.9)	46.8^{a} (43.2)
	1.0	39.8	52.3^{a} (46.8)	40.2^{b} (39.3)
	1.5	51.9	41.8^{b} (40.3)	32.1^{c} (34.5)
	2.0	67.5	29.8^{c} (33.1)	22.5^{d} (28.3)
	2.5	71.2	23.4^{c} (28.9)	19.6^{d} (26.3)

Figure in parentheses are arc sin transformation values. Subgroup columns sharing the same letter are not significantly different ($P < 0.05$)

peruviana did not show any IGR properties against two mosquito species (LAPCHAROEN et al., 2005). Our findings are in contrast with the earlier study. IGR activity was recorded in first instar larvae of *S. litura*. Feeding detergency was also observed in the first instar larvae of *S. litura* and the IGR activity might be attributed to the antifeedancy of the extracts. Probable mode of action is likely to be juvenile hormone mimics like juvocimenes of sweet basil, miroesterol derivatives of *Pueraria mirifica* etc.. Though we could not present the antifeedant data but feeding detergency was observed, which is in agreement with REED et al., 1982. Glycoside from *T. nerifolia* like neriifolin, thevetin A & B, peruvoside was found to be major ingredient for acting as feeding deterrent to the striped cucumber beetle, *Acalymma vittatum.* Glycosides like triterpenic saponins were found to have antifeedant and IGR activity against *Spodoptera litura* (SAHA et al., 2010).

Larval, pupal and adult survival was influenced by the persistence of the active molecules in the insect hemolymph (Tab. 4). IGR activity was characterized by initial larval mortality followed by mortality of pupa and adult. Elongation of pupal period was not clearly observed, though minor changes in the pupation were recorded as compared to control insects. Larval and pupal mortality during the respective stages, and molting could either be due to the presence of toxic ingredients in the extract or the imbalances between growth stimulating and growth-inhibiting hormones. Further studies are needed to establish a more exact explanation for the cause of death.

It is not clear whether this is due to mimicking of the juvenile hormone, to blocking hormone degradation, or to some other physiological interference. Various functions occurring during molting and metamorphosis may be affected, such as cuticle tanning and

hardening, but actual target of action needs more study to confirm. With the present state of knowledge, it can be concluded that IGR actions of the methanol extract was clear and might be attributed to the glycosides present in the extract.

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