



BIOCIDAL ACTIVITIES OF CERTAIN PLANT EXTRACTS AGAINST TOBACCO CATERPILLAR *SPODOPTERA LITURA* FAB.

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Abstract

Spodoptera litura, commonly known as the Oriental leaf worm moth, tobacco cutworm, and tropical armyworm, is causing economic damage in various crops under favourable climatic conditions throughout the world and this insect developed resistance against various groups of insecticides which are available right now in the market. Researchers are considering botanical insecticides as one of the viable alternatives; because of its complex phyto-chemical constituents. In this line, a study was carried out with the leaf extracts of eleven plants belonging to nine different families. Extracts were obtained by using solvents viz., acetone, ethyl acetate and benzene each separately. Bio-assays were conducted to judge the antifeedency, IGR activities and insecticidal actions of the extracts against third instar of *S.litura*. The results showed that the acetone based extracts of *Rheum rhabbarbarum* and *Citrullus colocynthis* were exerted strong feeding inhibition. The highest insecticidal action was observed in acetone based extract of *Adenanthera pavonina*. *Nelumbo nucifera* recorded astonishing insect growth regulatory activity.

Key words : *Spodoptera litura*, Antifeedency, IGR and Insecticidal action.

Introduction

Botanical insecticides are not a new component in insect pest management. But renewing the interest of this age-old concept is necessary in the present day pest management because of the ill effects caused by the over or improper use of insecticides by the farming community. There are several thousand investigations on botanicals worldwide but still there is scope for getting new compounds. In the present study, eleven plants belonging to the families such as Polygonaceae, Apiaceae, Fabaceae, Nymphaeaceae, Asteraceae, Apocynaceae, Cucurbitaceae, Euphobiaceae and Oxiladaceae were selected. The plants viz., *Rheum rhabbarbarum*, *Conium maculatum*, *Adenanthera pavonina*, *Nelumbo nucifera*, *Ageratina altissima*, *Cerbera odollam*, *Citrullus colocynthis*, *Plumeria rubra*, *Pedilanthus tithymaloides*, *Oxalis corniculata* and *Croton bonplandianum* were subjected to antifeedant, insecticidal and IGR studies using *S.litura* as test insect. Out of the eleven plants, *Ageratina altissima* (Asteraceae), *Conium maculatum* (Apeaceae) and *Nelumbo nucifera* (Nymphaeaceae) are unexplored and however the literature pertaining to the biocidal and

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medicinal properties of other plants are also scanty. Phanatchakon somsroi and Sukanda chai yong, (2016) reported antifeedancy and insecticidal action of fruit extract of *Cerbera odollam*. Emodin a phytochemical in Rhubarb was reported to have a deterrent effect on a large spectrum of organisms from invertebrates to vertebrates (Mohammad and Mazen, 2018). Oleic and linoleic acids in *Citrullus colocynthis* was known to be more effective against aquatic insects and isolated fraction 'C' obtained from *C.colocynthis* showed 94.4, 96 and 98.4% mortality against third instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinque fasciatus* at 100 ppm and LC50 values were 18.57, 23.48, 19.26 ppm and LC90 values were 98.65, 92.26, 84.84 ppm respectively (Subramanian Arivoli *et al.*, 2015). Silver nano particles synthesized by using *Plumeria rubra* plant latex was highly toxic than the aqueous crude latex extract against *A.aegypti* and *A.stephensi* (Chandrashekar *et al.*, 2012). *Conium maculatum* was reported as poisonous for sheep (Keeler *et al.*, 2008). The seed coat extract of *Nelumbo nucifera* was found to possess larvicidal action against *A.stephensi* (Anushree *et al.*, 2015). In the light of the above literature bio-assays were conducted to judge the antifeedency, IGR activities and insecticidal actions of the extracts against third instar

of *S.litura*.

Materials and Methods

Culturing of *Spodoptera litura*

Larvae of tobacco caterpillar *Spodoptera litura* (Noctuidae: Lepidoptera) were reared on castor leaves (natural host) up to second instar and then on Bengal gram flour based semi synthetic diet up to pupation. Culturing was initiated with egg masses collected from the field in and around Annamalainagar. Larvae hatched out from the collected egg masses were transferred on castor leaves *Ricinus communis* L. and kept aside a plastic container of 10 L. capacity and covered with muslin cloth. Fresh leaves of castor were supplied daily and the old leaves were removed. When the caterpillars became third instar, they were transferred onto semi synthetic diet.

Third instar onwards larvae of *S. litura* were maintained on Bengal gram based semi synthetic diet. Cut pieces of semi synthetic diet were placed on iron stands and kept inside the plastic containers (10 L. capacities) which were filled with sterilized sand up to 15 cm. height. Twenty five larvae were released per container and covered with the lid fitted with muslin cloth (Selvamuthukumar, 2009).

Pieces of fresh diet were provided once in three days and pupation took place in the sand layer provided. Pupae were collected and washed with 1% formaldehyde solution, sexed and transferred into oviposition cages (1'×1'×1') at the rate of ten pairs per cage. After adult emergence, 10% honey solution fortified with vitamin E provided as adult food in a cotton wig. Then a *Nerium oleander* twigs (petiole immersed in water) were kept inside the cage which acted as oviposition substrate. Eggs laid were collected daily, washed with 0.05 % sodium hypochlorite solution and incubated. The hatched neonates were transferred to castor leaves and recycled. Rearing was done at 24 ± 2°C and 70 ± 5% RH.

Extraction of selected plants

Leaves (1kg) of the eleven plants table 1 were collected and shade dried for two weeks. Then the dried leaves were powdered using electric blender (mixer) each separately. Then 1 kg of powdered plant sample was packed as 25g pockets using Whatman No.40 filter paper. These pockets were refluxed in soxhlet apparatus for 48h with acetone (BP 64-65°C), ethyl acetate (BP 77.1°C) and benzene (BP 70.1°C) each separately. Then the extracts were concentrated under reduced pressure by using rotary vacuum evaporator (Buchi Roto evaporator R200) to obtain solid or semisolid misilla. The misilla

collected at various periods, pooled and stored in a deep freezer at -20°C. This was considered as stock material and used in bioassays at various dilutions.

Anti feeding assay

Leaf discs (78.5cm² area) intact with petioles cutoff from fresh castor (*Ricinus communis* L.) leaves were used in the antifeedent-leaf disc bioassay. Five per cent concentration of each leaf extract was applied on both the surfaces of the leaf disc and smeared using a blunt glass rod (0.5 ml/ side). Then the treated leaf discs were air-dried. Petiole of the disc was inserted into glass vial (20 ml capacity) containing water and secured tightly by using non absorbent cotton wool and kept in transparent plastic containers (500ml capacity) @ one disc/container. On each disc, newly moulted third instar larvae of *S.litura* (pre starved for 3h) were introduced (@ one/disc) five such discs were used in a replication. Mouth of the containers were covered by muslin cloth and tightly secured by using a rubber band. Cannibalism was noticed in *S.litura* in our earlier bioassays, thus larvae were maintained each separately. All the containers were kept at 20±2°C and 75% RH. Experiments were started by 6 pm. Each treatment was replicated at three times. When leaf discs in control completely fed, the treated leaf discs were collected. The leaf area unfed in the treatments were measured by using leaf area meter (Systronics Leaf Area Meter 211) and per cent leaf area protection over control was computed and graded by referring the following formula and grading scale.

Percent leaf area protection over control =

$$\frac{\text{Percent leaf area protection in control} - \text{Percent leaf area protection in treatment}}{100 - \text{Percent leaf area protection in control}} \times 100$$

Rating Scale	
Per cent leaf area protection	Grade
> 80	Strong Inhibition (++++)
50-80	Medium Inhibition (+++)
20-50	Weak Inhibition (++)
< 20	Insignificant inhibition (+)

(Rani and Arivudainambi, 2013)

Insecticidal and IGR assays

The method described in antifeedent assay was followed with little modification. Instead of 5% concentration, 2% was used. After complete feeding on leaf discs, the exposed larvae were supplied with fresh leaves and reared until adult emergence. Observation on per cent mortality, pupal mortality and adult emergence were made at 24h interval.

Results and Discussion

Out of eleven plants tested acetone based extracts of two plants viz., *Rheum rhabbarbarum* (85%) and *Oxalis corniculata* (80.83%) (Table 2) exhibited strong antifeedency. The report of Tripathi *et al.*, (2012) is in accordance with present result; who investigated *Rheum* sp. for anti insect properties against *S. litura* and revealed that emodin rich fraction of *Rheum* sp. was responsible for IGR and antifeedent activity (58.69%) @ 0.4 %. *Oxalis corniculata* was also reported more toxic and showed to have antifeedent action against *Tribolium casteanum* (57%) and *Ephestia cautella* (49%) @

50mg/ml respectively by Azizur Rehman *et al.* (2015). *Rheum rhabbarbarum* and *Oxalis corniculata* exerted medium antifeedancy while extracted with ethyl acetate that was 61.33 and 61% respectively table 3. These plants had shown weak antifeedency while extracting with the benzene. It shows that, acetone extracts of *Rheum rhabbarbarum* and *Oxalis corniculata* are superior in exhibiting antifeedant activity. Thus out of 33 various treatments excluding control, only two extracts were shown strong antifeedency.

Appreciable insecticidal action of 68.88 and 55.55% were exerted by acetone based extracts of *Adenanthera*

Table 1: Details of the plants selected.

S.No.	Botanical Name	Common name	Family	Parts used for extraction
1.	<i>Rheum rhabbarbarum</i> M.	Rhubarb	Polygonaceae	Leaves
2.	<i>Conium maculatum</i> L.	Hemlock	Apiaceae	Leaves
3.	<i>Adenanthera pavonina</i> L.	Red bead tree	Fabaceae	Leaves
4.	<i>Nelumbo nucifera</i> G.	Lotus	Nymphaeaceae	Leaves
5.	<i>Ageratina altissima</i> L.	White snake root	Asteraceae	Leaves
6.	<i>Cerbera odollam</i> G.	Suicide tree	Apocynaceae	Leaves
7.	<i>Citrullus colocynthis</i> L.	Bitter apple	Cucurbitaceae	Leaves
8.	<i>Plumeria rubra</i> L.	Frangipani	Apocynaceae	Leaves
9.	<i>Pedilanthus tithymaloides</i> L.	Devil's back bone	Euphobiaceae	Leaves
10.	<i>Oxalis corniculata</i> L.	Creeping wood sorrel	Oxiladaceae	Leaves
11.	<i>Croton bonplandianum</i> L.	Rush foil	Euphobiaceae	Leaves

Table 2: Efficacy of leaf extracts (acetone) against *Spodoptera litura*.

S.No.	Treatments	Anti-insect Activities					
		Leaf area protection over control	Antifeedent grading	Insecticidal Action (% mortality) (At 5%)	IGR activities (at 2%)		
					Larval malformation & mortality	Pupal malformation & mortality	Normal adult emergence
1.	<i>Rheum rhabbarbarum</i>	85.00 ^b (67.20)	++++	2.20 ^a (4.96)	0.00(0.00)	0.00 ^a (0.00)	91.11 ^f (72.85)
2.	<i>Conium maculatum</i>	10.30 ^e (18.71)	+	55.55 ^b (48.18)	0.00(0.00)	11.07 ^c (19.21)	37.78 ^c (37.89)
3.	<i>Adenanthera pavonina</i>	11.17 ^f (19.51)	+	68.88 ^a (56.101)	0.00(0.00)	6.6 ^b (14.88)	24.44 ^b (29.57)
4.	<i>Nelumbo nucifera</i>	2.37 ^b (8.85)	+	0.00 ^f (0.00)	100(90.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
5.	<i>Ageratina altissima</i>	13.20 ^a (21.30)	+	17.78 ^c (24.84)	0.00(0.00)	0.00 ^a (0.00)	46.66 ^d (43.06)
6.	<i>Cerbera odollam</i>	14.70 ^b (22.54)	+	44.44 ^c (41.77)	0.00(0.00)	6.6 ^b (14.88)	48.89 ^d (44.32)
7.	<i>Citrullus colocynthis</i>	52.53 ⁱ (46.434)	+++	0.00 ^f (0.00)	0.00(0.00)	0.00 ^a (0.00)	93.33 ^f (77.85)
8.	<i>Plumeria rubra</i>	6.50 ^d (14.76)	+	24.44 ^d (29.57)	0.00(0.00)	0.00 ^a (0.00)	53.33 ^{de} (46.92)
9.	<i>Pedilanthus tithymaloides</i>	17.07 ⁱ (24.40)	+	0.00 ^f (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
10.	<i>Oxalis corniculata</i>	80.83 ^k (64.01)	++++	28.89 ^d (32.28)	0.00(0.00)	0.00 ^a (0.00)	31.11 ^{bc} (33.86)
11.	<i>Croton bonplandianum</i>	4.23 ^c (11.87)	+	17.78 ^c (24.84)	0.00(0.00)	0.00 ^a (0.00)	60.00 ^e (50.78)
12.	Control	0.90 ^a (5.44)	+	0.00 ^f (0.00)	0.00(0.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
13.	Positive control – Neem 1500 ppm azadiractin commercial formulation	91.33(73.37)	++++	70.27(56.93)	0.0(0.00)	0.0(0.00)	15.10(22.86)
	CD	0.65	-	6.101	0.00	1.84	6.899

Table 3: Efficacy of leaf extracts (Ethyl acetate) against *Spodoptera litura*.

S.No.	Treatments	Anti-insect Activities					
		Leaf area protection over control (at 5%)	Antifeedent grading	Insecticidal Action (% mortality) (at 5%)	IGR activities (at 2%)		
					Larval malformation & mortality	Pupal malformation & mortality	Normal adult emergence
1.	<i>Rheum rhabbarbarum</i>	61.33 ^a (51.53)	+++	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
2.	<i>Conium maculatum</i>	8.30 ^d (16.73)	+	40.00 ^b (39.18)	0.00(0.00)	8.83 ^b (17.05)	51.11 ^c (45.62)
3.	<i>Adenantha pavonina</i>	4.10 ^b (11.67)	+	46.66 ^c (43.06)	0.00(0.00)	13.30 ^c (21.38)	44.44 ^b (41.78)
4.	<i>Nelumbo nucifera</i>	0.93 ^a (4.51)	+	0.00 ^a (0.00)	100(90.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
5.	<i>Ageratina altissima</i>	6.30 ^c (14.53)	+	8.83 ^c (17.05)	0.00(0.00)	0.00 ^a (0.00)	75.55 ^c (60.39)
6.	<i>Cerbera odollam</i>	14.70 ^e (22.54)	+	28.88 ^c (32.47)	0.00(0.00)	11.07 ^{bc} (19.21)	60.00 ^d (50.75)
7.	<i>Citrullus colocynthis</i>	32.93 ^f (35.01)	++	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
8.	<i>Plumeria rubra</i>	4.23 ^b (11.87)	+	11.09 ^c (19.23)	0.00(0.00)	0.00 ^a (0.00)	79.99 ^d (63.61)
9.	<i>Pedilanthus tithymaloides</i>	15.10 ^e (22.86)	+	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
10.	<i>Oxalis corniculata</i>	61.00 ^e (51.33)	+++	24.44 ^c (29.45)	0.00(0.00)	0.00 ^a (0.00)	44.44 ^b (41.78)
11.	<i>Croton bonplandianum</i>	2.50 ^{ab} (9.10)	+	13.33 ^d (20.97)	0.00(0.00)	0.00 ^a (0.00)	79.99 ^d (63.64)
12.	Control	0.60 ^a (4.43)	+	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
13.	Positive control	90.23 (71.76)	++++	70.43(57.04)	0.00	0.00	15.33(23.04)
	CD	1.904		5.194	0.00	2.60	3.871

Table 4: Efficacy of leaf extracts (Benzene) against *Spodoptera litura*.

S.No.	Treatments	Anti-insect Activities					
		Leaf area protection over control (at 5%)	Antifeedent grading	Insecticidal Action (% mortality) (at 5%)	IGR activities (at 2%)		
					Larval malformation & mortality	Pupal malformation & mortality	Normal adult emergence
1.	<i>Rheum rhabbarbarum</i>	32.03 ^f (34.45)	++	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
2.	<i>Conium maculatum</i>	6.30 ^c (14.53)	+	24.44 ^a (29.24)	0.00(0.00)	6.60 ^b (14.88)	73.33 ^d (59.01)
3.	<i>Adenantha pavonina</i>	2.20 ^{ab} (8.53)	+	22.22 ^a (28.06)	0.00(0.00)	20.00 ^d (26.32)	80.00 ^c (63.64)
4.	<i>Nelumbo nucifera</i>	1.17 ^a (5.06)	+	0.00 ^a (0.00)	100(90.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
5.	<i>Ageratina altissima</i>	4.53 ^{bc} (12.288)	+	4.40 ^a (9.92)	0.00(0.00)	0.00 ^a (0.00)	80.00 ^c (63.61)
6.	<i>Cerbera odollam</i>	2.50 ^{ab} (9.09)	+	20.00 ^{ab} (26.35)	0.00(0.00)	13.3 ^c (20.93)	66.66 ^c (54.78)
7.	<i>Citrullus colocynthis</i>	28.37 ^e (32.165)	++	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
8.	<i>Plumeria rubra</i>	2.50 ^{ab} (9.09)	+	11.09 ^{cd} (19.23)	0.00(0.00)	0.00 ^a (0.00)	80.00 ^c (63.61)
9.	<i>Pedilanthus tithymaloides</i>	14.60 ^d (22.45)	+	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	100.00 ^a (90.00)
10.	<i>Oxalis corniculata</i>	30.30 ^{ef} (33.38)	++	15.55 ^{bc} (23.12)	0.00(0.00)	0.00 ^a (0.00)	60.00 ^b (50.78)
11.	<i>Croton bonplandianum</i>	1.10 ^a (4.92)	+	6.66 ^{de} (14.95)	0.00(0.00)	0.00 ^a (0.00)	86.66 ^f (69.98)
12.	Control	0.33 ^a (3.30)	+	0.00 ^a (0.00)	0.00(0.00)	0.00 ^a (0.00)	0.00 ^a (0.00)
13.	Positive control	90.43 (71.96)	++++	70.10(56.83)	0.00	0.00	15.43(23.12)
	CD	2.97		6.236	0.00	3.713	5.822

pavonina and *Conium maculatum* at 2% concentration and this was highest among all the 33 treatments. Maria *et al.*, (2004) conducted an experiment with *A.pavonina* Kunitz type of inhibitor (ApTi) against *Callosobruchus maculatus* and they observed 50 per cent mortality at 0.25 and 0.5% of ApTi inoculated artificial diet. *Conium maculatum* is a poisonous plant, but very few works have been carried out against invertebrates. As found earlier, ethyl acetate and benzene based leaf extracts of

A.pavonina and *C.maculatum* recorded lower larval mortality. Maximum pupal mortality was observed in benzene based extract of *Adenantha pavonina* (20%) followed by *Cerbera odollam* (13.31%) and acetone based extracts of *Conium maculatum* (11.07%). Phanatchakon Somsroi and Sukanda Chaiyong (2016) reported antifeedant and insecticidal action of ethanolic fruit crude extract of *C.odollam* at 30.0% (w/v) and 5.0% (w/v) respectively and the concentration used in

that study was too high. In the present study *C. odollam* leaf extract possessed insect growth regulatory activity rather than antifeedant and insecticidal. Complete adult emergence was noticed in the treatments with *Pedilanthus thithimaloides*.

Among the eleven plants, *Nelumbo nucifera* showed 100 per cent insect growth regulatory activity. It was astonishing to see the results; because the treated third instars did not convert to fourth instar. But the treated third instars were continuously engaged in moulting without melanization. They lived for about nine days and then died. There were no earlier reports related to the IGR of the leaf extract of *N. nucifera*. But, Anushree *et al.*, (2014) reported that crude extract of *N. nucifera* seed coat was larvicidal against *A. stephensi*. Thus it is concluded that acetone extracts of *Rheum rhabbarum* and *Oxalis corniculata*, *Adenanthera pavonina* and *Nelumbo nucifera* were identified for their strong antifeedant, good insecticidal and very strong IGR activities in our preliminary screening. The research related to decode the IGR activities of *Nelumbo nucifera* are under progress.

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