Dose dependant differential anti insect activity of lactone glycoside, a potent plant derived molecule

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ABSTRACT

Lactone glycoside, the active ingredient present in *Cleistanthus collinus* (Roxb.) Benth. possessing variety of anti insect properties like antifeedant, insecticidal and insect growth regulatory activity was bioasayed on third, fourth and fifth instar *Spodoptera litura* Fab. larvae to determine its median effective concentrations. Median deterrency index (DI_{50}), median lethal concentration (LC_{50}) and median emergence inhibition (EI_{50}) were worked out for antifeedant, insecticidal and insect growth regulatory activity respectively through regression and probit analysis. The anti insect activity was dose dependant. The activity varied with increase in concentration in the following order *viz.,* insect growth regulatory, insecticidal and antifeedant action while Median emergence inhibition was at 0.17 ppm, 0.26 ppm and 0.38 ppm, median lethal concentration and median deterrency index were at 1.02 ppm, 1.71 ppm, 2.045 ppm and 60.66 ppm, 68.47 ppm, 71.10 ppm for third, fourth and fifth instars respectively. Further, the activity was also found to be age dependant. As the larval age increased, the required median effective dose also increased. Multi pronged anti insect activity of the lactone glycoside was compared with other botanicals and discussed in detail.

Keywords: *Cleistanthus collinus***,** Lactone glycoside, anti insect activity, median effective concentrations.

INTRODUCTION

Insecticides of plant origin have been exploited from time immemorial for the management of insect pests of crop plants and stored produce (Jacobson and Crosby, 1971 and Karl Maramorsch, 1991). Though they exerted coherent management over insect pests, invention of synthetic organic insecticides replaced botanicals from insect management scenario. But, excessive reliance on synthetic molecules created challenges *viz.*, residue, resistance and resurgence development. With the introduction of integrated pest management concept, botanicals again acquired importance. However, their large-scale utilization in pest management is obstructed by non-availability of effective formulations. This can be surmounted through structural elucidation, recognizing the mode of action, synthesis and formulation of the active principle.

One of the earlier research work carried out at our Institute on screening various plant species for their pesticidal value revealed the supremacy of leaf extracts of *Cleistanthus collinus* (Roxb.) Benth (Euphorbiaceae) as a potent extract. The active ingredient responsible for variety of anti insect properties like antifeedant, insecticidal and insect growth regulatory activity was structurally elucidated as “Lactone glycoside” and was found to impart very significant antifeedant activity (Arivudainambi, 2001), emergence inhibition larval mortality against third, fourth and fifth instar *Spodoptera litura* larvae (Selvamuthukumaran and Arivudainambi, 2008 a and b). The present work aims at determination of median effective concentrations *viz.,* median deterrency index (DI_{50}), median lethal concentration (LC_{50}) and median emergence inhibition (EI_{50}) of lactone glycoside which would help in further determination of mode of action and development of formulation.

MATERIALS AND METHODS

Culture of *Spodoptera litura*

Tobacco caterpillar, *Spodoptera litura* was reared on bengal gram flour based semi synthetic diet (PDBC, 1998) under 25 ± 2°C and 70 to 80 % relative humidity continuously throughout the study period in the laboratory and utilized in the bioassays as test insect.

Lactone glycoside

The active principle lactone glycoside was obtained from the initial isolated stock available in the Toxicology laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University.

Concentrations used

One ppm concentration of lactone glycoside was prepared by dissolving one µg in 10 µl of acetone and mixed with 85 µl of double distilled water and 5 µl of Tween 80. 20 ppm, 40

ppm, 60 ppm, 80 ppm and 100 ppm concentrations were prepared to determine median deterrency index (DI_{50}) for antifeedant action and bio assayed against third, fourth and fifth instars. 0.625, 1.25, 2.5, and 5 ppm concentrations against third instar and 1.25, 2.5, 5 and 10 ppm concentrations against fourth and fifth instars were prepared to determine median lethal concentration (LC_{50}) for insecticidal action.0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.5 ppm concentrations were prepared to determine median emergence inhibition (EI_{50}) for growth regulatory action and bio assayed against third, fourth and fifth instars. 90 % antifeedancy, 90 % mortality and 60 % emergence inhibition were reported against fifth instar *S.litura* at 100, 10, 0.5 ppm concen trations of lactone glycoside respectively (Arivudainambi, 2001; Selvamuthu kumaran and Arivudainambi, 2008 a and b). Hence varied intervals of 0.1 – 0.5 ppm, 0.625 – 10 ppm and 20 – 100 ppm were prepared to found out respective median effective concentration. Moreover, 93 % mortality was reported at 5 ppm concentration itself against third instar *S.litura* (Selvamuthukumaran and Arivudainambi, 2008 b). Hence the dose interval for determining median lethal concentration (LC_{50}) for third instar was fixed as 0.625 – 5 ppm.

Bioassay

2 cm diameter castor leaf discs were treated on either side with 100 µl lactone glycoside. These leaf discs were kept individually in plastic containers (200 ml capacity) after air drying. Three third instar larvae pre starved for 4 hour were released per disc and the container was closed with muslin cloth. One solvent control and absolute control leaf discs treated with 100µl of acetone and emulsified water respectively were also maintained. Each treatment was replicated ten times. Similar bioassays were conducted with fourth and fifth instar larvae. Three such bioassays were carried out with respective concentrations of lactone glycoside as mentioned above to determine median effective concentrations for antifeedant, insecticidal and growth regulatory actions.

Median deterrency index

In the first bioassay, when the control leaf discs, whose leaf area pre determined, were completely fed (after 2 – 3 hours), leaf area fed in treated discs were determined graphically and per cent leaf area fed was worked out. Deterrency index was calculated using the formula

$$\text{Deterrency Index (DI)} = \frac{C - T}{C + T} \times 100$$

Where C = Per cent leaf area consumed in control discs

T = Per cent leaf area consumed in treated discs.

Using regression analysis, DI_{50} (concentration needed for 50 percent deterrency) was found out (Wheeler and Isman, 2001).

Median lethal concentration

In the second bioassay, after 48 hours, data on the number of dead larva was recorded. Mortality was corrected using abbott's formula (1925). Using Finney's method of probit analysis (1971) LC_{50} value and fiducial limits were worked out.

Median emergence inhibition

In the third bioassay, after the treated leaf discs were completely fed, fresh castor leaves were provided and larvae reared until adult emergence. Observations were made daily on per cent malformed insects emerged. Using regression analysis, median emergence inhibition (concentrations inhibiting 50 per cent emergence) was found out (Sivagnaname and Kalyanasundaram, 2004).

RESULTS

The results of the bioassays proved the presence of effective anti insect activities of lactone glycoside, of *C.collinus*. The imparted deterrency index varied from 11.28 to 89.42 against third instar at 20 to 100 ppm. Against fourth and fifth instar, the deterrency index varied from 6.36 to 80.60 and 3.92 to 77.16 respectively. Regression equations obtained through regression analysis of deterrency indices revealed that the DI_{50} was 60.66, 68.47 and 71.10 ppm for third, fourth and fifth instars respectively (Fig 01).

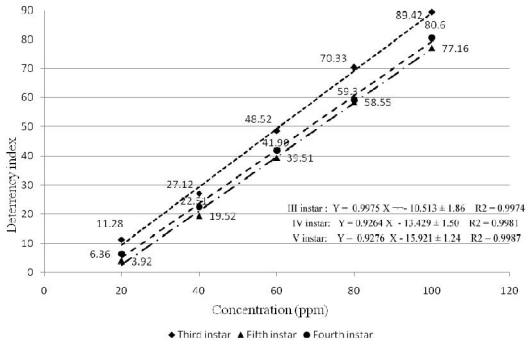

Figure 1. Linear regression curves of deterrency indices*of third, fourth and fifth instar *S.litura* larvae

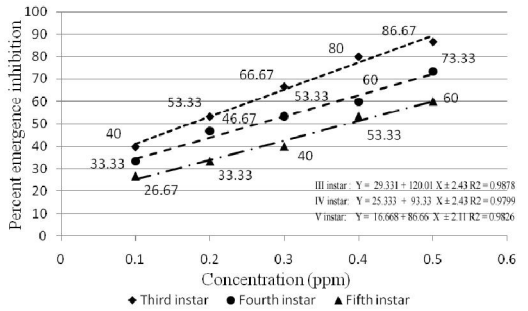

Figure 2. Linear regression curves of emergence inhibition*of third, fourth and fifth instar *S.litura* larvae

Table 1. Toxicity of lactone glycoside against third instar *S. litura* larvae

Concentration (in ppm)	Concentration (in %)	No. of insects	No. Dead	% larval mortality	Corrected per cent mortality
0.625	0.0000625	30	14	46.67	44.10
1.25	0.000125	30	19	63.33	62.10
2.5	0.00025	30	23	76.67	75.90
5	0.0005	30	29	96.67	96.60
Solvent control(Acetone)	0.00	30	1	3.33	0.00
Absolute Control	0.00	30	1	3.33	0.00

LC_{50} = 1.02 ppm (0.76 ppm – 1.36 ppm), Y = 7.2623 + 2.279 X, Slope(b) = 2.279, Intercept(a) = 7.2623, Table χ^2 = 7.8147278, Calculated χ^2 = 1.273965

The larval mortality recorded in the lowest concentration tested in the insecticidal bioassay was 46.67 percent, 40 percent and 33.33 percent against third, fourth and fifth instar larvae respectively. In the highest concentration tested the mortality increased to 96.67 percent, 96.67 percent and 90 percent. Through probit analysis, the LC_{50} values and fiducial limits of 1.02 (0.76 – 1.36), 1.71(1.19 – 2.45) and 2.45(1.77 – 3.41) ppm were obtained for third, fourth and fifth instar larvae respectively (Table I, II & III).

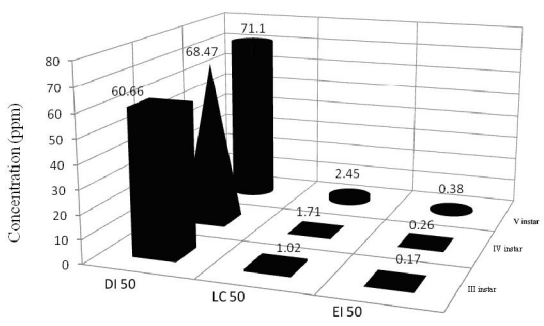

Figure 3. Comparison of median effective concentrations of third, fourth and fifth instar *S.litura* larvae

Percent emergence inhibition ranged from 40 to 86.67 per cent, 33.33 to 73.33 per cent and 26.67 to 60.00 per cent at 0.1 to 0.5 ppm against third, fourth and fifth instars respectively. When regression analysis was done on these percent emergence inhibition, EI_{50} values of 0.17, 0.26 and 0.38 ppm were obtained against third, fourth and fifth instars respectively (Fig 02).

DISCUSSION

The median effective concentrations had clearly shown that lactone glycoside possess varying anti insect activities at varying concentrations. At lower concentration up to 0.5 ppm, it imparted insect growth regulatory activity while upto 5 ppm and 75 ppm concentration it imparted insecticidal and antifeedant action respectively towards *S.litura* (Fig 03). The results reveals that the anti insect activity was dose dependent and it varied from growth regulatory to insecticidal and antifeedant action with increase in concentration.

Such, dose dependent anti insect activity is a common phenomenon in botanicals as reported by Garcia and Rembold (1984) in azadirachtin and they found that 600 times less concentration of azadirachtin was required to disrupt development in *Rhodnius prolixus* Stal. than was required to produce antifeedant effect. Schmutterer (1990) and Mordue and Blackwell (1993) suggested that azadirachtin treatment resulted in various morphogenetic defects as well as mortality depending on concentrations applied.

Similarlly, Nisbet *et al*. (1992) suggested that 100 to 1000 ppm azadirachtin induced significant primary antifeedant effects whereas Mordue and Blackwell (1993) and Ramachandran et al., (1989) reported that 50 ppm was enough to impart significant antifeedant action in Lepidoptera. Hence, lactone glycoside, whose median deterrency index (DI_{50}) for fifth instar *S.litura* was 70 ppm would be as effective as azadirachtin. LC_{50} value of 1.02, 1.71 and 2.45 ppm against third, fourth and fifth instar *S.litura* larvae were found to be more effective than the LC_{50} value of 2.5118 ppm for azadirachtin against *S.litura* as reported by Mukerjee and Sharma (1993).

Azadirachtin when treated against *Spodoptera litura* as injection @ 1.1 µg / g caused 50 per cent moult inhibition (Rao and Subrahmanyam, 1987). For first instar *S.litura*, 1 ppm was reported to result in 95 per cent moult inhibition (Kubo and Klocke, 1982) whereas for second instar 0.62 ppm was required for 50 percent moult inhibition (Mukerjee and Sharma, 1993). However, lactone glycoside was found to impart 50 percent emergence inhibition at lower concentrations of 0.17, 0.26 and 0.38 ppm against third, fourth and fifth instar larvae of *S.litura*.

Table 2. Toxicity of lactone glycoside against fourth instar *S. litura* larvae

Concentration (in ppm)	Concentration (in %)	No. of insects	No. Dead	% larval mortality	Corrected percent mortality
1.25	0.000125	30	12	40.00	37.9
2.5	0.00025	30	20	66.67	65.5
5	0.0005	30	25	83.33	82.8
10	0.001	30	29	96.67	96.6
Solvent control(Acetone)	0.00	30	1	3.33	0.00
Absolute Control	0.00	30	1	3.33	0.00

LC_{50} = 1.71 ppm (1.19 ppm – 2.45 ppm) Slope(b) = 2.023253, Intercept(a) = 6.553879, Y = 6.554 + 2.023 X, Table x^2= 7.8147278, Calculated x^2= 0.111387

The possible reason for dose dependant anti insect activity was that at higher concentration significant antifeedant action was imparted and might have prevented ingestion and further toxic effects. Such inability to ingest, resulting from the perception of the antifeedant at sensory level was reported as primary antifeedancy (Schmutterer, 1985). At lower concentrations, toxin would have been ingested and hence, manifestation of insecticidal and insect growth regulatory action occurred.

These findings suggested that the antifeedant action might be operating by reducing food consumption through its action on feeding receptors of *S.litura*. It was supported by reports of Schoonhoven and Jermy (1977), Schoonhoven and Liner (1994), Simmonds *et al*. (1995a, b) and Messchendrop *et al*. (1996) that stimulation of deterrent receptors and suppression of taste receptors in azadirachtin, toosendanin and drimane induced antifeedant effects. At lower concentrations, as the lactone glycoside was ingested it induced toxic and morphogentic effects by influencing various physiological systems.

The results indicated that the mortality was rapid. At 48 hrs 90 percent mortality was reported even against fifth instar suggesting effect on nervous system. This was supported by Arivudainambi and Baskaran (2004) who observed that hyper excitation, ataxia, tremors and unbalanced walking in *C. collinus* extract treated larvae of *S. litura* as in organophosphorus and organochlorine poisoned insects. Azadirachtin was known to affect juvenile hormone and ecdysteroid titres and cause severe abnormalities (Rembold *et al*., 1987; Min-Li and Shin-Foon, 1987 Martinez and Van Emden, 2001). Hence, appearance of severe abnormalities in lactone glycoside treatment at very low concentrations against *S.litura* indicated interference with neuro secretory system.

The median effective concentrations increased with the age of the larvae tested. Such variations of the effect of lactone glycoside along the age of the larvae was supported by the reports of Ramachandran *et al*. (1989) and Mukherjee (1992) who reported differential sensitivity of *S. litura* larvae based on age in azadirachtin treatment.

These findings revealed the potential of the active ingredient lactone glycoside and the possibility of developing it into an effective plant based insecticide.

Table 3. Toxicity of lactone glycoside against fifth instar *S. litura* larvae

Concentration (in ppm)	Concentration (in %)	No. of insects	No. Dead	% larval mortality	Corrected percent mortality
1.25	0.000125	30	10	33.33	31.0
2.5	0.00025	30	16	53.33	51.70
5	0.0005	30	20	66.67	65.50
10	0.001	30	27	90.00	89.70
Solvent control(Acetone)	0.00	30	1	3.33	0.00
Absolute Control	0.00	30	1	3.33	0.00

LC_{50} = 2.45 ppm (1.77 ppm – 3.41 ppm), Y = 6.103 + 1.808 X, Slope(b)= 1.808259, Intercept(a) = 6.103385,Table x^2= 7.8147278, Calculated x^2= 0.786635

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