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Insecticidal Efficacy of *Porteresia coarctata* (Roxb.) on Bio-chemical Alteration of *Spodoptera litura* (Fab.)

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Hexane extract of *Porteresia coarctata* (Roxb.) exhibits a toxic effect on the tissues of *Spodoptera litura* (F) while fed at the dose of 1000 and 2000 ppm thoroughly mixing with castor leaves (*Ricinus communis* L) after dissolving in DMSO at late fourth instar whereas only DMSO treated castor leaves were fed to control group. The larvae were put to rear at 28°C±1°C, 76±4% R.H. under 12 L+12 D photoperiodic regime. In test group insects substantial reduction of protein and DNA content was marked in fat body and midgut tissues compared to DMSO treated control group. The significant biochemical alterations in the midgut tissues and fat body of test group insects indicate the insecticidal property of the said plant extract that could be tested in facilitating the phenomenal stride in Integrated Pest Management.

Key words: Insecticidal efficacy, *Porteresia coarctata*, *Spodoptera litura*, Integrated Pest Management, Plant Metabolite, Insect tissue, Biochemical alteration, Sterol biosynthesis

Introduction

Plant possesses secondary metabolites and that can be utilized as environmentally safe insecticides for insect pest control. Several authors have observed the efficacy of some crude plant extracts on insects. Their observations revealed that the crude plant extracts possess antifeedant effect, growth retardation toxicity and oviposition deterrence (Hiremath *et al.*, 1997; Breuer and Schmidt, 1995; Kiepzig and Schlyter, 1999; Wheeler and Isman, 2001; Bhattacharya *et al.*, 2003). The crude plant extract also can reduce the fecundity and fertility (Muthukrishnan and Pushpalatha, 2001). Now a days, disinfection of pest insects with synthetic insecticides creates a series of problems. Obviously, introducing plant products as an alternative to synthetic chemical insecticides will be safe. Moreover, several plants are the important sources of insecticides (Sadek, 2003), as such, crude extracts of plants exhibits entomotoxic properties (Sadek, 1997; Rodriguez-Saona and Trumble, 1999; Tapondjou *et al.*, 2005). In this context we intend to observe the efficacy of *Porteresia coarctata* (Roxb.) which are abundantly distributed in Sunderban of West Bengal., India. The dry leaves of *Ricinus comunis* L mixed with extract of *Porteresia sp* were supplied as feed to *Spodoptera litura* (Noctuidae: Lepidoptera). Moreover the main emphasis of this work is to evaluate the effects of *Porteresia coarctata* leaf extracts on the feeding activity and biochemical alteration of the larval tissues of *Spodoptera litura*.

Materials and Methods

The cultures of test insect *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) were maintained in the laboratory on castor leaves (*Ricinus comunis* L.) under 12L: 12D photo regime at 28±1°C with 76±4% relative humidity. The culture had been perpetually enriched with wild moths captur-

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Table 1. Evaluate the summary statistics of four variables and the shape of the distribution of mean data. The data of the protein and DNA contents in the fat body and the midgut tissues of the late fourth instar larvae of *Spodoptera litura* (F.)

Variables	Control			Dose I			Dose II		
	Mean	SD	SE of mean	Mean	SD	SE of mean	Mean	SD	SE of mean
Var. A	7.74	0.15	0.05	6.71	0.27	0.08	2.24	0.20	0.06
Var. B	3.19	0.09	0.03	3.29	0.09	0.03	1.46	0.17	0.05
Var. C	5.47	0.05	0.01	5.34	0.29	0.09	1.66	0.29	0.09
Var. D	3.84	0.21	0.06	3.56	0.04	0.01	0.52	0.04	0.01

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA μg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA μg/insect
Dose I=1000 ppm; Dose II=2000 ppm; SD=Standard deviation; SE=Standard Error

Table 2. Shows the parametric comparison of means, considering the data from each of the groups i.e., control versus Dose I (1000ppm); control versus Dose II (2000 ppm) and Dose I versus Dose II

Variables	Control Vs Dose I			Control Vs Dose II			Dose I Vs Dose II		
	t- value	df	P value	t- value	df	P value	t- value	df	P value
Var. A	10.37	18	0.00005	67.90	18	0.00001	41.96	18	0.00005
Var. B	2.25	18	0.98	27.85	18	0.00009	29.63	18	0.00004
Var. C	1.42	9.55	0.09	40.92	9.55	0.00008	28.35	18	0.00002
Var. D	3.96	9.88	0.0014	46.48	10.27	0.00004	127.01	18	0.00003

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA μg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA μg/insect
Dose I=1000 ppm; Dose II=2000 ppm; df=Degree of freedom; t=T test value p-value

ing through light trap from the vicinity of agricultural farm of W B. Agricultural University, Mohanpur, Nadia, India.

Preparation of crude plant extracts

The wild rice plants, viz. *Porteresia coarctata* (Roxb.) [Takeoka]=*Oryza coarctata* (Roxb.)]. Cyperalae; Poaceae or Gramineae, were collected from Mayagoalini, situated on the west coast of Sagar Island of Sunderbans, West Bengal, India. The fresh leaves of *Porteresia coarctata* (Roxb.) weighing 4.68 kg. were rinsed in distilled water and allowed to dry in air for isolation of allelochemicals. Dried leaves were crushed and soaked in one-liter hexane for three weeks. Soaked leaves were filtered and the solvent hexane was distilled off over water bath to obtain the crude extract. The crude extract was dissolved in Dimethyl Sulfoxide (DMSO) and administered to *Spodoptera litura* to observe the insecticidal efficacy of the non- polar compounds from the said wild plant species.

Bio-efficacy studies

1000 and 2000 ppm of crude extracts of *Porteresia coarctata* (Roxb.) were sprayed on the leaves of *Ricinus comunis* L and fed to the late fourth instar larvae. In each experiment 20 larvae were put to rear in each replication maintaining three replications. Test larvae were fed for 72 hours along with control fed with only DMSO treated leaves. The live larvae from the control groups and two different treatment groups (viz., 1000 ppm and 2000 ppm), were dissected out in presence of ringer solution and homogenized the midgut and fat body tissues of said insect larvae by using potter Elvahjem homogenizer. Total

Table 3. Shows the Non-parametric comparison of location parameter, considering control versus Dose 1; control versus Dose II and Dose I versus Dose II. Non parametric comparison of locations parameter may consider as Ho : $\mu_1=\mu_2$

Variables	Control Vs Dose I	Control Vs Dose II	Dose I Vs Dose II
	P-value	P-value	P-value
	HI : $\mu_1>\mu_2$	HI : $\mu_1>\mu_2$	HI : $\mu_1>\mu_2$
Var.A	0.0001	0.0001	0.0001
Var.B	0.9833	0.0001	0.0001
Var.C	0.0862	0.0001	0.0001
Var.D	0.0001	0.0001	0.0001

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA μg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA μg/insect
Dose I=1000 ppm; Dose II=2000 ppm; P=Value; H_0=Null Hypothesis;
μ_1=mean of control; μ_2=Dose; H 1=alternative hypothesis

Table 4. Shows the Parametric Comparison of Mean among groups; Control; Dose 1 and Dose II. One way Analysis of Variance (ANOVA)

Variables	Sources of variation	Df	Sum of Sq.	Mean sum of Sq.	F Value	P Value
Var. A	Between groups	2	170.81	85.40	1849.44	0.00001
	Within groups	27	1.24	0.046		
Var. B	Between groups	2	21.21	10.60	666.58	0.00002
	Within groups	27	0.43	0.016		
Var. C	Between groups	2	93.66	46.83	821.39	0.00001
	Within groups	27	1.53	0.057		
Var. D	Between groups	2	67.84	33.92	1909.38	0.00001
	Within groups	27	0.48	0.017		

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA µg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA µg/insect
Dose I=1000 ppm; Dose II=2000 ppm; df=Degree of freedom F-value P-Value

Table 5. Shows the non parametric test for comparison of location of parameter among groups i.e., control; Dose 1 and Dose II

Variables	Kruskal-Walluis Chisq	df	P-value
Var. A	25.83	2	0.00004
Var. B	21.36	2	0.00005
Var. C	20.27	2	0.00004
Var. D	25.83	2	0.00001

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA µg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA µg/insect
Dose I=1000 ppm; Dose II=2000 ppm; df=Degree of freedom p-value

Table 6. Shows the multivariate comparison of means under normality assumption (between two groups in MANOVA TEST)

Groups	Sources of Variation	df	Pillai Trace	P-value
Control Vs Dose I	Between groups	1	0.89	0.00004
	Within groups	18		
Control Vs Dose II	Between groups	1	0.99	0.00001
	Within groups	18		
Dose I Vs Dose II	Between groups	1	0.99	0.00001
	Within groups	18		

Note: Groups are:Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA µg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA µg/insect
df=Degree of freedom; p-value

amount of DNA were measured following the method of Munro (1966) and protein was estimated by the method of Lowry *et al.*, (1951) using BSA as standard. All data recorded from the experiments were subjected to various statistical analysis, viz., ANOVA and MANOVA.

Results

Analysis of variance (ANOVA) was performed to observe the effects of different doses i.e., 1000 ppm (Dose-I) and 2000 ppm (Dose-II), of extract of *Porteresia coarctata* (Roxb.), over control group pertaining to four variables (A, B, C & D) groups (where A=Fat body protein mg/100 mg tissue; B=Fat body DNA µg/insect; C=Midgut protein mg/100 mg tissue; D=Midgut DNA µg/insect separately. The analysis of variance (ANOVA) test clearly denotes a significant change in the protein and the DNA content of the fat body and midgut of *Spodoptera litura* (Fab.) from that of control (Table 1, 2, 3, 4). A significant reduction in the midgut protein and DNA content in Dose-II from that of control is marked in the late fourth instar larvae of said insect. Moreover, The ANOVA test has been performed with the help of nonparametric (Table 5) and parametric (Table 6) processes. The p-values in both the tables for four variables implicate that there are significant difference, at about 1% level, mean values in three groups: control, in dose I (1000 ppm) and in dose II (2000 ppm) (Table 1 & 2). We have taken into account the t-test to find out in which groups (pair wise) the mean values differ significantly.

From Table 1, 2, 3 & 4 both parametric and non-parametric, clearly denote that variable A and D show significantly greater mean values in control group than dose I (1000 ppm) group (7.74±0.05; 3.84±0.06 in control and 6.7±0.08; 3.56±0.01 in Dose I). In case of variable B shows the reverse out put having less mean value in control group than In Dose I group (3.19±0.03 in control and 3.29±0.03 in Dose I). Moreover, variable C does not show any significant change from that of control versus Dose I group (5.47±0.01 in control and 5.34±0.09 in Dose I). Compar-

Table 7. Shows multivariate comparison of means under normality assumption (MANOVA) among groups Control, Dose I and Dose II

Sources of Variation	df	Pillai Trace	P-value
Between Groups	2	1.71	0.00001
Within Groups	27		

Note: Var. A=Fat body protein mg/100 mg tissue; Var. B=Fat body DNA μg/insect
Var. C=Mid gut protein mg/100 mg tissue Var. D=Mid gut DNA μg/insect
Dose I=1000 ppm; Dose II=2000 ppm; df=Degree of freedom; p-value

Dose I). Comparison of means in between control and Dose II, all the variables (A, B, C & D) show significant differences in their mean values. The control possesses larger mean values than the dose II groups (7.74±0.05; 3.19±0.03; 5.47±0.01; 3.84±0.06 in control and 2.24±0.06; 1.46±0.05; 1.66±0.09; 0.52±0.01 in dose II groups). Comparisons of mean in between Dose I (1000 ppm) and Dose II (2000 ppm) show very significant result. All the variables (A, B, C & D) show significantly greater mean values in Dose I group than those Dose II group (Table 1 & 2).

Moreover, if we can assume that the expression of four variables (A, B, C & D) is correlated to each other and also assumes that data are multivariate normal (Table 7).

Under this assumption, we intend to compare the mean among the groups i.e., control, dose II (pair wise and all at a time). To expedite the results we have gone through multivariate analysis and variance technique (MANOVA) with Pillai trace test. Here the test statistics is distributed as F. The results in the Table 7 show that the mean vectors are significantly different in between the groups (pair wise). Considering all the three groups at a time, the p-values in Table 7 also reveal that all the mean vectors in three groups are not in equal position.

Discussion

The crude extract of *Porteresia coarctata* (Roxb.) leaves were therefore, found to be effective in controlling *Spodoptera litura* (Fab.). The long-term effect of the said plant product might alter biochemical changes in different tissues of insect. Moreover, these marked changes might be manifested first in the midgut and then in fat body of the insect concerned. In recent years, several authors have given attention on the effect of the plant products in insect physiology (Venkatswarlu and Mukhopadhyaya, 1999; Dureja and Johnson, 2000; Jena, 200; Herit *et al.*, 2002; Bhattacharya *et al.*, 2003; Xingwei *et al.*, 2004; Deota and Upadhyay, 2005; Athanassiou *et al.*, 2005). Some biochemical alterations and some effect of plant products of *Spodoptera litura* (Fab.) have been studied by several authors (Prasada-Rao *et al.*, 1983; Ray *et al.*, 1984; Sridevi *et al.*, 1988; Bomford and Isman, 1966; Murugan *et al.*, 1988, Suganthy *et al.*, 2005; Sharma and Seth, 2005). The effect of *Porteresia coarctata* (Roxb.) in insect physiology is yet to evolve. So far, it is our first report to propose that leaf extract of *Porteresia coarctata* (Roxb.) has profound influence on gene inhibitory mechanisms.

The unique ecological adaptation of this particular plant, *Porteresia coarctata* (Roxb.) possesses unique phytochemicals, which will be of great interest to researchers in future. From the classical biochemical work of Fujimoto *et al.* (1980) it is known that phytophagous insect like *Spodoptera litura* (Fab.) lacks the capability of denovo biosynthesis of sterols in their body. These kinds of insects require exogenous phytosterols (e.g. sitosterol, campsterol, and stigmasterol) as precursor for making cholesterol. These phytosterols undergo side chain hormones dealkylation at the C-24 position to make sterol. In addition to making steroid cholesterol is also an integral component of insect cell membrane. Work from Misra *et al.*, 1988 showed that tri-terpenoids are present in *Porteresia coarctata* (Roxb.) like, B-Amyrin (9.9-12.0%), A-Amyrin (7.8-10.5%), Lupeol (28.0-40%), Betulin (11%), Oleanolic acid (18.5-27%) and Ursolic acid (4.5-11.7%). At this stage of our investigation the next logical step would be to understand the role of each secondary metabolite in *Porteresia coarctata* (Roxb.). Studies conducted by deSousa Mennezes *et al.*, 1999 showed interestingly that Lupeol along can induce the expression of oncoprotein and modulate the protein expression of various other signaling cascades involving molecules like PKC a/OC; P13K/Akt and MAPKs pathways along with a significant reduction in the activation of Nfk B signaling pathway, which finally leads to apoptosis. It is encouraging in the light of our preliminary observations in this experiment. Subsequently it is held to trace bioactive molecules, which can significantly reduce the total DNA and protein content in the primary (mid-gut) and secondary (fat body) contact foci of the *Spodoptera litura* (Fab.).

Therefore, the experimental observation in the present form clearly indicates that the crude extract of *Porteresia coarctata* (Roxb.) has consistent effects in altering the molecular physiology of the insects. Thus the said plant's secondary metabolites may be tested for insecticidal property, if any, in controlling the insect pest in near future. Further studies are under way to actual mechanism for inhibition of gene functions in mid gut and fat body cells of *Spodoptera litura* (Fab.).

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