Influence of *Thevetia peruviana* (Pers).K.schum on biochemical parameters of *Spodoptera litura* Fab.

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ABSTRACT
Botanical pesticides are used in a variety of pest control situations. Their less-toxic nature makes them a suitable candidate for IPM. They possess dose-dependentanti-insect activities starting from repellency and leading to the death of insects. Their large-scale utilization is obstructed by the non-availability of formulations. A potential botanical *Thevetia peruviana* (Pers).K.schum,whose influence on various physiological systems of *Spodoptera litura* was studied.Acetone fraction of hexane extract, acetone fraction of methanol extract, and acetone fraction of ethyl acetate extract were found to possess insecticidal activity. Among them,acetone fraction of hexane extract was the most promising extract with LC_{50} of 5 percent. At $LC_{50,}$ the carbohydrate, protein, and lipid contents were reduced to the tune of 80, 63, and 73 percent.
Keywords: Botanicals; *Thevetia peruviana; Spodoptera litura*; LC_{50}

INTRODUCTION
Botanicals are pesticidesderived from plants and are used in a variety of pest control situations as natural and simple extract rather than as formulations from time immemorial in India and elsewhere(Jacobson and Crosby, 1971 and KarlMaramorsch, 1991).Since botanicals are relatively less-toxicthey readily fit into IPM protocol(Ogunnika, 2007 and Oyun and Agele, 2009).They help in reducing pesticide usage, impact on the environment, and human and animal health hazards (Grainge and Ahmed, 1988).

Botanicals havedose-dependentanti-insect activities starting from repellency and leading to the death of insects. However, their large-scale utilization is obstructed bythe non-availability of formulations and can be surmountedby studying their influence on insect physiological systems(Yamamoto, 1998).
A potential botanical *Thevetia peruviana*(Pers).K.schum,(Prakash and Rao2006; Kestenholz *et al*. 2007 and Neoliya*et al*.2007)whose influence on various physiological systems of *Spodoptera litura*was studied.

Ray (2009) and Ray et al., (2012) reported larval malformation and mortality in methanol and ethyl acetate extract when evaluated against *S. litura* and attributed the activity to the glycosides present in the extract. Similarly, Shailja et al. (2013) reported the feeding deterrent effect on stored insect pests.Yadav et al. (2013) recorded delayed larvicidal action among mosquitoes and related it to insect growth inhibition.Dhivya and Manimegalai (2014) identified larvicidal bioactive compounds as peruvianoside1 and Thevetin A and reported that they blockthe odorant receptor proteins of mosquitoes.

MATERIALS AND METHODS
Mass culturing of *Spodoptera litura* Fab.
S. litura egg masses collected from the field were used to initiate the culture and the larvae were maintained in Bengal gram flour-basedsemi-synthetic diet(Priyanka, 2011).

Collection, extraction and fractionation
Drupes of *T. peruviana* collected from wild shrubs were shade dried, powdered, and extracted with hexane, petroleum ether, ethyl acetate, and methanol at room temperature for three days separately. The filtrate was evaporated, and the semisolid

extractive was obtained (Arivudainambi, 2001).The Semisolid extractive wascolumn chromatographed with acetone and fractionated (Upadhyay et al. 2011).

Bio-assay

Leaf disc no choice bioassay was done to evaluate the solvent fractions. Fresh castor leaf discs (3 cm^2) were dipped in various undiluted solvent fractions separately. Three pre-starved third instar *S. litura*larvae were released in individual Petriplates containing leaf discs and allowed to feed for 24 h. Solvent fractions showing insecticidal activity were selected for further studies. The effective solvent fractions were bio-assayed at 10, 20, 50, and 70 percent concentration as described above and the most effective solvent fraction identified.

Median lethal concentration of the most effective solvent fraction

Stock solution of the most effective solvent fraction was prepared. Thirty final instar larvae were treated with 20 µl of different concentrations by topical application with the help of Burkard Micro applicator. The median lethal concentration was worked out as described by Finney (1952) and simplified by Busvine (1971).

Influence of most effective solvent fraction

The effect of the most effective solvent fraction on total carbohydrate, protein and lipid content was studied by releasing three third instar *S. litura* larva separately into a Petridish (80 mm dia.) containing leaf disc (6 cm dia.) treated with 300 µl of median lethal concentration (LC_{50}) of the most effective solvent fraction. A solvent control and untreated absolute control were maintained. The experimental setup was replicated five times.

The total carbohydrate content was determined using glucose standard according to Singh and Sinha (1977). 24 hours after treatment one larva from each replication of respective treatment was weighed, homogenized, and centrifuged at 3000 rpm for 15 min. One ml of the supernatant was used instead of the standard glucose solution and carbohydrate content was estimated. Similarly, one larva from each replication of the respective treatment was weighed and homogenized separately in 10 percent TCA and the contents were centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted and the pellet was suspended in 1ml of sodium hydroxide. 0.5 ml of this solution was assayed for total protein content as described by Lowry et al. (1951). 24 hours after treatment one larva from each replication of respective treatment was weighed, homogenized in chloroform: Methanol (2:1) mixture, filtered through what man No. 40 filter paper and lipid content estimated as described by Pande *et al.*,(1963).

Statistical analysis

All the percentage data were subjected to arcsine transformation and the lethal concentrations were worked out using probit analysis. The data on carbohydrate, protein, and lipid content were subjected to standard error calculations. Analysis was done with ANOVA and the means were compared by following Duncan's multiple range test (DMRT) at $p = 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Estimation of toxicity

Among various solvent fractions evaluated, acetone fraction of hexane extract and acetone fraction of methanol extractimparted cent percent mortality, while acetone fraction of ethyl acetate extract imported 70 percent mortality. The remaining solvent fractions were on par with absolute control (Table 1).The effectiveness of these solvent fractions had been indicated by Ray (2009) who reported that hexane, ethyl acetate and methanol extract of T. peruviana clearly provided maximum larval mortalities. The earlier works of Bai and Koshy (1993) and Singhaet al. (2007) supported the toxicity of these solvent fractions.

At higher concentrations (70 %) all the three imparted significant mortality (100 % in acetone fraction of hexane extract and methanol, 66 % in acetone fraction of ethyl acetate extract). However, at 50 percent concentration acetone fraction of hexane extract alone imparted cent percent mortality and the same treatment exhibited 66.6 percent mortality even at the lowest dose of 10 percent. All other treatments failed to show mortality at lower doses (Table 2).The studies on confirmatory bioassay illustrated the dose-dependent toxicity of the acetone fraction of hexane extract. Acetone fraction of ethyl acetate and methanol extracts provided effects that were non-relativeto the dose applied. Dose-dependentanti-insect activity is a common phenomenon in botanicals as reported by Garcia and Rembold (1983) and Martinez and Van Emden (2001) in azadirachtin.

Determination of median lethal concentration

Acetone fraction of hexane extract bioassayed at 90, 70, 50, 20, 10, 5, 3, and 1 percent concentrations through topical application. The larval mortality recorded ranged from 96.6 percent to 6.66 percent at 90 and one percent concentrationrespectively. When mortality data had been subjected to probit analysis, LC_{50} of 5 percent with fiducial limits of 5 and 6.24 percent was obtained (Table 3).

Influence on various biochemical parameters

Acetone fraction of hexane extract when applied at median lethal dose on third instar S. litura larva was found to have a profound influence on total carbohydrate, protein, and lipid content. At LC_{50}treatment carbohydrate, protein, and lipid contents got drastically reduced. Carbohydrate content was 4.98 mg / g of body weight in LC_{50} treated insects while it was 6.21 mg / g of body weight in the untreated larva. Such similar results were noticed in protein and lipid content also. LC_{50} treated larva recorded a value of 3.23 mg/g and 411 µg/g respectively while untreated larva recorded 5.13 mg/g and 560 µg/g. The carbohydrate, protein, and lipid contents were reduced to the tune of 80, 63, and 73 percent (Table 4).

The profound effect of many botanicals on protein content leads to a more pronounced debilitating effect as it is one of the most complex and characteristic macromolecules. They are essential for many vital biological structures and functions. Such negative influence of botanicals on insect biochemical parameters was reported by Rosenthal (1977 & 1986), Rosenthal and Dahlman (1986), Berge and Rosenthal (1990), Padmaja and Rao (2000), and Josephrajkumar and Subrahmanyam (2000). Jbiloua and Sayah (2008) supported the present findings of reduction in lipid content due to botanical treatment.

Table 1. Preliminary evaluation of toxicity of *T. peruviana* solvent fractions on *S. litura*

Treatments	*Mean percent mortality
Acetone fraction of petroleum ether extract	0 (0)[c]
Acetone fraction of ethylacetate extract	70 (56.79)[b]
Acetone fraction of methanol extract	100 (90.0)[a]
Acetone fraction of hexane extract	100 (90.0)[a]
Solvent control	0 (0)[c]
Absolute Control	0 (0)[c]

S.Ed	6.051
C.D.	18.549

*Mean of three replications
Values with in parentheses are arc sine transformed values
Values with different alphabets differ significantly with in columns.

Table 2. Confirmatory toxicity bioassay of the promising solvent fractions of *T. peruviana* on *S.litura*

Concentrations (%)	*Mean percent mortality		
	Acetone fraction of hexane extract	Acetone fraction of methanol extract	Acetone fraction of ethyl acetate extract
10	66.6 (59.98)[b]	0 (0)[b]	0 (0)[b]
20	66.6 (59.98)[b]	0 (0)[b]	0 (0)[b]
50	100 (90.0)[a]	0 (0)[b]	0 (0)[b]
70	100 (90.0)[a]	100 (90.0)[a]	66.6 (59.98)[a]
Solvent control	0 (0)[c]	0 (0)[b]	0 (0)[b]
Absolute control	0 (0)[c]	0 (0)[b]	0 (0)[b]
S.Ed	9.264	6.323	6.541
C.D.	20.908	13.721	20.381

*Mean of three replications
Values with in parentheses are arc sine transformed values
Values with different alphabets differ significantly with in columns.

Table 3. Median lethal concentration of *T. peruviana* acetone fraction of hexane extract (most promising solvent fractions) on third instar S. litura larvae

Concentrations (%)	Number of larva used	Number of larva dead	Percent larval mortality	Corrected percent larval mortality
90	30	29	96.66	96.66
70	30	28	93.33	93.33
50	30	28	93.33	93.33
20	30	25	83.33	83.33

10	30	20	66.66	66.66
5	30	15	50.00	50.00
3	30	7	23.33	23.33
1	30	2	6.66	6.66
Solvent control	30	0	0	0
Absolute control	30	0	0	0

LC50 = 5 % Y = -0.01+01.63X

UL = 6.24 %

LL = 5 %

VAR = 0.101

Table 4. Effect of median lethal concentration of most effective solvent fraction of *T. peruviana* on various biochemical parameters of third instar S. litura larvae

Treatments	*** Total carbohydrate content (mg /g)**	*** Total protein content (mg /g)**	*** Total lipid content (µg / g)**
LC_{50}	4.98 ± 0.31[a]	3.23± 0.51[a]	411± 2.91[a]
Solvent control (Acetone)	6.16 ± 0.22[b]	5.07± 0.42[b]	549± 4.18[b]
Absolute Control	6.21 ± 0.11[b]	5.13± 0.15[b]	560± 3.12[c]
S. Ed.	0.14	0.19	4.07
C.D.(p = 0.05)	0.30	0.40	8.63

*Mean the replications
Values with different alphabets differ significantly.
Values following ± sign are standard error values.

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