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Toxicity of *Thevetia peruviana* (Pers) Schum. Extract to Adults of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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ABSTRACT

Leaf, stem and roots of Thevetia peruviana (Pers) Schum. were extracted in four organic solvents; petroleum spirit, ethyl acetate, acetone and methanol and tested against the adults of Callosobruchus maculatus F. All the tested extracts effectively produced mortality of C. maculatus and their toxicity was in order of solvents: petroleum spirit>ethyl acetate>acetone>methanol. Root extract was the most toxic to C. maculatus. Females were more tolerant than males.

Key words: Extract, mortality, solvent, *Thevetia peruviana, Callosobruchus maculatus*.

INTRODUCTION

Storage losses due to attack by insects cause a severe loss to small scale farmers in developing countries who rely on the stored cereals and legumes throughout the storage period to feed their families (Kestenholz 2001). There is an increasing dichotomy between the demands of the first world for quality food uncontaminated by insecticidal residues, and desperate need of third-world populations to maintain and protect their harvested grain from the deprivations of insects, so as to maintain a minimum level of food security (Donahaye 2000). In most developing countries, grain legumes are subject to attack of many insect pests especially at the small-scale farmer or trader level where storage conditions are usually inadequate to prevent or reduce insect attack (Rajapakse *et al*. 1998). Among insect of stored pulses, *Callosobruchus maculatus* F. is most destructive pest. During storage 100% seed infested and damaged by *C*. *maculatus* after 3-4 months was recorded in African countries (Osman *et al*. 1991, Thomas and Gasper 1994, Liuzhu 1998). Synthetic chemical control of stored product insect pests including *C*. *maculatus* has been the most efficient and effective means for protection of stored produce. But, the overuse of organic synthetic pesticides has led to widespread resistance in insect and other arthropod pests, and also caused irreparable damage to the ecosystem. Moreover, synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use (Keita *et al*. 2000). Therefore, development of economically feasible and socially acceptable alternative practices that may protect the ecosystem is one of the major objectives of pest management programme. Botanical insecticide, an alternative to synthetic insecticides and are biodegradable, often low mammalian toxicity and less hazardous to the environment. Recent search has focused on natural product alternatives for pest control in developing countries (Keita *et al*. 2001).

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The flora of Bangladesh includes several plant species having insecticidal properties. The plant *Thevetia peruviana* (Pers) Schum. (**Family Apocynaceae**), commonly known as **Karabi (yellow oleander)** is a large evergreen shrub grown in gardens and roadside as ornamental plant. Leaves of *T. peruviana* contain polyhydroxy-dimonoterpinoides and dimonoterpinoid apiosylglucosides (Abe *et al*. 1996). Leaf and seed extracts are toxic against *Tribolium castaneum* (Parveen *et al*. 1997; Khan *et al*. 1998; Khatun *et al*. 1999).

Much research has not been conducted so far to evaluate the toxic effects of *T. peruviana* leaf, stem and root extracts against *C. maculatus*. The present study was made to assess the toxic effect of *T. peruviana* leaf, stem and root extracted in four different solvents against adults of *C. maculatus*.

MATERIALS AND METHODS

The experiment was carried out at the Integrated Pest Management (IPM) Laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi 6205, Bangladesh, during April 2000- March 2004.

Callosobruchus maculatus F. was reared in the laboratory under controlled condition, to get the insects of uniform age. They were reared in Petri dish (15cm diam) and black gram, *Phaseolus mungo* L. seeds were used as food.

The pant parts such as leaves, stems and roots were collected and dried in an oven (40°C) and made into fine dust using a hand-grinding machine. The dusts were extracted in petroleum spirit, ethyl acetate, acetone and methanol separately by **Soxhlet's apparatus**. The extracts were dried in a vacuum rotary evaporator at 40°C under reduced pressure. The crude extracts were collected and stored in a refrigerator.

Four different doses i. e., 0.01875, 0.03750, 0.07500 and 0.15000g for petroleum spirit and ethyl acetate; 0.025, 0.050, 0.10 and 0.20g for acetone and methanol extract were weighted by an electronic balance and dissolved in requisite amount of respective solvents. The doses were transferred to $\mu g/cm^2$ by measuring the dry weight of extracted materials and divided by the surface area of Petri dish (9 cm diam). The test insects were exposed to the residual film technique. Extract was dispersed into each Petri dish for each dose. After evaporating the solvent from the Petri dish, 30 same aged (0-24h old) adult male and females of *C. maculatus* were released into each Petri dish. Control batch for males and females was maintained with respective solvent only. The experiments were replicated thrice. Mortality was recorded after 24, 48 and 72 hours exposure. The recorded mortality was subjected to probit analysis according to Finney (1947) and Busvine (1971). The experiments were conducted at 30±1°C and 70% relative humidity.

RESULTS AND DISCUSSION

The LD_{50}-values, 95% confidence limits, regression equations and χ^2-values have shown in **Tables 1, 2 and 3**. The mortality was dose dependent. χ^2-values showed no significant heterogeneity among exposure time, dose and mortality. The time 72 hours exposure exhibited lower LD_{50}-values than 24 and 48 hours. It was observed that the extracts of leaf, stem and root in petroleum spirit gave highest mortality in comparison with other solvents. Root extract offered highest mortality than that of leaf and stem. It was highest in solvent petroleum spirit after 72 hours exposure, where LD_{50}-value (317.6658$\mu g/cm^2$) was lowest. Males showed highest mortality in all treatments than that of females.

The results of the present study showed that extracts of *T. peruviana* possessed toxic effect against *C. maculatus* adults. No published information regarding *T. peruviana* leaf, stem and root extracts was found to compare the present result. However, Khan *et al*. (1998) showed, ethyl acetate extract of seed of *T. peruviana* was toxic to *Tribolium castaneum* adults which was followed by the extract prepared in petroleum spirit, acetone and methanol. The leaf extract of *T. peruviana* showed insecticidal effect against three strains of *T. castaneum* (CR1, CTC12 and FSS2) and their toxicity was in order: ethyl acetate>acetone>methanol>petroleum spirit (Khatun *et al*. 1999). Seed extracts of *Thevetia neriifolia* were toxic to the adult CTC12, a strain of *T. castaneum* (Parveen *et al*. 1997). Petroleum spirit, ethyl acetate, acetone and methanol extracted leaf, stem and root extracts of Dhutura (*Datura metel*), Akanda (*Calotropis procera*), Dhol kalmi (*Ipomoea maxima*), Custard apple (*Annona squamosa*) and Kamini (*Murraya paniculata*) were toxic against *C. maculatus* adults (Mollah and Islam 2001, 2002, 2003a, 2003b, 2005). Hundred per cent adult mortality of *C. maculatus* caused at the dose 1.2µl/l to 2.25µl/l of *Ocimum basilicum*, 1.62µl/l to 2.5µl/l of *Cymbopogon nardus* and 0.67µl/l to 1.25µl/l of *C. schoenanthus* (Ketoh *et al*. 2002). Two fractions of *Anethum sowa* produced 100% adult mortality of *C. maculatus* at the dose 3.0µl/l and fraction III was 100% ovicidal at 7.0 µl/l (Tripathi *et al*. 2001)

Table 1. Toxicity of *T. peruviana* leaf extract in different solvents tested against adult males and females of *C. maculatus* at different exposure times

Solvent	Sex	Exposure Time (h)	LD_{50}-values (μg/cm^2)	95% confidence limits		Regression equation	χ^2-values
				lower	upper		
petroleum spirit	Male	24	24542.58	2081.21	289417	Y=2.1919+0.6397X	0.0364
		48	1809.942	1180.27	2775.557	Y=1.7645+0.9932X	0.2096
		72	385.6062	308.544	481.9157	Y=0.0828+1.937X	1.8319
	Female	24	69689.59	1247.16	3894146	Y=2.4642+0.5215X	0.1581
		48	2759.195	1467.35	5188.376	Y=1.9482+0.8870X	0.9827
		72	461.8023	366.355	582.1163	Y=0.5853+1.6569X	0.3192
ethyl acetate	Male	24	49077.64	1443.02	1669149	Y=2.4345+0.5469X	0.0363
		48	1620.174	1014.63	2587.109	Y=2.2918+0.8438X	0.3026
		72	483.5824	395.849	590.7609	Y=-0.0646+1.887X	1.2117
	Female	24	96470.51	834.025	115864	Y=2.6377+0.4739X	0.0705
		48	2270.317	1195.22	4312.68	Y=2.4281+0.7663X	0.3253
		72	497.7887	399.090	620.9038	Y=0.5234+1.6598X	0.9151
acetone	Male	24	128614.2	1113.37	148572.4	Y=2.5779+0.4741X	0.0703
		48	2366.126	1396.53	4008.894	Y=2.3258+0.7926X	0.1254
		72	548.7568	430.95	798.767	Y=0.3387+1.7016X	3.4709
	Female	24	10393.72	7283.84	25217.94	Y=-0.4632+1.360X	0.3396
		48	3233.00	2432.15	4297.633	Y=-0.6140+1.885X	2.7614
		72	1263.162	1070.38	1490.663	Y=-1.0325+1.945X	2.1161
methanol	Male	24	55512.49	2719.32	1133236	Y=2.0242+0.6272X	0.0165
		48	3286.856	1965.46	5496.637	Y=1.4216+1.0175X	0.5885
		72	570.1389	443.326	733.226	Y=-0.6072+1.594X	3.6618
	Female	24	39324.08	363.71	425683.3	Y=1.5286+0.7555X	0.2369
		48	3934.777	2482.91	6235.609	Y=0.2958+1.3086X	1.0580
		72	1486.017	1306.17	1690.628	Y=-3.6562+2.729X	4.3161

Table 2. Toxicity of *T. peruviana* stem extract in different solvents tested against adult males and females of *C. maculatus* at different exposure times

Solvent	Sex	Exposure Time (h)	LD_{50}-values (μg/cm^2)	95% confidence limits		Regression equation	χ^2-values
				lower	upper		
petroleum spirit	Male	24	12499.82	2634.78	59301.10	Y=1.6045+0.8288X	0.0476
		48	1624.56	1886.85	2428.30	Y=1.8372+0.9851X	0.0395
		72	338.9586	257.449	446.2744	Y=0.6838+1.7058X	1.1509
	Female	24	26370.39	2188.90	317692.5	Y=2.1375+0.6475X	0.1102
		48	2265.79	1330.68	3858.01	Y=1.8941+0.9257X	0.2383
		72	409.9367	313.935	535.2963	Y=0.9685+1.5430X	1.2898
ethyl acetate	Male	24	18418.93	2432.11	139490.7	Y=1.9040+0.7558X	0.0852
		48	1617.506	1028.97	2542.66	Y=2.015+0.8721X	0.1373
		72	379.610	298.500	482.77	Y=0.3246+1.8126X	0.9496
	Female	24	21747.81	2344.74	201714.8	Y=1.9863+0.6948X	1.1509
		48	2100.102	1308.50	3370.59	Y=1.7069+0.9912X	0.0897
		72	411.1634	312.082	541.7021	Y=1.1080+1.4889X	0.9034
acetone	Male	24	172835.9	1062.55	2495442	Y=2.5035+0.4790X	0.2634
		48	2191.79	1452.11	3308.26	Y=1.7645+0.9684X	0.0897
		72	546.8861	437.218	684.062	Y=0.0484+1.8439X	3.6125
	Female	24	8040.10	3976.01	17258.28	Y=0.7822+1.4806X	0.3911
		48	3056.78	2264.86	4149.92	Y=-0.8903+1.689X	1.4315
		72	1211.11	1035.40	1416.64	Y=-1.4091+2.079X	3.2549
methanol	Male	24	150129.2	1039.74	2167720	Y=2.6206+0.4596X	0.2220
		48	3314.778	1871.78	5870.211	Y=1.7759+0.9158X	0.5104
		72	562.6129	444.372	712.3151	Y=0.2845+1.7145X	2.5749
	Female	24	17645.30	3990.03	78033.52	Y=1.6117+0.9038X	0.3207
		48	3482.225	2421.28	5008.04	Y=-0.4286+1.533X	2.1254
		72	1294.89	1140.32	1470.43	Y=-3.3446+2.681X	3.6479

Table 3. Toxicity of *T. peruviana* root extract in different solvents tested against adult males and females of *C. maculatus* at different exposure times

Solvent	Sex	Exposure Time (h)	LD_{50}-values ($\mu g/cm^2$)	95% confidence limits		Regression equation	χ^2-values
				lower	upper		
petroleum spirit	Male	24	19440.47	2146.21	176092.7	Y=2.2273+0.6465X	0.0592
		48	1529.44	989.752	2363.406	Y=2.2179+0.8736X	0.1323
		72	317.6658	237.830	424.3001	Y=0.7766+1.6880X	0.5905
	Female	24	19630.69	212.74	181540.8	Y=2.1517+0.6635X	0.0469
		48	1899.165	1148.55	3140.315	Y=2.1494+0.8695X	0.3451
		72	338.1755	243.299	470.0483	Y=1.4281+1.4123X	0.7744
ethyl acetate	Male	24	38119.88	1701.45	854046.2	Y=2.4160+0.5640X	0.0264
		48	1658.763	1027.15	2678.75	Y=2.3067+0.8365X	0.3236
		72	402.8395	317.392	511.290	Y=-0.4307+1.7539X	1.0977
	Female	24	32224.05	2100.6	494328.9	Y=2.1297+0.6367X	0.0301
		48	2301.72	1316.35	4024.703	Y=2.0055+0.8907X	0.3503
		72	514.854	410.508	605.7236	Y=0.6996+1.5459X	0.4507
acetone	Male	24	371182.2	359.748	38297940	Y=2. 7903+0.3967X	0.2279
		48	2214.298	1453.79	3372.631	Y=1.8091+0.9539X	1.3555
		72	632.9846	518.1351	772.9681	Y=-0.3019+1.893X	0.8488
	Female	24	10465.06	4240.36	25827.33	Y=-0.2480+1.306X	0.1046
		48	3134.605	2330.20	4216.689	Y=-1.1481+1.759X	1.7944
		72	1280.626	1112.54	1474.107	Y=-2.4698+2.404X	2.9613
methanol	Male	24	69029.73	2308.86	2063827	Y=2.0810+0.6032X	0.0515
		48	4534.101	2275.88	9033.022	Y=1.5726+0.9374X	0.337
		72	651.2729	534.305	793.847	Y=-0.3124+1.888X	0.4532
	Female	24	21053.45	4330.27	102360.2	Y=0.6397+1.0085X	0.7034
		48	4730.869	2699.68	8291.684	Y=0.5018+1.2240X	0.6343
		72	1314.78	1151.88	1500.714	Y=-3.0361+2.577X	3.0838

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