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Insecticidal Activity of Root Bark of *Calotropis gigantea* L. Against *Tribolium castaneum* (Herbst)

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Abstract: The residual film toxicity, fumigant toxicity and repellent effect of methanol extract of root bark of *Calotropis gigantea* (Linn) and its chloroform and petroleum ether (40-60°C) soluble fractions were evaluated against several inster of larvae and adult of *Tribolium castaneum*. In residual film toxicity, methanol extract and its chloroform and petroleum ether fractions showed insecticidal activity against *T. castaneum* and data were analyzed by probit analysis. Methanol extract showed lowest LD_{50} values against several inster of larvae and adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42 and 300.03 µg/cm^2, respectively) which indicates highest toxicity or insecticidal activity. Whereas LD_{50} values of petroleum ether extract were 407.69, 485.46, 437.38, 235.51, 256.25, 369.66 and 411.84 µg/cm^2 in case of 1st, 2nd, 3rd, 4th, 5th, 6th inster larvae and adult, respectively and for chloroform extract that were 291.83, 299.29, 382.98, 745.18, 637.71, 1259.42 and 739.87µg/cm^2, respectively. The order of toxicity on *T. castaneum* was methanol extract> petroleum ether fraction> chloroform fraction. No fumigant toxicity of test samples was found. In the treated filter paper repellency test, methanol extracts and also its chloroform and petroleum ether soluble fractions were repellent to *Tribolium castaneum* in mild to moderate range.

Key words: *Calotropis gigantea* · *Tribolium castaneum* · Residual flim toxicity · Fumigant toxicity · Repellency

INTRODUCTION

Tribolium castaneum (Herbst) is an important stored product insect in grain storage in Singapore [1] and other ASEAN countries [2]. *T. castameum* live on cracked grain on breakfast food or meal, rice, dried fruit, bleached and unbleached wheat flour, cornmeal, barley flour and atmeal [3]. In Bangladesh, *T. castaneum* is abundantly found in stored grain of different cereals. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, resistance to pesticides and lethal effects on non-target organism in addition to direct toxicity to users [4,5]. Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are rational alternatives to synthetic insecticides. Dyte reported that almost all of the strains of *T. castaneum* have become resistant to malathion and almost all organophosphorus insectidices [6]. The occurrence of resistance in different strains of *T. castaneum* has given an extra impetus to research for alternative way for the control of this pest. Yang and Tang reviewed the plants used for pest insect control in China and found that there was a strong connection between medicinal and pesticidal plants [7]. *Calotropis gigantea* (Linn) (Family: Asclepiadaceae), a common medicinal plant in Indian subcontinent, has purgative, alexipharmic anthelmintic, analgesic, anticonvulsant, anxiolytic, sedative and antipyretic effect [8, 9] and is used as a treatment for leprosy, leucoderma, ulcers, tumours, piles and diseases of the spleen, liver and abdomen [10]. Previous chemical studies on *C. gigantea* reported the isolation of many cardenolides, cardiac glycosides [11], flavonoids [12], giganticine (a novel nonprotein amino acid) [13] and other cytotoxic principles [14, 15] from this plant. Latex constituents from *Calotropis procera* (a another plant of Asclepiadaceae family) display toxicity upon egg

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hatching and larvae of *Aedes aegypti* (Linn.) [16]. Singh *et al.* reported the larvicidal properties of leaf extract of *Calotropis procera* against mosquito larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* [17]. Despite the previous studies on bioactivity of root bark of *C. gigantea* to various research, its activity against *T. castaneum* (Herbst) has not yet been determined. The present research was therefore under taken to investigate the effect of the methanol extract of root bark of *Calotropis gigantea* and its chloroform and petroleum ether soluble fractions against adults and several inster of larvae of *T. castaneum* (Herbst).

MATERIALS AND METHODS

Insects: Adults of *T. castaneum* were collected from the Crop Protection Lab of the Department of Agriculture and Environmental Science, University of Newcastle upon Tyne, UK and successfully reared for more than ten years in the Crop Protection and Toxicology Lab of the Institute of Biological Science, Rajshahi University. Mass cultures were maintained in Jars (1000 ml) containing food medium and kept in an incubator at 30±1°C and 70-80% (Relative Humidity). A stand ard mixture of sterilized (at 60°C for 24 hours) whole-wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium in the experiments [18-20].

Extraction: The plant *C. gigantea* L. was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi. Voucher specimen (No. 1A. Alam, collection date 15.08.2004) was kept in the Dept. of Botany, University of Rajshahi. Root bark of *C. gigantea were* collected, dried and ground to powder. It was then extracted with methanol in Soxhlets apparatus, the process described by Schmutterer [21]. The extract was then filtered through Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator under reduced pressure at 50° C to afford crude methanol extract (40 gm). This crude methanol extract (30 gm) was then fractionated into petroleum ether (3 g) and chloroform (10 g) by solvent-solvent partitioning [22].

Residual Film Method of Toxicity: Residual film method as described by Busvine, was used [23]. A preliminary screening of different doses was performed on several insters of larvae and adults to obtain 0% to 100% mortalities. Then 200 mg, 100 mg, 50 mg and 25 mg of each test sample (Methanol extract, chloroform fraction and petroleum ether fraction) were dissolved separately in 5 ml of corresponding solvent to get concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml respectively which were used as stock solutions. 1 ml of various concentrations for each sample was applied on petridishes (7 cm diameter) in such a way that it made a uniform film over the petridishes. For solvent evaporation, the petridishes were air dried leaving the extract on it. The actual extract present in 1ml mixture was calculated and the dose per square centimeter was determined by dividing the value present in one ml with the area of the petridish. So calculated doses were 1040.5 $\mu g/cm^2$, 520.0 $\mu g/cm^2$, 260.0 $\mu g/cm^2$ and 130.0 $\mu g/cm^2$. After drying 10 beetles were released in each petridish with three replication. A control batch was also maintained with the same number of insects after preparing the petridish by applying and evaporating the solvent only. The treated beetles were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the beetles were counted after 24 hour post-exposure [24].

Fumigant Toxicity: Each filter paper (diameter 2.0 cm) were impregnated with 0.2 ml of various concentrations (20 mg/ml, 10 mg/ml and 5 mg/ml) of each extract and fractions and placed on the underside of the screw cap of a glass vial (diameter 2.5cm, height 5.5 cm). Then calculated doses were 884.20 $\mu g/cm^2$, 442.10 $\mu g/cm^2$ and 221.05 $\mu g/cm^2$. The solvent was allowed to evaporate for 1 min. before the cap was screwed tightly on the glass vial containing 10 insects. Respective solvent was used as control. Six replicates were prepared for each treatment and control. Mortality counts were made after 24 hours of treatment.

Repellency Test: The repellency test used was adopted from the method McDonald [25] with some modifications by Talukder and Howse [26, 27]. Half filter paper discs (Whatman No. 40, diameter 9 cm) were prepared with 0.1 ml of various concentrations of each testing samples (methanol extract and its chloroform and petroleum ether soluble fractions) and allowed to air-dry for 10 minutes. Each treated half disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a petridish (dimeter 9 cm), the inner surface of which was smeared with flour to prevent insects escaping. The orientation of the seam was changed in the replicates to avoid the effects on any external directional stimulus offering the distribution of the test insects. Twenty adults insects were released in the middle of each filter-paper circle and a plastic cover with some small

holes was placed on the petri dish [29]. Each concentration was tested five times. Insects that settled on each half of the filter paper disc were counted after 1h and then at hourly intervals for 5 hours. No significant difference as detected between the repellency of solvent impregnated (methanol, chloroform and petroleum ether) and untreated filter papers in tests designed to check for any possible influence of solvents. The average of the counts was converted to percentage repellency (PR) using the formula of Talukder and Howse [26, 27]

$$PR= 2(C-50)$$

Where C is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values attractancy. The data were analyzed for percents repellency (PR) and were transformed them into arcsine percentage values.

RESULTS

Residual Film Toxicity: Residual film toxicity showed that methanol extract and its chloroform and petroleum ether soluble fractions of root bark of *C. gigantea* were found to be toxic to *T. castaneum* (Table 1). On the basis of LD_{50} and LD_{90} value, it was observed that among the tested materials methanol extract showed highest toxicity. However according to the intensity of toxicity they could be arranged in the following order methanol extract> petroleum ether extract> chlorform extract. It was also observed that younger larvae were more susceptible than older larvae and adults.

Repellency: Methanol extract of *C. gigantea* and its chloroform and petroleum ether fraction showed repellent toxicity in mild to moderate range (Table 2). Repellency increased with concentration and in case of methanol

Table 1: Residual Film toxicity of root bark extracts of *C. gigantea* against *T. castaneum* larvae and adults

Plant materials	Life stage	LD_{50} (µg/cm^2)	95% Con. Limit	LD_{90} (µg/cm^2)	95% Con. Limit	Slope±SE
Methanol	1st Inster	121.59	86.30-171.32	316.22	273.71-358.73	3.035+0.04
extract	2nd Inster	129.44	107.02-190.35	363.07	321.41-404.75	3.13+0.02
	3rd Inster	146.84	108.87-198.05	405.51	360.92-450.09	2.87+0.03
	4th Inster	235.65	154.61-266.48	645.65	589.72-701.58	2.53+0.001
	5th Inster	290.65	185.68-454.96	3090.29	2955.66-3224.92	1.24+0.002
	6th Inster	358.42	262.31-489.73	1905.46	1791.76-2019.16	1.77+0.005
	Adult	326.1	210.50-427.63	1905.46	1796.90-2014.02	1.625+0.04
Chloroform	1st Inster	253.05	208.34-408.78	1018.59	918.37-1118.81	2.39+0.02
fraction	2nd Inster	299.29	206.28-434.23	1230.27	1116.29-1344.24	2.43+0.34
	3rd Inster	382.98	257.65-569.26	2290.86	2135.06-2446.67	1.65+0.02
	4th Inster	745.18	585.39-948.58	2511.88	2330.29-2693.47	2.39+0.001
	5th Inster	541.62	452.68-898.37	3981.07	3758.23-4203.91	1.60+0.007
	6th Inster	605.87	758.88-2090.12	5397.61	10554.56-11885.80	1.375+0.03
Petroleum ether	Adult	739.87	529.11-1034.58	4365.15	4112.42-4617.88	1.66+0.02
fraction	1st Inster	407.69	302.38-549.67	1267.06	1143.42-1390.72	2.57+0.03
	2nd Inster	485.46	282.35-834.68	2818.38	2542.22-3094.55	1.69+0.04
	3rd Inster	437.38	346.40-552.26	1348.96	1246.04-1451.88	2.67+0.09
	4th Inster	235.51	171.22-323.93	1047.12	970.77-1123.47	1.96+0.003
	5th Inster	256.25	187.48-350.26	1174.89	1093.50-1256.28	1.91+0.0001
	6th Inster	369.66	283.02-482.83	1479.1	1379.20-1579.00	2.11+0.01
	Adult	411.84	287.42-590.11	2818.38	2667.04-2969.72	1.54+0.015

Table 2: Repellency of *T. castaneum* adults by methanol extract of root bark of *C. gigantea* and its chloroform and petroleum ether fractions

		Repellency percentage (arsine) at intervals				
Plant material	Dose µg/cm^2	1h	2h	3h	4h	5h
Methanol	1260	-13.4 (-21.47)	00 (00)	-20 (-26.56)	6.6 (14.89)	26.6 (31.05)
extract	630	-26.6 (-31.05)	-20 (-26.56)	-13.4 (-21.47)	6.6 (14.89)	6.6 (14.89)
	315	-26.6 (-31.05)	-40 (-39.23)	-20 (-26.56)	-26.6 (-31.05)	-6.6(-14.89)
	157	-26.6 (-31.05)	-13.4 (-21.47)	-26.6 (31.05)	-26.6 (-31.05)	13.4 (21.47)
Chloroform	1260	-13.4 (-21.47)	-20 (-26.56)	00 (00)	20 (26.56)	33.4 (35.30)
fraction	630	-6.6 (-14.89)	-6.6 (-14.89)	6.6 (-14.89)	-20 (-26.50)	13.4 (21.5)
	315	-33.4 (-35.30)	-13.4 (-21.47)	-6.6 (14.89)	00 (00)	00(00)
	157	-40 (-39.23)	-26.6 (-31.05)	6.6 (14.89)	00 (00)	00(00)
Petroleum	1260	46.6 (43.05)	26.6 (31.05)	13.40 (21.47)	-13.4 (-21.47)	-13.4(-21.47)
ether	630	20 (26.56)	00 (00)	-13.4 (-21.47)	-26.6 (-31.05)	-20 (-26.56)
fraction	315	26.6 (31.05)	00 (00)	-13.4 (-21.47)	-26.6 (-31.05)	-20 (-26.56)
	157	6.6 (13.89)	-6.6 (13.89)	-6.6 (13.89)	00 (00)	-26.6(31.05)

extract and chloroform fraction repellent effect increased with time but in case of petroleum ether fraction, the repellent effect decreased with time over the 5 experimental period.

Fumigant Toxicity: The extracts demonstrated no fumigant toxicity to *T. castaneum*.

DISCUSSION

The methanol extract of root bark of *C. gigantea* and its chloroform and petroleum ether soluble fractions were showed the insecticidal activity and repellent toxicity to adults and larvae of *T. castanium*. *T. castaneum* larvae were more susceptible than adults. Methanol extract proved to be more toxic than other fractions in all larval and adult stages. It is interesting to note that in all extracts LD_{50} values were more or less similar from 1st inster to 5th inster larvae but in the 6 th inster larvae LD_{50} values were jumped indicating more tolerant. In case of methanol and chloroform fraction, 1st inster larvae showed lowest LD_{50} and LD_{90} values indicating more toxic to this larval stage. In case of petroleum ether fraction LD_{50} values against the 4th and 5th inster larvae were dropped indicating the more toxic to these larval stages. This present result of mortality is similar to the work of M. Khalequzzaman, who reported that ethyl acetate extract of Neem (*Azadirachta indica*) leaf showed lowest LD_{50} value on 5th instar larvae indicating more susceptible to this larval stage [28].

Fumigation activity of the extracts were investigated but no mortality were found which indicate that the root bark extract did not contain such kind of compound or it might contain volatile compounds which was evaporated when treated filter paper were evaporated for 5 minutes or longer before being capped on the glass vials in the preliminary experiments.

The repellent effect of the extracts of root bark of *C. gigantea* was also investigated. Methanol extract and its chloroform and petroleum ether fractions showed mild to moderate repellent effect on *T. castaneum*. Similar observations on other plant extracts have also been made. For example., Talukder and Howse showed that crude seed extracts of pithraj, *Aphanamixis polystachya* Wall and Parker, strongly repelled *T. castaneum* [26, 27]. Liu and Ho showed that essential oil of *Evodia rutaecarpa* strongly repelled *T. castaneum* [29].

The repellent activity of petroleum ether fraction decreased with time, a trend similar to crude extracts of pithraj [26]. It is very likely that the repellent constituent(s) of the petroleum ether fraction have low molecular weights and high volatility [30]. This property of high volatility is further evident from the lack fumigation activity when treated filter papers were evaporated for 5 minutes or longer before being capped on the glass vials in the preliminary experiments.

This study demonstrates that the methanol extract of root bark of *C. gigantea* and its chloroform and petroleum ether soluble fraction had residual film toxicity and repellent toxicity against *T. castaneum* with different efficacies. These finding suggest that there may be different constituents in the methanol extracts and its chloroform and petroleum ether fraction possessing different bioactivities but their identities are yet to be determined. The isolation and identification of the bioactive c ompound(s) in the extracts of root bark of *C. gigantea* are of out most importance so that their potential application in controlling stored-product pest can be fully exploited.

REFERENCES

1. Pranoto, R.I., M.S. Sim, A.M.E. Howie and S.H. Ho, 1991. Grain Storage Pand control practices in Singapore. In Current Trends in Integrated Pest Management For Grain Storage in the ASEAN Region, Eds., Frio, A.S., ASEAN Grain Postharvest Programme, Thailand, pp: 67-80.
2. Semple, R.L., 1986. Problems relating pest control and use of pesticides in grain storage: the current situation in ASEAN and future requirements. In: Pesticides and Humid Tropical Grain Storage systems, Eds., Champ, B.R. and E. Highly. Proceedings of an International Seminar Manila, Philippines, pp: 46-75.
3. Chittenden, F.H., 1897. Some insects injurious to stored grains and their habits and control. 4th edn, Mc Graw Hill book Company, New York, pp: 925.
4. Jembere, B., D. Obeng-Ofori, A. Hassanali and G.N.N. Nyamasyo, 1995. Productsderived from the leaves of *Ocimum kilimand scharicum* (labiatae) as post-harvest grain protestants against the infestation of three major stored product insect pests. Bulletin of Entomol. Res., 85: 361-367.

5. Okonkwo, E.U. and W.I. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maije grain against infestation by *Collosobruchus maculatus* (Fabricius) Colcoptera: Bruchidae) and *Sitophilus zeamais* (Mots chulsky) (Coleoptera: Curculionitae) in Nigeria. Intl. J. Pest Management, 42: 143-146.
6. Dyte, C.E. and D. Halliday, 1985. Problems of developments of resistance to phosphine by insect pests of stored grains. Bull Organization Europeanne et Mediterraneenne pour la protection des plantes, 15: 51-57.
7. Yang, R.Z. and C.S. Tang, 1988. Plants used for pest control in China: A literature review. Economic. Botany, 42: 375-406.
8. Argal, A. and A.K. Pathak, 2006. CNS activity of *Calotropis gigantea* roots. J. Ethnopharmacol., 106(1): 142-145.
9. Chitme, H.R., R. Chand ra and S. Kaushik, 2005. Evaluation of antipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. Phytotherapy Research, 19(5): 454-6.
10. Kartikar, K.R. and B.D. Basu, 1994. Indian Medicinal Plants, Vol. 3, Edn 2nd, Allahabad, India, pp: 1606-1609.
11. Lhinhatrakool, T. and S. Sutthivaiyakit, 2006. 19-Nor- and 18, 20-epoxy-cardenolides from the leaves of *Calotropis gigantea*. J. Natural Product, 69(8): 1249-51.
12. Sen, S., N.P. Sahu and S.B. Mahato, 1992. Flavonol glycosides from *Calotropis gigantea* L. Phytochem., 31(8): 1919-1921.
13. Pari, K., P.J. Rao, C. Devakumar and J.N. Rastogi, 1998. A novel insect antifeedant nonprotein amino acid from *Calotropis gigantea*. J. Natural Product, 61(1): 102-104.
14. Kiuchi, F., Y. Kukao, T. Maruyama, T. Obata, M. Tanaka, T. Sasaki, M. Mikage, M.E. Haque and Y. Tsuda, 1998. Cytotoxic principles of a Bangladeshi crude drug, akond mul (roots of *Calotropis gigantea* L.). Chemical Pharmaceutical Bulletin (Tokyo), 46(3): 528-30.
15. Kitagawa, I., R.S. Zhang, J.D. Park, N.I. Baek, Y. Takeda, M. Yoshikawa and H. Shibuya, 1992. Indonesian medicinal plants. I. Chemical structures of Calotroposides A and B, two new oxypregnane-oligoglycosides from the root of *Calotropis gigantea* (Asclepiadaceae). Chemical Pharmaceutical Bulletin (Tokyo), 40(8): 2007-13.
16. Ramos, M.V., G.P. Band eria, C.D. de Freitas, N.A. Noqueira, N.M. Alencar, P.A. de Sousa and A.F. Carvalho, 2006. Latex constituents from *Calotropis procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn). Memorius Do Instituto Oswaldo Cruz, 101(5): 503-10.
17. Singh, R.K., P.K. Mittal and R.C. Dhiman, 2005. Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family-Asclepiadaceae) against mosquito larvae. J. Communicable Diseases, 37(2): 109-13.
18. Park, T. and M.B. Frank, 1948. The fecundity and development of the flour beetle *Tribolim castaneum* and *Tribolium confuseum* at three constant temperatures. Ecol., 29: 368-373.
19. Zyromska, R.H., 1966. Abundance and empigrations of *Tribolium castaneum* in a laboratory model. Ekologia Polska, 14: 491-518.
20. Khalequzzaman, M., M. Khanam and D. Talukdar, 1994. Growth of *Tribolium confusum* Duv. on wheat flour with various yeast levels. International Journal of Pest control, 36: 128-130.
21. Schmutterer, H., 1982. Higher plants as sources of novel pesticides. In Insecticides, Mechanism of Action and Resistance. Intercept Ltd. and over, pp: 3-15.
22. Bahl, B.S. and A. Bahl, 1992. A text Book of Organic Chemistry. Edn 13th, Schand and Company Ltd, New Delhi, pp: 5-14.
23. Busvine, J.R., 1971. A critical review of the techniques for testing insecticides. Commonwealth Agricultural Buereux, London, pp: 345.
24. Islam, H., K. Farhana and N. Islam, 2004. Sereening of spices for biological activity against *Tribolium castaneum* (Herbst) adualts. University J. Zool. Rajshahi University, 23: 65-68.
25. McDonald, L.L., R.H. Guy and R.D. Speirs, 1970. Preliminary evaluation new cand idate materials as toxicants, repellents and attractants against stored product insects. Marketing Research Report No. 882 Agricultural Research Service, U.S. Department of Agriculture, Washington.
26. Talukder, F.A. and P.E. Howse, 1993. Deterrent and insecticidal effects of extracts of pithraj, *Aphanamixis polystachya* (Meliaceae), against *Triboloum castaneum* in storage. J. Chemical Ecol., 19: 2463-2471.

27. Talukder, F.A. and P.E. Howse, 1995. Evaluation of *Aphanamixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolum castaneum* (Herbst). J. Stored Product Research, 31: 55-61.
28. Khalekuzzaman, M. and M. Khanom, 2000. Effect of Neem (*Azadirachta indica*) leaf extracts on larvae and adults of *Tribolium castaneum* (Herbst). J. Biol. Sci., 8: 83-91.
29. Lie, Z.L. and S.H. Ho, 1999. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook Thomas against the grain storage insects, *Sitophilus* zeamais motsch and *Tribolium castaneum* (Harbst). J. Stored Product Research, 35: 317-328.
30. Jilani, G. and R.C. Saxena, 1990. Repellent and feeding deterrent effects of Tarmaric oil, sweet flag oil, neem oil and neem based insecticide against the lesser grain borer (*Coleoptera: Bostrychidae*). J. Economic Entomol., 498: 1-57.