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Chemical characterization and insecticidal activity of *Calotropis gigantea* L. flower extract against *Tribolium castaneum* (Herbst)

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ABSTRACT

Objective: To test the insecticidal activity of ethyl acetate extract of *Calotropis gigantea* L. flower (designated as EECF) against stored grain pest *Tribolium castaneum* (Herbst) of different larval and adult stages.

Methods: Residual film method was used here to study the toxicity of EECF against *Tribolium castaneum* and gas chromatography-mass spectrometer analysis was also performed to characterize the chemicals of EECF.

Results: In residual film bioassay, EECF showed lowest LD₅₀ (0.134 mg/cm²) against 1st instar larvae of *Tribolium castaneum* and this finding ultimately revealed that the insect of initial stage was more susceptible than other stages. From the results of this study, it was found that with the increasing of age, *Tribolium castaneum* showed some extent of resistance against the toxicity of EECF. Moreover, chemical profiles of EECF identified by gas chromatography-mass spectrometer analysis were also found to consistent with its insecticidal activity.

Conclusions: So, the overall results suggested that extracts of *Calotropis gigantea* L. flower have potential insecticidal effect which might be used in pest control.

1. Introduction

Most of the chemicals that are used as insecticides in agricultural sector for crop protection, have undesirable effects on living beings particularly animals and human. These chemicals are considered as one of the reasons of environmental pollution. Therefore, the development of environment-friendly insecticides is an urgent need. In recent years, bioinsecticides have drawn a special attention of researchers as a viable pest control strategy[1-3]. Plant extracts and phytochemicals have already been recognized as antifeedants, repellents, growth inhibitors or as insecticides. The trend to utilize botanical pest control agents led to the study of the efficacy of many plant extracts as insecticides[4,5]. A common and most destructive pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is found throughout the world. This species has been found associated with a wide range of commodities including grain, flour, peas, beans, cacao,

nuts, dried fruits and spices[6]. This pest attacks the germ part (embryo portion) of the grain and their presence in stored foods directly affects both the quantity and quality of the commodity[7]. Currently, there are different kinds of preventive and curative control measures to get protection from this pest and chemical pesticides are one of them. But the use of chemical pesticides has serious drawbacks such as direct toxicity to beneficial insects, fishes and human due to their effects on non-target organisms[8,9]. However, few plant materials that are locally available in Bangladesh, have been investigated to determine their efficacy against *Tribolium castaneum* (*T. castaneum*). We have already demonstrated the insecticidal activity of root bark of *Calotropis gigantea* L. against *T. castaneum* (Herbst)[10]. As a continuation, the chemical constituents present in ethyl acetate extract of *Calotropis gigantea* L. flower (EECF) were determined in this study and the insecticidal activity of EECF was tested against the *T. castaneum* of different larval and adult stages.

2. Materials and methods

2.1. Plant material

For this study, the plant materials (flower's petal) were collected from

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the Rajshahi University campus. Professor A. T. M. Naderuzzaman, Department of Botany, University of Rajshahi, identified this plant and a voucher specimen (No. 1A. Alam, Collection date 15.08.2004) was deposited in the same department.

2.2. Extraction

After complete shade drying, the dried flower was pulverized into a coarse powder and it was stored in an airtight glass container. At room temperature, the powder (1.0 kg) was extracted with ethyl acetate (Carl Roth, Germany; 1.5 L) in an aspirator bottle for 7 days. To remove the solvent completely, rotary vacuum evaporation was then applied and 38 g crude EECF was found. It was designated as EECF and preserved in a vacuum desiccator for further use.

2.3. Gas chromatography-mass spectrometer (GC-MS) analysis of EECF

The chemical composition of EECF was established by GC-MS/quadrupole detector analysis using GCMS-QP2010S (Shimadzu Kyoto, Japan) spectrometer. They equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas at a flow of 14 psi (split 1:10), and the injection volume of each sample was 1 μL. The database used for the identification of chemical compounds and measurements of peak areas obtained is that of NIST/EPA/NIH MS LIBRARY (NIST 05) and also AMDIS version 2.0 d.

2.4. Insects

The Department of Zoology, University of Rajshahi, Bangladesh, had provided us different adult and larval stages of *T. castaneum* to examine the pesticidal activity of EECF. It was previously reported that (30 ± 1) °C, 65% relative humidity and 12:12 h. dark/light photoperiod were essential for rapid growth of this insect[11] and it was maintained strictly during this study. Insects were reared on a diet mixture of whole meal flour with Bakers yeast (19:1) in a Jar[12].

2.5. Toxicity assay

Residual film method had been applied here to determine the toxicity of EECF against *T. castaneum*[13]. As a preliminary screening, different doses of EECF were applied on adult *T. castaneum* to check 0% to 100% mortalities. Then five stock solutions of EECF were prepared in such a way that their concentrations became 80, 40, 20, 10 and 5 mg/mL. To make a uniform film over the petridishes, 1 mL of each stock solution of EECF was applied on petridishes

of 7 cm diameter. The petridishes were then air dried to evaporate the solvent and the extract was left on the petridishes. Here, the dose was calculated by dividing the actual amount of EECF (in mg) present in 1 mL with the area of the petridish and it was expressed as amount per square centimeter (mg/cm²). By this way, we got five doses that were 2.079, 1.040, 0.520, 0.260 and 0.130 mg/cm². When the petridishes were dried, 10 beetles were released in each petridish with three replication. A negative control was also performed with using the same number of insects and solvent only. Insect mortality was recorded 24 h and 48 h after treatments[13].

2.6. Statistical analysis

The mortality data were subjected to Probit analysis[14] for the determination of LD₅₀ values using the computer software SPSS of 14 version. Results with *P* < 0.05 were considered to be statistically significant.

3. Results

This study described the toxic effect of EECF against both larvae and adults of *T. castaneum*. From the probit analysis of mortality rate, several statistical data like LD₅₀, 95% confidence limit and Chi-square values were determined and shown in Table 1. After 24 h of exposure, 0.206, 0.199, 0.705, 0.738, 0.754, 0.451 and 1.371 mg/cm² were found as the LD₅₀ values of EECF against 1st, 2nd, 3rd, 4th, 5th, 6th instar larvae and adult *T. castaneum*, respectively (Table 1). As the effectiveness of EECF was increased with the increase of exposure time, the maximum residual toxicity was observed with LD₅₀ of 0.134, 0.174, 0.455, 0.440, 0.559, 0.390 and 0.716 mg/cm² for 1st, 2nd, 3rd, 4th, 5th, 6th instar larvae and adults *T. castaneum*, respectively, after 48 h of exposure (Table 1). No mortality was observed in control.

Table 1

Insecticidal activity of EECF against *Tribolium castaneum* (Herbst).

Sample	Life stage	Exposure time (h)	LD ₅₀ (mg/cm ²)	95% CL		Chi-square; χ^2 (degree of freedom)
				Lower	Upper	
EECF	1st instar	24	0.206	0.131	0.324	1.022 (2)
		48	0.134*	0.071	0.254	0.219 (1)
	2nd instar	24	0.199*	0.106	0.372	0.398 (2)
		48	0.174	0.097	0.313	0.136 (2)
	3rd instar	24	0.705	0.484	1.027	0.558 (3)
		48	0.455	0.329	0.628	1.525 (3)
	4th instar	24	0.738	0.573	0.952	2.932 (3)
		48	0.440	0.324	0.598	1.338 (2)
	5th instar	24	0.754	0.574	0.990	1.368 (3)
		48	0.559	0.409	0.763	0.238 (3)
	6th instar	24	0.451*	0.315	0.647	2.801 (3)
		48	0.390*	0.255	0.597	0.232 (2)
Adult		24	1.371	0.708	2.653	0.183 (3)
		48	0.716	0.493	1.052	3.624 (3)

*: Values were based on four doses with 20 insects each. *: Significant at *P* < 0.05 level. CL: Confidence limits.

Table 2

Chemical composition of EECF analyzed by GC-MS.

Retention time (min)	Compounds	Area (%)
4.44	1,2,3-Propanetriol	1.03
7.29	Methoxy-4-vinyl phenol	25.18
9.00	1,2,3-Propanetriol monoacetate 2-	0.84
9.65	Triacetin	0.68
12.63	2,5-Cyclohexadiene-1,4-dione	0.72
15.50	1-Hexadecene	0.86
20.06	1-Octadecene	2.64
21.08	2,4-Diphenyl-4-methyl-2(E)-pentene	0.66
21.78	1,2-Benzenedicarboxylic acid	1.10
22.87	5-Amino-6-nitroquinoline	1.06
24.25	Cyclohexadecane	3.97
25.44	S-Indacene-1,7-dione	0.73
28.11	Di-(2-ethylhexyl) phthalate	2.94
31.75	A/Neogammacer-22(29)-en-3-ol	1.04
33.48	Urs-20-en-3-ol	2.91
33.76	Anhydrosophoradiol-3-acetate	7.10
34.12	Neryl acetate	13.08
36.52	Lupa-12,20(29)-dien-3b,28-diol	1.15
42.70	2-Ethyl acridine	1.06
43.20	5-Methyl-2-phenylindolizine	1.24
44.14	2-Methyl-7-phenylindole	2.36
44.87	Urs-20 (29)-en-3-ol	0.85
52.06	Olean-12-ene	3.88
56.04	Urs-12-en-24-oic acid	5.39
Total area = 82.12		

The chemical composition of EECF is shown in Figure 1 and Table 2. Based on GC-MS chromatogram of EECF, 24 compounds were identified which were representing 82.12% of the total

relative content of EECF (Table 2). The major components of EECF were determined to be methoxy-4-vinyl phenol (25.18%), neryl acetate (13.08%) urs-12-en-24-oic acid (5.39%), cyclohexadecane (3.97%) and olean-12-ene (3.88%). All of the other components were present in lower relative amounts based on peak area (*i.e.*, 0.03%–2.57%).

4. Discussion

In this bioassay, the mortality rate of *T. castaneum* of different larval and adult stages was found to be increased with the increase in concentration of EECF. When make a comparison with other larval instars, it was found that the highest mortality of the 1st instars larvae was caused by EECF after 48 h exposure thereby producing lowest LD₅₀ value (0.134 mg/cm²). But the adult *T. castaneum* (after 24 h exposure) were less susceptible with the highest LD₅₀ values (1.371 mg/cm²). Results of this study demonstrated that toxicity of the plant extracts was decreased with the increase of age of the larvae. This may clearly support of others that insect’s age play an important role in influencing susceptibility[15]. The more or less similar type of results has been reported by Upadhyay[16] where the insecticidal properties of *Piper nigrum* were tested against *T. castaneum*.

Previous studies proved the potent insecticidal activity of

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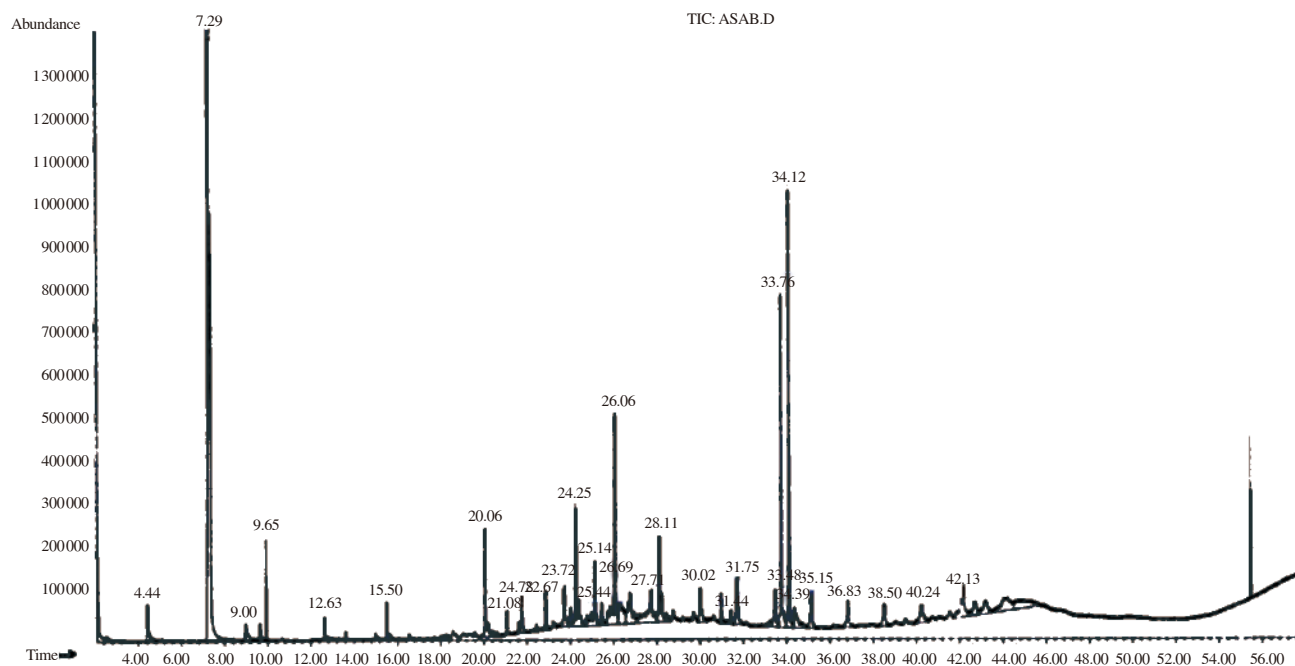


Figure 1. GC-MS chromatogram of EECF.

methoxy-4-vinyl phenol[17], neryl acetate[18] and urs-12-en-24-oic acid[19]. Here, the presence of these compounds in EECF was confirmed by GC-MS analysis. Moreover, 1,2-benzenedicarboxylic acid which was identified as a component of EECF, exhibited insecticidal activity against *Galleria mellonella* in a dose-dependent manner[20,21]. Therefore, the chemical profile of EECF has also justified its effective insecticidal activity against *T. castaneum*.

Based on the results as described above, EECF has a merit for using in the sustainable pest management in the stored products. However, further studies also need to be conducted to evaluate the its cost, efficacy and safety on wide range of pests in commercial store.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Govinda B. An overview of agrochemicals and their effects on environment in Nepal. *Appl Ecol Environ Sci* 2014; **2**: 66-73.
- [2] Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues and risk assessment indicators. *Int J Environ Res Public Health* 2011; **8**: 1402-19.
- [3] McGraw EA, O'Neill SL. Beyond insecticides: new thinking on an ancient problem. *Nat Rev Microbiol* 2013; **11**: 181-93.
- [4] Leng PF, Zhang ZM, Pan GT, Zhao MJ. Applications and development trends in biopesticides. *Arf J Biotech* 2011; **10**: 19864-73.
- [5] Grzywacz D, Stevenson PC, Mushobozi WL, Belmain S, Wilson K. The use of indigenous ecological resources for pest control in Africa. *Food Sec* 2014; **6**: 71-86.
- [6] Campbell JF, Runnion C. Patch exploitation by female red flour beetles, *Tribolium castaneum*. *J Insect Sci* 2003; **3**: 20.
- [7] Qasim MU, Hassan MW, Wang JJ, Jamil M, Iqbal J, Mansoor-Ul-Hasan. Management of *Tribolium castaneum* (Coleoptera: Tenebrionidae) with phosphine fumigation in relation to packaging materials and food types. *Pak J Zool* 2013; **45**: 1639-45.
- [8] Shukla S, Tiwari SK. Insecticidal activity of *Dryopteris filix-mas* (Linn.) Schott ethanolic extract against *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae). *J Biopesticides* 2011; **4**(2): 138-43.
- [9] Fry JP, Love DC, MacDonald GK, West PC, Engstrom PM, Nachman KE, et al. Environmental health impacts of feeding crops to farmed fish. *Environ Int* 2016; **91**: 201-14.
- [10] Alam MA, Habib MR, Nikkon F, Khalequzzaman M, Karim MR. Insecticidal activity of root bark of *Calotropis gigantea* L. against *Tribolium castaneum* (Herbst). *World J Zool* 2009; **4**: 90-5.
- [11] Kundu BR, Ara R, Begum MM, Sarker ZI. Effect of Bishkatali, *Polygonum hydropiper* L. plant extracts against the red flour beetle, *Tribolium castaneum* Herbst. *Univ J Zool Rajshahi Univ* 2007; **26**: 93-7.
- [12] Rustamani MA, Khatri I, Sultana R, Laghari MH. Population fluctuation of red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) on different cereal foods in laboratory. *Pak J Zool* 2014; **46**: 1511-4.
- [13] Mondal OA, Haque E, Haque J, Khan AR. Insecticidal activities of *Abroma augusta* (L.) chloroform and methanol extracts against *Tribolium castaneum* (Herbst) adults. *J Life Earth Sci* 2013; **8**: 11-5.
- [14] Finney DJ. *Probit analysis*. London: Cambridge University Press; 1971, p. 333.
- [15] Rohani A, Aziz I, Zurainee MN, Rohana SH, Zamree I, Lee HL. Current insecticide susceptibility status of Malaysian *Anopheles maculatus* Theobald to malathion, permethrin, DDT and deltamethrin. *Trop Biomed* 2014; **31**: 159-65.
- [16] Upadhyay RK, Jaiswal G. Evaluation of biological activities of *Piper nigrum* oil against *Tribolium castaneum*. *Bull Insectol* 2007; **60**: 57-61.
- [17] Sun ML, Wang YM, Song SQ, Feng GZ. Insecticidal activities and active components of the alcohol extract from green peel of *Juglans mandshurica*. *J Forestry Res* 2007; **18**: 62-4.
- [18] Chen HP, Yang K, You CX, Du SS, Cai Q, He Q, et al. Chemical constituents and biological activities against *Tribolium castaneum* (Herbst) of the essential oil from *Citrus wilsonii* leaves. *J Serb Chem Soc* 2014; **79**: 1213-22.
- [19] Chandramu C, Manohar RD, Krupadanam DGL, Dashavantha RV. Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. *Phytother Res* 2003; **17**: 129-34.
- [20] Ullah I, Khan AL, Ali L, Khan AR, Waqas M, Lee IJ, et al. An insecticidal compound produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. *Molecules* 2014; **19**: 20913-28.
- [21] Khalil NM, Shalaby EA, Ali DMIA, Ali EM, Aboul-Enein AM. Biological activities of secondary metabolites from *Emericellanidulans* EGCU 312. *Afr J Microbiol Res* 2014; **8**: 2011-21.