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Efficacy of the plant *Centella asiatica*(L.) Urb.(Apiaceae) on *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae: Bruchinae)

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ABSTRACT: Effect of aqueous, ethanol and acetone extracts of leaves of *Centella asiatica was* tested against the beetle *Callosobruchus chinensis.* The efficacy of the extracts on the test insect was dose-dependent. The results showed that higher doses of the extracts were significantly more toxic to *C. chinensis* compared to lower doses. LD50 value was assessed using probit analysis. After 48 hours of extract exposure of the plant, total body protein, glycogen and total free amino acids and the enzyme transaminase of the insects analysed indicated that total body protein and glycogen was found increased compared to control, while total free amino acid decreased. The enzyme transaminase was found increased.

KEY WORDS: *Callosobruchus chinensis,* protein, glycogen, *Centella asiatica*, transaminase, insecticidal activity.

INTRODUCTION

The pulse beetle, *Callosobruchus chinensis* L. (Chrysomelidae: Bruchinae) is a widespread and destructive major insect pest of stored legumes (Park et al., 2003). This insect is considered as a notorious pest of green gram, chickpea, black gram, peas, cowpea, lentil and pigeon pea. Female beetle lays eggs on the seeds. Hatching larvae bore inside and spend their life within the seed. Thus the pulses become completely hollow and unsuitable for human consumption.

Various methods have long been used for controlling insects including the use of chemicals. But pesticides cause toxicity to humans and warm-blooded animals (Salma Mazid and Jogen Ch. Kalita, 2011). There comes the importance of biological pesticides as they do not lead to

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resistance because of their degradable nature. Biopesticides are a group of naturally occurring, often slow-acting protecting agents that are usually safer to humans with minimal residual effects to the environment than conventional pesticides. Biopesticides can be biochemical or microbial.This study is an attempt to analyze the effective repellent and toxicant properties of the plant *C. asiatica* against the stored product pest *C. chinensis*. The test plant being medicinal, would yield environmentally sound chemicals having no harmful effects on the non target organisms. The biological activity of the plant extract might be due to the various compound, including phenolics, terpenoids, and alkaloids, exist in plants (Kashmira et al., 2010). Keeping this in view, the present study was carried out to test the efficacy of the leaf extracts of the plant *C. asiatica*. It is a herbaceous annual plant, growing in tropical swampy areas and has mild anti bacterial, anti viral, anti inflammatory and anti ulcerogenic properties (Newall et al., 1996)

MATERIALS AND METHODS

Culturing of test insects:

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College, Thiruvananthapuram. *C. chinensis* L. adults were obtained from naturally infested green gram seeds from local markets. The adult male and female beetles were reared on clean and un-infested green gram (*Vigna radiata* L). The seeds were made pesticide free by washing with clean water. Three jars each of 300 g were used. Each jar was filled with 200g chickpea grains and about 100 beetles were added to each jar. The jars were then covered.

Preparation of aqueous extract of plants:

The plant leaves were collected and washed well with distilled water. The leaves were ground without adding water. 25 g of the ground mass was then transferred into a beaker containing 100 ml of distilled water. Then it was mixed well and kept for three days. After three days the mixture was filtered. Then this mixture was kept in a water bath at 60-70^{0}C. After drying, this residue is dissolved in water and made up to different concentrations (Talukdar and Howse, 1993).

Preparation of acetone and ethyl alcohol extracts of plants:

For the extraction, soxhlet apparatus was used. 25g powder of plant leaves were extracted with 250ml ethyl alcohol and acetone. The extraction of each plant sample was done in about 12 hrs. After soxhlet extraction; the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept in a water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Treatments:

The extracts were applied at different doses (0.2%,0.4%,0.6% and 0.8%) on Whatmann No. 1 filter paper and air-dried for an hour. The controls were treated with acetone, ethyl alcohol or distilled water respectively for acetone, ethanol and aqueous treatments respectively. The treated and control filter paper discs were placed singly at the bottom of plastic jars and 200g of green gram seeds were placed on the papers. Hundred test insects *were* released in each plastic container. There were three replicates for each treatment and control. Observations were recorded on the seventh day of treatment.

Biochemical and enzyme assay:

Bioassay of protein (Lowry et al., 1951), total free amino acids (Spies,1957), glycogen (Dubois et al., 1956) and enzymes transaminases (AsAT [E.C.2.6.1.1.] and AIAT [E.C.2.6.1.2]) (Reitman and Frankel, 1957) were assayed on both control and insects exposed to sub lethal doses of *C. asiatica* .

Electrophoresis of whole body protein of adult beetles subjected to sub lethal dose was conducted according to the method devised by Laemmeli (1970). Electrophorogram obtained was subjected to GEL-DOC analysis using Lab Image Platform software.

Statistical analysis of data:

The data obtained are recorded as mean ± standard deviation. For testing the significance of the data obtained, statistical analysis were carried out using ANOVA (pd"0.05) using SPSS software (Daniel, 2006). LD 50 was calculated using probit analysis (Muhammad Akram Randhawa, 1944).

And standard error of LD 50 was calculated using the formula,

$$\text{Approx. S.E of LD 50} = \frac{\text{Log (LD84-Log LD16)}}{\sqrt{2N}}$$

Probit values were plotted against log doses and the dose corresponding to probit 5 that is 50% was found out.

RESULTS AND DISCUSSION

Effect of plant extracts on mortality of insects:

The total number of adult insects surviving after the treatment was recorded for seven days consecutively. Acetone and Ethanol extracts of the plant showed significant mortality compared to the aqueous extract. No mortality was seen in the case of control. Table 1,2 and

3 shows effect of aqueous, ethanol and acetone extract of plant leaves on LD 50 against the insects of *C. chinensis* respectively.

In the case of aqueous extract maximum mortality was seen in higher doses compared to lower ones (Table 1). In the probit analysis Log LD 50 was 2.8 and LD 50 was 56 mg in the case of aqueous extract.

Table 1. Effect of aqueous leaf extract on LD 50 against adult insects of *C. chinensis*

Group	Dose (wt of plant/wt of feed) mg kg^{-1}	Log dose	% dead	% corrected	probit
1	200	2.3	26	26	4.36
2	400	2.6	45	45	4.87
3	600	2.7	52	52	5.05
4	800	2.9	60	60	5.25

In the case of ethanol extract it was clear that the mortality percentage was more in higher doses compared to aqueous extract (Table 2) and Here Log LD 50 was 2.75 and LD 50 was found as 50.5 mg.

Table 2. Effect of ethanol leaf extract on LD 50 against adult insects of *C. chinensis*

Group	Dose (wt of plant/wt of feed) mg kg^{-1}	Log dose	% dead	% corrected	probit
1	200	2.3	20	20	4.16
2	400	2.6	40	40	4.75
3	600	2.7	50	50	5.00
4	800	2.9	60	60	5.25

In the effect of acetone extract on treated insects the percentage of mortality was higher compared to aqueous and ethanol extracts (Table 3). In the probit analysis Log LD 50 was 2.6 and LD 50 was 48mg. Effectiveness of the extracts was in the order acetone > ethanol > aqueous.

Table3. Effect of acetone leaf extract on LD 50 against adult insects of *Callosobruchus chinensis*

Group	Dose (wt of plant/wt of feed) mg kg^{-1}	Log dose	% dead	% corrected	probit
1	200	2.3	28	28	4.42
2	400	2.6	48	48	4.95
3	600	2.7	56	56	5.15
4	800	2.9	64	64	5.36

Lethal and sub lethal doses of different extracts of the plant *C. asiatica* on mortality of *C. chinensis* were respectively 56mg and 54mg in the case of aqueous extract, 50.5mg and 48.5 mg in ethanol extract and 48 mg and 46 mg in acetone extract (Table 4). 50 percentage of mortality was obtained in lethal doses.

Table4. Effect of sub lethal and lethal dose of aqueous, ethanol and acetone extract of *Centella*

Aqueous extract Dose (mg)	Mortality rate (%)	Ethanol extract Dose (mg)	Ethanol extract rate (%)	Acetone extract Dose (mg)	Mortality rate (%)
(sub lethal) 54	34 ±0.02	(sub lethal) 48.5	38±0.00	(sub lethal) 46	42±0.00
(lethal) 56	50±0.01	(lethal) 50.5	50±0.01	(lethal) 48	50±0.01

Values are mean ±SE; all values are significant at $p \leq 0.05$ level of significance

Effect of plant extracts on bio molecules of insects:

After exposure of sub lethal doses of different extract of the plant extract protein, glycogen and the enzyme transaminase was found to be increased while total free amino acids was found to be decreased when compared to the control insects (Table 5). Electropherogram of aqueous, ethanol and acetone treated insects showed the appearance of new and thick bands compared to control (Fig.1).

The use of plant extracts to control stored products insects is an ancient practice and known to possess bioactive compounds that are either toxic to insects, *Sitophilus zeamais* and *Tribolium castaneum* at various stages of life or elicit anti-feedant properties (Huang et al.,

Table5. Effect of sub lethal dose of different extracts of *C. asiatica* on glycogen, protein, amino acid, GPT and GOT contents in *C. chinensis*

Bio chemical Parameters	Aqueous extract		Ethanol extract		Acetone extract	
	Control insect	Treated insect	Control insect	Treated insect	Control insect	Treated insect
Glycogen (μg mg^{-1})	0.593±0.002	1.34±0.003	1.34±0.003	1.45±0.002	0.592±0.002	1.73±.006
Protein (μg mg^{-1})	9.27±0.001	9.40±0.006	9.40±0.006	9.52±0.003	9.28±0.006	10.16±.005
Total free aminoacids (μg mg^{-1})	9.03±0.004	8.44±0.004	8.44±0.004	8.19±0.001	9.06±0.001	7.84±.002
GPT	5.12±0.021	6.50±0.004	6.50±0.004	7.23±0.024	5.21±0.012	7.60±.004
GOT	3.86±0.011	5.23±0.009	5.23±0.009	5.43±0.031	3.89±0.008	5.69±.003

Values are mean ±SE; all values are significant at $p \leq 0.05$ level of significance

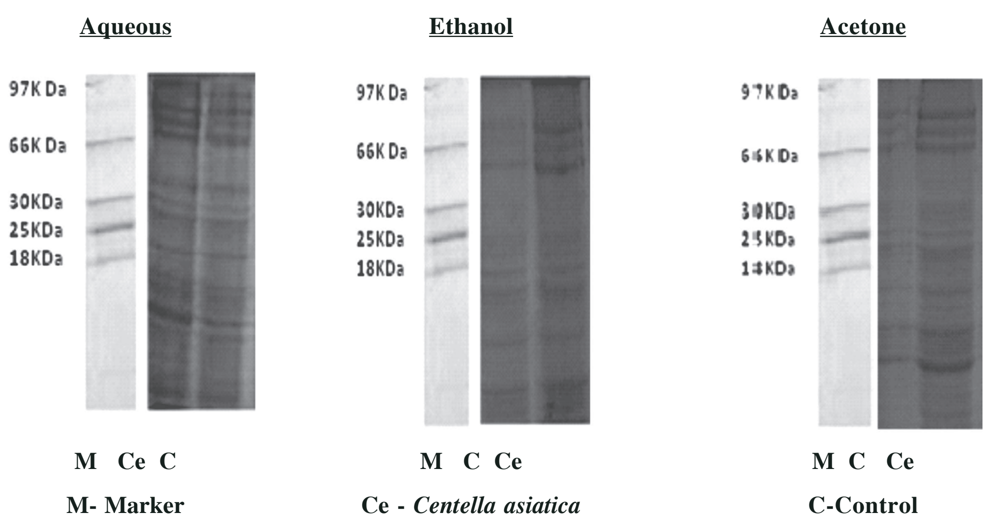

Fig. 1. SDS-PAGE protein patterns of total body of *C. chinensis* treated with aqueous, ethanol and acetone extract of *C. asiatica*

2000). Zia et al. (2011) studied the effect of aqueous extracts from ten plant species on mortality of chickpea beetle, *C. chinensis*. The plant *C. asiatica* contains primary metabolites like saponins also called triterpenoids which are known to be insect deterrent and toxic to insects. Toxic and deterrent modes of action have been suggested as responsible for the activity of several triterpenoid (Ortego et al., 1999). Results obtained in the present investigation clearly demonstrated that both solvent and aqueous extracts of *C. asiatica w*ere toxic to *C. chinensis.* Maximum mortality was obtained in acetone extract of the plant.

Elevated levels of protein indicate the de novo synthesis of novel proteins to overcome stress. Appearance of new protein bands and excess production of proteins as evidenced by SDS PAGE electrophorogram indicated that the insect responded effectively to stress conditions like inability in breathing or feeding deterrence that aroused due to plant extract treatment. Total free amino acids level in the control insect was higher compared to treated insect. The low level of total free amino acids in the test insect indicated a disturbance in the metabolism of insect. This decrease can be because of increased neuromuscular activity of treated larvae which resulted in higher demands for energy. Because of it, high amount of free amino acids entered into the Tricarboxylic acid cycle and oxidized, resulting free amino acid reduction (Chen, 1966). Decrease in free amino acid content indicated the possibility of active catabolism of amino acids through transamination and subsequent entry of corresponding ketoacids into Kreb's cycle to meet the emergent energy requirement as well as their utilization in the production of some new proteins synthesized to cope up with the stress. Hyperglycemia may be due to the inhibition of glycolytic enzymes on exposure to different physical, chemical and biological stress occurred due to treatment. It is also reported that crude plant extracts increase the whole body glycogen content in *S. litura* (Sahayaraj and Agnul, 2004). The increased GOT and GPT activity suggests the mobilization of amino acids during the insecticidal stress exerted by certain components present in the extract to meet the energy demands. Elevation or reduction in enzyme level is associated with physiological imbalance in insects (Saleem and Shakoori, 1985). Changes in the levels of certain enzyme during the course of insecticidal effect of malathion and carbaryl is also reported (Chanda, 1991) .

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