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Effect of ethanol extract of two medicinal plants on total body protein profile of adult *Callosobruchus chinensis* (Coleoptera: Chrysomelidae: Bruchinae)

Bindhu VR, Ganga S, Susha Dayanandan

Department of Zoology, University College, Thiruvananthapuram, Kerala, India

Abstract
Effect of two medicinal plants were tested against the stored product pest *Callosobruchus chinensis*. Sub lethal dose of the ethanol extract of the plant was used and the results showed increase in the amount of total body protein, PAGE and GEL DOC analysis convincingly proved the result of increased protein. Thus the plants are proved to be effective in making changes in the bio molecule of the pest and thereby causing mortality.

Keywords: page, GEL DOC, *Callosobruchus chinensis,* boerhavia, bacopa, electropherogram

Introduction
Proteins are known biological compounds which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. Proteins play a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants when entering into the animals (Wilkinson, 1976) [15]. Wigglesworth (1979) [14] has stated that the fat body in insect is the main site for protein synthesis as well as the intermediating site of metabolism of amino acids, which are utilized for the production of hormones and enzymes and the composition of protein in the body as a whole may be greatly modified.

Plant derived extracts and phytochemicals have long been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impacts. Plant derived insecticides are reported to have the ability to influence the proportion of various biochemical components (carbohydrates, lipids, proteins etc.) in the body of insects, thus disturbing the internal metabolism of the insect, causing their reduced activity or mortality. The potential effects of botanical insecticides on biochemical milieu of insects is of great interest in biological control applications. Senthilkumar *et al.,* (2009) [13] reported that *A. annua* and *A. indica* can affect biochemistry and physiology of insects.

In this study the total body protein profiling is done in adult insects of *Callosobruchus chinensis* adult insects to find out the influence of different plant leaf extracts on the protein content.

Materials and Methods
Test insects
Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, and University College Thiruvananthapuram. The pulse beetle, *Callosobruchus chinensis* L. adults were obtained from naturally infested green gram seeds purchased 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. For the study third instar larvae and adults were used.

Plants used for the study
Two medicinally important plants were used for the study namely *Boerhavia diffusa* and *Bacopa monnieri*. *Bacopa monnieri* is a perennial, creeping herb native to the wetlands of southern India and Australia. Bacopa is an important medicinal herb used in Ayurveda, where it is also known as "Brahmi". Bacopa has traditionally been employed as a neurological tonic and cognitive enhancer, and it is currently being studied for its possible neuroprotective properties. The best characterized compounds in *Bacopa monnieri* are dammarane types of triterpenoid saponins

Boerhavia diffusa is a perennial creeping weed and found throughout the warmer parts of India *Boerhavia diffusa* has shown antibacterial activity, mainly against Gram-negative bacteria Extracts of. *Diffusa* leaves have shown antioxidant and heap to protective properties in pharmacological models. Punarnavine (an alkaloid isolated from *B. diffusa*) has shown some anticancer, ant estrogenic, immunomodulatory and ant amoebic activity (particularly against *Entamoeba histolytica*). *Boerhavia diffusa* is a source of antioxidants, and may be effective against arsenic trioxide (an effective drug used against acute promyelocytic leukemia) induced cardio toxicity. *B. diffusa*thus also possess cardio protective properties. Boeravinones G and H are two retinoid isolated from *B. diffusa*.

Preparation of ethanol extract
Ethanol extract was prepared using the sox let apparatus. 20 gm. of powdered leaves of the plants were weighed and tied in a thin cloth separately and placed in extraction tube.200 ml ethanol was taken in the glass flask and was boiled at 55°C continuously. Boiling was continued for six to eight hours till the extract became pale green. On completing the boiling, the extract was allowed to cool and stored in air tight containers for further use under refrigerated condition. The ethanol extract obtained were treated as 100% concentration.

Treatment of adult insects
The effect of ethanol extracts was analyzed by using residual film method. No.1 What man filter paper were cut in round

shape and placed in the plastic containers. Sub lethal doses of the extract (2%) (Done by probity analysis) was applied to these filter papers using a micropipette and allowed to dry so that the solvent may evaporate completely. Then 20 gm. of feed was weighed out and ten one day old adult insects were placed in the containers so that each would get about 2gm of feed.
For each treatment control were also set up without applying plant extract. Solvent (ethanol) alone was used in the control. Six replicates were kept for each treatment and its control.

Protein assay
To determine the concentration of protein by Lowry's (1951) method.
Electrophoresis was carried out using the method of Laemmeli (1970) [6].
The molecular weight of each protein band and difference in the width of each bands were analyzed using GEL-DOC.

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 ($p \leq 0.05$) using SPSS software (Daniel 2006). LD 50 was calculated using probit analysis, (Muhammad Akram Randhawa, 1944) [11] Probit values are plotted against log doses and the dose corresponding to probit 5 that is 50% was found out.

Observation and Results
Total body protein content was found to be increased in the treated larvae and adults. Significant difference was observed in the level of protein in the control and treated insects (Table 1)
When adults of *Callosobruchus chinensis* was treated with ethanol extract the total body protein was found to be 8.34µg/mg in the control adults. The total body protein of Boerhavia, and Bacopa ethanol extract treated adult insects was 11.6µg/mg and 11.4µg/mg. (Table).
In electropherogram study, a significant change in the number of protein bands, width of the bands and the molecular weight of the proteins was observed in the case of ethanol extract treated adult insects of *Callosobruchus chinensis* when compared to that of the untreated adult insects. Figure.1
Gel Doc analysis also showed difference in the molecular weight of proteins of control and treated insects. Table 2

Table 1: Estimation of protein (µg/mg) in *Callosobruchus chinensis* adult insects when treated with the plant extracts

Extract (Dose %)	Control (µg/mg)	Test (µg/mg)	
		Boerhavia diffusa	***Bacopa monnieri***
Ethanol (2%)	8.34±0.03	11.6±0.03	11.4±0.05

Amount of protein is expressed in µg/mg of tissue. All values are mean ±S.E of six replicates

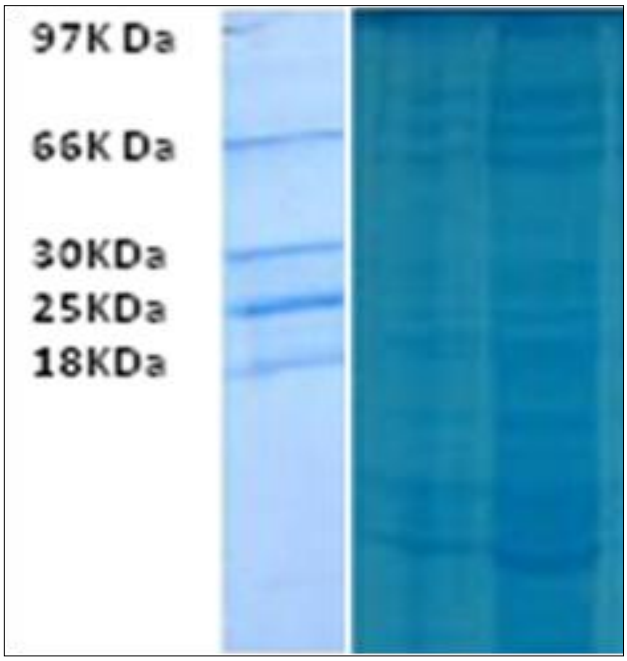

Fig 1: Electropherogram showing effect of ethanol extract on adults of *Callosobruchus chinensis*

Detection of protein bands using Gel Doc Analysis
Gel Doc: *Callosobruchus chinensis* treated with ethanol extracts
The SDS PAGE Gel electrophoresis was done using the control and treated one day old adult insect samples of *Callosobruchus chinensis.* In electrophoresed gel 6 lanes can be observed. The lanes 1 and 2 represent the marker and control respectively. The lanes 3and 4 represent insects treated with ethanol leaf extract of, *Boerhavia difusa* and *Bacopa moneri*. Each lane consists of bands of different molecular weight.
In lane 2, the band of control insects consists of the proteins of molecular weights 102.50 Kda, 95.00 Kda, 87.69 Kda, 68.54 Kda and 55Kda, 40.23 Kda, 29.81Kda and 25.40 Kda respectively.
Lane 3 represents the protein bands in the *Boerhavia difusa* treated insects. It consists of 7 bands of molecular weights 120.94Kda, 91.01Kda, 76.90Kda, 54.08Kda, 39.85Kda, 23.63Kda, 20.94Kda respectively. The lane shows significant increase in the number of protein bands when compared to control. This clearly indicates the increase in the total body protein of treated insects.
Lane 4 represents the protein bands in the *Bacopa monneri* treated insects. It consists of 6 bands of molecular weights 115.79Kda, 49.09Kda, 36.04Kda, 21.32Kda, 18.35Kda and 10.87Kda respectively. This shows a significant increase in the protein in the treated insects.

Table 2: Gel Doc analysis of ethanol extract treated insects
ROI 1: Lane 2

Band No	Band Name	R f	Band area(pixel)	Mole. Wt. (KD a)
1	Band 1	0.06	450	102.5
2	Band 2	0.17	405	95
3	Band 3	0.21	495	87.69
4	Band 4	0.24	450	68.54
5	Band 5	0.39	405	55
6	Band 6	0.44	315	40.23
7	Band 7	0.48	315	29.81
8	Band 8	0.52	368	25.40

ROI 1: Lane 3

Band No	Band Name	R f	Band area(pixel)	Mole. Wt. (KD a)
1	Band 1	0.08	504	120.94
2	Band 2	0.17	336	91.01
3	Band 3	0.24	378	76.90
4	Band 4	0.38	420	54.08
5	Band 5	0.49	630	39.85
6	Band 6	0.57	420	23.63
7	Band 7	0.67	462	20.94

ROI 1: Lane 4

Band No	Band Name	R f	Band area (pixel)	Mole. Wt. (KD a)
1	Band 1	0.17	495	115.79
2	Band 2	0.19	135	49.09
3	Band 3	0.24	540	38.04
4	Band 4	0.38	420	21.32
5	Band 5	0.49	630	18.35
6	Band 6	0.57	420	10.87

Discussion

The results showed that level of protein in the treated insects increased significantly. The increased level of protein may be due to the effect of different compounds like flavonoids, alkaloids, steroids, triterpenoids, and lignins, present in the plants. The increase in protein content with different extracts may be attributed to the increased activity of protein biosynthesis using amino acids. El-Bermawy and Abdel Fattah (2000) [2] explained that the protein type has specific biological role. Due to this role the DNA secretes enzymes which act as catalyst to produce specific type of protein, this protein is responsible for specific biological process, due to the difference in protein band between treated samples and control the biological process may be different too.

The appearance of new protein bands upon treatment may be due to liberation of free radicals which affect nitrogenous compounds directly; this in turn leads to breakdown of the peptide linkage, causing fragmentation of protein molecules. Protein is responsible for specific biological process, due to the difference in protein band between treated samples and control, the biological process may be affected (Megahed 1996, Gehad and Shaurub 1997) Protein fractions that fluctuated in number and relative concentrations might be due to protein breakdown and incorporation into other protein or both.

El-Naggar and Abdel-Fattah (1999) [3] showed that, either Eucalyptus oil alone or its combination with gamma radiation caused quantitative and qualitative changes in protein of larvae and pupae of *S. littoralis*. El-Bermawy and Abdel-Fattah (2000) [2], found qualitative changes in protein pattern in larvae and pupae of *T. confusum* after treating fourth instar larvae with plant oil (Vetiver). Electrophoretic analysis of total proteins, lipoproteins and glycoproteins revealed inhibitory action of the used plant extract of the Myrrh, namely; oil and oleo resin on the protein contents of *Culex pipiens* larvae (Massoud *et al*., 2001) [8]. Also, electrophoretic analysis of total protein showed appearance and disappearance of some protein bands in the treated *Culex pipiens* larvae by Lemongrass, Red basil, citronella and peppermint as compared with control group (Mohammed *et al.*, 2003). Renuga and Sahayraj (2009) [12] has reported that treatment of *Spodoptera litura*by the dried plant *Ageratum conyzoids* lowered the total protein content of the head of the third and fourth larval instars.

Kinnear *et al*., (1971) [5] suggested that increased protein levels in *Calliphora stygia* were due to increased synthesis of new proteins by the fat body, haemolymph and other tissues of the larvae. Al Qahtani *et al.*, (2012) [1] reported that the electropherogram obtained after treatment with ginger extract in *Oryzaephilus surinamensis* shows the changes of body protein and extract treatment was found to divide body proteins in to smaller subunits and alter the protein configuration after treatment.

In the present study ethanol extract of the two plants caused significant increase in the amount of protein in adults and it is convincingly analysed by PAGE and GEL DOC analysis. Thus proved to be effective in disturbing the biomolecule content of the pest thereby probably leading to mortality.

References

1. Al Qahtani AM, Al Dhafa, ZM, Rady MH. Insecticidal and biochemical effect of some dried plants against *Oryzaephilus surinamensis* Coleoptera; Sylvanidae Journal of entomology and Zoology studies 2012; 3(2):421-428
2. El-Bermawy SM, Abdel-Fattah HM. Changes in protein electrophoretic pattern of *Tribolium confusum* 4th instar larva after treatment with volatile plant oil Vetiver. J. Egypt-Ger. Soc. Zool. 2000; **31**(E):167-182
3. El-Naggar SEM, Abdel-Fattah HM. Effects of gamma radiation and plant oil extract of *Eucalyptus globules* on the cotton leaf worm, *Spodoptera littoralis* Boisd. Bull. Ent. Soc. Egypt, Econ. Ser. 1999; 26:59-69.
4. Gehad MA, Shaurub EH. Effect of two radiomodifiers on protein pattern of gamma irradiated blow fly, *Chrysomya albiceps* WIED. J. Egypt. Ger. Soc. Zool. 1997; 22**:**105-116.
5. Kinnear JF, Martin MD, Thomas JA. Developmental changes in the late larva of *Calliphora stygia*. 3. The occurrence and synthesis of specific tissue proteins. *Aust*. J. Biol. Sci. 1971; 24: 275-289.
6. Laemmeli UK. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature, 1970; 227: 680-685.
7. Lowry OH, Roseborough NT, Farr A, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry193. 1951; (1):265-275.
8. Massoud AM, Labib IM, Rady M. Biochemical changes of *Culex pipiens* larvae treated with oil and oleo resin extracts of *Myrrh commiphora molmol*. J. Egypt. Soc. Parasitol. 2001; 31(2):517-529.
9. Megahed FM. Effect of gamma radiation on the ultrastructure of ovaries and protein and esterase patterns of female *Culex pipiens*. Ph. D. Thesis, Fac of science, Cairo University, Egypt. 1996
10. Mohamed MI, El-Mohamady RH, Mohamed HA. Larvicidal activity and biochemical effects of certain plant oil extracts against *Culex pipiens* larvae Diptera: Culicidae. J. Egypt. Acad. Soc. Environ. Develop. A. Ent. 2003; 3(1): 75-93
11. Muhammad Akram Randhawa. Calculation of LD50 values from the method of Miller and Tainter, Department of Pharmacology, College of Medicine, University of Dammam, Dammam, Saudi Arabia. 1944

12. Renuga FB, Sahayraj K. Influence of botanicals in total head protein of *Spodoptera* litura Fab Journal of bio pesticides. 2009; 2(10):52-55
13. Senthilkumar N, Varma P, Gurusubramaniam G. Larvicidal and adulticidal activities of some medicinal plants against the malaria vector, *Anopheles stephensi Liston.* Parasitol Res 2009; 104: 237- 244.
14. Wigglesworth VB. The principles of insect physiology. 7th edition, Chapman and Hall. 1979
15. Wilkinson CF. Insecticide Biochemistry and Physiology. Plennum, New York 1976