International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Toxic Effects of Four Medicinal Plants on the Blood Sugar Trehalose of the Pulse Beetle *Callosobruchus chinensis* (Coleoptera: Bruchide)

Ganga .S[1], Bindhu .V .R.[2], Susha Dayanandan[3]

[1]Department of Zoology, University College, Thiruvananthapuram, Kerala-34, India

[2, 3]Assistant Professor, Department of Zoology, University College, Thiruvananthapuram, Kerala-34, India

Abstract: ***Different extracts of four medicinal plants Vetiveria zizanoides, Asparagus racemosus, Hyptis sauveolens and Citrus limon were assessed to demonstrate their toxic effects on the stored insect pest Callosobruchus chinensis. The four plants caused significant effects on the beetle and the effect was dose-dependent. In the study, insect blood sugar trehalose was analysed to check the effects of the treated plants. Trehalose was found to be decreased compared to control. Aqueous, ethanol and acetone extracts were used for the study. Acetone extracts of Citrus limon could produce more significant effects indicating the presence of more active components.The four medicinal plantsthus proved to have strong insecticidal activity against the insect pests.***

Keywords: *Vetiveria zizanoides,Asparagus racemosus,Hyptis sauveolens* and *Citrus limon,Callosobruchus chinensis*, Trehalose

1. Introduction

The disaccharide trehalose is the main blood sugar (Wyatt, 1967; Steele, 1985; Becker *et al.*, 1996; Candy *et al*., 1997; Elbein *et al*., 2003; Thompson, 2003). Trehalose is a non-reducing disaccharide also known as mycose or tremalose comprising two glucose molecules. It is synthesized and released into haemolymph by the fat body, the central organ of intermediary metabolismin insects. It has been shown that trehalose can protect proteins and cellular membranes from inactivation or denaturation caused by a variety of stress conditions, including desiccation, dehydration, heat, cold, and oxidation. Trehalose served as a storehouse of glucose for energy and or for synthesis of cellular components. The fact that trehalose is so resistant to acid hydrolysis may be key to understanding the use of trehalose in insect blood or hemolymph (Mirth and Riddiford, 2007).Glycogen is mobilized for use by other tissues, mostly in the form of trehalose.

The secretion of trehalose by adipocytes involves a membrane transporter. The identification and characterization of the first insect trehalose transporter has been recently reported (Kurita *et al.*, 1979, 1981). Apart from the use of trehalose for maintenance of energy metabolism during fasting or nonfeeding periods (Bloomquist, 1996), it act as a substrate for insect flight in general. Long-term flyers, such as locusts (Lewis *et al*,. 1993) and mosquitoes, subjected to several hours of flight (Pereira and Gurudutt, 1990) start flying using trehalose and after some time switch to lipids. Short-term flyers such as the cockroach Periplaneta americana (Frazier, 1986) use mostly trehalose.

The relation between trehalose and insects was ignored until the substance was rediscovered in insects in the mid fifties (Athenstaedt and Daum, 2006) and its metabolism was extensively studied in the following years. The reaction involved in the synthesis and degradation of trehalose in insects are now well understood however despite much effort over almost forty years, it is still poorly understood how these processes are regulated. Trehalose is present in all insects (at least in the adults) studied in this respect, and in many insects it is present in high concentrations and constitutes the major haemolymph (blood) sugar and can be stored in relatively high concentration in body fluids (Raja *et al*., 2005).

The present study investigates the changes in the trehalose content in adult *Callosobruchus chinensis* when treated with the plant leaf extracts of *Vetiveria zizanoides, Asparagus racemosus, Hyptis sauveolens* and *Citrus limon.*

2. Materials and Methods

Test Insect

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College Thiruvananthapuram. The pulse beetle, *Callosobruchus 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. The adult insects separated from the initial stock were introduced into the fresh green gram. The culture was maintained in the laboratory in plastic containers at room temperature (±28°C). The cultures were cleaned every day to avoid contamination. Newly emerged adults were used for the study.

Plants used for the study

Four medicinally important plants were used for the study namely *Vetiveria zizanoides, Asparagus racemosus, Hyptis sauveolens* and *Citrus limon.*

Preparation of aqueous leaf extracts

50 grams of powdered leaves were weighed and dissolved in 100 ml distilled water and kept for 24 hours. After 24 hours the mixture was heated at low heat for 2-3hours continuously in a hot plate. While boiling the solution was

mixed thoroughly with a glass rod at regular intervals to prevent overflow. After boiling the mixture was filtered through What'smann No. 1 filter paper. The supernatant was collected and centrifuged for 10 minutes at 2000 rpm. The concentration of prepared extract was considered as 100%. Finally it was stored in air tight glass containers under refrigeration.

Preparation of Ethanol extract

Ethanol extract was prepared using Soxhlet apparatus. 50gms of each leaf powder were weighed and tied in a thin cloth and placed in the extraction tube. 500ml ethanol was taken in the glass flask. Ethanol was boiled at 55ºC continuously. Boiling was continued for six to eight hours till the extract became pale green. After the extraction is over the extract is allowed to cool, and stored in air tight containers. The concentration of prepared extract was considered as 100%. Finally it was stored in air tight glass containers under refrigeration for further use.

Preparation of acetone extract

Acetone extracts were prepared according to the method of Talukder and Howse (1993) with modifications. Twenty grams of ground leaves of *vetiveria, asparagus, hyptis* and *citrus* were separately mixed with 200ml acetone and stirred for 30 minutes using a magnetic stirrer and then left to stand for 24 hours. The mixture was then filtered through Whatman No.1 filter paper and the solids were stirred again for 15 minutes with 50 ml of acetone and filtered and the filtrates were combined. The solvent from the pooled filtered solution was evaporated in a water bath at 65ºC. After complete evaporation of solvents, the final cooled extracts were weighed and preserved in sealed bottles in a refrigerator at 5°C until used for insect bioassays.

Treatment of adult insects

The effect of aqueous, ethanol and acetone extracts were analysed by using residual film method. No.1 Whatman filter paper were cut in round shape and placed in the plastic containers. Plant leaf aqueous, ethanol and acetone extracts with the doses (2.4 %, 2.2 % and 1.6%) were applied to these filter papers separately using a micropipette and allowed to dry so that the solvent may evaporate completely. Ten one day old adult insects were placed in each of the containers along with twenty grams of feed (green gram seed) so that each adult would get about two grams of feed. The feed was weighed out using a weighing balance Control was also prepared for each experiment by adding solvent (distilled water, ethanol and acetone) alone instead of leaf extracts. Five replicates were kept for each treatment and its control.

After three days of treatment, adult insects were separated from the treated culture and homogenated in phosphate buffer. The homogenate was centrifuged at 6000rpm for 10 minutes. Supernatant obtained was used for the estimation of trehalose.

Procedure

1. The insect tissues were homogenized using glass homogenizer in Ringer's solution
2. Added 0.5 ml of 2%NaOH
3. Shaken well and keep in boiling water bath for 10 minutes
4. Cooled in ice bath
5. Added 5 ml Anthrone reagent.
6. Kept in boiling water bath for 15 minutes.
7. Read at 620 nm.

3. Observation and Results

Results from the Table (Table.1) showed that the trehalose content of the treated adults were decreased compared to control insects. Each extract and all the doses tested showed significant differences in the amount of trehalose.

Estimation of Trehalose in adult *C.chinensis*

When the insectswere treated with the aqueous extract, the trehalose content in control insects was estimated as 10.27mg/ul In all the treated samples trehalose was significantly less when compared to the control and it was estimated as 8.46 mg/ul, 7.58 mg/ul, 7.22 mg/ul and6.69mg/ul when trated with *Vetiveria,Asparagus,Hyptis* and *Citrus.* When the treatment was done with the ethanol extract, the control insects had shown the trehalose content as 10.22mg/ul In all the treated samples trehalose was significantly less when compared to the control and it was estimated as 8.22mg/ul, 7.12mg/ul, 7.09mg/ul and 6.65mg/ul when trated with *Vetiveria,Asparagus,Hyptis* and *Citrus.* The treatment with acetone extract showed the trehalose content in control insects as 10.01mg/ul respectively.All the treated insects had shown a decrease in the trehalose content. The values were estimated as 7.13mg/ul, 7.11mg/ul, 6.42mg/ul and 5.98mg/ul. Also it was noticed that the trehalose content decreased in the higher doses tried and it can be said that the treatment was dose-dependant.

4. Discussion

It has been observed that the trehalose content had been reduced in all the treatments when compared to the control. This may be due to the stress condition that occured in the insects during the treatment.

In most insects, carbohydrates reserves are present as glycogen and trehalose and both the reserves can be ready converted into glucose for the support of all life processes. Metamorphic changes in insect are usually accompanied by substantial depletion of their carbohydrate reserves. During this period, glycogen and treholase supply glucose which provides an energy source and a substrate for the synthesis of pupal and adult tissues, especially the cuticle. In addition to this, the stress condition that occured in the insects during the adverse condition causes a drastic reduction in the amount of trehalose. In this experiment, the changes are probably due to the treatment with plant extracts. The obtained results show that the activity of trehalose was affected in treatments and was lower than that obtained with untreated insects. Change in trehalose indicates the role of plant extracts in altering the physiological balance in insects.

Islam and Roy (1981) noticed depletion of trehalose in *Lohita grandis* due to the action of various hormones. Bouayad *et al.,* (2013) noticed enhanced activity of hydrolytic enzymes and decreased amount of trehalose in Plodia interpunctella on treatment with *Moroccan* plant extracts . Hatem Mohamed Al-shannaf , Hala Mohamed

Mead and Al-Kazafy Hassan Sabry (2012) repoted the effect of some bio insecticides and Igrs on American Bollworm, Helicoverpa armigera and noticed a drastic increase in the activity of the enzyme trehalose and reduced amount of trehalose.

It can be concluded that the plant extracts used in this study probably exhibit a significant reduction in carbohydrates content, associated with general disturbances in carbohydrates metabolism, which probably lead to the reduction in trehalose content of the insects.

Table 1: Estimation of trehalose (mg/ul) in adult *C. chinensis*

Extract	Dose (%)	Control insects (mg/ul)	Treated insects (mg/ul)			
			Vetiveria	*Asparagus*	*Hyptis*	*Citrus*
Aqueous	2.4	10.27±0.04	8.46±0.04	7.58±0.04	7.22±0.01	6.69±0.02
Ethanol	2.2	10.22±0.02	8.22±0.00	7.12±0.00	7.09±0.04	6.65±0.04
Acetone	1.6	10.01±0.00	7.13±0.03	7.11±0.03	6.42±0.01	5.98±0.01

Amount of trehalose is expressed in mg/ul.

All values are mean ±SE of five replicates and significant at p≤0.05 level of significance

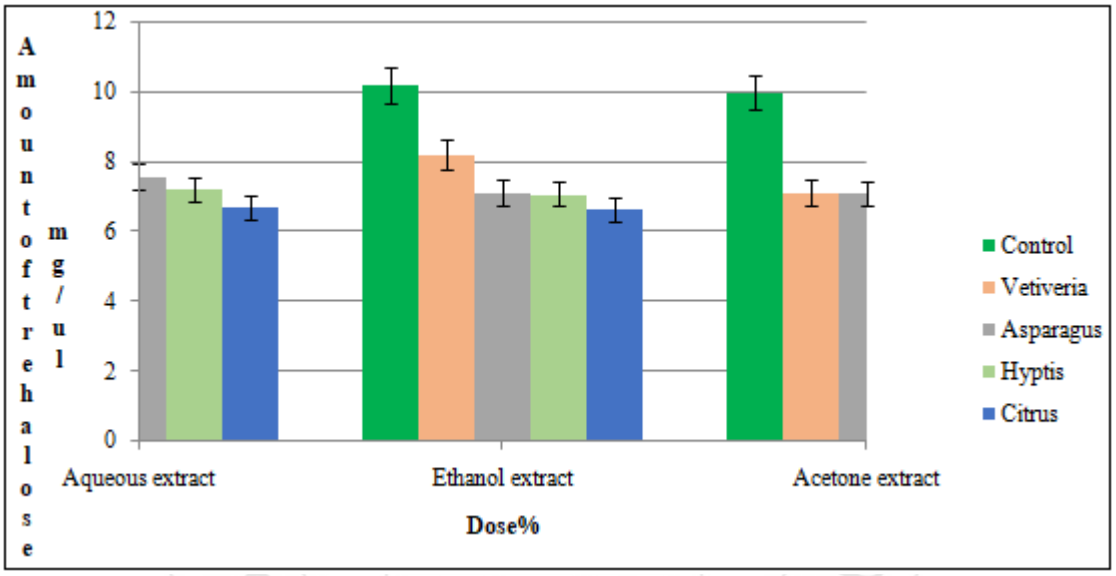

Figure 1: Graph showing amount of trehalose (mg/ul) in adult *C. chinensis*

Amount of trehalose is expressed in mg/ul.

All values are mean ±SE of five replicates and significant at p≤0.05 level of significance

5. Acknowledgement

We express our heartful thanks to HOD, University College, Dept. of. Zoology, Thiruvananthapuram for providing necessary facilities for the study.

References

[1] Athenstaedt, K. and Daum, G. 2006. The life cycle of neutral lipids: synthesis, storage and degradation. *Cell Mol Life Sci*. **63**: 1355-1369.

[2] Becker, A., Schloeder, P., Steele, J.E., and Wegener, G. 1996. The regulation of trehalose metabolism in insects. Experientia, **52**, 433-439.

[3] Bloomquist, J.R. 1996. Ion channels as targets for insecticides. *Annual Review of Entomology*, **41**, 163-190.

[4] Bouayad, N., Rharrabe, K., Ghailani, N.N., Jbilou, R., Castanera, P. and Ortego, F. (2013). Insecticidal effects of Moroccan plant extracts on development, energy reserves and enzymatic activities of *Plodia interpunctella*. *Spanish Journal of Agricultural Research*. **11(1)**: 189-198.

[5] Candy, D.J., Becker, A., Wegener, G. 1997. Coordination and integration of metabolism in insect flight. *Comp Biochem Physiol***117**B: 497-512.

[6] Elbein, W.A., Reitman, A., Frankel, S.A. 2003. Recent developments in controlling insect and plant pathogens of agricultural and medical importance by *Artemissia annua* L. (Asteraceae).

[7] Frazier, J.L. 1986. The perception of plant allelochemicals that inhibit feeding. In: *Molecular aspects of Insect-Plant Associations*, edited by L.B. Brattsten, S. Ahmad (Plenum Press, New York, USA). 1-42.

[8] Hatem Mohamed AI-Shannaf, Hala Mohamed Mead and AI-Kazafy Hassan Sabry (2012). Toxic and Biochemical Effects of some Bioinsecticides and Igrs on American Bollworm, *Helicoverpa armigera* (Noctuidae: Lepidoptera) in Cotton fields.

[9] Islam, A. and Roy, S. 1981. Effects of epinephrine, JH and Corpora cardiaca extract on the level of various sugars in *Lohita grandis* Gray (Hemiptera: Pyrrhocoriedae).

[10] Kurita, N., Miyaji, M., Kurane, R., Takahara, Y. and Ichimura, K. 1979. Antifungal activity and molecular orbital energies of aldehyde compounds from oils of higher plants. *Agriculture and Biological Chemistry*. **43**, 2365-2371.
[11] Kurita, N., Miyaji, M., Kurane, R., Takahara, Y. and Ichimura, K. 1981. Antifungal activity of components of essential oils. *Agriculture and Biological Chemistry*. **45**, 945-952.
[12] Lewis, M.A., Arnason, J.T., Philogene, J.R., Rupprecht, J.K. and McLaughlin, J.L. 1993. Inhibition of respiration at site I by asimicin, an insecticidal acetogenin of the paw paw, *Asimina triloba* (Annonaceae). *Pesticide Biochemistry and Physiology*. **45**, 15-23.
[13] Mirth, C.K. and Riddiford, L.M. 2007. Size assessment and growth control: how adult size is determined in insects. *Bio. Essays*. **29**. 344-355.
[14] Pereira, J., Gurudutt, K.N. 1990. Growth inhibition of *Musca domestica* L. *and Culex quinquefasciatus* (Say) by (levo)-3-epicaryoptin isolated from leaves of *Clerodendron inerme* (Gaertn) (Verbenaceae*). Journal of Chemical Ecology*. en**16**. 2297-2306.
[15] Raja, N., Jayasankar, A., Venkaimnactesan., Jayakumar, S. and Ignacimuthu, S. 2005. Of *H. sauveolens* against Lepidopteran pests. *Current Science*, volume **88 (2).**
[16] Steele, T.E. and Hall, S. 1985. Trehalose synthesis and glycogenolysis as site of action for the *Corpora cardiacum* in *Periplanata americana* . *Insect Biochem*., **15**: 529-536.
[17] Talukder, F.A., Howse, P.E. 1993. Deterrent and insecticidal effects of Pithraj, *Aphanamixis polystachya* against *Tribolium castaneum* in storage. *Journal of Chemical Ecology*. **19(11):** 2463-2471.
[18] Thompson, S.N. 2003. Trehalose: the insect 'blood' sugar. *Adv Insect Physiol*. **31**:205–85.Comprehensive review on trehalose in insects.
[19] Wyatt, G.R. 1967. In Insect Biochemistry and function (Candy and Kilby) 89-154, Chapman and Hall, London.