Biospectra : Vol. 10(1), March, 2015. Spl. issue, pp.65-68

An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Effect of rogar on the free amino acids in the haemolymph of *Nezara viridula* (male) (hetroptera : pentatomidae)

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Received , 5th December, 2014; Revised: 11th January, 2015

Abstract : In insects *Nezara viridula* (Male) free amino acids of haemolymph were determined qualitatively by two-dimensional paper chromatography and there after quantitatively by Spectrophotometer. Fifteen amino acids were detected of which Glutamine occurred in high concentration followed by Glycine. The concentration of all free amino acids decreased after one hour Rogar treatment and also after the two hour stage.

Keywords: free amino acid, Rogai, haemolymph, *Nezara viridula.*

INTRODUCTION

The present investigation is to determine the effect of Rogar on the concentration of the free amino acids in the haemolymph of male *Nezara viridula* at fixed time intervals.

Insect have a high concentration of free amino acid in their haemolymph, which form useful metabolites for insect tissues. The amino acid also play an important role in the osmoregulation Bishop et al. (1926)[1] and Beadle and Shaw, (1950)[2].

MATERIAL AND METHODS

The adults *Nezara viridula* (Male) were collected locally from the potato plants during the period of Norember to February. They were kept in the laboratory under suitable conditions in an insect box, which contained tubers potato plats (*Solanum tuberculosum*) which was changed every alternate days.

The haemolymph was collected by cutting the coxae from the body wall. In all, 0.5 ml. of haemolymph was pooled and deprotenized according to the method of Pant & Agrawal (1964)[3]. All chemicals used were of analytical reagent grade. Extracts were applied as compact spots on whatmen. No-1, filter paper sheet 40 (cm. × 40 cm).

Two dimensional paper chromatography was done according to the method of Smit & Agiza (1951)[4]. The chromatograms were first developed with n-butanol: glacial acetic acid: distilled water (4 : 1 : 5) for 6 hours. The second solvent was made with Phenol saturated with distilled water. Spots were developed by ninhydrin and identified with the Predetermined Rf. values of the known amino acids.

The concentration of free amino acids were determined by elution. The nihydrin coloured spots were placed in separate tubes to which was added one ml. NaOH (neutralized with phenolphthalene), one ml. citrate buffer (pH_5) and two ml. ninhydrin solution.

The tubes were then placed in boiling water and into them was added one ml. stannus chloride solution, this produced red colour which after fifteen minutes change to purple. The tubes were then kept in a dark place for 15 minutes, 5 ml. n-butanol was added in each tubes. They were then shaken and kept ready for estimation. The optical density of solution was measured on Boush and Lomb spectronic-20. Spectrophotometer (Moel VSU-1) at 550µ and at 440µ (only for proline) against a blank extract prepared from a piece of the same filter paper of the same area. Concentration of the separated amino acids was calculated from standard curves previously prepared using

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standard amino acid solutions chromatographed for the same period using the same solvent.

The LC_{50} value of Rogar for the insects under investigation was determined using graded concentration of Rogar solution.

Known quantity (LC_{50}) of Rogar was sprayed on the insects and haemolymph was pooled after the lapse of one hour of insecticial treatment by cutting the coxae. Again after the lapse of two hours the haemolymph was collected for chromatographic separation and quantitative estimation was carried out by processes described above.

OBSERVATIONS

Fifteen amino acids were observed by two dimensional paper chromatogrphy in *Nezara viridula* (Male). Their concentration is mentioned in Table-I. Glutamine, Glycine, serine and Glutamic acid showed higher concentratioin. Among them cystine occurred in lower concentration. The concentration of all free amino acids changed by applying Rogar. After one hour of insecticidal treatment, fifteen free amino acids showed less hours of Rogar poisoning, it was found that all the free amino acids showed further decline in concentration.

Table 1: Concentration of free amino acids in the haemolymph of *Nezara viridula* (Male) (Concentration = µ mol./ 100 ml. of haemolymph)

Sl.No.	Amino acids	Normal	After one hour insecticidal treatment	Two hours insecticidal treatment
1.	Cystine	22.13	20.72	16.54
2.	Alanine	171.07	156.14	84.35
3.	Glycine	202.53	141.05	104.01
4.	Glutamic acid	159.40	127.23	99.31
5.	Lysine	121.46	26.01	22.19
6.	Histidine	96.79	83.54	72.26
7.	Proline	161.40	139.47	109.05
8.	Arginine	118.83	104.81	96.74
9.	Ornithine	79.48	62.39	58.16
10.	Valine	156.25	144.62	113.41
11.	Glutamine	206.55	192.41	174.52
12.	Serine	195.90	178.34	169.07
13.	Leucine	146.25	134.21	124.64
14.	Tyrosine	28.84	25.31	20.09
15.	Phenylalanine	36.34	34.28	28.46

DISCUSSION

Proteins are the most important among the cells components. The words has been derived from the Greek word Proteas means “first rank”. Proteins are present everywhere in the cell, cell membrane; enzymes and some of the hormones which control the biochemical process are all made of Proteins. According to Abbot (1926)[5], Beckwith and Rose (1929), the proteins make up about half the body weight in insects.

Free amino acids in the haemolymph of insects are sixty times higher than in the human blood and all those commonly found in proteins have been indentified in the insect haemolymph, although some of them such as cystine, tyrosine, phenylalanine, ornithine and histidine either occur in low concentration Gilmour (1965)[6]. The concentration of different amino acid in *Nezara viridula* (Male) has been reported in present study. The males of this insect show high concentration of Glutamine.

Leven Boak (1966)[7] reported glutamine plus glutamate in *Phormia regina* amounting to 10 to 20 percent of the total concentration in the fat body and the haemolymph whereas the value is closer to 50% in the Gut. Although their relative concentration in insect tissues are considerably more variable, the ratio of glutamine over glutamate in locust haemolymph is 9 : 1 (Kilby, 1958).

Cystine is present in low concentration; in *Nezara viridula*. According to Wyatt (1956) and Stevens (1961) both cystine and cysteine are absent in the haemolymph of silk worm and American Cockroaches. Gilmour (1961) both the amino acids are interconvertable by oxidation and

reduction. Because cystine is easily oxidised aerobically to cystine and cysteic acid. According to Robinson (1963) isolation of these last two is no proof that they exist in the living organisms. Belongun (1974) has suggested that proline plays the role of a readily available metabolizable energy reserve for flight and movement. Besides, a great number of amino acids like alanine, Glutamine, Glycine and proline are known to play important role in the synthesis of cuticle proteins, chitin and other constituents of cuticle.

In present work, proline concentration was much higher than that of the other amino acids. The same situation has been reported by Bursell (1953)[8] in *G. morsitans* (Diptera) and Barret (1974) in *R. prolixus*. It has been also found abundantly in the body of female mosquito. *A. aegypti* (Thayer Terian, 1970). It has been proposed as an energy source during the flight in *G. morsitans* (Bursell, 1963)[9]. A great number of amino acid like alanine, glutamine, glycine and proline are known to have important role in synthesis of cuticle proteins, chitin and other constituents of cuticle.

Winteringham & Harrison (1956)[10] reported depletion of glutamate and proline in DPP poisoned houseflies, the liberated ammonia being trapped by glutamine. Corrigan & Kearns (1963)[11] observed that the concentration of proline and glutamine in the haemolymph of D.D.T poisoned cockroaches varies inversely with increase in toxicity. Present observations suggest that the level of free amino acids in the treated insects are influenced by the amount of insecticides used for poisoning (lethal concentration) and the time of evaluation of the toxic effects.

The correlation between the degree of toxicity and reduction of amino acids indicate, that the depletion of amino acids was the direct effect of poisoning.

Thus it is concluded that the depletion of amino acids was mainly a consequence of the higher metabolic activity of the poisoned insects. The decrease in the level of amino acids is due to an imbalance between the rates of anabolism and catabolism in them.

REFERENCES

1. **Bishop, G.H., Briggs, A.P. and Ronzani, E. 1926**. Body fluids of the honey larva. J. Biol. Chem., **66**: 77-78.
2. **Beadle, L.C. & Shaw, J. 1950**. The retention of salt and regulation of the non-protein nitrogen fraction in the blood of the aquatic larva sielis lutroia. J. Exp. Bio., **27**: 96-109.
3. **Pant, R. and Agarwal, H.C. 1964.** Free amino acids of the haemolymph of some insect. J. Insect. Physiol., **10**: 443-446.
4. **Smit, A.K. and Agiza, A.H. 1951.** Free amino acids and related compounds in the tissues of aging-female aedes aegypti mosquitoes. J. Insect. Physiol., 16: 1-5.
5. **Abbot. 1926.** Digestion and absorption in the crop of cockroach. J. of Erptl. Zoology, **44**: 219
6. **Glimour, D. 1965**. The metabolism of insects. Oliver & Boyd. London.
7. **Leven Book, L. 1966**. Haemolymph amino acid and peptides during larval growth of the blowfly. *Phorima regina*. Comp. Biochem. Physiol. 18: 341-351.
8. **Bursell, E. 1963.** Aspects of the metabolism of amino acids in the tse tse fly: Glossina (Diptera). J. Insect. Physiol., **9**: 439-452.
9. **Bursell, E. 1966**. Aspect of the flight metabolism of the tse tse fly (Glossina). Com. Biochem. Physiol., 19: 809-818.
10. **Winteringham, F.P.W. & Harrison, A. 1956**. Study of anti cholinesterase action in insects by a labelled pool technique. Nature. Lond., 178: 81-82.
11. **Corrigan, J.J. and Keams, C.W. 1963**. Amino acids metabolism in DDT poisoned American Cockroaches. J. Insect. Physiol. **9**: 1-12.
12. **Balagun, R.A. 1974**. Some aspect of amino acid metabolism in the tse tse fly during flight activity. Nigerian. J. Ent., **1**: 17-20.
13. **Man Singh, A. 1964.** The free amino acids of the haemolympth of malathion resistant, Baltella germanica L. Indian J. Ent., **26**: 247-251

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