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Impact of plant extracts on total free amino acids alterations in the haemolymph of *Pericallia ricini*

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ABSTRACT
The biochemical changes in the haemolymph of *Pericallia ricini* larvae as a result of treatment with different plant extracts showed that the total protein concentrations were significantly changed. The total amino acids showed quantitative difference in the haemolymph of normal and treated larvae. Analysis of the haemolymph revealed the presence of 17 aminoacids. Asparagine, Glutamine, Arginine and Methionine exhibited quantitative decrease while Alanine, Phenylalanine, Isoleucine, Leucine showed an obvious increase after treatment with the plant extracts. The results showed that the plant extracts are promising alternative to chemical pesticides and can be used as a biopesticide against *P. ricini.*

Keywords: *Pericallia ricini*, Haemolymph, Amino acids

1. Introduction
Pericallia ricini is found all over India and is commonly known as castor hairy caterpillar. It is a serious pest of oil seed plant, castor and cucurbitaceous crops. The female moths lay eggs in large number on the lower surface of leaf. The larvae feed on young and full grown plant leaves and fruits. In heavy infestation only stem and branches are left behind [1]. However, when the larval population is controlled, the crop yield may extend up to 70 to 80 percent [2].
The chemical insecticides are very effective against the target insect pest but brutally eliminate other non-target arthropods in the field [3]. Recently, *H. armigera* is reported to have developed resistance to many commonly used insecticides [4, 5]. Therefore, plant extracts were used cautiously for controlling *P. ricini.* Many plants extracts treated lepidopteran larvae showed a significant reduction in the body protein [6] and the formation of adultoids [7].
In recent years there is revival of interest in amino acid analysis in insects. Amino acids are important constituent of insect body. These amino acids occur as free amino acids having high concentration in insect haemolymph. The high concentration of amino acids is believed to play an important role in osmoregulation [8, 9] energy production for flight and cocoon construction [10]. It is also important for buffering blood to some extent with predominant function of unit for protein synthesis [11]. In the present investigation the effect of the extracts of five different plants namely *Catharanthus roseus, Datura metel, Delonix regia, Eucalyptus globules* and *Pongamia glabra* on free amino acids in the haemolymph of the last instar larva of on *P. ricini* was studied.

2. Materials and Methods
2.1 Insect Culture
The eggs and the freshly emerged I instar larvae of *P. ricini* were collected from the castor plants cultivated in the vicinity of Madurai and kept in the laboratory at room temperature of 29±1 ºC relative humidity throughout the period of study. The larvae were fed with fresh castor leaves and allowed to hatch in to moths. These moths were fed with 10% sucrose solution soaked in a small piece of cotton. Male and female moths were kept in specially designed cages for mating. Castor leaves were introduced for egg laying and the moths were allowed to lay eggs. The egg hatching was completed on the leaf itself and the freshly emerged first instar larvae were collected and separated. These individuals were maintained in the laboratory for the experimental purpose.

2.2 Plants tested
Five plants namely *Catharanthus roseus, Datura metel, Delonix regia, Eucalyptus globules* and *Pongamia glabra* were selected for the present study. Their leaf extracts were used for the present investigation.

2.3 Extraction Method
The plant leaves collected from the local garden were dried at room temperature in shade and then pulverized in a grinder. About 25 g of powdered material was extracted in soxhlet apparatus, using acetone as the solvent for 8 hours at 55 °C. The extract was again redistilled and concentrated on water bath weighted accurately in a monopan balance and then stored in refrigerator.

2.4 Application of plant extract
Different concentrations such as 200 ppm, 400 ppm, 600 ppm, 800 ppm of the plant extract were prepared by dissolving known weight of the concentrated extract in acetone which was further diluted with distilled water for application.

2.5 Administration
Newly moulted third instar larvae of *P. ricini* were introduced in separate containers. They were fed with *R. communis* leaves soaked in different concentrations of plant extracts. The larvae were maintained till pupation.

2.6 Assessing ED $_{50}$ Values
The effective Dosage level to reduce larval growth to 50% of control weights (ED $_{50}$ values) was calculated for each extract. For each instar the initial weight was taken and then they were fed with castor leaves soaked in different concentrations of the extracts. At the end of each larval instar the final weight was taken just few hours before moulting and the difference in weight was recorded. It indicated growth and was compared with weight of the control insects. The dosage required for growth inhibition of 50% control weight is chosen as ED 50 value.

2.7 Estimation of Protein content
The protein content of control and plant extract treated samples was determined spectrophotometrically by Lowry's method using bovine serum albumin (BSA) as standard [12].

2.8 Identification and quantification of amino acids
HPLC analysis of samples was done for Identification and quantification of Amino acids.

3. Results
C. roseus leaf extract treatment resulted in drastic depletion of Protein content (Table 1) and most of the total amino acids as compared to control (Fig 1). Histidine was found to show amounting to 13.19 µg/g in treated fifth instar larva. The aspartic acid, glutamic acid, arginine, showed a reduction in amount. A lesser degree of depletion was noticed for phenylalanine (Table 2, Fig 2).

Table 1: Estimation of protein content in Haemolymph plant extracts treated larvae.

S. No	Treatment	Quantity of protein in sample (g/100 ml)	Decreased Percent of protein over control
1.	*C. roseus*	29.07	45.44
2.	*D. metel*	46.09	13.50
3.	*D. regia*	29.29	45.03
4.	*E. globulus*	33.64	36.87
5.	*P. glabra*	35.63	33.13
6.	Control	53.29	-

Table 2: Free amino acid compositions (µg/g) in the hemolymph of fifth larval instar of *P. ricini* treated with plant extracts

			µg/g of amino acids in *P. ricini* samples				
S. No	Name of the amino acids	Control	*C. roseus* Extract treated sample	*D. metel* Extract treated sample	*D. regia* Extract treated sample	*E. globulus* Extract treated sample	*P. glabra* Extract treated sample
1.	Aspartic Acid	32.09	10.08	14.40	13.94	08.8	14.10
2.	Glutamic acid	34.59	07.33	10.88	12.16	13.20	17.50
3.	Asparagine	-	04.57	02.76	07.07	01.67	-
4.	Serine	04.76	-	-	-	-	02.64
5.	Glutamine	-	-	01.15	00.87	-	-
6.	Histidine	83.17	13.19	31.60	35.36	14.30	-
7.	Glycine	13.88	-	-	13.97	06.20	-
8.	Threonine	-	09.45	06.40	13.54	-	14.44
9.	Arginine	61.57	31.53	50.64	14.96	10.58	09.89
10.	Alanine	04.28	15.12	05.09	03.94	08.49	11.12
11.	Tyrosine	-	-	-	-	02.66	04.96
12.	Methionine	23.69	26.48	17.36	16.10	10.11	14.60
13.	Valine	-	-	-	-	-	03.08
14.	Phenylalanine	7.67	08.16	05.69	15.55	03.49	14.46
15.	Isoleucine	13.83	19.15	11.62	26.20	05.48	-
16.	Leucine	16.96	26.28	12.44	38.42	06.65	-
17.	Ornithine	-	-	-	-	-	326.10
18.	Lysine	-	-	-	-	-	-

Protein content was decreased slightly as compared to other samples when treated with *D. metel* leaf extract (Table 1). A drastic decrease in glutamic acid content was noticed (10.88 µg/g). However, leucine, methionine and alanine content was increased (Table 2, Fig 3)

D. regia leaf extract showed 45 percent decreased protein level which evaluated next to *C. roseus* leaf extract treatment sample. Mount and decrease of amino acids were noticed in the fifth instar larva fed with 800 ppm dosage of *D. regia* leaf extract (Table 1). When the amino acids were taken as a whole, a lesser degree of depletion was noticed for alanine and also higher quantity of depletion was observed in arginine when compared with control (14.96 µg/g). On the other hand, Phenylalanine, Isoleucine, Leucine was increased during this treatment (Table 2, Fig 4).

E. globulus leaf extract showed 36 percent decreased protein level over control. A noticeable difference was achieved in this treatment. Arginine and histidine showed evidence of the maximum reduction (Table 1). It is clearly manifest that the *E. globulus* extract induced a quantitative reduction in amino acids (Table 2, Fig 5).

33 percent decreased protein levels over control were observed in the *P. glabra* treatment. Asparagine, Glutamine, Histidine, Glycine, Isoleucine, Leucine were completely absent. Among them, arginine showed a steep fall in quantity (9.89 µg/g). Valine, Tyrosine, Ornithine was observed but it was absent in control (Table 2, Fig 6).

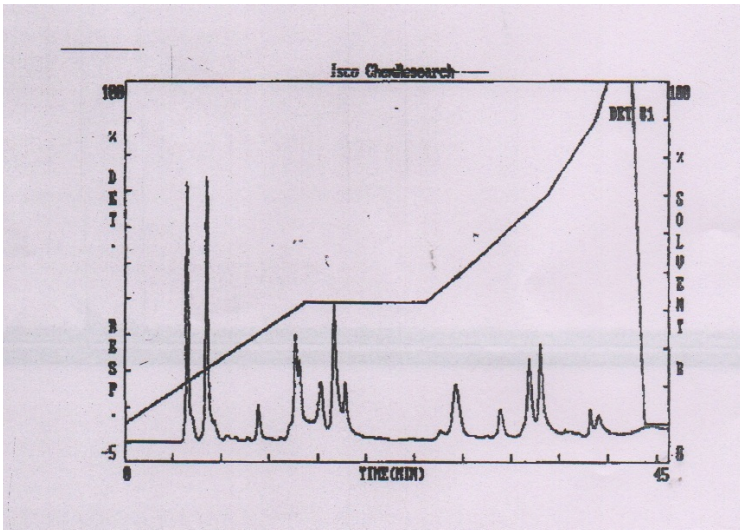

Fig 1: HPLC column profile of amino acids in the untreated fifth instar larvae of *P. ricini*

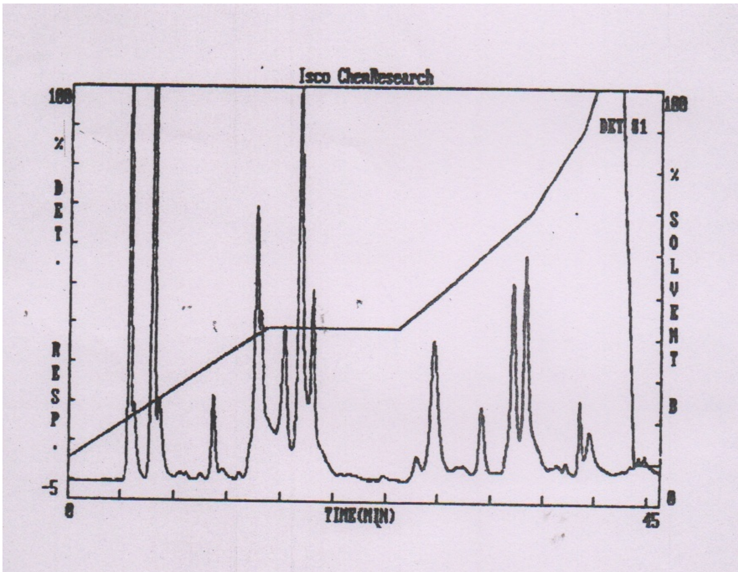

Fig 2: HPLC column profile of amino acids in *C. roseus* treated fifth instar larvae of *P. ricini*

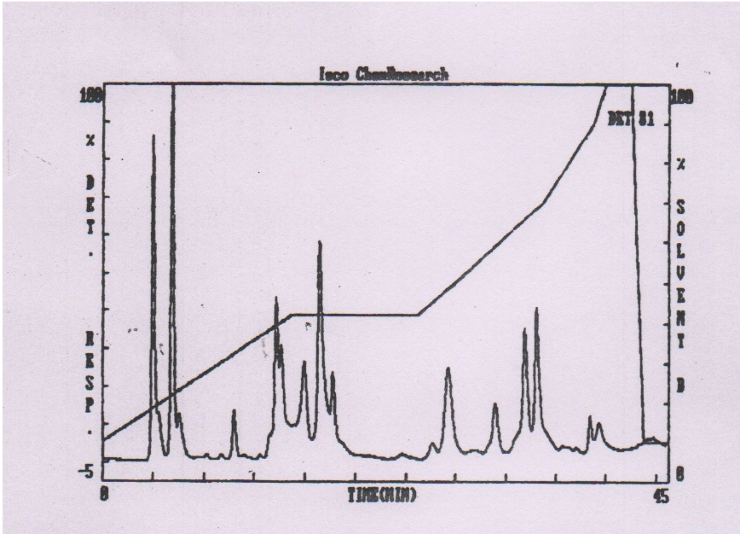

Fig 3: HPLC column profile of amino acids in *D. metel* treated fifth instar larvae of *P. ricini*

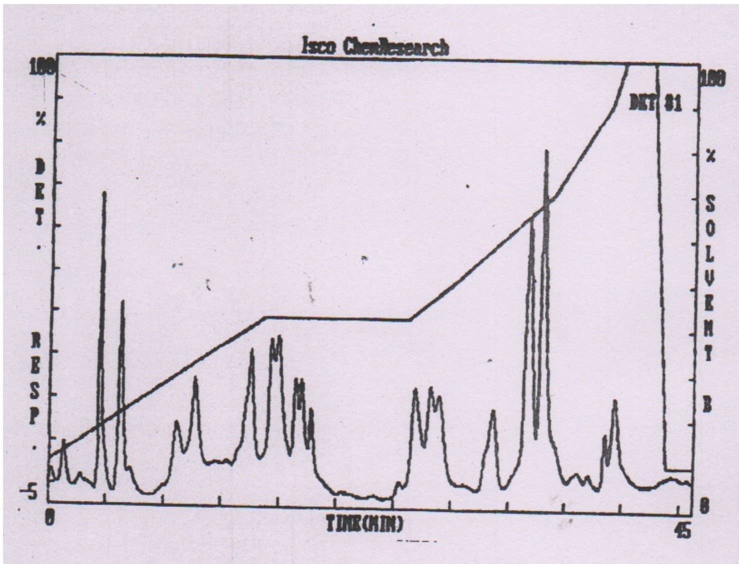

Fig 4: HPLC column profile of amino acids in *D. regia* treated fifth instar larvae of *P. ricini*

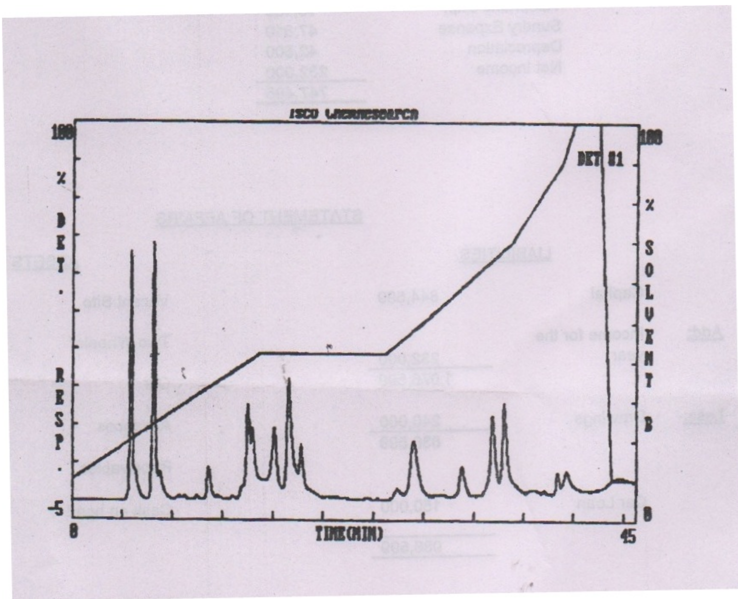

Fig 5: HPLC column profile of amino acids in *E. globules* treated fifth instar larvae of *P. ricini*

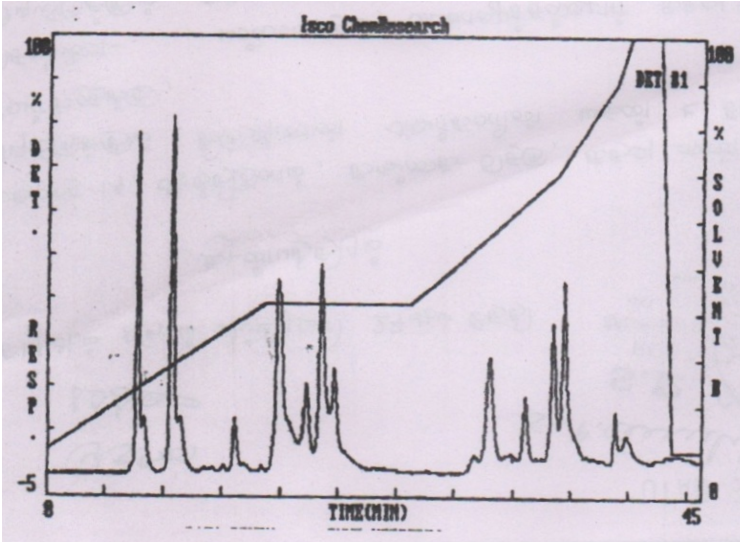

Fig 6: HPLC column profile of amino acids in *P. glabra* treated fifth instar larvae of *P. ricini.*

4. Discussion

Insect blood plasma is characterized by very high levels of free amino acids. The total concentration in plasma is usually more than 6 mg ml-1 in endopterygotes, and lesser in exopterygotes. Insects have largest qualitative and quantitative diversity of amino acids. It performs additional metabolic function in insects apart from protein synthesis. Most of the protein amino acids are present, but their concentrations vary greatly from insects to insects. The concentration of amino acids may change at different stages of the life cycle [13]. The amino acids were usually present in the diet as proteins, and the value of any ingested protein to an insect depends on its amino acids content and the ability of the insect to digest it [14]. Chapman (2002) [15] reported that proteins contain 20 different amino acids of which 10 of these are essential in the diet; absence of any one prevents growth. Although the other 10 amino acids are not essential, but they are necessary for optimal growth.

Marked changes occurred in the *P. ricini*, when it was treated with different plant extracts. The most predominant amino acid observed in *P. ricini* was histidine followed by arginine Chapman demonstrated glutamate as neurotransmitters. Moreover, histidine is an essential amino acid linked to the pathway of purine synthesis, while threonine was considered as one of the end products of succinyl coenzyme A catabolism. Serine is the precursor of glycine and cystine [16]. But Serine was completely lost in the haemolymph of insects treated with plant extracts. Along with the depletion of such amino acids, there was also a drastic decrease in the protein content. Variation in the amino acid content affects directly the protein turnover resulting thereby in retardation of growth and development of the insect [17]. It is evident that the plant extracts induced significant reduction in protein content and amino acids. However, some amino acids were found in plant extract treated sample but it were absent in control.

Certain amino acids showed a decrease in the haemolymph of larvae treated with different plant extracts. Greater decrement of essential amino acids (Asparagine, Glutamine, Arginine and Methionine) was observed in larvae fed with *C. roseus* and *E. globules* plant extracts. This decrease can be attributed to 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. It results that the free amino acid of haemolymph is reduced drastically. Amino acids are reported to have a role in accelerating moulting in insects [18]. Depletion of such amino acids showed a definite impact in physiology, and moulting process producing morphological abnormalities in treated insects. This view is also supported by Pandey *et al*., (1986) [19].

On the other hand, some amino acids (such as Alanine, Phenylalanine, Isoleucine, Leucine) showed an obvious increase in the haemolymph of the larvae after treatment with different plant extracts. An increase in the amino acid content indicates either low transaminase activity [20] or high proteolytic activity of enzymes. Moreover, this increase may be due to low food intake, reduction in protein synthesis or higher mobilization of protein. Same results were observed in tasar silkworm [21]. Fatma (2009) [22] observed that both essential and non-essential amino acids were higher in the haemolymph of the last larval instars of *S. littoralis* reared on castor oil leaves.

In conclusion, present results revealed that all plant extracts affected the concentration of amino acids in haemolymph. Especially were mainly decreased the essential amino acids. A noticeable decrement was detected in the amino acid level of larvae treated with *C. roseus* and *E. globulu* extracts. The results showed that the plant extracts are promising alternative to chemical pesticides and can be used as a biopesticide against *P. ricini*.

5. References

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