Haemolymph amino acids alterations in pyridalyl treated desert locust, *Schistocerca gregaria* in relation to age

Zahia, K. Mostafa[1], Eman, M. Rashad[2] and Salam, S. Teleb[2]

[1]Deparment of Entomology, Faculty of Science, Ain Shams University, Egypt.
[2]Department of Zoology, Faculty of Science, Zagazig University, Egypt.
salamteleb@gmail.com

Abstract: Studies were undertaken to investigate haemolymph amino acids alterations by amino acid analyzer in the 5th nymphal instar and adult *Schistocerca gregaria* in preoviposition and oviposition periods before and after pyridalyl treatment. The concentrations of these amino acids in both control and treated haemolymph samples of the 5th nymphal instars showed a wide range of variations. The pattern of amino acids showed an obvious increase in the haemolymph of the nymph after treatment with pyridalyl. Methionine and tryptophan were completely lost in untreated and treated nymphs. The pattern of some amino acids in 1-day old adult exhibited obvious increase in the haemolymph after treatment with pyridalyl, while proline, threonine, glycine and arginine showed a decrease in their concentrations after treatment. On the other hand, most of the amino acids exhibited an obvious decrease in the haemolymph of the 10 days old adult females after treatment with pyridalyl. Also, the amino acids in the haemolymph of the 18 days old adult females exhibited an obvious decrease after treatment with pyridalyl except threonine, serine, phenylalanine, glutamic acid and alanine.

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1. Introduction

The haemolymph, in most insects, is a greenish yellowish fluid containing a large number of cells or haemocytes and various organic and inorganic constituents. The insect haemolymph serves as a bathing medium for various tissues and organs as they lack an epithelial lining of a true coelom. Therefore, the haemolymph forms the meeting place of both the raw materials required and the products of various physiological activities of the insect body. Since the haemolymph is not directly connected with the external environment any change in it can be taken as a measure of the physiological state of the internal environment of the intact animal. Analysis of the haemolymph, therefore, may provide one of the most reliable data which can be used as an index of the physiological activity (Buck, 1953; Wyatt, 1961). The insect haemolymph is noted for its high titer of free amino acids (Chen, 1962). The most important functions of amino acids, the building blocks of the proteins that are derived from the insect diet, include the synthesis of structural proteins of the integument and the synthesis of hormones and enzymes that participate in the synthesis nucleic acids (Klowden, 2007). Amino acids are also required by insects for transport and storage, and as receptor molecules. In addition, some amino acids are involved in morphogenesis (Chapman, 2002). The pattern of haemolymph free amino acids can be modified by development, oogenesis, feeding, cuticular tanning, silk production, or flight activity (Blum, 1985). Therefore, the present work aims to study the haemolymph amino acids alterations in the 5th nymphal instar and adult of *Schistocerca gregaria* in preoviposition and oviposition periods before and after pyridalyl treatments.

2. Material and Methods

Insects:

The colony of *Schistocerca gregaria (*S. *gregaria)* was maintained from Anti Locust Research Center Dokki –Giza, Egypt. The insects were maintained in the laboratory under crowded conditions at 32±2ºC and 65-80% RH and reared according to Hunter-Jones (1961) and Hassanein (1965).

Administration of the insecticide:

Groups of 50 hoppers of 1- day old 4th nymphal instars were treated with 500 p.p.m. of pyridalyl® which is a novel insecticide that has a phenoxy-pyridaloxy derivative structure (S-1812; 2, 6-dichloro-4-(3, 3-dichloroallyloxy) phenyl 3- (5-(trifluoromethyl)-2-pyridyloxyl propyl ether). The concentration was chosen depending on some preliminary trials carried out on the present insect species. Feeding technique was applied using fresh clean lettuce (*Lactuca sativa)* after dipping for 3 minutes. Feeding on treated food plant was allowed for 24 hrs. Controls were fed for 24 hrs on lettuce

leaves dipped in tap water for 3 minutes and dried in open air. After feeding for 24 hrs, the treated nymphs were daily fed on untreated fresh lettuce and kept under the same laboratory conditions.

Sample preparation for the assay of total amino acids in the haemolymph:

Haemolymph from the 5-days old 5th nymphal instars, 1-day old adult females, 10-days old adult females and 18- days old adult females was drawn by a fine capillaries through a puncture in the neck membrane, pooled into Eppendorff tubes, stored in ice, containing few milligrams of phenylthiourea to prevent tanning or darkening and then diluted five times with saline solution 0.7%. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until used.

Analysis of amino acids in the haemolymph with amino acid analyzer:

The haemolymph from the 5-days old 5th nymphal instars, 1-day old adult females, 10-days old adult females and 18-days old adult females of *S. gregaria* treated and untreated were collected and kept at -20˚C. Amino acid analysis was carried out by amino acid analyzer. The samples were hydrolyzed in sealed, evacuated ampoules in an oven at 110˚C for 16 hrs. The extraction and analysis were performed in Cairo University Research Park (CURP) at Amino Acids Lab. according to the method described by Rashad and Abdel Zaher (2008).

3. Results

1. Total amino acids in the haemolymph of the 5th nymphal instar:

The total amino acid profile of haemolymph of *S. gregaria* nymph was analyzed using the Amino Acid Analyzer and the results are presented in figure (1). The analysis detected the amino acids in control and treated nymphs. Comparison of the concentrations of these amino acids in both control and treated haemolymph samples showed a wide range of variations. It appears that the pattern of amino acids showed an obvious increase in the haemolymph of the nymph after treatment with pyridalyl. The relative abundance of total amino acids in the haemolymph of the control nymph was as follows: glycine > glutamic acid > aspartic acid > leucine > alanine > valine > lysine > serine > tyrosine > histidine > threonine > arginine > isoleucine > phenylalanine. The concentration of proline was much lower.

The relative abundance of total amino acid profile in pyridalyl treated nymph was as follows: glutamic acid > glycine > aspartic acid > leucine > valine > alanine > tyrosine > lysine > histidine > isoleucine > arginine > phenylalanine > threonine > serine. Proline showed no obvious quantitative changes in the haemolymph of the nymph after treatment with pyridalyl. Methionine and tryptophan were completely lost in untreated and treated nymphs. The difference between untreated and treated nymphs showed a significant increase in individual free amino acids except proline.

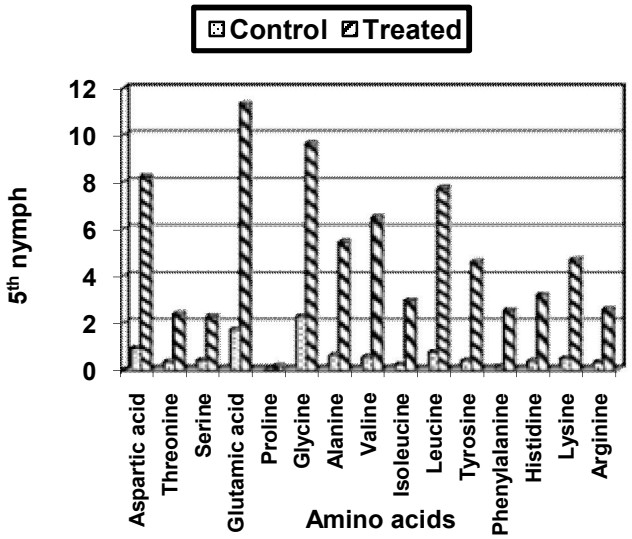

Figure (1): Effect of pyridalyl on the haemolymph amino acid (g/100ml) in the control and treated nymphs of *S. gregaria*.

Total amino acids in the haemolymph of the adult *S. gregaria* in relation to age:

2.1. Total amino acids of 1day old adult:

The data presented in figure (2) shows that the 15 amino acids were also detected in control and treated 1day old adult of *S. gregaria.* It appears that the pattern of some amino acids exhibited an obvious decrease in the haemolymph of the adult after treatment with pyridalyl; these amino acids were proline, glycine and arginine. On the other hand, some amino acids showed an obvious increase in the haemolymph of the adult after treatment with pyridalyl, e.g. aspartic acid, leucine, alanine, valine, lysine, histidine, phenylalanine, tyrosine, isoleucine, serine, and glutamic acid. Threonine showed no obvious quantitative changes in the haemolymph of the adult after treatment with pyridalyl.

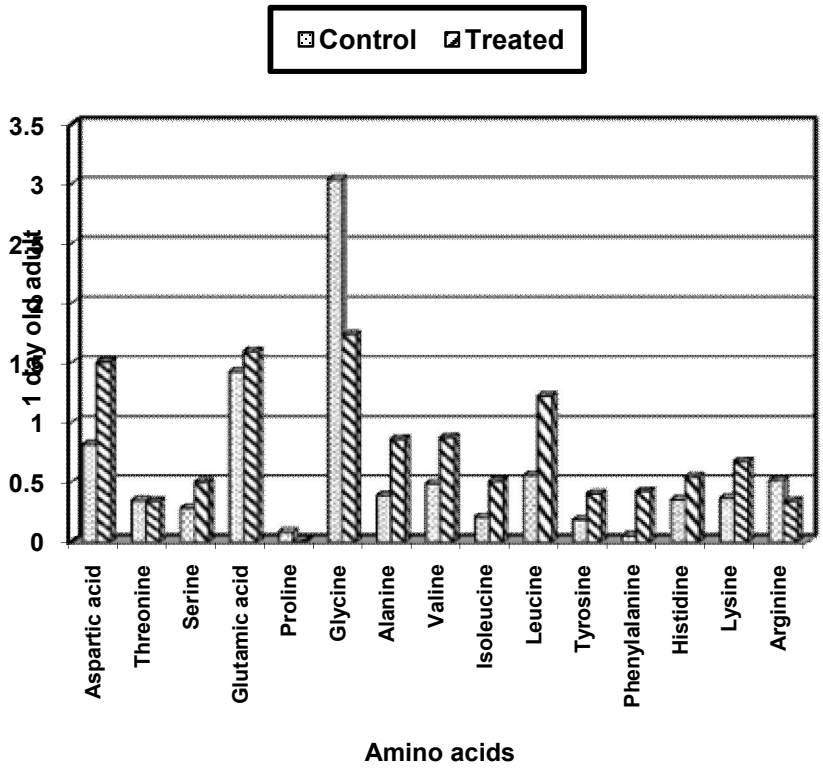

Figure (2):Effect of pyridalyl on the haemolymph amino acid (g/100ml) in the control and treated 1day old adult of *S. gregaria.*

2.2. Total amino acids of 10 days old adult:

The data presented in figure (3) shows that most of the amino acids exhibited an obvious decrease in the haemolymph of the 10 days old adult females after treatment with pyridalyl; these amino acids were glycine, glutamic acid, aspartic acid, leucine, valine, histidine, lysine, tyrosine, isoleucine, serine and threonine. Alanine, phenylalanine and arginine showed no obvious quantitative changes in the haemolymph of the adult after treatment with pyridalyl. Proline exhibited some increasing in treated 10 days old adult females.

2.3. Total amino acids of 18 days old adult:

Data presented in figure (4) shows that all the amino acids exhibited an obvious decrease in the haemolymph of the 18 days old adult females after treatment with pyridalyl except threonine, serine, phenylalanine, glutamic acid and alanine. Threonine, serine and phenylalanine showed an obvious increase after treatment while glutamic acid and alanine were completely lost.

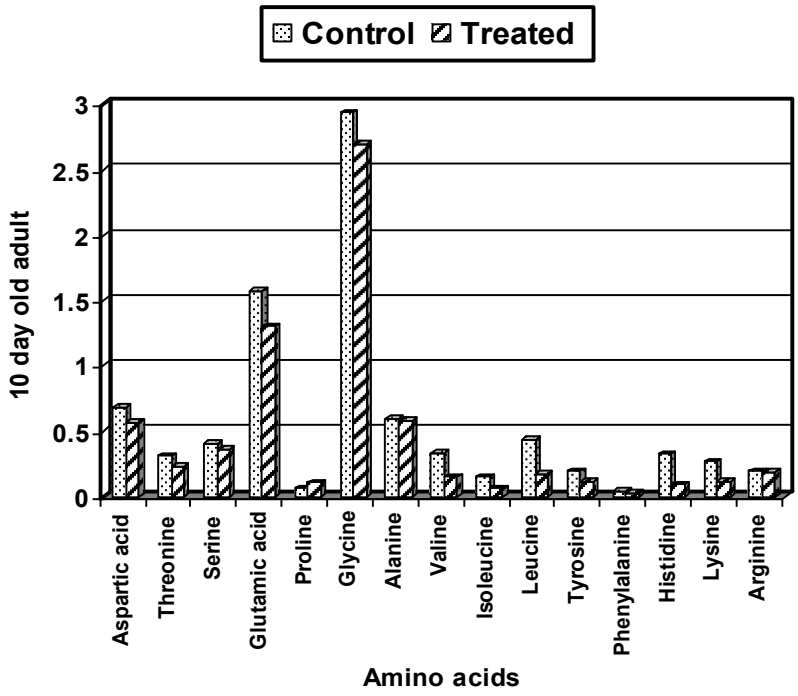

Figure (3): Effect of pyridalyl on the haemolymph amino acid (g/100ml) in the control and treated 10 days old adult of *Schistocerca gregaria.*

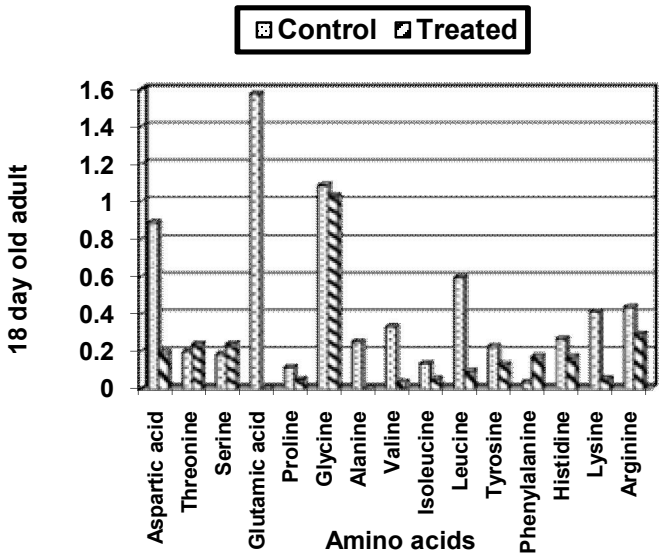

Figure (4):Effect of pyridalyl on the haemolymph amino acid (g/100ml) in the control and treated 18 days old adult of *S. gregaria.*

Comparison between the pattern of haemolymph amino acids in the chosen experimental stages showed that the concentration of glycine was the highest value in the normal 1-day old adult (3.0399 g/100ml) followed by a decrease in 10-days old adult (2.937 g/100ml) then 5th nymphal instar and 18-days old adult as shown in table (1). Glutamic acid showed no obvious change between the chosen stages except it's highly increase in the 5th nymphal instar (11.339 g/100ml) and it's lost in 18-days old adult after treatment with pyridalyl. Aspartic acid showed the lowest value in the normal 1-day old

adult (0.815 g/100ml) than the 5th nymphal instar (0.931 g/100ml). Its value was decreased in normal 10-days old adult (0.682 g/100ml) then was increased in18-days old adult (0.889 g/100ml). After treatment with pyridalyl, aspartic acid was increased in both 5th nymphal instar (8.260 g/100ml) and 1-day old adult (1.508 g/100ml) and decreased in 10-days old adult (0.563 g/100ml) followed by in18-days old adult (0.196 g/100ml). The other amino acids were fluctuated as shown in table (1) between increasing and decreasing after treatment with pyridalyl.

Table (1): Total amino acid profile of haemolymph in pyridalyl control and treated 5th nymphal instar, 1-day old adult, 10-days old adult and 18-days old adult of *Schistocerca gregaria*.

No.	Amino acids	5th nymph control	5th nymph treated	1day old adult control	1day old adult treated	10days old Adult control	10days old adult treated	18 days old adult control	18days old adult treated
1	Aspartic acid	0.931	8.260	0.815	1.508	0.682	0.563	0.889	0.196
2	Threonine	0.362	2.401	0.346	0.339	0.314	0.232	0.199	0.241
3	Serine	0.423	2.291	0.282	0.498	0.403	0.358	0.186	0.243
4	Glutamic acid	1.728	11.339	1.427	1.595	1.577	1.303	1.577	-
5	Proline	0.038	0.177	0.082	0.01	0.064	0.108	0.117	0.053
6	Glycine	2.270	9.652	3.039	1.737	2.937	2.697	1.089	1.033
7	Alanine	0.630	5.454	0.389	0.860	0.601	0.584	0.253	-
8	Valine	0.543	6.510	0.488	0.876	0.338	0.150	0.335	0.039
9	Isoleucine	0.255	2.980	0.206	0.508	0.151	0.065	0.138	0.055
10	Leucine	0.752	7.756	0.558	1.223	0.439	0.176	0.596	0.098
11	Tyrosine	0.390	4.609	0.185	0.403	0.197	0.117	0.229	0.136
12	Phenylalanine	0.116	2.552	0.052	0.423	0.040	0.032	0.037	0.175
13	Histidine	0.377	3.196	0.354	0.545	0.325	0.091	0.268	0.173
14	Lysine	0.504	4.704	0.366	0.676	0.267	0.120	0.415	0.058
15	Arginine	0.327	2.592	0.514	0.341	0.193	0.190	0.439	0.298

4. Discussion

Analysis of haemolymph of 5th nymphal instar, 1day old adult, 10 days old adult and 18 days old adult of *S. gregaria* revealed the presence of 15 amino acids, the concentration of which in normal and treated samples showed a wide range of variation. All amino acids showed an obvious increase in the haemolymph of the 5th nymphal instar after treatment with pyridalyl. The disappearance of methionine as an essential amino acid may be due to its consumption in the methylating intermediary pathways (Boctor, 1978). The fact that arginine increased after treatment was related to its proposed function in the metabolic process in the healthy nymph or to its accumulation as guanidine derivative (Pant & Agrawal, 1964). In the present study, increasing the amino acids in the treated 5th nymphal instar of

S. gregaria may be due to the increase representing the compensation for the loss of chlorides and other inorganic ions which fall during starvation when osmotic pressure falls (Wigglesworth, 1972) as well as it might be interpreted as being due to the inhibition of protein formation (Bakr *et al.*, 1991). They added that the variations in quantity of the free amino acids content may interfere in the transcript of DNA during the process of protein synthesis which may be the reason of the observed abnormalities.

The high levels of amino acids in treated insects may cause a great disturbance in biochemical activities as protein metabolism as well as in the regulation of osmotic pressure (Enan, 2004). Blum (1985) reported that the use of amino acids for osmoregulation may at times be passive process in which all amino acids increase or decrease proportionally, but the change in specific amino acid titers in some insects indicates active regulated processes.

The remarkable increase of glycine, glutamic acids and aspartic acid in the chosen stages indicated that these are the predominant amino acids of *S. gregaria.* Blum (1985) stated that glutamate, proline and glycine are the predominant amino acids in most insect haemolymph. In the present study, proline was present in low concentration, this may be due to it does not used as a source of energy. Chapman (2002) explained that locusts depend mainly on fat as a fuels providing energy for flight but use carbohydrates during short flight and the early stages of sustained flight. Fat is more suitable than carbohydrates as a reserve for insects that make long flight because it

produces twice as much energy per unit weight. The lower concentration of proline and highest concentration of glutamic acid and alanine may be due to the conversion of proline to glutamate as mentioned by Klowden (2007). He stated that the proline is converted to glutamate by the enzyme proline dehydrogenase, which is activated by high level of pyruvate, the transamination of glutamate with pyruvate produce the alanine. Ray (1964) stated that the loss of free proline was balanced by an increase in free alanine and glutamine.

On the other hand, the massive production of certain kinds of proteins alters the haemolymph amino acids pattern (Blum, 1985). In species like locusts and cockroaches, with a discontinuous oviposition rate, there is a cyclic rise and fall of haemolymph vitellogenins concentration corresponding to the extent of ovarial maturation of eggs (Blum, 1985). These statements of Blum explain the increasing of glycine in 1-day old adult followed by decreasing in10-days old adult then increased again at 18-days old adult. The 1-day old adult represents the beginning of the first cycle followed by the end at 10-days old adult. The beginning of the second cycle was represented in 18-days old adult.

Collett (1976) recorded that glycine was active in the synthesis of several proteins. Although the results from this study did not conclusively show that pyridalyl interferes with cell function, it would be worthwhile to investigate its effect on cell function in a future study.

Corresponding author
Salam, S. Teleb
Department of Zoology, Faculty of Science, Zagazig University, Egypt.
salamteleb@gmail.com

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