Biochemical Effects of Cyromazine on *Culex Pipiens* Larvae (Diptera: Culicidae).

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Abstract: The current work was carried out to evaluate the biochemical effects of the insect growth regulator (cyromazine) as chitin synthesis inhibitor (CSI) against, 4th larval instar of *Culex pipiens* , treated as 2nd larval instar with 0.1 and 1 ppm to determine the effect of this CSI on glucose , protein and amino acids content as well as the phosphatase , transaminase and phenoloxidase enzymes. The obtained results indicated that the tested IGR significantly decreased the glucose, the amino acids, the alkaline phosphatase (ALP) and the phenoloxidase in the homogenate of 4th larval instar of *C. pipiens* ,while protein content and the activity of acid phosphatase increased . Also, the tested IGR elicited inhibitory effect on alanine amino transferase (AST) and aspartate aminotransferase (ALT) at 0.1 ppm, while induced significant stimulatory effect at 1 ppm.
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Key words: Cyromazine, *Culex Pipiens* Larvae, ALT, AST, ALP, Amino acids and Phenoloxidase

1. Introduction

Mosquitoes are vectors of serious human diseases all over the world Mosquito-borne pathogens infect more than 600 million people annually (**Kolberg, 1994**). Many species can act as vectors of *Wuchereria bancrofti,* Nile virus and Japanese encephalitis. In urban areas, the vector is commonly *C. pipiens* but in rural areas, it is often an anopheline mosquito and occasionally a species of *Mansonia. C. pipiens* considered as the main vectors of human diseases such as the Rift valley fever virus (**Darwish and Hoogstraal, 1981**), viral encephalitis and bird malaria (**Kettle, 1995**); elephantiasis (**Gad *et al.,* 1996**), and Western Nile virus (**Pelah *et al.,* 2002**).

Many insecticides have been used directly or indirectly in the control of *C. pipiens*. Throughout the world, mosquitoes have developed resistance to these insecticides. Furthermore, resistance has been recorded for most conventional insecticides.

As a consequence, it provides impetus to study new alternatives and more ecologically acceptable methods of insect control. The insect growth regulators (IGR's) have been used in a variety of practical applications and were described as agent that elicit their primary action on insect metabolism, ultimately interfering and disrupting the process of growth, development and metamorphosis of the target insects, particularly when applied during the sensitive period of insect development (**Ishaaya and Horowitz, 1997**).

The biochemical effect of IGR's on the carbohydrate (glucose), protein, the amino acids, the phosphatase, transaminase and phenoloxidase enzymes on insects was studied by several authors but little or no work was done on *C. pipiens*.

Cyromazine a triazine derivative is an insect growth regulator that is used in veterinary medicine for the protection of animals against insects. This application concerns the external use of cyromazine for the prevention of blowfly strike (*Lucilia sericata*) on sheep and lamps. Cyromazine is an insecticide that interferes with the first dipteran larval moult and possibly with metamorphosis. Larvae and pupae undergo typical morphological transformations before they die (**Emea, 2001**).

Cyromazine has been used since 1984 as a feed - through - although no study has been carried out to assess the biochemical effect of cyromazine on *C. pipiens.*

The present study was carried out to evaluate the impacts of cyromazine as chitin synthesis inhibitor on the glucose, protein and the amino acids content as well as the phosphatase, transaminase and phenoloxidase enzymes on *C.pipiens* larvae.

2.Material and Methods

Insect culture and bioassays:-

The mosquito, *C. pipiens* used in the present study was obtained from susceptible reared strain of Research Institute of Madical Entomology, Dokki, Egypt. The colony was maintained under laboratory conditions of 27±2C^0 and 75± 5% R.H. according to El-bokl and Moawad (1996). The 2nd instar larvae were collected for broassay tests. The chitin synthesis inhibitor (cyromazine) was dissolved in the water to make the different concentrations (0.001, 0.01, 0.1, 1 and, 0 ppm).

In each test, 25 larvae were put in a plastic cup with 100 tap water and then treated with cyromzine . Each test was replicated four times. Control

experiments were performed using water only A mixture of ground dried bread and Brewer's yeast pellets (3:1) were added daily as food for the larvae. After 72 hours from treatment with 0.01 and 0.1 ppm, some larvae were separated (used) to biochemical studies .

A- The compound used : (Insecticide)

IGR, cyromazine (Trigard) is (N- cyclopropyl 1, 3, 5- triazine - 2, 4, 6, - triamine).

B- Biochemical studies

C. pipiens larvae were collected after 3 days of treatment and homogenized in distilled water. Homogenates were centrifuged at 5000 rpm for 15 min. The supernantant was placed in tubes for analysis.

1- Estimation of total glucose content :-

Total glucose content in larval homogenate was estimated according to Trinder (1969).

2- Estimation of total protein content:-

Total protein content in larval homogenate was estimated according to Bradford (1976).

3- Estimation of amino acids:-

The samples were hydrolyzed according to (Ibraim and El-Eraqy, 1996) and analyzed by Lc 3000 Amino Acid Analyzer.

4- Estimation of acid and alkaline phosphatases and phenoloxidase:-

In larval homogenates, they were estimated according to Powel and Smith (1954).

5- Estimation of transaminase enzymes:-

Aspartate aminotransferase (AST/GOT) and alanine aminotrasferase (ALT/ GPT) were determined according to the method of Reitman and Frankle (1957).

D- Statistics:-

All experiments contained 3-4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using costat statistical software (cohort-software-Bekeley). When the ANOVA statistics were significant (p<0.01), means were compared by the Duncan's multiple range test.

3. Results and Discussion

1- Effect of cyromazine on the amino acids :-

The chemical analysis of larval body using the Amino Acid Analyzer indicated that the body of *C. pipiens* larvae contained 15 different free amino acids (Table 1 and figs.1-3).

Results presented in table (1) showed the most abundant amino acids in the body of untreated and treated larvae of *C. pipiens* were glutamic, aspartic and proline, this was followed by alanine, lycine, histidine valine, arginine, serine, however threonine, tyrosine, isoleucine and glycine were the lowest.

It is clear from results obtained in table (1); the total concentration of all 15 amino acids was greatly decreased by treatment of 2nd larval instar of *C. pipiens* larvae with cyromazine as compared with untreated larvae. The total concentration of all 15 amino acids tesed was 1680.40, 1125.76 and 2999.40 µg/ml at 0.1, 1 ppm and control, respectively.

The data in Table (1) shows that glutamic acid was the highest value; its concentration was 274.72 and 230.32 µg/ml at 0.1 and 1 ppm, respectively. The glutamic acid value with the control was 465.40 µg/ml. The leucine value was 165.48, 121.56 and 311.84 µg/ml at 0.1, 1 ppm and control respectively. The aspartic acid value was 183.52, 91.20 and 302.12 µg/ml at 0.1, 1 ppm and control, respectively. The proline value was 156.04, 71.08 and 303.24 µg/ml at 0.1, 1 ppm and control, respectively.

It is clear from the data in table (1), the tested compound decreased the values of free amino acids and the total concentration of amino acids in the larval body of *C. pipiens*. This effect was dose dependant. These result agree with those obtained by Bakr (1986) who stated that dimilin , BAY SIR 8514 and altosid decreased some amino acids of *Musca domestica* larvae. Valine decreased by dimilin and altosid , it increased by BAY SIR 8514 .On the other hand , dimilin, BAY SIR 8514 and altosid increased the values of the majority of the free amino acids.

Data presented in Table (1) show the percent reduction of all tested amino acids. This reduction was dose dependent. The percent reduction at 1 ppm was 76.55, 73.05, 69.81, 67.89, 67.60, 67.40, 67.21, 66.94, 62.10, 61.01, 58.82, 58.66, 55.85, 50.51, and 35.19% with proline, arginine, aspartic, serine, threonine, valine, histidine, isoleucine, glycine, leucine, lycine, phenyalanine, alanine, glutamic and tyrosine, respectively.

According to Hackman (1953), the cuticular proteins have a high content of proline and tyrosine. Tyrosine as the precursor for the formation of polyphenols and quinones necessary for the formation of the darkening and hardening of the larval cuticle which gives rise to the pupation the accumulation of these two amino acids suggests therefore the preparation of the larva for the synthesis of cuticular proteins and the associated tanning.

Several experiments indicated that the free amino acids play an important role in detoxication. In *Bombyx,* glycine is found to conjugate with benzoic acid to form hippuric acid , a detoxication mechanism similar to that in higher animals . The site of hippuricase which regenerates glycien from hippurate has been detected in both fat body and silk gland . Also , hisidine like glycine serves as a detoxicating agent in insects (Shyamala, 1964).

Proline is known to be a possible energy

reserve since it is a derivative of glutamic acid and could enter the citric cycle after deamination to α-ketoglutaric acid (Brusell, 1963). The varying concentration of proline at the different dose levels is probably due to the varying rates of utilizing the amino acids as a source of energy in repair mechanism.

Chen (1974) reported that alanine is a very active transaminase and plays an important role in glucose production from pyruvic acid through transamination. The glutamic alanine transaminase system serves as the main pathway in both the deamination of glutamic acid to ketoglutaric acid and the conversion of pyruvic acid to alanine Krap (1979) reported that there are 20 different amino acids commonly in dipeptides and polypeptide chain proteins. Proteins are composed either wholly of amino acids or of amino acids bound together with some types of molecules. There are miscellaneous functions that require specific proteins. Also, proteins provide structure support both within the cell and in the extracellular space. Therefore the effect and function of the used materials on protein via presence, increase or/ and reduction of these amino acids.

Amino acids are required for the production of structural proteins and enzymes (they are present in the diet as proteins). Proteins or amino acids are always essential in the diet. Although some 20 amino acids are needed for protein production, only 10 are essential in the diet, the others can be synthesised from these ten as in other animals. Insects cannot synthesize certain amino acids and many other organic compounds they need, but obtain them by easting other living or dead organisms or green plants. The ten essential amino acids are arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine. In gernal the absence of any one of these essential acids prevents growth. Athough other amino acids are not essential, they are necessary for optimal growth Glutamic and aspartic acids are necessary in addition to the essential amino acids for good growth (Chapman, 1988).

Abou EL- Ela *et al.* (1993) showed that treatment of *Synthesiomyia nudiseta* larvae with dimilin, BAY SIR and altosid induced some variations in the amino acids of the resulting pupae. Zeenath and Nair (1994) concluded that when the sixth instar larvae of *Spodoptera mauritia* were treated with juveinle hormone anologue hydroprene, the total amino acid concentration increased.

Abdel Hafez *et al.* (1988) stated that when *S.littoralis* larvae treated with diflubenzuron and triflumuron, the level of free amion acid reduced.

Ahmed and Mostafa (1989) reported that, the free amino acids of *S. littoralis* larvae reduced with treatment of triflumuron and chlorfluazuron. Besides, glutamic acid in chlorfluazuron treated larvae and tryptophan in triflumuron treated larvae were highly decreased.

Bakr *et al.* (1991) mentioned that the total pool of free amino acids in the larvae and pupae of *M. domestica* was increased by treatment with dimilin, BAY SIR 8514. The increase of free amino acids in the treated stages might be interpreted as being due to the inhibition of protein formation.

2-**Effect of cyromazine on the total protein content:-**

The data obtained (Table 2) shows that cyromazine significantly decreased the total protein content in the homogenate of 4th instar larvae of *C.pipiens* treated as 2nd larval instar. The total protein was 463± 17.58, 459.30±0.09 mg/g. b.wt at 0.1 and 1 ppm , respectively, while, it was 387± 14.14 mg/g.b.wt in the control group. Similar increase in the protein content of *M. domestica* with BAY SIR and altosid was reported by Bakr (1986). The protein content was increased by pyriprxylen and chlorfluazuron in other insect species by (El-Sokkary, 2003) against *Schistocerca gregaria* and by Farag (2001) and Abdel Aal (2002) against *S. littoralis*.

On the contary, the protein content was unaffected by diflubenzuron and triflumuron (El-Kordy, 1985). While, the protein content was decreased by pyriproxyfen (El- Bermawy, 1994) and by methoxyfenozide (Assar and Abo-Shaeshae, 2004), match and cosult (Assar *et al.,* 2010) against *M. domestica.*

El-Bermawy (1994) verified that treatment of *M. domestica* larvae with variable level of IKI, BAY SIR and sumilarv resulted in a reduction in the total protein content of 3rd instar larvae of *M. domestica* , while an increase in total protein content of 1st and 2nd larvae was recorded. The author attributed this reduction to the inhibitory role of the tested IGR's on tissue protein synthesis, whereas , the high levels of total protein in tissues of 1st and 2nd larval instars may be referred either to a special stimulatory effect of the tested IGR's or unaffected protein synthesis.

Guneidy *et al.* (2011) stated that the chitin synthesis inhibitor (lufenuron) decreased the total protein in *M. domestica* eggs gradually reaching their minimum level (1.17 mg proteins /10 mg eggs) at 7 hrs postoviposition (late embryogenesis).

Proteins are essential constituents of the general animal cells and in the maintenance of different activities. Because protein is essential to chitin synthesis, the depletion of these metabolic macromolecules indicates that chitin production must be inhibited. In addition, proteins are essential for energy production. The insect body contains thousands of different types of proteins, each with a very specific purpose. A protein may be merely structural giving form and strength to the exoskeleton or binding cells together into biochemical reaction, the storage and transport of a nutrient or waste product of the

movement of a specific molecule across cell membranes. Most insecticides currently in use act on target proteins involved in nervous system signaling (neuroactive agents), cellular respiration (respiration disruptors), or growth and development (Insect growth regulators). Some target proteins contain more than target site to which insect control products bind to cause their detrimental effects. The effect of binding on the target protein (inhibition, activation, etc.) and how this effect leads to symptoms known as the mode of action. Mode of action of an insect control product is important because it helps determine safety, speed of action and resistance (Salgado, 1997).

3- Effect of cyromazine on the total glucose content:-

Results given in Table (2) indicated that cyromazine decreased the total glucose content of 4th larval instar of *C. pipiens* treated as 2nd larval instar. The increasing effect was dose dependant. The glucose content was 70.70 ± 2.36 and 68.90± 2.69 mg/ g. b. wt. at 0.1 and 1 ppm, respectively. Whereas, the glucose content was 79.06± 3.08 mg/g b.wt in control gruop. The reduction in glucose content in the present study is similar to the results reported by El-Kordy (1985) using triflumaron and diflubenzuron and Assar *et al.* (2010) using applaud against *M. domestica* larvae. Pyriproxyfen induced reduction in glucose content of mosquitoes (Ranjit and Dash, 1994); on *S. littoralis* (Abdel-Aal, 2002) and on *Agrotis ipsilon* (El-Sheikh, 2002).

JHA decreased the total glucose or (carbohydrate) content in Chrysocoris stollii (Saha *et al.*, 1986), and diflubenzuron, pyriproxyfen and flufenoxuron in *S. littoralis* (Farag, 2001 and Abdel - Aal , 2002).

4- Effect of cyromazine on phosphatase enzymes:-

Results presented in Table (3) showed the activity of acid phosphatase (Acp) of 4th instar larvae of *C. pipiens* treated as 2nd instar larvae with 0.1 and 1 ppm of cyromazine . The tested IGR significantly increased the activity of acid phosphatase. The activity of acid phoshatase was 964.33 and 1102.66 µ× 10^3 /g. b.wt at 0.1 and 1 ppm respectively as compared to 460. µ×10^3/g.b.wt in the control group. Similar increase in Acp activity was reproted by different IGR'S such as JHA and ecdysone on *Chrysocoris stollii* (Saha *et al.,* 1986) ; pyriproxyfen on *C. pipiens* (El-Bassal , 1993) ; pyriproxyfen on *Pectinophora gossypiella* and *Earias insulana* (Anan *et al.*, 1993) ; hexaflumuron on *S. littoralis* (Sokar, 1995) ; Chlorfluazuron, flufenoxuron and pyriproxyfen on *S. littoralis* (Abdel- Aal, 2002) ; pyriproxyfen on *A. ipsilion* (El-sheikh, 2002) and applaud, consult, match, mimic and admiral on *M. domestica* larvae (Assar *et al.*, 2010).

On the other hand , there are IGR'S decrease the activity of Acp in other insect species, such as JHA (Sokar, 1995) against *S. littoralis* ; BAY SIR 8514 (El-Bermawy, 1994) and mimic (Assar *et al.*, 2010) on *M. domestica*.

The tested compound induced a significant decrease in the activity of alkaline phosphatase (ALP) as compared to the control (Table 3). The activity of ALP was 12-66 and 10.76 µ/g. b.wt at 0.1 and 1 ppm, respectively as compared to 15.61 µ/g. b.wt in the control group. These results agree with those obtained by El-Sheikh (2002) using pyriproxyfen against *A. ipsilon.*, while ALP activity was not affected by pyriproxyfen against *C. pipiens* (EL- Bassal, 1993) .

Acid and alkaline phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and kanehisa, 1984). Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. It is known that acid phosphatase hydrolyzes a variety of or the phosphorylation reactions (Hollander, 1971). Ecdysone is responsible for increase in the number of lysosomes (Radford and Misch, 1971) and of the activity of acid phosphatase (Van-pelf verkuil, 1979). This indicates that the increased activity of acid phosphatase in the present study may be due to increased number of lysosomes.

Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes during development is reflected in increase or decrease in acid - soluble phosphorus content.

5- Effect of cyromazie on transaminase enzymes:-

Results given in Table (3) clarified the effect of cyromazine on the activity of aspartate aminotransferase (AST or GOT) and alanine aminotransferase (ALT or GPT) of 4th instar larvae of *C. pipiens* treated as 2nd instar larvae. The obtained results revealed that cryomazine induced inhibitory effect on the total AST and ALT at 0.1 ppm and induced. a significant stimulatory effect on total AST and ALT at 1 ppm.

The total activity of AST was 230 and 287.33 µg.b.wt at 0.1 and 1 ppm, respectively, while the total activity was 272.66 µg.b.wt in the control group. The total activity of ALT was 2338.33 and 3196.66 × 10^3 µg. b.wt at 0.1 and 1 ppm, respectively while the total ALT was 2441.66 µ× 10^3 /g. b.wt in the control group.

The inhibitory effect of some IGR'S on the acitivity of AST and ALT on *C. pipiens* larvae was in accordance with those obtained by Saha *et al.* (1986) using THA against *Chrysocoris stollii*; Abdel- Hafez *et al.* (1988) using diflubenzuron and triflumuron against *S. littoralis* ; Ahmed *et al.* (1990) using chlorofluazuron against *S. Littoralis* ; Sokar (1995) using hexaflumuron against *S.littoralis* ; Abdel -Aal (2002) using chlorfluazuron ,flufenoxuron and

pyriproxyfen against *S. littoralis.* EL-sheikh (2002) using pyriproxyfen against *A. ipsilon* and Assar *et al.* (2010) using consult and match against *M. domestica* .

The stimuatory effect induced on the total AST and ALT by cryomazine in the present study agree with the results obtained by JHA against *Chrysicoris stollii* (Saha *et al.,* 1986); pyroproxyfen against *P. gossypiella* and *E. insulana* (Anan *et al.*, 1993) ; pyriproxyfen, flufenoxuron and chlorfluazuron against *S. littoralis* (Abdel- Aal , 2002), hexaflumuron against *S. littoralis* (Sokar, 1995) ; and mimic , applaud and admiral against *M. domestica* (Assar *et al.*, 2010) .

Specific types of proteins are sythesized in the haemolymph from precursors of amino acids by enzymatic transformation reactions. Glutamic acid is formed by amino transfer from aspartic acid by aspartate aminotransferase (AST) or from alanine by alanine aminotransferase (ALT). It is probably a very significant enzymatic activity in the final stage of development (Gowda and Ramaiah, 1976).

Transaminase enzymes were considered as key enzymes in the formation of non essential amino acids, which if formed inside the body not taken from outside in metabolism of nitrogen waste and gluconeogensis (Mordue and Goldworthy, 1973). The same authors stated that the change in transaminase levels have been correlated with anabolism or catabolism of protein. Maintainance of the balanced " amino acid pool " in insects is the result of various biochemical reactions carried out by a group of enzymes called amino- transferases (Meister, 1957). In addition, Glbert (1967) reported that the level of ALT varies with the amount of synthesized protein. The amino transaminase alanine is one of the components of oxidative metabolism of proline, which is utilized during the initial periods of flights; it acts as a catalytic agent in the carbohydrate metabolism.

Azmi *et al.* (1998) stated that the transaminases (ALT and AST) enzymes help in the production of energy and serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions.

6- Effect of cyromazine on phenoloxidase:-

Results presented in table (3) showed the activity of phenoloxidase of 4th instar larvae of *C. pipiens* treated as 2nd instar larvae with 0.1 and 1 ppm of cyromazine. The tested IGR significantly decreased the activity of phenoloxidase. The activity of phenoloxidase was 4.56 and 3.36 O.D units / min/g. b.wt at 0.1 and 1 ppm, respectively as compared to 4.64 O.D units/ min/g.b.wt in the control group. On the other hand, the activity of phenoloxidase increase was obtained by chlorfluazuron, flufenoxuron and teflubenzuron against *S. littoralis* (Abd El-Khalek, 1990) and by diflubenzuron and pyriproxyfen against *S. littoralis* (Farag, 2001).

Nigm *et al.* (1997) stated that the phenoloxidase is important component of insect immuno system. In addition, phenoloxidase correlate with resistance to some parasites and pathogens across species.

The effects of CSIs on insects vary according to species, the developmental stage at the time of application, the kind of compound and the administered dose (Mulla *et al.* 2003).

Table (1) Effect of Cyromazine on the amino acids of the 4th instar larvae of *Culex pipiens* treated as 2nd larval instar.

Amino acids	Control		0.1 ppm	1 ppm	% Reduction	
	Conc. µ g / ml		Conc.µ g /ml	Conc. µ g / ml	0.1 ppm	1 ppm
Acidic	**Aspartic**	302.12	183.52	91.20	39.28	69.81
	Glutamic	465.40	274.72	230.32	40.97	50.51
Basic	**Arginine**	146.64	93.28	39.52	36.35	73.05
	Histidine	187.88	109.60	61.64	41.67	67.21
	lycine	224.96	131.64	92.56	41.48	58.82
Neutral	**Alanine**	233.80	131.28	103.20	43.84	55.85
	Glycine	63.96	43.68	24.24	31.70	62.10
	Isoleucine	99.36	53.12	32.84	46.53	66.94
	Leucine	311.84	165.48	121.56	46.93	61.01
	Phenyl alanine	124.60	92.88	51.52	25.45	58.66
	Proline	303.24	156.04	71.08	48.54	76.55
	Serine	143.28	47.20	46.04	67.06	67.89
	Threonine	106.24	37.88	34.44	64.31	67.60
	Tyrosine	103.72	86.64	67.20	16.48	35.19
	Valine	177.16	73.44	58.40	58.57	67.40
Total		**2994.20**	**1680.40**	**1125.76**		

Figure (1): Amino acids pattern in the whole body tissue of untreated 4th instar larvae of *Culex pipiens*.

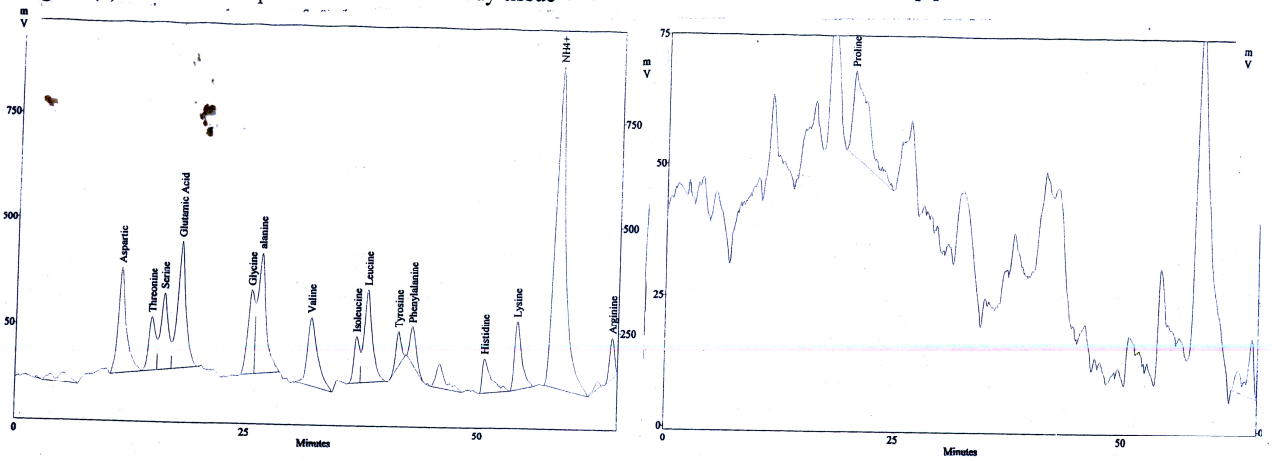

Figure (2): Amino acids pattern in the whole body tissue of 4th instar larvae of *Culex pipiens* treated with 0.1 ppm cyromazine.

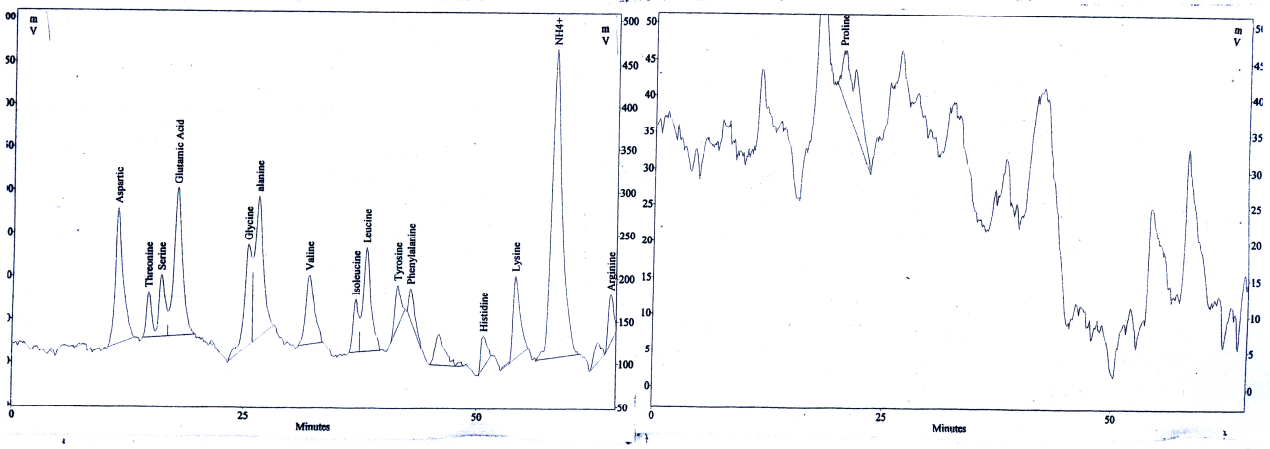

Figure (3): Amino acids pattern in the whole body tissue of 4th instar larvae of *Culex pipiens* treated with1 ppm cyromazine.

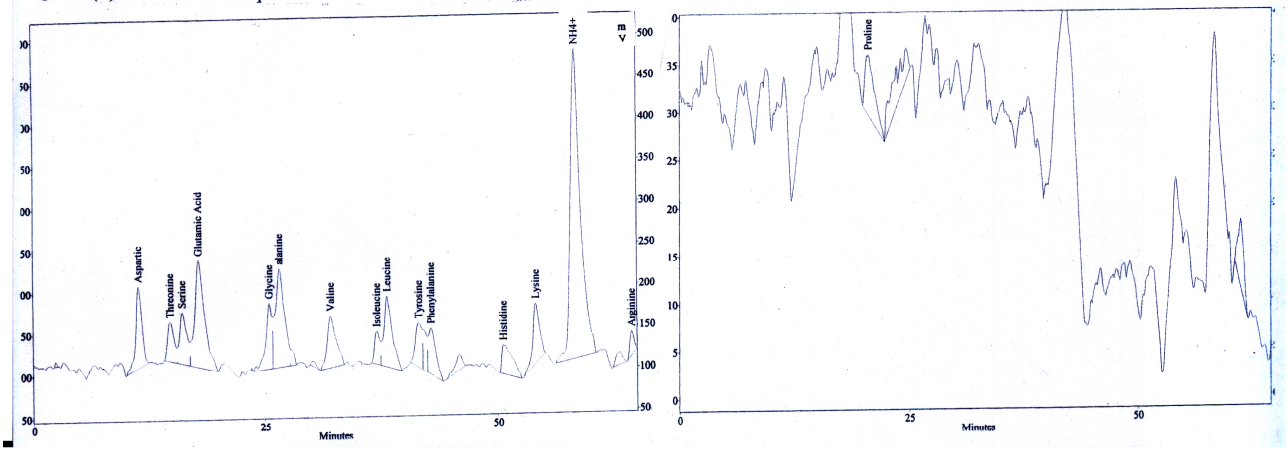

Table (2): Effect of Cyromazine on the total glucose and total protein contents of the 4th instar larvae of *Culex pipiens* treated as 2nd larval instar

Conc. (PPm)	Mean total glucose content (mg/g.b.wt.) ±S.E.	Mean total Protein content (mg/g.b.wt.) ±S.E.
0.0	79.06±3.08	387.00±14.11
0.1	70.70±2.36	463.00±17.58
1.0	68.90±2.69	459.30±0.09
F-value	20.305	19. 047
P.	*< 0.005	*< 0.005

Table (3): Effect of cyromazine on the activity of phosphatases (ACP and ALP.), transaminases (AST. and ALT) and phenoloxidase of the 4th instar larvae of *Culex pipiens* treatment as 2nd larval instar

	Phosphatases		Transaminases		Phenoloxidase
Conc. PPm.	Mean activity of ACP. ($\mu x10^3$/g.b.wt.)±S.E.	Mean activity of ALP. ($\mu x10^3$/g.b.wt.)±S.E.	Mean activity of AST. ($\mu x10^3$/g.b.wt.)±S.E.	Mean activity of ALT. ($\mu x10^3$/g.b.wt.)±S.E.	Mean activity of phenoloxidase. (O.D.units/mini/g.b.wt.)±S.E.
0.0	460.00± 22.61	15.61± 1.05	272.66± 9.29	2441.66± 72.86	4.64 ± 0.11
0.1	964.33± 31.37	12.66± 0.69	230.00± 9.54	2338.33±42.52	4.56± 0.28
1	1102.66± 23.76	10.76± 0.53	287.33± 6.43	3196.66± 98.66	3.36± 0.28
F-value	8.589	27.321	8.034	10.108	24.716
P.	* < 0.005	* < 0.005	* < 0.005	* < 0.005	* < 0.005

ACP: Acid phosphatase **ALP:** Alkaline phosphatase
ALT: Alanine aminotransferase (ALT/GPT) **AST:** Aspartate aminotransferase (AST/GOT)
O.D. : Optical density **U:** International unit

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