Insecticidal and Biochemical Activities of Pleo 50%Ec and Nomolt 15%Sc on Some Mosquito Species

Salam S. Teleb ,Eman M. Rashad and Farag A. Ahmed

Zoology Department, Faculty of Science, Zagazig University, Egypt
salamteleb@gmail.com

Abstract: The insecticidal action of Pleo and Nomolt at various dosages were investigated against the 4th larval instar of *Anopheles pharoensis, Culex pipens* and *Culista longiareolata* . The Pelo and Nomolt were highly toxic against *Anopheles pharoensis* larvae (LC_{50} were 0.6±0.1 and 7.8±0.7ppm respect.) followed by *Culex pipens* (LC_{50} were 5.5±1.0 and 2200±346ppm respect.) and then *Culista longiareolata* (LC_{50}were 37.7±6.0 and 2500±173.2 respect.).The obtained results indicated that LC_{50} values from Pleo and Nomolt increased the total protein content in the homogenates of the 4th larval instar of *Anopheles pharoensis, Culex pipens* and *Culista longiareolata* larvae after 24 and 48hrs. post treatments. Moreover, treatment of the 4th larval instar of *Culex pipens* by both Pleo and Nomolt induced a noticeable increase in the concentration of each free amino acid detected after 24 hrs. of treatment except histidine which is decreased in larvae treated by Pleo. While, Pleo caused a remarkable increase in the total pool of free amino acids and the concentration of each free amino acid detected except threonine which is decreased in *Cx. pipiens* larvae after 48 hrs. of treatment.

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Introduction

Mosquitoes transmit diseases to more than 700 million people annually (Aregawi *et al.*, 2008). Therefore, the control of mosquitoes is an important public health concern around the world. For example, *Culex pipens*) is a pantropical pest and urban vector of *Wuchereria bancrofti*, Plasmodium (avian malaria) and other diseases in some parts of the world (Holder, 1999).

Chemical control is an effective strategy used extensively in daily life. Synthetic insecticides are today in the forefront of mosquito-controlling agents. Pyridalyl (experimental code: S-1812) is a novel synthetic insecticide (S-1812; 2, 6-dichloro-4-(3, 3-dichloroallyloxy) phenyl 3- (5 (trifluoromethyl)-2-pyridyloxyl propyl ether], that has a phenoxy-pyridaloxy derivative structure. It functions via new action mechanisms that are completely different from those utilized by existing agents, such as organophosphates. The compound was reported effective on the pests of order Lepidoptera and Thysanoptera on cotton and vegetables, without any phytotoxicity. Pleo has been verified as an environmentally friendly insecticide that is suitable for use in IPM systems, for the following reasons: it possesses a high level of safety for humans, animals and fish; and it has minimal impact upon natural pest predators, such as parasitic wasps, pirate bugs (*Orius strigicollis*), lacewing ,ladybugs, predatory mites and spiders Its efficacy was also reported against populations of tobacco budworm, *Heliothis virescens*, cotton bollworm, *Helicoverpa zea* (Johnson *et al*., 2000) and *Plutella xylostella* (Umela and Strickland, 1999) which are resistant to various currently used insecticides.

Insect growth regulators (IGRs) affect hormonal regulation of immature mosquito growth and development and have little environmental impact on non target organisms. The primary effect of IGRs is the reduction of adult emergence, but reproduction and ecdysteroid production in surviving females are also affected (Fournet *et al.,* 1993and 1995). Moreover, the chitin synthesis inhibitors (CSI) are grouped with IGRs, and Nomolt (Teflubenzuron) belong to benzoyl-phenyl urea compound that inhibit the production of chitin (Williams, 1967). The presence of an IGR during the larval - pupal and pupal – adult molts results in the production of abnormal or intermediate forms that fail to molt normally and soon die (Bstid, 1973 and Retnakaran, *et al*., 1985).). In general, IGRs have high levels of activity and efficacy against various species of mosquitoes and can be applied in a variety of habitats (Mulla *et al*., 1989). Additionally, IGRs have shown a good margin of safety to personal Applying them and to non-target biota, including fish and birds (Miura and Takahashi, 1975; Mulla *et al*., 1985). These attributes of IGRs increases the potential for them to be used more widely for mosquito control, in addition to the much used microbial larvicides, pyrethroids and organophosphorus larvicides (Mulla *et al*., 1989).

The present work was carried out to clarify the toxic effect of Pleo (Pyridalyl) and Nomolt

(Teflubenzuron) against the 4th larval instars of *An. pharoensis, Cx. pipiens* and *Cs. longiareolata* and to determine their effect on the whole protein content and the free amino acid level in treated and untreated *Cx.pipiens* larvae.

Material and Methods

1- Mosquito rearing

A laboratory colony of the mosquito larvae was established in the Zoology Department, Faculty of Science, Zagazig University. Larval bioassays were conducted on 4th instar larvae of *Anopheles pharoensis (An. pharoensis), Culex pipiens (Cx.pipiens)* and *Culista longiareolata* (*Cs. longiareolata)*.The colonies of mosquitoes were maintained at 25 ± 2°C and 60 ± 5 % relative humidity. Larvae were daily fed on a mixture of fish food-dried yeast (3:1 ratio). Adults were provided with a 10% sucrose solution and blood.

2- Larvicidal activity

Mosquito larvicidal trials were carried out according to WHO standard procedures (1996), with slight modifications. Twenty five fourth instars larvae of mosquitoes were exposed to different concentrations from Pleo and Nomolt. Control larvae were reared on food. The insecticidal assay was conducted with four replicated. Mortality was determined after 24 hrs of exposure, during which no food was offered to the larvae

3- Control agents

The Pleo50%Ec and Nomolt15%Sc were obtained from Shoura chemicals.

Pleo50%Ec (active ingredients: pyridalyl, (S-1812; 2, 6-dichloro-4-(3, 3-dichloroallyloxy) phenyl 3- (5-(trifluoromethyl)-2-pyridyloxyl propyl ether], is a novel insecticide that has a phenoxy-pyridaloxy derivative structure. The Nomolt15%Sc (active ingredients: Teflubenzuron **(**IUPAC: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6- difluorobenzoyl urea)

4- Biochemical studies

A-Preparation of samples for biochemical studies.

The 4th instar larvae of *An. pharoensis , Cx.pipiens* and *Cs. longiareolata* were treated with LC_{50} of the tested insecticides to study the effect of these insecticides on total protein, in the whole larval body extracts. Larvae were collected after 24 and 48hrs of treatment and homogenized in distilled water. The homogenate was centrifuged at 3500 r.p.m. for 10 min. at 4 °C and the supernatant was frozen till use.

B-Total protein

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

C-- Amino acid composition

The 4th instar larvae of treated and untreated *Cx.pipiens,* were collected after 24 and 48 hrs of treatment by Pleo and Nomolt and kept at -20˚C until 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).

5-Data analysis

Data obtained from eachdose larvicidabioassay (total mortality) were subjected to probit analysis (Finney, 1971); LC50 and LC9 values were calculated. All results were expressed as mean ± standard error, and the data were analyzed using student T-test.

Results and Discussion

1-Toxicity of Pleo and Nomolt on the4th instar larvae of *An. pharoensis, Cx.pipiens* and *Cs. Longiareolata* .

Results of treating the early 4th instar larvae of *An. pharoensis, Cx.pipiens* and *Cs. longiareolata* with different concentrations of Pleo 50% Ec (Pyridalyl) and Nomolt (Teflubenzuron) were presented in table (1). Pleo exhibited highly insecticidal activity (LC_{50} were 0.6±0.1, 5.5±1.0 and 37.7 ± 6.0 for *A. pharoensis, Cx.pipiens* and *Cs. longiareolata*, respectively) than Nomolt (LC_{50} were 7.8 ± 0.7, 2200 ± 346.0 and 2500 ± 173.2 for *A. pharoensis, Cx.pipiens* and *Cs. longiareolata*, respectively). At the level of LC_{90} values, Pleo showed the highest larval toxicity (1.3 ± 0.1 for *A. pharonesis*); followed by 39.7± 11.0 for *Cx.pipiens* while 108.3 ± 10.1 for *Cs. longiareolata*. The slope of regression lines ranged between 1.9 - 4.2 for the three species indicating different degree of homogeneity or heterogeneity of the tested insects.

The results showed that Pleo was the most toxic insecticide against the 4th larval instars of mosquito species. Saito *et al.,* (2004) stated that pyridalyl is an insecticide with a novel chemical structure that is different from any other existing insecticide class. It suppresses populations of *Spodoptera litura* even at lower dose rates. Also, Miyamoto and Katagi, (2010) reported that pyridalyl is a new insecticide which has an acute toxicity in the larval midge *Chironomus yoshimatsui.* They determined the LC_{50} of it to be 1.1 mg/l. On the other hand, Nomolt belong to benzoylphenyl ureas that interact with chitin synthase in such a way to prevent /or inhibit chitin synthesis (Deul *et al.,* 1978). Moreover, it seems likely that the greater the amount of IGR absorbed by, or reaching the site of action, the greater is the toxicity of the compound (Shaurub *et al*., 1999).

2- Total protein

Results in table (2) showed that application of LC_{50} of Pleo and Nomolt to the 4th larval instar of mosquitoes' species induced a significant increase in the whole body homogenates protein content after 24

and 48 hrs of treatment. In case of Pleo, the whole body protein level after 24 hrs of treatment was 0.876 mg/g then changed to 0.114 mg/g after 48 hrs of treatment for *An. pharonesis*, was 1.145 mg/g after 24 hrs then increased to 1.86 mg/g after 48 hrs of treatment for *Cx.pipiens* and was 1.347 mg/g at 24 hrs post treatment then increased to 1.42 mg/g at 48 h post treatment for *Cs. longiareolata*, respectively as compared to 0.115 and 0.070 mg/g for untreated *A. pharoensis*., 0.149 and 0.71 mg/g for untreated *Cs. longiareolata*, respectively.

In case of Nomolt, the protein content in the whole body after 24 and 48 hrs of treatment were 0.583 and 0.453 mg/g for *An. pharonesis*, 1.261 and 1.591 mg/g for *Cx. pipiens* and 0.503 and 0.25 mg/g for *Cs. longiareolata,* respectively. The percent change of protein content at 24 hrs post treatment was higher in Pleo than Nomolt treatment in *An. pharonesis* and *Cs. longiareolata* while in *Cx. pipens,* percent change of protein content was higher in Nomolt than Pleo, on the other hand, at 48 hrs post treatment, the percent change of protein content was higher in Pleo than Nomolt treatment for *Cx.pipiens* and *Cs. longiareolata* but it was higher in Nomolt treatment than in Pleo for *An. pharonesis* as shown in table (2).

Many workers obtained similar findings on other insects as Wilkinson (1976) stated that protein helps to synthesize microsomal detoxifying enzyme which assists in detoxification. Also, Shakoori and Saleem (1991); attributed the greater protein synthesis with insecticidal treatment to synthesis of the proteinases needed for insecticide detoxification.

3- Amino acids composition

Table (3) revealed that the total pool of free amino acids in the 4th larval instars of *Cx. pipiens* was 22.56mg/g; also the presence of 12 free amino acids where threonine was the most predominant free amino acid while isoleucine was the least one. The total pool of free amino in *Cx. pipiens* after 24 hrs of treatment by Pleo and Nomolt increased to reach 68.952 and 87.618 mg/g, respectively as compared to the control and detection of 12 amino acids. These treatments caused the appearance of glutamic acid in high level (17.174 mg/g) and disappearance of alanine. Moreover, treatment of larvae by both Pleo and Nomolt induced a noticeable increase in the concentration of each free amino acid detected in treated larvae except histidine which is decreased in larvae treated by Pleo as shown in table (3).

In table (4) the total pool of free amino acids in the control of the 4th larval instars was 39.121 mg/g with the appearance of free amino acids where aspartic acid, glutamic and arginine where the most abundant ones. Meanwhile, the remaining free amino acids were present in small amounts ranging between 0.684 to 3.920 mg/g. Also, results depicted that Pleo caused a remarkable increase in the total pool of amino acids and the concentration of each free amino acid detected except threonine which is decreased in *Cx.pipiens* larvae after 48 hrs of treatment. Pleo induced the appearance of proline, glycine, valine and methionine (1.288, 55.146, 22.576 and 26.046 mg/g, respectively). However, Nomolt induced the appearance of glycine (3.24 mg/g) and the absence of arginine with a marked increase in the concentration of each free amino acid detected except asparagine which is decreased and it caused a slight decrease in the total pool of free amino acids in *Cx.pipiens* larvae after 48 hrs of treatment as compared to the control (Table 4).

Insects in general contain an unusual high concentration of free amino acids in both tissue and hemolymph (Chen, 1977), which perform a number of physiological functions in morphogenic processes (Schaub *et al*., 1990), reproduction (Uchida *et al.,*1990), detoxification of insecticides (Rajender and Rao, 1989; Bounias *et al.,* 1989)and cold hardiness (Su *et al*., 1993).

In the present work, the increase of free amino acids in treated insects 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 protean formation (Baker *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.

Also, Yi and Adams (2000) and Chapman (2002) stated that insects generally have high concentrations of free amino acids during periods of high protein synthesis and low amino acid pools during periods of low protein synthesis. The high levels of amino acids in treated insects may cause a great disturbance in biochemical activities as protein metabolism as well as in regulation of osmotic pressure (Enan, 2004).

The results obtained showed that the level of the free amino acids was fluctuated according to the role of each one. Tyrosine was essential for cuticular sclerotization, while, histidine was an essential amino acid, linked to the pathway of purine synthesis. Methionine was known to supply sulphur to cystine and to contribute in the production of eggs in several insect species (Barrett and Friend, 1975; Mostafa *et al*., 2003and Rashad *et al*., 2003). Arginine acted as energy stored in muscles known as phosphogen (Chapman, 2002), while leucine, isoleucine and valine play a role in the pupation process. Alanine and glycine were deaminated and converted to glycogen, acting as a reserve substance and is deposited in the fat body for the formation of chitin (Goto *et al*., 1998).

The detection of proline in larvae treated with Pleo might be due to the varying rates of utilizing the amino acids as a source of energy in the repair of mechanisms. On the other hand, Buscarlet *et al*., (1986) stated that threonine and lysine concentrations increased by IGR might be explained as muscle proteins were hydrolyzed and provide the extracellular fluid and the hemolymph with new chlorides. Collett (1976) recorded that glycine was active in the synthesis of several proteins, thus maintained at lower level. He attributed this to the regulation of the free amino acid pool affecting the level at which amino acids were maintained. He also, added that while several of the essential amino acids were present in large amounts, others were clearly active in more than protein synthesis and, thus, were maintained at low levels. Thus, this might explain the appearance of glycine as the insects treated by Pleo. Enan (2004) stated that the high levels of amino acids in treated insects might cause a great disturbance in biochemical activities as protein metabolism. Chapman (2002) revealed that arginine and proline were synthesized from glutamic acid which considered as neurotransmitter in insects and this in turn might lead then to low value or disappearance as obtained in the current study. Lefevere *et al*., (1989) found that proline was a cryoprotective agent in plant cells and protect membranes against desiccation and toxic solutes. Since, proline appeared in insects exposed to stress, it has been suggested that proline might have a cryoprotective function in insects too. Moreover, Micheu *et al*., (2000) reported that proline was used to supply a rapid demand for energy required in locomotion or flying.

Table (1): Toxicity of Pleo and Nomolt on the 4th larval instars of *An. pharonesis*, *Cx.pipiens* and *Cs. Longiareolata.*

Mosquito species	Toxicity index Mean ± S.E*					
	Pleo			Nomolt		
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope
Anopheles pharonesis	0.6±0.1	1.3±0.1	1.9±0.0	7.8±0.7	24.3±2.0	2.4±0.0
Culex pipiens	5.5±1.0	39.7±11.0	4.2±1.9	2200±346	4200±462	1.0±0.4
Culiseta longiareolata	37.7±6.0	108.3±10.1	2.3±0.13	2500±173.2	5350±938	1.9±0.03

*Results are the means of 4 replicates.

Table (2): Effect of LC_{50} of the tested Pleo and Nomolt on the total protein content of the different mosquito species.

Insecticides	Mean protein content mg/g tissue ± S.E*					
	Anopheles pharonesis					
	Control	24hrs	%change	Control	48 hrs	%change
Pleo	0.115±0.002	0.876±0.04	+647.8	0.070±0.003	0.114±0.01	+62.6
Nomolt	0.115±0.002	0.583±0.02	+406.9	0.070±0.003	0.453±0..02	+547.1
Culex pipiens						
Pleo	0.149 ±0.001	1.145 ±0.002	+688.4	0.71±0.02	1.86 ±0.023	+162
Nomolt	0.149 ±0.001	1.261±0.001	+751.4	0.71±0.02	1.591±0.002	+124
Culiseta longiareolata						
Pleo	0.298±0.01	1.347±0.03	+352.0	0.188±00.01	1.42±0.01	+655.3
Nomolt	0.298±0.01	0.503±0.03	+ 68.8	0.188±00.01	0.25±0.01	+33.0

*Results are the means of 4 replicates.

Table (3): The free amino acids level (mg/g) in the 4th larval instar of *Cx.pipiens* after 24 hrs of treatment by Pleo and Nomolt .

Amino acids / Insecticides	Control	Pleo	
Aspartic acid	1.422	5.82	9.852
Threonine	3.870	13.136	10.448
Serine	1.062	5.368	6.838
Glutamic acid	-	17.174	22.282
Proline	-	-	-
Glycine	2.718	3.908	4.550
Alanine	1.916	-	-
Valine	-	-	-
Methionine	-	-	-
Isoleucine	0.756	2.246	3.115
Leucine	1.452	5.106	9.529
Tyrosine	1.7	2.74	2.301
Phenylalanine	1.856	3.446	5.322
Histidine	2.62	2.08	4.528
Lysine	2.252	5.114	6.864
Arginine	0.936	2.814	1.989
Total pool	22.56	68.952	87.618

Table (4): The free amino acids level (mg/g) in the 4th larval instar of *Cx.pipiens* after 48 hrs of treatment by Pleo and Nomolt .

Insecticides / Amino acids	Control	Pleo	Nomolt
Aspartic acid	10.956	11.662	8.383
Threonine	2.770	0.477	14.268
Serine	1.452	23.154	8.034
Glutamic acid	6.653	20.241	18.804
Proline	-	1.288	-
Glycine	-	55.146	3.24
Alanine	-	-	-
Valine	-	22.576	-
Methionine	-	26.046	-
Isoleucine	1.202	21.202	3.286
Leucine	2.854	25.567	9.12
Tyrosine	0.684	31.117	2.059
Phenylalanine	0.854	26.026	2.467
Histidine	1.848	34.497	3.888
Lysine	3.920	31.005	6.598
Arginine	5.928	21.669	-
Total pool	39.1	351.673	80.147

Conclusion:

Pleo and Nomolt has insecticidal action at various dosages against the 4th larval instar of *Anopheles pharoensis, Culex pipens* and *Culista longiareolata* .The LC_{50} values increased the total protein content in the different mosquito species . Pleo and Nomolt disrupted many physiological processes in *Cx.pipiens* after treatment of the 4th larval instars. The difference in their effect may be due to their chemical nature and their mode of action. The biochemical mechanisms of pyridalyl's insecticidal action have not been identified to date. It might interferes with cell function since it resembles substances (validoxylamine A and diafenthiuron) which is known as an inhibitor of trehalase and of mitochondria ATP synthesis, respectively (Saito *et al*.,2004).

However, Nomolt is a chitin synthesis inhibitor (CSI) affecting moulting and belong to benzoylphenyl ureas. Moreover, Ishaaya and Casida (1974) and Mayer *et al*., (1990) have reported that benzoylphenyl urea may affect other physiological systems, such as hormone system, that activates the chitinase and phenol oxidase. Accordingly, it may affect the endocrine system and consequently affect the metabolic processes performed under its control.

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

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