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Biological effects of a benzoylphenylurea derivative (Novaluron) on larvae of *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Novaluron is a chitin synthesis inhibitor belonging to the class of benzoylphenylurea. A commercial formulation of novaluron (MCW-275) was tested at different concentrations, ranging between 0.2 and 1.6µg/l, against third and fourth-instar larvae of Culex pipiens. Treatments were done on newly moulted larvae for 24 h (WHO) under standard laboratory conditions. Mortality was observed for both stages, after incomplete development and the LC_{50} and LC_{90} were estimated at 0.32 and 1.2 µg/l for the third-instar larvae, respectively, while these respective values were 0.58 and 2.2µg/l for the fourth-instar larvae. Furthermore, the compound was applied at LC_{50} and LC_{90} against the fourth instars larvae and its effects was investigated on development duration, biochemical composition of larval body and body weight. Metabolite analyzes showed that novaluron affected significantly the amount of carbohydrates, lipids and proteins of fourth instars larvae starting day three following treatment. For the same treated series a significant decrease was also recorded in the body weight with an increase of development duration. The final step of the chitin biosynthesis pathway is inhibited by the novaluron and the precursor is not converted in to chitin and this leads to death or the increase in life time of the larvae.

Keywords: chitin synthesis inhibitor, benzoylphenylurea, novaluron, *Culex pipiens.*

INTRODUCTION

Mosquitoes are medically and veterinary important vectors, responsible for the transmission of many diseases [1] *Culex pipiens* represents the most interesting mosquito species in Algeria, particularly in urban areas and is generally controlled by conventional insecticides [2, 3]. Because of the secondary effects of conventional neurotoxic insecticides on the environment, the insect growth regulators (I.G.Rs) seem promising because of their specific mode of action on insects and their lower toxicity against non-target organisms than conventional insecticides. Thus, the insect growth regulators (I.G.Rs) such as chitin synthesis inhibitors affect the hormonal regulation of molting and development processes in many orders of insect [4]. Novaluron is a chitin synthesis inhibitor belonging to the class of benzoylphenylurea, showing a high toxicity level and effectiveness against several dipteran species [5, 6, 7]. Laboratory bioassays were carried out against second and fourth-instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* using Novaluron [8,9] and the results indicated that novaluron was highly effective against mosquito larvae and pupae. *Aedes aegypti* was found to be more susceptible to novaluron than *Culex quinquefasciatus*. *Anopheles* species were less susceptible than the other two genera. Field studies in artificial and natural habitats showed also that novaluron 10% EC was effective against populations of *Aedes aegypti*, *Anopheles* species and *Culex quinquefasciatus* [5].

In insects, the haemolymph undergoes metabolic modification during the developmental stages and the principal forms of storage of energy are represented by carbohydrates and lipids. The carbohydrates, as energy elements play

a crucial role in the physiology of the insects, the rates of glycogen and in tissues are closely related to the physiological events such as the flight, the moult and the reproduction [10]. The exposure of an organism to xenobiotic product can modify the synthesis of certain metabolite [11].

In the present study, we assessed the toxicity of novaluron against third and fourth larval stages of *Culex pipiens*, and the duration of both stages were determined. In addition, its effects at LC_{50} and LC_{90} were studied on biochemical composition and on the body weight of the fourth larval stage in order to provide better insights in the physiology of its mode of action.

MATERIALS AND METHODS

Mosquito rearing

The larvae of *Culex pipiens* (Diptera: Culicidae) were obtained from a stock colony of the laboratory and reared as previously described [2]. Each 25 larvae were kept in Pyrex storage jar containing 500 ml of stored tap water and maintained at temperature between 25-27°C and a photoperiod of 14L:10D. Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight), and water was replaced every four days.

Toxicity bioassay

A commercial formulation of novaluron (MCW-275) was tested at various concentrations prepared in distilled water. Appropriate aliquots (0.1-1ml) were added to treatment beakers to give the following final concentrations of 0.2, 0.4, 0.8 and 1.6 µg of active ingredient per liter. Newly ecdysed third and fourth-instars larvae of *Culex pipiens* were exposed to the different concentrations for 24 hours when the control larvae were exposed to water only. After the exposure time of 24 hours, according to the World Health Organization (12), the larvae were removed, and placed in clean breading water. The test was carried out with three repetitions containing 25 larvae each. The growth and development was examined and mortality was registered daily until adult emergence. The mortality percentage recorded at various developmental stages was corrected [13] and toxicity data were studied by probit analysis [14], and LC_{50} (50% lethal concentration), confidence limits (95%). and slope of the concentration-mortality lines were calculated by the method of Swaroop et al. [15].

Biochemical procedure

Newly moulted fourth instar larvae of *Culex pipiens*, were treated as above with two concentrations (LC_{50}=0.58µg/l and LC_{90}=2.22µg/l) and sampled during the larval development at 1, 3, 5 and 7 days. Each pooled sample (10 individuals, starting with more than 150 larvae), was weighted and subjected to extraction in trichloracetic acid (TCA 20%) [16]. After a first centrifugation, the supernatant was used to evaluate the carbohydrates, as described by [17], and to the pellet was added a mixture of ether and chloroform (1V/1V) for making a second centrifugation. The lipids were quantified from the supernatant 2 [18]. Therefore the protein bioassay was carried out from the dissolved pellet 2 in NaOH (0.1N) [19]. Data were expressed in µg/mg whole body; means ± SD were analysed based on three replicates per treatments each using a Student's t test at p = 0.05. Analyses were conducted on the survivors of the treatment.

RESULTS

Insecticidal Activity

Dose-response relationship was determined for novaluron applied for 24 h to newly ecdysed third and fourth-instar larvae of *Culex pipiens*, and the mortality was recorded up to adult emergence. The product exhibited a larvicidal activity against the treated mosquito larval stages. The highest concentration tested, 1.6µg/l caused 97% and 83% mortality against the third and the fourth instar larvae of *Culex pipiens*, respectively (Fig. 1 and 2). With probit for the stage L3, the LC_{50} was calculated as 0.32 µg/l (N= 261; 95%; CL = 0.27-0.37 µg/l; Slope= 2.50) and the LC_{90} was 1.16 µg/l (95% CL = 0.98-1.36 µg/l), and for the stage L4 the LC_{50} was found as 0.58 µg/l (N=180; 95% CL = 0.47-0.71 µg/l; Slope= 2.81) and the LC_{90} was 2.22 µg/l (95% CL = 1.80-2.73 µg/l). The observations of death larvae after treatment showed that most mortality occurred after an incomplete moult. The chitin synthesis inhibitor proved to affect growth and development in mosquito *Culex pipiens*. After about 2 days of treatment, intoxicated larvae showed a change in their behaviour by sinking to the bottom of the jar and remain stable until they died. Morphological examination of intoxicated larvae and pupae of the mosquitoes revealed the absence of the exocuticle, and only the presence of the endocuticle, of the previous stage, that still attached to the epidermis (Fig. 3). The new cuticle synthesis novaluron-treated larvae in this cases was completely inhibited (Fig. 3) and as a consequence larvae or pupae died trapped within their old exuvium. In some cases the treated larvae reached the pupal stage but without or with incomplete formation of the new cuticule and the absence of the color that could explain the absence of the chitin synthesis process and the melanization. The figure 3 shows a larva that did not shed

the old head capsule nor completed ecdysis after 5 days following treatment with novaluron. Further on during the development, novaluron presented toxicity up to adult emergence because treated insects failed to ecdyse into pupae and adults and died within the respective exuvium.

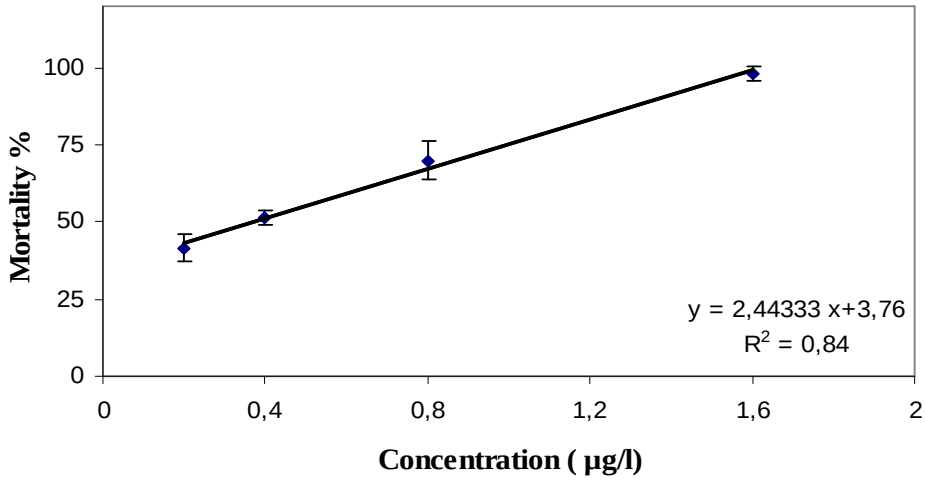

Fig. 1: Dose-response relationship for treatment of Novaluron, applied for 24 h to newly ecdysed third- instar larvae of *Culex pipiens* (R^2 = coefficient of determination)

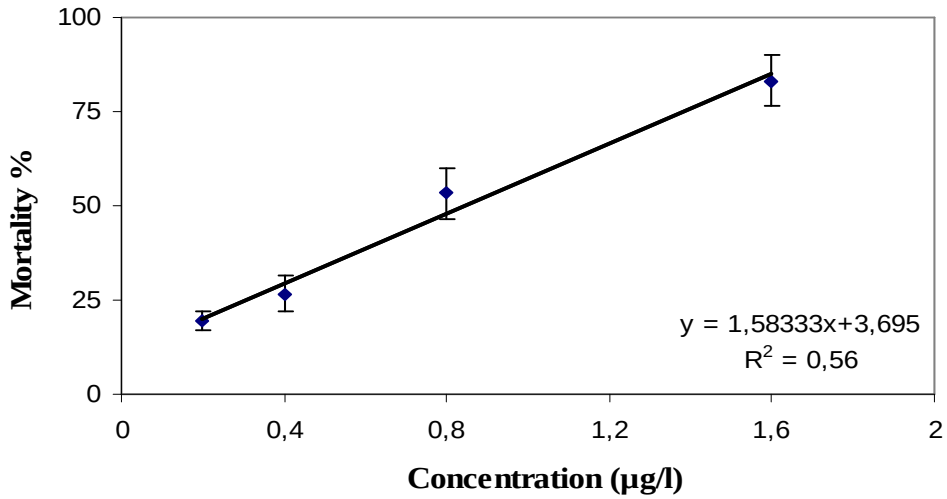

Fig. 2: Dose-response relationship for treatment of Novaluron, applied for 24 h to newly ecdysed fourth- instar larvae of *Culex pipiens* (R^2 = coefficient of determination)

Fig. 3: Treated larva that did not shed the old head capsule nor completed ecdysis after 5 days following treatment with novaluron (left). Comparison of the appearance of a pupae of the control of *Culex pipiens* (middle) with pupae of a larva treated with novaluron (right). Note the absence of the melanization and the arrows indicate the old cuticle attached to the epidermis and the absence of new cuticle.

Effects on larval development duration

The results of developmental duration, of the third and the fourth instars larvae of *Culex pipiens* after treatment with lethal concentrations, (LC_{50} = 0.32 µg/l, LC_{90} = 1.16 and LC_{50} = 0.58µg/l, LC_{90} = 2.22 µg/l) respectively (Table 1), showed that Novaluron inhibits the growth, by extending the age of the larval development, for both stages with the highest concentrations (CL_{90}). The effect on development duration of the third larval stage was recorded only with CL_{90}, the duration was 5.53 days compared with control that was 4.35 (P>0.05). However the effect, on the fourth instar larvae was significant for the both lethal concentrations (CL_{50} P>0.05 and CL_{90} P>0.005) with a duration of 7.33 days for LC_{50} and 9.16 days for LC_{90} whereas for the control the age was 6.80 days.

Table 1: Effect of Novaluron administered at lethal concentrations (CL_{50} & CL_{90}) on the developmental duration of the third and the fourth instar larvae of *Culex pipiens*. (Data are expressed as means ± SD, n=15-42)

For each duration and each instar, means values followed by different letters are significantly different (P<0.05)

Duration (days)			
Third instar larvae		**Fourth instar larvae**	
Control	4.35 ± 0.3**a**	**Control**	6.80 ± 0.40**a**
LC_{50}=0.32 µg/l n=42	4.53± 0.49**a**	**LC_{50}**=0.58 µg/l n=30	7.33 ± 0.51**a**
LC_{90}=1.16 µg/l n=20	5.53±0.58**a**	**LC_{90}**=2.22 µg/l n=15	9.16 ± 0.61**a**

Effects on the larval body weight

The whole body weight measurement of *Culex pipiens* larvae showed that the compound applied at two concentrations (LC_{50} and LC_{90}) to newly ecdysed fourth instar larvae affected their weight. In control, the larval body weight was 2.92 ± 0.13 mg and increased to reach a maximum at day 7 with 4.60 ± 0.17 mg weight coinciding with the pupal ecdysis (Table 2). In treated series, a significant decrease in the body weight start at day 3 for the two tested concentrations, and the highest body weight recorded at day 7 was only 3.40 ± 0.26 mg for LC_{50} (P = 0.012) and 3.30 ± 0.20 mg for LC_{90} (P = 0.003) as compared with controls.

Table 2: Effect of novaluron administered at lethal concentrations (LC_{50}= 0.58 and LC_{90} = 2.22 µg/l) on the fresh body weight (mg), during the fourth instar larvae of *Culex pipiens* (means ± SD; n=10)

*Asterisks indicate a significant difference with control of the same time; ** =P<0.005*

Time (days)	**Body weight** (mg)		
	Control	**LC_{50}**	**LC_{90}**
1	2.92 ± 0.13	2.71 ± 0.25	2.83 ± 0.76
3	3.76 ± 0.25	2.60 ± 0.17 **	2.86 ± 0.11 **
5	4.26 ± 0.25	3.23 ± 0.32 **	3.03 ± 0.05 **
7	4.60 ± 0.17	3.40 ± 0.26 **	3.30 ± 0.20 **

Effects on biochemical composition

In a second series of experiments novaluron was tested at two concentrations (LC_{50} = 0.58µg/l and LC_{90} = 2.22 µg/l) on the changes of metabolite amounts, carbohydrates, lipids and proteins, of the fourth instar larvae of *Culex pipiens* during different times of the developmental stage (1, 3, 5 and 7days) (Fig. 4). The results showed that the carbohydrates and lipids level did not change at day 1 but an increase was recorded (P = 0.032, P = 0.014 and P = 0.013, P = 0.061) starting from the day 3 for the carbohydrates and lipids respectively for both concentrations. The differences in amounts were significantly higher (P = 0.004) at day 7 compared to control for both concentrations (Fig. 4A and 4B). Whereas novaluron-treated-larvae of *Culex pipiens* was found to undergo a high significant (P< 0.05) suppressed protein level as compared to control, during the periods of bioassay 3and 5days. For the day 7 the decrease of protein amount was significant (P = 0.018) only for CL_{90} compared to control series. In control, the changes in protein amounts presented a single peak that occurred at day 5 during the larval development (Fig. 4**C**). The bioassay measurements of metabolites in the whole body of larvae revealed that Novaluron at the two tested concentrations shifted the moment of this peak (day 3), and it increased their values. Indeed, during day 3, the values of components of LC_{50} and LC_{90} treated series were significantly different compared to control of the same age.

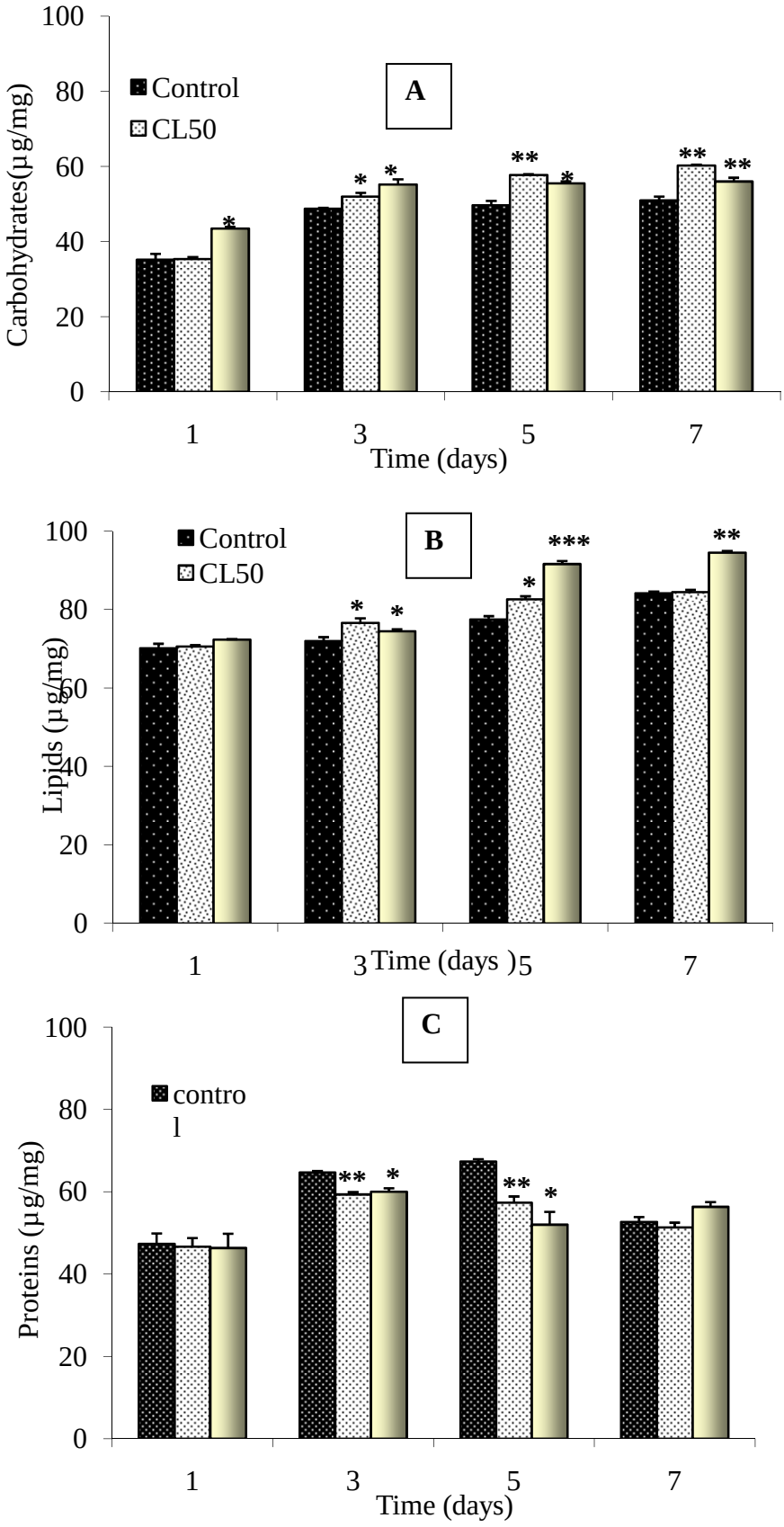

Fig. 4: Comparison of the effect of Novaluron at two concentrations (LC_{50} = 0.58µg/l and LC_{90} = 2.22 µg/l) during the fourth larval stage development of *Culex pipiens* on the amount of the metabolites in the larval body (µg /mg body weight). (A)=Concentrations of carbohydrates. (B) = Lipids. (C) = proteins

DISCUSSION

The potency of Insect growth regulators (I.G.Rs) for mosquito control has been the subject of intensive investigations [20, 6, 21]. A few reports documented the larvicidal efficacy of novaluron under laboratory conditions against the larvae of *Aedes aegypti* [8] and *Culex* mosquitoes [9, 22]. The present study was to evaluate the effects and the mechanism of an insect growth regulator novaluron, which is considered too as an endocrine disrupting compound (E.D.Cs) for insect species, on *Culex pipiens*. These results showed that the novaluron exhibits toxic effects via the disturbing the growth and the development in *Culex pipiens* and this is similar to diflubenzuron when used against the same species [21]. The toxicity assays conducted under laboratory conditions on *Culex pipiens* larvae indicated that novaluron presented a larvicidal activity when applied to newly ecdysed larvae. The same results were found when the novaluron was used against *Culex quinquefasciatus*, in small and medium-scale Trials, [7] and *Aedes aegypti* [8, 9].

In insects, the haemolymph undergoes metabolic modification during the developmental stages [23, 24]. Energy expenditure while flying, running and swimming requires the mobilization of metabolites in insects, and the storage and release of carbohydrates, lipids, proteins and amino acids are strongly under endocrine control [25]. The exposure of an organism to xenobiotic product can modify the synthesis of certain proteins (enzymes of biotransformation, proteins of stress) and it contributes in the melanization of the insect when the cuticule takes place and then used for the tannin synthesis [11]. Indeed, beside the exposure to a chemical product, any fluctuations would happened, are related to the various physiological process of insect such as the moult, the nymphosis, the diapauses and metamorphosis process [26].

The bioassays of the mean metabolites (carbohydrates, lipids and proteins) in this study reveal a modification of the amounts in the whole body of treated larvae of *Culex pipiens* compared to control during different times of larval development (1, 3, 5 and 7 days). Biochemical analyses performed on fourth instar larvae of *Culex pipiens* after treatment with two lethal concentrations of novaluron reveal an increase in the contents of carbohydrates and the lipids in the treated larvae, compared to the control. Similar results were observed using other growth regulators against *Culiseta longiareolata* [3]. The quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as reproduction, maintenance and growth, and this balance is disturbed by any toxic product [27]. Carbohydrates are mobilized mainly from glycogen reserves in the fat body, under neuropeptide induction, resulting in increased level of soluble carbohydrates in haemolymph [25]. They form a group of important elements, some represent the energy source for the living organisms, either immediately usable, or in the form of reserves, others have a structural role like cuticle synthesis. The carbohydrates, as energy elements play a crucial role in the physiology of the insects, the rates of glycogen in tissues are closely related to the physiological events such as the reproduction, the moult and the flight [10] which are infected by the chitin synthesis inhibitor. The lipids represent the independent source of energy in insects and are transported via the haemolymph; from their synthesis site of storage, to be used at the time of vitellogenesis [26] and cuticular synthesis [28].

A biomarker represents a biological response of the impact or presence of xenobiotic in the organism, and not the direct evidence of this one. This response must be measured in an organism or its products and indicate a change compared to the normal state. This reaction cannot be detected at a healthy organism. The chitin synthesis inhibitor Novaluron is considered too as endocrine disrupting compounds, consequently it acts on the hormonal level in the haemolymph to announce the synthesis degradation or the inhibition of the metabolites. In this case the metabolite modification recoded is related to the presence of novaluron in the organism. Therefore the energy reserves are can to be considered as biomarkers that give information on the health condition of the organism [29]. In this case the metabolite modification recoded is related to the presence of novaluron in the organism. The high value of proteins recorded, during the larval stage at day 3 (Fig. 3C) is probably in relation with the transport of these proteins in the haemolymph, released from the mobilization of the reserves, destined for the synthesis of the new cuticule [30]. The proteins would also come, from the digestion of procuticulaire deep layers of the old cuticule and from an exogenic origin (31). The proteins enter at various reactions such as the hormonal regulation and are integrated in the cell as a structural element at the same time as the carbohydrates and the lipids [23]. The modification in the concentration of the proteins results from the volume of haemolymph changed under stress of the insecticide [24] and this explain the decrease in protein content of the treated larvae. This induction of proteins could be used as biomarker of exposure which is the response to an interaction between a xenobiotic agent and a molecule or target cell; like the athrocytes, in insect, which have been suggested to function in protein synthesis and secretion in the haemolymph [32].

Our results show that the total protein contents decrease after treatment by novaluron during the tested larval stage of the mosquito species, *Culex pipiens*. A fall of the proteinemy is observed also at *Leptinotarsa decemilneata* after

application of other growth regulators like 20E, RH-5849 and the RH-5992 [33] and RH-0345 reduced with the same way the rates of proteins of *Tenebrio molitor* pupae [34]. Azadirachtin, a phytogenic insect growth inhibitor, exhibited the same effects on the treated L4 and the L5 larvae of *Helicoverpa armigera* [35]. These results confirm the observed drop in protein amounts in treated series of fourth instar larvae of *Culex pipiens*. Whereas, a topical application of a juvenile hormone analogue, methoprene, on fly *Bactrocera cucurbitae* exhibit an increase level of the proteins [36].

In fact the metabolites essays did not explain clearly the mode of action of the chosen substance and it remains unclear if the observed effects are linked to endocrine disruption. Insect growth and morphogenesis is strictly dependent on the capability to remodel chitin-containing structures [37]. In the presence of chitin synthesis inhibitors, the final step of the chitin biosynthesis pathway is inhibited and the precursor is not converted in to chitin. Haemolymph volume changes under insecticide stress resulting in alteration in protein concentration [23]. These arguments support and explain the decrease in the body weight of the treated larvae and extend the duration of development stages.

Beyond the toxic effect, the novaluron may either act on the hormonal level in the haemolymph to announce the synthesis, degradation or the inhibition of proteins or on the neuro-secretion cells which control endocrine glands. It can also change the behaviour feeding of larvae towards action to avoid food and to maintain the metabolism of the body to the expenditure of storage out of cellular proteins. In most insects, the chitin synthesis inhibitor, novaluron exhibits a toxic effect by preventing metamorphosis at each of the larval molts and has a good potential in formulating novel IGR-based control agents against mosquitoes.

REFERENCES

[1] K. Gerch, L.A. Maung, A.F. Read. *Malaria Journal*, **2007**, 6(130): 1-9.
[2] N. Rehimi, N. Soltani**.** *Rev. Sci. Tech.*, **2002**, 18: 106-110.
[3] Tine-Djebar F, Soltani N. *Synthèse*, **2008**, 18: 23-34.
[4] T.S. Dhadialla, A. Retnakaran, G. Smagghe. Comprehensive Insect Molecular Science. L.I. Gilbert, I. Kostas, & S. Gill (eds). Pergamon Press, New York, **2004**.
[5] J.I. Arrendondo–jimenez, K.M. valdez-delgado. *Medical Veterin. Entomol.*, **2006**, 20: 377- 387.
[6] H. Cetin, F. Erler, A. Yanikoglu. *J. Insect Science*, **2006**, 6: 344-351.
[7] P. Jambulingam, C. Sanadanandane, N. Nithiyananthan, S. Sbramanian, M. Zaim. *J Am. Mosq. Control Assoc.*, **2009**, 25(3): 315-322.
[8] M.S. Mulla, U. Thavara, A. Tawatsin. *J. Vector Ecol.*, **2003**, 28: 241-254.
[9] T. Su, M.S. Mulla, M. Zaim. *J. Am. Mosq. Control Assoc.*, **2003**, 28: 241-254.
[10] C. Kaufmann, M.R. Brown. *J. Insect Physiol.*, **2008**, 54: 367-377.
[11] M.J. Rodriguez-Ortega, B.E. Grosvik, A. Rodriguez-Ariza, A. Goksoyr, J. Lopez-Barea. *Proteomics*, **2003**, 3(8): 1535–1543.
[12] WHO. Report of the eight WHOPES working group meeting. Review of: Novaluron 10% EC. WHO/*CDS/WHOPES*/**2005**.*10*.
[13] W.B. Abbott. *J. Econ. Entomol.*, **1925**, 18: 265-267.
[14] D.J. Finney. Probit analysis 3rd eds, Cambridge University Press, London, G.B, 1971.
[15] S. Swaroop, A.B. Gilroy, K. Uemura. *World Health Organisation*, **1966**.
[16] S. Shibko, P. Koivistoinen, C. Trayneck, A. New Hall, L. Freidman. *Analyt. Biochem.*, **1966**, 19: 415-528.
[17] G. Duchateau, M. Florkin. *Archs. Insect Physiol. Biochem.*, **1959**, 66: 573-591.
[18] G.J. Goldsworthy, W. Mordue, G. Guthkelch. *Gen. Comp. Endocr.*, **1972**, 18: 545-551.
[19] M.M. Bradford. *Analyt. Biochem.*, 1976, 72: 248-254.
[20] H. Boudjelida, A. Bouaziz, T. Soin, G. Smagghe, N. Soltani. *Pest. Biochem. Physiol.*, **2005**, 83: 115-123.
[21]H. Cetin, A. Yanikoglu, J.E. Silek. *J. Am. Mosq. Control Assoc.*, **2006**, 22(2): 343-345.
[22] A. Tawatsin, U. Thavara, P. Bhakdeenuan, J. Chompoosri, P. Siriyasatien, P. Asavadachanukorn, S.M. Mulla. *Southeast Asian J. Trop. Med. Public Health*, **2007**, 38: 434-441.
[23] E. Cohen. *Adv. Insect Physiol.*, **2010**, 38: 5-74.
[24] M. Sugumaran. *Adv. Insect Physiol.*, **2010**, 39: 151-209.
[25] G. Gäde. *Annual Rev. Entomol.*, **2004**, 49: 93-113.
[26] G. Zhou, R.L. *J. Insect Physiol.*, **2009**, 55: 40-46.
[27] L.E. Canavoso, Z.E. Jouni, K.J. Karnas, J.E. Pennington, M.A. Wells 1). *Annual Rev. Nutr.*, **2001**, 21: 23–46.
[28] L. Dapporto, D. Lambardi, S. Turillazzi. *J. Insect Physiol.*, **2008**, 54: 89-95.
[29] L.K. Lowry. *Toxicology Letters*, **1995**, 77: 31–38.
[30] J.H. Willis. *Insect Biochem. Molec. Biol.*, **2010**, 40: 189-204.
[31] N.C. Papandreou, V.A. Inconomidou, J.H. Willis, S.J. Hamodrakas. *J. Insect Physiol.*, **2010**, 56: 1420-1426.

[32] C. Owa, F. Aoki, M. Nagata. *J. Insect Physiol.*, **2010**, 56: 108-117.
[33] G. Smagghe, E. Vinuela, H.V. Limbergen, F. Budia, L. Tirry. *Entomol. Exp. Appl.*, **1999**, 30: 220-230.
[34] N. Soltani, N. Aribi, H. Berghiche, S. Lakbar, G. Smagghe. *Pestic. Biochem. Physiol.*, **2002**, 72: 83-90.
[35] N.K. Neoliya, S. Dwijendra, R.S. Sangwan. *Current Science*, **2007**, 92(1): 94-99.
[36] I.H. Haq, C. Caceres, J. Hendrichs, P. Teal, V. Wornoayporn, C. Stauffer, A.S. Robinson. *J. Insect Physiol.*, **2010**, 56: 1503-1509.
[37] H. Merzendorfer, L. Zimoch. *J. Exp. Biol.*, **2003**, 206(24): 4393–4412.