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STUDY OF THE TOXIC EFFECT OF CHEMICAL PESTICIDE ACTELLIC 50EC AGAINST *CULISETA LONGIAREOLATA* MOSQUITO LARVAE

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ABSTRACT

In this study, the toxicity of an insecticide, Actellic 50EC, was tested against L3 and L4 instars of mosquito larvae *Culiseta longiareolata* (Macquart). The newly exuviated larvae were subjected to various concentrations (25, 40, and 75 µg/ l) for 24 hr and out until the adult stage. Probit analysis for L3 stage showed that following obdsevations continued to adult stage LC_{50}=18.70 µg/ l and LC_{90}= 60.81 µg/ l. On the other hand, L4 stage shows these were LC_{50}= 36.42 µg/ l and the LC_{90}= 102.45 µg/ l, respectively, indicating that it is more toxic to L3. This study reveals that lipids and carbohydrates have decreased, while there is an increase in proteins, which has been associated in some cases with morphological deformations.

Key words: Organophosphate, pirimiphos-methyl, biochemical metabolite, Culicidian, L3 stage larvae, L4 stage larvae, lipids, carbohydrates, proteins, LC_{50}, LC_{90}, diseases, chemical stressors, morphological deformations.

Because of its role in the transmission of viral and parasitic diseases that can affect humans and animals, the Culicidian fauna has taken center stage in entomological news around the world (Salavati, et al., 2021). Several mosquito species are vectors of medical and veterinary zoonoses, responsible for the transmission of many human and animal diseases, including malaria, yellow fever, West Nile fever, dengue, and other arboviruses, causing millions of deaths (Abutaha et al., 2023; Das and Deobhankar, 2023). Controlling mosquito populations with insecticides is a key component of malaria prevention campaigns. Strategic approaches to vector control frequently rely on the use of multiple insecticides to avoid or overcome the recurrent emergence of resistance in natural populations (WHO, 2018). In this regard, various classes of insecticides have been developed (Ranson, 2011). Organophosphate pesticides are organic compounds containing phosphorus and are used as insecticides. They break down rapidly in sunlight and in contact with air and soil, although small amounts can be detected in food and drinking water (Grau-Bové et al., 2021). Pyrimiphos-methyl is a cheap organophosphate pesticide extensively used in mosquito control interventions for crop and food protection, particularly in Africa (Grau-Bové et al., 2021; Sherrard-Smith et al., 2018). Moreover, Pirimiphos-methyl is a fast-acting insecticide with less toxicity to humans and the environment (Tabbabi et al., 2017). Actellic 50EC is within pirimiphos-methyl, with excellent properties for protecting stored foodstuffs. It acts on the larval and adult forms of insects by contact, ingestion, and inhalation. This study evaluates the toxic effect of Actellic 50EC (pirimiphos-methyl 50%) against L3 and L4 Culiseta. *longiareolata* (Macquact) larvae. Additionally, the impact of lethal concentrations (LC_{50} and LC_{90}) on the metabolites of L4 mosquito larvae were estimated.

MATERIALS AND METHODS

Cs. longiareolata eggs and larvae were collected from untreated areas located in Annaba city (Algeria). The larvae were reared as previously described (Aissaoui et al., 2022). Each batch of 25 larvae being kept in small plastic bowl having 500 ml of tap water. They were fed daily with a diet of finely ground brewer yeast and Biscuit Petit Regal-dried yeast (3:1). Pirimiphos-methyl is a chemical compound belonging to organophosphates that inhibits acetylcholine esterase, an enzyme involved in the regulation of nerve impulses (Rambabu and Rao, 1994). It belongs to class III according to WHO classification, i.e. an insecticide of little danger to humans under normal conditions of use

(WHO, 2005a). The WHO has evaluated pirimiphos-methyl as a mosquito larvicide and adulticide. The trade name of pirimiphos-methyl is Actellic 50EC. Its chemical formula is O-[2-(diethylamino)-6-methyl-4-pyrimidinyl] O, O-dimethyl phosphorothioate. The larvicidal efficacy of pirimiphos-methyl has been determined against *Cs. longiareolata*. The toxicological tests were carried out with three concentrations (25, 40, and 75 µg/ l) of pirimiphos-methyl against newly exuviated larval stages (L3 and L4) of *Cs. longiareolata*, under laboratory conditions. Bioassays and control series were performed with three replicates of 25 larvae for each used concentration, prepared in a separate jar containing 500 ml of breeding water. After 24 hr of larvae exposure, according to the recommendations of the World Health Organization (WHO, 2005b), the water is changed, and the food is added every three days until when adults emerge. The mortality of the control series and the treated series is recorded daily and followed during the other stages of development until the emergence of the adults.

Carbohydrates, proteins, and lipids were extracted according to the method described by Shibko et al. (1966). Pooled samples (20 individuals of L4 larvae per pool) were weighed and extracted in 1 ml of trichloroacetic acid (20%). Briefly, protein quantification was performed by Bradford method (Bradford, 1976). Carbohydrates were determined by the anthracite method (Duchateau and Florkin, 1959). Lipids were measured by the vanillin method (Goldsworthy et al., 1972). The rate of mortality was corrected using Abbott's formula (Abbott, 1925) when mortality was observed in the control series. Subsequently, the toxicity data were subjected to probit analysis (Ficher, 1957) to determine the lethal concentrations (LC_{50} and LC_{90}) for the treated larvae of the tested species. The analysis included the calculation of confidence limits (95%) for the upper confidence limit values (LSC) and lower confidence limit (LSC), as well as the determination of the slope and regression equation of the concentration-mortality lines (Veerakumar et al., 2014). All statistical analyses were performed using MINITAB Software, and significance level of $p< 0.05$ was considered for determining statistically significant differences.

RESULTS AND DISCUSSION

Actellic 50EC exhibited a significant larvicidal effect ($p < 0.05$) against *Cs. longiareolata,* expressed by a relatively high mortality compared to control. The cumulative mortality rate varied between 64.4% (25 µg/ l) to 93.33% (70 µg/ l) in L3 larvae and from 34.66% to 80% in L4 larvae with a dose-response relationship. Both sublethal and lethal concentrations exhibited variation according to the periods after treatment (Table 1). The effectiveness of pirimiphos-methyl 50EC has been demonstrated in several studies across several mosquito species, including larvae of *Anopheles Culicifacies*, *Anopheles stephensi, Cx. quinquefasciatus*, and *Cx. pipiens* (Ansari et al., 2004). Kolaczinski et al. (2000) reported significant effectiveness of pirimiphos-methyl 50EC against adults of *Anopheles gambiae* and *Cx. quinquefasciatus*. Studies indicate that the combination of pirimiphos-methyl with *Bacillus thuringiensis* effectively killed both larvae and adult mosquitoes. Whether used alone or in combination with a biolarvicide, pirimiphos-methyl exhibits promising potential in the combatting *Ae. aegypti* (Lee et al., 2014). However, pirimiphos-methyl caused high levels of mortality in larvae and adults of *Tenebrio molitor* (Nickolas et al., 2019). In contrast, the utilization of carbamates, DDT, and pyrethroids has demonstrated substantial insecticidal activity against *An. gambiae, Culex* sp (Koumba et al., 2018). A similar observation was made by Aksorn and Mayura (2018) on *Aedes aegypti* larvae and nymphs, where mortality was found to be correlated with doses.

The effect on the development time of third and fourth instar larvae are shown in Table 2. The results show that Actellic 50EC interferes with larval growth in both L3 and L4 stages, leading to an extended duration of larval development. A significant difference was observed between the control and treated series, particularly with the concentrations (40 and 90 µg/ l) for the third and the fourth instar larvae ($p < 0.001$). Some morphological deformities were also observed in treated individuals

Table 1. Lethal concentrations (LC_{50} and LC_{90}, Fiducial limits (FL), µg/ l) of pirimiphos-methyl 50EC against third and fourth instar larvae of *Cs. longiareolata*

Larval stage	LC_{50}		LD_{90}		Hill slope	R^2	Regression equation
	µg/ l	95% FL	µg/ l	95% FL			
L3	18.70	15.84-21.87	60.81	51.53-71.75	2,48	0,97	y = 2,5049x + 1,8204
L4	36.42	32.51-40.79	102.45	91.47-114.74	1.91	0.99	y = 2,855x + 0,5534

Table 2. Effect of Actellic 50EC on the duration of development of larvae of *Cs. longiareolata*

Concentration (µg/ l)	Third instar	Fourth instar
Control	3.91± 0.28	6.86 ± 1.60
25	4.13± 0.40	7.53± 0.64 P=0.013*
40	5.43± 0.40* p=0.013	9.35± 0.76 P=0.016*
70	5.86± 0,11**p=0.008	11.16± 0.76 P=0.003**

Data means ± standard deviation (n= 10). Values on the same row with asterisk significantly different based on student tests ($p<0.05$)

(Fig. 1). This aligns with studies on novaluron and RH-0345, which reported effects on *Cs. longiareolata* and *Cx. pipiens* larvae development, including various morphological aberrations (Bouaziz et al., 2011; Djeghader, 2014; Bouaziz et al., 2017; Bouabida et al., 2017). Alouani et al., (2018) showed an extension in the duration of larval and pupal development of *Cx. pipiens* after treatment with azadirachtin. Similar effects are indicated, after novaluron treatment against *Palpita unionalis* larvae (Ghoneim et al., 2017), Morphological transformation stopped, and abnormalities appeared during treatment with a chitin formation inhibitor on the pupa of *Lobesia botrana* (Saenz-de-Cabeson et al., 2006). Similar deformities were recorded during the moulting of a lufenuron-treated *Schistocerca gregaria* pupa in the fifth instar larvae, resulting in adult color change and the inability of adults to shed the old cuticle with deformed wings (Bakr et al., 2009).

Actellic 50EC was tested on the fourth instar larvae of *Cs. longiareolata* with two lethal concentrations (LC_{50}= 32.42µg/ l and LC_{90}= 102.45µg/ l). Its effects were assessed on the biochemical composition (carbohydrates, lipids, and proteins) throughout the whole body at 2, 4, and 6 days after treatment. The comparison of mean values as given in Table 3 shows a significant increase in the total protein content after treatment with the highest concentration (controls vs LC_{50}; p=0.0002, control vs LC_{90} p=0.001) with a dose effect (LC_{50} vs LC_{90}: p=0.0034). Various studies have indicated that exposure to an organism to the xenobiotic product can modify the synthesis of certain proteins (biotransformation enzymes, stress proteins) (Curtis et al., 2010). Proteins play a crucial role in the development, growth, and accomplishment of vital activities of insects, as they are essential for enzymes that carry out the cascades of metabolic activities in organisms (Preet and Sneha, 2011). Proteins, such as chitin production and cuticle development, play a crucial role in insect metamorphosis. When insects are subjected to plant-based biopesticides, their whole-body protein levels may vary due to down regulation or up regulation (Parthiban et al., 2021; Parthiban et al., 2019). Lawrence and Olorunfemi (2019) reported an increase in tissue protein levels in adult *Cx. quinquefasciatus* following treatment with sublethal concentrations of cypermethrin. Previous studies suggest that the synthetic pyrethroid deltamethrin and the insect neurotoxin imidacloprid could increase protein content in *Nilaparvata lugen*s (Ge et al., 2009). Similar results were observed in *Sitophilus granarius* treated with azadirachtin (Guettal, 2021). Moreover, Lawrence and Olorunfemi (2019) reported an increase in tissue protein levels in *Cx. quinquefasciatus* adults following treatment with the highest sublethal concentrations of cypermethrin. An elevation in protein levels was revealed in *Cx. pipiens* following treatment with *Rosmarinus officinalis* essential oil (Zeghib et al., 2020). In contrast, treatment of *Cx. pipiens* larvae with entomopathogenic fungi, *Origanum glandulosum* EO and *Ocimum basilicum* EO led to a drop in total protein concentrations (Bouguerra and Boukoucha, 2021; Hamama et al., 2021). Variations in protein content likely reflect the balance between synthesis, storage, transport, and degradation of structural and functional nutrients during ontogenesis as well as the response to particular physiological conditions (Shoukry et al., 2003).

(A) (B) (C)

Fig. 1. The morphological aberrations observed on the larvae after treatment A: Incomplete metamorphosis (L3- l4), B: Incomplete metamorphosis (larva - pupa), C: The adult undergoes partial pupation limited to the anterior part

Regarding carbohydrate content, the treatment causes a significant decrease with a dose-response effect (controls vs LC_{50}: p=0.0406; controls vs LC_{90}: $p<0.001$; LC_{50} vs LC_{90}: $p<0.001$). The results show a significant reduction in whole-body carbohydrate levels of treated larvae (Table 3**)**. Similar results were noticed by Lawrence and Olorunfemi (2019), who inferred that tissue carbohydrate levels decreased in larvae and adults of *Cx. quinquefasciatus* treated with cypermethrin. Ali Mohamadi et al. (2014) reported reduced glycogen content in fourth-instar larvae of *Hippodamia variegate* after treatment with hexaflumuron and spirodiclofen.

Table 3. Effect of Actellic 50EC (LC_{50}= 32,42µg/ l and LC_{90}= 102, 45µg/ l) on proteins, carbohydrates, and lipids amounts (µg/ individual) in the fourth instar larvae of *Cs. longiareolata*

Content (µg/ individual)	Time (days)	Control	LC_{50}=32,42 µg/ l	LC_{90}=102, 45 µg/ l
Proteins	2	123,5± 12,61[a]	185,336± 4,86[b]	230,5± 9,55[c]
	4	118,60± 1,50[a]	175,03± 1,86[b]	218,66± 2,36[c]
	6	113,16± 4,04[a]	144,33± 3,51[b]	216,66± 3,05[c]
Lipids	2	75,66± 5,13[a]	55,16± 3,01[b]	44,16± 3.83,01[c]
	4	63,11± 11,51[a]	54,66± 2,75[a]	50,33± 2,51[a]
	6	54,33± 6,02[a]	49,33± 1,52[a]	48,33± 3,21[a]
Carbohydrates	2	123,33± 11,93[a]	117,33± 4,53[a]	86,66± 9,01[b]
	4	118,83± 7,11[a]	111,66± 7,09[a]	79,33± 8,50[b]
	6	102,7± 7,26[a]	95,16± 10,20[a]	71,66± 11,71[b]

Data expressed as means ± standard deviation (n = 3 pools each containing 20 individuals of L4 larvae). Significance at $p<0.05$. Values on the same row with different letters (a, b, c) are significantly different.

Bouabida et al. (2017) showed a significant reduction in carbohydrate levels only with the highest dose. A significant increase in carbohydrate content was reported after treating *Cx. pipiens* larvae with *Tecoma stans* ethanolic extracts (Hafsi et al., 2022). The depletion of glucose may be attributed to the stress conditions, requiring more energy to cover energy expenditure via induction by neuropeptides (Yazdani et al., 2014).

The lipid content shows a significant decrease after treatment with Actellic 50EC with the two applied concentrations without dose effect (controls vs LC_{50}: $p=0.0245$; controls vs LC_{90}: $p=0.0036$; LC_{50} vs LC_{90} ($p>0.05$) (Table 3). Treatment of adult *Cs. longiareolata* with Actellic 50EC induced a significant decrease in lipid content. The same observations were made in *S. granarius* treated with lemon and azadirachtin (Guettal, 2021), The effects of spirodiclofen and hexaflumuron have also been studied on certain physiological changes of late-stage larvae of *H. variegate* by measuring total lipid contents (Ali Mohamadi et al., 2014); and after the treatment of *Cx. pipiens* and *Cs. longiareolata* larvae with methoxyfenozide (Draouet et al., 2020), and larval treatment with *Foenilucum vulgare* oil extracts (Keffous and Aissaoui, 2023). A similar reduction in these energy reserves has been observed in previous studies on different types of stressors: environmental (Muturi et al., 2011), nutritional or botanical (Vantaux et al., 2016), and chemical stressors (Preet and Sneha, 2011). The depletion of this biochemical component after treatment is due to the stress induced following exposure to an insecticide (Sancho et al., 1998), resulting in alteration in synthesis (Klowden, 2007), hormonal dysfunction controlling lipid metabolism, the use of this metabolic reserve (Sak et al., 2006), and increased lipolysis to provide energy (Lohar and Wright, 1993).

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AUTHOR CONTRIBUTION STATEMENT

BS and AB conceived and designed research. RC and HG conducted experiments. AB, BS, RC and HG analyzed data. AB and BS wrote the manuscript. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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