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Activity of a lipid synthesis inhibitor (spiromesifen) in *Culiseta longiareolata* (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the activity of spiromesifen against the most abundant and investigated mosquito species, *Culiseta longiareolata* Aitken, 1954 (Diptera, Culicidae).
Methods: *Culiseta longiareolata* larvae were collected from untreated areas located at Tébessa (Northeast Algeria). A commercial formulation of spiromesifen (Oberon® 240 SC) was tested at different concentrations ranging between 238 and 1 428 µg/L on newly molted fourth-instar larvae under standard laboratory conditions according to Word Health Organization recommendations. The effects were examined on the mortality, the morphometric measurements, two biomarkers (catalase and malondialdehyde), and the biochemical composition of larvae, respectively.
Results: The compound exhibited insecticidal activity. Moreover, it disturbed growth and several morphological aberrations were observed. It also affected body volume, biomarkers and contents of carbohydrates, lipids and proteins. A marked effect on lipids and malondialdehyde was noted, confirming its primary mode of action on lipid synthesis.
Conclusions: Spiromesifen appears less potent than other insecticides tested such as the insect growth disruptors.

1. Introduction

Vector control is an essential requirement in control of epidemic diseases that are transmitted by mosquitoes [1]. These diseases that cause morbidity, mortality, economic loss, and social disruption are well-documented [2]. *Culiseta longiareolata* (*C. longiareolata*) is the most interesting mosquito species in Algeria, particularly in Tébessa area [3]. The control of mosquito larvae by chemical substances is not safe at present because of environmental imbalance and insecticide resistance [4]. Spiromesifen is a systemic insecticide/acaricide belonging to the class of spirocyclic tetronic/tetramic acid derivatives. It acts on lipid synthesis by inhibiting acetyl CoA carboxylase [5] and causes a significant decrease in total lipids [6,7]. This compound has been introduced in several countries over the last few years and is becoming an important compound for controlling whiteflies and mites in resistance management programmes, along with other effective insecticides such as neonicotinoids and diafenthiuron. Because of its high selectivity, good residual activity, minimal risk to pollinators and predatory mites [8,9] combined with a novel mode of action make spiromesifen as an excellent new tool for many integrated pest management programs [10].

Several recent studies have shown the effectiveness of spiromesifen against a variety of insect pests [11,12]. The carbohydrates play a crucial role in the physiology of insects and the rates of glycogen in tissues are closely related to the physiological events such as the flight, the moult and the reproduction [13]. In addition, lipids play an important role in general metabolism and reproduction [14]. Moreover, the fatty acids constitute precursors of cuticular hydrocarbons and pheromones [15]. Malondialdehyde (MDA), a product of lipid peroxidation, has been widely used as a marker of free radical damage to lipid molecules [16].

Previously, it was reported that spiromesifen was found to reduce the amounts of body lipids and to enhance the rate of

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MDA [7] and to affect the amounts of carbohydrates, glycogen and the activity of lactate dehydrogenase [17] in *Drosophila melanogaster* pupae. In the present study conducted under laboratory conditions on *C. longiareolata*, a medically important mosquito species, the lethality parameters of spiromesifen against fourth-instar larvae were determined. In a second series of experiments, the metabolic responses were investigated following spiromesifen exposure by measuring catalase (CAT) activity and MDA rate, biomarkers of oxidative stress and lipid peroxidation, respectively. In addition, its effects on morphometric measurements and on main biochemical components (carbohydrates, proteins and lipids) in whole body were investigated. The data obtained provide better insights on its mode of action and give information on its potential for use as a mosquito control agent.

2. Materials and methods

2.1. Mosquito rearing

C. longiareolata originated from eggs and larvae were collected in 2016 from untreated areas located at Tébessa (Northeast Algeria). Larvae specimens were morphologically identified according to Brunhes *et al.* [18] and kept as previously described [19]. Pyrex storage jars (80 mm × 100 mm) containing 150 mL of tap water were maintained at temperature 25 °C and a photoperiod of 14:10 (L:D). 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.

2.2. Toxicity bioassays

The insecticidal assay was conducted in 2016 as previously described by Boudjelida *et al.* [20]. A trade formulation of spiromesifen (Oberon® 240 SC, Bayer Crop Science) courtesy of Pr. G. Smagghe (Ghent University, Belgium) was added to treatment beakers at different final concentrations (238, 476, 714, 952 and 1428 µg active ingredient per litre). Newly molted fourth-instar larvae of *C. longiareolata* (<8 h) were exposed to the different concentrations for 24 h in accord with World Health Organization criteria [21]. Controls were exposed to water only. After the exposure time of 24 h, larvae were removed, washed with untreated water and placed in clean water. The test was carried out with 4 replicates containing each 25 larvae per concentration. Growth was examined and mortality was registered daily until adult emergence. The mortality percentage obtained was corrected [22] and toxicity data was studied by probit analysis [23]. Lethal concentrations (LC_{50} and LC_{90}) and 95% confidence limits (95% *CL*) were estimated, and slope of the concentration–mortality lines were calculated [24].

2.3. Determination of CAT activity and MDA rate

CAT activity was measured by determining the decomposition of its substrate H_2O_2 as described by Claiborne [25]. Each sample (3 pools each containing 10 individuals) was conserved in buffer phosphate (100 mM; pH 7.4). After sonication and centrifugation (15000 rpm for 10 min), the supernatant was collected and used for the determination of the CAT activity. The protein amount in the total homogenate was quantified according to Bradford [26]. The absorbance was red at 240 nm. The assay was conducted with 6–8 repeats and data expressed as µmol/min/mg protein.

The rate of MDA was determined as lipid peroxidation index according to Draper & Hadley [27]. This method was based on a spectrophotometric measurement of the reaction of thiobarbituric acid with MDA at 532 nm. The protein content was evaluated according to Bradford [26] using bovine serum albumin as standard (BSA, Sigma). The rate was expressed as µmol/mg protein.

2.4. Morphometric measurements

As above, newly moulted fourth instar larvae were treated with spiromesifen at its LC_{50} and LC_{90} as determined before. The morphometric measurements were performed following the procedure of Timmermann & Briegel [28]. The body volume corresponds to cubic value of width.

2.5. Biochemical composition of body

Protein, carbohydrate and lipid were extracted following the procedure of Shibko *et al.* [29] and quantified as previously described [30]. Newly moulted larvae were collected. Pooled samples (10 individuals per pool) were weighed and extracted in 1 mL of trichloracetic acid (20%). In brief, quantification of proteins was carried following the Coomassie Brilliant Blue G-250 dye-binding method [26] with bovine serum albumin as a standard. The absorbance was measured at 595 nm. Carbohydrates were determined according to Duchateau & Florkin [31] using anthrone as reagent and glucose as standard. Lipids were measured by the vanillin method [32] and the table oil (99% triglycerides) used as a standard. Data were expressed in µg per individual and assays conducted with 3 replicates per treatment.

2.6. Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as mean ± SD. The significance between different series was tested using Student's *t* test. All statistical analyses were performed using MINITAB Software (Version 16, PA State College, USA) and $P < 0.05$ was considered to have statistically significant difference.

3. Results

3.1. Insecticidal activity

Dose–response relationship was determined for spiromesifen and applied for 24 h to newly molted fourth instar mosquito larvae. The mortality was scored up to adult emergence. The highest concentration tested (1428 µg/L) caused 95.88% ± 4.01% mortality. With probit, LC_{50} was calculated as 555.37 µg/L (*CL* 95% = 437.93–650.80 µg/L), slope = 2.01 and LC_{90} was 1366.70 µg/L (*CL* 95% = 1062.85–1757.40).

3.2. Effects on MDA and CAT

The results summarized in Table 1 show that the rate of MDA in control and treated groups increased during the fourth-

Table 1
Effect of spiromesifen (LC_{50} and LC_{90}) on the MDA and CAT in the fourth instar larvae of *C. longiareolata* (mean ± SD, $n = 3$ pools each containing 20 individuals).

Treatment	MDA (μM/mg)			CAT (μM/mg)		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.120 ± 0.000	0.122 ± 0.001	0.126 ± 0.000	5.190 ± 0.100	5.340 ± 0.010	5.110 ± 0.090
LC_{50} spiromesifen	0.183 ± 0.025	0.195 ± 0.000	0.207 ± 0.000	5.990 ± 0.080*	6.200 ± 0.100*	7.380 ± 0.050*
LC_{90} spiromesifen	0.235 ± 0.001*	0.251 ± 0.003	0.260 ± 0.000*	6.000 ± 0.020*	8.510 ± 0.210*	11.460 ± 0.070*

*$P < 0.05$ compared with control group.

Table 2
Effect of spiromesifen (LC_{50} and LC_{90}) on body weight, body volume, proteins, carbohydrates and lipids in fourth instar larvae of *C. longiareolata* (mean ± SD, $n = 3$ pools each containing 10 individuals).

Treatment	Body weight (mg)	Body volume (mm^3)	Protein content (μg)	Carbohydrate content (μg)	Lipid content (μg)
Control	5.23 ± 0.15	6.97 ± 0.92	87.91 ± 7.96	170.46 ± 14.53	60.57 ± 2.31
LC_{50} spiromesifen	4.13 ± 0.15*	4.47 ± 0.20	73.08 ± 6.37*	167.94 ± 6.47	45.39 ± 2.73*
LC_{90} spiromesifen	3.20 ± 0.26*	3.78 ± 0.28*	65.69 ± 8.56*	160.10 ± 6.59	29.23 ± 1.77*

*$P < 0.05$ compared with control group.

instar larval stage but in a non-significant manner ($P > 0.05$). The comparison between control and treated series (LC_{90}) revealed a significant increase in the rate of MDA at 24 h ($P = 0.03$) and 72 h ($P = 0.001$).

As regards the activity of CAT, the values present a significant increase ($P < 0.05$) in control and treated series. There was a significant difference between control and treated series at all ages. The increase in the activity of CAT was also significant at 24 h (control *vs* LC_{50} $P = 0.016$ and control *vs* LC_{90} $P = 0.006$), 48 h (control *vs* LC_{50} $P = 0.007$ and control *vs* LC_{90} $P = 0.003$) and 72 h (control *vs* LC_{50} $P < 0.001$ and control *vs* LC_{90} $P < 0.001$), respectively (Table 1).

3.3. Effects on weight and volume of body

Changes in whole body weight showed a significant reduction in weight of fourth instar larvae (control *vs* LC_{50} $P = 0.007$ and control *vs* LC_{90} $P = 0.038$). Also, spiromesifen significantly ($P = 0.042$) reduced the body volume of fourth instar larvae only with the highest concentration (LC_{90}) compared to controls (Table 2).

3.4. Effects on biochemical composition of bodies

The levels of carbohydrates, lipids and proteins have been estimated in the whole body extracts from fourth larval stage using two lethal concentrations (LC_{50} and LC_{90}). The comparison of mean values shows that the protein content decreased significantly with the two concentration (LC_{50} and LC_{90}) (control *vs* LC_{50} $P = 0.014$ and control *vs* LC_{90} $P < 0.001$). No effect of the product on the carbohydrate content was reported with the two doses applied ($P > 0.05$). Lastly, the lipid content was reduced significantly with the two tested concentrations (control *vs* LC_{50} $P = 0.027$ and control *vs* LC_{90} $P = 0.009$) (Table 2).

4. Discussion

The intensive use of synthetic pesticides has caused secondary effects on the environment [33]. The application of spiromesifen and buprofezin on *Bemisia tabaci* and *Bemisia argentifolii* caused a reduction in the number of population particularly in the nymphal stage [34,35]. In addition, the same product used at different doses (0.0024, 0.024, 0.24, 2.4 and 24 mg/L) exhibited an insecticidal activity on *Bactericera cockerelli* (Hemiptera: Triozidae) nymphs [36]. In the current study, spiromesifen tested on fourth-larval instar of *C. longiareolata* was found to present an LC_{50} of 555.37 μg/L and an LC_{90} of 1 366.70 μg/L. The study of Djeghader *et al.* [37] conducted in *Culex pipiens* using novaluron, an inhibitor of chitin synthesis showed the following values LC_{50} and LC_{90} were 0.32 and 1.2 μg/L for the third-instar larvae; while these respective values were 0.58 and 2.20 μg/L for the fourth-instar larvae. In addition, the chitin synthesis inhibitors appeared more potent against *Culex pipiens* larvae as compared to molting hormone agonists, juvenile hormone analogues or spiromesifen against *C. longiareolata*. Spiromesifen and acetamiprid were effective in reducing the sucking pests of chilli *viz.* mites and thrips, without significantly affecting the natural enemies [38]. The research of Halder *et al.* [39] revealed that maximum reduction in mite and thrips population was obtained in treatment (0.6 ml/L) with spiromesifen (57.43% and 58.29% respectively). The study of Ghadim Mollaloo *et al.* [40] revealed that treating *Neoseiulus californicus* (Acari: Phytoseiidae) with increasing lethal concentration of spiromesifen decreased population numbers, the estimated values of LC_5, LC_{10} and LC_{15} are found to be 5.834, 9.529 and 13.267 ppm, respectively.

The effects of different newer acaricides were evaluated on the life stages of *Tetranychus urticae* (Acarina: Tetranychidae), spiromesifen was found to be toxic to the adults ($LC_{50} = 12.53$ ppm) [41]. Diafentheuron and Spiromesifen were found to be most effective in reducing population of whitefly and also provide a good response to the yield over control [42].

To contribute to an understanding of these mechanisms, we assessed the effect of the spiromesifen on the activity of a biomarker of oxidative stress (MDA, CAT) in *C. longiareolata*. The results show a significant increase in the rate of MDA following exposure to spiromesifen (LC_{50} and LC_{90}) compared to controls confirming our previous experiment made in *Drosophila melanogaster* [7]. Spiromesifen treatment also induced an increase in the CAT activity in the fourth instar larvae of *C. longiareolata*. This increase in activity reflects an

establishment of the process of detoxification, which is a form of defence of the insect against the pesticide [43].

The body size is an important trait for mosquitoes because of its influence on the blood-feeding ability, host attack rate and fecundity. All of these traits are determinants for their potential to transmit diseases [44]. In the present study the application of spiromesifen (LC_{50} and LC_{90}) induced a significant decrease in the weight and the volume of larva body of the fourth stage in *C. longiareolata* compared to controls. Novaluron was also found to reduce the body weight significantly in third and fourth larval stage of *C. longiareolata* [45].

In insects, the hemolymph undergoes metabolic modification during the developmental stages [46,47]. The exposure of an organism to xenobiotic products can modify the synthesis of certain metabolite and disturb its functionality [48]. Biochemical analyses revealed a decrease in the levels of lipids and proteins in whole body extracts in spiromesifen treated larvae as compared to control series. The reduction in protein levels observed in *C. longiareolata* larvae might be due to their degradation for metabolic ways or to an impaired incorporation of amino acids into polypeptide chains or inhibition of protein synthesis. As reported by Ghasemi *et al.* [49] pyriproxyfen caused a significant decrease in protein contents in *Plodia interpunctella* (Lepidoptera). Similarly, treatment of *Bemisia tabaci* with spiromesifen was found to affect the lipid contents [35]. The effects of spirodiclofen and hexaflumuron were also investigated on some physiological changes of the last instar larvae of *Hippodamia variegate* by measuring total lipid contents [50]. Our results showed no effect in carbohydrate levels. Ali Mohamadi *et al.* [50] reported a reduction in glycogen contents in fourth instar larvae of *Hippodamia variegate* after treatment with hexaflumuron and spirodiclofen.

This study was conducted to offer a preliminary understanding of the role played by spiromesifen against *C. longiareolata*. The results obtained using spiromesifen applied at two lethal concentrations (LC_{50} and LC_{90}) on fourth instar larvae showed that treatment disrupt the biochemical composition, as well different morphometric measurements. Moreover, this compound constitutes a good alternative to neurotixic insecticides for mosquito control.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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