Original Article

Effect of Three Insect Growth Regulators on The Avian Malaria Vector-Culiseta longiareolata Larvae- Field Population

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ABSTRACT

A number of species within the Culicidae family are responsible for the transmission of pathogens to animals and humans. The study of these species and the fight against these natural enemies represent a significant area of concern for scientists in the present era. An inventory of Culicidae in the M'chouneche region (34° 56' 59.99" N, 6° 00' 0.00" E) (Biskra, southeastern Algeria) was conducted in various breeding sites between November 2022 and May 2023. Four species of Culicidae were identified: Culiseta longiareolata, Culex pipiens, Culex theileri, and Anopheles multicolor. To assess the efficacy of three insect growth regulators (Lufenuron, Teflubenzuron, and Spirotetramat) on the fourth larval stage of Cs. longiareolata, control tests were conducted under experimental conditions. Lufenuron demonstrated a markedly higher toxic effect, with a mortality rate of 57% (ranging from 0 to 100%), compared to Spirotetramat, which exhibited an average mortality rate of 37.71% (ranging from 0 to 80%), and Teflubenzuron, which showed an average mortality rate of 12.08% (ranging from 0 to 45%). The mortality rates demonstrated an increase from one concentration to the next over time. Furthermore, the correlation coefficient between the two factors (time and concentration) and the mortality rates was found to be low at 30%. Individuals that were treated after reaching the adult stage exhibited a notable delay in their development. For concentrations of 20 mg/L and 40 mg/L, the delay duration was approximately two days ± 12 hours. In contrast, the third concentration (80 mg/l) resulted in a development delay of approximately three days±15 hours.

Keywords: M'chouneche, Lufenuron, Teflubenzuron, Spirotetramat, Culiseta longiareolata.

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1. Introduction

Biting insects are the vectors for numerous vector-borne diseases in humans and animals. Typically, these vectors are arthropods that ingest pathogenic microorganisms from an infected host during a blood meal and inject them into a new host (1). Indeed, the disparate roles these vectors play in epidemiology present significant challenges to public health and the global economy (2). Some of these diseases, including malaria, leishmaniasis, and dengue, can be fatal in the absence of treatment (3). As reported by the World Health Organization, these diseases represent 17% of the estimated global burden of all infectious diseases and result in more than one million deaths annually (1). The most effective means of controlling these conditions is to gain as comprehensive an understanding as possible of the vectors that transmit them. Among these vectors, mosquitoes are the most well-known, belonging to the Culicidae family (4). The Culicidae are the most harmful to human populations and are feared for their role in transmitting parasitic diseases during their bites. They act as vectors for a number of pathogens. The pathogens transmitted by mosquitoes include Plasmodium, Filaria, bacteria, and numerous arboviruses (5). Over the past two decades, numerous studies have been conducted on the Culicidae fauna in Algeria, with particular emphasis on systematic, biochemistry, morphometrics, chemical and biological control measures (6, 7). These studies have been conducted by various researchers in the country. Insect growth regulators are chemical compounds that alter the normal developmental profile of insects, causing metabolic errors and asynchronous development, which ultimately results in the death of the insect. The objective of this study is to assess the impact of three insect growth regulators (Lufenuron, Spirotetramat, and Teflubenzuron) on the development and behavior of Cs. longiareolata larvae collected from the M'chounech region over a five-month period (December 2022 to April 2023).

2. Materials and Methods

2.1. Sampling of Mosquitoes

Over a five-month period, a comprehensive survey of mosquito sample collections was conducted in the M'chnounech Valley. The valley is characterized by clear waters and a large palm grove, which provides abundant shade. The landscape is punctuated by prominent breaks in rock and clay. The immature individuals were collected using the dipping method (9). The collection was conducted using a ladle with a capacity of 500ml. Some of the collected individuals were preserved in 75% ethanol, while the remaining samples were transferred to the laboratory for breeding after morphological identification (Figure 1).

2.2. Insects Regulators Growth Preparation

The preparation of sub-lethal doses for the three growth regulators (lufenuron, spirotetramat, and teflubenzuron) commenced with preliminary tests to ascertain suitable doses for the toxicity tests. The dilution was conducted by combining 50 grams of the raw material with 100 milliliters of distilled water.

2.3. Toxicological Tests

The larval stage L4 of Cs. longiareolata is employed for the purpose of evaluating the impact of these insect growth regulators on the development of exposed individuals. The toxicological parameters for the three products under investigation were monitored using the method of logarithmic regression of decimal concentrations (X) against probits (Y) in accordance with the Fisher and Yates technique (1957) (10), thereby enabling the estimation of the lethal doses LD50 and LD90 as per Finney (1944). The data were analyzed using the statistical software package SPSS V19.00.

2.4. Statistical Tests

The statistical analyses of the data presented in this study commenced with a Shapiro-Wilk test to ascertain the normality of the data. The Kruskal-Wallis test, the Spearman-Kappa correlation, and graphical representations were tested using SPSS V19.0.

3. Results

The species collected in the surveyed sites of the M'chounech region and in the valley of this region have been found to exhibit the presence of two subfamilies (Culicinae and Anophelinae) distributed among four species. The species identified were Culex pipiens, Culex theileri, Culiseta longiareolata, and Anopheles multicolor. With regard to the toxicological tests, the mortality of Cs. longiareolata larvae exposed to the three products (teflubenzuron, spirotetramat, and lufenuron) was statistically tested and demonstrated through the Shapiro-Wilk test that the P values were less than 0.05. Accordingly, the data are not normally distributed, irrespective of the three exposure times, doses employed, or the three products utilized, as evidenced by the normality table. The Kruskal-Wallis test was employed to assess the three products and the three doses, yielding a statistically significant result ($\chi2$ =36.44; df=3; P≤ 0.000) and ($\chi2$ =16.37; df=2; P≤ 0.000), respectively. Nevertheless, the Kruskal-Wallis test applied for the four exposure times yielded a low, statistically significant difference ($\chi2$ =6.45; df=2; P= 0.040). The mortality rates exhibited elevated values for lufenuron, with an average rate of 57%, spanning a range of 0 to 100% (with maximum and minimum values). In the second position, Spirotetramat exhibited an average mortality rate of 37.71%, with a range of 0 to 80%. In the third position, Teflubenzuron exhibited an average mortality rate of 12.08%, with a range of 0 to 45% (Figure 2). As illustrated in Figure 2, the data indicate that lufenuron was the most efficacious product, followed by spirotetramat, and finally teflubenzuron across all concentrations tested (20, 40, and 80 mg/L). Mortality rates demonstrated a positive correlation with both dose and exposure time, which ranged from 24 to 72 hours (Figure 3). In consideration of the calculated toxicological parameters (LD50 and LD90) and their associated

confidence intervals, a ranking of the three products according to their toxicities against individuals of Cs. longiareolata can be established. Lufenuron is the most toxic, followed by Spirotetramat, and finally, Teflubenzuron, which has a lesser effect than the others on the molting and morphological transformation of mosquito bodies (Table 1). The linear regression curve between the mortality rate, exposure time, and concentration revealed a Pearson correlation coefficient (calculated for non-normally distributed data), indicating low R-squared values. In particular, the R-squared value was approximately 24.6% for the concentrations employed and 8% for the exposure time (Figure 4).

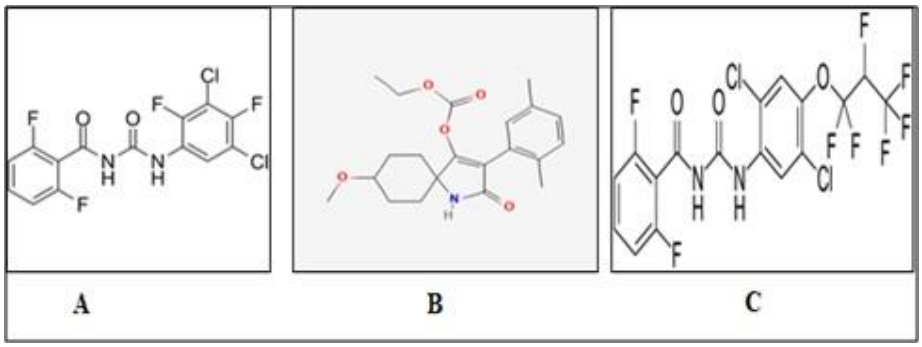

Figure 1: Chemical structure of A: Teflubenzuron; B: Spirotetramat; and C: Lufenuron.

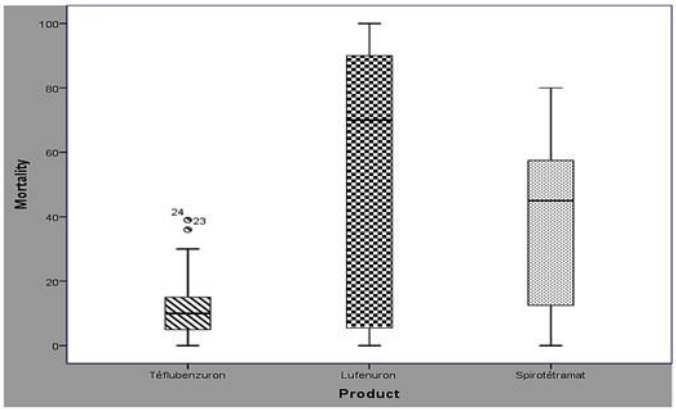

Figure 2: Mortality rate of the three Insect growth regulator.

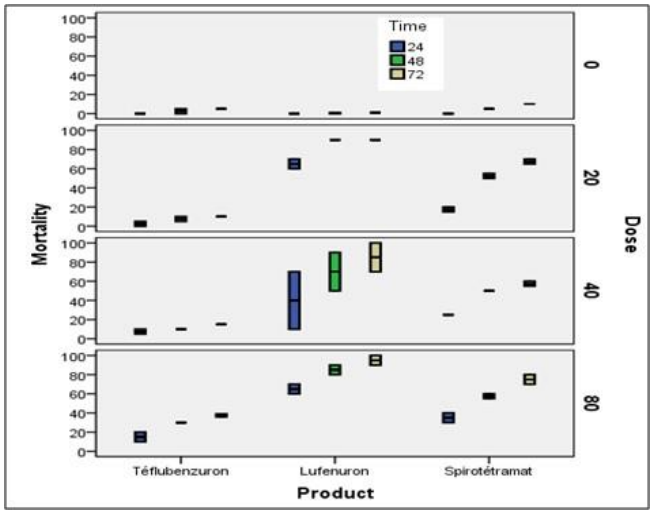

Figure 3: Mortality rate of the concentrations used of the three Insect growth regulators

Table 1. Toxicological parameters of the three Insect growth regulators.

Product	$LLLD_{50}<LD_{50}<ULLD_{50}$ mg/l	$LLLD_{90}<LD_{90}<ULLD_{90}$ mg/l
Lufenuron	24.76<6.65<40.19	54.95<43.16<71.86
Spirotetramat	4.58<24.09<50.92	79.72<60.94<106.93
Teflubenzuron	34.58<39.07<79.15	103.81<90.94<126.07

LLLD: low limit of lethal dose; **LD**: lethal dose; **ULLD**: upper limit of lethal dose.

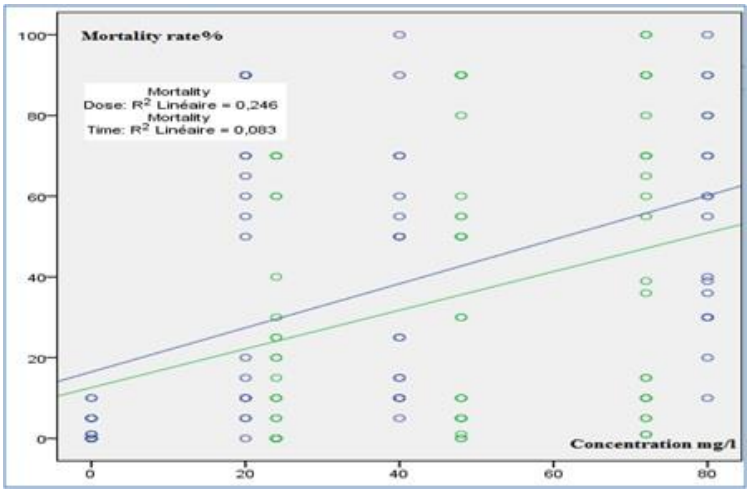

Figure 4: linear regression between mortality rate, times and concentration.

4. Discussion

Insect growth regulators (IGRs) are chemical agents that interfere with the typical developmental and growth processes of insects. These chemicals are frequently employed in pest control programs to target specific pests while exerting minimal influence on non-target organisms, including humans and other animals. In the context of mosquito control, IGRs have demonstrated efficacy in regulating mosquito populations by interfering with their life cycle. Mosquitoes undergo a four-stage life cycle, which can be described as follows: egg, larva, pupa, and adult. The larval stage, which occurs in water, is the typical target of IGRs. The molting process of mosquito larvae is disrupted by IGRs. Mosquito larvae undergo a process of molting, or exoskeleton shedding, as they develop. The process of molting is disrupted by IGRs, preventing the larvae from developing into pupae and subsequently into adult mosquitoes. The manner in which insect growth regulators (IGRs) act is contingent upon the targeted developmental stage. They can be classified into two principal categories: "juvenile" IGRs and "chitin-synthetase" IGRs (11). Insect growth regulators (IGRs) are frequently employed in vector control programs targeting mosquitoes. Such agents can be deployed in areas of stagnant water where larvae develop, including marshes, ponds, and reservoirs. The deployment of IGRs can serve to diminish the mosquito population by impeding their capacity to reproduce and develop (12). It is crucial to acknowledge that the utilization of insect growth regulators (IGRs) for vector control purposes necessitates the adherence to specific guidelines, which vary across different geographical regions (13). The results of our tests indicate that Lufenuron exhibits greater toxicity than the other products when used against Cs. longiareolata. The results of this test are presented herein. The mortality of larvae exposed to lufenuron and spirotetramate was found to be significantly higher at the three doses of 20 mg/l, 40 mg/l, and 80 mg/l, in contrast to teflubenzuron, which exhibited lower mortality than the first two. Therefore, elevated mortality rates are observed when the treatment concentration is increased and the exposure time is extended. This can be interpreted as an indication that a greater quantity of the product is being administered over an extended period of time. Conversely, the mortality rate declines as the concentration decreases. The specific effect of regulators of growth (IGRs) on larval growth has been observed to result in either a slowdown or cessation of growth at low concentrations, in comparison to control groups. In the study conducted by Piri et al., the impact of lufenuron was examined in conjunction with specific biological and biochemical treatments of Glyphodes pyloalis. Lufenuron demonstrated potent toxicity against G. pyloalis larvae, exhibiting lethal effects (LC50=19 ppm) and sublethal effects (LC10=3.74 and LC30=9.77 ppm) when compared to the fourth instar larvae of G. pyloalis (14). In a study conducted by Butter in 2003, the toxicity of lufenuron was evaluated against Helicoverpa armigera on

cotton. The potency of the IGR against the larval stages of the pests was demonstrated, with the CL90 values for larvae in the 1st, 2nd, 3rd, 4th, and 5th stages being 5.63, 7.89, 8.03, 11.39, and 14.76 mg/L, respectively. Nevertheless, no significant difference was observed between the different larval stages in terms of CL50 and CL10. The degree of head swelling in the larvae treated with the IGR was markedly diminished (1.5-2.3 mm) in comparison to the untreated controls (2.9 mm). The weight of the larvae was significantly reduced, from 190 mg in the control group to 50-70 mg in the group treated with lufenuron (15). In 2011, Acheuk presented a series of studies investigating the insecticidal activity of teflubenzuron and its impact on the chitin and cuticular protein content in the L5 larvae of the migratory locust, Locusta migratoria cinerascens. The product was administered orally at doses of 2.5, 5, 10, 15, 20, and 25 µg/larva. The results demonstrated that the product exhibited good larvicidal activity (16). The findings of the study conducted by Fansiri et al. indicated that teflubenzuron exhibited a reduction in egg hatching rates among wild mosquito species, in comparison to laboratory species. The administration of teflubenzuron (1-5 ppm) resulted in a reduction of Culex quinquefasciatus larvae in ditches by 40-90% (17). In a separate study, Assar et al. investigated the impact of three insect growth regulators on the second larval instar of Culex pipiens at varying concentrations. Their findings indicated that Novaluron exhibited superior efficacy, followed by teflubenzuron and hexaflumuron. Rumbos and Athanassiou (19) corroborate the efficacy of teflubenzuron against larvae of Culex pipiens pipiens and Culex pipiens molestus in laboratory settings. The impact of insect growth regulator insecticides on fourth-instar nymphs and adults of the neotropical brown stink bug Euschistus heros was assessed under laboratory and greenhouse conditions. These findings corroborate the efficacy of teflubenzuron in reducing stink bug fecundity and egg viability (20). In their study, Liang et al. investigate the impact of spirotitramat on Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae). The findings indicate that the sublethal concentrations employed (30 mg/L) resulted in a reduction of one day in the duration of the egg stage, in comparison to the control cohort. The preadult duration, adult longevity, pre-oviposition period, and total pre-oviposition period of the spirotetramat-treated cohort were all found to be shortened (21). Following a 24-hour exposure period, the gross fertility of treated females was reduced by 2.4–64.7%, and the net fertility by 12.4–88.8%, in comparison to the control. The series of concentrations of spirotitramat applied included 200, 60, 18, 5.4, and 1.62 mg/L. With the exception of the lowest concentration, all concentrations resulted in a significant reduction in net fertility and female longevity (22). The available literature on mosquitoes treated with spirotitramat is limited. However, the resistance rate of Culex quinquefasciatus to this insect growth regulator is 0.01–0.07, which is comparatively lower than the resistance rates observed in other products, such as imidacloprid, acetamiprid, and emamectin benzoate, which have resistance rates of 0.09–11.18, 0.39–8.00, and 0.002–0.020, respectively. Additionally, the resistance ratio (RR) to indoxacarb is 3.00–118.00. The subjects who underwent testing after reaching adulthood exhibited a notable delay in their developmental trajectory. The delay duration for the doses of 20 mg/l and 40 mg/l was approximately two days ±12 hours. In contrast, the third dose resulted in a development delay of approximately three days, with a standard deviation of 20 hours. Insect Growth Regulators (IGRs) have the potential to interfere with the synthesis of chitin or to inhibit the enzymes that are essential for this process in insects (24). By inhibiting the formation of a robust exoskeleton, IGRs disrupt the life cycle of insects, reduce their fertility, and can ultimately result in their demise. In the case of mosquitoes, insect growth regulators (IGRs) can be employed to target larvae and nymphs in their aquatic habitats. By disrupting their development and molting, IGRs can impede the ability of larvae to transform into biting and reproducing adults, thereby contributing to a reduction in mosquito populations (17). It is therefore imperative to undertake dedicated research to evaluate the impact of IGRs on local mosquito populations and to implement integrated strategies within the context of anti-vector control programs. In conclusion, the toxicological tests of three growth regulators (lufenuron, teflubenzuron, and spirotetramat) at three doses (20 mg/l, 40 mg/l, and 80 mg/l) on fourth-stage larvae of the savage population of Cs. longiareolata demonstrated a significant toxic effect for all three products. Lufenuron demonstrated the highest mortality rate, followed by Spirotétramat, with Téflubenzuron exhibiting a lower mortality rate than the first two. The toxicity ranking based on LD50 and LD90 values corroborated the toxicity classification of the three growth inhibitors. In comparison to the control group, the treated groups exhibited a developmental delay and a reduction in longevity of the adult stage. A notable delay in the development of the tested individuals was observed in the adult stage. The delay in development for the 20 mg/l and 40 mg/l concentrations was approximately two days, with a standard deviation of 12 hours. In contrast, the third concentration (80 mg/l) resulted in a development delay of approximately three days ± 15 hours. It can thus be concluded that the treatment with these products has a significant effect on the development of C. The specimens of Longiareolata.

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Authors' Contribution

M.B. made significant contributions to the study's overall design, methodology, and the interpretation of findings. Additionally, they were instrumental in the manuscript's preparation and translation. D.I. and L.I. were responsible for harvesting and monitoring the organic material, as well as preparing the three products. Z.C. and O. M. were instrumental in the identification of mosquito species and the monitoring of specimen treatment. O.M.L provided assistance in the correction of the manuscript.

Ethics
The advancement of this study was situated within the broader context of environmental protection, with the research team, whether in the field or in the laboratory, dedicated to this objective.

Conflict of Interest
The authors have no competing interests to declare that are relevant to the content of this article.

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Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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