

Efficacy and Residual Activity of Novaluron on *Anopheles coluzzii* and *Anopheles arabiensis* in Laboratory and Semi-Field Conditions in Senegal

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Abstract

Vector control methods have significantly reduced malaria transmission. However, challenges such as insecticide resistance and behavioral adaptation of vectors highlight the urgent need for alternative control measures. Novaluron, an insect growth regulator, was evaluated following the World Health Organization Pesticide Evaluation Scheme. Laboratory bioassays were carried out using third (L3) fourth instar (L4) larvae and pupae of *Anopheles coluzzii* and *Anopheles arabiensis*. At the experimental station, efficacy and residual activity were assessed exclusively on L3, with multiple diagnostic doses (DD) for each species. In the laboratory, emergence inhibition (EI) 50% and 99% values on L3 were 0.145 and 0.29 µg/l for *An. coluzzii* and 0.37 and 0.69 µg/l for *An. arabiensis*, on L4, 1.95 and 3.61 µg/l for *An. coluzzii* and 2.76 and 5.02 µg/l for *An. arabiensis*. For pupae, EI₅₀ and EI₉₉ values were 145 and 265 µg/l for *An. coluzzii* and 428.37 and 714.13 µg/l for *An. arabiensis*. At the experimental station, *An. coluzzii* showed respectively 86% and 77.08% cumulative mortality (CM) and emergence inhibition rate (EIR) by day 33 at 6 µg/l. For *An. arabiensis*, 84% CM and 77.08% EIR were observed by day 40 at 15 µg/l. *Anopheles coluzzii* and *An. arabiensis* are susceptible to Novaluron at low concentrations under laboratory conditions. At optimal doses, Novaluron effectively inhibits both pupation and adult emergence.

Keywords

Insect Growth Regulator, Novaluron, Vector Control, Semi-Field Evaluation, Mosquito Larvae

1. Introduction

Mosquitoes are vectors of numerous pathogens responsible for major diseases such as malaria, which remains one of the leading cause of morbidity and mortality worldwide [1]-[3]. In developing countries, particularly across intertropical Africa, malaria continues to represent the most significant public health challenge [4]. In 2023 alone, an estimated 263 million new cases and 597,000 deaths were recorded globally, with 95% of cases occurring in Africa [5]. Furthermore, malaria also imposes a major economic burden, with Africa losing approximately £12 billion annually due to healthcare costs, reduced productivity, and lost economic opportunities [6]. Investments in malaria control reached US\$4.1 billion in 2022, compared to US\$3.5 billion in 2021 and US\$3.3 billion in 2020 [5]. According to the World Health Organization (WHO): World Malaria Report 2024, the five countries with the highest malaria burden are Nigeria (25.9%), the Democratic Republic of the Congo (12.6%), Uganda (4.8%), Ethiopia (3.6%) and Mozambique (3.5%). Together, these countries account for more than half of all malaria cases worldwide. Their contribution to malaria mortality is equally alarming: Nigeria alone accounts for 30.9% of global deaths, nearly 40% of which occur in children under 5 years, while the DRC and Mozambique account for 11.3% and 3.0%, respectively [7]. Vector control remains the cornerstone of malaria prevention, relying primarily on long-lasting insecticide-treated mosquito nets (LLINs) and indoor residual spraying (IRS), as recommended by the World Health Organization [8]. Throughout sub-Saharan Africa, where over 90% of the global malaria burden is concentrated, LLINs and IRS have significantly reduced vector densities and malaria incidence [9]. Although these insecticide-based interventions have produced encouraging results, their effectiveness is increasingly challenged by three major issues [10]: inadequate coverage and/or insufficient use of LLINs; behavioral changes in vector populations (e.g., outdoor biting, early biting); widespread and growing insecticide resistance. These limitations highlight the urgent need for alternative or complementary vector control strategies. One promising approach is larval source management (LSM), which includes the use of chemical or biological larvicides to target mosquito immatures [11] [12]. This technique aims to reduce vector density by killing larvae and pupae or preventing the emergence of adult mosquitoes [12] [13]. Although larviciding has proven consistent success and has gained renewed interest across Africa [14] [15], its large-scale implementation is often hindered by operational challenges, including resistance development, high costs and the difficulty of achieving sufficient coverage of breeding habitats. Chemical larvicides such as temephos or *Bacillus thuringiensis* (*Bti*) have

shown declining effectiveness due to resistance and limited residual activity [16] [17]. Consequently, insect growth regulators (IGRs) have emerged as a promising class of alternatives for LSM [18]. Insect growth regulators exhibit high specificity and a favorable safety profile most non-target organisms, offering important advantages for integration into mosquito control programs [19]. Several IGR compounds have demonstrated strong larvicidal activity against mosquito species of the genera *Culex* [20], *Aedes* [21] and *Anopheles* [22]. Novaluron, a benzoylurea IGR, inhibits the synthesis of chitin and disrupts the moulting process, thereby preventing successful development of mosquito larvae. According to WHO, Novaluron has a wide safety margin for mammals, birds, earthworms and aquatic plants, although it is highly toxic to crustaceans [23]. It has a demonstrated strong activity against several insect orders including Lepidoptera, Coleoptera, Hemiptera and Diptera [18]. Despite its potential advantages for managing mosquito populations and reducing malaria transmission, no studies have yet evaluated in Senegal within the context of malaria vector control. This study was therefore designed to assess the efficacy and residual activity of Novaluron under laboratory and semi-field conditions, following standardized procedures of the WHO Evaluation Scheme (WHOPES).

2. Materials and Methods

2.1. Study Site

The study was conducted at the Laboratoire d'Ecologie Vectorielle et Parasitaire (LEVP) of Cheikh Anta Diop University (UCAD), Dakar. The laboratory operates an insectary maintaining several *Anopheles* strains, including *An. coluzzii* strain from Cameroon, *An. arabiensis* strain from Dakar, maintained for several generations, and the *An. gambiae* s.s. (Kisumu) strain from Kenya. The facility also includes a controlled experimental station for larvicide evaluation.

2.2. Insect Growth Regulator Used for Efficacy Testing

Novaluron, 1-[chloro-4-(1, 1, 2-trifluoro-methoxyethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea] is an IGR developed by Makhteshim-Agan Industries Ltd and supplied by Syngenta [24]. It acts as a chitin synthesis inhibitor (CSI) belonging to the benzoylurea chemical class.

2.3. Laboratory Tests

2.3.1. Biological Material

Stage III and IV larvae, as well as pupae from two *Anopheles* species were used in laboratory bioassays: *An. coluzzii*, a laboratory strain maintained for several generations and known to be susceptible to DDT and pyrethroids, *An. arabiensis*, a laboratory-maintained strain. To determine the activity range of the product, larvae and pupae were initially exposed to concentrations ranging from 0.01 µg/l and 1000 µg/l. Next, sixteen (16) concentrations were selected: five (5) doses for stage III larvae (0.01 µg/l; 0.05 µg/l; 0.1 µg/l; 0.5 µg/l and 1 µg/l); 5 doses for stage IV

larvae (1 µg/l; 2 µg/l; 3 µg/l; 4 µg/l and 5 µg/l) and 6 doses for pupae (100 µg/l; 200 µg/l; 300 µg/l; 600 µg/l; 700 µg/l and 750 µg/l ug/l).

2.3.2. Preparation of Test Solutions

A stock solution was prepared following WHO recommendations [23]. A 1% stock volume was obtained by adding 10 ml of the product stock solution to 990 ml of distilled water, yielding a concentration of 1000 mg/L. This solution was then diluted ten times by mixing 2 ml of the stock solution with 18 ml of distilled water. Serial dilutions were subsequently prepared, and aliquots (100 - 1000 µl depending on the target concentration) were added to enamel-coated trays containing 250 ml of tap water and 25 larvae, or plastic cups containing 100 ml and 25 pupae. Disposable pipettes were used for transfer, with separate pipettes for treating and control batches to avoid cross-contamination. Each concentration was tested with four replicates, and an equivalent number of controls. The entire experiment was repeated three times on separate days. Larvae were fed every two days with Tetramin® fish food and development status (larvae, pupae and adults) were recorded daily until all individuals had either emerged or died. Live pupae from each tray were transferred to plastic cups containing 25 ml of water from the original tray for emergence monitoring.

2.3.3. Environmental Conditions

During the tests, temperature ranged from 24 to 27°C, with a relative humidity of 80% ± 10% and a 12:12 light-dark photoperiod.

2.3.4. Effect of Novaluron on Pupation Rate

The effect of novaluron on larval mortality was assessed to determine pupation inhibition rates (PI%). Moribund larvae, dead larvae and pupae that had not fully completely separation from the larval exuviae were classified as affected by novaluron. Data from all replicates were pooled to calculate the mean number of larvae impacted per concentration.

2.4. Experimental Station Trial (Semi-Natural Conditions)

The semi-natural tests were conducted at the experimental station located on the premises of the former Geological and Mining Research Office (GMRO), which currently hosts the Laboratoire d'Ecologie Vectorielle et Parasitaire (LEVP).

The station consists of a 20 m² enclosed structure, delimited by a 1 m high wall topped with a plastic mosquito net extending to a transparent plastic roof. This setup allows natural sunlight to penetrate while preventing the containers from being filled by rainwater and avoiding contamination from external mosquitoes or other insects. Inside the enclosure, ten white-bottomed plastic batches (48 cm × 37 cm × 23 cm) were arranged on the ground in two rows of five, spaced at least 1 m apart. Each bin was covered with a removable mosquito net equipped with a sleeve to facilitate the collection of pupae and emerging adults during follow-up.

Following the two laboratory experiments, DD was established (double of EI₉₉),

and two monitoring cycles were initiated at the experimental station using third-instar larvae of the two *Anopheles* species tested. For each species, three concentrations, expressed as multiples of the DD, were evaluated. Monitoring of *An. coluzzii* was carried out for 33 days at concentrations of 0.6 µg/l, 3 µg/l and 6 µg/l and that of *An. arabiensis* was carried out for 40 days at concentrations 1.5 µg/l, 7 µg/l and 15 µg/l.

2.4.1. Collection of Wild Larvae

Anopheles coluzzii larvae used for the tests under semi-natural conditions came from the insectarium. The wild larvae used at the experimental station are of the *An. arabiensis* species, collected from the Dakar suburbs. In fact, entomological studies to characterize larval habitats, as well as the molecular identifications carried out annually by the LEVP, always revealed the exclusive presence of *An. arabiensis* in Dakar [25] [26]. Larval surveys were conducted at larval breeding sites to collect aquatic stages of wild *An. arabiensis* used during the trials. Larvae were sampled using the dipping method [27], which consists in approaching the water body with caution, visually inspecting it for the presence of *Anopheles* larvae, and dipping a ladle or tray into calm, sunlit, shallow areas where larvae are most likely to concentrate. The characteristics and geographical coordinates of each surveyed breeding site were recorded. The *Anopheles* larvae collected were sorted by developmental stage and transferred into containers filled with water from their original breeding site for transportation to the experimental station.

2.4.2. Experimental Setup

Under semi-natural conditions, a total of eight batches were used, *i.e.* four batches per species (one for each concentration and one for control). Each treated batch contained 3 liters of dechlorinated tap water, 50 larvae and the volume of Novaluron solution required to achieve the desired concentration. The two control batches contained only 50 larvae and 3 liters of dechlorinated tap water. New lots of 50 larvae were introduced into the containers whenever the previous lots were depleted due to adult emergence or mortality. No additional water was added to the containers during the monitoring period, although a slight drop in the water level was noted.

2.5. Test Validation Criteria

In both laboratory and semi-natural conditions, test validity was determined based on larval or pupal mortality and pupation rates observed in the control groups. The test was considered invalid if the pupation rate in the control was greater than 10% within 24 hours following treatment, or control mortality was $\geq 20\%$. The test was considered valid if control mortality was $< 5\%$ and the pupation was $< 10\%$. When control mortality ranged between 5% and 20%, emergence in the treated groups was corrected using Abbott's formula (1925).

$$\text{Adjusted mortality} = \frac{\% \text{ survival in control group} - \% \text{ survival in treatment group}}{\% \text{ survival in control group}} \times 100$$

2.6. Analysis and Interpretation of Results

As with all insect growth regulators, the efficacy and persistence of Novaluron were assessed by calculating the emergence inhibition rates, cumulative mortality and pupation inhibition rate using the following formulas:

Emergence Inhibition Rate (EIR)

$$EIR(\%) = 100 - \frac{T - 100}{C}$$

T is the percentage survival in the treated group and C is the percentage survival in the control group

Cumulative Mortality (CM)

$$CM(\%) = LM + PM + AM$$

LM is the Larval mortality; PM the pupal mortality and AM the Adult mortality
Pupation Inhibition (PI)

$$PI(\%) = 100 - \frac{T - 100}{C}$$

T is the percentage of pupation in the treated batch and C is the percentage of pupation in the control group.

The concentrations causing 50% and 99% inhibition (IC_{50} and IC_{99}) were determined using dose-response curve analysis in XLStat version 2022.

A chi-square test was used to compare emergence inhibition, larval, pupal and adult mortality rates, as well as the percentage of adult emergence between treatment groups. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Laboratory Tests

Larvae tests: Dose-response assays showed that Novaluron affected adult emergence in *An. coluzzii* and *An. arabiensis* at very low concentrations. For L3, inhibition of emergence was observed at doses ranging from 0.01 to 0.5 µg/l, whereas for L4, effective concentrations ranged from 1 to 6 µg/l ($P = 0.337$ and $P = 0.957$), respectively.

The minimum doses that completely inhibited adult emergence were estimated at 0.03 - 0.07 µg/l for L3 and 3.65 - 5.05 µg/l for L4.

Pupation inhibition rates were generally high for all concentrations tested on both species at the L3 and L4, ranging from 58.33 to 100%, except for the 0.01 µg/l concentration in *An. arabiensis* (25%, $P < 0.05$). However, none of the concentrations tested on L4 achieved complete inhibition of pupation (**Table 1**).

Emergence inhibition rates (EIR) were also high for both species, ranging from 54.54 to 100%.

The data from the three independent dose-response series showed consistent trends for both species. They were therefore pooled by concentration to estimate overall emergence inhibition (EI); EI_{50} , and EI_{99} and DD (**Tables 1-2**). For L3, EI_{50} and EI_{99} values were low: 0.145 and 0.29 µg/l for *An. coluzzii*; 0.37 and 0.69 µg/l

for *An. arabiensis*. For L4, higher concentrations were required: 1.95 and 3.61 µg/l *An. coluzzii* strain; 2.76 and 5.02 µg/l for *An. arabiensis* (Table 2).

Table 1. Pupation and adult (inhibition rate) of pupation in *Anopheles coluzzii* (insectary strain) and *Anopheles arabiensis* (Dakar population) exposed at the L3 and L4 stages to different concentrations of Novaluron.

Larval stages		L3				L4					
Doses (µg/l)		0.01	0.05	0.1	0.5	1	1	2	3	5	6
Pupation inhibition (%)	<i>An. arabiensis</i>	25	75	83.33	87.5	100	58.33	70.83	75	70.83	84.16
	<i>An. coluzzii</i>	66.66	87.5	95.83	100	100	75	79.16	66.66	79.16	91.66
Emergence inhibition (%)	<i>An. arabiensis</i>	62.5	82.96	91.48	91.48	100	54.54	72.72	81.81	72.72	100
	<i>An. coluzzii</i>	73.91	86.95	95.65	100	100	85.71	90.48	80.95	100	100

Table 2. EI₅₀, EI₉₉ values, 95% confidence intervals (CI) and diagnostic doses (DD) for L3, L4 and pupal stages of *Anopheles coluzzii* and *Anopheles arabiensis* exposed to Novaluron.

Stages		EI ₅₀ (µg/l)	CI ₉₅ (%)	EI ₉₉ (µg/l)	CI ₉₅ (%)	DD (µg/l)
<i>An. coluzzii</i>	L3	0.145	0.052-0.246	0.29	0.0929-0.447	0.58
	L4	1.95	0.822-3.077	3.61	1.53-7.59	7.22
	Pupae	145	107-182	260	193-326	520
<i>An. arabiensis</i>	L3	0.37	0.195-0.54	0.69	0.357-1.21	1.376
	L4	2.76	1.852-3.349	5.02	3.41-6.07	10.04
	Pupae	428.37	372.33-520	714.13	650-788.5	1428.26

Pupae test: The pupal bioassay demonstrated the contact mode of action of Novaluron, with activity observed at concentrations ranging from 100 to 750 µg/l (Figure 1). The EI₅₀ and EI₉₉ values pupae were higher than those for larvae: 145 µg/l and 260 µg/l for *An. coluzzii*, 428.37 and 714.13 µg/l for *An. arabiensis*. Overall, *An. coluzzii* was significantly more susceptible to Novaluron than *An. arabiensis* (P = 0.03).

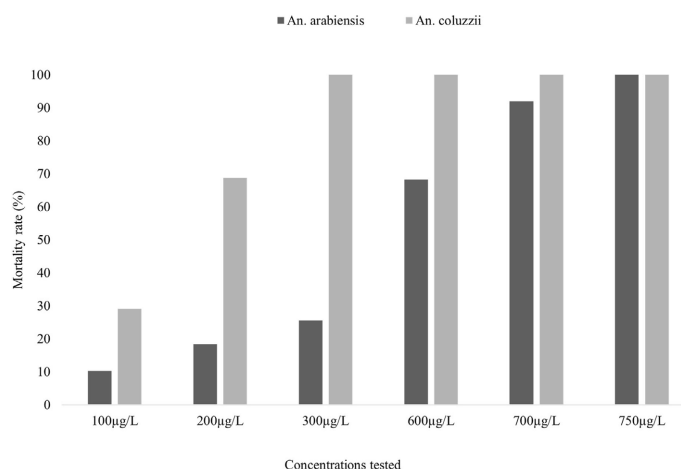


Figure 1. Cumulative mortality of *Anopheles coluzzii* (insectary strain) and *Anopheles arabiensis* (Dakar population) pupae and adults following exposure to Novaluron.

3.2. Experimental Station Trial

***Anopheles coluzzii*:** The CM in the control batches was very low throughout the monitoring period. By day 11, all concentrations tested (0.6 µg/l, 3 µg/l and 6 µg/l) resulted in 100% CM and complete emergence inhibition (EIR = 100%). No pupation was observed at any of these doses.

By day 21, only the 6 µg/l concentration maintained complete CM. At 0.6 µg/l and 3 µg/l, it reached 86% and 94%, respectively. Larval mortality ranged from 68% at 0.6 µg/l to 82% at 6 µg/l, PM from 12% to 20%, and AM from 4% to 6%. Emergence inhibition rates ranged from 78.26% to 95.65% at 0.6 µg/l (P between 0.057 and 0.924) (**Figure 2** and **Table 3**).

At day 33, CM ranged between 68 and 86%. Larval and pupae mortality were similar respectively between the 0.6 µg/l and 3 µg/l doses (LM: 34% and 38%; PM: 24% and 28%, P = 0.91). Emergence Inhibition rate varied between 56.25% and 77.08%. No significant difference was detected between CM across the tested concentrations (P = 0.674) during the entire monitoring period. However, a significant difference was observed in the LM values for the 0.6 µg/l dose between day 11 and day 33 (100% vs. 34%, P < 0.001) (**Figure 2** and **Table 3**).

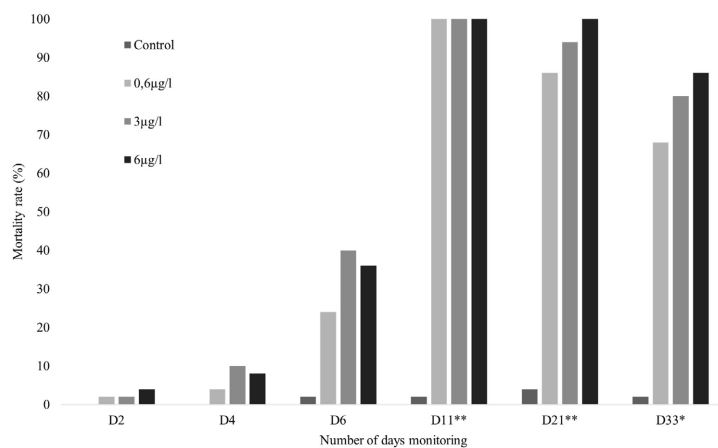


Figure 2. Cumulative mortality (CM) of L3 larvae, pupae and adults of *Anopheles coluzzii* (insectary strain) following exposure to Novaluron in the experimental station trial. **: End of monitoring of the 50 larvae and reintroduction of 50 new larvae; *: End of monitoring of the 50 larvae.

Table 3. Mortality of *Anopheles coluzzii* (insectary strain) L3 larvae, pupae and adults after exposure to Novaluron over 33 days.

Doses (µg/l)	Parameters (%)	Days (D)		
		D11	D21	D33
Control	LM	2	0	2
	PM	0	2	0
	AM	2	0	2
	LA	96	98	96
	EIR	-	-	-

Continued

0.6 µg/l	LM	100	68	34
	PM	0	12	24
	AM	0	6	10
	LA	0	14	32
	EIR	100	78.26	56.25
3 µg/l	LM	100	70	38
	PM	0	20	28
	AM	0	4	14
	LA	0	6	20
	EIR	100	89.13	64.58
6 µg/l	LM	100	82	58
	PM	0	14	20
	AM	0	4	8
	LA	0	0	14
	EIR	100	95.65	77.08

LM: Larval mortality; PM: pupal mortality; AM: Adult mortality; LA: Living adults; EIR: Emergence Inhibition Rate.

***Anopheles arabiensis*:** As with *An. coluzzii* strain, mortality in the control batches was very low throughout the monitoring period. On Day 10, CM and an EIR of 100% were achieved with all tested concentrations (1.5 µg/l, 7.5 µg/l, and 15 µg/l). No pupation or emergence was observed (**Figure 3** and **Table 4**).

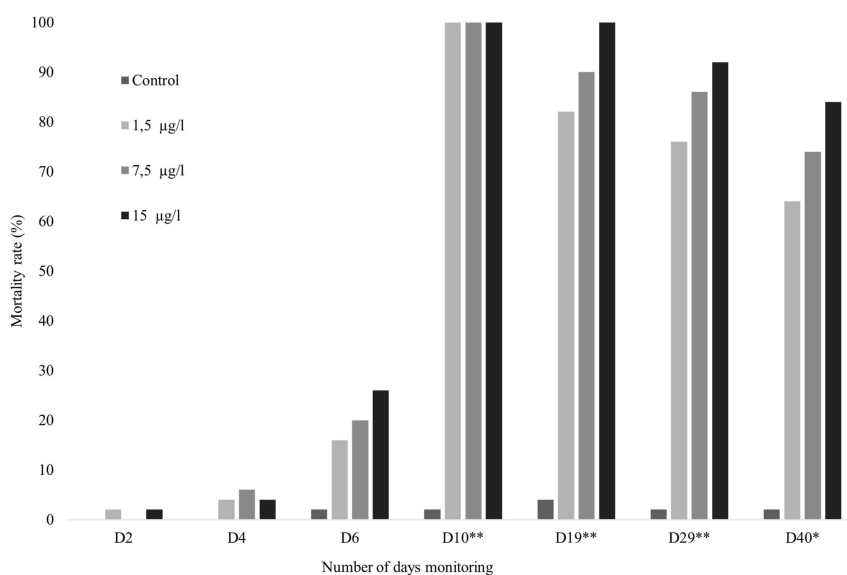


Figure 3. Cumulative mortality (CM) of L3 larvae, pupae and adults of *Anopheles arabiensis* (Dakar population) following exposure to Novaluron in the experimental station trial. **: End of monitoring of the 50 larvae and reintroduction of 50 new larvae; *: End of monitoring of the 50 larvae.

On Day 19, only the 15 µg/l concentration provided complete CM; for the 1.5 µg/l and 7.5 µg/l, it was, respectively, 82% and 90%, with EIR ranging from 76.16% to 93.75%. Larval mortality ranged from 68% at 1.5 µg/l to 80% at 15 µg/l; PM, from 12 to 16%; and AM, from 6% to 20% were observed. No significant difference was observed between the CM and the EIR values for the three doses (P ranging from 0.058 to 1) (**Figure 3** and **Table 4**).

On Day 29, CM ranged from 76 to 92%, LM, 42% at 1.5 µg/l to 68% at 15 µg/l; PM was similar, from 20 to 28%. The EIR ranged from 63.82 to 87.23%. Larval mortality was very high compared to PM ($P < 0.01$) (**Figure 3** and **Table 4**).

On Day 40, CM ranged from 64 to 84%, and EIR ranged from 52.08 to 77.08%. Larval mortality were comparable, ranging from 40% at 1.5 µg/L to 50% at 7.5 µg/l; PM were also similar, ranging from 20 to 28%. The LM and EIR values were not significantly different ($P = 0.052$) (**Table 4**).

No significant difference was observed between the CM of the tested doses ($P = 0.965$) throughout the monitoring period (**Figure 3**).

Table 4. Mortality of L3 larvae, pupae and adults of *An. arabiensis* (Dakar population) after exposure to Novaluron over 40 days.

Doses (µg/l)	Parameters (%)	Days (D)			
		D10	D19	D29	D40
Control	LM	0	2	0	2
	PM	2	0	2	0
	AM	2	0	2	0
	LA	96	98	96	98
	EIR	-	-	-	-
1.5 µg/l	LM	100	68	42	40
	PM	0	12	24	14
	AM	0	6	10	10
	LA	0	14	24	36
	EIR	100	79.16	63.82	52.08
7.5 µg/l	LM	100	74	52	50
	PM	0	16	28	20
	AM	0	4	6	4
	LA	0	6	14	26
	EIR	100	89.58	78.72	68.75
15 µg/l	LM	100	80	68	46
	PM	0	14	20	22
	AM	0	6	4	6
	LA	0	0	8	12
	EIR	100	93.75	87.23	77.08

LM: Larval mortality; PM: Pupal mortality; AM: Adults mortality; LA: Living adults; EIR: Emergence Inhibition Rate.

4. Discussion

Although studies on *Bacillus thuringiensis* have been conducted in both experimental stations and natural environments in Senegal [28], its effectiveness is limited due to the development of resistance and a short residual effect [17] [29] [30]. There is therefore a constant need to identify alternative tools and diversify control strategies to synthetic insecticides. The aim of this study was to evaluate the effects of Novaluron on larvae and pupae in the laboratory and to determine its efficacy and residual activity under semi-field conditions.

Laboratory assays showed that *An. coluzzii* and *An. arabiensis* larvae were highly susceptible to Novaluron, with doses inducing complete mortality ranging from 0.01 to 6 µg/l. These values are lower than those reported in Chiapas, Mexico, where effective doses ranged from 15.18 to 67.23 µg/l for *An. albimanus* and *An. pseudopunctipennis*, respectively [31]. Similarly, in Tanzania [22], stage 1 larvae of *An. gambiae*, *An. arabiensis* and *An. funestus* were treated with concentrations of 2.001 mg/l; 2.013 mg/l and 5.58 mg/l, respectively. In comparison, *An. arabiensis* from Dakar was more susceptible than the same species in Tanzania. As a result of large-scale MILDA and PID release campaigns, *An. gambiae* and *An. funestus* have developed a high level of resistance to insecticides [32]-[34]. Pupation inhibition was very high (75 to 100%) for concentrations between 0.05 and 1 µg/l, confirming the lethal effect of Novaluron, confirming the lethal effect of novaluron on the larvae by interfering with the normal moulting process. Although the precise mode of action is not fully clearly elucidated, Novaluron is a chitin inhibitor that disrupts moulting by causing of an abnormal endocuticle [35]. This is reflected in the prolonged duration required for stage 3 and 4 larvae to reach pupation (approximately 9 and 11 days, respectively).

Our laboratory tests showed that pupae were significantly less susceptible than larvae, requiring higher doses to induce mortality for both two species (EI₉₉: 260 and 714,13 µg/l) compared to EI_{99%} of L3 and L4 which were less than 6 µg/l. At the same time, studies carried out in Mexico on *An. albimanus*, *An. pseudopunctipennis*, *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* have shown the opposite, namely that pupae were more susceptible than larvae [31]. Furthermore, these studies confirmed that Novaluron acts in two modes: through ingestion by the larvae and through contact with the pupae,

During our study, the efficacy and residual activity of Novaluron were further evaluated under semi-natural conditions. The duration which emergence inhibition exceeds 80% is considered the period of effective residual activity [36]. Two semi-field trials were conducted over 33 and 40 days, respectively on L3 of *An. coluzzii* and *An. arabiensis*,

For *An. coluzzii* concentrations of 3 and 6 µg/l maintained high efficacy throughout the 21-day follow-up. For *An. arabiensis*, only 15 µg/l remained effective until 29 days of the follow-up. In Sri Lanka efficacy against *Aedes aegypti* and *Ae. albopictus* larvae was observed for nearly 2 months at 0.001 µg/l and 3 months at 0.01 µg/l, with EIR of 89% - 95% [37]. These findings suggest that Novaluron was

more effective against *Aedes* species. These results support WHO recommendations for semi-field trials, which suggest using doses between 10 and 50 µg/l [23]. However, comparable studies, conducted in Mexico reported that formulated Novaluron (Rimon 10EC) maintained activity for up to 4 months at 166 µg/l and 550 µg/l on *An. albimanus* and *Cx. Coronator* [31]. There is one possible explanation regarding the difference in concentrations: the mosquitoes from Mexico are less susceptible because they are subject to constant insecticide challenges because of permanent dengue vector control operations and agriculture.

Integrating IGR with different modes of action can help delay or counteract the development of insecticide resistance in mosquito populations [38]. Our findings highlight the potential of Novaluron to reduce adult mosquito emergence in breeding habitats and support its in rotation with other IGRs, such as pyriproxyfen, to manage insecticide resistance [22].

Unfortunately, our study presented some limitations, in fact, the concentrations tested at the experimental stations could not be replicated and adult females emerging from the treated batches were to be monitored to verify the sublethal effect of Novaluron (potential vertical transmission).

Our work was limited to laboratory and semi-field conditions, field studies are needed to validate these findings in real-world settings. Furthermore, the long-term impact on mosquito populations, as well as the potential development and management of resistance—which are crucial for assessing the sustainability of this vector control strategy require further research [37].

5. Conclusion

This study demonstrates that *An. coluzzii* and *An. arabiensis* are highly susceptible to Novaluron at low concentrations under laboratory conditions. At optimal doses, Novaluron effectively inhibits both pupation and adult emergence. Its efficacy and residual activity under semi-field conditions supports its potential as a valuable tool for integrated malaria vector control strategies. These results provide insights into optimizing application frequency and operational costs for potential field deployment.

Author's Contributions

Mame Fatou Tall., Seynabou Mocote Diédhiou, Ousmane Faye, Lassana Konaté and Ibrahima Dia. conceived and designed the study; Mame Fatou Tall, Moussa Diop, Abdoulaye Kane Dia, Mouhamadou Bassir Faye, Abdoulaye Konaté, Khady Sylla., El Hadji Diouf, Omar Thiaw and Ousmane Sy collected the samples. Mame Fatou Tall performed the laboratory and experimental trials; Mame Fatou Tall, El Hadji Amadou Niang, Ibrahima Dia, Seynabou Mocote Diédhiou Oumou Kalsom Gueye, Moussa Diop and Ousmane Faye analyzed the data, wrote the manuscript with contributions from Abdoulaye Niang, Oumou Kalsom Gueye, Badara Samb, Lassana Konaté. and Ibrahima Dia. All authors read and approved of the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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