

Bio-efficacy and persistence evaluation of Dimilin[®] GR-2% and Mosquiron[®] 10 EC insect growth regulators against *Anopheles gambiae* s.l (diptera: Culicidae) larvae

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ABSTRACT

Expansive chemical-based vector control has resulted in development of vector resistance to different insecticides harnessed for prevention of disease transmission in public health. The environmentally safe insect growth regulators and microbial larvicides provide potential tools for insecticide resistance management. The efficacy and persistence of Dimilin[®] GR-2% and Mosquiron[®] 10 EC insect growth regulators were evaluated against *Anopheles gambiae* s.l larvae under laboratory and simulated field conditions. In laboratory bio-efficacy trials, complete emergence inhibition was achieved at higher concentrations with 96 hours post exposure of mosquito aquatic stages to the two larvicides. In simulation field trials, persistence of both larvicides at higher concentrations increased gradually with complete inhibition attained at 7 days and maintained up to 21 days. In the quest of deploying non insecticide based interventions for a sustainable environment, insect growth regulators can be recommended for operational scale larviciding for mosquito larval control in the context of integrated vector management.

Keywords: Evaluation; Insect Growth Regulators; Integrated Vector Management; Malaria Control

1. INTRODUCTION

Globally malaria remains a major public health problem and this is epitomized by sub-Saharan African countries [1]. The huge burden of the disease is maintained by strong vectorial potential of *Anopheles gambiae* Giles,

An. arabiensis Patton and *An. funestus* Giles [2,3]. Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are expansively being deployed as frontline tools to prevent malaria transmission [4,5]. However, chemical-based vector control tools have resulted in development of vector resistance to different insecticides in use for public health and agriculture in addition to adverse environmental concerns [6].

To optimize the use of limited available resources for vector control, implementation of the World Health Organization (WHO)-led integrated vector management (IVM) strategy is being advocated to rationalize decision making [7]. To this effect, a Global Plan on Insecticide Resistance Management for Malaria Vectors (GPIRM) has been developed to maintain effectiveness of malaria vector control [8]. The WHO also recommends larviciding with *Bacillus thuringiensis* var. *israelensis* (Bti) and the insect growth regulators (IGR) for the purposes of malaria vector control, as a supplementary tool, within the context of IVM in sub-Saharan Africa [9]. The IGRs and Bti, unlike the chemical larvicides, are strictly arthropod-specific, environmentally safe and potential tools for insecticide resistance management [10,11].

There are two types of IGRs, juvenile hormone (JH) analogues (Juvenoids) and the chitin synthesis inhibitors. JH analogues interfere with the transformation of late instar larvae to pupae and then to adult, whereas chitin synthesis inhibitors inhibit cuticle formation and affect all instars and immature stages of the mosquito [12]. Dimilin[®] GR-2% (Diflubenzuron) and Mosquiron[®] 10 EC (Novaluron) are benzoyl urea based chitin biosynthesis inhibiting stomach poison compounds that have successfully passed the WHO's pesticide evaluation scheme (WHOPES's) and recommended for mosquito larvae control [13]. Many laboratories and simulated field trials

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have been conducted with chemical larvicides and Bti to evaluate their efficacy and persistence [14,15].

The present study assessed and compared the efficacy of Dimilin® GR-2% and Mosquiron® 10 EC against *An. gambiae* s.l larvae under laboratory and simulated field conditions. This was an initial step towards programmatic deployment of larviciding as a supplementary vector control intervention in Zambia.

2. MATERIALS AND METHODS

2.1. Experimental Site

Zambia is a landlocked country in southern Africa with an estimated population of 13 million people, 45% are children below 15 years of age [16]. The study was conducted at National Malaria Control Centre (NMCC) using field collected *An. gambiae* s.l larvae between September and October, 2007. During the study period, annual ambient temperatures varied between an average minimum of 26°C and maximum of 28°C and Relative humidity ranged from 47 to 54 (Meteorological Stations).

2.2. Mosquito Collections and Laboratory Processing

Anopheles mosquito larvae were collected from breeding sites using WHO standard 250 mL dippers [17], transported to the NMCC in Lusaka for the evaluation assays. Some immature stages were reared to F0 adults under standard laboratory conditions with a temperature range of 25°C ± 2°C and 70% - 80% relative humidity. Mosquitoes were identified morphologically using standard keys for anophelines of southern Africa [18,19].

2.3. Evaluated Products

Dimilin® GR-2% is a granular larvicide, 100 grams of this slow acting product is composed of 2 grams Diflubenuron and 98 grams Coformulants. The product is manufactured by Rea Industrie Chimiche SRL, SS 87 Km 20.700, 81025 Marcianise (CE), Italy and the Registration Holder is Crompton (Uniroyal Chemical) Registrations Ltd., Ankerweg 18, 1041 Amsterdam, The Netherlands. Dimilin® GR-2% inhibits the synthesis of chitin and, hence, interferes with molting; to a lesser extent, a contact poison and acts by disturbing the molting process of all stages of larvae instars of mosquitoes [20]. Application rates for *Anopheles* larvae control in clear water is 1.25 - 2.50 kg/Ha.

Mosquiron® 10 EC is an emulsifiable concentrate. 100 grams of this product is composed of Novaluron. The Registration Holder is Makhteshim-Agan SA (Pty) Ltd. Registration number 1992/001741/07 Posbus 498 Brackenfell 7561 Israel. Mosquiron® 10 EC acts by in-

hibiting chitin biosynthesis resulting in interference with the formation of the cuticle, thereby halting the normal growth and development of larvae. Application rates for *Anopheles* larvae control in clear water is 10 l water/200 m² up to 50 cm deep (0.1 ml - 0.5 ml/m³).

The supplies of Dimilin® GR-2% and Mosquiron® 10 EC used in this trial were manufactured in October 2007 with a two-year lifespan.

2.4. Entomological Evaluation

The evaluation was done in accordance with the WHO standard protocol [21]. Biological activity was determined using stock solution (1%) prepared by dissolving 200 mg in 20 ml. Test concentrations were diluted with water and serial dilutions (0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%, 1.0% and 10%) were made in the same manner. The assays were in two different stages; the Laboratory testing phase to determine the inherent bio-potency of the technical materials and the small scale field trials to establish the efficacy levels of the products against target mosquito larvae, under simulated field conditions. Mortality was assessed and recorded. Larvicide formulations were tested at different concentrations and phase one studies guided the dosages chosen for use in phase two trials [21].

2.5. Laboratory Trials

During the laboratory trials, bioassays were conducted for *Anopheles* pre-imagos. Batches of 25 larvae were introduced in test vessels (0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%, 1.0% and 10%) and fed on a mixture of finely ground yeast extract (one part) and dog biscuit (three parts) at a concentration of 10 mg/l at two-day intervals until mortality counts were made. The food powder was suspended in water and two drops added per test cup including the control. A mixture of second- and third-stage larvae were selected for the experiment as they were large enough and can easily be counted and had three to two stages before becoming adults and therefore, giving investigators enough time to make follow-ups of their development. All the test and control cups were covered with netting to prevent successfully emerged adults from escaping into the environment [21].

The test containers were held at 25°C - 28°C for a photoperiod of 12L: 12D. Mortality or survival was counted every other day until the complete emergence of adults. At the end of the observation period, the impact was expressed as IE% based on the number of larvae that do not develop successfully into viable adults. In recording IE% for each concentration, moribund and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as "affected". The experiment stopped when all the larvae or pupae in

the controls had died or emerged as adults. The testing room was maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 75% - 80% relative humidity [21].

2.6. Simulated Field Trials

In simulated field trials, multiple plastic tubs of water were placed under imitation field conditions. Plastic tubs with diameter of 60 cm and depth of 15 cm were used with 10 litres of water from the breeding sites where the larvae were collected. The water-filled containers were given at least 24 hrs for conditioning or ageing. A batch of 30 third instar *An. gambiae* s.l larvae was re-leased into each replicate and fed on alternate days. After 2 - 3 hrs of larval acclimation, separate containers were treated with Dimilin[®] GR-2% and Mosquiron[®] 10 EC dosages using pipettes. The containers were covered with netting material to prevent other mosquitoes or other insects from laying eggs and to protect the water from falling debris. The water level in the containers was sustained and a minimum of four replicates of each dosage and four controls were utilized.

To test residual activity, Larval survival was assessed every third day post addition until all larvae had pupated and emerged and pupal skins were counted seven days or more after addition to assess emergence. This process continued until no mortality was noted. Adults not freed from pupal skins were considered dead. The observations were performed at 1, 3, 7, 14 and 21 days post-treatment, by which time all larvae would have pupated and emerged as adults. The test was terminated when there was no statistically significant residual activity in terms of larval mortality or inhibition of adult emergence when comparing the treated (at the highest dosage tested) batches and the untreated controls. The weekly settings of larvae continued until no difference in mortality was recorded between untreated controls and treated batches. Percentage mortality or emergency inhibition (IE %) was calculated [21].

2.7. Data Management and Statistical Analysis

Data were collected from both laboratory and simulation field experiments for Dimilin[®] GR-2% and Mosquiron[®] 10 EC, entered in Excel spread sheets (Microsoft Corporation) and statistically analyzed by employing Epi-Info version 3.2.2. The data on the number of live and dead larvae and pupae from all replicates of each dosage on one day was combined and percentage mortality or IE% calculated. The significance of the difference in the mosquito mortalities was analyzed by Chi-square (X^2) test using a computer software [22] and Odds ratios (OR) and 95% confidence intervals (CI) to assess the efficacy of the given concentration of each larvicide.

3. RESULTS

The effect of Dimilin[®] GR-2% and Mosquiron[®] 10 EC at various concentrations within their activity range, on field-collected 2nd and 3rd instar *An. gambiae* s.l larvae was expressed in terms of the percentage of larvae that do not develop into successfully emerging adults or adult emergence inhibition (IE %).

3.1. Laboratory Trials of Dimilin[®] GR-2% and Mosquiron[®] 10 EC

While the 0.01, 0.001, 0.0001 and 0.00001 concentrations (v/v) had exhibited appreciable emergence inhibition effect, a marked percentage reduction effect was observed at 1.0% and complete killing effect was observed at 10.0% concentration (v/v) for both larvicides. At 1.0% and 10.0% conc. (v/v), 72 hrs and 96 hrs post-application, the inhibition rate was 90% and 100% for Dimilin[®] GR-2% (**Figure 1(a)**) and 93% and 100% for Mosquiron[®] 10 EC (**Figure 1(b)**).

3.2. Simulated Field Trials of Dimilin[®] GR-2% and Mosquiron[®] 10 EC

Emergence inhibition (IE %) at 0.1%, 1.0% and 10% conc v/v was effective in three weeks. For both Dimilin[®] GR 2% and Mosquiron[®] 10 EC percentage emergence inhibition increased gradually from <10% (0.1% and 1.0% conc. v/v) and from <40% (10% conc. v/v) to 90% (0.1% conc. v/v) and 100% (1.0% and 10% conc. v/v) 24 hrs post application (**Figures 1(c)** and **(d)**). At 14 and 21 days post-treatment, complete killing effect for 1.0% and 10% conc. v/v was maintained for both larvicides. The 0.1% concentration remained at 98% at 14 days and only killed the larvae fully at 21 days after application (**Figures 1(c)** and **(d)**).

3.3. Comparison Efficacy of Dimilin[®] GR-2% and Mosquiron[®] 10 EC

According to the simulation field results, there was marked effect of Dimilin[®] GR-2% and Mosquiron[®] 10 EC at both 10 and 1.0% from the first day of application to the last day of the study as exemplified by the computed odds ratios and Chi-square P-values of the 0.1% concn. v/v (**Table 1**) and as determined by the student t-test ($t = -0.768$, $df = 4$, $P = 0.485$).

4. DISCUSSION

Expansive utilization of chemical insecticides in mosquito vector control has not only resulted in the development of vector resistance to different insecticides but also have adverse environmental and ecological effects [11]. In view of this, there have been aggressive efforts

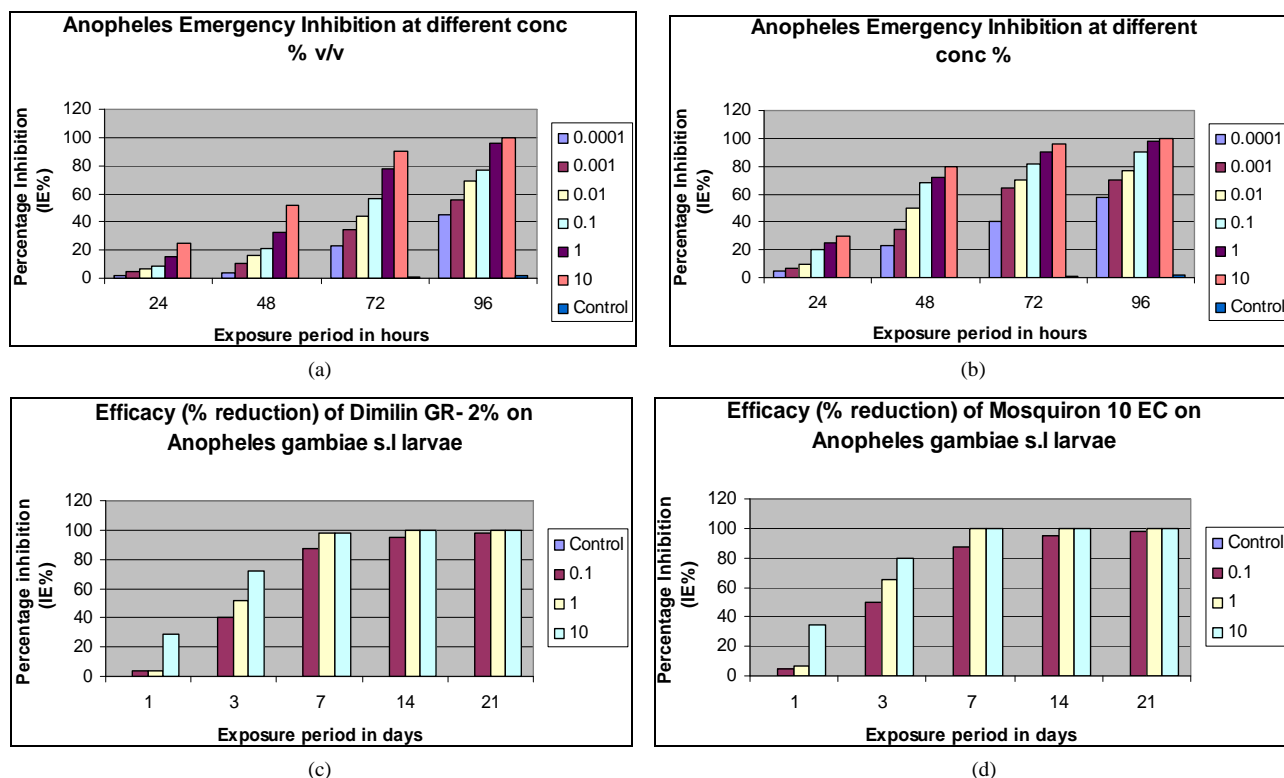


Figure 1. Laboratory and simulation field efficacy percentage (%) inhibition results for Dimilin GR-2% and Mosquiron® 10 EC. (a) Dimilin GR-2% laboratory efficacy % inhibition 24, 48, 72 and 96 hrs post-application at different concentrations (v/v); (b) Mosquiron® 10 EC laboratory efficacy % inhibition 24, 48, 72 and 96 hrs post-application at different concentrations (v/v); (c) Results of the simulation field efficacy trial indicating the % inhibition at 0.1%, 1.0% and 10% in three weeks; (d) Results of the simulation field efficacy trial indicating the % inhibition at 0.1%, 1.0% and 10% in three weeks.

Table 1. Residual activity of Dimillin® GR-2% and Mosquiron® 10 EC and percentage (%) reduction of emergency of *An. gambiae* s.l. larvae.

Duration (Days)	Dimillin® GR-2%			Mosquiron® 10 EC			OR [95% CI]*	P-Value*
	10	1	0.1	10	1	0.1		
	Treatment (concn v/v of product)			Treatment (concn v/v of product)				
1	(30) 25%	(6) 5%	(6) 5%	(34) 28%	(8) 7%	(7) 6%	1.18 (0.38 - 3.61)	0.763
3	(85) 71%	(59) 49%	(52) 43%	(96) 80%	(77) 64%	(60) 50%	1.31 (0.79 - 2.17)	0.468
7	(120) 100%	(120) 100%	(108) 90%	(120) 100%	(120) 100%	(106) 88%	0.84 (0.37 - 1.90)	0.882
14	(120) 100%	(120) 100%	(115) 96%	(120) 100%	(120) 100%	(114) 95%	1.21 (0.36 - 4.08)	0.944
21	(120) 100%	(120) 100%	(116) 97%	(120) 100%	(120) 100%	(118) 98%	2.04 (0.37 - 11.32)	0.944

*Computations based on the 0.1 concentration (v/v).

in research towards alternative interventions, particularly the use of biological larvicides such as *B. thuringiensis* var. *israelensis* and *B. sphaericus* including Insect Growth Regulators [14,15]. Although only a few operational field studies have been conducted, various laboratory and simulated field studies have been conducted

with Afro-tropical malaria vector species [11]. With increased funding for malaria vector control, re-evaluating the potential of antilarval measures tailored to local conditions in sub-Saharan Africa is critical [22-24] and has been supported by the increasing interest in integrated vector management [7,25,26].

Examination of data from laboratory trials demonstrated effective bio-potency of Dimilin® GR-2% and Mosquiron® 10 EC for the control of *An. gambiae* s.l at dosages of 10% and 1% concentration (v/v) achieving very high emergence inhibition rates. The mean larval mortality rates reached 95% and 100% at 24 hrs and 48hrs post-treatment respectively. Although high mortality of larvae and pupae was observed at 24 h after treatment in laboratory trials, daily results indicated that significant mortality of larval *An. gambiae* s.l was delayed up to 96 and 72 h and after treatment. Delayed effectiveness against larvae of *Anopheles* species has also been reported [27].

In simulated field trials, both products were found to be effective against *An. gambiae* s.l at dosages of 0.1% and 1.0% concentration (v/v), with percent emergence inhibition persisting up to 3 wks (336 hrs) post-treatment. The delayed action of IGRs on treated larvae means that mortality is assessed every other day or every three days until the completion of adult emergence. Delayed mortality of larvae appears to be due to the molting of larvae to higher instars and to pupae at various intervals, which may have obscured the mortality associated with individual instars similar to the effect produced by certain IGRs [28].

Although field trials with larvicides in natural breeding places are important than experiments under laboratory conditions due to the difficulty in simulating all the factors that influence their action in the laboratory [29], trials are an inevitable pre-requisite. This efficacy study demonstrates that Dimilin GR-2% and Mosquiron® 10 EC are effective and persistent in simulation field trials and persisted longer at higher doses. For the products to provide effective control their application should be repeated every 3 - 4 weeks. Earlier studies have demonstrated prolonged efficacy of IGRs on malaria vectors [28]. The WHO interim statement on Larviciding in sub Saharan Africa clearly stipulates that this tool should be deployed as a supplementary intervention and confined to areas where breeding sites are few, fixed and findable [9].

In response to the international calls to protect both human health and the environment from DDT through the United Nation Environment Programme (UNEP), Global Environment Facility (GEF) and WHO, Zambia has halted the use of DDT for IRS [30]. In the quest of deploying non insecticide based interventions for a sustainable environment, Larval source management has been integrated in the national Malaria Control Programme [31,32]. Evaluations for larviciding with *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) have been conducted both at large scale under a collaborative project between the Ministry of Health and a Cuban company Labiofarm [33], and on a

small scale under the auspices of Biovision [34]. Operational deployment of Dimilin GR-2% and Mosquiron® 10 EC as supplementary vector control tools in the context of IVM could be useful in Zambia, particularly in urbanized areas.

5. CONCLUSION

The IGRs, Dimilin GR-2% and Mosquiron® 10 EC demonstrated effective and persistent post-treatment activity both in the laboratory and simulation field assays. They are recommended as ideal larvicides for operational malaria control in the context of the WHO-recommended IVM approach.

6. AUTHORS' CONTRIBUTION

E. Chanda designed and coordinated the study, participated in data collection, analyzed the data, and drafted the paper. A. Kandyata and J. Chanda assisted with data collection, analysis and interpretation. K.S. Baboo critically reviewed the paper.

7. CONFLICT OF INTEREST

The author of the paper does not have a direct financial relation with the commercial identities mentioned in the paper that might lead to a conflict of interest for any of the authors. The authors declare that they have no competing interests.

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