

Biological Activity of Sumilarv 0.5G against *Anopheles gambiae* sensu stricto and *Anopheles arabiensis* in Northern Tanzania

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ABSTRACT

Background: Sumilarv 0.5G (Sumitomo Chemical Co., Ltd., Tokyo, Japan) is a granular insecticide developed for the control of mosquito and fly aquatic stages. The active ingredient is pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether), a juvenile hormone analogue that acts as an insect growth regulator. Sumilarv 0.5G functions by inhibition of adult emergence from pupae. In this study, the Tropical Pesticides Research Institute in Tanzania carried out laboratory, semifield, and full-field evaluation on a new candidate of pupicide, Sumilarv 0.5G. The present study, therefore, sought to test the bio-efficacy of Sumilarv 0.5G in laboratory, semifield, and full-field conditions in Mabogini, northern Tanzania.

Methods: Standard World Health Organization laboratory bioefficacy evaluations of Sumilarv 0.5G and untreated microcosms were prepared and monitored for inhibition of the larvae introduced to the habitats, while field plots were monitored for 5 weeks after the introduction of Sumilarv 0.5G using manufacturer-recommended doses.

Results: Sumilarv 0.5G biolarvicide was highly efficacious in its pupicidal effect, with an adult emergence inhibition rate of up to 90% in all conditions. In both laboratory and semifield experiments, the emergence inhibition was dose-dependent, with the lowest adult emergence being recorded in association with the highest Sumilarv 0.5G dose of 0.03 ppm of active ingredient. Under field conditions, the application rate recommended by the manufacturer – 5 mg ai per m² – reduced the adult emergence rate by 90% to 96% for up to 5 weeks.

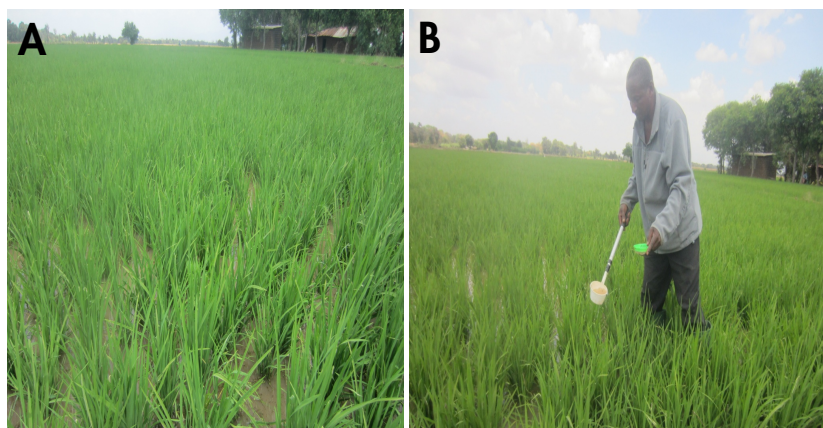
Conclusion: We demonstrated the long-lasting biological activity of Sumilarv 0.5G under field conditions. Notably, the field efficacy was attained using the recommended dose of 5 mg per m², thus making it economical to apply this product, which is capable of inhibiting mosquito productivity in natural habitats for longer periods than achieved by existing products, the efficacy of which is usually about 1 week.

INTRODUCTION

In Africa, the main malaria vectors are members of *Anopheles gambiae* sibling species complex and the *Anopheles funestus* complex.^{1,2} The *An. gambiae* complex consists of *An. gambiae* sensu stricto (s.s.), *Anopheles arabiensis*, *Anopheles merus*, *Anopheles melus*, *Anopheles bambwae*, *Anopheles colluzzii*, and *Anopheles ahmaricus*.^{1,2} The *An. funestus* complex vectors are *An. funestus* s.s., *Anopheles leesoni*, *Anopheles rivulorum*, and *Anopheles vaneedeni*.³⁻⁸ The main frontline malaria vector control tools are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS).⁹ Insecticide resistance has emerged as an urgent threat to these tools¹⁰⁻¹² that requires alternative or complementary solutions. Larval source management (LSM) is of paramount importance

in the fight against malaria, through vector control, to complement LLINs and IRS. Larval control is a new avenue which, if effectively implemented, can have an impact on malaria epidemiology.^{13,14} LSM has been shown to be more effective when combined with other tools that target adult vectors.^{14,15} Insecticide resistance among disease vectors is achieved via a variety of mechanisms at different vector developmental stages,¹⁶ but the main advantage of LSM is that it targets the immobile immature stages of mosquito vectors, thus controlling both outdoor and indoor resting and biting vectors.^{14,17}

Commercially available chemical and microbial larvicides are highly effective for short-lasting control of the aquatic stages of the main malaria vectors.^{13,14,18-20} The major challenge of existing biolarvicides is their short duration of activity in environmental conditions,

FIGURE 1. The Irrigated Rice Fields

(A) During site selection and (B) during larvae density estimation

which means that they require weekly reapplication.^{18,21} Labour and larvicide supply are the major costs associated with large-scale vector management, which aims to reduce costs by maximising the reapplication intervals.²² Also, the toxicity of larvicides to untargeted aquatic insects limits the practicality of regular larvicide programmes.²³

Sumilarv 0.5G (Sumitomo Chemical Co., Ltd., Tokyo, Japan) is a granular insecticide that was developed to control the pupal stages of mosquitoes and flies.²⁴⁻²⁷ The active ingredient is pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether), a juvenile hormone analogue that acts as an insect growth regulator.²⁸ Pyriproxyfen generally inhibits the adult emergence of target insects species.^{29,30}

However, pyriproxyfen causes delayed effects on reproduction among female adult mosquitoes exposed to sublethal doses at the larval³¹ or adult stage.^{32,33} Sumilarv 0.5G has exceptional residual activity of up to more than 1 month for the control of mosquito species in their natural breeding sites.^{30,33,34} Furthermore, pyriproxyfen has been evaluated as a safe insecticide for application in drinking water,³⁵ with limited impact on untargeted aquatic insects and the environment.^{36,37} Nevertheless, Sumilarv 0.5G has never been evaluated for the control of *An. arabiensis*, the major malaria vector in Tanzania.

This study aimed to evaluate the efficacy of Sumilarv 0.5G as a pupicide against *An. gambiae* s.s. in laboratory and semifield conditions, and against *An. arabiensis* under field conditions.

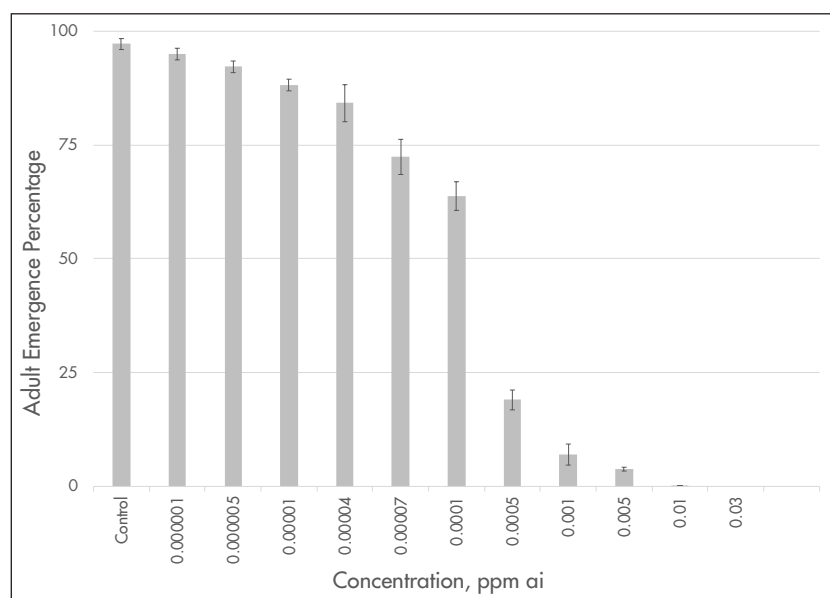
METHODS

Study design

This was laboratory and field experimental study.

Sumilarv 0.5G Formulation

Sumilarv 0.5G was applied only to mosquito breeding habitats that did not drain into natural water bodies. According to the manufacturer's instructions, the amount of Sumilarv 0.5G applied was determined by the volume of water in the respective habitats (width×length×depth), based on a target concentration of 0.01 to 0.05 ppm of active ingredient (ai) or (0.2-1.0 oz/100 ft³). The targeted habitats were temporary or permanent water holding sites amenable to treatment: ornamental ponds, fountains, cesspools, abandoned swimming pools, gutters, construction site depressions, septic tanks, flooded basements, gutters, animal waste lagoons, livestock runoff lagoons, sewers, sewage effluent, tire tracks, waste water impoundments associated with organic pollutants and industrial run off, waste water and settling ponds, and vegetation-choked phosphate pits. Other potentially treatable sites include natural and artificial water holding containers: hollow trees and tree holes, potted plants, bird baths, tire dumps, landfills, rain barrels, flooded roof tops, flower pots, buckets, salvage yards, abandoned vehicles, vehicle impounds, and junkyards. The targeted mosquito species for Sumilarv 0.5G are *Aedes aegypti*, *Aedes albopictus*, *Aedes vexans*, anophelines, *Culex pipiens*, *Culex quinquefasciatus*, *Culex tarsalis*, and *Culex restuans*.

FIGURE 2. Percentage Emergence of *Anopheles gambiae* sensu stricto in Laboratory Trials for Different Concentrations

Mosquito Rearing

Mosquitoes originated from a colony of *An. gambiae* s.s., established in Kisumu, Kenya, in 1992, and were reared at the Tropical Pesticides Research Institute TPRI. The laboratory larval rearing of protocol is described elsewhere.^{38,39} In the insectary, larvae were fed TetraMin (Spectrum Brands Pet, Blacksburg, VA, USA) fish food at rate of 0.003 g/larva. Third instar larvae were used for trials, as recommended by the World Health Organization protocol.⁴⁰ The photo phase in the insectary was 12 light:12 darkness, with a temperature of $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and a relative humidity of $78\% \pm 2\%$.

Dose-Response Bioassays

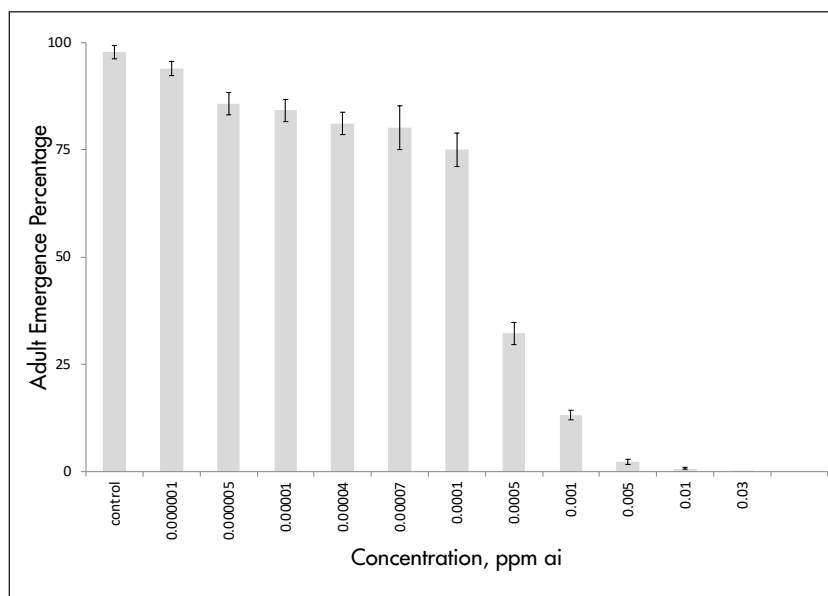
Experiments were done in the laboratory and in semifield conditions – experiments conducted in controlled conditions outside the laboratory but with restricted interaction with nature.⁴¹ Before the dose-response experiments, a range-finding test was implemented by exposing test larvae to a wide range of test concentrations and a control. This was used to find the activity range of the insecticide for tested species.

A range of concentrations between 10 ppm ai and 1.0×10^{-7} ppm ai were tested. After determining the emergence inhibition of the larvae in a broader range, concentrations causing emergence inhibition of between 10% and 95% were chosen and used in dose-response bioassays.⁴⁰ Fifteen serial dilutions were made, and the best 11 doses causing emergence inhibition for use in laboratory and semifield trials were selected. A stock solution was prepared by grinding the

granular formulation into a fine powder, following the procedure described Sihuincha et al.³³ Using a pestle and mortar, 5 g of SumiLarv 0.5 G (25 mg ai) was ground and added to 500 ml of unchlorinated tap water. This produced a stock solution of 10,000 ppm SumiLarv 0.5G (50 ppm ai). The top of the vial was covered with aluminium foil, and the solution was left to agitate for 1 hour on a shaker. The mixture was left overnight to allow the active ingredient to be released into the solution. The next morning, the mixture was again agitated on a shaker for 30 minutes to prepare a homogenous mixture, as some of the inert ingredients of the formulation – potentially still containing some active ingredient – had settled overnight. Serial dilutions were made immediately after shaking in unchlorinated tap water to produce the test concentrations.

The laboratory-reared colony of *An. gambiae* s.s. was evaluated against different concentrations of Sumilarv 0.5G. Each test concentration and a control were replicated 6 times, and 200 ml of each test solution was set up in 300 ml glass bowls. The test was repeated 3 times for each concentration. Separate batches of 25 insectary-reared third instar larvae of test species were introduced into each test concentration and the control.

Larvae were fed with TetraMin (Spectrum Brands) fish food only when the experiments were monitored for more than 24 hours. Bowls were covered with netting to prevent any emerging adults from escaping. The pupae were monitored until emergence or death. The number of dead larvae, pupae, and emerging adults were recorded until the end of the experiment, when all pupae had emerged or died. Live

FIGURE 3. Percentage Emergence of *Anopheles gambiae* sensu stricto in Semifield Trials for Different Concentrations

pupae from each bowl were transferred into a separate bowl containing 20 ml of water from the habitats. These bowls were covered with netting for monitoring adult emergence. Separate pipettes were used to collect pupae from treated and control bowls to avoid cross-contamination.

Semifield Studies

Semifield trials were carried out in an open field with artificial microcosms.^{40,42} Six artificial microcosms were made up using small washing basins (diameter, 21.5 cm; depth, 10 cm) filled with 1 kg of soil and 1,500 ml of water to resemble a natural larval habitat. Microcosms were paired between treatments and controls at 3 m intervals. Monitoring of the microcosms was conducted daily until the first pupa was observed, then monitoring was conducted twice a day. All pupae were collected with some water from each microcosm. Batches of 25 insectary-reared third instar larvae were introduced into each microcosm.

Field Trials

Two pairs of rice plots were selected for the field trials, with larval abundance evaluated before treatments (Figure 1). Two were control plots, and the other 2 were treatment plots. The plots measured 70 m × 20 m. Larvae were sampled 3 times per week (Monday, Wednesday, and Friday) for 5 weeks. All collected control and treatment pupae were kept in labelled paper bowls and monitored for adult emergence. The effect of Sumilarv 0.5G was measured as adult emergence inhibition

in all treated plots, while for the control, emergence inhibition was considered as natural mortality.

Parameters Measured

The percent inhibition of adult emergence (%IE) was calculated following the World Health Organization guideline,⁴⁰ using the following formula:

$$IE (\%) = 100 - (T \times 100 / C)$$

Where,

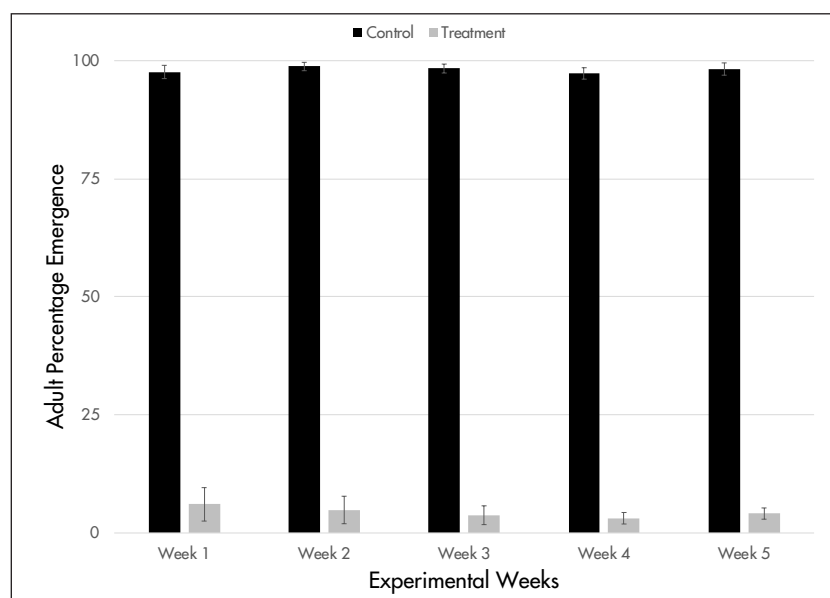
T = percentage survival or emergence in treated batches

C = percentage survival or emergence in control batches

Data Analysis

Data were analysed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Descriptive statistics were deployed for data analysis to obtain the confidence intervals and mean differences. Excel (Microsoft Corp., Redmond, WA, USA) spreadsheets were used to calculate percentage inhibition of larvae. The comparison between treatment and control was done using paired samples t-tests. Probit analysis was used to calculate the LC_{50} and LC_{95} . *P* values less than .05 were considered statistically significant.

FIGURE 4. Percentage Emergence of *Anopheles gambiae* sensu stricto in the Field Trials for 5 Weeks After a Single Treatment



Ethical Considerations

Approval for this study was granted by the Tanzania Pesticides registrar's office (experimental permit number 2310, issued in 2015).

RESULTS

Laboratory Trials

A series of doses were evaluated, and those with emergence inhibition effects were considered for laboratory trials. Selected trial doses ranged from 10^{-6} to 0.03 ppm ai. Percentage inhibition was dose-dependent (Figure 2). The dose of 0.03 ppm ai caused 100% emergence inhibition. The percentage inhibition among treatment doses was statistically significant ($df=11$, $F=242.9$, $P<.001$).

Semifield Trials

Similar results to those observed in laboratory studies were found in the semifield experiments. Emergence inhibition was dose-dependent, and the highest inhibition levels resulted from the highest doses (Figure 3). Inhibition percentage was statistically different between the groups, with more adult emergence observed at lower doses ($df=11$, $F=367.86$, $P<.001$).

Field Trials

In the first week of sampling after the plots were treated, the emergence rate for pupae differed significantly between

treated and control plots, with more adults emerging from the pupae sampled in the control than the treated plot arms ($df=4$, $t=74.1$, $P<.001$). The same was observed in weeks 2 ($df=4$, $t=70.5$, $P<.001$), 3 ($df=4$, $t=70.5$, $P<.001$), 4 ($df=4$, $t=69.7$, $P<.001$), and 5 ($df=4$, $t=81.1$, $P<.001$), as shown in Figure 4.

DISCUSSION

Our findings shown that wild *An. arabiensis* and laboratory *An. gambiae* s.s. populations had similar responses to Sumilarv 0.5G under different environmental conditions. Similar findings were presented by a study conducted in Kenya by Mbare et al.⁴³ Sumilarv 0.5G inhibited over 90% of the total adult emergence over a period of 5 weeks in irrigated rice fields at an application rate of 5 mg ai per m². This level of inhibition is consistent with observations of a study targeting the control of *An. gambiae* sensu lato (s.l.) in Kenya, where a concentration of 0.003 ppm pyriproxyfen was enough to completely inhibit emergence for up to 1 month.⁴³ In the present study, however, weekly emergence rates remained low for up to 5 weeks, even at the lower doses. Weekly emergence inhibition was frequently higher than 90%, the threshold recommended by the World Health Organization Pesticide Evaluation Scheme for the successful control of immature mosquito stages.⁴⁰ Application rates in field conditions were increased up to several times the minimum dose instructed by the manufacturer to obtain sufficient control under field conditions.²⁸ The findings were consistent with those observed in previous studies.^{40,44} The higher dose of 5 mg ai per m² in field conditions inhibited over 80% of adult emergence for 5 weeks.

Further field tests to establish the optimum dose for operational control in a variety of different habitats are necessary. However, based on the results presented in this study, it is likely that the optimum dose lies between the doses tested here. This concurs with the maximum dose recommended by the manufacturer (0.05 ppm ai) for field operational control of anopheline mosquito species.²⁸ Kawada et al.⁴⁵ previously found emergence inhibition for *An. gambiae* to be 4 times less than the level found in the present study. The same dose for *An. gambiae*, however, was found to be in the range recommended for culicine and *Aedes* species.^{46,47} The observed differences in these studies were attributed to factors such as differences in container types and pyriproxyfen formulations.^{34,45} The study by Kawada et al.⁴⁵ used an emulsifiable concentrate (5%) formulation, while the present study used a granular formulation. Moreover, in the present study, the plastic bowls used during the bioassays were found to have retained high larvicide amounts leading to a higher residual effect relative to the aluminium bowls used by Kawada et al.⁴⁵ Plastic materials are known to retain substantial amounts of active ingredient and to release it slowly, which could explain the higher emergence inhibition found in this study at all doses used.^{36,37,48} Our experiments have shown that Sumilarv 0.5G is effective at low active ingredient concentrations. The required concentration of pyriproxyfen is substantially lower than that of other microbial agents used as larvicides.¹⁹ A previous study on culicine mosquitoes also demonstrated that pyriproxyfen operates effectively at very low concentrations.⁴⁹ The efficacy of lower concentrations will lower the operational costs for larvicide programmes that use Sumilarv 0.5G.²² The observed residual impact on *An. arabiensis* emergence rates was similar to what was found in previous studies on other mosquito species.^{34,46}

In previous studies under field conditions, Sumilarv 0.5G at 0.02 ppm ai and 0.05 ppm ai effected complete emergence inhibition for 6 weeks for *Anopheles quadrimaculatus*, *Culex nigripalpus*, *Aedes taeniorhynchus*, *Ae. albopictus*, and *Ae. aegypti*.⁵⁰ This granular formulation is released and produces its effects relatively slowly compared to other bio-larvicides, exhibiting extended residual effects, particularly when applied to mosquito breeding containers. It was very effective against *Aedes* larvae even when the habitats were flushed with untreated water.³⁴ In Sri Lanka, a single dose of 0.1 mg/l was shown to be sufficient for six months against anopheline malaria vectors in pots and small pits, meaning that 2 applications per annum were sufficient.³⁰ In Peru, it was observed that 0.003 g ai pyriproxyfen/m² was sufficient to extend emergence inhibition for 5 months in water tanks housing *Ae. aegypti*.^{33,51}

Overall, it can be concluded from previous and our own study that the efficacy and residual activity of different pyriproxyfen-containing products depends on the formulation, dose, habitat types, and vector species.^{36,46}

We did not observe that the efficacy of Sumilarv 0.5G is reduced in turbid water, as reported by Mbare et al.⁴³ Some

of the turbidity observed in that study might have been due to algae and bacteria growing in the established habitats. It is possible that the debris absorbed some of the active ingredients of the Sumilarv 0.5G, reducing its efficacy.⁵² Debris in aquatic habitats is an important parameter that is often associated with the abundance, development, and survival of *An. gambiae* s.l. larvae.^{42,53,54} In the recent past, anopheline larvae have been found to exploit aquatic habitats with varying degrees of water turbidity and pH, from sunlit and ephemeral to permanent, large water bodies in both urban and rural areas.⁵⁴⁻⁵⁶ Debris and other decaying materials provide mosquitoes with food particles that enhance their aquatic survival, thus increasing adult emergence from turbid water bodies.^{42,53,54,57,58} This condition in natural habitats needs to be considered and monitored in field operations for the effective control of aquatic stages.⁴⁰ At 5 mg ai per m², reproduction by female mosquitoes declined by over 90% as a consequence of the sublethal effect of Sumilarv 0.5G on emergence for 5 weeks. Similar effects of pyriproxyfen have been shown for *Anopheles*, *Aedes* and *Culex* species in both laboratory and field conditions.^{30,31,33,59} Another effect is to suppress the viability of eggs, thus reducing emergence rates and subsequently reducing intervention costs. The outcome of stress caused by growth regulators is known to affect the adult sex ratio and reduce blood feeding ability.⁶⁰ The same phenomenon is observed among adult mosquitoes exposed to pyriproxyfen.³² Insect growth regulators have also been found to suppress ovarian and egg development.^{61,62} Caution should be taken when considering the continuous use of pyriproxyfen, as resistance might develop, as has occurred with other insecticides.⁶³⁻⁶⁶ Examining the population of insects surviving after exposure for tolerance against pyriproxyfen must be built into malaria vector control strategies.

CONCLUSION

We have shown that *An. arabiensis* and *An. gambiae* s.s. are highly susceptible to Sumilarv 0.5G at low doses. This product would, therefore, be useful for targeting productive natural habitats of malaria vectors and help control wild mosquito populations. Such reduction in population size can happen within a relatively short period, as this study has shown that Sumilarv 0.5G significantly inhibits adult emergence and egg viability. We recommend further studies to better understand and standardize re-treatment intervals in both dry and rainy seasons.

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