

## Preplanned Studies

## Laboratory and Semi-Field Evaluation on S-Methoprene Formulations Against *Anopheles sinensis* (Diptera: Culicidae) — Yuxi City, Yunnan Province, China

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### Summary

#### What is already known about this topic?

*Anopheles sinensis* (*An. sinensis*) is the predominant malaria vector in China. The impact of S-methoprene on the emergence process of mosquito larvae suggests its potential as a control method for vector mosquitoes. However, the efficacy of S-methoprene in controlling *An. sinensis* has not yet been demonstrated.

#### What is added by this report?

The effectiveness of S-methoprene against *An. sinensis* was assessed in laboratory and semi-field conditions in Yunnan Province.

#### What are the implications for public health practice?

These results offer valuable options and guidance for utilizing S-methoprene products in malaria reimportation prevention areas within Yunnan Province.

Malaria is an infectious disease transmitted by mosquitoes, specifically through the bite of *Anopheles* mosquitoes or by blood transfusion from an infected person. In 2021, there were approximately 247 million malaria cases reported in 84 countries endemic to malaria (1).

The most common mosquito species responsible for malaria transmission in China is *Anopheles sinensis* (*An. sinensis*) Wiedemann (2). Chemical control is currently a primary method for comprehensive vector management due to its simplicity, effectiveness, and ease of use. However, the emergence of pesticide resistance among malaria vector mosquitoes has become a significant concern. Yunnan Province, located in the Greater Mekong subregion (GMS), is particularly vulnerable as a malaria hotspot (3). Studies have demonstrated that *An. sinensis* mosquitoes in Yuxi City, Yunnan Province have developed resistance to traditional chemical insecticides like organophosphorus, carbamate, organochlorines, and pyrethroids (4).

In recent years, biological control has gained attention in the field of entomology and vector control as a potential solution to pesticide resistance. One notable biological insecticide is the juvenile hormone analogue S-methoprene, which has been used in the United States since the 1970s and is recognized for its efficacy against various vector species while maintaining environmental safety and non-target organism protection (5). Several formulations of S-methoprene, including emulsifiable concentrate, microcapsule suspensions, granules, pellets, water-soluble pouches, and briquets (6).

S-methoprene technical material and its products were tested in a laboratory against late-instar larvae of *An. sinensis*. Following this, an experimental site in Yuxi City, Yunnan Province (Figure 1) was selected to evaluate the impacts of different formulations of S-methoprene products on *An. sinensis* under semi-field conditions (Supplementary Material, available at <https://weekly.chinacdc.cn/>). The bioassays showed that the formulated products and technical material had similar efficacy in inhibiting adult emergence at three different IE levels. Under semi-field conditions, a 20% microcapsule suspension at a dosage of 0.025–0.1 mL/m<sup>2</sup> provided 100% efficacy for at least 3 days, while 1% granules at 9.09 g/m<sup>2</sup> and 4.3% granules at 2.0 g/m<sup>2</sup> provided more than 85% efficacy for at least 14 days. The results confirmed the strong biological activity and safety profile of S-methoprene, supporting its recommendation as a standard larvicidal tool for controlling *An. sinensis* in various habitats while adhering to local regulations.

In the concurrent bioassays, we tested the effectiveness of S-methoprene technical material, microencapsulated suspension, and two granules against *An. sinensis*. The results showed that all formulations exhibited high activity in inhibiting adult emergence. Interestingly, there were no significant differences observed between the formulated products and the pure S-methoprene technical material at three different application levels. This data is summarized in

TABLE 1. Laboratory bioassays on S-methoprene technical material and products against *Anopheles sinensis*.

Product	IE 10 (μg/L) (95% CI)	IE50 (μg/L) (95% CI)	IE 90 (μg/L) (95% CI)
Technical S-methoprene	0.055 (0.012–1.060)	0.220 (0.119–0.303)	0.883 (0.640–1.529)
microencapsulated suspension	0.046 (0.019–0.076)	0.236 (0.017–0.322)	1.199 (0.744–2.893)
1% granule	0.052 (0.009–1.065)	0.221 (0.116–0.331)	1.238 (0.885–3.840)

Note: Mortality data was corrected by factoring the mortality in untreated control (6.4%–8%) using Abbott formula (Abbott, 1925) before probit analysis.

Abbreviation: CI=confidence intervals; IE=inhibition of emergence.

Table 1.

During the testing period, the infection rate (IE) of *An. sinensis* against IE in untreated control (UTC) was as low as 0–4%. The experimental endpoint was considered achieved when the IE provided by the insecticidal preparations was less than 85%. There were dose-dependent and time-related effectiveness trends observed within the intended range of 0.025–0.1 mL/m<sup>2</sup>. On the third day of the evaluation period, all three selected concentrations showed 100% efficacy. However, on the 7th day of assessment, the IE decreased to 48% at 0.025 mL/m<sup>2</sup> and 52% at 0.038 mL/m<sup>2</sup>. For the highest concentration of 0.1 mL/m<sup>2</sup>, assessments were conducted from day 14 until the experimental endpoint (Figure 2). The IE% showed a highly significant difference among the UTC and treatment groups ( $\chi^2=193.2$ –250.0,  $P<0.001$ ), as well as among the doses on days 3, 7/14 ( $\chi^2=10.4$ –18.9,  $P<0.01$ ).

Dose-dependent and time-related effectiveness trends were observed within the intended range of 1.60–9.09 g/m<sup>2</sup>. On the third day of the evaluation period, all three selected concentrations demonstrated 100% efficacy. However, at a concentration of 1.60 g/m<sup>2</sup>, the effectiveness persisted for less than 7 days. By the 14th day of assessment, the effectiveness decreased to 38% at 5.13 g/m<sup>2</sup> and 86% at 9.09 g/m<sup>2</sup>. For the highest concentration of 9.09 g/m<sup>2</sup>, assessments were continued from day 21 until the end of the experiment (Figure 2). The effectiveness percentage (IE%) showed significant differences among the UTC and treatment groups ( $\chi^2=150.9$ –250.0,  $P<0.001$ ), as well as among the different doses on days 3, 14–21 ( $\chi^2=9.33$ –11.27,  $P<0.01$ ).

Dose-dependent and time-related trends in effectiveness were observed within the intended range of 0.4–2 g/m<sup>2</sup>. By the third day of evaluation, all three selected concentrations showed 100% efficacy. At a concentration of 0.4 g/m<sup>2</sup>, the duration of effectiveness was less than 7 days. On the 7th day of evaluation, all concentrations showed 100% efficacy. On the 21st day, the effectiveness decreased to 76% at 1.0 g/m<sup>2</sup> and 80% at 2.0 g/m<sup>2</sup>. The effectiveness percentages



FIGURE 1. Semi-field testing site in Yuxi City, Yunnan Province. (A) Microcosms; (B) Assembled sentinel cage.

were significantly different among the untreated control group and the various treatments ( $\chi^2=145.7$ –250.0,  $P<0.001$ ) and among the doses on days 3, 14–21 ( $\chi^2=27.78$ –31.86,  $P<0.01$ ).

## DISCUSSION

The Greater Mekong Subregion (GMS), which includes Yunnan Province of China, Cambodia, Lao PDR, Myanmar, Thailand, and Vietnam, is a significant malaria hotspot. The introduction of the Mekong Malaria Program (MRP) by the World Health Organization (WHO) has led to notable improvements

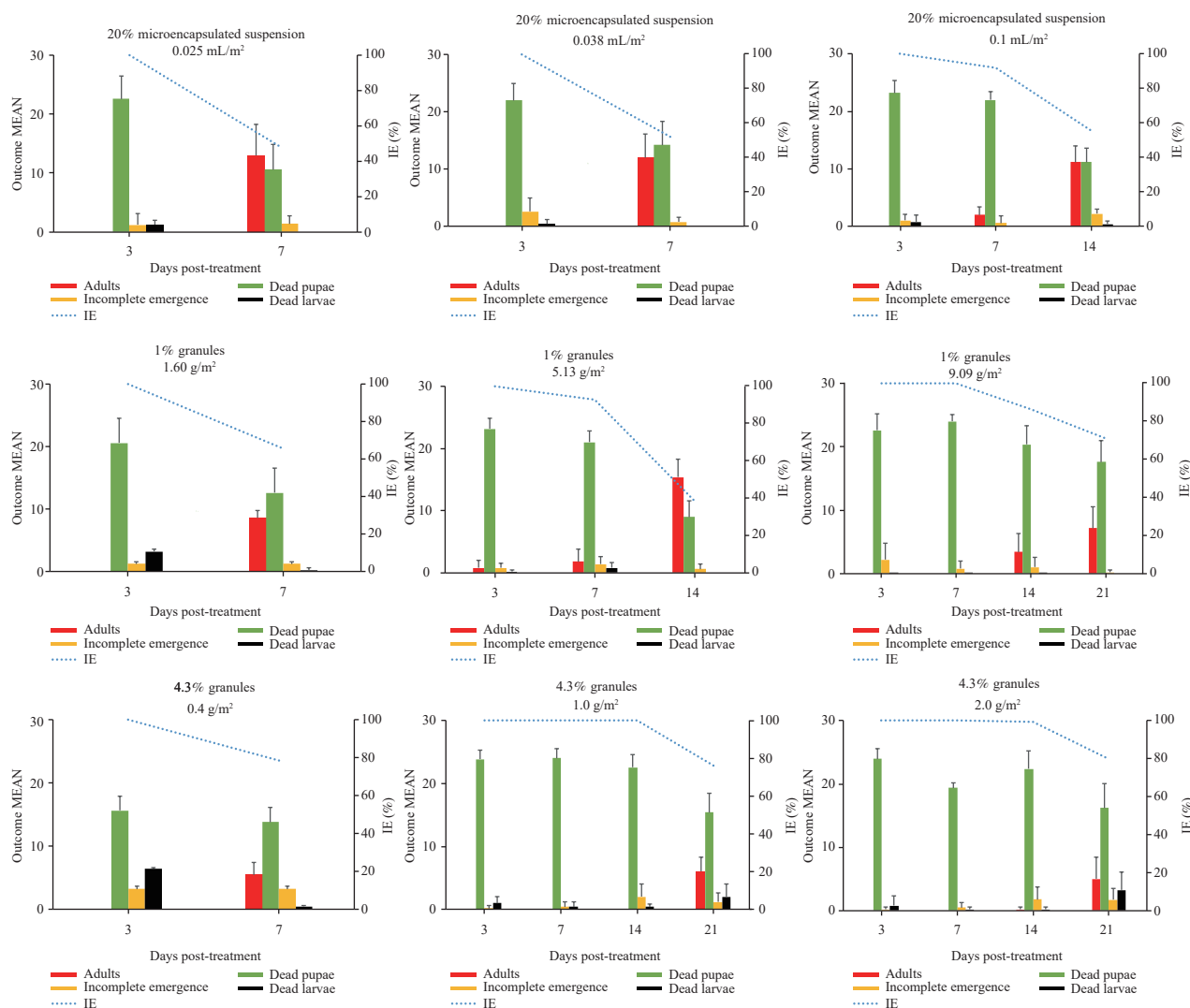


FIGURE 2. Inhibition of emergence (IE) against *Anopheles sinensis* by S-methoprene products with a water depth of 13.0 cm.

in malaria control in the region, with a consistent decrease in annual malaria incidence and deaths (4).

Integrated mosquito control, combining environmental management and targeted pesticide use, is essential for reducing mosquito populations and controlling mosquito-borne diseases. Larviciding, which targets the aquatic stages of mosquitoes, is a cost-effective approach in mosquito control compared to adulticiding, which targets adult mosquitoes.

For example, in a study by Zhou et al., it was found that *An. sinensis*, the malaria vector, has developed high resistance to conventional chemical insecticides such as beta-cypermethrin and propoxur in Yunnan Province (7).

Over the past 50 years, the USA has established the advantages of S-methoprene against economically significant pests in public health, livestock, stored goods, and agriculture (6). However, S-methoprene is

rarely used in China, with only the Synergetica Life Science Changzhou company producing S-methoprene products. It rapidly degrades in soil, particularly under sunlight, with a half-life of 10–14 days. The primary microbial degradation product is carbon dioxide (8).

To cater to various application scenarios, S-methoprene products have been developed in multiple forms, with granules and microencapsulated suspensions being the most popular. The milky white turbid microcapsule suspension, diluted with water, was utilized with spraying equipment. The granules used granulated as a natural carrier with a diameter of 1.0–2.0, which was doubled using a specialized binding technique. Both products were extensively evaluated against the index species, utilizing late fourth instar larvae in laboratory and field studies. These larvae, prior to pupation, exhibit high susceptibility to external JHAs due to low levels of internally present

juvenile hormone III. The comparable efficacy of the formulations with the S-methoprene technical materials in bioassays justified further field evaluation for both the granules and microencapsulated suspensions.

The formulated S-methoprene product showed the desired initial and residual efficacy against *An. sinensis*. Microcapsules were used at concentrations of 0.025 mL/m<sup>2</sup>, 0.038 mL/m<sup>2</sup>, and 0.1 mL/m<sup>2</sup>, effectively controlling *An. sinensis* for a minimum of 3 days at a water depth of 13 cm. Granules, on the other hand, exhibited longer persistence. The 1% granules provided over 85% control for at least 14 days at a concentration of 9.09 g/m<sup>2</sup>. Similarly, at doses of 1.0 g/m<sup>2</sup> and 2.0 g/m<sup>2</sup>, the 4.3% granules maintained at least 85% control for 14 days. It is worth noting that microencapsulated suspension and granules have distinct characteristics and are suited for different scenarios. The effectiveness observed can be attributed to the special carrier system, which ensures proper binding, preservation, and transport of the active ingredient. In the case of microcapsule suspension, the methoprene ester is coated within the capsule shell, providing protection against light damage and extending the effectiveness period (9). The granules, composed of fossilized diatoms, possess a high water absorption capacity and a large surface area, reducing exposure of the active ingredient to ultraviolet radiation and microbial activity in aquatic ecosystems (10).

This study has several limitations primarily due to the semi-field evaluation approach. First, the study excluded factors such as rainfall and sunlight exposure and instead created more controlled microcosms. Additionally, the capture of *An. sinensis* larvae in the field may introduce inevitable systematic errors, as less motile larvae are more likely to be captured.

There is an urgent need to find alternatives to conventional chemical pesticides to address the problem of mosquito resistance, specifically with larvicides that are effective and cost-efficient. S-methoprene has been shown to be safe for non-target organisms and environmentally friendly. Considering its proven performance against *An. sinensis* and its ability to adapt to different scenarios, it would be reasonable to recommend S-methoprene as a standard larvicidal tool for various mosquito habitats, while complying with local regulations.

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## SUPPLEMENTARY MATERIAL

The S-methoprene technical material used in this study was provided by Synergetica Life Science Changzhou Co., LTD. (Jiangsu, China). The S-methoprene 20% microencapsulated suspension (WP20210196 by China Ministry of Agriculture) was also used. The 1% and 4.3% granules used in the experiment were obtained from the same supplier. These granules are yellowish/brownish in color and have a diameter ranging from 1.0 to 2.0 mm, with various shapes including round and angular.

The mosquito species *An. sinensis*, a malaria vector, was obtained from the insectary of the National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases at the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The insectary maintains a stable and susceptible population of this species. The *An. sinensis* larvae used in the field trial were collected from Yuanjiang County, Yuxi City, Yunnan Province. For laboratory bioassays and on-site insecticide efficacy evaluation, late 4th instar larvae, which were about to pupate, were selected.

Laboratory bioassays were conducted to validate the quality of microencapsulated suspension and granules, as well as the technical grades, using previously established techniques. The S-methoprene technical material was dissolved in pure acetone (Sinopharm Chemical Reagent Co., Ltd, China) and then serially diluted with the same solvent. The microencapsulated suspension was dissolved in dechlorinated water. The granules were powdered in a high-speed blender (Bear Electric Co., Ltd, China) at the maximum speed in interruption mode, and then suspended in tap water by vortexing for 3 minutes.

For the bioassay, five concentrations within the dose range that caused approximately 5%–95% cumulative mortality at adult emergence were used. Each concentration and the untreated control (UTC) were replicated three times. The UTC had the same conditions as the experimental group, except that no insecticide was added.

Each replicate consisted of 25 late 4th instar larvae placed in 200 mL of tap water in a 250 mL disposable Styrofoam cup. The insecticide was administered according to the planned concentration and continuously observed. To promote larval growth until pupation, approximately 150 mg of tropical fish food (Desai Limited, Germany) was added to each bioassay cup. The bioassays were carried out within a temperature range of 27.0 °C–29.0 °C.

The inhibition of emergence was recorded when all exposed larvae exhibited outcomes. The concentration-response data were analyzed using SPSS2020 and Probit regression to calculate the concentrations that caused 10%, 50%, and 90% inhibition of emergence (referred to as IE10, IE50, and IE90) respectively, along with their corresponding 95% confidence intervals. In the control group, if the IE was greater than 95%, no correction was performed. For values between 80% and 95%, Abbott's formula was used for correction. If the IE was less than 80%, the experiment was repeated.

The test site chosen for this study was located in Ganzhuang Street, Yuanjiang County, Yuxi City, Yunnan Province. In order to create microcosms, blue plastic boxes were placed in shaded areas. The microcosms measured 0.74 L × 0.54 W × 0.41 D meter. Sandy loam soil, approximately 1.5 cm deep, was added to the microcosms. This soil was collected from a nearby paddy field that had no history of pesticide or herbicide applications. To mimic the breeding environment, the water was maintained at a depth of 13 centimeters. Larval food, in the form of tropical fish food pellets, was added at a rate of approximately 6 grams per microcosm, both after flooding and on a weekly basis as organic enrichment. Daily records were kept for local temperature and humidity. To prevent oviposition by natural mosquito populations, primarily *Culex* spp., the microcosms were covered with window screens (1.4 mm) during non-sampling periods. Each treatment and UTC was replicated five times. Treatment was initiated on the fifth day after flooding, when the organic enrichment had properly fermented and the soil had settled. The microcapsule suspension was administered at dosages of 0.025 mL/m<sup>2</sup>, 0.038 mL/m<sup>2</sup>, and 0.100 mL/m<sup>2</sup>. The 1% granules were applied at dosages of 1.60 g/m<sup>2</sup>, 5.13 g/m<sup>2</sup>, and 9.09 g/m<sup>2</sup>. The 4.3% granules were applied at dosages of 0.4 g/m<sup>2</sup>, 1.0 g/m<sup>2</sup>, and 2.0 g/m<sup>2</sup>.

In this study, late 4th instar larvae from *An. sinensis* colonies, previously reared in an insectary, were introduced into sentinel cages on days 3, 7, 14, 21, and 28 for testing. Each microcosm contained approximately 50 larvae (Table 1). The sentinel cage was a 1,000 mL square plastic tub with a 2×4 cm window on each of its four sides. The windows were covered with 0.3 mm screen to allow water to flow freely while retaining the larvae and pupae. The cage lid was perforated for ventilation and to prevent debris from entering. To serve as a floater, a plastic foam

floater was attached underneath the rim of the cage. Two days after the introduction of larvae, 25 pupae were collected from each sentinel cage and placed in a 250 mL Styrofoam cup filled with 200 mL water from the same microcosm. The remaining larvae and pupae in the sentinel cages were disposed of properly. The cup containing pupae was covered with a window screen (1.4 mm) to confine emerged adults. Different outcomes, such as mortality (in the form of dead pupae mostly, and occasionally incompletely emerged adults with attached wings and/or legs) and successful emergence (only free pupal exuviae were considered), were recorded separately. Once recorded, the adult mosquitoes in the cup were disposed of properly.

The mean IE% for each treatment on each sample day was calculated as follows:

$$\text{IE}\% = 1 - \frac{\text{number of successfully emerged adults}}{\text{total number of pupae isolated}}$$

The statistical analysis used for determining the significance of the IE% among treatments and the UTC was the Chi-square test, with  $\chi^2$  values of 3.84 and 6.63 for the significance levels of  $P=0.05$  and  $P=0.01$ , respectively.