

EVALUATION ON THE ACTIVITY AND EFFICACY OF OMNIPRENE™ 20CS AGAINST *Aedes*, *Anopheles* AND *Culex* MOSQUITOES IN OUTDOOR MICROCOSMS

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ABSTRACT

Mosquito control by pesticides remains the main intervention to reduce the burden of mosquito nuisance and mosquito-borne diseases. In mosquito control, larviciding by application of environmentally friendly larvicides has been one of the routine operations. Larvicides based on microbial agents and insect growth regulators (IGRs) account for most of the available products. Ones that are formulated using S-methoprene ranged from microencapsulated concentrate (or capsule suspension - CS), various granules, and pellets to briquets for different habitats. The CS formulation has been among the traditional products based on S-methoprene due to its advantages of easy application, fast action, and even area coverage. The current paper evaluated a newly developed CS formulation OmniPrene 20CS™ containing 20% S-methoprene against mosquitoes of public health importance. High inhibition of emergence activity was indicated against the test species, and *Aedes* and *Anopheles* mosquitoes showed higher susceptibility than *Culex* in laboratory bioassays. The performance under field conditions exceeded the label specification of the currently available products with the same application pattern. Over the 21-day evaluation period, the control levels ranged 86.4-100%, 89.8-100% and 79.8-100% against *Aedes aegypti*, *Anopheles hermsi* and *Culex quinquefasciatus* respectively when it was applied at the intended label dose of 54.8-73.1 mL/ha. This product is expected to be used as one of the viable tools along with other available products to combat mosquitoes of public health concerns.

Key Words: S-methoprene, OmniPrene™, mosquitoes, microcosm, field efficacy

INTRODUCTION

To reduce the impact of mosquitoes and mosquito-borne diseases on public health and well-being, sustainable mosquito control remains one of the main interventions. In mosquito control operations, combating the aquatic immature stages is more feasible and cost-effective by biorational larvicides based on microbials (Antonio-Nkondjio et al. 2018, Derua et al. 2019) and insect growth regulators (IGRs). Due to high cost of research, development and registration, strict regulations, narrow market niches and resistance development, available larvicides are very limited. To meet the need of safe and effective mosquito control products due to existing, emerging, and resurging mosquitoes and mosquito-borne diseases (Gratz 1999, Chala and Hamde 2021), innovated formulations based on available active ingredients and searching for novel active ingredients are equally viable solutions.

S-methoprene, a synthesized chiral form out of the early mixture of R- and S- enantiomers, is the only IGR that is recognized as a biopesticide by US Environmental Protection Agency (US EPA 2021), and has been playing

a crucial role since the early 1970s to control insects of economic importance, mainly mosquitoes, dipteran flies, stored product pests, etc. (Henrick 2007, Su 2018). Numerous products from microencapsulated concentrate (or capsule suspension – CS), emulsifiable concentrate (EC), several granules, pellets and briquets have been developed and applied to combat numerous target species. As to the CS and EC, the advantages have been recognized such as relatively fast release of active ingredient, reduced sublethal exposure risk as compared with slow-release long residual formulations, and minimal persistence in the environment. Along with the availability of the CS formulations under another trademark (Central Life Sciences 2022), the current paper is aimed to evaluate a new product, OmniPrene™ 20CS containing 20% S-methoprene, that is formulated with innovated proprietary microencapsulation technologies, against *Aedes*, *Anopheles* and *Culex* species. High inhibition of emergence activity was indicated in laboratory bioassay, and the performance under field conditions exceeded the label specification of the currently available counterpart products with the same application pattern.

MATERIAL AND METHODS

Test Materials. The technical S-methoprene (US EPA 73487-1) was provided by Synergetica International Inc. (Marlboro NJ, USA). The sample had a Lot# MT08-218 and the purity was 98.06% by high performance liquid chromatography (HPLC). The OmniPrene 20CS (lot# SC-L210810), an innovated CS formulation containing 20% S-methoprene (US EPA registration pending 73487-3), was provided by the same supplier.

Mosquitoes. The yellow fever mosquito *Aedes aegypti* L. was supplied by Benzon Research (Carlisle, PA) from its long-term susceptible colony. The southern California malaria mosquito *Anopheles hermsi* Barr & Guptavanij originated from field-collected larvae and host-seeking female adults from southern California. The southern house mosquito *Cx. quinquefasciatus* Say was from a long-term in-house susceptible colony. The late 4th instar larvae, that were about to pupate, were used in laboratory bioassays and field efficacy evaluations.

Laboratory bioassay. Laboratory bioassay was conducted according to the previously published protocols (Su et al. 2018) against all test species to validate the quality of the test materials prior to field evaluation. The technical S-methoprene was dissolved in and serially diluted by ACS pure acetone (Cole Parmer, Vernon Hills, IL). The OmniPrene 20CS was diluted in deionized water. In the bioassays, 5 concentrations (0.025, 0.5, 2.5, 10, and 25 ppb) within the concentration range resulting in approximately 5–95% mortality, plus untreated control (UTC) were used, with 3 replicates at each concentration and UTC. Each aliquot with the appropriate dilution in the amount of 100 to 500 μ L was added to 100 mL tap water, where the volume increase was negligible. In technical S-methoprene, the acetone in the bioassay water evaporated quickly without an observable impact on the Styrofoam polystyrene cup as was demonstrated with the test subject and the UTC that had the solvent only. For each replicate, 25 late instar larvae were placed in 100 mL tap water in a 120-mL disposable cup. A small piece (approximately 100 mg) of rabbit pellets was added to each bioassay cup to have slow release of the nutrients to support larval growth to pupation. In mosquito species, late instar larvae are more susceptible than the early instar larvae to external JHAs such as S-methoprene (Noguchi and Ohtaki 1974). Young larvae have low susceptibility to JHAs due to high internal juvenile hormone. The bioassay using young larvae would underestimate the activity of JHAs, take longer to complete or end with inconclusive results because the peak concentration of JHAs and susceptibility window of larvae miss each other upon degradation of active ingredient and larval growth.

Therefore, late instar larvae, sometimes called pupating larvae were used in the current studies to ensure the maximized and synchronized inhibition of emergence in the laboratory bioassays. Bioassays were conducted at 27.0-29.0°C. The mortality was recorded when all exposed individuals died or emerged as adults. Only those that fully separated from their pupal exuviae were considered to have successfully emerged.

Field evaluation. *Test Facility and Treatment* Test was carried out in outdoor microcosms that were black color plastic tubs located at a protected, semi-shaded area with trees and other vegetation in suburban Riverside, California. The microcosms measured L0.81 x W0.51 x D0.41 meter. A 1.3-cm deep substrate of sandy loam soil was added to each microcosm to simulate the natural habitat. The soil was collected from a nearby orchard, where there were no known recent records of pesticide and herbicide applications. Water depth was maintained at 0.305 meter for each microcosm (approximately 118 liters) with a surface area of 0.41 square meter. Rabbit food pellets were added to each microcosm at 0.005% (about 6 g each microcosm) as larval food and organic enrichment, after flooding and on a weekly basis thereafter. A minimum-maximum thermometer was put at bottom of one tub that was in the middle of the microcosm layout to monitor water temperature range during each sampling interval. The microcosms were covered by a window screen (1.4 mm) to prevent oviposition by the natural mosquito populations (mostly *Culex* spp.) during non-sampling times. Five replicates (tubs) were made of each treatment and UTC.

The treatment was made on day 5 post-flooding, when enrichment was well fermented, and sediment had settled out. The application doses were 54.8 and 73.1 mL/ha as recommended on intended product label, equivalent to 2.125 μ L and 2.834 μ L of original product per microcosm. The product was diluted by adding 500 μ L product to 499.5 mL of deionized water resulting in 1000-time dilutions for ease of application and even coverage. Each microcosm was treated by pipetting 2.125 mL and 2.834 mL of the diluted product over the surface.

Efficacy assessment The previously insectary-reared late 4th instar larvae of the three test species were introduced on day 0, 2, 4, 7, 14 and 21 post-treatment, where day 0 referred to day of treatment. The late instar larvae were used here for the same reason as described previously. To collect 25 pupae after introduction on one occasion, 50 larvae were introduced to each sentinel cage in each microcosm for each of the three test species. The sentinel cage was made of 946.4 mL square plastic tub, 5.1 x 5.1 cm square window was made in the center of each side, the window was covered by 0.30 mm mesh to allow

water running through freely but to retain the larvae and pupae. The same window was also made in the center of the lid to ensure ventilation and to keep debris from falling into the cage. A plastic foam belt was attached underneath the rim of the cage as a floatation device. Twenty-five (25) pupae were collected from each of five (5) replicated microcosms 2-3 days (varied from cage to cage) after larval introduction when most introduced larvae had pupated. Collected pupae were held in 100-mL of water from the same microcosm in a 177.4-mL Styrofoam cup. In total, 125 pupae from each treatment or UTC were considered an adequate number for percentage calculation of emergence inhibition. After a one-time pupal collection, the remaining larvae and pupae in the sentinel cages were rinsed to the ground and disposed of. Cups with pupae were covered by a plastic dome with a 2.5-cm diameter screened top (1.4 mm mesh) and emerged adults were confined under this dome. Around day 3-4 after pupal collection, when all pupae died or emerged, surviving adults were released to a 30.5 x 30.5 x 30.5 cm screened mosquito cage, and only mosquitoes that were free from their pupal exuviae were counted and considered as successfully emerged. Observation on inhibition of emergence was conducted at 27.8-29.4°C. Adults released to the mosquito cage during result reading died later due to sugar and water deprivation and were disposed of properly later.

DATA ANALYSIS

Concentration–response data in laboratory bioassays were corrected by Abbott formula to factor the mortality in UTCs (Abbott 1925), then analyzed using POLO Plus (Robertson et al. 2006) for probit analysis to calculate the concentrations causing 10%, 50% and 90% inhibition of emergence (IE) and their 95% confidence intervals (95% CIs). Significant differences in IE were indicated by separate 95% CIs (Su et al. 2018). In field test, the mean IE% for UTC and each treatment on each sampling day was calculated: $IE\% = 1 - (\text{number of successfully emerged adults} / \text{total number of pupae collected})$. The IE% between the doses and among the sampling intervals were analyzed by *Chi* square test after correction by Abbott formula for the significance at $\chi^2 > 3.84$, $p < 0.05$ and $\chi^2 > 6.63$, $p < 0.01$ levels.

RESULTS

Laboratory bioassay. Concentration-response was established in the concurrent bioassays using technical S-methoprene and OmniPrene 20CS against all test species, with low mortality in all UTCs (5.3-9.3%). High

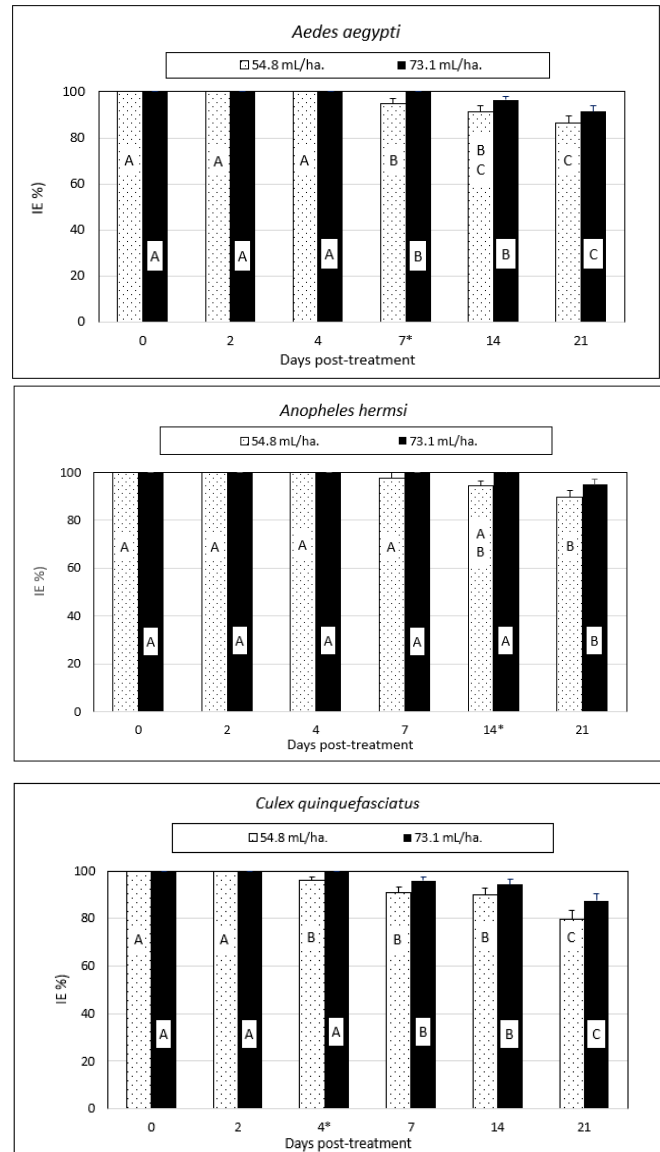


Figure 1. Corrected inhibition of emergence (IE) against *Aedes aegypti* (Top), *Anopheles hermsi* (Middle) and *Culex quinquefasciatus* (Bottom) by OmniPrene 20CS in microcosms. The significance between two doses was indicated by “*” associated with a given sampling day. The significance in efficacy decline over time was indicated by different capital letters inside the columns for each of two doses ($\chi^2 > 3.84$, $p < 0.05$; $\chi^2 > 6.63$, $p < 0.01$).

activity of inhibition of adult emergence was indicated in all test species after data correction by Abbott formula. By comparing the IE_{10} , IE_{50} and IE_{90} , *An. hermsi* appeared more susceptible than *Ae. aegypti*, but the differences did not reach the significant levels, as their 95% CIs overlapped. However, both *Ae. aegypti* and *An. hermsi* were more susceptible than *Cx. quinquefasciatus* as indicated by their separate 95% CIs. There were no significant differences between the technical S-methoprene and the formulated product OmniPrene 20CS against all test

Table 1. Laboratory bioassays on technical S-methoprene and OmniPrene™ 20CS containing 20% S-methoprene against the test species*.

Products	IE ₁₀ (ppb) (95% CI)	IE ₅₀ (ppb) (95% CI)	IE ₉₀ (ppb) (95% CI)
<i>Aedes aegypti</i> †			
Technical S-methoprene	0.059 (0.032-0.123)	0.932 (0.699-1.731)	9.532 (6.512-13.980)
OmniPrene 20CS	0.060 (0.036-0.152)	0.831 (0.389-1.342)	8.881 (6.211-12.363)
<i>Anopheles hermsi</i> †			
Technical S-methoprene	0.014 (0.009-0.039)	0.459 (0.245-1.034)	4.331 (2.834-7.945)
OmniPrene 20CS	0.022 (0.010-0.044)	0.434 (0.255-0.906)	4.692 (2.912-6.883)
<i>Culex quinquefasciatus</i> †			
Technical S-methoprene	0.189 (0.137-0.432)	2.408 (1.855-4.012)	17.587 (14.344-30.321)
OmniPrene 20CS	0.192 (0.156-0.445)	2.138 (1.357-3.122)	16.823 (13.832-32.345)

*Mortality data was corrected using Abbott formula (Abbott 1925) to factor the mortality in UTCs (5.3-9.3%) before probit analysis.

† The susceptibility of *Ae. aegypti* and *An. hermsi* was significantly higher than that of *Cx. quinquefasciatus* as indicated by separate 95% confidence intervals (CIs).

species at three (3) IE levels as shown by overlapped 95% CIs (Table 1). Mortality occurred in an overall pattern of incomplete adult emergence, dead intermediate form of larvae and pupae (also known as “puparvae”) (Su et al. 2020), dead pupae and dead larvae, upon the increases of S-methoprene concentrations.

Field evaluation. *Efficacy during 21-day evaluation* The mortality as represented by dead pupae and incompletely emerged adults were low at all UTCs, ranging 2.4-6.4% in the three test species. The corrected efficacy levels were as follows. Against *Ae. aegypti*, the low dose 54.8 mL/ha. provided 86.4 – 100% control, while the efficacy increased to 91.5 – 100% at the high dose 73.1 mL/ha. The IE was significantly higher at the high dose than that at the low dose on day 7 ($\chi^2 = 6.15$, $p < 0.05$). The efficacy at the low dose declined initially on day 7 ($\chi^2 = 6.15$, $p < 0.05$), and further on day 21 ($\chi^2 = 5.79$, $p < 0.05$). The efficacy decline at the high dose was delayed to day 14 and 21 ($\chi^2 = 4.07$ - 5.79 , $p < 0.05$). Against *An. hermsi*, the low and high doses resulted 89.8-100% and 94.9-100% inhibition

of emergence, respectively. The high dose outperformed the low dose on day 14 ($\chi^2 = 7.20$, $p < 0.01$). The efficacy declined on day 21 at both doses ($\chi^2 = 6.15$ - 6.68 , $p < 0.05$ - 0.01). Lastly, the efficacy against *Cx. quinquefasciatus* was slightly lower, being 79.8-100% for the low dose and 87.4-100% for the high dose. The high dose was significantly more efficacious than the low dose on day 4 ($\chi^2 = 5.10$, $p < 0.05$). The efficacy declined initially on day 4 ($\chi^2 = 5.10$, $p < 0.05$) and further on day 21 ($\chi^2 = 5.36$, $p < 0.05$) at the low dose, this initial decline was delayed to day 7 ($\chi^2 = 5.10$, $p < 0.05$) and followed by further decline on day 21 at the high dose ($\chi^2 = 3.88$, $p < 0.05$) (Figure 1).

Water temperature Water temperatures ranged 17.8°-19.9°C (average 18.9°C) for the minimums, and 24.3°-27.1°C (average 26.3°C) for the maximums.

DISCUSSION

Among the biopesticides categorized by the US EPA including microbials, biochemicals and plant

incorporated protectant (PIP), S-methoprene and its sibling compounds S-hydroprone and S-kinoprene are the only IGR biopesticides (US EPA 2021). These compounds have similar molecular structure and identical function to the natural juvenile hormones, doubled with their benign environmental and non-target profile (Henrick 2007, Su 2018). S-methoprene has very high bioactivity as indicated by low IE concentration at parts per billion (ppb) scale, low risk of resistance development and lack of cross-resistance to other larvicides. For example, field-collected mosquitoes that showed high levels of resistance to *Bacillus sphaericus* and pyriproxyfen, remained susceptible to S-methoprene. On the other hand, mosquitoes that have acquired high levels of resistance to S-methoprene through laboratory selection still showed high susceptibility to other commonly used pesticides except *B. sphaericus*, an obvious case of cross-resistance (Su et al. 2019a, b, 2021). Taking the advantages of advancement in formulation technologies, different products ranging from CS (5% and 20%), various granules (0.3% SBG and 4.25% P35), pellets (4.25%), briquets (8.62%) and extended residual briquets (2.1%) under the trade name of Altosid® (Central Life Sciences 2022) that are customized for habitats and mosquito species have been developed and used in mosquito control operations since early 1970s. While slow-release formulations provide extended period of control and save labor cost in field operations, the sublethal exposure, one of the leading causes to resistance development (Su et al. 2021), seems unavoidable. While the resistance risk is low in S-methoprene, its actual status in the field populations of mosquitoes may have been overlooked after many years of applications.

The laboratory bioassay is critical to evaluate prototype or commercial products to prompt the subsequent field evaluations. In this paper, all actual, not projected, IE_{10} , IE_{50} and IE_{90} with their 95% CIs were obtained from dose-responses covering approximately 5-95% cumulative mortality. The significantly higher activities in *Ae. aegypti* and *An. hermsi* than in *Cx. quinquefasciatus* may imply higher effectiveness against the same species under field conditions. The species-dependent susceptibility to S-methoprene was also observed by others at various extents, where data showed generally higher susceptibility in floodwater mosquito species than others (Lowe et al. 1975, Ritchie et al. 2017, Su et al. 2019a, b). The species-dependent variations in susceptibility also exists in other commonly used mosquito larvicides, for instance, the species in *Stegomyia* group namely *Ae. aegypti* are much less susceptible to *B. sphaericus* (Su et al. 2019b). The formulated suspension showed the similar adult emergence inhibition as the technical grade S-methoprene, indicating the microencapsulating process did not appear to interfere

with its insecticidal activity. In terms of stage-specific mortality across the dose-response range in laboratory bioassay, there seemed less mortality at “puparvae” as compared with that in pyriproxyfen (Su et al. 2020), because mortality mostly occurred at pupal stage, plus minors during larval stage at the higher concentrations and some mortality as incomplete emergence at the lower concentrations.

For ephemeral habitats or univoltine species, short-persistent formulations with adequate longevity are desired. A couple of CS formulations that have the similar product profiles and use patterns as the OmniPrene 20CS were made available decades ago such as Altosid liquid larvicides (A.L.L.) with 5% and 20% active ingredient. However, the labels of these products do not provide the efficacy longevity, rather advise to repeat the treatment as breeding sites become reinfested or when monitoring indicates an increase in adult populations (Central Life Sciences 2022). The field evaluations on the efficacy longevity of A.L.L. are quite meager (McCarry 1996, Webb et al. 2012). The new product OmniPrene 20CS is formulated with innovated proprietary microencapsulation technologies and provided 86.4-100%, 89.8-100% and 79.8-100% control against *Ae. aegypti*, *An. hermsi* and *Cx. quinquefasciatus*, respectively, over a 21-day evaluation period. This CS formulation, under the proprietary right and trademark protection, encapsulated the S-methoprene in a novel polymer shell suspended in water with a dispersant and wetting agent (Gimeno 1996, Dubey et al. 2009, Lam et al. 2010).

In mosquito species, late instar larvae are more susceptible than the early instar larvae to external JHAs such as S-methoprene and pyriproxyfen due to the significant decline of internal juvenile hormone III (JH-III) when completing larval growth and approaching pupation (Noguchi and Ohtaki 1974). Young larvae have low susceptibility to JHAs as their internal JH-III remains high to regulate larval growth. When treating a habitat with mixed larval instars at the label dose of a S-methoprene product, only old larvae that are approaching pupation will be mostly affected, ending up with dead pupae or incompletely emerged adults. The younger larvae however are considered being exposed to the sublethal dose of the active ingredient, a leading cause of resistance evolution. Hence, resistance monitoring for S-methoprene product is highly encouraged before and after applications. In contrast to direct kill larvicides, either conventional synthetic pesticides or ones with microbial origins, the unique mode of action of S-methoprene, i.e., allowing normal larvae growth and development, and only interrupting the pupal development and adult emergence, would allow the minimum impact to the trophic webs in

aquatic habitats. However, this mode of action can mislead to a perception of low efficacy as normal-looking larvae do prevail after treatment, this scenario can be an issue for the public before the mode of action in S-methoprene and other JHAs is understood.

Currently, the CS formulations remain one of the most advanced formulation types for pesticide products worldwide. When CS formulations are diluted with water in the spray tank, a spontaneous suspension is formed, with particles in the size range of 0.1 to 20 µm. When sprayed, the dilute emulsion gives a uniform and accurate application of active ingredient onto the water surface, which is essential for effective control of target mosquito species. It is desirable to provide controlled release of S-methoprene as well as prevent degradation of the active ingredient by the innovated CS formulation technology. One can anticipate that the new product OmniPrene 20CS will soon be added to the toolbox that is at a historical all time low as we are facing the demand of more sustainable mosquito management.

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