Article

Laboratory and field evaluation of Sumilarv 0.5G, a commercial Pyriproxyfen on the larval strains of *Aedes aegypti* and *Aedes albopictus*

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Abstract

This study was conducted to evaluate the laboratory and field bioassay of 4th instar larvae of *Aedes aegypti* and *Aedes albopictus* to Sumilarv 0.5G, an insect growth regulator larvicide that is conventionally used for dengue vector control. We used three treatments of Sumilarv 0.5G (1 ppm, 2 ppm, and 4 ppm) against the dengue vector mosquito larvae. Laboratory bioassay showed that dosages of Sumilarv 0.5G at 2 mg/L (2 ppm) and 4 mg/L (4 ppm) were adequate to exert 95.7% and 98.9% inhibition of emergence (%IE) in Cagayan de Oro strains of *Ae. aegypti* larvae. The same doses yielded 96.9% and 99.1% percent emergence inhibition (%IE) on *Ae. albopictus* larvae. The dosage of 1 mg/L (1 ppm) of Sumilarv 0.5G yielded 92.2% IE in *Ae. aegypti* and 80.1% IE in *Ae. albopictus.* Field bioassay at 2 ppm and 4 ppm Sumilarv 0.5G doses showed 70% and 91% inhibition of emergence in *Ae. aegypti* while *Ae. albopictus* yielded 97% and 98% inhibition of emergence for the same doses. One ppm dosage of Sumilarv 0.5G produced 71% IE in *Ae. aegypti* and 82.4% IE in *Ae. albopictus.* Based on the results, the lowest recommended application dosage (2 ppm) was found effective, but the most significant efficacy obtained was from the 4 ppm dosage. Sumilarv 0.5G qualifies as a tool for vector control on *Ae. aegypti* and *Ae. albopictus.*

Keywords Pyriproxyfen; dengue; Sumilarv; efficacy.

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1 Introduction

Most mosquitoes on earth are in larval or immature stages (Smith, 2008). The larva's main activity is to feed and grow. Mosquito larvae grow by consuming microorganisms such as algae and bacteria that decompose decaying plant material, so at any one time, they contribute to the transfer of matter in the aquatic ecosystem (Peach, 2019; Kaufman et al., 1999). When aquatic predators consume them, they contribute to the aquatic

food chain by serving as a food source.

Larval development is critical to mosquito emergence and health because they amass body resources that are most needed at their adult stage. Unlike adults that avoid intervention by changing their location (in flight), larvae are strictly aquatic and confined to the body of water where they were hatched. It is at this stage, therefore, when they are most vulnerable to the elements of nature.

For these reasons, the larval stage of the mosquito life cycle is an ideal target for applying intervention methods (Smith, 2008). Chemical control is the primary control measure exercised on mosquito vectors in the Philippines. According to the head of sanitary inspectors of the Cagayan de Oro City Health Office, the larvicide they have been using for some time is a commercially granulated Pyriproxyfen called Sumilarv 0.5G (manufacturer: Sumitomo Chemical Company, Japan). Pyriproxyfen is a synthetic juvenile hormone analog with low mammalian toxicity (Ohba et al., 2013). It is a growth regulator that inhibits metamorphosis and embryogenesis in several species of insects. Pyriproxyfen inhibits morphogenesis and embryogenesis (Dhadialla et al., 1998). In *Ae. aegypti* and *Ae. albopictus,* Pyriproxyfen 0.5% (Sumlarv 0.5G) has been claimed to be highly effective in inhibiting adult emergence (Lau et al., 2018; Lau et al., 2015; Ritchie et al., 2013; Kwekaet al., 2019; Estrada and Mulla, 1986; Kawada et al., 1988; Hatakoshi et al. 1988; Hirano et al., 1998).

In Cagayan de Oro, pyriproxyfen granules (Sumilarv 0.5G) are routinely sprinkled on swampy areas prone to flooding during the rainy months. But, based on a narrative of the City's Sanitary Inspectors, the impact of larviciding has never been checked.

Since there is a need to evaluate the efficacy of the Sumilarv 0.5G on Cagayan de Oro strains of dengue vector mosquitoes, a larval bioassay was carried out. The main objective of this bioassay was to determine the percent inhibition of emergence (%IE) in *Ae. aegypti* and *Ae. albopictus* larvae upon exposure to the insect growth regulator larvicide (Sumilarv 0.5G).

2 Materials and Methods

2.1 Preparation of Sumilarv 0.5G

Packets of Sumilarv 0.5G granules were provided by the Cagayan de Oro City Health Office. Pyriproxyfen granules were repacked in 1, 2, and 4 mg by analytical balance and dissolved in separate liters of rainwater to obtain appropriate dosages of 1, 2, 4 ppm solutions of Pyriproxyfen. The dosage choice was based on the application rate recommended by Sumitomo Chemical, the Sumilarv 0.5G. According to their recommendation, Sumilarv 0.5G can be applied at 2-10 gms/m^3. Since the experimental containers used in the experiment could accommodate only 1 liter per treatment, the volume, m^3, was converted to liter. Since 1 m^3 = 1000 liters, the transmutation of the lowest recommended concentration (2 gms/m^3) was therefore converted to 0.002 gm/liter or two ppm. Two other concentrations in the treatment groups were arbitrarily chosen based on the recommended two mg/L dosages: 0.001 g/l (1 ppm) and 0.004 g/l (4 ppm), to check the response of the larvae to concentrations lower or higher than the lowest recommended dose. There were therefore 3 treatments all-in-all: (a) treatment-1: 1 ppm, (b) treatment-2: 2 ppm, (c) treatment-3: 4 ppm, and (d) 0 ppm as control. There were four (4) replicates for each treatment and control. The 1-liter replicates of Sumilarv 0.5G treatments, and control (i.e., 0, 1, 2, and 4 ppm) were stored for a week or two in 20 cm x 20 cm white polyethylene boxes, covered with netting before bioassay was started. No probit analysis was done in this study since the main dosages, 2 and 4 ppm, were within standard recommended dosages.

2.2 Preparation of *Ae. aegypti* and *Ae. albopictus* larvae

Wild-caught *Ae. aegypti* larvae were collected from tire habitats that were positioned at 200 meters apart in a 7-hectare open, vegetated field on the outskirts of a suburban village in Cagayan de Oro City. *Ae. albopictus*

larvae, on the other hand, were collected from giant bamboo (*buntong*) larval traps installed at thickets at the periphery of the same village. These larvae were then allowed to grow to adulthood in white, shallow plastic pans which were enclosed in tulle nets inside a small, makeshift insectary. Larval samples obtained from these field collections were subsampled for microscopic examination of morphology to confirm the correct species were collected. As adults of *Ae. aegypti* and *Ae. albopictus* emerged from the rearing pans; these were then segregated based on species and sex in separate insect cages and sustained by 10% sucrose soaked in flat cotton that was laid on the cage tops (Fig. 1). Weeks before the larvicide study was to commence, adult *Ae. aegypti* and *Ae. albopictus* females and males were transferred and joined in cages exclusive to their species and fed with chicken blood to induce egg-laying. White coffee filter papers were laid inside small glasses filled with 10-15 ml water to provide substrate for egg-laying. The eggs were collected and stored in clean and dry cotton-plugged plastic tubes. Two to three days in storage, the eggs were induced to simultaneous hatching by immersing the filter paper with eggs in 24 hours old deoxygenated solution of distilled water mixed with grounded dogfood for 12-14 hrs. This simultaneous hatching of *Aedes*eggs into larvae ensured that a homogenous supply of larvae was used to evaluate the Sumilarv 0.5G larvicide (Novak and Shroyer, 1978). Following WHO protocol on Pesticide Evaluation Scheme, only 4th instar larvae were used in the larvicide bioassay (WHO, 2005).

Fig. 1 Preparation of Sumilarv 0.5G treatments (A,B); harvesting pupae for rearing (C); extracting dead pupae (D) & exuviae from tire (E,F,G); dead pupae from treatments 1, 2 & 3.

2.3 Laboratory trial

In the laboratory, batches of thirty (30) 4th instar larvae were selected from the larval cohorts of *Ae. aegypti* and *Ae. albopictus* and transferred into the treatment and control boxes for bioassay. The top of the plastic "rearing" boxes was firmly secured with tulle netting to keep any emerged adult from flying off. Following the WHOPES procedure, the larvicide bioassays were run in a series 1 to 2 weeks. The rearing medium of the larvae was added with tap water when the water level in the bioassay box reached half the volume, but no additional Sumilarv 0.5 solutions were added. About three days after larval transfer, the rearing medium was sprinkled with 4 grams of powdered dogfood and yeast every 5 days. The scum that developed on the water's surface was removed using a flat nylon strainer an hour before feeding.

The live and dead pupae of *Aedes* mosquitoes were counted every day until the last pupa had eclosed or died. Percent emergence (%E) in control was based on the formula:

$$\% E = \left[\frac{\text{No.of emerged adults}}{\text{Initial No.of larvae}}\right] x\ 100$$

$$\% IE = 100 - \left[\frac{T \times 100}{C}\right]$$

where T1 - percent survival or emergence in treatment group, and C - percentage of survival or emergence in control group.

% R= 1-[(C1/T1) x (T2/C2)] x 100

where, T1 - pretreatment treated, T2 - post-treatment sprayed area, C1 - pre-treatment unsprayed control, C2 - post-treatment unsprayed control.

The quotient of the pupal exuviae (pupal skin) count against the total number of starting larvae (i.e., 30) was multiplied by 100 to obtain the percentage of eclosed pupae in the control group. Percent inhibition of emergence (%IE) in the treatment groups was calculated by deducting the percentage of (dead) unenclosed pupae from 100. Data were recorded in an excel data sheet according to the WHOPES larvicide evaluation protocol (WHOPES, 2005). The excel format for larvicide results recording was very convenient for determining the number of data. However, no sorting and calculation of ratios between emerged male and female adults were done in this study.

2.4 Field trial

To limit confounding effects of microclimatic factors, only two kinds of habitats were chosen for the Sumilarv 0.5G field trials: used tires and giant bamboo stumps (*buntong*). This selection was based on unpublished field observations in past surveillances in the city that *Ae. aegypti* commonly bred in used tires in urban areas, while *Ae. albopictus is* more frequently bred in giant bamboo cuttings in suburban areas.

Sixteen (16) unused tires were obtained from tire storage cluttered around Cagayan de Oro, and sixteen (16) bamboo stumps from giant bamboo plantations near the city. The tires and bamboo stumps were transferred and installed in RCBD fashion at about 200 meters apart in a 7-hectare unused open field in a suburban location in Cagayan de Oro City. At their open field locations, sixty (60) 3^{rd} - 4^{th} instar larvae and water were loaded to their respective tire and bamboo habitats. *Ae. aegypti* larvae were placed in tire habitats, and *Ae. albopictus* were placed in bamboo stumps.

A week or two were allowed to pass before Sumilarv 0.5G treatments were applied. Sampling for dead pupae and/or pupal exuviae commenced one (1) week after larvicide application. A sampling of larvae and pupae was done on two groups of four (4) habitats. Sampling was done weekly until Day 38, after which sampling was conducted biweekly. During sampling, the entire larval contents were temporarily transferred in shallow white plastic trays were alive, and dead immatures could easily be observed and counted with handheld counters. Only 4^{th} instar larvae were counted; all other younger immatures were estimated. Dead pupae and exuviae were recorded and were alcohol-preserved in 4 ml sealable sampling tubes. The live immatures (larvae and pupae) were then returned to their respective habitats. Unlike in the laboratory bioassays, the percent emergence (%E) in control was determined based on the count of pupal exuviae/skin. The percent inhibition of emergence (%IE) of treatment groups was measured based on the number of dead pupae that were sampled from the water using a kitchen baster and/or 4ml pipettes. The same %IE formula was used to calculate the percentage inhibition of emergence (%IE) in the laboratory phase. No replacement of treatment media was done. Only water was replenished when the habitats were about to start drying up. Microclimatic pH, electroconductivity (EC) of habitat waters, and rainfall (RF) were recorded every week. These were then analyzed in multiple linear regression for any association with the *Aedes*populations in tire or bamboo stump habitats.

3 Results and Discussion

3.1 Laboratory evaluation on Sumilarv 0.5G on *Ae. aegypti* and *Ae. albopictus*

The control groups of wild-caught 4^{th} instar *Aedes* larvae in rearing boxes showed maximum adult emergence between the 5^{th} to 8^{th} day in *Ae. aegypti* (Fig. 2), between 7^{th} to 8^{th} day in *Ae. albopictus* (Fig. 3). Maximum pupation of 4^{th} instar *Ae. aegypti* larvae were reached between the 3^{rd} to 4^{th} day of laboratory rearing at an average water temperature of 28.6°C (Fig. 2). In comparison, *Ae. albopictus* larvae reached maximum pupation

between the 5th to 8th day at an average water temperature of 28.7°C (Fig. 3). Percent of emergence (%E) of *Ae. aegypti* and *Ae. albopictus* in all control groups in the laboratory tests showed very similar values with average means of 99.2%. *Aedes*pupae usually take only 1½-3 days to become an adult in the control group.

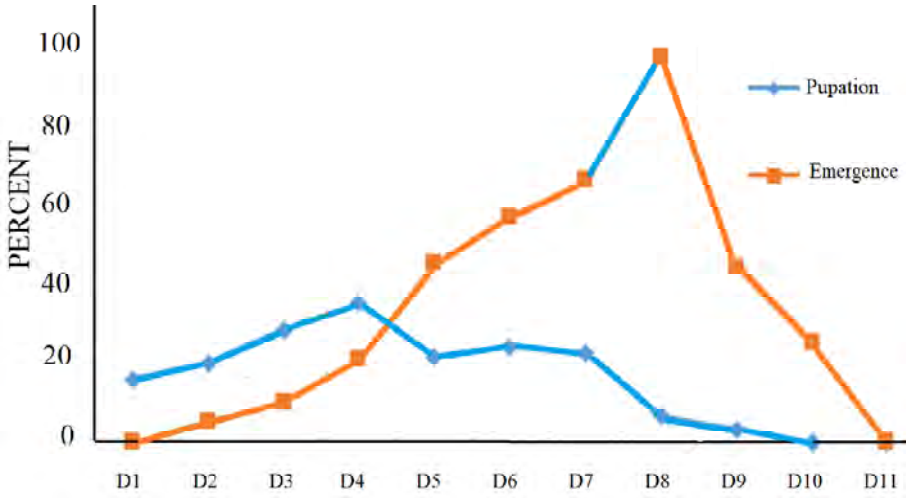

Fig. 2 Daily mean pupation and emergence of laboratory-reared *Ae. aegypti* in control groups.

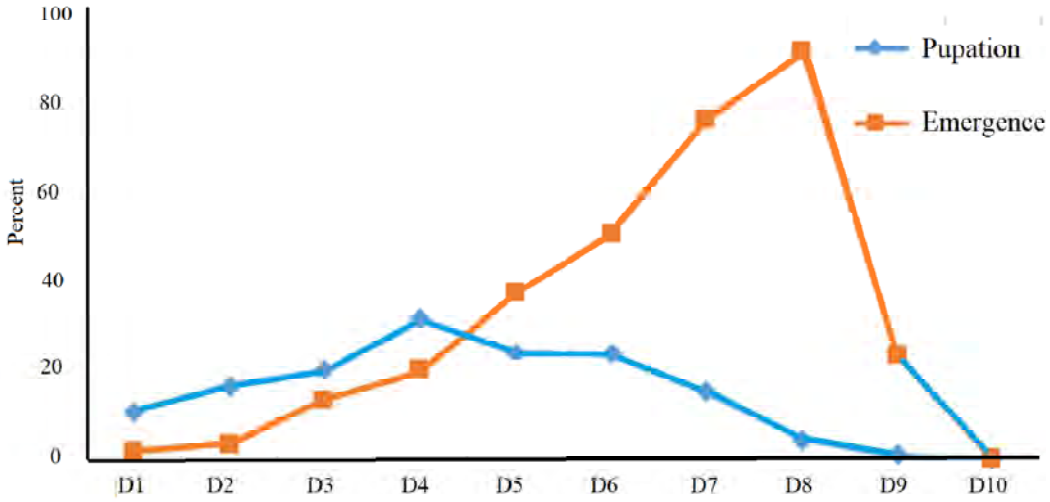

Fig. 3 Daily mean pupation and emergence of laboratory-reared *Ae. albopictus* in control groups.

Laboratory-reared *Ae. aegypti* exposed to pyriproxyfen treatments (2 ppm and 4 ppm) showed substantial percent emergence inhibition (%IE) based on the count of stunted and dead pupae found in each rearing box. The control, on the contrary, showed no physical manifestation of maldevelopment or dead pupae as compared to treatments (Fig. 4).

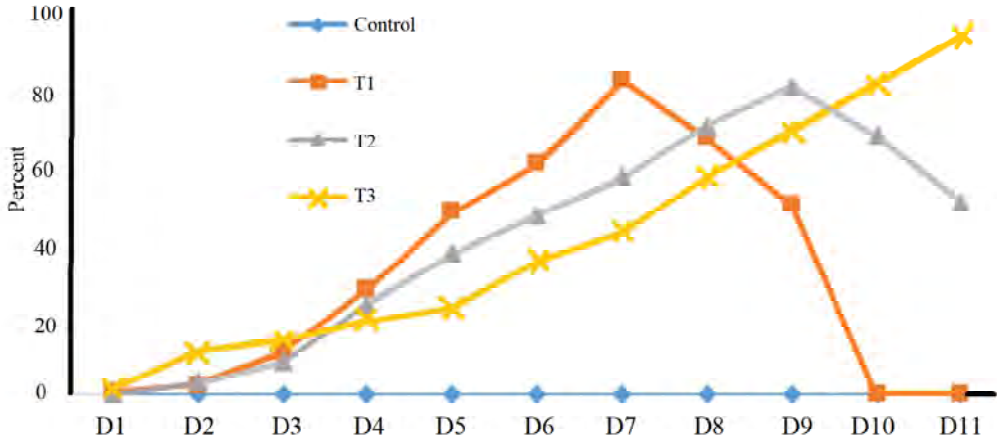

Fig. 4 Mean percent inhibition of emergence (%IE) in *Ae. aegypti* various doses of Sumilarv 0.5G and control.

Analysis of percent inhibition of emergence (%IE) between different treatments (except control) in *Ae. aegypti* showed a very significant difference in treatment-3 (4 ppm) compared to treatments 1 (1 ppm) and 2(2 ppm) (p=0.003<0.05), indicating that 4 ppm of Pyriproxyfen (0.004 g/liter) exerted the most potent inhibitory effect on eclosion of pupae. The percent inhibition of emergence (%IE) in *Ae. albopictus* also showed significant means differences in treatments 2 and 3 compared to treatment 1 (p=1.3x10^{-8}<0.05). Percent emergence of pupae in *Ae. aegypti* and *Ae. albopictus* were observably disrupted upon exposure to Sumilarv 0.5G at their recommended dose (Fig. 5, 6).

Fig. 5 (A) Normal pupa from control; (B) dead, unenclosed pupa as affected by Pyriproxyfen in most treatments; (C) pupal exuviae (skin) common in control; (D) dead, maldeveloped adult due to Pyriproxyfen common in treatment-1.

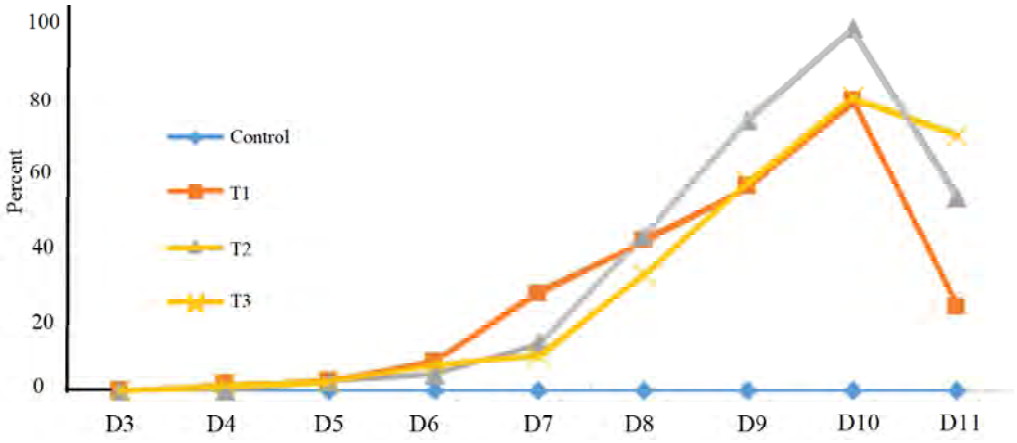

Fig. 6 Mean percent inhibition of emergence (%IE) in *Ae. albopictus* under various doses of Sumilarv 0.5G and control.

The mortality scores of laboratory-reared *Aedes*larvae under treatments-2 and -3 of Sumilarv 0.5G showed that 2 ppm and 4 ppm were effective in producing %IE of 95% and 98%, respectively, in *Ae. aegypti* and %IE of 96% and 99%, respectively, in *Ae. albopictus*. It was further observed that treatment-1 (1 ppm), the lowest Sumilarv 0.5G concentration, could only achieve %IE=92% control in *Ae. aegypti* and %IE=80% in *Ae. albopictus*. The percentage emergence (%E) and percent inhibition of emergence (%IE) of control groups and treatment groups are shown in Tables 1 and 2.

Table 1 Total Percent Emergence of *Ae. aegypti* and *Ae. albopictus* in Control Group and Treatment Groups.

Average % Emergence	Control	T1	T2	T3
Ae. aegypti	99.2	7.8	4.3	1.1
Ae. albopictus	99.2	19.9	3.1	0.9

Table 2 Mean Percent Inhibition of Emergence (%IE) in *Ae. aegypti* and *Ae. albopictus* under Sumilarv 0.5G.

Mosquito	T1 (1 ppm)	T2 (2 ppm)	T3 (4 ppm)	Control
%IE *Ae. aegypti*	92.2	95.7	98.9	0.8
%IE *Ae. albopictus*	80.1	96.9	99.1	0.8

Most pupal death observed during laboratory tests of Sumilarv 0.5G were observed between the 4th to the 9th day in *Ae. aegypti* (Fig. 6)and from the 5th to the 11th day in *Ae. albopictus* (Fig. 7). It was also observed that the higher the larvicide concentration used, the longer it took for the larvae to reach the pupal stage. Some mosquito larvae exposed to Sumilarv 0.5G never even reached the pupal stage. This may be an effect characteristic of a juvenile hormone analog (Aribi et al., 2006). Synthetic Pyriproxyfen mimics the effects of juvenile hormones. Natural juvenile hormones have been biochemically documented to inhibit the genes that promote the development of adult characteristics, causing the insect to remain as a nymph or larva (Subramanian and Shankarganesh, 2016). The Sumilarv 0.5G does not kill larvae but stops morphogenesis by keeping pupae from turning into adults. From the observed longer metamorphosis of 4th instar larvae into pupae, it can even be surmised that Sumilarv 0.5G may also interfere with changes involved in larval development to pupae.

3.2 Field evaluation of Pyriproxyfen on *Ae. aegypti* and *Ae. albopictus* populations

Although the percent adult emergence in the *Aedes*mosquitoes in the field fluctuated every week, the average percent of emergence in *Ae. aegypti* control group based on count of pupal exuviae was 58.6% in 11 weeks of field surveillance with no dead, unenclosed pupae. In comparison, *Ae. albopictus* showed 52.1% average emergence in the control group in 11 weeks. Upon application of Sumilarv 0.5G at Day 10 (second week), the number of dead pupae dramatically increased over the number of pupal skin or exuviae (Fig. 7). This was most pronounced beginning in the third week (Day 17).

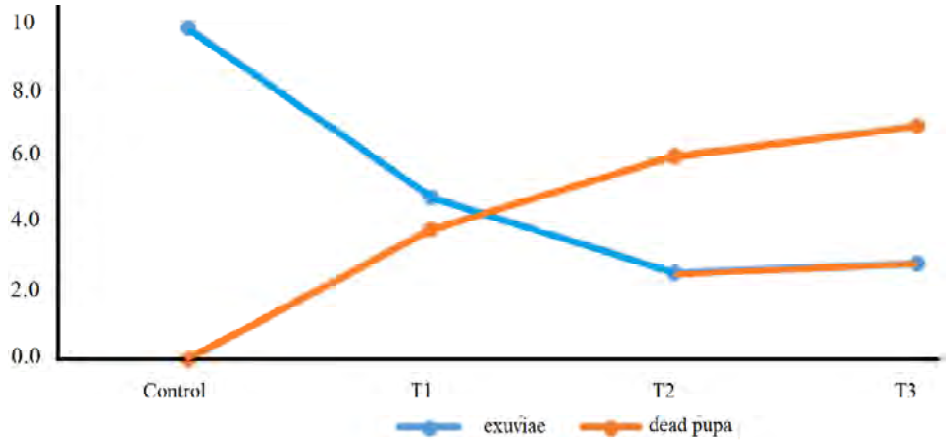

Fig. 7 Mean pupal exuviae & dead pupae from field surveillance of control and treatment groups in experimental habitats.

Test for equal means on the count of pupal skins per treatment showed significant differences (p=1.99x10^{-07}<0.05). The same response was observed in *Ae. albopictus* bamboo stump populations. Although the populations of live larvae remained in substantial numbers in the experimental field habitats under treatment and control due to continuous oviposition and colonization by the *Aedes*mosquitoes, almost maximum inhibition of emergence resulted in a week after application of the Sumilarv 0.5G (p=1.15x10^{-05}<0.05) (Day 17). But this drastic inhibition of emergence appeared to last only until the sixth week (Day 38) for treatment 1 and up to the seventh week (Day 66) for treatments 2 and 3. As shown in Figs 8 and 9, the percent inhibition of emergence began to plunge by the fifth week.

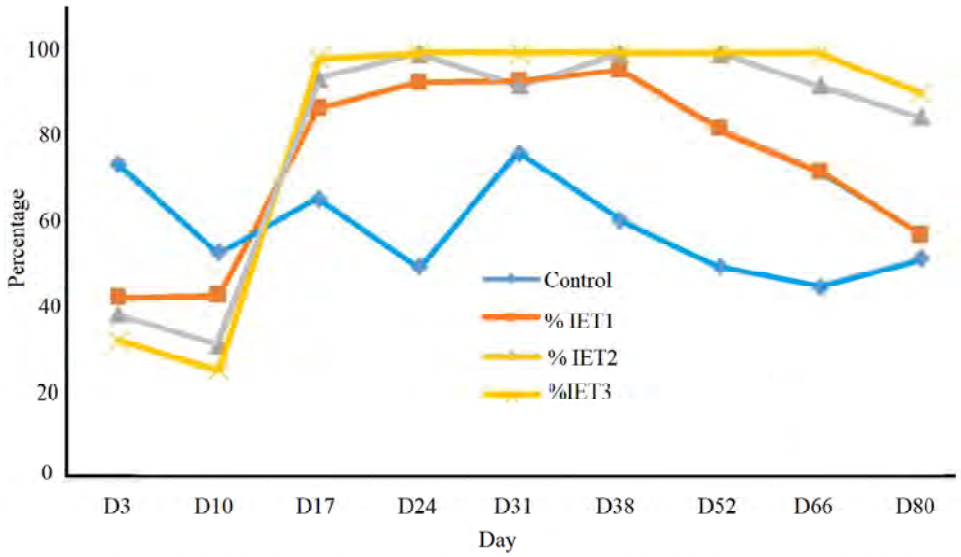

Fig. 8 Percent Inhibition of Emergence (%IE) of *Ae. aegypti* in tire bamboo habitats with Sumilarv 0.5G and control.

This indicates that Sumilarv 0.5G is effective only for several weeks amid microclimatic changes in rainfall, pH, and electroconductivity. The findings of previous studies reported that percent inhibition of emergence (%IE) by similar treatments under Sumilarv 0.5G with regular replacement of water could last for four (4) months against *Ae. aegypti* and *Ae. albopictus* reared in earthen jars (Vythilingam et al., 2005). The current findings in the field evaluation of recommended dosage without replenishment of treatment show effectivity of Sumilarv 0.5G start to decrease around the second month (Day 66).

Exploring pretreatment and posttreatment populations based on live larvae of the *Aedes* mosquitoes using Abbott's formula for population reduction (R) as described by Fleming and Retnakaran (1985), yielded calculations that indicated treatment 3 (4 ppm) caused the highest percent reduction (-81%) in *Ae. aegypti* and *Ae. albopictus* (Table 3). Calculated percent inhibition of emergence (%IE) for *Ae. aegypti* was highest in treatment 3, while %IE of *Ae. albopictus* was highest in both treatments 2 and 3 (Table 4).

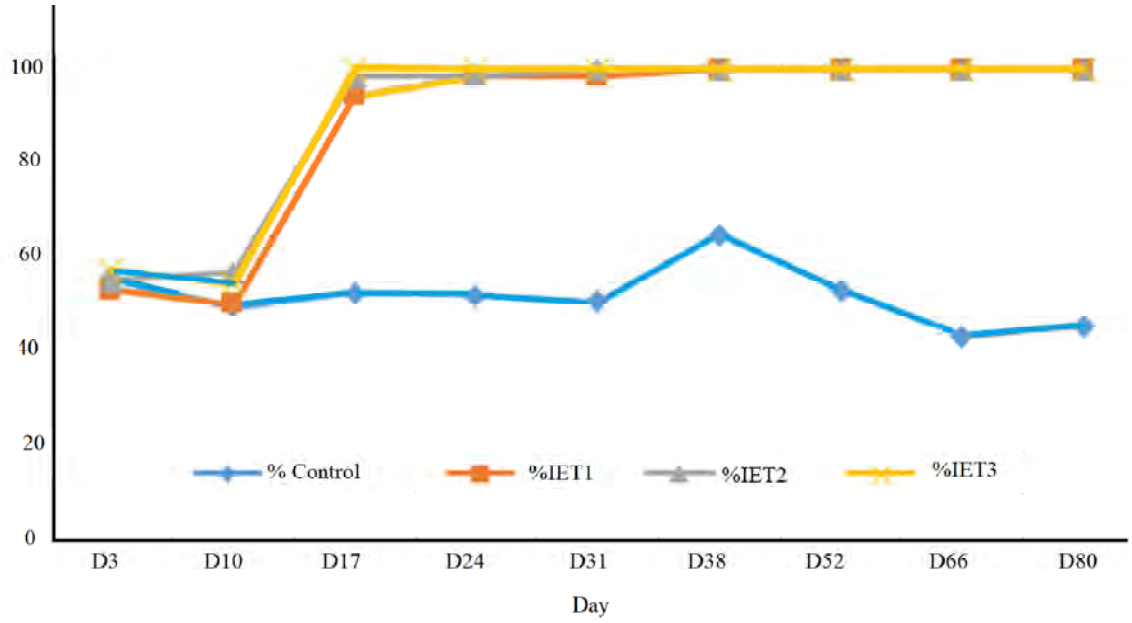

Fig. 9 Percent Inhibition of Emergence (%IE) in *Ae. albopictus* in bamboo habitats with Sumilarv 0.5G and control.

Table 3 Population reduction in pretreatment and posttreatment of *Ae. aegypti* and *Ae. albopictus* in the field in response to Sumilarv 0.5G treatments.

Mosquito species	T1 (1 ppm)	T2 (2 ppm)	T3 (4 ppm)
Ae. aegypti	23.5	-27	-81
Ae. albopictus	-17	-27	-81

Table 4 Percent inhibition of emergence in *Ae. aegypti* and *Ae. albopictus* in response to Sumilarv 0.5G treatments in field bioassay.

Mosquito species	T1 (1 ppm)	T2 (2 ppm)	T3 (4 ppm)
%IE *Ae. aegypti*	71	70	91
%IE *Ae. albopictus*	82.4	97	98

It was noted that the correlation coefficient in treatment 3 to rainfall in *Ae. aegypti* and *Ae. albopictus* showed weakly positive r (0.28293 and 0.12919, respectively) (Figs. 10 and 11). The slight positive correlation of the *Aedes* populations to rainfall may be due to the continuous colonization of these habitats by *Aedes*mosquitoes. Rainfall has always been cited as an important influence on *Ae.*dengue vectorpopulations, but only in areas where it is markedly seasonal (Barrera *et al.,* 2006). Cagayan de Oro City lies in type III regional climate classification, characterized by a poorly pronounced maximum rain period with a dry season lasting from one to three months, either from December to February or from March to May (PIDS, 2005). Rainfall was not significantly seasonal during that year, 2019, and breeding places for the anthropophilic *Aedes*mosquitoes abound in the surrounding human villages of the area. Likely, rainfall was not heavily influential to *Aedes*population dynamics in the area, as was indicated by r (Fig. 10). Linear correlation has also been calculated between larval abundance and pH, and most pH measurements were alkaline at the start of monitoring. As weeks wore on, a decrease in pH was generally observed, a phenomenon easily attributable to accumulations of organic debris, especially of leaves, insects, and in certain cases, pieces of fruits that fell from old overarching mango and java plum trees in the areas. R values of *Ae. aegypti* larval abundance to pH in treatment 3 correlated slightly positive (r= 0.15835) *Ae. albopictus* showed slight positive correlation in treatment 2 (r= 0.11842), but slightly negative correlation in treatment 3 (-0.30015) (Fig. 11).

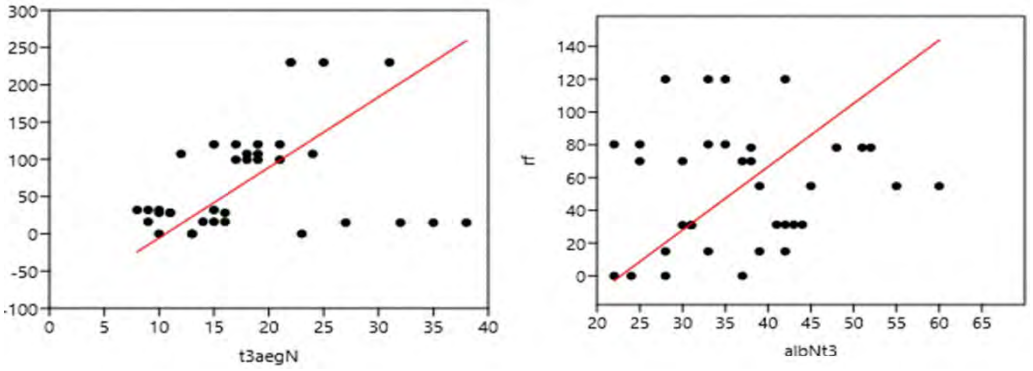

Fig. 10 Linear correlation plot of *Ae. aegypti* abundance in treatment 3 (4 ppm Sumilarv 0.5G) to rainfall.

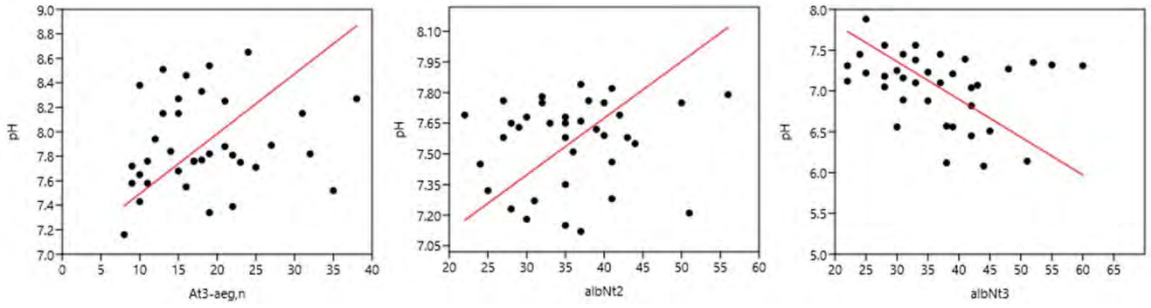

Fig. 11 Linear correlation plot of abundance and pH in treatment 3 in *Ae. aegypti* and treatments 2 and 3 in *Ae.Albopictus*.

The use of pyriproxyfen IGR larvicides has been found to contaminate gravid female mosquitoes when they have alighted on chemically-treated water in the field. It strategically recruits the mosquito to disperse a small but adequate quantity of the larvicide to habitats that are hard to find or reach (Chande et al., 2016). For

this reason, Pyriproxyfen has been used in a mosquito control method called "autodissemination."This is a new strategy of mosquito control by which adult female mosquitoes are lured to an oviposition device where they are contaminated with the insect growth regulator, Pyriproxyfen (PPF) microgranules, and transfer PPF to their aquatic habitats. The method has already been demonstrated for *Aedes* and *Anopheles* mosquitoes (Lwetoijera et al., 2014). This approach could potentially enable high coverage of aquatic mosquito habitats, including those hard to locate or reach via conventional larviciding.

4 Conclusions

Based on the results of laboratory and field evaluation of Sumilarv 0.5G, the recommended doses of 2 mg/L (2 ppm), and 4 mg/L (4 ppm) doses of Sumilarv 0.5G were adequate to produce indoor mortality of 96%-99%, and outdoor mortality of 70-91% in local strainsof *Ae. aegypti* larvae. The same doses of the same insect-growth-regulator larvicide could also induce indoor mortality of 97%-99% and outdoor mortality of 97-98% in *Ae. albopictus* larvae. The dosage of 1 mg/L (1 ppm) of Sumilarv 0.5G has not been found recommendable based on results that could produce only 92% indoor and 71% outdoor mortality in *Ae. aegypti*. *Ae. albopictus* produced only 80-82% mortality from indoor to outdoor conditions.

The best dosage tested was 4mg/L or 4ppm of Sumilarv 0.5G which produced more than 95% mortality in indoor or outdoor locations. It is recommended that its application in autodissemination should be explored as an upgrade of its use in the locality.

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