Article

Evaluating the efficacy of Olyset® nets in the laboratory and trap surveillance of *Aedes* mosquitoes

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Abstract

With Chikungunya and Zika in the Philippines, there is an urgent need for effective methods to control Aedes aegypti and Aedes albopictus in as much as these mosquitoes are not only vectors of Dengue but also of Chikungunya and Zika. Evaluation of the efficacy of Olyset® nets was done in two approaches: (1) laboratory WHO cone bioassay on Olyset® net and (2) comparative mosquito trap surveillance of dengue vector mosquitoes in DepEd classrooms with and without Olyset® nets. During laboratory cone bioassay, % knockdown and % mortalities were recorded for Ae. aegypti and Ae. albopictus. Penetrability tests were also done on Olyset® nets. During comparative mosquito trap surveillance, the total Aedes capture in classrooms with Olyset® nets was compared to Aedes capture in classrooms without Olyset® nets. Ae. aegypti showed A 54% percent knockdown and 63% mortality Ae. albopictus showed 77% knockdown, and 79% mortality was shown in cone bioassay of Olyset® nets. Penetrability test on Olyset® nets showed 35% Ae. aegypti and 34% Ae. albopictus crossed the nets, but only 10% in Ae. aegypti and 6% in Ae. albopictus survived the crossing. Female adult and larval Aedes capture differed significantly between classrooms with Olyset® nets and those without Olyset® nets. But the observed lack of screen doors and poor physical integrity of Olyset® nets in classrooms might compromise its efficacy. Based on WHO 98% susceptibility test criterion, there was probable insecticide resistance in Aedes mosquitoes to 2% permethrin in Olyset® nets. In the classroom mosquito surveillance, significant differences of Aedes capture between classroom groups may have been obtained, but other observations indicated compromised efficacy of the Olyset® nets.

Keywords Olyset® nets; knockdown; penetrability; surveillance.

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1 Introduction

With the emergence of Chikungunya and Zika in the Philippines, there is an urgent need for effective methods

tocontrol the mosquito vectors *Aedes aegypti* and *Aedes albopictus* (Lambrechts et al., 2010; Ritchie, 2014; Higgs and Vanlandingham, 2015; Cevallos et al., 2017; Powell, 2018). The traditional community-based clean-up drives and fogging for controlling *Aedes* mosquito vectors are not enough interventions against *Aedes*-borne diseases in the Philippines. These types of interventions only provide intermittent control of the *Aedes* mosquito vectors. During these Pandemic times of rising cases of Dengue and other vector-borne diseases, what is needed is a practical, long-lasting intervention against the *Aedes* mosquito vectors of Dengue, Chikungunya, and Zika viruses. Several control methods for mosquito control and surveillance were available such as ovitraps which can attract *Aedes* females to oviposit (WHO-WPRO, 1995; Fay and Eliason, 1966; De Las Llagas and Medina, 2002; Lenhart et al., 2005; Obenauer, 2009; Wan Norafikah et al., 2011; Silver, 2013; Mackay et al., 2013; Barrera et al., 2014; Chaves and Friberg, 2021). Ovitraps were known to be sensitive methods that can detect mosquito populations even at low infestation levels, some of which were found to be better than the standard ones (Lee, 1992; Ritchie et al., 2003; Cheng et al., 1982; Rawlins et al., 1998; Focks, 2003; Lenhart et al., 2005; Martin et al., 2019; Garcia-Luna et al., 2019; Ahmad-Azri et al., 2021; Autry et al., 2021; Juarez et al., 2021; Khater et al., 2022; Gualberto and Demayo, 2022a, b; Lenhart et al., 2020).

In the Philippines in 2010, the DOH-Philippines rolled out insecticide-treated screens (ITS, brand name: Olyset® Net) to reduce *Aedes*adult mosquito populations. Olyset® Net is a resin-based fiber produced by Sumitomo Chemical Company of Japan impregnated with a synthetic insecticidal pyrethroid called Permethrin that persists. It slowly diffuses to its surfaces for highly extended periods (WHO, 2001). Olyset® Net was initially manufactured as a mosquito net intervention against malaria mosquito vectors in Africa (Okuno and Iko, 2006; Tami et al., 2004). It was proven successful in malaria control, so it was considered for dengue mosquito vector intervention (Igarashi, 1997; Nguyen et al., 1996). To date, Olyset® nets have been famous as a mosquito vector control tool because it was the first of their kind as a long-lasting insecticidal net on the market. Likewise, it is wash-resistant, its use as an intervention against malaria and Dengue is cost-effective, and its efficacy can last 4 to 5 years (Okuno and Ito, 2006; Tsunoda et al., 2013; Dev et al., 2010).

In 2015, the DOH-Philippines joined forces with the Department of Education-Philippines (DepEd) and the Department of Social Welfare and Development (DSWD) in its anti-dengue campaign through a costly nationwide distribution of Olyset® nets in public schools. The ITS was installed in public school windows and doors to slowly release the insecticide, pyrethroid, against *Aedes* vector mosquitoes. Insecticide-treated materials (ITS), such as Olyset® nets, have recently shown promising results in reducing household-level Dengue vector infestations (Lambrechts et al., 2010). Unlike most dengue vector control strategies, ITS targets the adult mosquito, the most critical vector stage. It is postulated that the likelihood of adult vectors exposed to ITS during host-seeking reduces their life expectancy, effectively altering the age structure of the vector population. Fewer mosquitoes live long enough to become infected with the dengue viruses.

Olyset® nets or ITS continue to be deployed and used in public schools all over the Philippines. The DOH-Philippines procurement budget for insecticide-treated screens (Olyset® Net) alone in FY 2020 has been ₱115,235,620.50 (www.doh.gov.ph/procurement/app2020). But strangely, no evaluation has ever been done on its efficacy in the country. In the most recent review of dengue-related research in the Philippines, not one kind of evaluation on the effectiveness of insecticide-treated screens has been cited (Agrupis et al., 2019). Upto-date monitoring and assessment of dengue interventions are vital to successfully implementing the antidengue strategy. According to WHO guidelines, monitoring and evaluation of interventions guide the planning and implementation of control strategies, assess their effectiveness, identify areas for improvement, and optimize the use of resources (WHO, 2012).

The main objective of this study was to conduct an entomological evaluation of the effectiveness of

Olyset® Net (ITS) in public schools as an intervention against *Aedes* vector mosquitoes. According to DOH-RX, Olyset® nets were first supplied to DepEd elementary schools in 2009 as a pilot program for the first evaluation of the insecticide-treated screens.

2 Materials and Methods

2.1 Establishment of insectary

Adult Ae. aegypti and Ae. albopictus were required for the cone bioassay of Olyset® in this study; hence adult mosquitoes were continuously reared from wild-caught larvae and pupae collected from tire and bamboo ovitraps installed in an open field. While at their pupal stages, Ae. aegypti and Ae. albopictus were laid in shallow trays, separated by sex and species, and then transferred to different rearing boxes equipped with screened tops and sleeves for easy extraction of adults. The adult Aedes mosquitoes were collected from hatching boxes using a battery-operated vacuum cleaner and separately transferred based on species and sex to 12 in x 12 in cages. The method used a handheld bulb aspirator with clear tubes to confirm the appropriate species based on scutal markings and other morphological traits by Savage' method (Savage and Smith, 1994). The Aedes adult mosquitoes were sustained with 10%-sucrose soaked in flat cotton pads that were laid at the cage tops. To ensure a continuous supply of adult mosquitoes, the male and female adults who emerged from wild-caught larvae were bred in cages in a makeshift insectary. Before egg-laying induction of Aedes females, these were sugar-deprived for 2-3 days in their separate cages, feeding them only water while maintaining the 10%-sucrose feeding for the males. At late afternoon hours, the female Aedes mosquitoes were blood-fed with immobilized chicken and, after an hour, transferred to another cage for breeding with males of the same species. Small and shallow water-filled glassware with filter paper cones were installed in the same enclosure to collect viable eggs. The eggs were then induced in simultaneous hatching in deoxygenated 10% nutrient broth, and the larvae were transferred to rearing bottles. After roughly one-week, cohorts of emerged adult Aedes mosquitoes were sorted by sex, and the females were transferred to separate cages to be used for cone bioassay.

2.2 Laboratory cone bioassay of Olyset®

The cone bioassay, a WHO method of insecticide evaluation (WHO, 2013), was used to assess the effectiveness of the Olyset® nets in public elementary classrooms of Cagayan de Oro City. The test, therefore, aimed to evaluate the insecticidal efficacy of Olyset® on *Ae. aegypti* and *Ae. albopictus* strains of mosquitoes in Cagayan de Oro when they came into contact with it compared to the same species exposed to untreated nets. The null hypothesis tested here was that the Olyset® nets had the same physically limiting effect on *Ae. aegypti* and *Ae. albopictus* as ordinary mosquito nets produced.

Initially, the cone bioassay was intended to be done on-site. But after attempts of cone bioassay "on-site," it was surprisingly discovered that the laboratory-reared *Aedes* adult mosquitoes brought "on-site" had uncanny abilities to squeeze through the mesh-holes of the Olyset® nets and enter the classrooms! It was decided from then on that the cone bioassay of the Olyset® nets had to be done in a laboratory setting.

In the laboratory, cone bioassay was performed on 28 cm x 28 cm cuttings of Olyset® that were stapled to 25 cm x 25 cm frames of 2cmx2cm wooden sticks. To include observation of the "penetrant" adult mosquitoes, additional tulle nets with smaller mesh sizes were attached to the front and back of the framed Olyset® nets. Four WHO bioassay cones were fixed to the framed Olyset® by firmly connecting them to the Net using ordinary hairpins. Olyset® nets at 1, 4, and 6 years-old usages were tested with cone bioassay. The framed Olyset® nets were held upright by pairs of 4-inch long ½-in aluminum angle bars as a base. The bioassay setups were preferably held at 90° instead of the recommended 60° angle to simulate the actual orientation of the ITS in the classroom (Owusu et al., 2015). Getting the natural responses of the test animals was more

important than maximizing the results.

Using a handheld bulb aspirator, five 3-5 days old, non-blood fed *Ae. aegypti* and *Ae. albopictus* were separately introduced into each of the four WHO cones of the bioassay setup. Exposure of each batch of five *Ae. aegypti* and five *Ae. albopictus* to Olyset® were done separately for 3 minutes, according to the WHO cone bioassay procedure. After 3 minutes, the *Aedes* mosquitoes were removed using a handheld bulb aspirator and transferred to small screen-topped plastic containers with proper labels. "Penetrant" mosquitoes were transferred to separate containers that were also labeled. The mosquitoes were stored and sustained with 10% sucrose for 48-72 hours. After 60 minutes, percentage knockdown and mortality after 24 hours were observed and recorded. Mosquitoes were scored as "alive" if they could fly (irrespective of the number of legs still intact), "dead", or "knocked down", if immobile or incapable of flying or standing in a coordinated manner, and "penetrant" or "non-penetrant, if they crossed or not the Olyset® nets during cone bioassay.

Cone bioassays were conducted on 1, 3, and 6 years old Olyset® net material was obtained from property custodians through the school principal's approval. Untreated nets with identical mesh sizes were used as a control and were tested during each bioassay. The bioassays were performed at room temperature (25.9–29°C), and relative humidity ranges 58–80%. Interpretation of results was based on WHO bioassay guidelines, which consider that a population is (1) fully susceptible if the final percentage mortality is >98% or higher, (2) incipient resistant if 80-97% mortality is observed, and (3) resistant if mortality is <80%.

2.3 Aedes mosquito surveillance in Olyset® and non-Olyset® classrooms

After complying with entry protocols through the DepEd authorities, three (3) surveillance adult-larval mosquito traps were installed in each of five randomly selected classrooms that had Olyset® on their windows (ITS classrooms) and another set of 3 mosquito traps in each of five randomly selected classrooms without Olyset® (Non-ITS classroom). A total of seventy surveillance mosquito traps were installed in seven DepEd elementary schools, with a particular preference for kinder and grade one elementary classroom. The null hypothesis of this surveillance was that there was no difference in *Aedes* mosquito abundance between DepEd classrooms with and without Olyset® nets.

A sampling of mosquitoes (adult and larvae) was done twice for two months in each DepEd elementary school sentinel classroom (Fig. 1). After two months, the surveillance was moved on to another selected school for another two months until all seven schools were covered. The surveillance tool used in the ITS- and non-ITS classrooms were the innovative adult-larval traps.



Fig. 1 (A) Field cone bioassay on Olyset® at DepEd school, (B) Laboratory cone bioassay, (C) Mosquito containers after cone bioassay, (D) Mosquito sampling at DepEd school with adult-larval traps, and (E) Student orientation about mosquito trapping in classrooms.

The Adult-Larval Trap (aka ALTrap) was primarily constructed of black, polyethylene plastic dippers with handles sawn-off that was arranged one on top of the other, with their open brims facing each other. One unit of each trap consisted of two parts: (1) an upper adult mosquito-capturing portion and (2) a lower larvae-capturing part. The top portion of the trap contains non-drying glue laid over a stiff board (glue board) inside its walls. Any insect in flight or resting would get stuck to this glue board and would not be able to escape. The lower part of the trap is filled with 1.4 L of 2-3 wks old stored water, the surface of which is covered by a floating ring of sliced foam and screen. Any gravid (egg-laying) female mosquito will be able to deposit its eggs inside its lower walls. Still, when these larvae develop and emerge as an adult, the emerging adult

mosquitoes will be drowned under the floating sliced foam and screen. The novelty of this trap is that it can capture both adult mosquitoes and their larvae. It is beneficial for controlling dengue vector mosquitoes inside rooms and outdoors, but it is also helpful for surveillance.

During sampling from the mosquito traps, the glue board containing adhered adult mosquitoes was collected and stored in re-usable plastic cellophane and labeled appropriately. If present at the bottom portion of the adult-larval trap, mosquito larvae were also collected from the mosquito traps, transferred to 4 ml sealable tubes, and labeled appropriately.

In the laboratory, the adhered insects in the glue boards and mosquito larvae were observed, identified, and photo-documented under the Andonstar inspection microscope. Samples from the adult-larval mosquito traps were classified and recorded as 'males' and 'females' of either *Ae. aegypti, Ae. albopictus,* or non-target mosquitoes (mosquitoes not recognized locally and globally as dengue vector mosquitoes). Non-mosquito samples, such as leafhoppers, midges, termites, et cetera, were noted and recorded but were not included in the computation of mosquito abundance.

3 Results

3.1 Overall knockdown and mortality of Aedes mosquitoes exposed to OlysetTM

A total of 1,200 3-5 days old, unfed adult female *Aedes* mosquitoes were used for WHO cone bioassay on the Olyset® nets. The mosquitoes were segregated into four replicates of 5, each in 5 treatment groups and one control group. A total of 600 adults, female *Ae. aegypti*, and 600 *Ae. albopictus* were sequentially exposed in batches of five individuals to the permethrin-impregnated Olyset® nets for 3 mins cone bioassay. In all treatment groups of *Ae. aegypti*, 52.3% died 24 hours after a 3-minutes exposure to Olyset® nets and a 54% knockdown for the first 60 mins. In *Ae. albopictus*, 81.8% died 24 hours after 3-mins Olyset® exposure and a 75.8% knockdown at the first 60 mins. No Abbott adjustments were necessary for the control groups because mortality was less than 10%.

3.2 Knockdown and mortality of *Aedes* mosquitoes to OlysetTM at various lengths of usage

The average percent knockdown and percent mortality for *Ae. aegypti* and *Ae. albopictus* resulting from cone bioassay of Olyset® nets is given below (Table 1).

Table 1 Percent knockdown and mortality of Ae. aegypti and Ae. albopictus in cone bioassay of Olyset® nets at different years of usage.

Olyset® Bioassay	Ae. aegypti		Ae. albopictus	
Years of usage	% Knockdown	% Mortality	% Knockdown	% Mortality
1	52	51	79	81
3	57	76	85	76
6	53	62	67	81
Average	54	63	77	79

Two-way ANOVA of the mortality scores between treatments and control groups in *Ae. aegypti* and *Ae. albopictus* showed significant differences between species (average p=0.015<0.05). Two-way ANOVA of means of mortality scores of *Ae. aegypti* bioassayed to Olyset® net materials of different ages detected no significant differences except between 3- and 6-years old net material (p=0.033<0.05). The sampled Olyset® net materials used in the cone bioassay were clean but were never washed. As pointed out by others, the insecticide's availability did not persist over time (Erlanger et al., 2004; Tami et al., 2004; Vythilingam et al., 1996; N'Guessan et al., 2001). The significant difference in mortalities between 3 and 6 years of usage may likely be due to the time-dependent potency of the Olyset® nets. Many interactions were detected in the 2-way ANOVA test of means between species and years of usage. This lends evidence that the observed mortalities are due to the potency of the Olyset® nets and the inherent susceptibilities of the species in question.

3.2.1 Penetrability of Olyset® nets

During this preliminary evaluation of Olyset® Net, it was surprisingly observed that some individuals of *Ae. aegypti* and *Ae. albopictus* were quite capable of crossing the Olyset® nets (Figs. 2 and 3). During cone bioassay, 35% of *Ae. aegypti* and 34% of *Ae. albopictus* crossed the test nets. Out of these penetrant mosquitoes, 71% in *Ae. aegypti* and 83% in *Ae. albopictus* died.



Fig. 2 Penetrant and non-penetrant Ae. aegypti during cone bioassay of Olyset® nets.



Fig. 3 Penetrant and non-penetrant Ae. albopictus during cone bioassay of Olyset® nets.

In a study conducted in Malaysia, the penetrability test on the Olyset® Net yielded 45.2% of *Ae.aegypti* and 19% of *Anopheles maculatus* could cross the 3 mm x 3 mm mesh size of the Olyset® Net (Vythilingam et

al., 1996). Mortality in the penetrant *Ae. aegypti* was 25%, and in *Anopheles maculatus*, mortality was 8.9%. In a comparative study on mosquito-penetrability across five different commercial bed nets, penetration success of *Ae. aegypti* was only 13% while *Ae. albopictus* showed 46% crossing Olyset® bednets (Andrade and Cabrini, 2010). Mortality was 100% in all *Ae. aegypti* penetrants, while only 96% of *Ae. albopictus* penetrants died. In a video-aided study in the US, Dickerson et al. (2018) showed that free-flight void entry of *Ae. aegypti* was mainly limited to 10-mm holes because as the hole got smaller (mesh size <10 mm), frequency of access or penetration became impossible.

In the current study, data have shown that out of 1000 *Ae*. mosquitoes exposed to Olyset® nets in cone bioassay, 34.2% (342 individuals) of *Aedes* mosquitoes were able to cross the nets. They crossed the nets not by flying but by crawling erratically and squeezing themselves through the nets. The mesh size of Olyset® nets was only 3-millimeters, but it was not an absolute barrier for the Cagayan de Oro strains of *Ae. aegypti* and *Ae. albopictus*. An adult female *Ae. aegypti* or *Ae. albopictus* exhibited an uncanny way of probing the mesh holes with its proboscis, after which it inserted its head first. Then by pushing and squeezing its thorax across the narrow Olyset® mesh hole, it swiftly jettisons itself across the Net with its fore- and midlegs. In the older (6-years old) softer-fabric version of Olyset® nets, *Aedes* mosquitoes effortlessly crouched through the mesh. During cone bioassay, 5 *Aedes* mosquitoes' test-batch was lost to the other side of the Net in no time!

Following the fate of these Olyset® trespassers in separate holding containers, it was found that only 10% of *Ae. aegypti* and 6% of *Ae. albopictus* survived. Having come into contact with Permethrin throughout most of their body, 25% of *Ae. aegypti* and 28% of *Ae. albopictus* could not last long minutes after the permethrin exposure. The surviving Olyset® net trespassers could have theoretically acted as vectors. It would have been interesting to follow the biting frequency of these surviving *Aedes* mosquitoes were they not needed to be terminated. *Aedes* mosquitoes have uncannily evolved a behavioral strategy that enables them to reach their blood source despite physical and chemical barriers. And based on the concept paper of Sumitomo Chemical Company itself, their design of 4 mm mesh size of the Olyset® Net exploits this trespassing behavior of mosquitoes to maximize exposure to the insecticide released at the surfaces of the net fibers (Okuno and Ito, 2006).

The ratio of Olyset® net percent penetrations by *Ae. aegypti* and *Ae. albopictus* were indistinguishable, as shown in figures 3 and 4, attesting that the populations had equal abilities to cross the nets (p=0.7659>0.05). But as scores of survival to permethrin exposure by the penetrants were analyzed, results showed that *Ae. aegypti* survived more than *Ae. albopictus* did (p=0.04<0.05). This finding consistently shows the generally low mortality that *Ae. aegypti* exhibited in cone bioassay. Reductions in biting rate had been observed by other researchers in *Anopheles gambiae*, *Anopheles funestus*, and *Culex quinquefasciatus* (N'Guessan et al., 2001) 3.2.2 Surveillance results of mosquito trapping at selected classrooms with and without Olyset®.

A total of 210 adult-larval mosquito traps were installed in sentinel classrooms at seven various DepEd elementary schools in Cagayan de Oro City. About half of these mosquito traps were installed in classrooms with Olyset® nets, while the other half were installed in classrooms without Olyset® nets. After requesting entry from the school principal and teachers, the traps were installed at corners inside the classrooms for about two months. Sampling was done twice in each sentinel classroom throughout the surveillance period.

From mosquito trap surveillance in 70 elementary classrooms, 1452 adult mosquitoes and 806 larvae were caught in the mosquito traps. A mean capture of 4.9 adult *Aedes* mosquito/trap was calculated from classrooms with Olyset® nets in contrast to a mean of 10.2 adult *Aedes* mosquito/trap in classrooms without Olyset® nets (Fig. 4).



Fig. 4 Comparison of means of Aedes female counts between classrooms with and without Olyset® nets.

Test of equal means detected highly significant differences in mosquito capture between classrooms with and without Olyset® nets ($p=8.82 \times 10^{-06} < 0.05$). Larval capture in the traps also showed substantial differences between classrooms with and without Olyset® nets ($p=7.5 \times 10^{-05} < 0.05$) (Fig. 5). The breakdown of female and male *Aedes* mosquitoes and non-dengue mosquitoes are listed in Table 3.



Fig. 5 Comparison of means of Aedes larvae counts between ITS and non-ITS classrooms

Table 2 Counts of adult *Ae.aegypti*, *Ae.* albopictus and non-dengue mosquitoes caught during mosquito trap suveillance in DepEd classrooms.

	Ae. aegypti		Ae. albopictus		Non-target Mosquitoes	
	Female	male	Female	male	Female	male
With Olyset®	176	31	14	14	257	83
Without Olyset®	289	57	22	7	382	120

A discrepancy was also noticed between *Ae. aegypti* and *Ae. albopictus* capture in the traps ($p=1.1x10^{-17}<0.05$). There were two likely reasons for this difference in capture. One reason may be based on their blood-feeding habits. It was being considered zoophilic by feeding habit, *Ae. albopictus* tend to forage outdoors where various blood sources exist. In contrast, *Ae. aegypti*, is anthropophilic. It usually forages indoors, where its most preferred blood source is present (Delatte et al., 2010; Bonizzoni et al., 2013; Sullivan et al., 1997; Harrington et al., 2001; Ponlawatt and Harrington, 2005; Richards et al., 2006). The second reason for the

contrasting trap captures may be their breeding preferences. *Ae. aegypti* prefers clear and less polluted waters in domestic containers inside or near human dwellings. *Ae. albopictus* prefers natural containers or outdoor artificial habitats containing organic debris (Chareonviriyaphap et al.,2003; Dom et al., 2013). The water in the indoor mosquito traps was devoid of organic debris, so more *Ae. aegypti* were captured in the mosquito traps inside the classrooms than *Ae. albopictus*. Based on mosquito trap capture, *Aedes* adult females and larval abundances were significantly low in classrooms with Olyset® nets than in classrooms without Olyset® nets (p=1.1x10⁻¹⁷<0.05)(Table 4). This was unexpected since it was observed that many of the Olyset® nets need replacement due to the presence of holes and tears. Furthermore, many ITS classrooms' lack of screened doors made the intervention appear compromised. It was suspected that the mosquitocidal impact of Olyset® nets had already been significantly reduced. Many classrooms with Olyset® nets did not even have screen doors. And the doors were left open during classes because, according to teachers, air ventilation was severely limited by Olyset® nets. This may have allowed unimpeded entry or movement of *Aedes* mosquitoes and other nontarget mosquitoes between classrooms (Fig 6). It has also been observed that many of the Olyset® nets, especially those with 1-year-old usage already had tears and holes of various sizes. This status should have been attended by school authorities but was not because of many other concerns in the school.

 Table 3 Means and abundance of Aedes adult females and larvae in classrooms with and without Olyset® nets based on count in mosquito traps

	Classroom with Olyset® nets (ITS)	A classroom without Olyset® nets (Non-ITS)
Mean of adult female Ae.	4.97+4.97	10.22+0.67
Sum of Adult female Ae.	174	358
Mean of Ae.larvae	6.66+0.73	12.30+1.30
Sum of Ae. larvae	240	566



Fig. 6 Samples of adult mosquitoes caught in mosquito adult-larval traps: (A) *Ae. aegypti* (B) *Ae. albopictus*, (C) *Culex quinquefasciatus*, (D) *Cx. gelidus*

4 Summary and Conclusion

This study set out to conduct an entomological evaluation of Olyset® nets that have been installed in DepEd elementary classrooms. The methods used were laboratory-based WHO Cone bioassay and field surveillance of adult female *Aedes* and larval abundance in selected DepEd classrooms using mosquito adult-larval traps.

Based on WHO 98-100% insecticide susceptibility guidelines, the cone bioassay of *Ae*. results. *Aegypti* and *Ae*. *albopictus* on Olyset® nets showed these mosquitoes were not fully susceptible to 2% permethrin in the Olyset® nets. Therefore, the Olyset® nets are not fully effective in producing the desired 98-100% knockdown and mortality for intervention against dengue mosquitoes.

Mosquito adult-larval trap surveillance on Ae. aegypti and Ae. albopictus in seventy DepEd elementary classrooms showed significantly low mean capture of adult and larvae of dengue vector mosquitoes in

classrooms with Olyset® nets than in classrooms without Olyset® nets. Mean capture of 4.9 adult female *Aedes* mosquitoes per trap ere obtained in classrooms with Olyset® nets, compared to 10.2 *Aedes* mosquitoes/trap caughtin classrooms without Olyset® nets. Test of equal means detected highly significant differences in mosquito capture between classrooms with and without Olyset® nets (p= $8.82 \times 10^{-06} < 0.05$). Larval capture in the traps equally showed substantial differences between classrooms with and without Olyset® nets (p= $7.5 \times 10^{-05} < 0.05$).

Nevertheless, mosquito adult-larval trap surveillance showed that the Olyset® nets are ineffective as an intervention against *Ae. aegypti* and *Ae. albopictus*, because vector mosquitoes are still present with Olyset® nets in classrooms. If the Olyset® nets were practical, no *Aedes* mosquitoes or very few mosquitoes would have been present. But the results show that they are still a clear and present danger in the classrooms with Olyset® nets.

Furthermore, the lack of complete enclosures in the classrooms, like screen doors, has unfortunately compromised the efficacy of the intervention. Therefore, the material integrity of the Olyset® nets and other accessories needed to maintain the classroom enclosure against dengue vectors should be assessed and attended to sustain the intervention. It would be interesting to follow up on the reductive impact of Olyset® nets against the hole index in the nets. Furthermore, a Cone bioassay on *Ae* populations over a wider area with GIS mapping of percent mortality and knockdown would begin a comprehensive study of pyrethroid resistance in the *Aedes* mosquitoes.

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