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Outdoor residual spray for the control of monkey malaria vectors in Sarawak, Malaysia

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Abstract

The increase in cases of monkey malaria in humans, especially in Malaysia Borneo which is believed to be due to infection by the malaria vector that took place outdoor, shows the importance of outdoor residual spraying in controlling this vector. This study aims to evaluate the effectiveness of the new formulation deltamethrin K-Othrine® (PolyZone) and examine its suitability in the control of knowlesi malaria vector in Sarawak, in comparison to deltamethrin K-Othrine® WG250 wettable granule, the insecticide currently use in the vector control programme. The study was performed at three sites in Sarawak having similar type of houses made out of wood and concrete. Houses of two sites were sprayed outdoor with deltamethrin K-Othrine® (PolyZone) at two different dosages, one site with 25 mg/m² and the other site with 30 mg/m². Houses of the third site (control house) were sprayed indoor with deltamethrin K-Othrine® WG 250 wettable granule at 25 mg/m². Residual activity on different walls was assessed using WHO standard cone bioassay techniques. For vector surveillances, larvae and adult surveillance were conducted pre and post spraying. Nested and semi-nested PCR was conducted to detect the malaria parasite in adult mosquitoes. Outdoor residual sprayed of Deltamethrin K-Othrine® (PolyZone) presented higher efficacy compared to indoor residual sprayed of Deltamethrin K-Othrine® WG 250. The efficacy of different type of wall surfaces (wood and concrete) was found varied. Deltamethrin K-Othrine® (PolyZone) at 30 mg/m² was the most effective with regards to ability to cause higher mortality. Spraying activity conducted during this study, has resulted in malaria cases in Julau reduced significantly. Based on findings of this study, it is recommended to consider outdoor residual spraying on wall surfaces in the community for improved effectiveness of malaria vector control programme.

Keywords: Outdoor residual spray, *Knowlesi* Malaria, malaria vector control

1. Introduction

Malaria is one of the major public health concerned as it is one of the highest cases reported especially in tropical country. In 2017 alone, about 219 million malaria cases have been reported and the number of deaths was over 435,000 worldwide. Although in recent years the number of reported cases has been seen decreased, the effectiveness in the prevention and elimination of malaria especially in rural areas is far from perfect [1]. In Malaysia, 68% of malaria cases were reported in the rural area of Malaysian Borneo (Sabah and Sarawak) while the other 32% were in Peninsular Malaysia [2].

Intervention including insecticide-treated bed nets (ITN), indoor residual spraying (IRS), larval control, improved diagnostic testing and treatment by artemisinin combination therapy (ACT) have contributed to the decreasing numbers of malaria cases globally [3]. In Malaysia, indoor residual spraying (IRS) of insecticide is one of the widely used control measures. Yet, in area where malaria is persistent including aboriginal areas, tribal villages and rural hilly area, it has become a long-term struggle for the community living in these areas in combating malaria as these areas were difficult to access for IRS to be conducted six monthly routinely [4]. Furthermore, IRS focuses on mosquitoes that feed and rest indoor, causing mosquitoes that feed and rest outside the house are pretty much left unharmed. A counter measure is clearly needed and to be seriously taken into consideration in the fight to combat the spreading of malaria from the outdoor environment. A new formulation of pyrethroid known as deltamethrin K-Othrine® (PolyZone) has been produced for outdoor residual usage.

This insecticide has been improved with anti-rain property which makes it an ideal insecticide for outdoor usage^[4]. Malaria is caused by the protozoan parasite genus *Plasmodium* which resides inside the body of female *Anopheles* mosquitoes which then transmitted into human while feeding^[5]. *Plasmodium knowlesi* has been identified as one of the major species attributed to the number of malaria cases in Malaysia while a small number of cases were attributed by *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*^[2]. The climatic factors such as humidity, rainfall, and temperature can aid in development of parasite and vectors in transmitting the disease^[5]. Furthermore, the challenge posed by mosquitoes that develop resistant to insecticides used in controlling malaria has become an additional factor that hamper the malaria control^[1].

Hence, the purpose of this study is to evaluate the effectiveness of the new formulation deltamethrin K-Othrine® (PolyZone) compared to the currently use vector control insecticide, deltamethrin K-Othrine® WG250 wettable granule sprayed on wood and concrete surfaces. It is hope that

findings generated from this study could help provide potential alternative approach in preventing the spreading of mosquito vector in rural area specifically and help controlling malaria in Malaysia generally.

2. Materials and methods

2.1 Study area

The study was conducted in Julau District, Sarawak. Field trial was focused in 3 study sites; Site A (Rumah Melaka, N1.759° 45.725' E111° 50.153'), Site B (consisting of Rumah Janting (N1° 55.433' E111° 53.469'), Rumah Berandah (N1° 55.210' E111° 53.600'), Rumah Mingang (N 1.9318° E111.896°) and Site C (Rumah Saong N2° 01.254' E111° 49.586'), (Fig. 1). The sites were selected based on the highest reported cases of malaria in the area. Most of the sites were situated in a remote area that accessible only using four-wheel vehicle. The houses in these sites were long houses which are built using different materials which include wood and concrete.



Fig 1: The location of the study sites

2.2 Insecticides and spraying methods

Specifically, two types of insecticides were used in this study: (1) the new formulation new formulation deltamethrin K-Othrine® (PolyZone) (a.i. 62.5 g/L deltamethrin) and (2) currently used vector control insecticide, deltamethrin K-Othrine® WG 250 wettable granule. For the new formulation insecticide, deltamethrin K-Othrine® (PolyZone), two doses were evaluated: 25 mg/m² and 30 mg/m². This new product has been approved for usage by WHO. For the currently used insecticide, deltamethrin K-Othrine® WG 250 wettable granule, only one dosage, 25 mg/m² was used which is the current dosage employed for malaria vector control.

Spraying was conducted outdoor (ORS) on shaded and unshaded surface area for Site A and B (test sites) and indoor spraying was performed for Site C (control site). The area sprayed was selected based on its potential as resting area for mosquitoes. The formulation and dosage used for spraying at study sites were as follows: Houses in Site A was sprayed with 30 mg/m² of deltamethrin K-Othrine® (PolyZone), houses in Site B was sprayed with 25 mg/m² of deltamethrin K-Othrine® (PolyZone) while houses of Site C was sprayed

with 25 mg/m² deltamethrin K-Othrine® WG 250 wettable granule as control site for this study. The spraying process was conducted by Ministry of Health (MoH) trained and experienced staffs using Hudsons Compressor Sprayer according to WHO guidelines and manufacturer instructions. Two sprayings were conducted for the study: 1st spraying was performed in October 2018, while 2nd spraying was in July 2019.

2.3 Experimental Design

Three factors were highlighted upon analysing the data of the spraying activity: (a) spraying location - outdoor (shaded and unshaded areas) and indoor, (b) type of insecticide - 25 mg/m² of deltamethrin K-Othrine® (PolyZone), 30 mg/m² of deltamethrin K-Othrine® (PolyZone) and 25 mg/m² deltamethrin K-Othrine® WG 250 wettable granule) and (c) type of wall – wood and concrete. The response variable of this study is the mortality rate of the mosquitoes.

Residual activity on the walls was assessed using the standard World Health Organization (WHO) cone bioassay techniques. For this study, *Anopheles cracens* laboratory strain was used

for the purpose of adult bioassay replacing the *Anopheles latens* which was the primary vector at the study area. *An. cracens* was used due to unavailability of laboratory strain *An. latens*. Furthermore, very small number of adult *An. latens* was captured during the study. The residual activity was observed every six weeks post spraying. Briefly, cones were placed onto the sprayed wall. A bioassay of five replicates for each dose and wall was conducted. Twenty sugar-fed 2–5 days old laboratory-bred *An. cracens* female mosquitoes were released into each cone through the aperture which was then clogged with cotton bud. Mosquitoes were exposed continuously for 30 minutes to the sprayed wall. Once completed, the live mosquitoes were transferred to paper cups where 10% sugar solution and Vitamin B complex were supplied to the mosquitoes. They were then kept for 24 hours where the mortality rate was recorded and then statistically analysed using ANOVA.

2.4 Larval surveillance

Larval surveillance was conducted on four occasions from between October 2018 to November 2019. All potential breeding sites within the study sites were inspected. Larvae were collected and identified. Briefly, a dipper was used to collect the larvae which then placed inside a white plastic tray. Mosquito larvae were collected with a pipette and transferred into labelled bottle. Type of breeding site and their location were recorded using GPS. Environmental parameters were obtained by collecting the data of rainfall, temperature, and humidity by installing via installation of localise weather station at the study sites.

2.5 Mapping of mosquito population

The coordinates of mosquito breeding sites were marked using hand-held Geographic Positioning System (GPS), (Garmin GPSMAP® 60CSx; Garmin, Olathe, KS) and processed with MapSource® software (Garmin). The coordinates where mosquito larvae were identified were marked on the map using GIS database software (ArcGIS 9.3; Environmental Systems Research Institute, Redlands, CA).

2.6 Adult survey and parasite identification

Adult mosquitoes from the sites were caught using Human landing catching (HLC) technique. The collection routine starts from 6:00pm to 12:00am (6 hours duration) for three straight days for each site. The collected mosquitoes were identified to species level using the keys of Reid ^[6] and Sallum *et al.*, ^[7]. Specimens morphologically identified were

further confirmed by PCR followed by sequencing analysis of ITS2 ^[8]. Briefly, the DNA was first extracted using the DNeasyR Blood & Tissue Kit (Qiagen, Valencia, CA). Mosquitoes were then undergoing the detection of malaria parasite using semi-nested and nested PCR techniques followed by sequencing analysis of the small-subunit (SSU) rRNA gene ^[9].

2.7 Ethics

Informed consent was obtained from all collectors performing Human Landing Catching (HLC). Community consent had been obtained beforehand from all selected sites/villages. This study was approved by Medical Research & Ethics Committee, Ministry of Health, Malaysia (NMRR-18-202-40239).

3. Result and Discussion

3.1 Bio efficacy of the insecticide

The result of residual activity of the insecticides at different concentrations sprayed on wood is presented in Fig. 2 and those sprayed on concrete is presented in Fig. 3. Based on Fig. 2, the mortality rate of mosquitoes exposed to outdoor shaded, outdoor unshaded and indoor wood surfaces sprayed with different type of insecticide and at different concentration demonstrated a decrease trend of mortality rate for all doses of insecticides. Almost all mortality rates for mosquitoes exposed to the new formulation were found higher than mortality rate of mosquitoes exposed to control insecticide. 100% mortality rate was observed up to week 25 for mosquitoes exposed to shaded and unshaded wood surface sprayed with 30 mg/m² of deltamethrin K-Othrine® (PolyZone). Observation on week 35 showed a drastic reduction in the mortality rate where it went down to around 55% for mosquitoes exposed to shaded wood surface and to around 50% for mosquitoes exposed to unshaded wood surface. After 2nd spraying was performed, the mortality rate recorded on week 43 demonstrated an increase back to 100% mortality for both group of mosquitoes. Observation performed six weeks later (week 48) indicated slight reduction (around 90%) for both groups of mosquitoes. Gradual reduction was observed during the rest of the observation weeks (week 56, 63, and 67) with mortality rate of mosquitoes exposed to unshaded wood surface was always lower than those exposed to shaded wood surface. The final reduction observed on week 67 did not exceed below the 50% mortality rate for both groups.

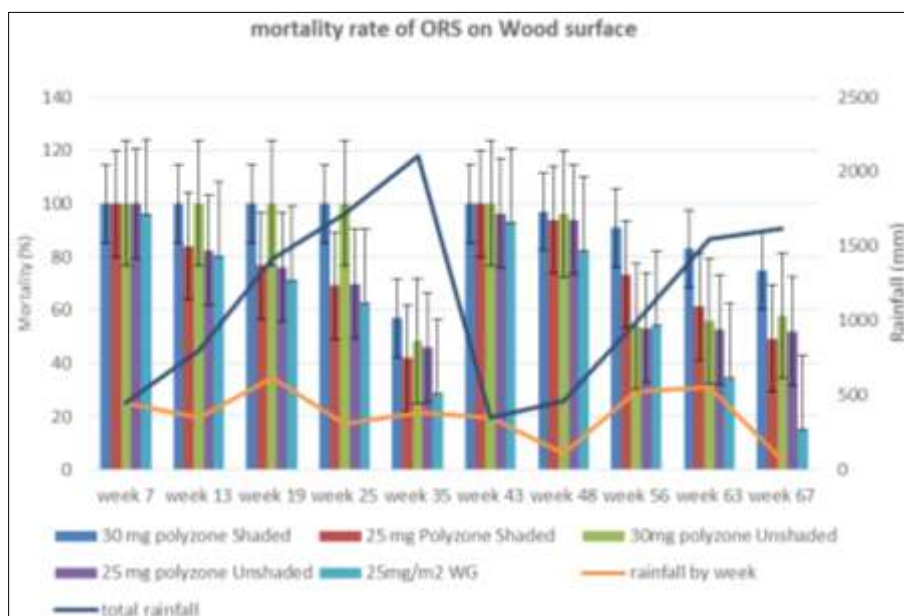


Fig 2: Mortality rate of mosquitoes exposed to outdoor (shaded and unshaded) wood surface sprayed with different type of insecticide and at different concentration

As for mosquitoes exposed to shaded and unshaded wood surface sprayed with 25 mg/m² of deltamethrin K-Othrine® (PolyZone), the effectiveness of the insecticide on the mosquitoes was found to be less than that presented by wood sprayed with 30 mg/m² of deltamethrin K-Othrine® (PolyZone). Wood sprayed with 25 mg/m² of deltamethrin K-Othrine® (PolyZone), caused 100% mortality rate at week 7. The mortality rate was seen reducing gradually during the following observation (week 13, 19, 25 and 35) to as low to almost 40%. After 2nd spraying was performed, the mortality rate recorded on week 43 was increased back to 100% for mosquito exposed to shaded wood and around 95% for

mosquito exposed to unshaded wood. In the following observation (week 48, 56, 63 and 67) the mortality rate shown to reduce gradually to as low as around 50% for both groups of mosquitoes.

As for the control group (mosquitoes exposed to indoor wood surface sprayed with 25 mg/m² deltamethrin K-Othrine® WG 250 wettable granule) the mortality rate recorded did not achieved the 100% mark at any time of observation. The mortality rate went down gradually from around 90% to around 25% at week 35 and went back up to 90% after the second spraying was performed at week 43 and gradually reduced again to as low as 18% at week 67.

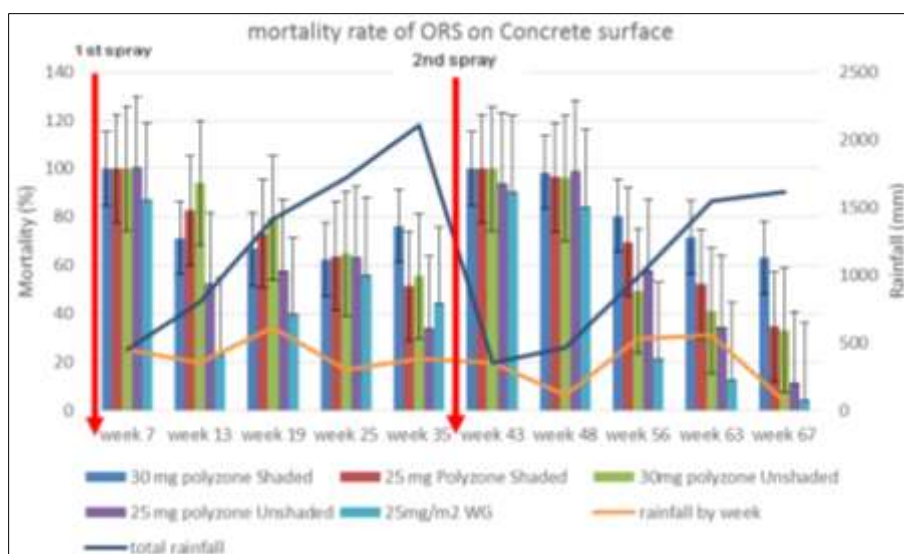


Fig 3: Mortality rate of mosquitoes exposed to outdoor (shaded and unshaded) concrete surface sprayed with different type of insecticide and at different concentration

Based on Fig. 3, the mortality rate of mosquitoes exposed to outdoor shaded, outdoor unshaded and indoor concrete surfaces sprayed with different type of insecticide and at different concentration also demonstrated a decrease trend of mortality rate for all mosquitoes exposed to different doses of

insecticides. Similar to results in Fig. 2, almost all mortality rates for mosquitoes exposed to the new formulation were found higher than mortality rate of mosquitoes exposed to control insecticide.

As shown in Fig. 3, 100% mortality rate was observed on

week 7 for mosquitoes exposed to shaded and unshaded concrete surface sprayed with 30 mg/m² of deltamethrin K-Othrine® (PolyZone). Observation on week 13 showed that both groups of mosquitoes already experiencing reduction in their mortality rate to 70% for mosquito exposed to shaded concrete surface and to 90% for mosquito exposed to unshaded concrete surface. It was then followed by a gradual reduction during following observation (week 19, 25 and 35) although for mosquito exposed to shaded concrete surface, an increase on the mortality rate was observed on week 35. After 2nd spraying was performed, the mortality rate on week 43 was increased back to 100% for both group of mosquitoes. Observation done six weeks later (week 48) however indicated some reduction (around 90%) for both groups of mosquitoes and much lower reduction was detected during the rest of the observation (week 56, 63, and 67) with mortality rate for mosquito exposed to unshaded concrete surface was lower than those exposed to shaded concrete surface. The reduction for mosquito exposed to shaded concrete surface on week 67 was around 60% while for mosquito exposed to unshaded concrete surface was around 35%.

As for mosquitoes exposed to shaded and unshaded concrete surface sprayed with 25 mg/m² of deltamethrin K-Othrine® (PolyZone), the effectiveness of insecticide on the mosquito was found to be lower than that of 30 mg/m² of deltamethrin K-Othrine® (PolyZone). At 25 mg/m² of deltamethrin K-Othrine® (PolyZone), 100% mortality rate was observed at week 7, six weeks after the 1st spraying was performed. The mortality rate was seen gradually reduced during the following observation (week 13, 19). At week 25 both groups of mosquitoes presented about the same mortality rate (around 60%) and at week 35 both groups indicated mortality reduction to as low as 50% for mosquito exposed to shaded concrete surface and to as low as around 35% for mosquito exposed to unshaded concrete surface. After 2nd spraying was performed, the mortality rate recorded at week 43 for both group of mosquitoes was increased back to 100% for mosquito exposed to shaded concrete surface and slightly less

than 100% for mosquito exposed to unshaded wood surface. For the following observation (week 48, 56, 63 and 67) the mortality rate shown to reduce gradually to as low as around 35% for mosquito exposed to shaded concrete surface and around 10% for mosquito exposed to unshaded concrete surface at week 67.

For the control group mosquitoes 100% mortality rate was not detected at any time of observation. After 1st spraying mortality rate shown to decrease gradually from around 90% to around 50% at week 35 and went back up to 90% at week 43 after the 2nd spraying was performed and gradually reduced to as low as 5% at week 67.

Statistically, the differences in the mosquito mortality rate between 30mg/m² and 25mg/m² deltamethrin K-Othrine® (PolyZone) insecticides sprayed on shaded area of wood and concrete, and on unshaded wood compared to control insecticide were found to be significantly different (Table 1) indicating that concentrations, formulations and surface types can influence the effectiveness of insecticides in killing mosquitoes. This finding is supported by Rohani *et al.*,^[4] who stated that efficacy of deltamethrin can be varied depending on the concentration used, formulation, surface of the area, humidity, temperature, and method of evaluation. Significant variation in residual efficacy between different types of wall surfaces was also reported by Desalegn *et al.*,^[10] using propoxur and Mutagahywa *et al.*^[11] using lambda-cyhalothrin who observed significant differences in mortality rate of *An. gambiae* exposed to different wall surface types sprayed with the same insecticide. In addition, study by Corrêa *et al.*,^[12] using etofenprox, alpha-cypermethrin, and lambda-cyhalothrin, deltamethrin, bendiocarb, and pirimiphos-methyl insecticides against wooden and concrete wall also found significant difference for all the insecticide tested. In contrast, no significant difference in mean mortality rates of *An. gambiae* among the three different wall surfaces [(a) mud but not plastered (b) mud and plastered (c) mud, plastered and painted] was reported by Hailu *et al.*,^[14]

Table 1: Summary of significant value of insecticides at different dosage sprayed on wood and concrete surfaces compared to control insecticide

Factors	Wood	Concrete
Control vs 30mg/m ² deltamethrin K-Othrine® (PolyZone) shaded	15.773**	8.647**
Control vs 25mg/m ² deltamethrin K-Othrine® (PolyZone) shaded	5.830*	4.521*
Control vs 30mg/m ² deltamethrin K-Othrine® (PolyZone) unshaded	7.811*	3.781
Control vs 25mg/m ² deltamethrin K-Othrine® (PolyZone) unshaded	4.737*	1.023

** Correlation is significant at the level 0.05 level (2-tailed)

* Correlation is significant at the level 0.01 level (2-tailed)

The differences in the mosquito mortality rate between 30mg/m² and 25mg/m² deltamethrin K-Othrine® (PolyZone) insecticides sprayed on unshaded concrete compared to control insecticide however were not significantly different indicating that sunlight exposure could affect the efficacy of the insecticides. Barlow *et al.*,^[14] emphasised that UV rays in sunlight may degrade any synthetic pyrethroid deposited because natural pyrethrum is biodegradable and undergoes photo-degradation when exposed to UV light.

The correlation between different concentration of insecticides sprayed on wood and concrete surfaces was demonstrated in Table 2 and Table 3 respectively. As presented in Table 2, significant correlation was observed for all concentration of insecticides sprayed on wood surface (shaded and unshaded), suggesting that change in the concentration of the insecticides used and change in wood

surface (shaded and unshaded) could affect the performance of the insecticides.

As for different concentration of insecticide sprayed on concrete surface (shaded and unshaded) the significant in correlation obtained however differ slightly compared to wood surface (Table 3). Significant correlation was not observed between 30mg/mm² (PolyZone) sprayed on shaded surface and 30mg/mm² (PolyZone) sprayed on unshaded surface; and between 30mg/mm² (PolyZone) sprayed on shaded surface and 25mg/mm² (PolyZone) sprayed on unshaded surface. These findings could suggest that when it comes to concrete wall, change in the concentration of insecticides used and change in concrete surface (shaded and unshaded) might not have much effect on the effectiveness of the insecticide.

Table 2: Correlation between different concentration of insecticides sprayed on shaded and unshaded wood surface

Wood	Control (WG250)	30mg/mm ² (PolyZone) shaded	25mg/mm ² (PolyZone) shaded	30mg/mm ² (PolyZone) unshaded
30mg/m ² (PolyZone) shaded	.808**			
25mg/m ² (PolyZone) shaded	.973**	.772**		
30mg/m ² (PolyZone) unshaded	.731*	.945**	.694*	
25mg/m ² (PolyZone) unshaded	.988**	.821**	.985**	.769**

** Correlation is significant at the level 0.05 level (2-tailed)

* Correlation is significant at the level 0.01 level (2-tailed)

Table 3: The correlation between different concentrations of insecticides applied on shaded and unshaded concrete surface

Concrete	Control (WG250)	30mg/mm ² (PolyZone) shaded	25mg/mm ² (PolyZone) shaded	30mg/mm ² (PolyZone) unshaded
30mg/m ² (PolyZone) shaded	.596			
25mg/mm ² (PolyZone) shaded	.754*	.683*		
30mg/m ² (PolyZone) unshaded	.888**	.610	.939**	
25mg/m ² (PolyZone) unshaded	.830**	.553	.869**	.845**

** Correlation is significant at the level 0.05 level (2-tailed)

* Correlation is significant at the level 0.01 level (2-tailed)

The correlation study summarised that changes in concentration of insecticide and surface type (shaded and unshaded) effected wood more than concrete in relation to the effectiveness of the insecticide and this is most likely could be due to surface porosity. According to Lo *et al.*, [15] high porosity of a wall structure could influence the efficacy of insecticides sprayed. His study demonstrated that mud wall has better insecticide efficacy compared to cement wall. Study by Desalegn *et al.*, [10] also showed that differences in the efficacy of insecticides sprayed are related to porosity between smooth and rough mud walls. The low residual bioefficacy of the pyrethroids on the cement surface compared with the wooden surface due to the rapid degradation on porous surfaces with a high absorption was also found in the study by Corrêa *et al.*, [12].

The correlation between different concentration of insecticides used to spray wood/concrete surface and the total of rainfall in the study sites was also examined (Table 4). Significant correlation was detected between shaded and unshaded wood surface sprayed with 25mg/m² (PolyZone) with the total rainfall. Significant correlation was observed for all insecticide concentration sprayed on shaded and unshaded concrete wall with the total rainfall. Based on both findings it could be suggested that wood surface is slightly better than concrete surface in retaining the effectiveness of insecticide against rainfalls particularly when higher concentration was applied.

Table 4: Correlation between insecticides and rainfall

Type of insecticides	rainfall	
	wood	concrete
30mg/m ² (PolyZone) shaded	-.575	-.742*
25mg/m ² (PolyZone) shaded	-.948**	-.924**
30mg/m ² (PolyZone) unshaded	-.498	-.778**
25mg/m ² (PolyZone) unshaded	-.891**	-.709*

** Correlation is significant at the level 0.05 level (2-tailed)

* Correlation is significant at the level 0.01 level (2-tailed)

3.2 Profile of *Anopheles* collections

Larvae surveillance conducted at all sites confirms the presence of *Anopheles* species in several habitats namely water pocket, puddle, pool, slow moving stream, fishpond, and tire track (Table 5). The most common habitat detected was water pocket.

Table 5: Larvae habitat detected in study areas

Habitat type	Number of habitats
Water pocket	9
Puddle	3
Pool	3
Slow moving stream	5
Fishpond	3
Tire track	1

Fig. 4 presents maps of study sites illustrating the distribution of all habitats. At site A, three water pocket, two slow moving stream and one puddle habitats were detected. At site B, breeding habitats detected were water pocket (1), slow moving stream (2), puddle (2), pool (3) and fishpond (3) while at site C, breeding habitats detected were water pocket (5), slow moving stream (1) and tyre track (1). Table 6 shows the *Anopheles* species captured in the study sites. A total of nine species were identified comprising of *An. An. donaldi*, *An. latens*, *An. introlatus*, *An. barbumbrosus*, *An. hodgkini*, *An. tessellatus*, *An. roperi*, and *An. pujutensis*. The highest number of species collected was *An. donaldi*. Some of these mosquitoes were captured before and some were capture after spraying. The number of mosquitoes captured pre spraying was slightly higher that mosquito captured after spraying (Table 7). Almost similar number of infected mosquitoes, were captured pre and post spraying (Table 7). *An. donaldi* was incriminated as the *P. knowlesi* vector in Lawas, northern Sarawak [16]. While *An. latens* and *An. introlatus* were incriminated as the *P. knowlesi* vector in Kapit, central Sarawak [17], and Hulu Selangor [18], respectively.

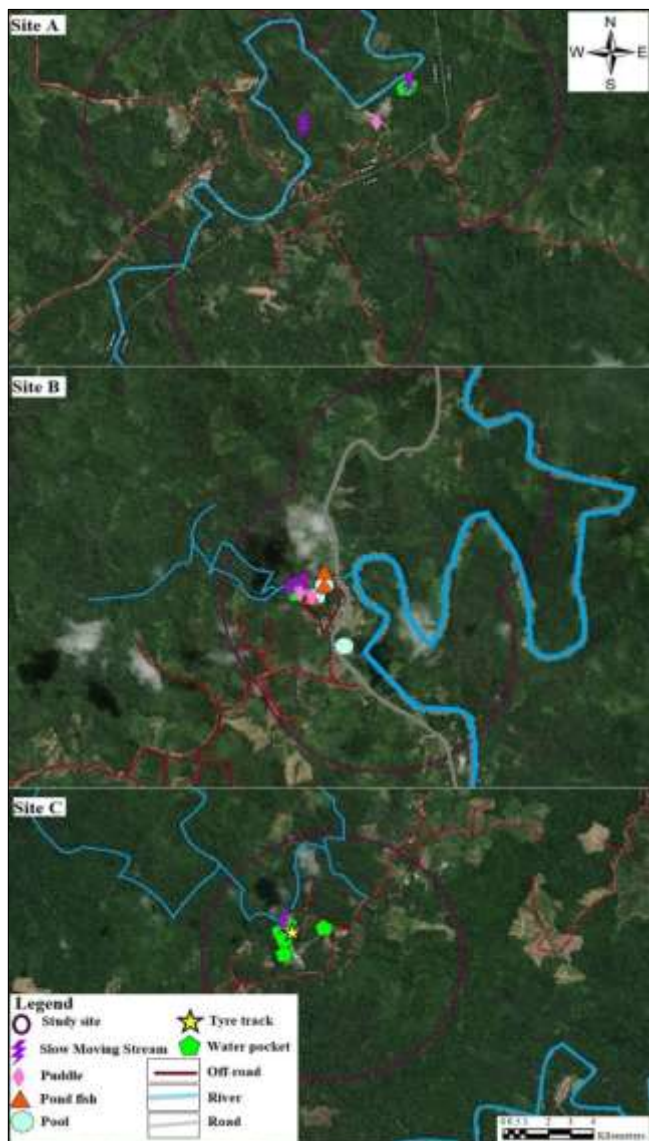


Fig 4: The distribution of *Anopheles* spp. Habitats

Table 6: Mosquito species captured in study sites

Species of mosquito	Number of mosquitoes captured
<i>An. donaldi</i>	72
<i>An. latens</i>	31
<i>An. introlatus</i>	8
<i>An. barbumbrosus</i>	3
<i>An. hodgkini</i>	3
<i>An. tessellatus</i>	2
<i>An. roperi</i>	1
<i>An. pujutensis</i>	1
Total	121

Table 7: Number of mosquitoes captured post and pre spraying during the study

Number of mosquitoes captured =121	
Pre-spraying	Post-spraying
not infected with malaria parasite	not infected with malaria parasite
41	31
infected with malaria parasite	infected with malaria parasite
27	22
Total mosquito captured	Total mosquito captured
68	53

The number of mosquitoes captured and positive for the

malaria based on study sites were illustrated in Fig. 5 while the malaria species infecting the mosquito based on sites is shown in Fig. 6. Malaria positive mosquitoes were detected in all study sites. The highest number of mosquitoes occurred in Site C (control site) and the lowest was in Site A. The highest malaria species detected was *Plasmodium knowlesi* (46.77%), followed by *P. vivax* (29.03%) and *P. faciparum* (24.19%) (Fig. 6). This study also confirmed that 22.45% of mosquitoes were detected with more than one parasite in the body.

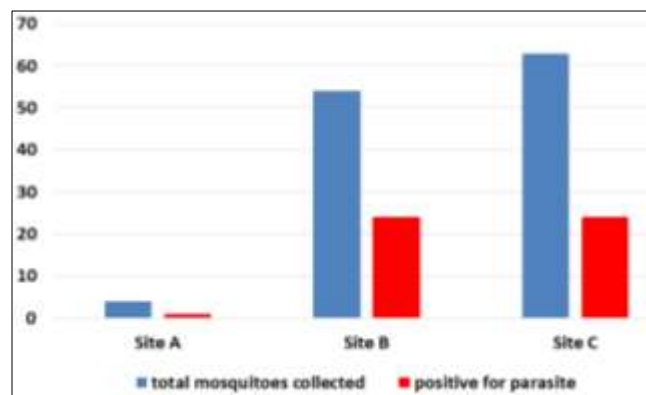


Fig 5: Number of mosquitoes captured and infected with malaria parasite based on sites



Fig 6: Species of malaria parasite detected in mosquito based on study site

To date, the highest malaria case in Malaysia is the *knowlesi* malaria [1, 19, 20]. Hussin *et al.*, [1] highlighted the occurrence of high incidence of *knowlesi* malaria in Sabah and Sarawak based on cases reported in 2017 despite the decreasing number of cases cause by *P. vivax* and *P. faciparum*. In addition, reviewed study by Rahim *et al.*, [21] emphasized a strong pattern of declines in all human malaria species with rising *P. knowlesi* incidence rates in Borneo and Peninsular Malaysia from 1980 to 2019. This current study performed in three sites in Sarawak around 2018-2019 clearly in accordance to report by Hussin *et al.*, [1] as the presence of *P. knowlesi*, was detected in mosquitoes captured in this study (Fig. 6). In addition, *P. vivax* and *P. faciparum* species were also detected in mosquitoes captures. Cases of malaria recorded from 2013 to Jun 2020 (Fig. 7) in Julau presented fluctuating pattern with two peaks were observed one in 2014 and the other in 2017. Drastic decrease in number of cases was observed after 2018 could be due to the effect from spraying activity performed during this study, first one in 2018 and the second one in 2019.

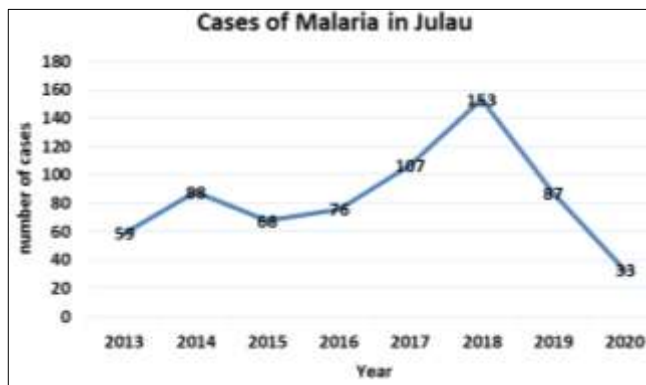


Fig 7: Number of malaria cases reported in Julau from 2013-2020

Fig. 8 shows distribution of malaria cases in Julau based on species. Clearly, *knowlesi* malaria was the dominant type in Julau where 95% of cases reported there was *knowlesi* malaria. This finding is in accordance with report highlighted by Hussin *et al.*,^[1].

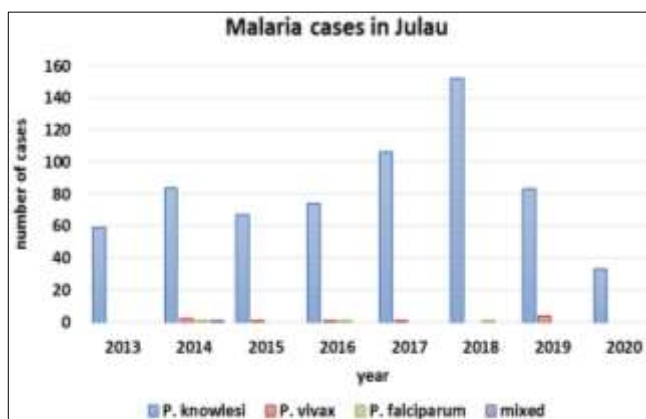


Fig 8: Malaria cases based on species reported in Julau from 2013-2020

4. Conclusion

This study provides the evidence that 30 mg/m² of deltamethrin K-Othrine® (PolyZone) sprayed on shaded wood and concrete surfaces works better than 25 mg/m² deltamethrin K-Othrine® WG 250 wettable granule currently use as the primary insecticides. 30 mg/m² of deltamethrin K-Othrine® (PolyZone) was retained longer (up to 67 weeks) on shaded surfaces and able to give rise to almost 60-100% of mosquito mortality. Therefore, deltamethrin K-Othrine® (PolyZone) at 30 mg/m² can be used as an alternative approach in combating malaria cases.

In conclusion, it may be best to apply deltamethrin K-Othrine® (PolyZone) at 30 mg/m² specifically on outdoor shaded wood or concrete surfaces. Interestingly this area is the area where most of the house occupants like to spend their time gathering, doing daily chores, eating, or just simply relaxing after working for long hours in the jungle. Based on complain made by house occupants, ORS should also be conducted on wall surface of the toilet to avoid massive mosquito attack while using the toilet.

In this study some difficulties were encountered which caused delays in completing the study such as (1) road condition at the sites. The condition of the road in most of the sites was somewhat bad making it hard to pass through especially during raining season to the point that some visits had to be

postponed. (2) Absence of house occupants. House owner was not available during the assessment period as most of them leave the house for work at early morning and only come home late in the evening. Therefore, more than one visit sometimes is required to assess one house.

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