A study of residual bio-efficacy of Inesfly 5AIGR and Inesfly 5AIGRNG insecticidal paints on Adult Anopheles gambiae complex mosquitoes on treated wall surfaces in Nigeria Communities: Masaka and Gidan-Zakara

Yako AB
Nasarawa State University
SC Hassan
Nasarawa State University
Olayinka MD
Nasarawa State University
I Sunday
Nasarawa State University
Asaga Mac Peter (pasaga123x@gmail.com)
INSTITUTE OF HUMAN VIROLOGY https://orcid.org/0000-0003-0038-8896

Research

Keywords: Bioassay cone test, Inesfly 5AIGR/5AIGRNG insecticidal paint, Anopheles gambiae mosquito

Posted Date: February 25th, 2020

DOI: https://doi.org/10.21203/rs.2.24523/v1

Abstract

Background

Since insecticides are commonly used in agriculture/cultivation and in vector control, they are frequently found in agricultural water bodies, where mosquito larvae are exposed. Although their concentration is often so low as not to kill the larvae, they affect the development of the mosquitoes. In particular, their effects may be transmitted to adults to influence their characteristics of life-history and their vectorial competence for arboviruses infection and malaria. Such effects on vectorial competence of sub-lethal doses of insecticides are likely to be related to their impact on immune response. Insecticide exposure influences the immune response of insects in several ways. Organophosphates and organochlorines affect the number of hemocytes (for phagocytosis) However, it is not known whether larvae exposure will impact the immune response of adults.

Method

Assessment of the bio-efficacy and residual activities of insecticides sprayed, wall surface using CDC wall cone bioassay test. Entomological indices with *Anopheles species* collection was measured to determined vectorial involvement and residual bio-efficacy of of Inesfly 5AIGR/ Inesfly 5AIGRNG insecticidal paints on Adult *Anopheles gambiae* complex mosquitoes on treated wall surfaces in at both communities (Masaka and Gidan Zakara) from January to April and May through June, 2018. A bio-assay cone test was carried out at various wall surfaces/height treated with above named insecticidal paints at parameters of 0.5meter, 1meter, and 1.5meter respectively.

Result

In both communities the knockdown/mortality threshold fluctuates (94%-99%.) indicating the state of resistance and susceptible, however, in the month of May and June at Gidan-Zakara, the knockdown/mortality of mosquitoes after 24hrs was 90%-100%.The seasonal abundance of mosquito population was generally observed to decrease between the month of January through April, 2018 and increases during the onset of rains in the months of April through June, 2018. There was no significance difference in the seasonal abundance of mosquitoes and the efficacy of Inesfly 5AIGR and Inesfly 5AIGRNG insecticide paints (F= 0.958> 0.435 and F 1.515>0.293. Similarly no significant difference in the residual efficacy and the malaria vector mortality (F=2.286>0.183) and in June (F1.549>0.287). The residual Inesfly paints were effective, given that 98%-100% malaria vector were susceptible.

Conclusion

The malaria vectorial competence of *Anopheles* species were 98% - 100% susceptible to residual ‘1NESFLY’ paints (5AIGR and 5AIGRNG) at various wall parameters of 0.5meters, 1meter and 1.5meters in the studied communities in central Nigetia. It is also imperative to state that the trial paints were in compliance with animals and human tolerance levels/standards during the during the trial periods, and fulfils the WHO criteria of insecticidal bio-efficacy (mortality >80%).

Background

Mosquito vectors ' immune system underlies their resistance to infections, and thus their capacity to spread pathogens to humans [1, 2]. Although their immune response intensity has a strong genetic component [3], it's also affected by the climate. The male *Anopheles gambiae* if undernourished as larvae, have a less successful melanisation response [4], correspondingly, environmental effects on the ability to transmit pathogens. The vectorial competence of mosquitoes is influenced by the bacterial microbiota the mosquitoes acquire as larvae [5], and that the susceptibility of *Aedes* mosquitoes to arboviruses and of *Anopheles* mosquitoes to malaria parasites depend on the temperature and food conditions during larval development[6,7,8]

The important aspect of the ecosystem is the use of insecticides. Since insecticides are commonly used in agriculture/cultivation and in vector control, they are frequently found in agricultural water bodies [9], where mosquito larvae are exposed. Although their concentration is often so low as not to kill the larvae, they affect the development of the mosquitoes. In particular, their effects may be transmitted to adults to influence their characteristics of life-history [10, 11, 12, 13] and their vector competence for arboviruses infection [14, 15] and malaria [16, 17].

Such effects on vectorial competence of sub-lethal doses of insecticides are likely to be related to their impact on immune response [18]. Insecticide exposure influences the immune response of insects in several ways [18]. Organophosphates and organochlorines [19] affect the number of hemocytes (for phagocytosis) However, it is not known whether larvae exposure will impact the immune response of adults [20].

Malaria remains one of the most important tropical diseases [21, 22]. It is estimated that 352 to 450 million people still lived in malaria endemic areas where there is little or no control of the disease [23]. Malaria has been eradicated from the USA, northern Australia, Israel, Cyrus and few other tropical Islands [24]. It was however, noticed in 1969 by the world health organization that the eradication was unrealistic [25].

The world health organization (WHO) declared that the main objective should be to control malaria and possibly reducing it to a point where it no longer constitute a major public health problem [26]. Malaria is widespread and common in sub-Sahara Africa. From the forgoing, it has become very imperative to implement control strategies that are effective in controlling the disease. In this study we evaluating the performance of wall cone bioassay code name Inesfly 5AIGR/ Inesfly 5AIGRNG insecticide paints for residual treatment against Anopheles mosquitoes infested (Malaria Endemic communities) areas in some selected local government area of Nasarawa State, Nigeria.

Methods

Study area

The trial of wall cone bioassay was carried out at two different locations, Masaka and Gidan Zakara communities of Keffi and Karu local government area of Nasarawa State in the north- central, Nigeria. Keffi is about 53km away from the Federal Capital Territory, Abuja and is located on longitude 8050' (South) and latitude 7050' (North) and is 630m above sea level.

Sampling of Houses

Ten (10) houses were randomly selected at Masaka and Gidan Zakara for the trials, the houses were painted with lnesfly 5AIGR (Chlorpyrifos 1.5%, dsiazinon 1.5% and Pyriproxyfen 0.063%) and lnesfly 5AIGRNG (Alphacypermethrin 0.7%, d-allethrin 1.0%, and Pyriproxyfen 0.063%) paints on the plastered walls. Pyrethrum spray collection (PSC) of mosquitoes was conducted randomly before the paints were applied at both locations for a period of six (6) months. The procedure was conducted in order to determine the residual efficacy of the insecticide embedded admixture against the wild mosquitoes.

Mosquitoes Larval Sourcing

Mosquito larvae were sampled out every three (3) days within a 6 months period at both locations [Masaka and Gidan Zakara]. *Anopheles gambiae* mosquitoes larvae were reared to adult stage in the insectary laboratory of Nasarawa State University, Keffi.

Two (2) to three (3) days old non-blood fed female mosquitoes were used in the field for the cone bioassay testing on the painted walls.

Procedure

The twenty (20) houses were randomly selected in each sentinel site, PSC method was applied as described by the WHO (1975) to sample indoor-resting mosquitoes. The houses were sampled by two people, one inside, and the other one outside the confinement of the room using an aero 01 insecticide (Raid) containing the active ingredients of 0.250% Allethrin, 0-150% tetramethrin, 0.015% deltamethrin, and 99.5 85% inert ingredients.

Two sprayers sprayed the room in opposite directions with all the doors closed for 15 minutes; the technicians later entered and collected the mosquitoes that were knocked down from on a white piece of cloth which was spread on the floor prior to spraying.

Mosquitoes were collected using a pair of forceps and they were then placed in petri dishes containing damp filter paper. The anopheles mosquitoes were then kept on an absorbent paper in a cool box. The mosquitoe species were later identified and counted according to (Gilles and Medellin, B. 1968, Gilles and Coetzee, 1987., Kent, 2006).

Residual Activity of Insecticide

The evaluation assessment of the bio-efficacy and residual activities of 'lnesfly' insecticide paint on the walls were carried out from January through June, 2018 to ascertain the mortality of the exposed wild

mosquitoes from the breeding sites within a time frame. The bio-efficacy as well as the decay rate of the insecticide paints on walls was measured using standard WHO cone test in the 20 randomly selected houses. The unpainted houses were used as controls. The cone test commences 24 hours post painting and it was carried out continually on a monthly bases for a period of six (6) months. The monitoring and evaluation of the insecticide paint residual efficacy was then determined.

Wall Cone Bioassay Test

Three cones were fixed using masking tapes on the interior walls at varied parameters, the lower point of 0.5m, middle point at 1.0m and the upper point at 1.5m. Three to five days-old unfed females of *An. gambiae* s.l. from the wild were used for the test as ten mosquitoes were gently transferred into each cone by an aspirator and exposed for 30 minutes and then observed for a period of 60 minutes. At the end of exposure time, the mosquitoes were transferred into insecticide free holding paper cups for further observation of 60 minutes and 24hrs holding period. The mosquitoes were fed with 10% sugar solution at the insectary laboratory.

The paper cups were kept in a cooling box and covered by damp towel to create favorable temperature and humidity in the insectary laboratory. Mortality was observed 24 hours post exposure and mosquitoes classified as dead if they are immobile or unable to stand or fly in coordinated manner or direction.

Results

Residual efficacy using cone bioassay test on Inesfly 5AIGR and Inesfly 5AIGRNG paints.

The residual efficacy of inesfly paints using wall cone bioassay was carried out to affirm the durability and efficacy of the paint applied to houses at Masaka and Gidan Zakara (Communities) both in keffi and Karu local government areas of Nasarawa state, Nigeria.

Monthly, the cone bioassay test were carried out on the wall parameters, mounted on the wall (0.5meters; 1.0meters; and 1 .5meters) respectively. At 0.5m 1.0m and 1 .5meters the cone bioassay test knockdown (KD) mortality status [For January] after 24hours showed a residual efficacy of 100% in the three households (Table 1).

In February through April, 2018, a 100% knockdown/mortality of mosquitoes after 24hours was comparably observed across same parameters heights. However in May, 2018, 0.5meters had 90% knockdown in household I, follow by household II (97%) mortality rate and household III 98% mortality rate after 24hours. In June, 0.5meters in household 1, showed a 90% knockdown while household II and household III showed a knockdown mortality of 97% and (96%) respectively. Household I and II at I.0meters showed a knockdown morality of 94% as against house III where 99% knockdown mortality of mosquitoes was chronicled. In a Similar manner, at 1.5meters a knockdown mortality rate of 98% was observed in household I and II, while household III showed a 93% mortality on the malaria vector (Table 1, Figure 1).

Table 1 Residual efficacy of inesfly paint using wall cone bioassay test from January to June 2018

Location	Months of cone Bioassay test	Cone Bioassay wall parameters	House hold numbers and percentage of mosquitoes knockdown/Mortality status after 24hours		
			Number one	Number two	Number three
Masaka	January	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	February	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	March	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	April	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	May	0.5m	90%	96%	94%
		1.0m	96%	90%	98%
		1.5m	98%	93%	98%
	June	0.5m	90%	97%	96%
		1.0m	94%	94%	99%
		1.5m	98%	93%	98%
-	-	-	-	-	-

Wall Cone Bioassay test in Gidan-Zakara

In order to measure transmission dynamics and entomological indices, *Anopheles* species samples collection were essential to determined vectorial capacity and residual efficacy at Gidan Zakara from January through April, 2018. Inesfly paints had a susceptibility mortality threshold of 100% at 0.5meters at house I while at house II 97% efficacy was observed against 100% susceptible at 1.0m in house II. Residual efficacy at house I and II was 96% and that of house III was 98%.

The result of the test from May through June, 2018 showed that at 0.5meters, 97% residual efficacy was observed in house I, 97% in house II and 100% in

III. At 1.0meters, 90% and 96% were observed in house I and III and 100% was recorded in house II. At I .5meters, 98% and 95% was observed in house I and III respectively and 100% residual efficacy was recorded in house II (Table 2, Figure 2).

Table 2 Residual efficacy of Inesfly paint using wall cone bioassay test from January to June, 2018, Nasarawa state university, Keffi.

Location	Months of cone Bioassay test	Cone Bioassay wall parameters	House hold numbers and percentage of mosquitoes knockdown/Mortality status after 24hours		
			Number one	Number two	Number three
Gidan Zakara	January	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	February	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	March	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	April	0.5m	100%	100%	100%
		1.0m	100%	100%	100%
		1.5m	100%	100%	100%
	May	0.5m	97%	97%	100%
		1.0m	90%	100%	99%
		1.5m	96%	98%	96%
	June	0.5m	96%	97%	100%
		1.0m	90%	100%	96%
		1.5m	98%	100%	95%

Discussion

Residual efficacy of Inesfly 5AIGR and Inesfly 5AIGRNG insecticide paint

The study investigated and evaluated the efficacy of insecticide paint using wall cone bioassay test from January through April, 2018 at Masaka and Gidan Zakara. The results showed a 100% efficacy of the Inesfly 5AIGR in reference to the wall parameter at 0.5m, l.0m, and 1.5m cone bioassay applied in houses sampled during the study period (Table 1 and Figure 1). *Anopheles gambiae* s.l were the most observed species as well as the most collected indoors compared to other species of mosquitoes this is in consonance with the results of Killeen *et al*., (2001) who reported similar cases of *An. gambiae* s.l that are typically endophagic, these trait appears to vary between locations. It was also observed during the study that *An. gambiaense* were more endophagic from the January periods through April (dry-season). This is in contrast to the wet season periods of May and June, 2018, where *An. gambiae* s.l. enter homes/houses in the early hours and exit in the morning hours. [Table 1]. The above findings shows the behavioral pattern of the female mosquitoes with respect to feeding activities that helps in laying of eggs and the rate of fecundity. The seasonal relative abundance of mosquitoes population generally showed a

decrease from January to April and a precipitous increase during the onset of rains from April to June, 2018. These could ascribed to extrinsic and intrinsic factors of malaria vectors, environmental factors and human activities which are interplay in the abundance and fecundity of the malaria vectors. There was statistical no significant difference in the seasonal abundance of mosquitoes and the efficacy of 5AIGR insecticide paint knockdown (F = 0.9580>0.435, df=2) and (F=1.5.57>0.293, df = 2).

At Gidan Zakara, a similar results were observed [Table 2 & figure 2]. From January through April, 2018 a 100% knockdown mortality were observed after 24hrs. The cone bioassay test ambient temperature and relative humidity were carefully observed to ensure that the bioassay agrees with the WHO standards. Survey and sourcing of mosquitoes larvae drastically reduced with changes in weather, in May through June, 2018 there was low abundance of larvae this may be attributed to rainfall which washed away most of the mosquitoe larva. Similar findings were observed by Githeko *et. al.,* [2001] who reported that climate change affect the development and abundance of malaria larvae and vector.

A 100% mortality of malaria vector was observed at parameters 0.5meters and 1meters respectively at House I and House II after a 24hrs bioassay test in June. This result does not negate that, *An. gambiae* s.1. will not develop resistance or become susceptible. This notion could be attributed to variation in strains therefore enabling resistance or susceptibility. There was however no significant difference in the efficacy of the residual Inesfly 5AIGRNG paint and the malaria vector mortality (F=2.2 86>0.183, df=2) and June (F=1 .549>0.287, df=2).

Conclusion

The malaria vectorial competence of *Anopheles* species were 98% - 100% susceptible to residual '1NESFLY' paints (5AIGR and 5AIGRNG) at various wall parameters of 0.5meters, 1meter and 1.5meters in the studied communities in central Nigetia. It is also imperative to state that the trial paints were in compliance with animals and human tolerance levels/standards during the during the trial periods, and fulfils the WHO criteria of insecticidal bio-efficacy (mortality >80%). The lack of malaria vector control and elimination(s) is recumbent in hands of various governments of the world as different vectors strains are emerging and re-emerging as a result of climate change. Human activities are contributing enormously to this change and if these trends are not scrutinized most especially in Sub-Saharan Africa, then the disputation and swedge against the control and elimination(s) of malaria will remain a mirage.

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of supporting data

Data is not restricted

Competing interests

The authors declare that they have no competing interests

Funding

CDC/PMI Grant No. 2345/pmi/2019

Authors' contributions

YAB: Design the study, collected the data and contributed to the analysis.

HSC: Design the study and contributed to the data analysis.

OMD: Contributed to data Collection and analysis.

SI: Contributed to data collection and analysis.

PAM: Wrote the manuscript and contributed to data analysis.

Acknowledgements

We are grateful to the insectary Laboratory Nasarawa State University, Keffi in collaboration with the PMI (US President Malaria Initiative) and the CDC fo. The Inesfly Corporation for financing the project and support from the Federal Ministry of Health (FMOH) G Abuja.

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Figures

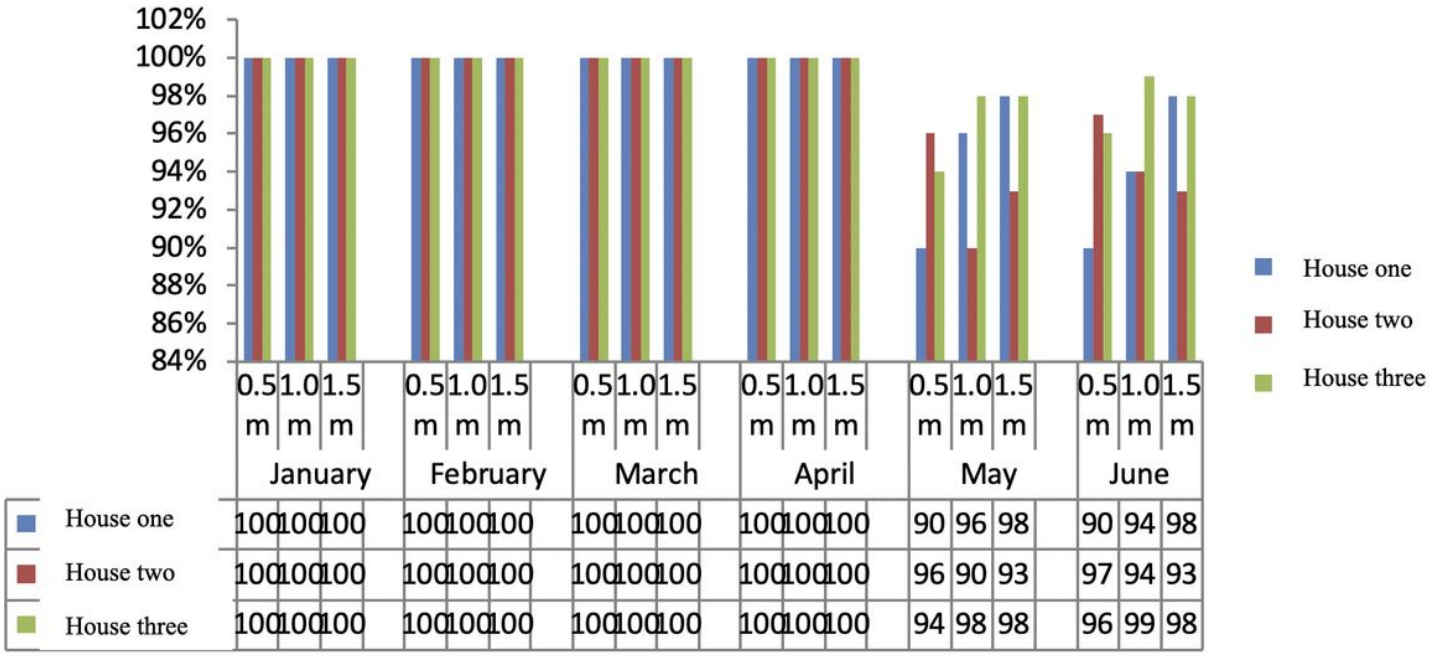
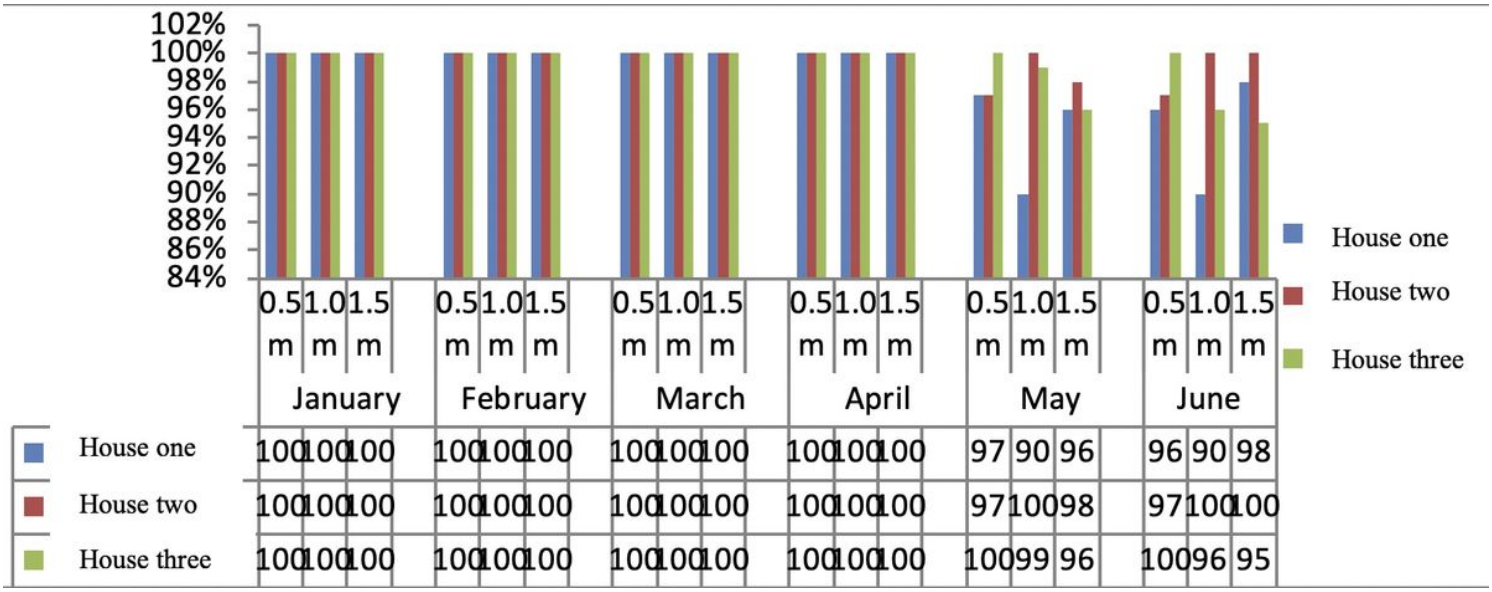


Figure 1

Estimated frequency of the susceptibility and Bio-efficacyof Inesfly 5IAGR &5IAGRNG insecticidal paints at Masaka community against malaria vector.



	January			February			March			April			May			June		
	0.5 m	1.0 m	1.5 m	0.5 m	1.0 m	1.5 m	0.5 m	1.0 m	1.5 m	0.5 m	1.0 m	1.5 m	0.5 m	1.0 m	1.5 m	0.5 m	1.0 m	1.5 m
House one	100	100	100	100	100	100	100	100	100	100	100	100	97	90	96	96	90	98
House two	100	100	100	100	100	100	100	100	100	100	100	100	97	100	98	97	100	100
House three	100	100	100	100	100	100	100	100	100	100	100	100	100	99	96	100	96	95

Figure 2

Estimated frequency of the susceptibility and residual bio-efficacy of Inesfly 5IAGR &5IAGRNG insecticidal paints at Gidan-Zakara community against the malaria vector.