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The Bio-efficacy of Inesfly vesta 50 (Transfluthrin 0.5% w/w) insecticidal paint: An alternative vector control intervention against *Anoheples gambiae* mosquitoes under laboratory condition, Keffi, Nigeria

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Abstract
Malaria remains a major public health concern. Particularly in Nigeria where more than 80% of the cases are reported and locally among natives in Nigeria. There are serious indication of insecticide resistance in Nigeria. Under PMI/Vectorlink laboratory condition, cement plaster surface and wooden board were brushed with a primer within 24-48hours before the paint application. The inesfly vesta 50 (transfluthrin 0.5% w/w) insecticide paint was applied at the dosage of $8m^2/L$ for cement and wooden board drying at room temperature for 5 days. The plastic cones were fixed on the treated walls and wooden board at 0.5m, 1.0m, and 1.5m parameters height, after which 10 non-blood fed females mosquito were gently introduced into the cone chamber for 30- 60 minutes observation. The holding paper cups were kept under favourable temperature of 27^{o}_{c} and humidity at 70%. In a dose trial finding, the data obtained were analyzed in IBM SPSS version 23.0 using ANOVA and the results presented as mean ± standard deviations of triplicate observations showed 100% yield (100.0±0.00*) mortality of malaria vector of novel trial with 0.5% w/w transfluthrin painted substrates in 24hours post exposure to 6months post application, except that, at l.5m in the month of July, 98% knockdown/mortality was recorded which follow same sequence of method and procedure in the application on the substrate absorbency, though, results showed high level of susceptibility from February to July (100% mortality rate). Results from wall cone and wooden board bio-assay residuality was alarming, with very high kill rates observed after 24hours post exposure. From the treated bio-assay of inesfly vesta 50 (Transfluthrin 0.5% w/w) by possible means showed that, this formulation is effective on malaria vector and possibly other insects. This suggests that, the transfluthrin 0.5% w/w can be tried alongside other known application with organophosphate, pyrethroids and the carbamate group, and can reduce human mosquito contact and impact on the perennial malaria transmission.

Keywords: Bio-efficacy, Inesfly vesta 50, transfluthrin, insecticide, paint

Introduction
Background
Malaria is still one of the most important public health concern in term of its morbidity and mortality, causing more than 200 million cases and 655,000 deaths every year [41]. Majorly, transmission events occur in the central highlands and along, international boarders where there has been substantial deforestation [37, 14, 36].

Radical approach to intensive pyrethroid based malaria vector control intervention program in the past decade in Africa according to Diabete (9), has led to rapid/rise to pyrethroid resistance in malaria vectors across Africa. Not only the wholistic approach, in addition to being geographically widespread, knockdown resistance (kdr) mutations have reached to extremely high frequency levels for *Anophels gambiae* throughout Africa [30, 32, 33, 34, 35].

Critical assessment with alternative to strategies, a novel trial of transfluthrin 0.5% w/w insecticidal paint was tried out as a new intervention towards integrated vector management to be used against malaria vector as it is known today that, new interventions towards integrated

vector management has been a challenge against adult mosquito [41]. Therefore, this novel trial will assess the impact of the Inesfly vesta 50 (Tansfluthrin 0.5% w.w) insecticidal paint against a high insecticide resistance.

Insecticide resistance is widely known among the pyrethroids vis-a-vis adult malaria vector proven highly resistance. This in other words poses a great dangerous threat to public health. To enhance further new-age strategies and intervention, Inesfly vesta 50 (Transfluthrin 0.5% w/w) is a new product introduced, which target *Anopheles gambiae* species at different stages of their life cycle. Both indoor and outdoor residual insecticidal paint (Transfluthrin 0.5% w/w) at a dosage of $8m^2/L$ should be a choice in preference to other intervention malaria vector control strategy as this brings to the treatment of resting sites for mosquitoes at both indoor and outdoor painted wall surfaces. The facts still remain that, the cheapest interventions are insecticide based, resistance to insecticide remain a formidable threat to vector control programs and in most glaring research program, control programs have to be evidence based. However, there are gaps in data gathering and management of insecticide resistance data in Nigeria which requires information on insecticide resistance to be harnessed for effective, timely and evidence based interventions.

Therefore, the use of insecticidal paints as an approach to pest and vector control based on the Inesfly paint technology, including microencapsulated insecticides are active ingredients embedded in the paint matrix and gradually released on the surface of the dried paints and so provide long -lasting insecticidal and or spatial repellent efficacy on mosquitoes and other pests.

Because of the big challenge to successful strategies and intervention, this calls for new alternative intervention control tools towards enhancing susceptibility [7]. Inesfly vesta 50 (Transfuluthrin 0.5.% w/w) has proven that is a long-lasting efficacy product against malaria vector.

Material

Study Site

Keffi, Nasarawa State Nigeria lies between latitude $7^0 45^0$ and $9^0 37^1$E. Nasarawa state, flanks up to the west. Keffi town is situated around the plain of undulating hills at the float, the central Plateau of century the relief ranges from about 277mm towards the north, to 430m to the South-East. The height, Keffi is about 30m with the lowest paint slightly less than 290m and highest point about 32m above sea level.

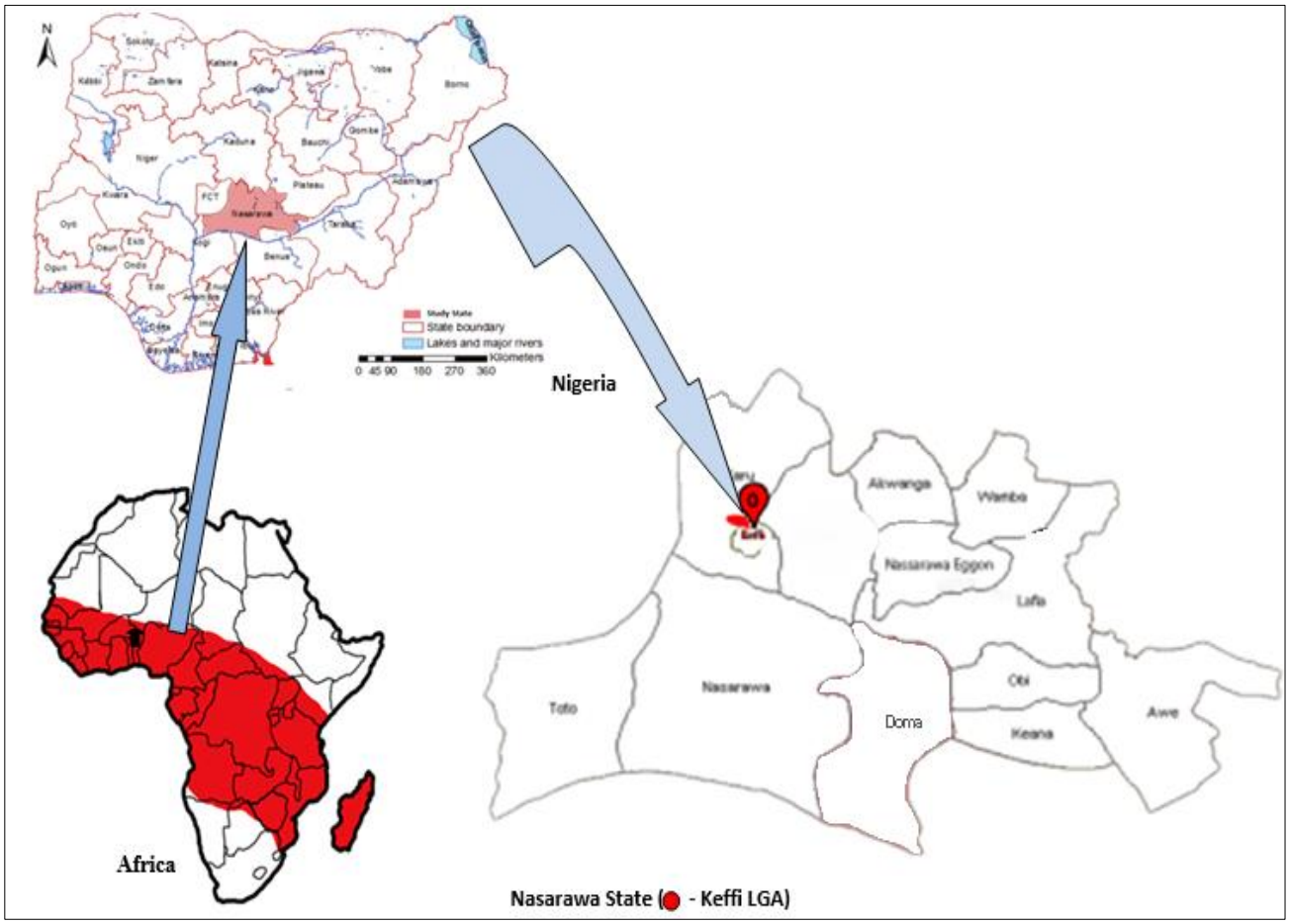


Fig 1: Map of Nasarawa State Showing Keffi LGA.(Local Government Area).

Biological Materials
Anopheles gambiae sensu latus from the field collected larvae reared in laboratory to first generation of adults were used (Plate 1 & 2).

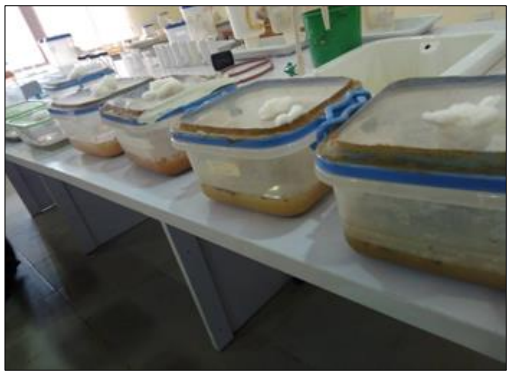


Plate 1: Collection of wild larvae (Bowl's)

Plate 2: Reared adults mosquitoes (Plastic cage)

Substrate
Cement plaster and wooden board were painted with a primer 24-48hours before the paint application. The primes was allowed to get dried within the given time frame before the insecticide paints were applied.

Dosage
$8m^2/L$ dosage was used for cement plaster surface and wooden board, brushing the surface in a single layer. Drying at room temperature for 5 consecutive days.

Formulation
Inesfly vesta 50 (Transfluthrin 0.5.% w/w) residual insecticidal paint was used for pilot trial.

Method
WHO wall cone bioassay test
The plastic cones were fixed using masking tapes on the treated walls and wooden board at varied wall cone-parameters, the lower point of 0.5m, middle point 1.0m and upper point 1.5m. Three to five days add of 10 non-blood fed females of mosquito were gently released into the cones chamber that is attached to the treated wall and wooden surface for 30 minutes which was observed up to a period of 60minutes.

1. After this time, mosquitoes were removed from the cone and placed into insecticide free holding paper cups for further observation of 60minutes and 24hours holding period with sucrose solution to prevent starving.
2. Control was used, the assay was performed with the same procedure but with the substrate painted with a regular paint, 3 as replicates.
3. The holding paper cups were kept in a cooling box with covered damp towel to create favourable temperature (27 ^{0}C) and humidity (70%).

Status of the female mosquitoes was registered at minute 30 (end of exposure), 60m from the beginning of the assay and 24hours after the beginning of the assay. The possible status was determined *viz*-a-vis;

Knockdown (*kd*) status
Insects present movility but without capacity to fly or land in a normal way.

Mortality
Insect in this case do not present movility to stimulus.

Control
Control assay was registered in the same manner.

Ethical Clearance
The Inesfly vesta 50 (transfluthrin 0.5% w/w) was approved (INESFLY CORPORATION) for trial in the Nigeria state (Nasarawa state), the research board review committee of the federal ministry of health (FMOH), Abuja, 2018 was sought.

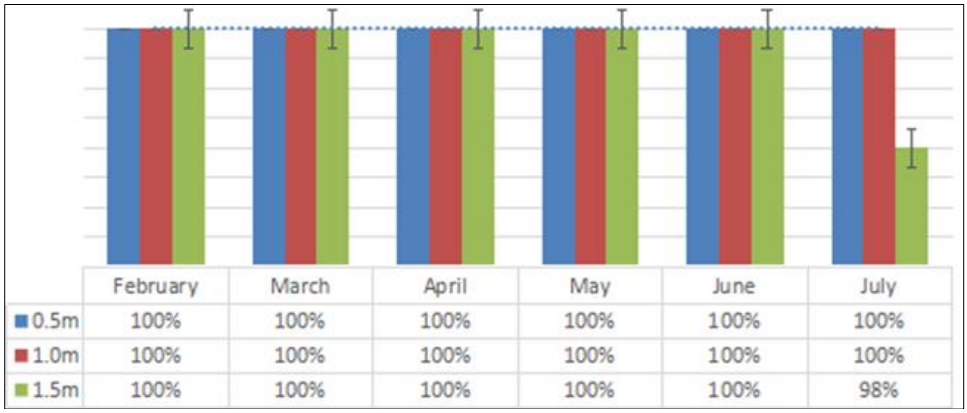
Statistical analysis
The data obtained were analyzed in IBM SPSS version 23.0 using ANOVA and the results presented as Mean ± standard deviations of triplicate observations. The level of significance was determined by comparing the percentage mortality to the total number of mosquitoes exposed to the insecticide.

Results
The detection of insecticide resistance in natural population or anopheles vector is absolutely necessary and a major prerequisite for implementation of an insecticide-based vector control operation. Resistance testing is one of the key fundamental components of malaria vector control.
In table 1, showed the trial of Inesfly vesta 50 (Transfluthrin 0.5% w/w) residual efficacy using the wall cone bio-assay from January to July, 2018 under laboratory condition, parameters heights varied with 0.5m, 1.0m, and 1.5m were considered to determine the state of absorbency unveiling the effect of transfluthrin 0.5% w/w on the painted substrates (Cement plaster and wooden board). For the *kdr*% mortality at different timings under observation, at 30m-1hr showed 70%Resistance and 90% suspected to be resistance. Lower in 30m with 60%Resistance and 94% above in 1hr when cone 2 bio-assay was used. Sixty-five (65% Resistance) relegate to the effect of residuality of the substance, against 96% that was suspected to be resistance at 1.5m height of the bio- assayed (30m-1hr timing of reading).
In March, it was observed at 30m-1hr post application, 55% - 60% was resistant at 0.5% parameter height. At 1.0m and 1.5m was an indicator at 70%Resistance and 90% (<90, >90-97%, WHO-Standard) suspected to be resistance. Though, in April at 1hr of 0.5m, 91% was recorded against 94%Suspected Resistance at 1.5m suspected kdr % mortality. Between May-July, the kdr % mortality limit was 73%Resistance (<90%, WHO-Standard) and the upper limit of susceptibility was 98%Susceptible (>98%-!00%, WHO-Standard) at 1.5m after 24hrs paper cup's holding. However, in all the cone bio-assay tested at varied parameter heights, after 24hrs post exposure to the residuality of transfluthrin

0.5% w/w susceptibility, was 100% with no dead of mosquito at the control when acetone was used alone.

The wall cone bioassay result obtained from February to July (6months) post exposure and application of the mosquito population *Anopheles gambiae* from the site of the novel trial of Transfluthrin 0.5.% w/w was highly susceptible after 24hours of paper cups holding. The control in three replicates, no knockdown/mortality observed during this period 24hours of post application. Field mosquitoes collected could not survive to Inesfly vesta 50 (Transfluthrin 0.5.% w/w) embedded paint residual and encapsulated on the prime cement plaster wall and the wooden board as it was the case (Table, 2).

Figure 2, showed *Anopheles gambiae* S.L. mosquitoes were exposed to prime cement plaster painted with a primer treated with Inesfly vesta 50 (Transfluthrin 0.5.% w/w) and 100% knockdown/mortality was obtained from the first month until the end of 6months experiment between the interval of 30minutes beginning to 60minutes and 24hours post exposure up to 6months post application except at 1.5m height, 98% knockdown/mortality was observed using transfluthrin based paint in July. The results observed significantly, high efficacy of the cones across all the months and time of the tests compared to the control with no evidence of mortality. Periodic comparison showed that there was significant efficacy (100.0±0.00*) within 24 hours of the test compared to other timings.



	February	March	April	May	June	July
0.5m	100%	100%	100%	100%	100%	100%
1.0m	100%	100%	100%	100%	100%	100%
1.5m	100%	100%	100%	100%	100%	98%

Fig 2: Estimated Frequency of Inesfly Vesta 50 (Transfluthrin 0.5%) insecticidal paints to determined susceptibility status of *An. ganbiae* s.1. using pyrethroids based paints (24hrs of paper cups holding).

Though, with increased in those at risk from malaria with indoor residual spraying (IRS) Inesfly vesta 50 insecticides have proven positive result with greater impact to public health, and decreasing in cases of malaria transmission.

Though, the increasingly insecticide resistant population of *Anopheles gambiae* s.1. mosquitoes in Nigeria could eventually degrade the tools currently used for vector control. Resistance to every currently used insecticide is been found as many factors are believed to increase vector prolific resistance though, it must be understood that, extensive use of the classes of insecticides by the Agricultural and public health sectors calls for a concern. For this imminent reason we conduct a core bio-assay trial and data anlysis to establish cases of resistance and the bio-efficacy of Inesfly vesta 50 (Transfuthrin 0.5% w/w) insecticidal paint (Appendix, 1).

Analysis of Residual efficacy of Ines fly vesta 50 (transfluthrin 0.5 w/w) paint using wall cone bio as test from February to July, 2018 at Nasarawa State University, Keffi, Nigeria.

Therefore, exploiting anopheles responses to insecticide bioassay and visual stimuli to improve surveillance and control of malaria is a major concern to accelerate action towards elimination of malaria cases by 2030. This calls for new alternative intervention control tools towards obtaining susceptibility and critical for decision making body for the elimination of malaria vector in Nigeria, and other parts of Africa.

Discussion and Conclusion

The inesfly vesta 50 (Transfluthrin 0.5.% w/w) insecticidal paint was found to be effective against mosquitoes and possibly other insects of medical importance. Though, chemical insecticide and pesticide control have greatly been in the past against mosquitoes nuisance [4] and otherwise today, widely used insecticide include majorly the Organochlorines, Organophosphate as well as the Pyrethroids. Under laboratory strain condition, complete knockdown/mortality of mosquito was observed using the diagnostic dose of transfluthrin 0.5.% w/w with high level of human tolerance though, the exposure levels and likely risk of pyrethroids base transfluthrin during applications yet still remain unknown. Similarly, long term exposure to pyrethroids base MRs indoor environment causes chronic neurotoxicity causing brain damage [32] and cholinergic dysfunction causing learning and memory deficiencies [33].

However, in this finding observation were carefully made as other mild side effects such as skin and eye irritation which reduces due to their relative low toxicity to man. Nonetheless, to the best of knowledge no comprehensive toxicokinetic study of transfluthrin has been reported. In other words, the availability of a sensitive analytical method for transfluthrin has been reported. Again, the availability of a sensitives analytical method allow the conduct of essential studies of its disposition in the body and its propensity to accumulate in mammals. This is because, the increasing popularity of transfluthrin in commercial household insecticide products has caused concern about risks of long term exposure, since both the exposure level and the duration of use of indoor products are significant [38, 11, 5].

Table 1: Residual efficacy of Inesfly vesta 50 (Transfluthrin 0.5 w/w) paint using wall cone bioassay test from February – July, 2018, Nasarawa state university, Keffi, Nigeria

	Kd (%)	February			March			April			May			June			July			control
		30 min	1hr	24 hrs	30min	1hr	24 hrs	30min	1hr	24hrs	30min	1hr	24 hrs	30min	1hr	24hrs	30min	1hr	24 hrs	24hrs
Wall cone Bioassay	Cone 1	0.5m	70	90	100	60	55	100	66	91	100	67	90	100	70	89	100	74	84	100	0
	Cone 2	1.0m	60	94	100	70	90	100	75	89	100	73	88	100	76	78	100	78	93	100	0
	Cone 3	1.5m	65	96	100	65	95	100	80	94	100	75	90	100	79	74	100	79	90	98	0

Table 2: Residual efficacy of Inesfly vesta 50 (Transfluthrin 0.5%) insecticide paint using wall cone bioassay test from February to July, 2018, Nasarawa State University, Keffi, Nigeria

	Months of Cone Bioassay test (24hrs)							Control		
	February		March	April	May	June	July	30m	1hr	24hr
Wall Cone Bioassay Parameters	Cone 1	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m
	Cone 2	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m
	Cone 3	1.5m	1.5m	1.5m	1.5m	1.5m	1.5m	1.5m	1.5m	1.5m
Cone bio-assay and % of mosquitoes knockdown/mortality status after 24 hours.	Cone 1	100%	100%	100%	100%	100%	100%	0%	0%	0%
	Cone 2	100%	100%	100%	100%	100%	100%	0%	0%	0%
	Cone 3	100%	100%	100%	100%	100%	100%	0%	0%	0%

Table 3: Analysis of Residual efficacy of Ines fly vesta 50 (transfluthrin 0.5 w/w) paint using wall cone bio as test from February to July, 2018 at Nasarawa State University, Keffi, Nigeria.

Materia		Month and time of test																	
		February			March			April			May			June			July		
	Parameter	30m	1hr	24hr	30m	1hr	24hr	30m	1hr	24hr	30m	1hr	24hr	30m	1hr	24hr	30m	1hr	24hr
Cone 1	0.5 m	70.0±0.0	90.0±0.0	100±0.0*	55.0±0.0	60.0±0.0	100±0.0*	66.0±0.0	91±0.0	100±0.0*	67.0±0.0	90.0±0.0	100.0±0.0*	70.0±0.0	89.0±0.0	100.0±0.0*	74.0±0.0	84.0±0.0	100.0±0.0*
Cone 2	1.0 m	60.0±0.0	94.0±0	100±0.0*	70.0±0.0	90.0±0.0	100±0.0*	75.0±0.0	89±0.0	100±0.0*	73.0±0.00	88.0±0.0	100.0±0.0*	76.0±0.0	78.0±0.0	100.0±0.0*	78.0±0.0	93.0±0.0	100.0±0.00*
Cone 3	1.5 m	65.0±0.0	96.0±0.0	100±0.0*	65.0±0.0	95.0±0.0	100±0.0*	80.0±0.0	94±0.0	100±0.0*	75.0±0.0	90.0±0.0	100.0±0.0*	74..0±0.0	79.0±0.0	100.0±0.0*	76.0±0.0	90.0±0.0	98.0±0.0*
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0

Appendix 1: Results are presented as Mean ± Standard deviation of triplicate observations. Mean value with * are considered statistically significant.

Here, the transfluthrin works as a potent dipteran sodium channel against and can elicit effects such as repellency, restlessness, knockdown and death in mosquitoes [18, 1, 11, 19, 3, 4]. The way forward is the choice alternative to the new product inesfly vesta 50 (Transfluthrin 0.5.% w/w) with low toxicity and of human tolerance be used to address the challenge of insecticide resistance generally.

The World Health Organization (WHO) gave an update on insecticide resistance test procedure to include resistance intensity assay [40]. Again, encourage a global plan for insecticides resistance management (GPIRM) [21, 38] to outlived series of strategies to combat resistance [23]. Though, this compound is a synthetic pyrethroids (transfluthrin) selected due to its volatile and relatively low toxicity to mammals [1, 3, 24]. Therefore, mosquitoes encountering these chemicals can be knockdown. In ortherwords mosquito can be killed depending on the exposure and chemical in question [4].

Recent field studies suggest this may be of limited importance for volatilized transfluthrin based approaches [14, 16]. Transfluthrin as a residual is being developed for use to control malaria vector populations that are poorly targeted by the current vector control tools and strategies [22, 21].

Multiple studies evaluating the impact of spatial repellents on mosquito behaviour and mortality have been conducted on various species of mosquitoes, including *Aedes agypti, A. albopictus, A. canadensis, A. vexans, culex quinquefasciatus* and several species of *Anopheles* mosquitoes [26, 30, 17, 19, 8, 9]. Though, indoor resting density (IRD) of Anopheles mosquitoes were investigated in some parts of Nigeria [31, 27, 28, 2, 29, 26, 25] to showcase the present study to investigate the IRD of female Anopheles mosquitoes to guide IRS intervention in a novel trial of inesfly vesta 50 (transfluthrin 0.5% w/w) to determine the residuality and its effect. Although in another relative view, indoor residual spraying with expensive alternatives to Pyrethroids is recommended for insecticide resistance management [38] and can improve malaria vector control impact, [42] it has proven too expensive to scale up [43].

However, this trial finding, have focused on a single mosquito species and were conducted on a prime cement plaster and wooden board with no other mosquito population tested under a laboratory strain condition.

This is critical to understand the effectiveness of inesfly vesta 50 transfuluthrin 0.5.% w/w though, with significant kill rate among the *Anopheles gambiae* population exposed from this result, this study demonstrate that, inesfly transfluthrin 0.5.% w/w becomes effective knockdown of *Anopheles* species and here suggest future studies which should examine the efficiency and subsequent effectiveness of these tools work in field settings to further attest a wider range of susceptibility. Again, agree if new products are to be adopted fast enough to delay the emergence of insecticide resistance [44], it is essential that review and recommendation policies take a bolder stance to emphasise pre-emptive action.

Competing Interests

The authors declared that, they have no competing interests.

Authors Contributions

1. 'Yako AB' with the collaborators proposed the study and contributed to the setting-up of the Research approach, drafting and written of the manuscript.
2. 'Hassan SC' and 'Olayinka MD' coordinate the study and implement research of the laboratory settings and data analysis.
3. 'Igboanugo SI' contributed to the research at the laboratory site and monitoring the *kd* after 24hours of paper cup's holding post exposure.
4. All authors read and accept the final copy of the manuscripts and no conflicting interest.

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