Research Article
Volume 12:5, 2023

Malaria Control & Elimination

ISSN: 2470-6965 Open Access

Testing the Bio-efficacy of Inesfly Carbapaint 10 (propoxur 1.0% w/w): Household Insecticide Repellent Paint against Anopheles Mosquitoes in Keffi, Nasarawa State, Nigeria

Yako Andrew Bmibmitawuza[1,2*], Hassan Suleiman Chuntah[1,2] Olayinka Michael Daniel[2] and Igboanugo Sunday Iwunor[2]

[1]*Department of Zoology, Nasarawa State University, Keffi, Nigeria*
[2]*USAID/Abt/PMI/Vectorlink Insectary/Laboratory, Nasarawa State University, Keffi, Nigeria*

Abstract

The increasing insecticide resistant population of *A. gambiae* S.l. mosquitoes in Nigeria and most of West Africa and other parts of the sub-Saharan regions of Africa is becoming worrisome and currently, it's becoming a threat to the tools widely used for vector control. Though, resistance developed faster in most areas where pyrethroids previously, has been tried alone than those places tried with other earlier applications such as Organophosphates, Organochlorine and the Carbamates group inclusive. The higher coverage of Indoor Residual Spraying (IRS) is suggestive of effective susceptibility in vector species of Anopheles tried in Nasarawa state, Nigeria using Inesfly Carbapaint 10 (Propoxur 1.0 w/w) insecticidal paint. The wall cone bio-assay used, a primer was employed to prime a cemented plaster surface and wooden board under laboratory condition within the 24–48 hours before the paint application. The primer was allowed to get dried up within a given time frame before the Inesfly Carbapaint 10 (Propoxur 1.0% w/w) insecticidal paint was applied at the dosage of 8 m2/L for cement plaster surface and wooden board drying at room temperature for 5 consecutive days. The wall-cone bio-assay and the varied wall cemented plaster surface parameters of 0.5 ml, 1.0 m and 1.5 m and wooden board were both treated with 1.0% propoxur painted substrates in 24 hours post exposure to validate 6 months post application. The bio-efficacy and durability of indoor residual treatment with propoxur 1.0% w/w insecticidal paint from February to July, 2018 (6 months) were shaded on the primer substrate. The wild *A. gambiae* mosquitoes after 24 hours of observation, showed 100% knockdown/mortality at varied cone-bioassay wall parameters height. Monthly distribution gave susceptibility result treatment with low toxicity effect and the results presented as Mean ± standard deviations of triplicate observations (100.00 ± 0.00). From the tried result under laboratory strain condition, with increased in those risk from malaria with Indoor Residual Spraying (IRS) admixed microencapsulated insecticidal paint have proven positive result activities and broad spectrum against insects and agricultural pests with greater hope to public health and decreasing in cases of malaria transmission. Indeed, the carbamates based insecticides is on the most preferred in the fight against malaria vector as such, should further paired or synergize with other affluent pyrethroids based to intensify were possible, high level of susceptibility, knockdown, hoping that new-age additional classes of insecticides (Pyrrole and Neonicotinoid) will become available for the control of adult mosquito.

Keywords: Bio-efficacy • Carba-paint 10 • Propoxur 1.0% w/w • Anopheles mosquitoes • Insecticide repellent

Introduction

Interestingly, malaria remains the most important scourge that has affected millions of persons in the world, malaria in order to achieve human and animal health and other social and economic targets, human needs to control pests [1] and still, people live in highly malaria areas where malaria is no longer a major public health problem for a country, this still remains the general philosophy towards malaria and insecticides, the principal component of the integrated management approach for the control of vector borne diseases [2]. In sub-Saharan Africa is a widespread and common today as it ever was though, the high scale up of the interventions in the last 20years has been associated with major reductions in diseases burden [3].

***Address for Correspondence:** Yako Andrew Bmibmitawuza, Department of Zoology, Nasarawa State University, Keffi, Nigeria, E-mail: 66yakoa@gmail.com*

Received: 01 September, 2023, Manuscript No. mcce-23-114634; **Editor Assigned:** 04 September, 2023, PreQC No. P-114634; **Reviewed:** 18 September, 2023, QC No. Q-114634; **Revised:** 23 September, 2023, Manuscript No. R-114634; **Published:** 30 September, 2023, DOI: 10.37421/2470-6965.2023.12.229

It is been puts, insecticide resistance in Africa which threatens, the largest genetic study of mosquitoes is been found, their ability to resist insecticides which is evolving rapidly and spreading across Africa, putting millions of people at higher risk of contracting malaria which account for high share of global malaria burden with 93% of cases and 94% deaths [4]. Currently, pyrethroids are the only class of insecticides approved for use against vector control intervention due to their high efficacy, excito-repellent properties and low toxicity to mammals [5,6].

Kate Kellan London Renters, the increasingly insecticide resistant population *A. gambiae* S.l. mosquitoes in Nigeria could eventually degrade the tools currently widely used for vector control intervention. Resistance to every currently used insecticides 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 Agricultural and Public Health sectors calls for a concern [7,8]. Though, it has been suggested that irrigated agriculture and crop spraying has subjected mosquito vectors to selection in the larval stages, especially with pyrethroids [9,10]. Hence, the resistance to the pyrethroids indeed has been suggested that pyrethroids should not be used for indoor residual spraying particularly in the major African malaria vector *A. gambiae* S.l. [11], though, from the knowledge, organophosphate used for indoor residual spraying entirely belong to different class of insecticide.

For this imminent reason we conduct a core bio-assay trial to establish cases of resistance and bio-efficacy of Inesfly Carbapaint 10 (Propoxux 1.0% w/w) insecticidal paint. Though, the pyrethroids offer several advantages over other insecticides in term of cost, safety (less toxic to mammals with less effect to humans) and duration of residual action. Unfortunately, resistance to pyrethroids in *A. gambiae* S.l. and *A. funestus* has emerged and it is spreading rapidly [12]. Hence, insecticide resistance does undermine the effects of malaria vector control and that tackling it with insecticides or insecticide combinations to which local vectors remain susceptible to [13]. Fortunately, Inesfly Carbapaint 10 (Propoxux 1.0% w/w) have proven to be effective against local malaria vector, the control measures of mosquitoes transmitted diseases rely on contained mosquito susceptibility to various insecticides and its discovery, chemical insecticides have presented the most widely method used to control mosquito borne vectors [14,15].

Materials

Study site

Nasarawa State University, Keffi lies between 7045I and 90371E. Keffi LGA of Nasarawa State, Nigeria, share boundaries with Kaduna State in the North, while Plateau State in the far East, Taraba and Benue States in the South, with Kogi State and Federal Capital City Abuja. Flank up to the west, Keffi town is situated around the plain of undulating hills at the float the central Plateau of the century, the relief ranges from about 277 m towards the north, to 430 m to the South East. The height of Keffi is about 30 m with lowest point slightly less than 290 m and the highest point above 32 m sea level (Figure 1).

Biological materials

gambiae sensu lato from field collected larvae were reared in laboratory to first generation of adults and other accessory tools (Figure 2).

Substrate

Cemented plaster surface and wooden board was primed with zicli screeding paint within 24-48 hours before the Inesfly Carbapaint application. The primes were allowed to perfectly get dry within the given time frame before the insecticidal paints were applied.

Dosage

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

Formulation

Inesfly Carbapaint 10 (Propoxux 1.0% w/w) insecticidal paint (Inesfly Corporation S.L., Paiporta, Spain).

Methodology

WHO wall cone bio-assay test

The plastic cones were fixed using masking tapes on the treated walls and wooden board at varied wall parameters height (The lower point 0.5 m, middle point 1.0 m and upper point 1.5 m). Three to five days old of 10 non-blood fed females mosquito were gently released into the cone chamber that is attached to the treated wall and wooden surface for 30minutes which was observed up to a period of 60minutes. After this time, mosquitoes were removed from the cone and placed into insecticide free holding paper cups for further observation of 60 minutes and 24 hours holding period with sucrose solution to prevent starving.

Control was used, the assay was performed with the same procedure but with the substrate painted with a regular zicli screeding paint, three (3) as replicates. The holding paper cups were kept in a cooling box with covered damp towel to create favourable temperature (27 °C) and humidity (70%).

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

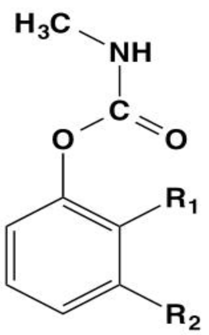
Knockdown; insect present movility but without capacity to fly or land in a normal way.

Mortality; insect in this case do not present movility to stimulous. Control assay was registered in the same manner.

Results

The high coverage of Indoor Residual Spraying (IRS) is suggested of effective susceptibility in vector species of Anopheles tried using Inesfly Carbapaint insecticide, interestingly, experimental carbamates possessed a significant reduced toxic rate. Though, as it is been reported from previous studies in Africa, it is been observed that, resistance developed faster in places where pyrethorids has been used alone than in those tried alongside mosaic application with Organophosphate, Pyrethroids, Organochlorine and the Carbamates group.

Here, Inesfly Carbapaint 10 (Propoxux 1.0% w.w.) paint showed from the mortality percentage (%) of mosquitoes knockdown after 24 hours post exposure present 100% mortality for the period under observation and for subsequent months. This evaluation were succinctly determined, using wall cone bio-assay parameter to yield 100% at 0.5 m, 1.0 m and 1.5 m susceptibility. Substrate



Compound	R_1	R_2
1	H	*tert*-Butyl
2	H	$Si(Me)_2Et$
3	$SCH_2CMe{=}CH_2$	H
4	SCH_2CHEt_2	H
5	OCH_2CHEt_2	H

Figure 1. Structures of experimental methylcarbamates used in this study.

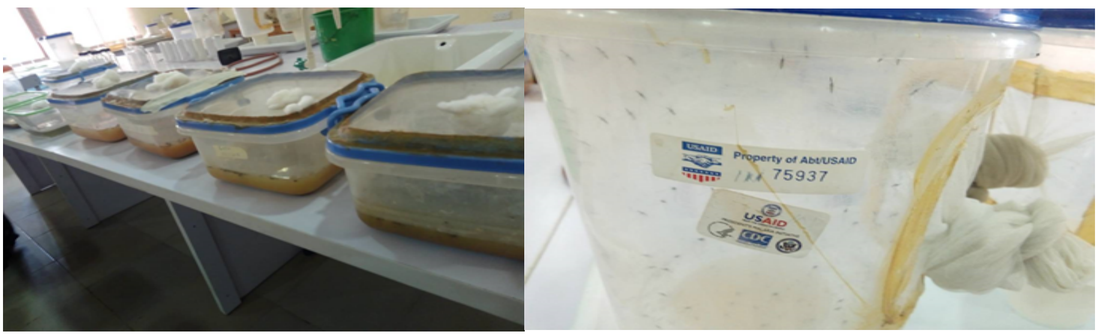


Figure 2. Collection of wild larvae (bowl's) and reared adults mosquitoes (plastic cage).

absorbency showed high level of susceptibility, 60 minutes from the beginning of the assay, with very high kill rates been observed 24 hours after the beginning of the assay (Table 1 and Figure 3).

Propoxur was shown to possess relatively high selective toxicity for *A. gambiae*, despite intensive selection in the laboratory of the wild strain of *A. gambiae* S.I., resistance ratio was difficult to obtain. The carba-paint insecticide substrate absorbency indicate differential level of tolerance of *A. gambiae* S.I. based on the wall parameters (0.5 m, 1.0 m and 1.5 m) height. In contrast, resistance does not impact much on the species of Anopheles mosquito under laboratory condition. This indicate sound knowledge of the mechanism of resistance which should be understood because, the outcome of this findings should allow impact on vector control strategies *viz*; high susceptibility substrate absorbency with Carbpaint 10 insecticide.

As shown in Table 2, the residual efficacy of Inesfly carbapaint 10 (propoxur 1.0 w/w) insecticide using wall cone bio-assay test from February to July was significantly higher in all the test cones when compared to the control. There was 100 percent efficacy within 24 hours of the test (Table 2 and Figure 4).

Discussion

This data on mosquito susceptibility could guide the implementation of adapted policy and strategy to control malaria resistance in Nigeria by high profile use of Inesfly Carbapaint 10 (Propoxux 1.0% w/w) would be terms of reference to policy decision making. However, Talisuna AO, et al. [16] reported the limited data on mosquito behaviour in southern Ugandan reference to policy body.

In similar trend, Hartsel JA, et al. [17] prepared an experimental carbamate propoxur and purified by column chromatography and/or re-crystallization and are >95% pure by 1H NMR analysis which was compared to commercial carbamate propoxur 99% purity to determine the activity of these experimental carbamates to other mosquito disease vectors and agricultural pests, in an effort to further

Table 1. Residual efficacy of inesfly carbapaint 10 (propoxur 1.0%) insecticides paint using wall cone bioassay test from february to july, 2018, Nasarawa State University, Keffi, Nigeria.

		Months of Cone Bioassay test						Control		
		February	March	April	May	June	July	30m	1hr	24hrs
Wall Cone Bioassay Parameters	Cone 1	0.5 m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m	0.5m
	Cone 2	1.0 m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m	1.0m
	Cone 3	1.5 m	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%

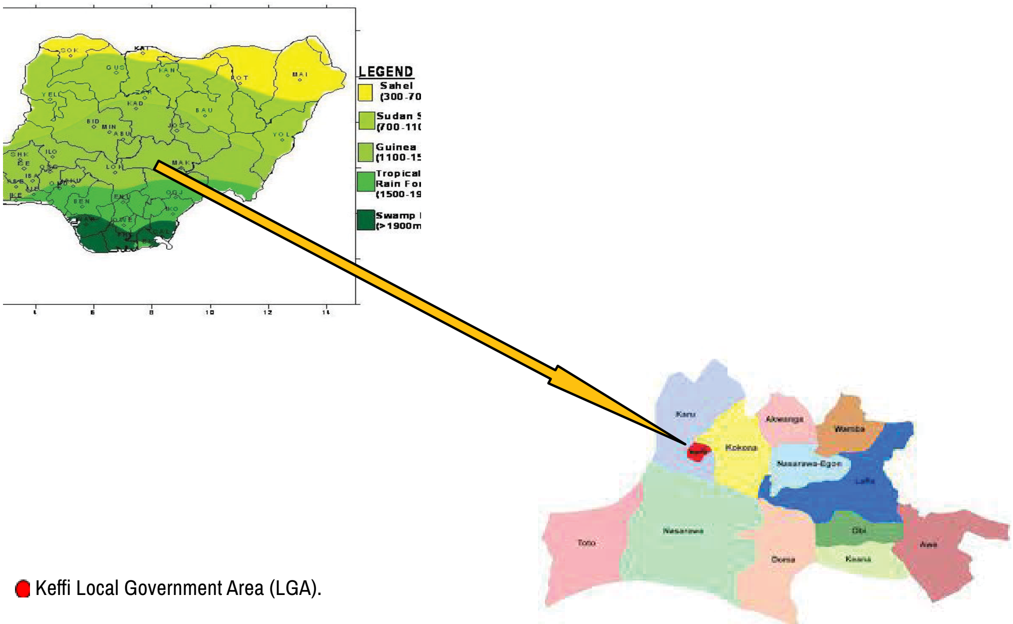
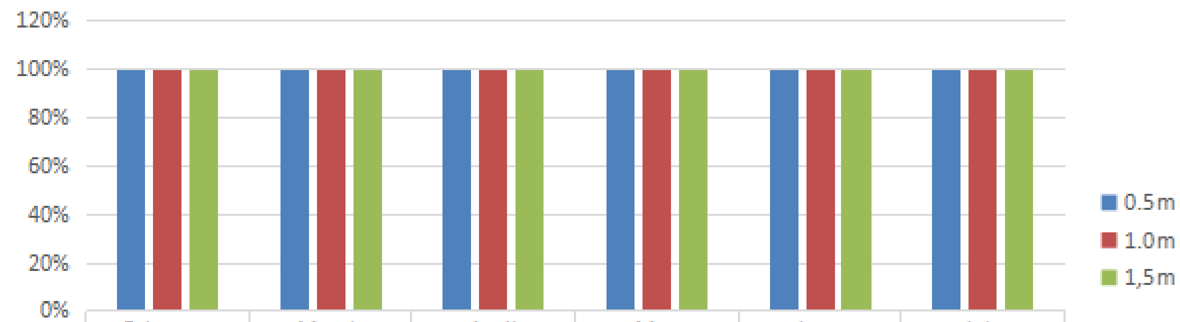


Figure 3. Map of Nigeria showing geographical zones and Nasarawa state.

Table 2. Residual efficacy of ines fly vesta 10 (propoxur 1.0 w/w) insecticide paint using wall cone bio as test from february to july, 2018 at Nasarawa State University, Keffi, Nigeria.

		Month and Time of Test																	
		February			March			April			May			June			July		
Material used	Param-eter	30 min	1 Hr	24 Hrs	30 min	1 Hr	24 Hrs	30 min	1 Hr	24 Hrs	30 min	1 Hr	24 Hrs	30 min	1 Hr	24 Hrs	30 min	1 Hr	24 Hrs
Cone 1	0.5 m	80.0 ± 0.0 100.0 ± 0.0*	99.0 ± 0.0	100 ± 0.0*	90.0 ± 0.0	100.0 ± 0.0	100 ± 0.0*	89.0 ± 0.0	98 ± 0.0	100 ± 0.0*	90.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0*	99.0 ± 0.0	100.0 ± 0.0*	100.0 ± 0.0*	94.0 ± 0.0	99.0 ± 0.0	100.0 ± 0.0*
Cone 2	1.0 m	75.0 ± 0.0 100.0 ± 0.00*	89.0 ± 0.0	100 ± 0.0*	80.0 ± 0.0	85.0 ± 0.0	100 ± 0.0*	0.0 ± 0.0	96 ± 0.0	100 ± 0.0*	88.0 ± 0.00	94.0 ± 0.0	100.0 ± 0.0*	98.0 ± 0.0	100.0 ± 0.0*	100.0 ± 0.0*	90.0 ± 0.0	96.0 ± 0.0	100.0 ± 0.00*
Cone 3 (%)	1.5 m	67.0 ± 0.0 100.0 ± 0.0*	80.0 ± 0.0	100 ± 0.0*	74.0 ± 0.0	89.0 ± 0.0	100 ± 0.0*	84.0 ± 0.0	98 ± 0.0	100 ± 0.0*	78.0 ± 0.0	90.0 ± 0.0	100.0 ± 0.0*	90.0 ± 0.0	97.0 ± 0.0	100.0 ± 0.0*	98.0 ± 0.0	100.0 ± 0.0*	100.0 ± 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

Results are presented as Mean ± Standard deviation of triplicate observations. Mean values with * are considered statistically significant (100 percent) mortality



	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%	100%

Figure 4. Estimated frequency of inesfly carbapaint 10 (propoxur 1.0%) insecticidal paint to determined susceptibility status of *A. gambiae sensu lato* using carbamates based paints.

explore species selectivity and to evaluate any advantageous properties for resistance management and present structures of the experimental carbamates propoxur for its proven and broad spectrum insecticidal activities;

All the mosquito species studied were sensitive to the experimental carbamates, found to be toxic at low nanogram dose, which suggests could be useful for controlling a variety of mosquito borne diseases.

This is why it is been proven that, with the huge measure up in vector control and fast emergence for insecticides resistance majorly of the pyrethroids generally prevailing all over Nigeria and consequently too the use of Agriculture and past use of pesticides activate to developed resistance, though, most insecticides used in agriculture are of the same chemical classes and have the same targets and models of action as those used for vector control. General studies suggested that the use of insecticides on agricultural lands contributed to the selection of resistance in mosquitoes, threatening the efficacy of vector control programmes in all the study sites of Nasarawa State. Although, indoor residual spraying with expensive alternatives to pyrethroids is recommended for insecticide resistance management and can improve malaria vector control impact. It has proven too expensive to scale up [18].

Chemical insecticides and pesticide control have greatly been in the past against mosquitoes nuisance and indeed the widely use of insecticides which include the organochlorines, organophosphate and pyrethroids, this led to an increase rate insecticide resistance of Anopheles mosquito in Africa and resistance which have spread across different regions [19,20]. Skeptically, the use of carbamates remains meager against mosquitoes [21,22]. Though, these chemicals were found very effective against mosquitoes but regular and over use of these insecticides according to Wolansky MJ and Tornero-Velez R [23]. remain harmful for the environment and for non target organisms within the atmospheric creeping land ecosystem including man therefore, the Carbapaint 10 formulation with a low level of toxicity to impact on man is been tried and tested although, the general use of insecticides for agricultural pests, human vector and livestock diseases has make insecticides resistance difficult in the control of malaria [24]. Recent work suggests that the environment is drastically modified when insects become resistant to insecticides [25,26].

As Carbapaint 10 serves an alternative vector control intervention strategy, the increase in report of pyrethroids resistance in a large scale, the notion on the metabolic-based resistance and targets site mutation of strain in Anopheles is been neglected or given the little attention, in similar preview and analysis as the case may be [27] considered the operational impact of metabolic based to have been overlooked. Though, the use of insecticidal paints as an approach to pest and vector control based on the Inesfly paint technology, including micro-encapsulated insecticides are active ingredients embedded in the paint matrix and gradually released on the surface of the dried paints provide long-lasting insecticidal and or spatial repellent efficacy on pests [28].

However, increased coverage of those at risk from malaria with Indoor Residual Spraying (IRS) admixed insecticide micro-encapsulated have proven susceptible with enormous impact to public health thereby decreasing cases of malaria transmission against the well-known establish pyrethroids with increase state of resistance in Nigeria and most other parts of Africa. Essentially, the introduction of IRS should be well hernex despite cost intensiveness though, it might be suggestive that, the carbamate class should synergise with the pyrethroids at a very low level of toxicity to alternate the intensification of the impact on resistance of Anopheles mosquitoes, needless the resistance on the use of pyrethroids however, is a hope that additional classes of insecticides will become available for the control of adult mosquito.

Conclusion

This research indeed open new alternative approach to vector control intervention of *A. gambiae* sensu latoThe elevated levels of P450 together with GST likely to be responsible for high pyrethroid resistance (Awolola, et al.), this is a useful information to ascertain mechanism which is develop of the Inesfly Carbapaint 10 (propoxux 1.0% w/w), for its active susceptibility. Currently, vector control interventions depend heavily on pyrethroid insecticides and here, suggests the new-age introduced insecticide such as chlorfenapy and clothianidine be added or synergised with Inesfly Carbapaint 10 to fight against malaria vectors.

Declaration

Ethical approval

The Inesfly Carbapaint 10 (Propoxur 1.0% w/w) was approved for trial in the Nigeria state (Nasarawa state), the research board review committee of the federal ministry of health (FMOH), Abuja, 2018 was sought.

Consent for publication

NA.

Availability of data and materials

All data are contained within the manuscript.

Data 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 (Mean value of 100% mortality (100.00 ± 0.00).

Competing Interests

The authors declared that, they have no competing interests.

Funding

Inesfly Corporation S.L., Paiporta, Spain.

Authors Contributions

YAB: With the collaborators, proposed the study and contributed to the setting-up of the Research approach, drafting and written of the manuscript.

HSC and OMD: Coordinate the study and implement research at the laboratory settings and data analysis.

ISI: Contributed to the research at the laboratory site and monitoring the kd after 24 hours of paper cups holdings post exposure.

All authors read and accept the final copy of the manuscripts and no conflicting interest.

Acknowledgment

We thank all the technical crew of the Entomology Laboratory /Insectary for their time and contribution despite stress. We also appreciate immensely, the Inesfly Corporation S.L. for their tremendous support to the research, working tools and financing the project.

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How to cite this article: Bmibmitawuza, Yako Andrew, Hassan Suleiman Chuntah, Olayinka Michael Daniel and Igboanugo Sunday Iwunor. "Testing the Bio-efficacy of Inesfly Carbapaint 10 (propoxur 1.0% w/w): Household Insecticide Repellent Paint against Anopheles Mosquitoes in Keffi, Nasarawa State, Nigeria." *Malar Contr Elimination* 12 (2023): 229.