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Efficacy of Chlorfenapyr and Clothianidin Insecticides against Permethrin Resistant *Anopheles gambiae* s.l. in Gidan Yaro Village, Sokoto, Nigeria

[1]Yahaya Muhammad Abdullahi, [2]Sani Abdullahi Fana and [1]Usman Salisu Batagarawa
[1]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

Background and Objective: The continued spread of insecticide resistance is becoming a threat to effective vector control programmes. Chlorfenapyr and clothianidin with distinct modes of action have been included in vector control programmes. This work aims to assess the susceptibility status of *Anopheles gambiae* s.l. to chlorfenapyr and clothianidin. **Materials and Methods:** Chlorfenapyr and clothianidin were tested, using CDC bottle bioassay and modified WHO susceptibility tests, respectively, against *Anopheles gambiae* s.l. collected from Gidan Yaro Village. Cross-resistance to permethrin, alphacypermethrin, bendiocarb and pirimiphos-methyl was assessed in the same mosquito populations using CDC bottle bioassays. **Results:** Complete mosquito mortality was observed using the recommended diagnostic doses for chlorfenapyr (100 µg/bottle) and clothianidin (2%/filter paper). The laboratory-reared and wild-captured mosquitoes were resistant to permethrin with mortality ranging from 62-82% and survived 2× and 5× diagnostic doses. The field population of *An. gambiae* s.l. was significantly more susceptible to clothianidin, reaching 100% mortality by day 3 compared to the laboratory strain which recorded 100% mortality by day 4 post-exposure. In contrast, the wild population was less susceptible to chlorfenapyr, with the highest mortality of 99% at 72 hrs using 100 µg/bottle compared to the laboratory colony, which recorded complete mortality at 100 µg/bottle by 24 hrs. **Conclusion:** The slow mode of action of the clothianidin and chlorfenapyr and the absence of cross-resistance make them appropriate for inclusion in the Malaria Control Programme particularly in this part of the country where malaria incidence is high.

KEYWORDS

Anopheles gambiae s.l., permethrin, chlorfenapyr, clothianidin, resistance, susceptibility

INTRODUCTION

Human beings have been struggling against malaria for countless generations. However, malaria remains a major global problem of public health concerns. Within the last two decades, remarkable achievements have been made in the fight against malaria which has led to a decrease in morbidity and mortality. These

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achievements are largely due to the rapid scale-up of diagnosis, treatment and vector control interventions, using indoor residual spraying (IRS) in tandem with Long-Lasting Insecticidal Nets (LLINs)[1]. Unfortunately, the adoption of IRS and LLIN as a primary form of malaria vector control intervention has placed high levels of selection pressure on *Anopheles* mosquito populations leading to the evolution of resistance to the four main classes of insecticides approved by WHO for public health use.

The increase in insecticide resistance among malaria vectors has become a serious challenge to effective malaria control. Major malaria vectors in Africa developed resistance to pyrethroids[2-3]. Resistance has now spread to every country in Sub-Saharan Africa[4]. This threat led to the development of new insecticides with novel modes of action against the malaria vector. Reports have shown that insecticides to be used in IRS and LLINs should have chemical and physical properties that facilitate effective uptake upon contact, long residual efficacy, specificity to mosquito species easily applicable, stability with low volatility and mammalian toxicity[1].

Chlorfenapyr is a slow-action insecticide belonging to the pyrrole group and was reported to be one of the best ovicidal agents that provide effective control against a variety of insects and mites by inhibiting oxidative phosphorylation[5]. It has a unique mode of action and has so far shown no evidence of cross resistance[6]. It was also reported to interrupt the synthesis of ATP by disrupting the proton gradient across mitochondrial membranes, ultimately resulting in the death of the organism[7]. Along with alphacypermethrin, it was incorporated into the long-lasting net (interceptor) recommended by WHO[6].

Clothianidin is one of seven insecticides within the neonicotinoid which is chemically similar to nicotine[6]. It has been reported to be used primarily against piercing-sucking insects of major crops and it has a low mammalian toxicity[8]. The basic mode of action is to target the Nicotinic Acetylcholine Receptor (nAChR) in the insect central nervous system[9]. Compared with chlorfenapyr, this class of insecticide has been through less rigorous study concerning vector control. At a molecular level, each neonicotinoid has been characterized by differential activity against the nAChR protein subunit of *An. gambiae*, suggesting that these compounds may have differential efficacies against target insects[9].

The study aimed to determine the susceptibility of permethrin, alphacypermethrin, pirimiphos-methyl, bendiocarb, chlorfenapyr and clothianidin, against *An. gambiae* s.l. collected from Gidan Yaro Village. This will provide a critical starting point to monitor future resistance and define the suitability of these insecticides for intervention deployment.

In addition, an improved understanding of the cross-resistance between these novel chemicals and currently used insecticides will aid the NMEP and other stakeholders in making informed choices regarding the most appropriate tools for malaria vector control and insecticide resistance management.

MATERIALS AND METHODS

Study area: The study was carried out in Gidan Yaro Village of Wamakko, Local Government Area, Sokoto State between June and September, 2020. Gidan Yaro is located at longitude 13°7'16.72"N and latitude 5°11'40.78"E. The people of the area are mostly farmers and a few fishermen. The horticultural activities include millet, guinea corn, beans and rice cultivation.

Mosquito collection and processing: Mosquito larvae and pupae were collected from stagnant water bodies using standard dippers (350 mL). The collected sample in a white plastic container was brought to the malaria Entomology Laboratory of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The larvae were fed with yeast tablets grounded with biscuits and reared to adults. Pupae were placed in separate containers. All collections were done between 8:00 and 10:00 hrs. The

emerging laboratory-reared (LR) adult mosquitoes were transferred into cages and fed with a 10% sugar solution from cotton wool. The indoor mosquitoes termed wild mosquitoes (WD) were obtained by aspiration using a prokopack aspirator and only unfed mosquitoes were used. They have acclimatised 24 hrs and sugar-fed before usage. The adult holding room temperature for all the mosquitoes was 28±2°C and relative humidity of 70-80%.

CDC bioassays: CDC bottle bioassays were conducted according to published guidelines[10]. Briefly, each Wheaton 250 mL bottle was coated with 1 mL of insecticide solution by rolling and inverting the bottles. In contrast, the two control bottles were coated with 1 mL of acetone following which all bottles were covered with a blanket sheet and left to dry in the dark. Twenty-five sugar-fed, 3-5 days old female *An. gambiae* s.l. were aspirated and introduced into each of the coated bottles. Mosquitoes were exposed to permethrin, alphacypermethrin, pirimiphos-methyl and bendiocarb for 30 min and chlorfenapyr (100 µg) for 60 min and at 10 min intervals, knock-down was recorded respectively. Following exposure, mosquitoes were transferred to a paper cup covered with an untreated net, provided with lightly moistened cotton wool containing 10% sugar solution and monitored at 24 hrs, for both the two insecticides. Mortality records are however extended to 48 and 72 hrs for chlorfenapyr post-exposure.

Clothianidin bioassays: Clothianidin was tested using WHO susceptibility tests, with minor modifications to the standard guidelines. Whatman No. 1 filter papers measuring 12 cm by 15 cm were treated with a diagnostic dose of clothianidin diluted in distilled water. A stock solution was prepared by diluting 264 mg in 20 mL distilled water. Two millilitres of the prepared solution were pipette and distributed evenly onto each filter paper and allowed to air dry. The filter paper was then wrapped with aluminium foil and stored at 4°C until use. Filter paper treated with 2 mL of distilled water was used as the negative control. Exposure time for clothianidin was set at 60 min. A total of 100 each of the laboratory strain and wild *An. gambiae* s.l. (3-5 days old) in 4 batches of 25 mosquitoes, each was tested. Additional 2 replicates of 25 mosquitoes were used as control. Following exposure, mosquitoes were transferred to holding tubes and provided with lightly moistened cotton wool containing 10% sugar solution. Knock-down was recorded at 15, 30 and 60 min. Mortality was recorded 1-7 days after exposure.

Mosquito identification: All the tested mosquitoes were identified as species based on morphological characteristics as described[11].

Data analysis: The results of the bioassays were interpreted based on the overall percent mortality after the period of exposure to each insecticide across all four exposed replicates expressed as a percentage of the total number of exposed mosquitoes. Susceptibility interpretation was done based on the WHO recommended criteria where 98-100% mortality indicates susceptibility, 90-97% mortality suggests possible resistance and required further confirmation and <90% mortality indicates the presence of resistance[12].

RESULTS

The susceptibility status of each of the insecticides tested against *An.gambiae* s.l. is shown in Fig. 1-3. It is clear from Fig. 1 that both wild-captured and laboratory-reared mosquitoes were resistant to permethrin with a mortality of 62 and 46% respectively. Alphacypermethrin, though a pyrethroid recorded full susceptibility with 100% mortality across the board. Pirimiphos-methyl and bendiocarb also resulted in complete mortality. Since permethrin happens to be resistant to the mosquito tested intensity assay was conducted. However, exposure of the same population of mosquitoes to 2× and 5× diagnostic doses resulted in the mortality effect of 78-86 and 69-81% of laboratory-reared and wild or field-collected mosquitoes, respectively. Thus exposed mosquitoes still survive these diagnostic doses exhibiting resistance to the insecticide (Fig. 1).

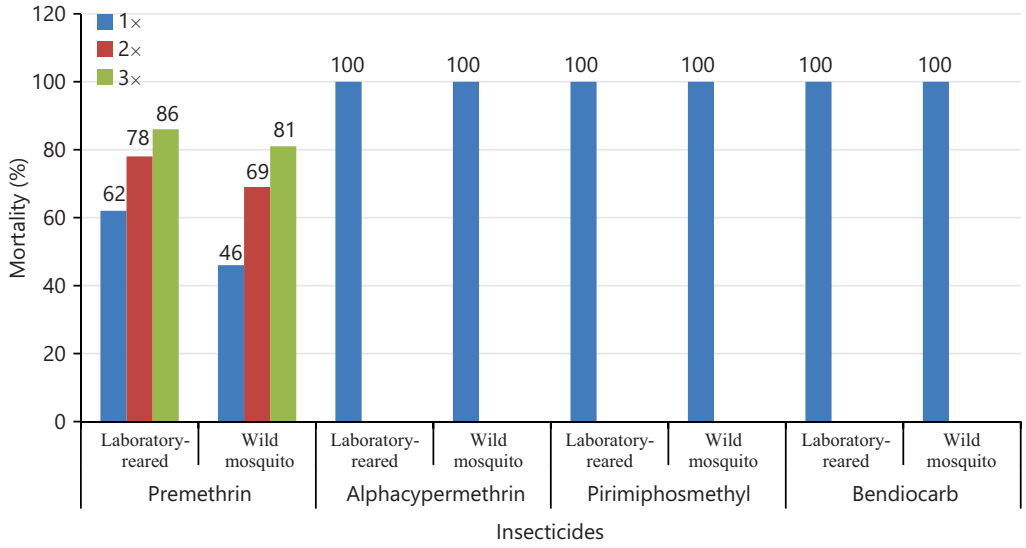

Fig. 1: Percent mortality results after exposure to different diagnostic doses

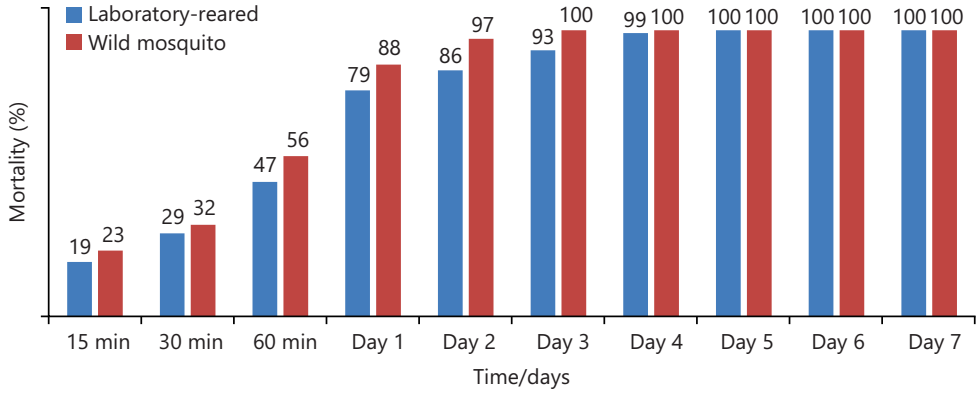

Fig. 2: Mortality effect of clothianidin on exposed mosquitoes

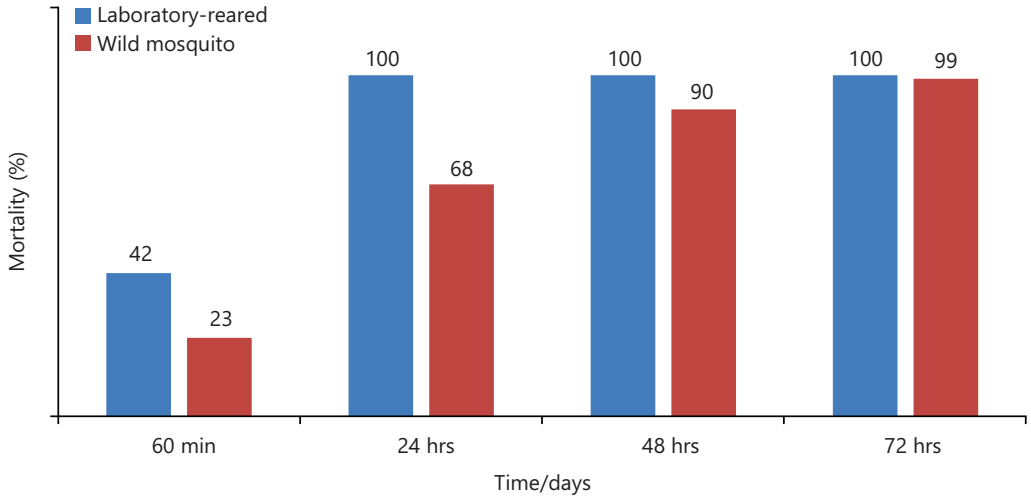

Fig. 3: Percent mortality of *Anopheles* exposed to chlorfenapyr

The knockdown rate for both LR and WM against clothianidin is shown in Fig. 2. It is clear from the table that the knockdown rates of 47 and 56% were obtained against the laboratory-reared and wild mosquitoes respectively. Thus mortality effect was higher in wild mosquitoes after 60 min of exposure. However, 24 hrs after mortality increases with 78 and 88% mortality in both the laboratory and wild

mosquitoes, respectively. Mortality rate increases with continued exposure to mosquitoes resulting in full susceptibility with 100% mortality recorded against the wild mosquitoes on the 3rd day post-exposure. Laboratory strain also shows susceptibility with 99% mortality 4th day after exposure. However, 100% mortality was reached on the 5th day after exposure. Wild mosquitoes are more susceptible to clothianidin than laboratory-reared mosquitoes.

The number of knocked-down mosquitoes after 60 min of exposure to chlorfenapyr was 42 and 23% on LR and WM mosquitoes, respectively (Fig. 3). This indicates that the knockdown effect was more on laboratory-reared mosquitoes than the wild type. However, 24 hrs after exposure the mortality rate increases resulting in full susceptibility with 100% mortality against laboratory-reared mosquitoes. The wild mosquitoes recorded 68% mortality indicating resistance. However, since chlorfenapyr is a slow-action insecticide, exposure continued for 72 hrs when 99% of the wild mosquitoes were found dead suggesting the susceptibility nature of the insecticide.

DISCUSSION

This study determined the susceptibility status of permethrin, alphacypermethrin (pyrethroids), pirimiphos-methyl (organophosphate), bendiocarb (carbamate), clothianidin (neonicotinoid) and chlorfenapyr (pyrrole) against both the laboratory-reared and field-collected *An. gambiae* s.l. in Gidan Yaro Village. Mortality rates of 62-86% obtained on mosquitoes exposed to permethrin revealed the resistance status of both the laboratory-reared and field-collected mosquitoes. Mosquitoes that were exposed to the diagnostic doses of alphacypermethrin, pirimiphos-methyl and bendiocarb resulted in full susceptibility with 100% mortality each. The resistance recorded is not surprising as various reports have shown resistance of *An. gambiae* s.l. not only to pyrethroid but also to other insecticides used in public health[13-15]. Reports have also shown that resistance to permethrin was recorded in all the geographical zones[13,16].

The ability of the exposed mosquitoes to survive the discriminating doses of permethrin 2× and 5× agreed with reported cases of resistance intensity reported when mosquitoes were exposed to 2×, 5× and 10× diagnostic doses[1,15]. The result further confirmed resistance to deltamethrin, a pyrethroid when *An. gambiae* was exposed to WHO-impregnated papers[17]. The susceptibility to alphacypermethrin recorded contradicted[17] but confirmed the susceptibility status to bendiocarb and pirimiphosmethyl[1,17].

Diagnostic doses for chlorfenapyr at 100 µg/bottle and clothianidin at 2%/filter paper against the laboratory-reared *An. gambiae* s.l. achieved full susceptibility by 24 and 96 hrs post-exposure, respectively. In contrast, however, the wild mosquitoes achieved full susceptibility 72 hrs after exposure to both chlorfenapyr and clothianidin. Similar observations were earlier made by Dagg *et al.*[1] who reported complete mosquito mortality using clothianidin and chlorfenapyr. The finding was not far from the 98% susceptibility against *An. gambiae* exposed to clothianidin[18]. The ability of these insecticides to show susceptibility to the permethrin resistance mosquitoes is an indication of the potential of the duo in fighting malaria vectors. This earlier corroborated the report that chlorfenapyr has the potential to prevent malaria transmission in pyrethroid-resistant areas better than pyrethroid[6,7].

The time taken to reach complete mortality is an indication of the slow nature of the chlorfenapyr which is believed to impose less selection pressure for resistance and may be able to reduce malaria transmission[6]. Since the mode of action of both chlorfenapyr and clothianidin differs from that of other insecticides there is minimal risk for cross-resistance to evolve[19]. Ngufor *et al.*[7] reported that chlorfenapyr has the potential to prevent malaria transmission in pyrethroid-resistant areas better than pyrethroids. Trials in Tanzania and Benin have highlighted the effectiveness of chlorfenapyr as an adjunct to pyrethroid-treated nets against a variety of vectors[20,21] and as a candidate for IRS in Benin[22]. Complete mosquito mortality with the laboratory strain using the recommended diagnostic doses for clothianidin (2%/filter paper) and chlorfenapyr (100 µg/bottle) was also reported[1].

Exposure to clothianidin initially shows a very low level of activity which increases with continued exposure giving varying levels of mortality. Clothianidin is one of seven insecticides with low mammalian toxicity and is primarily used against piercing-sucking insects of major crops[9]. The basic mode of action is to target the Nicotinic Acetylcholine Receptor (nAChR) in the insect central nervous system[9]. Mortality rates recorded with clothianidin whether alone or in the mixture demonstrated a delayed effect lasting up to 5 days[21]. The findings of this work further confirmed the delayed effect with complete mortality after 4-5 days of exposure.

Several factors might have played a role in insecticide resistance. The inhabitants are engaged in farming activities therefore, there is the continuous and indiscriminate use of agricultural chemicals. These activities might expose the mosquitoes or their larvae to different dosages of the chemicals thereby enhancing the development of resistance. Widespread use of insecticides in both agricultural and public health sectors contributed to the development of insecticide resistance[23,24]. This was believed to be the main driving force behind the development of resistance[25]. Insecticide selection pressure from agriculture is believed to be an important source of insecticide resistance in malaria vectors[26]. There are also reported cases of IRS and ITNs leading to insecticide resistance which has been demonstrated to reduce the effectiveness of the IRS and ITNs[27,28].

Because pyrethroids are being commonly used, larvae of *An. gambiae* s.l. might be exposed to selection pressure in their breeding sites which will enhance their resistant status. Thus, the development of resistance by *An. Gambiae* s.l. in these places should not be a surprising thing as continuous contact with substrates treated with insecticides will enhance resistance development against such insecticides.

CONCLUSION

The results also show that insecticide resistance in the malaria vector population is increasing therefore serious attention needs to be given to restore insecticide susceptibility and help put the menace of Malaria and the vector to a halt. Further studies on the effects of these two insecticides are desirable as the detailed investigation may reveal more information on the nature susceptibility or otherwise of the malaria vectors. The findings may also guide the agency for the control of malaria in the area for proper planning and implementation of their control programmes.

SIGNIFICANCE STATEMENT

This study discovered that insecticide resistance to permethrin is becoming widespread in the area. The results also revealed the susceptibility nature of the other insecticides tested. The findings of this study will serve as a basis for a detailed investigation of the nature and mechanisms of insecticide resistance in the area.

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