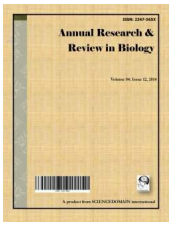

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Pyretheroids Resistance and Detoxifying Enzymes Activities of Malaria Vector (*Anopheles gambiae*) Breeding in Auyo Irrigation and Residential Sites, Jigawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author MS participated in sample collection, specie identification, designed of the study and WHO bioassay. Author AJA managed the biochemical and data analysis. Author AAI interpreted the results and critically reviewed the manuscript. Author HA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to evaluate the resistance status and detoxification enzymes activities of important malaria vector (*Anopheles gambae*) to WHO recommended pyretheroids insecticides in a highly malaria endemic country like Nigeria.
Study Design: Mosquitoes larvae collected from Auyo residential (AR), and Auyo irrigation (AI) sites were reared to adults and adult *gambae* were specifically exposed to Permethrin and Deltamethrin. The insecticides resistant and susceptible mosquitoes of AR and AI were respectively redistributed as ARr, ARs, AIr, and AIs.
Place and Duration of Study: Residential site (AR) and Rice irrigation sites (AI) of Auyo town, in Auyo LGA Jigawa State Nigeria, between July and October, 2014.
Methodology: Pyretheroids resistance status was studied using WHO adult mosquito

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bioassay protocols. Specific activities of insecticides detoxifying enzymes; GST, esterase and monooxygenase of the resistant and susceptible vectors were determined using standard methods.

Results: The results of the study established high resistant status of malaria vectors to both insecticides tested based on WHO interpretation (< 90% mortality). Significant elevated activities ($P<0.05$) of GST, esterase and lower activity of monooxygenase was recorded in permethrin resistant strain compared to susceptible strain of Auyo irrigation sites. Also a significant higher ($P<0.05$) activities of GST, esterase and monooxygenase was established in Deltamethrin resistant strain of both AR and AI, except for esterase in AR.

Conclusion: The findings of the study established resistance in both residential and irrigation sites, which could be associated to indiscriminate use of insecticides in residential sites against malarial vector and other flying insects as well as agrochemicals in the irrigation sites. Based on this finding it may be concluded that selection pressure that confers resistance to malarial vector is not restricted to agricultural activities alone.

Keywords: Pyretheroids resistance; bioassay; malarial vector; detoxification enzymes.

1. INTRODUCTION

Nigeria bears up to 25% of total malaria burden in Africa, hence contributing significantly to the one million lives lost per year in the region, which mostly consist of children and pregnant women in addition to its negative impact on nation economy. The disease accounts for annual loss of 132 billion naira, as payment for treatment and prevention as well as idle hours of unproductivity [1]. Increasing incidence of malaria transmission in urban and peri urban areas may not be unconnected with farming practices [2,3]. Farming activities provides favorable breeding environment for the vectors and agrochemical spray serves as a source of selection pressure that trigger the emergence of insecticides resistant vectors. This have been documented to have significant impact on malaria spread [4,5]. Increasing activities of detoxification enzymes have been reported to account for insecticides resistance through metabolizing them before reaching their target sites of action or as a result of reduced target site sensitivity of pyretheroids binding site sodium ion channel [6] and carbamate binding site acetylcholinesterase [7]. Increased activity of esterase is associated with amplification of corresponding structural gene [8]. GSTs, a multigenic family `of dimeric proteins are important in metabolism of organochlorine and organophosphate [9]. Many works reported increased GSTs activity in crude supernatant of insecticides resistant insects, which suggested the possible roles of the enzyme in conferring resistance [10,11]. Resistant insects usually show very high activity of esterase [12,13] for the ability of the enzyme to hydrolyses ester linkage in organophosphates, carbamates and pyretheroids [14]. This work aimed at evaluating the pyretheroids susceptibility or otherwise and level of detoxifying enzymes in *Anopheles gambae* collected from Auyo residential and irrigation sites.

2. MATERIALS AND METHODS

2.1 Materials

All reagents used are of analytical grade obtained from BDH, spectrafuge by Labnet 24d and micro plate reader by Nortek Genesis – MR 6000 were used for the study.

2.2 Study Area

The study area is predominantly rice cultivation site in Auyo Local Government Area of Jigawa State, Nigeria. The town lies between latitude 12^0 21'' 36' N and longitude 9^0 59'' 8' E, situated in northeastern part of the state, bordered in the east by Hadejia, west by Kafin Hausa and Bauchi State and with a shared boarder in the north east with Malammadori Local Government. It has a total land mass of about 740 square kilometer mainly made of Sudan savannah. The inhabitants are mostly farmers and traders. Common trade and occupation include fishing, rice farming and establishment of irrigation based activities.

2.3 Larval Collection and Rearing

The larvae collected from different points in both residential sites (AR) and agricultural sites (AI) in Auyo were reared to adult.

2.4 WHO Bioassay

Mosquitoes insecticides diagnostic kit was used to establish susceptibility and resistant status using 0.05% deltamethrin and 0.75% permethrin

impregnated paper according to WHO procedure [15]. For each insecticide, adult mosquitoes were divided into batches of 20 – 25 per test (four replicate) and exposed to insecticides treated paper for 1hr. the effect of paper treated only with carrier oils were assayed in parallel as control. The knock down rate was recorded at every 10 minutes for 1 hour before they were transferred back to the resting tubes for 24 hours when percentage mortality was recorded. Mortality rate between 98 -100% indicate full susceptibility, 80 -97% indicate possible resistance and less than 80% is considered resistant to the tested insecticides. The resistant and susceptible mosquitoes were separately placed as ARr, ARs, AIr and AIs in labelled effendof tubes and stored at -80℃ for subsequent enzyme assay.

2.5 Enzyme Analyses

Individual mosquitoes were analyzed for protein, esterase, GST and monooxygenase. The mosquitoes were individually homogenized using glass rod in 150 µl ice cold distilled water and homogenate was centrifuged at 13000 g for two minutes.

2.5.1 Esterase assay

Esterase was determined by spectrophotometric method described by Faiz et al. [16]. The enzyme hydrolyses paranitrophenylacetate to acetate and a yellow colour product paranitrophenol was formed. A quantity of ten microliter of each homogenate was mixed with 200 µl of 1 mM paranitrophenyl acetate working solution (100 mM paranitrophenyl acetate: 50 mM sodium phosphate buffer pH 7.4, 1:99) in a microtitre plate well. The absorbance was read at 405 nm after ten minutes incubation. An extinction coefficient of 6.53 $mM^{-}cm$ and a path length of 0.6 cm was used to convert the absorbance to moles of product. Esterase specific activity was reported as µmolproduct/ min/ mgprotein.

2.5.2 GST assay

Glutathione S transferase (GST) was determined following the method described by Habig et al. [17]. The enzyme catalyses the conjugation of glutathione and chloro 2,4 dinitrobenzene to form 2- chloro-4-nitrophenyl glutathione. A quantity of ten microliter of each homogenate was mixed with 200 µl reduced glutathione (GSH/l-chloro -2,4 dinitrobenzene working solution {95 parts of 10 mM reduced glutathione in 100 mM phosphate buffer pH 6.5 + 5 parts of 63 mM chloro-2,4 dinitrobenzene diluted in methanol} in a microtitre plate well. The absorbance was read at 340 nm after 10 minutes incubation. An extinction coefficient 5.76 $mM^{-}cm$ and a path length of 0.6 cm was used to convert absorbance to moles of product. Gst specific activity was reported as CDNB conjugated µmole product min- mg- protein.

2.5.3 Monooxygenase (Cytochrome P450) assay

This was measured by the method of Borgdon [18]. The monooxygenase catalyses the reduction of hydrogen peroxide and oxidation of tetramethylbenzedine to form water and oxidized blue color tetramethylbenzidine. Twenty microliter of homogenate was mixed with 80 µl of potassium phosphate buffer pH 7.2 +200 µl of 6mM tetramethylbenzidene (TMBZ) working solution {(0.01 g TMBZ was dissolved in 5 ml methanol and then in 15 ml of sodium acetate buffer pH 5.0) +25 µl of 3% v/v H_2O_2 solution} in a microtitre plate well. After two hours incubation at room temperature, the absorbance was read at 630 nm. By using a standard curve of cytochrome C, a crude estimate of the amount of monooxygenase present was obtained and expressed as equivalent units of cytochrome P450/mg protein.

2.6 Statistical Analysis

Enzyme activity were subjected to statistical test (P=0.05) to determine statistical differences and deviation using ANOVA.

3. RESULTS AND DISCUSSION

The results of the study established high resistant status of malaria vectors to both insecticides tested based on WHO interpretation (< 90% mortality). Significant elevated activities (P<0.05) of GST, esterase and lower activity of monooxygenase was recorded in permethrin resistant strain compared to susceptible strain of Auyo irrigation sites. Also a significant higher (P<0.05) activities of GST, esterase and monooxygenase was established in Deltamethrin resistant strain of both AR and AI, except for esterase in AR.

3.1 Results

Figs. 1 and 2 depict one hour knocked down rate per 10 mins exposure to insecticides

impregnated papers of anopheles mosquitoes collected from auyo residential and irrigation sites. The percentage mortality to both insecticides ranges from 20% and 38% irrespective of the sites. Tables 1 and 2 show the specific activities of detoxifying enzymes of anopheles mosquito (resistant and susceptible) of Auyo residential and irrigation sites respectively exposed to permethrin and deltamethrin.

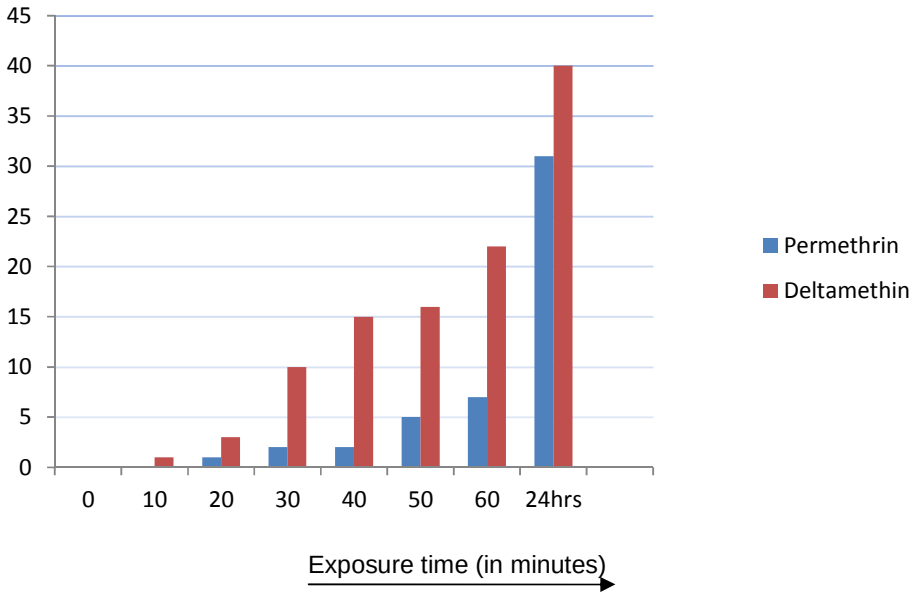

Fig. 1. % knock down (10-60 mins) and % mortality (24 hrs) of *Anopheles* mosquitoes bioassay to permethrin 0.75% and deltamethrin 0.05% collected from Auyo residential site

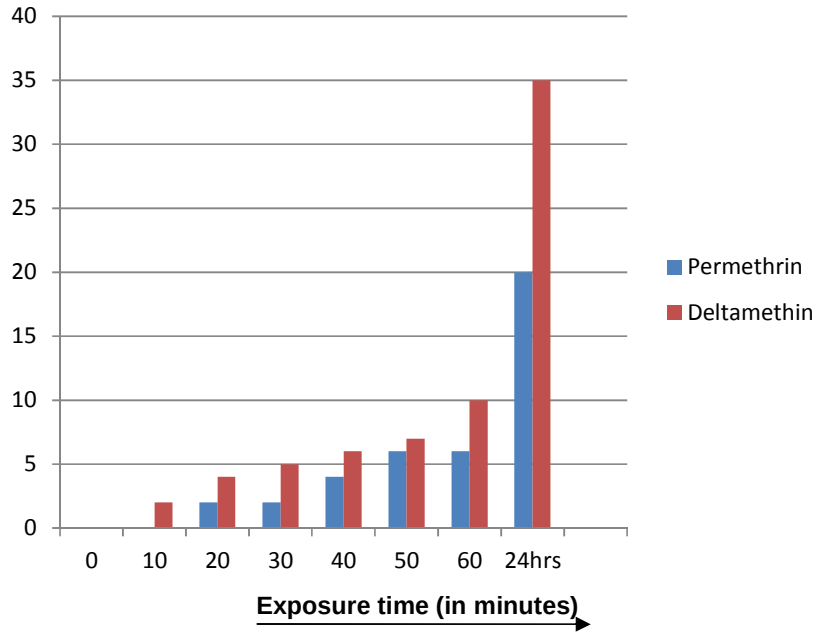

Fig. 2. % knock down (10-60 mins) and % mortality (24 hrs) of *Anopheles* mosquitoes bioassay to permethrin 0.75% and deltamethrin 0.05% collected from Auyo irrigation site

Table 1. GST, esterase and Monooxygenase specific activities (mean ± SD) in *Anopheles* mosquitoes exposed to Permethrin collected from Auyo irrigation and residential sites

Group	No tested	GST (µmole/min/mg)	Esterase (umole/min/mgprotein)	Monoooxygenase (nmol/min/mg protein)
ARr	12	0.0387±0.0049	0.0426±0.0088	0.4315±0.1360
ARs	12	0.0345±0.0058	0.0370±0.0047	0.4763±0.1808
AIr	12	0.0346±0.0054^{h}	0.0344±0.0071^{h}	0.0417±0.0086^{m}
AIs	12	0.0205± 0.0065^{h}	0.0222 ± 0.0057^{h}	0.4855 ±0.1233^{m}

Values with similar superscript indicates significant difference (P<0.05) when the groups were compared
Key: ARr: Auyo residential site resistant strain
ARs: Auyo residential site susceptible strain
AIr: Auyo irrigation site resistant strain
AIs: Auyo irrigation site susceptible strain

Table 2. GST, esterase and Monooxygenase specific activities (mean ± SD) in *Anophles* mosquitoes exposed to Deltamethrin collected from Auyo residential and irrigation site

Group	No tested	GST (µmole/min/mg protein)	Esterase (umole/min/mg protein)	Monoooxygenase (nmol/min/mgprotein)
ARr	12	0.0127 ± 0.0044^{d}	0.0433±0.0134^{d}	0.4993±0.1686^{d}
ARs	12	0.0091 ± 0.0029^{d}	0.0213±0.0060^{d}	0.3213±0.1260^{d}
AIr	12	0.0365±0.0075	0.0464±0.0079^{i}	0.5597±0.1989^{k}
AIs	12	0.0373 ±0.0057	0.0386±0.0046^{i}	0.0434±0.0065^{k}

Values with similar superscript indicates significant difference (P<0.05) when the groups were compared
Key: ARr: Auyo residential site resistant strain
ARs: Auyo residential site susceptible strain
AIr: Auyo irrigation site resistant strain
AIs: Auyo irrigation site susceptible strain

3.2 Discussion

Irrespective of the collection sites, the adult mosquito bioassay revealed high resistance status to permethrin and deltamethrin according to WHO interpretation. However the pattern of resistance varies with insecticides and breeding sites. Vectors collected from residential sites (AR) showed percentage mortality of 30% and 38% and those collected from irrigation sites showed percentage mortality of 20% and 35% for permethrin and deltamethrin respectively. Following the failure of DDT to combat malaria epidemic, in 2010, Nigeria joined the team of other African countries to arrest the spread through free distribution of pyretheroids treated bed nets. In addition to government intervention, there is also individual use of pyretheriods in form of mosquito coils and liquid vaporizers. In Nigeria synthetic pyretheroids are currently been used not only for vector control but also against agricultural pest. The use of agrochemical to improve crop production may impart negative effect on vector control strategy as most of them share same target site of action with insecticides approved for vector control. Mounting evidence indicated that use of broad spectrum insecticides in agricultural sites contributes to insecticides resistance in malarial vectors [19]. The recorded resistance in this study may be attributed to mosquito exposure to these insecticides due to various government programmes and individual practices that warrant their use in environment [19]. It is believed that common Kdr target site resistance to pyretheroids in West Africa, actually arose as a result of heavy utilization of DDT in agricultural field. However, this cross resistance is not automatic phenomena as pyretheroids resistant *anopheles funestus* were found to be highly susceptible to DDT [20]. The finding of this work is similar to that of Elissa [21] who reported that pyretheroids resistance from Cote d ivoire. Two years later the resistance became widespread to a worried and alarming level. This may be as a result of increasing utilization of the two insecticides particularly deltamethrin and permethrin. Pyretheroids resistance became widespread not only in mosquitoes but also in other insects such as housefly and cockroach [22,23]. Report of resistance in *anopheles gambiae* to pyretheroids; delramethrin and permethrin were made available by Reidy et al. [24] and Grant et al. [25] which also correspond to finding of this study in Auyo town.

The results of enzyme analysis (Table 1) show a correlation between GST activity and permethrin resistance in irrigation site, suggesting the role of GST in permethrin resistance. This may be associated with the use of permethrin for crop pest control in agricultural field. The finding is in accordance to that of Josaine et al. [26], who reported elevated level of GST in Piota pyretheroids resistant mosquitoes. The results also echo well with the report correlating high of GST with high resistant to pyretheroids [24,25]. Induction of GST activity has been reported not only after exposure to oranophosphate and organochloride but also against pyretheroids [27].

The result of the study also shows a correlation between GST activity and deltamethrin resistance in the irrigation site (Table 2) which may be induced by excessive agricultural spray. The finding also echoes well with the reports correlating high level of GST with pyretheroids resistant in several insect species including mosquitoes [24,25]. The increase in esterase activity in resistant (Table 1) corresponds with finding of Desfintianes et al. [28], who reported elevating activity of GST and esterase in Duala town, Cameroun, where coil and mat treated with pyretheroids are extremely used for harvest protection and against mosquitoes bite. A relationship between high esterase activity and pyretheroids resistance has been established in insects other than mosquitoes [29]. Aruminal et al. [30] indicated significant role of ester hydrolysis in permethrin resistant strain of *cx-quiquifasciatus.* Increase in esterase and GST activities may be the major mechanism underlying permethrin resistance. The study (Table 2) also shows correlation between esterase activity and deltamethrin resistance in residential site. This may not be surprising because of the increasing use of insecticides by individual and government intervention including insecticides spray. The esterase activity may be induced by indoor residual spray and pyretheroids treated bed nets. The present data suggests that the development of resistance to deltamethrin and permethrin is largely due to increased activity and metabolism of GST and esterase.

Studies have demonstrated the role of monooxygynase mediated degradation of deltamethrin in conferring deltamethrin resistance in the larvae of *Ae. Aegypti, Cx quiquifasciatus and An stephensi* [31]. The present results (Table 2) show increasing activity of monooxygenase in deltamethrin resistant strain. Hemingway and Ranson [32] and Brooke et al. [33] reported the role of monooxygenase in conferring pyretheroids resistance. The result also corresponds to finding of Josaine et al. [26] who reported high monooxygenase activity in pyretheroids resistant mosquoitoes. But on contrary an increase in monooxygenase activity was seen in permethrin susceptible strain (Table 1) suggesting that the enzyme does not involves in permethin detoxification. The death of the insect may be due to high exposure to various chemicals that lead to generation of high levels of Reactive Oxygen Species (ROS). The ROS are cytotoxic and mutagenic due to their high chemical reactivity that produces substantial oxidative modifications in unsaturated lipids, proteins and DNA with loss of their function and cell viability [34,35].

4. CONCLUSION

The finding of the study established resistance in both residential and irrigation sites. This may be concluded as a result of higher activities of detoxifying enzymes; GST, esterase and monooxygenase induced by indiscriminate use of insecticides in residential site against malarial vector and other flying insects as well as agrochemicals in the irrigation sites. Based on this finding it may be concluded that selection pressure that confers resistance to malarial vector is not restricted to agricultural activities alone.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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