DETOXIFICATION ENZYMES ACTIVITIES IN DDT AND BENDIOCARB RESISTANT AND SUCEPTIBLE MALARIAL VECTOR (*Anopheles gambae*) BREED IN AUYO RESIDENTIAL AND IRRIGATION SITES NORTHWEST NIGERIA

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Abstract

Evaluating levels of detoxifying enzymes is informative to governmental, nongovernmental organization and insecticides producing industries. In this work DDT and bendiocarb resistance status of *Anopheles gambae* breeds in Auyo (May – September, 2014) was studied. Larvae collected from residential sites (AR) and rice Irrigation sites (AI) of Auyo town in Auyo LGA Jigawa State Nigeria reared to adult was studied by WHO adult bioassay. Standard methods were adapted to determine the specific activities of insecticides detoxifying enzymes; glutathione s transferase (GST), esterase and monooxygenase in the insecticides resistant (r) and susceptible (s) vectors. In addition those tested with bendiocarb were analyzed for acetylcholinesterase activities. The finding of the study established high resistance status of the malarial vectors to DDT in both sites, low resistance status to bendiocarb in residential site and possible resistance to bendiocarb in irrigation site according to WHO interpretation. Significant ($P<0.05$) elevation in the activity of esterase and GST were seen

in DDT resistant strain of residential site compared to susceptible (s). Significantly higher ($P<0.05$) specific activity of monooxygenase and acetyl cholinesterase was observed in bendiocarb resistant strain of both residential and irrigation sites, except for acetyl cholinesterase of irrigation site. Elevated activity of was observed in bendiocarb resistant strain of residential site. The finding of the study could be associated with indiscriminate use of insecticides in residential site against malarial vector and other flying insect and agrochemicals in the irrigation site. Base on this finding it may be concluded that conferring resistance to malarial vector is not restricted to agricultural area alone. Therefore challenge to researchers and chemical industries in fight against malarial should be redirected to producing specific irreversible and/or reversible inhibitors to these insecticides detoxifying enzymes.

Keywords: Insecticides, Bioassay, Malarial vector, detoxification enzymes

INTRODUCTION

Nigeria was included in WHO first large scale multilateral initiative for malaria control between 1955 and 1969. The initiative tagged malaria eradication programme, was based on indoor residual spraying of DDT. Aimed at eradication of malaria across the world but only succeeded in eradicating the disease in south Europe, former USSR, some countries of North Africa and the Middle East (Allilio *et al.,* 2004). Various measures are put in place to eradicate it but unfortunately the disease burden is still on the rise and some estimate that the number of cases could double in the next twenty years without the development of new methods for control (Sachs and Malaney, 2002). Increasing incidence of malarial transmission in urban and peri urban areas may not be unconnected with farming practices (klinkenberg *et al.,* 2001*;* Wang *et al.,* 2005). Agricultural field provide a favorable environment for the vectors to multiply and agricultural spray serves as a source of selection pressure that could trigger the emergence of insecticides resistant vectors. Insecticides spray has been documented to have significant impact on malaria spread (Hawley *et al.*, 2003; Mabaso *et al.*, 2004). However this is associated with major challenges such as growing insecticides resistance by malarial vectors to commonly used insecticides (WHO, 1992). Insecticides resistance is a complex evolutionary phenomenon, which continuously sabotage efforts to control agricultural insect pests and disease vectors. According to a database of arthropods resistant to pesticides maintained by the Center for Integrated Plant Studies, Michigan State University – there are at least 533 arthropod species resistant to one or more of the main groups of insecticides approved for malarial vector control (organochlorines, organophosphates, pyrethroids, and

carbamates) (Anonymous, 2005). In 1946 only two Anophelines were known to be resistant to Dichloro-Diphenyl-Trichloroethane (DDT) (Warrell and Gilles, 2002), but today at least 63 species of Anopheles are recorded as being resistant to insecticides (Anonymous, 2005). Insecticides resistant insect evolved as a result of unusual expression of one or more resistance gene following indiscriminate exposure to various insecticides. Natural selection and genetic drift act on genetic variation in the population that is created by mutation, genetic recombination and gene flow. Insecticide resistance mechanisms have a biochemical basis (Borgdon and McAllister, 1998). The two major groups of mechanisms involved in biochemical resistance in insects are target site resistance and detoxification enzyme resistance. The target sites include Acetylcholinesterase (AChE) breaks down the neurotransmitter acetylcholine in the nerve synapses. Increased activities of detoxification enzymes has been reported to account for insecticides resistance through metabolizing them before reaching their target site where toxicities are expressed or as a result of reduced target site sensitivity of DDT binding site sodium ion channel (Nwane *et al.*, 2009) and carbamate binding site acetylcholinesterase (Weill *et al., 2004*). Esterases detoxify organophosphate, carbamates and synthetic pyretheroids through hydrolysis of ester bond and binding of insecticides to the active site of esterase (Crow *et al*., 2007). Insect cytochrome p450 monooxygenase metabolize exogenous compounds including insecticides and plant toxins leading to insecticides resistance (Wen *et al*., 2003) and higher tolerance to plant toxins (Li *et al*., 2002; Wen *et al*., 2003). Resistant insects usually show very high activity of esterase (Yang *et al*., 2004; Wu *et al*., 2004).

MATERIAL AND METHODS

Material

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.

STUDY AREA

Area is predominantly rice cultivation site in Auyo local government area of Jigawa state. It lies between latitude 12 21 36 N and longitude 9 59 8 E. It is situated in northeastern corner of the state, it is bordered on east by Hadejia, west by kafin Hausa and Bauchi state and with a shared boarder on northeast with Malammadori local government. It has a total land mass of about 740 square kilometer which is 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.

Larval collection and rearing

The larvae collected from different points in both residential sites (AR) and rice Irrigation sites (AI) in Auyo and were reared to adult according to WHO (1998) recommendation.

WHO Bioassay

Mosquitos' insecticides diagnostic kit was used to establish susceptibility and resistant status using 4% DDT and 0.1% bendiocarb impregnated paper according to WHO procedure (WHO, 1998). 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, 90 -97% require further investigation, less than 90% is consider resistant to the tested insecticides. The resistant and susceptible mosquitoes were separately placed in labeled effendorf tubes and at - 80˚C for subsequent enzyme assay

Enzyme Analyses

Enzymes analyses were carried out u*sing procedure outlined by WHO (1998). Individual mosquitoes were analyzed for protein, esterase, glutathione S transferase and monooxygenase. In those tested with bendiocarb, acetylcholinesterase was also analyzed. The mosquitoes were individually homogenized using glass rod in 150µl ice cold distilled water, 20 µl of crude mosquito homogenate was taken for acetylcholinesterase assay while the remaining homogenate was centrifuged at 13000g for two minutes. To obtain specific activities of enzyme, the protein concentration of individual homogenate was determined by the method of Bradford (1976) using Coomasie blue reagent. A quantity of each homogenate (10µl) was mixed with 200µl of Coomasie blue reagent and absorbance was read at 630nm after five minutes incubation.*

Esterase assay

Esterase was determined by sprctrophotometric method (Faiz *et al*., 2007), the enzyme hydrolyszes paranitrophenylacetate to acetate and a yellow colour product paranitrophenol which maximally absorbs light at 405nm. A quantity of ten microliter of each homogenate was mixed with 200µl of 1mM paranitrophenyl acetate working solution (100mM paranitrophenyl acetate : 50mM sodium phosphate buffer *pH* 7.4, 1:99) in a microtitre plate well. The absorbance was read at 405nm after ten minutes incubation. An extinction coefficient of $6.53mM^{-}cm^{-}$ 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/ mg protein.

GST assay

Glutathione s transferase (GST) was determined following the method of (Habig *et al*., 1974).

The GST catalyzes the conjugation of glutathione and chloro 2,4dinitrobenzene to form 2- chloro-4- nitrophenyl glutathione which absorbs light at 340nm. A quantity of ten microliter of each homogenate was mixed with 200µl reduced glutathione (GSH/I-chloro -2,4 dinitrobenzene working solution{95 parts of 10mM reduced glutathione in 100mM 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 340nm after 10 minutes incubation. An extinction coefficient $5.76mM^{-}cm^{-}$ and a path length of 0.6cm was used to convert absorbance to moles of product. Gst specific activity was reported as CDNB conjugated µmole product min^{-} mg^{-} protein.

Cytochrome p450 (Monooxygenase) assay

This was measured by the method of (Borgdon *et al*., 1998). The monooxygenase catalyses the reduction of hydrogen peroxide and oxidation of tetramethylbenzedine to form water and oxidized blue color tetramethylbenzidine which absorbs light at 630nm. Twenty microliter of homogenate was mixed with 80µl of potassium phosphate buffer *pH* 7.2 +200µl of 6mM tetramethylbenzidene (TMBZ) working solution{(0.01g TMBZ was dissolved in 5ml methanol and then in 15ml of sodium acetate buffer *pH* 5.0) +25 µl of 3% v/v H_2O_2 solution} in a micrititre plate well. After two hours incubation at room temperature, the absorbance was read at 630nm. By using a standard curve of cytochrome c, a crude estimate of the amount of the amount of monooxygenase present was obtained and expressed as equivalent units of ytochrome p450.

Acetylcholinesterase assay

This was measured by the method of Ellman *et al (*1961). Twenty microliter of homogenate was mixed with 145µl of triton phosphate buffer (*pH* 7.8) and 25µl of 0.01M acetylthiocholine iodide followed by $10cm^3$ 0.01M dithiobis 2-nitrobenzoic acid. The kinetics of enzyme reaction was continuously monitored at 405 nm after 5 minutes incubation. An extinction coefficient of $13.6mM^{-1}cm^{-1}$ and path length of 0.6cm was used convert the absorbance to moles of product.

Statistical analysis

The data was statistically analysed using t-test with P value <0.05 considered extremely significant, a component of GraphPad Instat3 Software

(2000) version 3.05 by GraphPad Inc.

RESULTS AND DISCUSSION

Results

Figure 1 and 2 shows one hour knocked down rate at interval of 10min exposure to insecticide impregnated papers of *Anopheles* mosquitoes respectively collected from Auyo residential and irrigation sites. The percentage susceptibility to both insecticide (DDT and bendiocarb) ranges from 14% and 96%. Table 1 and 2 show the specific activities of detoxifying enzymes of *anopheles* mosquito (resistance and susceptible) of Auyo residential and irrigation sites respectively exposed to DDT and bendiocarb.

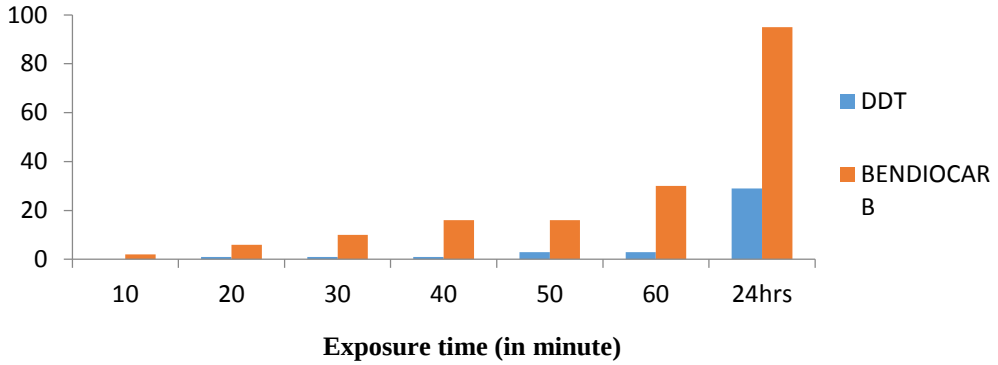

Figure 1: % knock down (10 – 60 mins) and % mortality (24hrs) of *Anopheles* mosquitoes bioassay to DDT 4%, permethrin 0.75%, Deltamethrin 0.05% and Bendiocarb 0.1% collected from Bichi residential site

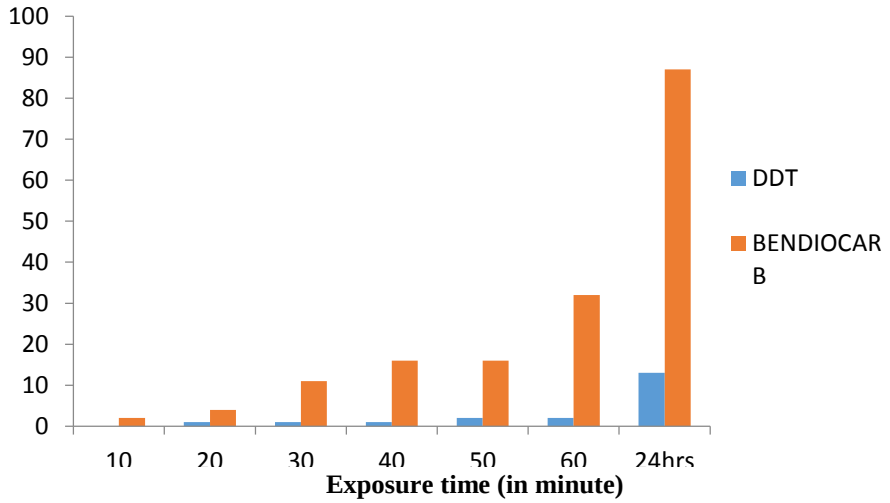

Figure 2: % Knock down (10 – 60 mins) and % mortality (24hrs) of *Anopheles* mosquitoes bioassay to DDT 4%, permethrin 0.75%, deltamethrin 0.05% and bendiocarb 0.1% collected from Bichi irrigation site

Table 1 Glutathione S transferase (GST), Esterase and Monooxygenase specific activities in *Anopheles mosquitoes* tested with DDT collected from Auyo irrigation and residential sites.

group	No tested	GST (µmole/min/mg protein)	Esterase (µmole/min/mg protein)	Monooxygenase (nmol/min/mg protein)
ARr	12	0.0610 ± 0.0088^{b}	0.0475 ± 0.0102^{a}	0.2308 ± 0.0613
ARs	12	0.0581 ± 0.0084^{b}	0.0234 ± 0.0064^{a}	0.2032 ± 0.1311
AIr	12	0.0473 ± 0.0060	0.0495 ± 0.0096	0.4950 ± 0.0096^{c}
AIs	12	0.0477 ± 0.0112	0.0524 ± 0.0071	0.4730 ± 0.0096^{c}

Values with similar superscript indicates significant difference (P<0.05) when the groups were compared

Table 2: GST, Esterase, Monooxygenase and Acetylcholinesterase specific Activity In *Anophles*mosquitoes population tested with Bendiocarb collected from Auyo residential and irrigation site.

Group	No tested	GST (µmole/min/mg protein)	Esterase (µmole/min/mg protein)	Monooxygenase (nmol/min/mg protein)	Acetylcholinesterase (µmole/min/mg protein)
ARr	12	0.0562 ± 0.0109	0.0485 ± 0.0066	0.5222 ± 0.2659^{f}	0.0387 ± 0.0119^{e}
ARs	12	0.0754 ± 0.0094	0.0489 ± 0.0089	0.2898 ± 0.0593^{f}	0.0227 ± 0.0113^{e}
AIr	12	0.0329 ± 0.0059	0.0377 ± 0.0079	0.3922 ± 0.0868^{i}	0.0305 ± 0.0128
AIs	12	0.0339 ± 0.0064	0.0403 ± 0.0089	0.2830 ± 0.0798^{i}	0.0314 ± 0.0069

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.

Discussion:

Irrespective of the collection sites this study demonstrates the occurrence of extreme resistance of malarial vector to DDT and possible resistance to bendiocarb according to WHO interpretation. However, the resistant level varied with the insecticides and breeding sites. Vectors collected from residential site (Figure 1) showed percentage mortality of 15% and 96% and those collected from irrigation site (Figure 2) showed percentage mortality of 14% and 80% with respect to DDT and bendiocarb. The extreme DDT resistance established by this work is in accordance with what prompted Nigeria to join the team of other African countries to arrest the spread through distributing Pyrethroid treated bet nets and indoor resident spray (IRS). However the efficacy of pyretheroids last shortly after its introduction, a situation that favors the introduction of alternative insecticides carbamate with different mechanisms of action (Oduola *et al*., 2012). In Nigeria, resistance of *Anopheles gambiae*, to common classes of

insecticides is well documented (Awolola *et al*., 2002; Oduola *et al*., 2012) but a little is known regarding the carbamate susceptibility and resistance mechanism. DDT resistance that has been reported since 1940s may presently be compounded by increasing reliance on pyretheroids treated bed nets since both share the same target sites of action. Bendiocarb resistance recorded in this study may be as a result of utilization of the insecticides in agricultural field and also in indoor residual spray either alone or in combination with pyretheroids. Corbel *et al* (2007) reported carbamate resistance in *Anopheles gambiae* with the mortality rate of 75%. The finding of this work is similar to that of Aikpon *et al* (2013) who reported bendiocarb resistance in *Anopehles gambiae* population in Nigeria neighbouring country Benin. Also the report of Nguessan *et al* (2003) shows carbamate resistance in 1990 and two years later the resistance level was reported to attain worried and alarming level, the observation echo well with finding of in Auyo, reported by this work. The intermediate resistance to bendiocarb reported in this study may be as a result of increased utilization of carbamate in agricultural field and IRS in residential sites. Oduola *et al.* (2012) first reported carbamate resistance in *Anopheles gambiae* ss resistant to DDT and pyretheroids in Lagos, Nigeria. DDT resistance in *Anopheles culicifaeces* was reported in India (Dash *et al.*, 2006). The elevated activity of GST (Table 1) in resistant strain of residential site suggests the involvement of GST in mediating DDT detoxification. Josaine *et al* (2007) reported high Level of GST in Duala DDT resistant population. The finding also echoes well with the studies of Hemigway *et al* (1991); Herath and Jayawardena (1988) that reported linear correlation between GST and DDT resistance. However in contrast to the vectors sample from irrigation site no significant difference ($p < 0.05$) in GST activity was seen in susceptible and resistant vectors. This may be as a result of variation in the insecticides commonly use in the two sites or the enzyme is induced enough to confer resistance even at statistically insignificant difference. In addition GST metabolism is not the only means of DDT resistance, mutation in voltage gated sodium ion channel protein, the target site of DDT and pyretheroids was reported to confer DDT resistance in *Anopheles subpictus* from anuradaphura (Karunartane and Hemigway 2001) which is similar to that found in Africa *Anopheles gambiae ss* (Martiez - torres *et al.*, 1998). Leu-phe mutation at sodium ion channel has been extensively documented from West African population of *Anopheles gambiae* (Diabete *et al.,* 2004; Awolola *et al.*, 2007). High level of esterase activity in DDT resistant population of residential site may not be surprising considering the indiscriminate use of pyretheroids by the house holders in protection against malarial vectors and other crawling insects. Desintianes *et al* (1989) reported elevated level of GST and esterase activities in Duala town, Cameroun,

where coils and mats containing pyretheroids are extremely used for crop protection and against mosquitoes bite. The elevated level of monooxygenase seen in resistant strain (Table 1) of irrigation site is probably not induced by DDT exposure alone but by individual utilization of different forms of insecticides and crop pest mainly in irrigation site. Elevated level of monooxygenase may confer DDT resistance since the enzyme is known to detoxify all classes of insecticides. The result (Table 2) shows significant increase ($p<0.05$) in monooxygenase activity in resistant strain of both residential and irrigation sites and increased Acetylcholinesterase activity in resistant strain of residential site. This suggests the role of monooxygenase and acetylcholinesterase in conferring resistance against bendiocarb. Devika *et al.* (2008) showed how monooxygenase contribute to multiple resistances in Tiassale population and couple with over expression of acetylcholinesterase resistant allele to produce extreme resistance to carbamate. The increased acetylcholinesterase in resistant strain may be as a result of low level inhibition by bendiocarb.

References:

Aikpon, R., Agossa, F., Osse R., Oussou, O., Aizon, N., Oke- Agbo, F., and Akogbeto M (2013). Bendiocarb resistance in *Anopheles gambiae* SI populations from Atacora department in Benin, West Africa: a threat for malarial vector control. *Parasite and Vector control 6:192.*

Allilio, M.S., Bygbjer, C., and Breman, J. C. (2004). Are the Multilateral malaria research and control programs the most Successful? Lesson from the past 100years in Africa. *American Journal of Tropical Medicine and Hygiene* 71:268 – 278.

Anonymous (2005).The Database of Arthropods Resistant to Pesticides. Michigan State University – Center for Integrated Plant Systems. http://www.cips.msu.edu/resistance.

Awolola, T.S., Brook, B.D., Hunt RH, and Cotzee, M (2002). Resistance of malaria vector *Anopheles gambiae ss* to pyretheroids insecticides, in southwest, Nigeria. *Journal of Tropical Medicine and Parasitology,* 96, (8,) Pp 849-852

Awolola, T.S., Oduola, A.O., Obansa, J.B., Chukwurar, N.J.and Unyimadu, J.P (2007). *Anopheles gambiae* s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *J. Vector. Borne.Dis*. 44:241-244.

Borgdon, W.G. and McAlister J.C. (1998). Insecticides resistance and vector control. *Emergin infectious disease 4:4*

Bradford, M.M (1976). A rapid and sensitive method for quantitation of microgram quantities of protein – dye binding. *Anal. Chem.* 72, 248 – 254.

Corbel, V., Nguessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbeto. M., Hougard, J.M., and Rowland M (2007). Multiple

insecticides resistance mechanisms in *Anopheles gambiae and Culex quinquifasciatus* from Benin West Africa *Acta Trop. 101:* 207 -216

Crow, J.A., Potter, P.M., Borazjani, A. and Rose ,M.K. (2007) Hydrolysis of pyretheroids by human and rat tissues. Examination of intestinal, liver and serum carboxylesterase. *Toxicology and applied pharmacology* 221: 1-12

Dash, A.P., Raghavendra, K., and Pillai, M.K.K. (2006). Combating Resistance to Insecticides in Malaria Control- Gains Made in India. *Bayer Environmental Science Journal* 18: 30-37.

Desfontaines, M., Gelas, H., Ghogoumu, A., Kouka Bemba, D.and Carnebell, P (1989). Evaluation des practiques et des couts de lute antivectorielli a lechelon familial en afrique central. I – ville de yaunde.*Bull. Soc .path*. Ex. 82: 558-565

Devika, M., Perera, B., Hemigway, J. and Karunaratne, P (2008). Multiple Insecticides resistance mechanisms involving metabolic changes and insensitive target sites selected in anophelene vectors of malaria in Sri Lanka. *Malaria journal 7:168*

Diabate, A., Brengues, C., Baldet, T., Dabire, K.R., Hougard, J.M., Akogbeto, M., Kengne, P., Simard, F., Guillet, P., Hemingway, J,and Chandre, F.(2004). The spread of the Leu-Phe kdr mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and de novo phenomena..*Pestic.Biochem. Physiol.*; *56* : 69-77.

Ellman, G.C., Courthy, K.D., Andres, V. and Featherstone R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity.*Biochemical pharmacology 7:*115 -275

Faiz, O.A., colak, N., Saglam, S, and Belduz, A.O (2007). Determination and characterization of thermostable esterolytic activity from a novel thermopilic bacterium.*Anoxybaccilus gonesis* A4 *J. Biochem. Mol. Biol.* 40(4): 588-594

Hawley, W.A., Phillips-Howard, P.A., TerKuile, F.O., Terlouw, D.J., Vulule J.M., Ombok, M., Nahlen, B.L., Gimnig, J.E., Kariuki, S.K., Kolczak, M.S. and Hightower, A.W.(2003). Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *American Journal of Tropical Medicine and Hygiene* 68: 121-127.

Hemigway, J., Miyamoto, J.and Herath P.J (1991). A possible novel link between organophosphorous and DDT insecticides resistance gene in *Anopheles* supporting evidence from fenithrothione metabolism studies.*Pesticide Biochemistry and Physiol 39: 49-56*

Herath, P.R.J., and Jayawardena K.G.J. (1988) DDT resistance in *Anopheles culicifacies* Giles and *A Subpictus* from Srilanka .a field study on the mechanisms and changes in gene frequency after cessation of DDT spraying. *Bull. Entomol. Res.* 78: 49-56

Josaine, E., Lucien, M., Jeane, C.T., Pierre, G., Etienne, F. and Fabric, C

(2007). Spectrum of metabolic based resistance to DDT and pyretheroids in *Anopheles gambiae* SI population from Cameroun. *Journal of Vector Biology 32: 123 -133*

Karautarne, S.H.P and Hemingway, J. (2001).Malathion resistance and prevalence of the malathion carboxyl esterase mechanisms in population of Sri lanka mosquito vectors of diseases. *-Bull world health organ* 79: 1060 - 1064

Li, X., Berenbaum, M.R., and Schuler, M.A (2002). Cytochrome P450 and actin genes expressed in *Helicoverpa zea* and *Helicoverpa armigera*: paralogy/orthology identification, gene conversion and evolution. *Insect.Biochem.Mol. Biol 32:* 311–320.

Mabaso, M.L., Sharp, B., and Lengeler, C. (2004).Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying.*Tropical Medicine and International Health*9: 846-856.

Martinez-Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Berge, J.B., Devonshire A.L., Guillet, P., Pasteur, N., and Pauron, D. (1998) Molecular characterization of pyrethroid knock-down resistance (kdr) in the major malaria vector Anopheles gambiae s. s. *Insect Molecular Biology* 7: 179-184.

Nguessan, R., Darriet, F., Gullet, P., Camevale, p., Traore- lamizana, M., and Corbel V,(2003).resistance to carbosulfan in anophless gambiae from ivory coast , based on reduce sensitivity of acetylcholinesterase. *Med. Vet. Entomol*. 17; 19-25

Nwane, P., Etang, J., Chouaibu, M., Toto, J.C., Kerah – Hinzombe, C., Mimfoundi, R., AwoAmbene H.P, Simard, F (2009). Trend in DDT and pyretheroids resistance in *Anopheles gambiae ss*. Population from urban and agroindustrial settings in southern Cameroon.*BMC Infect Dis 9: 163*

Oduola, A.O., Idowu, E.T., Oyebola, M.K., Adegun, A.O., Olojede, J.B., Otunbanjo, O.A. and Awolola, T,S. (2012). Evidence of carbamate resistance in urban population of *Anophels gambiae ss* mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South west Nigeria. *Parasite and Vectors* 5: 116

Sachs, J. and Malaney, P.(2002). The economic and social burden of malaria. *Nature,**415***, 680 -685 *Anal. Biochem.***82**: 334-341.

Wang, S. J., Len geler, C., Smith, T. A., Vounatsou, P., Diadie, D. A., Pritroipa, X., Convelbo, N., Kientga M., and Tanner, M. (2005). Rapid Urban Malaria appraisal (RUMA). Epidemiology of ueban malaria in Ouagadougou. *Malaria Journal* 4: 43.

Warrel, D. A., and Gillies H. M. (2002). Essential Malariology. 4th edition. London. Arnold. P348.

Weill, M., Malcolm, C., Chandre, F., Mogensen, K., Berthomieu, A., Marquine, M., and Raymond, M. (2004). The unique mutation in ace-1

giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Molecular Biology* 13: 1-7

Wen, Z., Pan, L., Berenbaum, M.B,, and Schuler, M.A. (2003) Metabolism of linear and angular furanocoumarins by *Papilio polyxenes* CYP6B1 co-expressed with NADPH cytochrome P450 reductase. *Insect.Biochem. Mol. Biol. 33: 937*–94

WHO (1992).Vector resistance to insecticides. 15th Report of the WHO Expert Committee on Vector Biology and Control. *World Health Organization Technical Report Series* 818: 1-62.

WHO (1998): test procedure for insecticides resistance monitoring in malaria vector, *bio efficacy and persistence of insecticides on treated surface.*

Winkenberg, E., Mccall, P. D., Hasting, I. A., Metaxakis, A., Wilson, M. D., Amerasinghe, F. P., Danelly, . J., Kostrapouleous, I., Papadopoulous, A. I., Boukouvala, E., Papadopoloulou, S., and Mourikidou, E. (2001). Glutathione S transferase in the defense against pyretheroids in insects. Insect Biochem. Mol. Biology 31: 313 – 319.

Wu, G., Jiang, S., Miyata, T (2004). Seasonal change methamidhopos susceptibility and Biochemical properties in *plutella xylostella* and it parasitoid *costellia plutella. Journal of economic entomology* 97: 1689-1698

Yang, Y., Wu, Y., Chen, S., Devine, G.J., Denholm, I.and Jewes, P. (2004). The involvement of microsomal oxidases in pyretheroids resistance in *Helicoverpa armigera* from Asia.*Insect Biochemistry and molecular Biology* 34: 763-773.