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Glutathione Levels and Variations in *Anopheles gambiae s.l.* from Agricultural and Residential Settings

[1*]Habibu Abdu, [2]Yayo Abdussalam, [3]Ado Abduljalal, [4]Ebere Nabueze, [5]Simona Hapca and [6]Yusuf Deeni,
[1]School of Science Education, Federal College of Education (Technical) Bichi, Kano, Nigeria
[2]Microbiology and Parasitology Department, Bayero University Kano, Nigeria
[3]Sustainable Healht and Malaria Control, Gyadi Gyadi Kano, Nigeria
[4]Animal and Environmental Biology Department, River State University, Portarcourt, Nigeria
[5]Division of Population Health Science, School of Medicine, University of Dundee, UK
[6]School of Science, Engineering and Technology, Abertay University, Dundee, UK

***Abstract*—**Glutathione (GSH) is widely distributed among all living organisms and associated with diverse functions that include maintaining redox homeostasis and detoxification of xenobiotic compounds. The level of GSH within the cell varies with stress often involving changes in GSH content, which is consumed in reactions that protect the cell leading to formation of oxidized GSH (GSSG). Mosquitoes breeding in habitats located at agricultural and residential ecosystems may encounter different kinds of pesticides. Enzyme recycling assay method was used to determine glutathione levels in adults *Anopheles gambiae* (*An. gambiae)* reared from larvae collected from agricultural (zone A) and residential (zone B) sites. More than 70% of the GSH occur in the reduced form across the sampling sites. Significantly, higher levels of the oxidized form of GSH were recorded in mosquitoes from agricultural compared to residential sites. This may possibly be an adaptive response to strong selection pressure due to higher concentration of pesticides in the agro-ecosystem. Under normal physiological condition, GSH exists mostly in the reduced form but is converted to the oxidized form during oxidative stress. GSH is a substrate for the glutathione S-transferases system and becomes rate limiting when organisms are exposed to large amount of xenobiotics. The ratio of reduced GSH (rGSH) to GSSG levels often remain above 99% but can change markedly during oxidative stress. Thus, the study highlighted glutathione status may be mediating the response and possibly adaptation of *An. gambiae* exposure to insecticide.

Keywords*—Anopheles gambiae, Agricultural and Residential settings, Glutathione, Oxidative stress, Variations*

I. INTRODUCTION

Glutathione (GSH) a tripeptide (γ-L-glutamyl-L-cysteinyl-glycine) is characterized by an N- terminal L-glutamyl moiety, a central cysteine residue and a variable C-terminal amino acid. It is widely distributed among all organisms and is associated with detoxification pathways and regulation of intracellular redox environment as well as an antioxidant [1, 2, 3]. Detoxification of toxic substances to water soluble excretable forms is catalyzed by the enzyme family glutathione S-transferases (GST) through nucleophilic addition of the GSH thiol group to electrophilic centres of xenobiotic substances [4, 5, 6]. GSH in natural physiological conditions exists mostly in the reduced form (rGSH) and serves as a substrate for the GST system [7].

During stress condition, cells are protected also by glutathione peroxidases forming the oxidized form (GSSG) [5, 8]. The oxidized can be converted back to the reduced form through the action of glutathione reductase and the ratio of levels of rGSH to GSSG which normally remain above 99% changed markedly during oxidative stress [1, 9, 10]. Several studies on measurement of GSH in insects have associated high levels of GSH with amount of exposure to xenobiotic and oxidative stress [9, 11]. Insects thriving in agro-ecosystems are reported globally to experience continuous oxidative stress due to protracted exposure to fertilizer, insecticides and herbicides, encountered in such habitats [12, 13].

Irrigation and rice production in some countries in Africa have caused proliferation in populations of *An. gambiae* and rise in malaria transmission [14, 15]. Such ecological scenarios, particularly irrigation and rice production in Africa have selected for pyrethroid resistant populations of the malaria vector *An. gambiae sl* [16, 17]. In Nigeria, populations of *An. gambiae ss* and *An. arabiensis* resistant to DDT, permethrin and deltamethrin have been identified from breeding sites in agricultural and residential areas[18].

Previously, we have detected differential levels of resistance to DDT, Deltamethrin, Alpha Cypermethrin and Bendiocarb and have shown significant increase in GST activity in the resistant individuals of *An. gambiae sl* from this area [19, 20]. In this study we compared variations in GSH levels between *An. gambiae sl* from agricultural and residential sites.

II. MATERIALS AND METHODS

A. Study area

Auyo and Bichi Local Government Areas (LGAs) are located between latitudes $12^0$20' N, $9^0$56' E and longitudes $12^0$13' N, $8^0$15' E and covers an area of about 512 km^2 and 612 km^2 respectively. Auyo is drained by rivers Hadejia Jama'are and Komadugu and Bichi is drained by the Kano river system. The annual rainfall ranges from about 400 – 800ml, 300-ml and mean annual temperature of about 27^0 in Auyo and Bichi respectively. In both areas, the vegetation is the Savannah type consisting of heat – resistant trees scattered across the grasslands, Farming, particularly production of rice by irrigation is the main economic activity. The settlements in the two areas comprise residential areas situated in the vicinity of large irrigation projects.

B. Larval prospection

A reconnaissance survey was undertaken in both LGAs using a cool guide to study the pattern of settlement in relation to the local ecological factors which may influence the relative risk of exposure to malaria transmission. The spatial locations of the settlements in relation to the existing rivers, streams and ponds draining areas were determined by GPS. The mosquito breeding sites in the agricultural and residential areas were identified. Anopheline mosquito larvae were collected from

rice fields, irrigation channels hoof prints and gutters using dippers, spoon and plastic cups as described [21]. After collection, larvae were processed to remove natural enemies and predators.

C. Mosquito rearing

The larvae were transferred into suitable containers, usually plastic jugs and transported to the Laboratory at AKTH. Larvae collected from different ecosystems were reared separately. The larvae were kept in bowls and fed with yeast following standard protocols [22]. The adults which emerge were kept in cages labelled accordingly.

D. Preparation of mosquito homogenate

Batches of 20-30 (50-60mg) adult *An. gambiae sl* randomly sampled from cages each containing mosquitoes reared from a known ecosystem were transferred to 1.5 ml Eppendorf tubes. Two hundred microlitre ice-cold potassium phosphate buffer (500mM / 5Mm EDTA, PH7.2) were added and the mosquitoes grinded and homogenized using plastic pestle. The homogenates were centrifuged at 10,000g for 10 min at 4^0 c using (E. Centrifuge S417R USA).

E. Protein assay

Protein standards were prepared in the same buffer as the samples, and a standard curve was made using Bovine serum albumin (BSA) of concentration 10mg/ml [23]. The protein concentration of each homogenate was determined by using Bradford Reagent according to the manufacturer's instructions.

F. Glutathione (GSH) assay

Sample preparation

The mosquito homogenates was deproteinized prior to glutathione assay 200 microliters 5% 5- Sulfosalicylic acid (SSA) solution was added to the homogenate and the mixture centrifuged at 10,000g for 10 m.

Total GSH and GSSG assays

An assay of total glutathione (tGSH) and oxidized glutathione (GSSG) was carried out on mosquitoes sampled from the five sampling sites (two residential.

and three agricultural) using Glutathione assay kit CS0260 by Sigma-Aldrich. Total GSH and GSSG were assayed by a modification of the enzymatic recycling method of [24] using glutathione assay kit CS0260 (Sigma–Aldrich), based on the manufacturer's protocol. In order to measure the level of tGSH, any GSSG present in the sample was converted to rGSH by glutathione reductase. In this method, the rate of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) reduction is proportional to the amount of either GSH or GSSG present. The enzymatic recycling reaction initiated by the addition of NADPH, and the rate of DTNB reduction was determined from the increase in the yellow product 5-thio-2-nitrobenzoic acid (TNB) at A_{412}. Measurement of the absorbance of the wells at 412 nm was done using a Micro plate reader (Modulus micro plate, Turner Biosystems, California USA). One measurement at 25 minutes was taken. This rate was corrected for the reaction of DTNB with glutathione reductase without the tissue sample according to [25]. The full assay was performed according to manufacturer'sprotocol.

To measure GSSG in samples, all reduced GSH present in the samples were removed by treatment with 2-Vinylpyridine before addition of the Ellman's reagent and glutathione reductase [26]. A solution of ethanolic 2-Vinylpyridine was made by adding 27µl of 2-vinylpyridine (2-VP) to 98µl ethanol in a fume hood. 5-Sulfosalicylic acid (SSA) treated samples, and GSSG standards stocks were then treated with 1µl of the ethanolic 2-VP solution for every 50µl of the sample and incubated for 1h at room temperature. The sample (supernatant) was diluted 1: 2.5 with assay buffer. GSSG standard solutions were prepared by serial dilution of a 50µM GSSG made from the 10mM GSSG stock solution. Measurement of GSSG was performed as described for total GSH after derivatization of GSH by 2-vinylpyridine. Reduced GSH was calculated from the difference between the total glutathione and the oxidized values. The difference between total and oxidized GSH was used to obtain the levels of reduced GSH. All the GSH levels were corrected for the milligrams of protein present in the sample and expressed as nmol/mg protein. In order to assess whether the GSH levels was related to resistance, SPSS statistical package was used to calculate the Coefficients of correlation.

III. RESULTS

Assessing the levels of the different forms of glutathione in An. gambiae from agricultural and residential zones

The levels and the distributions of the three forms of glutathione were measured from the same mosquito populations whose resistance statuses were reported by [20, 27] (Figure 1 and Figure 2). The levels and the distributions of tGSH, rGSH and GSSG in mosquito population sampled are shown in tables 1 and 2 respectively. The ratios of oxidized and reduced relative to the total GSH described. Based on the ratios obtained, more than 70% GSH is present in the reduced-form across the sampling sites.

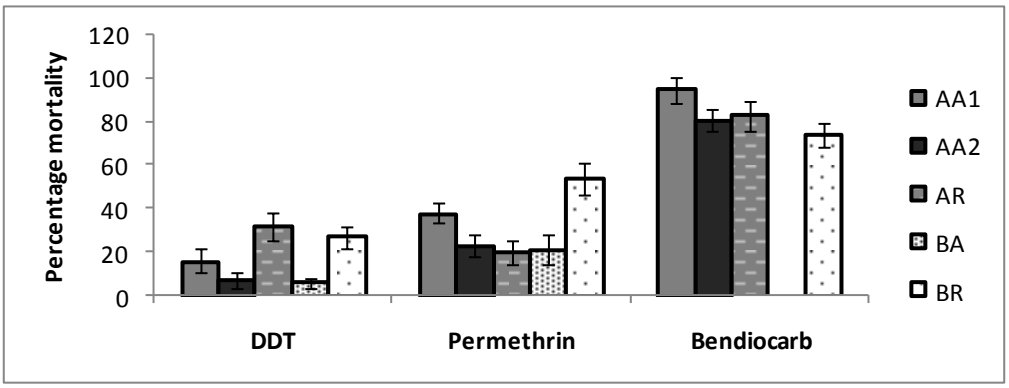

Figure 1 Insecticide mortality rate of *An. gambiae s.l.* 24 h post exposure in Nigeria collected in 2013 rainy season. Mosquitoes were exposed to 4% DDT, 0.75% permethrin and 0.1% bendiocarb in WHO susceptibility test at: Auyo agricultural 1(AA1); Auyo agricultural 2 (AA2); Auyo residential (AR); Bichi agricultural (BA) and Bichi residential (BR) sites

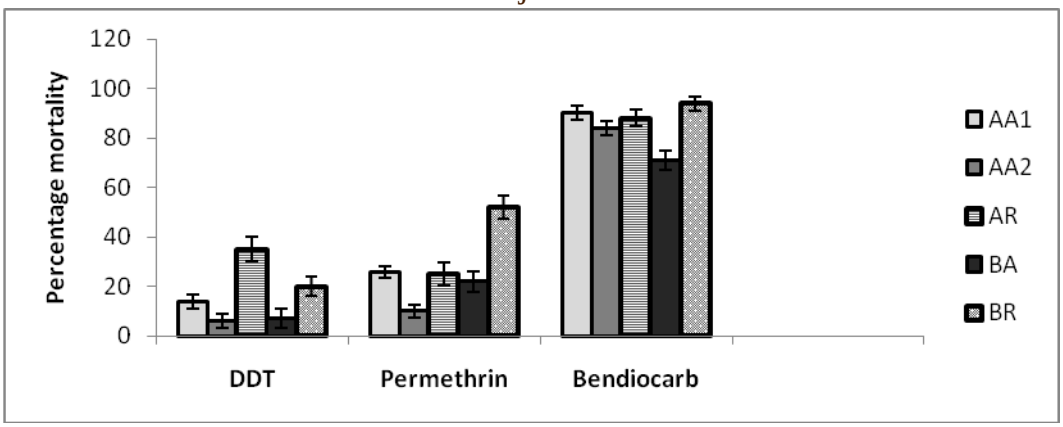

Figure 2 Insecticide mortality rate of *An. gambiae s.l.* 24 h post exposure in Nigeria collected in 2014 rainy season. Mosquitoes were exposed to 4% DDT, 0.75% permethrin and 0.1% bendiocarb in WHO susceptibility test at: Auyo agricultural 1(AA1); Auyo agricultural 2 (AA2); Auyo residential (AR); Bichi agricultural (BA) and Bichi residential (BR) sites

Table 1 Glutathione levels (nmol/mg protein) in the *An. gambiae* breeding in agricultural and residential sites in Northern Nigeria in 2013

Site	tGSH[a] (Total)	P=0.562	GSSG (Oxidized)	P=0.00	GSH (Reduced)	P=0.138	GSH: tGSH[b]	GSSG: GSH[c]	GSSG: tGSH[d]	Study Zone[e]
AA1	56.4 ± 8.9		12.6 ± 0.8		43.8 ± 8.1		0.78	0.29	0.22	A
AA2	116.4 ±10.1		26.2 ± 1.0		90.3 ± 9.1		0.78	0.29	0.22	
BA	96.1 ± 7.9		16.6 ± 0.1		79.5 ± 7.8		0.83	0.21	0.17	
AR	42.5 ± 5.7		8.9 ± 1.4		33.6 ± 4.3		0.79	0.27	0.21	B
BR	41.1 ± 4.3		9.3 ± 0.9		31.8 ± 3.4		0.77	0.29	0.23	

[a]Mean ± S.D for three determinations, [b, c, d] Ratios of reduced to total; oxidized to reduced and oxidized to total glutathione; AAI = Auyo agricultural 1; AA2 = Auyo agricultural 2; BA = Bichi agricultural; AR= Auyo residential; BR = Bichi residential; [e]Zone : A = intensive agriculture; B = residential breeding ecologies

Table 2 Glutathione levels (nmol/mg protein) in the *An.gambiae* breeding in agricultural and residential sites in Northern Nigeria in 2014

Sites	tGSH[a] (Total)	P=0.562	GSSG (Oxidized)	P=0.000	GSH (Reduced)	P=0.138	GSH: tGSH[b]	GSSG: GSH[c]	GSSG: tGSH[d]	Study Zone[e]
AA1	113.0 ± 0.3		25.4 ± 0.1		87.7 ± 0.2		0.78	0.29	0.22	A
AA2	111.3 ± 1.4		25.2 ± 0.0		86.1 ± 1.4		0.77	0.29	0.23	
BA	101.1 ± 4.5		25.3 ± 0.3		75.8 ± 4.2		0.75	0.33	0.25	
AR	114.3 ± 1.3		19.6 ± 0.1		94.7 ± 1.2		0.83	0.21	0.17	B
BR	116.0 ± 1.1		18.5 ± 0.2		97.5 ± 0.9		0.84	0.19	0.16	

[a]Mean ± S.D. for three determinations, [b, c, d] Ratios of reduced to total; oxidized to reduced and oxidized to total glutathione; AA1 = Auyo agricultural 1; AA2A = Auyo agricultural 2;; BA = Bichi agricultural; AR = Auyo residential; BR = Bichi residential; [e]Zone : A = intensive agriculture; B = residential breeding ecologies

The relative distribution of glutathione showed that the levels of tGSH and rGSH in Anopheline mosquitoes across the two study zones appeared to be similar while the levels of GSSG seemed to be higher in Anopheline mosquitoes from the study zone A (intensive agricultural study sites) compared to the study zone B (residential study sites). GSSG level in zone A in 2013 was about 2.03 fold higher than those of zone B. Similarly in Auyo GSSG level in zone A was about 2.21 fold greater than those of zone B while in Bichi GSSG level in zone A was about 1.82 fold higher than those of zone B. In 2014 the GSSG level in zone A was about 1.33 fold greater than those of zone B. In Auyo and Bichi for 2014 GSSG levels in zones A were 1.32 and 1.41 folds higher than those from zone B respectively.

To investigate the differential mean distribution of tGSH, GSSG and rGSH a one-way ANOVA in SPSS v 22 was used. The results show no significant differences (p=0.562 and 0.138) in the mean distribution of total and reduced glutathione levels across the study zones. However, the average distribution of oxidized glutathione across the study zones was statistically significant (p=0.000).

To evaluate the role of glutathione in insecticides resistance and the impact of its differential levels on insecticide resistance in *An. gambiae*, the relationship and correlations between the three forms of GSH levels and the mortality rates were examined. A statistical tool in SPSS v.22 was employed to investigate these relationships.

The results of the Pearson correlation analysis showed there were significant negative correlations between GSSG and mortality due to DDT exposure (P=0.050) in both agricultural and residential zones. Thus, increased GSSG levels observed correlate with higher insecticides resistance.

IV. DISCUSSION

The work determined the levels per mg protein of the different forms of GSH in *An. gambiae s.l.* collected from breeding sites located across the two study zones. The result obtained, shows more than 70% of the assayed GSH was present in the reduced-form across the sampling sites in zones A and B. Furthermore, no significant differences (p=0.562 and 0.138) in the mean distribution of total and reduced glutathione levels were observed across the study zones. However, the average distribution of GSSG across the study zones was higher in study zone A compared to zone B. Pearson correlation analysis showed that the GSSG was significantly correlated with mortality (p=0.050).

Extensive agricultural practice is suspected as a source of selection pressure for the adaptation to insecticides in *An. gambiae*. The higher resistance to insecticides by mosquito populations in agricultural sites could be due to the impact of agricultural pesticides. This is further supported by the higher levels of GSSG recorded in study zone A. This observation; higher GSSG levels is consistent with previous studies [15, 28, 29, 30]. In terms of mortality; lower mortality to insecticides implies high resistance to insecticides. The increased consumption of rGSH led to the build-up of higher levels of GSSG in zone A, which then correlated positively with resistance.

Observation from previous studies [9, 31, 32] have established increase in oxidative stress induced by xenobiotics overload as a source of generation and accumulation of GSSG, leading to lower GSH/GSSG ratio in various organisms. The high levels of the oxidized-form of GSH and lower-reduced form of GSH recorded, and the ratio of the reduced to oxidized-form of GSH indicate the redox state of the cell. It reflects the cells are under oxidative stress. Thus maintaining optimum GSH/GSSG ratio is essential to cell viability [3].

According to [33] the level of total and reduced glutathione may increase, reduce or may not change significantly under conditions of oxidative stress. However, levels of GSSG and the ratio between oxidized and reduced forms of glutathione is usually used as the more accurate indicator of the redox state of a cell, particularly in situations where no apparent and significant induction of the synthesis of glutathione occurred [31, 33]. Thus, finding from this study appeared to agree with these observations. Therefore, it could be argued that while significant induction of the synthesis of glutathione may not have occurred in *An. gambiae* sampled across the two zones; there was, however, a significant increase in utilization of rGSH in mosquito samples collected from breeding sites where higher environmental xenobiotics were recorded. The observed low changes in total and reduced glutathione contents in mosquitoes across the sampled sites despite differences in the levels of environmental chemical factors could probably be explained by the fact that glutathione is constitutively synthesized and abundantly available in all organisms (adjustable homeostatic balance). Moreover, it could be that the levels of reduced glutathione recorded in this study represent the normal threshold levels in *An. gambiae*. This implies that despite the recorded high resistance status in mosquitoes from agricultural sites where higher levels of xenobiotics were also recorded, the levels of the glutathione was sufficient for its role in the overall detoxification process. This finding is consistent with that reported by [30]. [34] highlighted that levels, availability and activities of glutathione responds to changes in oxidative stress induced by xenobiotics overload. It could be stated that the sources of the oxidative stress in the *An. gambiae* sampled may not be sufficient enough to cause the induction of glutathione synthesis above the threshold levels even though they were able to select for the emergence of *An. gambiae* that is highly resistant to most insecticides.

The rGSH is being converted to the GSSG form during oxidative stress and/or metabolic detoxification processes. The depletion of GSH due to oxidative stress reported in this study is consistent with previous study reported by [35] in the American cockroach and similar pattern were found in mammals [36, 37, 38] that the response of a cell to a stress often involves changes in GSH content, which may first be consumed in reactions that protect the cell leading to the formation of GSSG suggesting the process is highly conserved among organisms including *An. gambiae*.

It is not clear whether DDT is the only insecticide where glutathione can inform the resistance status because DDT resistance has settled in the areas under study; however the data of the bioassays shows that resistance to the other two insecticides especially Bendiocarb has not settled in these areas. So is this because resistance to these insecticides has not settled that is why significant effect of GSSG was not observed? So is it a transition or because glutathione can only tell resistance manifestation that has settled due to a particular class of insecticide like DDT? The correlation between resistance status and GSSG levels indicates resistance has settled while no correlation indicates resistance has not settled yet. Mosquito control in areas where resistance has settled due to a particular insecticide such as DDT would not yield the desired results. Therefore, similar studies covering larger area and longer period are needed to fully establish this.

CONCLUSION

The study highlighted glutathione status may be mediating the response, tolerance and possibly adaptation of *An. gambiae* exposure to insecticide. The results of this study suggest a close relationship between an increase in GSSG level and resistance status of *An. coluzzii* (*An. gambiae* M-form) from agricultural sites than in *An. coluzzii* from residential sites. Methods of detecting insecticide resistance are cumbersome involving WHO bioassay, biochemical and molecular assays. Our results indicate that measuring the levels of GSH in the mosquitoes sampled could possibly be used as a tool to assess and detect the insecticide resistance status of *An. gambiae s.l.* population.

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References

[1] Zhou, Y., Harrison, D.E., Love-Myers, K. et al., (2014). Genetic analysis of tissue GSH concentration Redoxbalance. Free Radical Biol Med 71: 157-164

[2] Montero, D., Tichibane, C., Winther, J. R., Appenzeller-Herzog (2013). Intracellular GSH Pools are heterogeneously concentrated. Redox Biology 1: 508-513

[3] Kalinina, E.V., Chernov, N.N., Novichkova, M.D. (2014). Role of GSH, GST and GrX in regulation of redox-dependent process. Biochemistry (Moscow), 79(13): 1562-1583
[4] Franco, R., Schoneveld, O. J., Pappa, A., Panayiotidis, M. I. (2007): The central role of glutathione in the pathophysiology of human diseases. *Arch Physiol Biochem* 113: 234–258
[5] Milne, G. L., Zanoni, G., Porta, A., Sasi, S., Vidari, G., Musiek, E. S., Freeman, M. L., Morrow, J. D. (2004):The cyclopentenone product of lipid peroxidation, 15-A (2t)-isoprostane, is efficiently metabolized by HepG2 cells via conjugation with glutathione. *Chem. Res. Toxicol*. *17*: 17 – 25
[6] Strange, R. C., Jones, P. W., Fryer, A. A. (2000): Glutathione S-transferase: genetics and role in toxicology. *Toxicol Lett,* 112 – 113: 357 – 363
[7] Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J. (2007): Free radicals and antioxidants in normal physiological functions and human disease, *Int. J. Biochem. Cell Biol.* 39*:* 44–84
[8] Sato, K., Yama, K., Murao, Y., Tatsunami, R., Tampo, Y. (2014): Epalrestat increases intracellular glutathione levels in Schwann cells through transcription regulation. *Redox Biology* 2: 15–21
[9] Babu, M.N., Padmaja, V. (2014). Changes in thiol content, GSH + H2O2 levels as ameasure of oxidative stress in Periplaneta Americana treated with metarhizium anisopliae. IJABPT, 5(1), 150- 157
[10] Caito, S.W., Schner, M.A. (2015).Quantification of GSH in C. elegans *Curr Protoc Toxicol* 64 (618):6.18.1- 6.18.6
[11] Albrechj, S.C., Barata, A.G., Grobhans, J. et al., (2011). In vivo mapping of H_2O_2 and oxidized GSH reveals chem + regional specificity of redox homeostasis. *Cell metabolism*, 14: 819-829
[12] Cutler, G. C. (2013). Insects, Insecticides and hormesis : evidence and considerations for study *Dose –Response*, 11 : 154- 177
[13] Duquesne, S., Lies, M. (2010). Indirect effects of pesticides on Mosquito larvae via alteration of community structure. *Israel J. Ecol Evolution*, 56: 433-447
[14] Mouchet, J. (1988). Agric + vector resistance. *Insect Sci Applic*, 9 (3): 297-302
[15] Mutura, E. J., Mwangangi, J., Shililu, J. et al., (2007). Mosquito species succession + physicochem factors affecting their abundance in rice fields in Mwea, *Kenya J Med Entomol* 44 (2) : 336-344
[16] Darriet, F., Rossignol, M., Chandre, F. (2012). The combination of NPK fertilizer + deltamethrin insecticide favours the proliferation of pyrethroid-resistant *An. gambiae. Parasites*, 19 : 156-164
[17] Reid, M. C., Mckenzi, F. E. (2016). The contribution of agic insecticide resistance in African Malaria Vectors. *Malar J*, 15 : 107
[18] Ibrahim, S. S., Yayo, A. M., Tukur, Z., Irving, H. and Wondji, C. S. (2014): High frequency of kdr L1014F is associated with pyrethroid resistance in *Anopheles coluzzii* in Sudan savannah of northern Nigeria. *BMC Infectious Diseases*, 14: 441
[19] Alhassan, A. J., Sule, M. S., Dan Gambo, M. A., Yayo, A. M., Safiyanu, M. and Sulaiman, D. (2015):Detoxification Enzymes Activities in DDT and Bendiocarb resistant and susceptible malarial vector (*Anopheles gambiae)* breed in Auyo residential and irrigation sites, northwest Nigeria *European Scientific Journal*, 11: 9
[20] Habibu, U. A., Yayo, A. M. and Yusuf, Y. D. (2017): Susceptibility status of *Anopheles gambiae* complex to Insecticides commonly used for Malaria control in Northern Nigeria. *International Journal of Scientific & Technology Research (IJSTR).* Vol. 6, Issue 6, 47-54 www.ijstr.org
[21] Service, M. W. (1999): Medical entomology for students. Chapman and Hall, London, 278pp
[22] Das, S., Garver, L., Dimopoulos, G. (2007): Protocol for mosquito rearing (*An. gambiae*). *J Vis Exp*, Issue 5: 221
[23] Abram, F., Gunnigle, E., Flaherty, V. O. (2009): Optimisation of protein extraction and 2-DE for metaproteomics of microbial communities from anaerobic wastewate treatment biofilms *Electrophoresis*, 30, 4149 4151
[24] Tietze, F. (1969): Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues, *Anal. Biochem*, 27: 502–522
[25] Hazelton, G. A., Lang, C. A. (1978): Glutathione determination in the mosquito. *Fed Proc* 37: 6, Abstr 2378
[26] Griffith, O. W. (1980): Determination of Glutathione and Glutathione Disulfide using Glutathione Reductaseand 2-Vinylpyridine, *Analytical Biochemistry*, 106, 207-212
[27] Habibu, U. A. (2015): A role for glutathione and its biosynthetic genes in *Anopheles gambiae* response to insecticides. A paper (Oral) presented at the European Biotechnology Congress 2015 held in Bucharest, Romania, from 7 – 9 May, 2015.
[28] Lipke, H. Chalkley, J. (1962): Glutathione, oxidized and reduced, in some Dipterans treated with 1, 1, 1-trichloro-2, 2-di-(p-chlorophenyl)-ethane. *Biochem J*, 85:104-109
[29] Oeriu, S., Tigheciu, M. (1964): Oxidized glutathione as a test of senescence, *Gerontologia*, 9: 9-17
[30] Imam, A. A. (2013): The role of physic-chemical environmental factors present in mosquito breeding ecologies as sources of selection pressure for the emergence and development of insecticides resistance in *Anopheles gambiae* from Northern Nigeria. *PhD thesis*, Abertay University Dundee, UK
[31] Araujo, A.R.T.S., Lucia, M.F.S. and Lima, L.F.C. (2008).Determination of the total and oxidize glutathione in human whole blood with a sequential injection analysis system. *Talanta* 74: 1511-1519
[32] Stephensen E., Sturve, J., Forlin, L. (2002): Effects of redox cycling compounds glutathione content and activities of glutathione-related enzymes in rainbow trout liver. *Comparative Biochemistry and Physiology part C, 133*: 435-442
[33] Chen, K. M., Gang H. J., Chen, G. C., Wang S. M., and Zhang C. L. (2004): Gradual drought under field conditions influences the glutathione metabolism, redox balance and energy supply in spring wheat. *J Plant Growth Regul*; 23:20-28
[34] Lu, S. C. (1999): Regulation of hepatic glutathione synthesis: current concepts and controversies, *FASEB J. 13*, 1169–1183
[35] Forgash, A. J. (1951):The effect of insecticides and other toxic substances upon reduced glutathione of *Periplaneta americana (L.) J. econ. Eat., 44*, 870-878.
[36] Dickinson, D. A., Levonen, A. L., Moellering, D. R., Arnold, E. K., Zhang, H. Q., Darley-Usmar, V. M. and Forman, H. J. (2004): Human Glutamate Cysteine Ligase Gene Regulation through the Electrophile Response Element, *Free Radical Biology and Medicine*, 37:8, 1152-1159
[37] Hashmat, U., Khan, M. F. and Hashmat, F. (2011): Determination of Glutathione concentration after its interaction with Cadmium Nitrate Tetra hydrate by using Ellman's modified method. *Gomal University Journal of Research*, 27: 2
[38] Khan, H., Khan, M. F., Khan, B. A., Abdul, W., Jan, S. U., Mukhtair, M., Ullah, N., Haque, N. and Farid, A. (2012): Oxidation of glutathione (GSH) in blood plasma due to oxidative stressors: A case study of Silver, *African Journal of Pharmacy and Pharmacology*, 6: 21