



HAL
open science

Larvicidal Activity of a Natural Botanical Biostop Moustiques® and Physiological Changes Induced in Susceptible and Resistant Strains of *Anopheles gambiae* Giles (Diptera: Culicidae)

Koffi M. Ahadji-Dabla, Jean-Luc Brunet, Guillaume Kétoh, Georges Y. Apétogbo, Isabelle A. Glitho, Luc Belzunces

► To cite this version:

Koffi M. Ahadji-Dabla, Jean-Luc Brunet, Guillaume Kétoh, Georges Y. Apétogbo, Isabelle A. Glitho, et al.. Larvicidal Activity of a Natural Botanical Biostop Moustiques® and Physiological Changes Induced in Susceptible and Resistant Strains of *Anopheles gambiae* Giles (Diptera: Culicidae). *Open Entomology Journal*, 2015, 9, pp.12-19. <10.2174/1874407901509010012>. <hal-02638221>

HAL Id: hal-02638221

<https://hal.inrae.fr/hal-02638221v1>

Submitted on 28 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Larvicidal Activity of a Natural Botanical Biostop Moustiques[®] and Physiological Changes Induced in Susceptible and Resistant Strains of *Anopheles gambiae* Giles (Diptera: Culicidae)

Koffi M. Ahadji-Dabla¹, Jean-Luc Brunet², Guillaume K. Ketoh^{1,*}, Georges Y. Apétogbo¹, Isabelle A. Glitho³ and Luc P. Belzunces^{2,*}

¹Unité de Recherche en Ecotoxicologie, Faculté des Sciences, Université de Lomé B.P.: 1515 (Togo); ²INRA, UR 406 A&E, Laboratoire de Toxicologie Environnementale, CS 40509, 84914 Avignon Cedex 9, France; ³Laboratoire d'Entomologie Appliquée, Faculté des Sciences, Université de Lomé, 01 B.P 1515, Lomé 01, Togo

Abstract: The larvicidal activity of Biostop Moustiques[®] (BM), a botanical biocide, was studied on susceptible and resistant strains of *Anopheles gambiae* s.s. at the concentrations of 1, 5, 10 and 20 mL/L of water. In addition to mortality and total protein concentration, the effect of BM on the activity of the following metabolic enzymes was evaluated in fourth instar larvae: glutathione-S-transferase (GST), alkaline phosphatase (ALP), superoxide dismutase (SOD), lactate dehydrogenase (LDH) and glucose 6-phosphate dehydrogenase (G6PDH). BM caused more than 90% mortality at different larval stages and had median lethal concentration (LC50) of 8.13 mL/L in susceptible Kisumu strain and 8.08 mL/L in resistant Acerkis strain. Protein concentration increased significantly in both strains when BM concentration was higher than 1 mL/L. GST and ALP activities increased significantly in both strains. LDH activity increased in Kisumu strain at 5 mL/L and decreased in Acerkis strain at all concentrations. G6PDH activity significantly increased with a maximum effect at 1 mL/L for Acerkis and 5 mL/L for Kisumu. BM completely suppressed SOD activity at 10 mL/L for Kisumu strain and 1 mL/L for Acerkis strain. This study showed that BM had a high larvicidal activity against both strains of *Anopheles gambiae* and it elicited a wide range of physiological changes.

Keywords: Biocide, Biostop Moustiques[®], larvae, metabolic enzymes, mosquitoes.

1. INTRODUCTION

Malaria, yellow fever, lymphatic filariasis and others are the most prevalent tropical vector-borne communicable diseases in West African sub-region where malaria is a major public health matter in children under five years and pregnant women [1]. In Togo, a mortality rate of 19% in patients hospitalized for malaria was reported in 2009 [2]. One of the World Health Organization (WHO) control programs on malaria vector (*Anopheles gambiae* s.l) is designed to prevent people against infective malaria mosquitoes by reducing vector longevity, vector density and human-vector contacts [1]. The most powerful and broadly applied interventions in vector control are long-lasting insecticide-treated nets (LLINs), indoor residual sprays (IRS) and the larval source control particularly by environmental management.

The control of larval stages of mosquitoes involves extensive and indiscriminate applications of synthetic insecticides that lead to environmental and health concerns, widespread development of resistance by mosquitoes and

unwarranted toxic or lethal effect on non-target organisms [3, 4]. Hence, more attention has been focused on botanicals that are likely not to induce adverse environmental and health effects. A large number of plant-based products has been reported to have larvicidal or repellent activity towards mosquitoes [5-7]. Many studies have demonstrated that they are effective, eco-friendly, easily biodegradable, cheap and seem to be one of the possible alternatives to synthetic insecticides [8, 9].

Botanicals with larvicidal activity such as seeds oils, kernel extracts, leaves, roots and bark of plants have been used against mosquitoes in India, Nigeria, Brazil and many other countries [6, 10-12]. However, in Togo, no report has been yet documented on larvicidal activity of botanicals against mosquito vectors.

This study was designed to investigate the larvicidal activity of a botanical insecticide, Biostop Moustiques[®] (BM), in susceptible (Kisumu) and resistant (Acerkis, resistant to organophosphate and carbamate insecticides) strains of the mosquito *Anopheles gambiae* s.s. The mode of action of this biocide was also approached by exploring physiological changes reflected by the modulation of five enzymes, superoxide dismutase (SOD), alkaline phosphatase (ALP), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH).

Superoxide dismutase converts the superoxide anion O₂⁻ into H₂O₂ and is considered together with catalase as a pri-

*Address correspondence to these authors at the INRA, UR 406 A&E, Laboratoire de Toxicologie Environnementale, CS 40509, 84914 Avignon Cedex - France; Tel: +33 (0)432 722 604; Fax: +33 (0)432 722 602; E-mail: luc.belzunces@avignon.inra.fr (Luc P. Belzunces)

Université de Lomé, Faculté des Sciences, Unité de Recherche en Ecotoxicologie, 01 B.P 1515, Lomé 01, Togo; Tel: +228 33 36 35 34 /22 25 50 94; Fax: +228 22 25 87 84 / 22 21 85 95; E-mails: gketoh@univ-lome.tg and guillaume.ketoh@gmail.com (Guillaume K. Ketoh)

mary defense against oxidative stress [13]. GST contributes to detoxification processes, by conjugating glutathione to xenobiotics, and to the defense against oxidative stress [14-18]. Alkaline phosphatase catalyzes the hydrolysis of phosphate monoesters and is involved in the transport of molecules and cell signaling [19-21]. G6PDH is the first and regulator key enzyme of the pentose phosphate pathway that contributes to the defense against oxidative stress and xenobiotic metabolism by generating NADPH (reduced nicotinamide adenine dinucleotide phosphate) [22]. NADPH is a biological reducer and the cosubstrate of glutathione reductase, that regenerates the reduced glutathione, and cytochrome P-450 reductase, that regenerates the detoxifying enzymes cytochromes P-450 [23]. LDH is involved in carbohydrate metabolism through conversion of pyruvate into lactate using NADH (reduced nicotinamide adenine dinucleotide) [24].

2. MATERIALS AND METHODS

2.1. Insecticide and Tested Organisms

The insecticide used in this study was Biostop Moustiques®, a 100% natural oil of coconut and some additional foodstuffs. It was developed in France in 1992 and was used as cutaneous insect repellent [25]. This biocide was kindly provided by Investekgroup Company in Lomé (Togo).

Two laboratory strains of *An. gambiae* s.s. were used for the experiments: Kisumu, a susceptible strain, and Acerkis, a resistant strain. The *An. gambiae* susceptible reference strain, Kisumu, was collected in Kenya in 1953 and has been maintained for many years under laboratory conditions [26]. The homozygous Acerkis strain, resistant to organophosphorous and carbamate insecticides, was obtained by introgression of the resistant *ace-1* G119S allele into the Kisumu's genome through successive backcrosses. *Ace-1* G119S allele was obtained from a sample of resistant *An. gambiae* population collected in Bobo-Dioulasso (Burkina Faso) in 2002 [27]. Eggs of both strains were provided by the "Laboratoire de lutte contre les insectes nuisibles" of IRD (Montpellier, France). Eggs and larvae were reared under laboratory conditions ($27 \pm 2^\circ\text{C}$; 70-75% relative humidity), under 12L:12D photoperiod cycles. The larvae were fed with TetraMin® Baby Fish food (from Tetra, Avignon, France). Second, third and fourth instar larvae were used for the larvicidal bioassay and the surviving fourth instar larvae were used to explore physiological modulations through enzyme activity.

2.2. Chemicals

Antipain, aprotinin, leupeptin, pepstatin A, soybean trypsin inhibitor, Triton X-100, reduced glutathione, 1-chloro-2,4-dinitrobenzene (DNCB), xanthine oxidase, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), nitroblue tetrazolium (NBT), *para*-nitrophenylphosphate (*p*-NPP), D-glucose-6-phosphate disodium, ethylenediaminetetraacetic acid tetrasodium salt (EDTA) and bovine serum albumin (BSA) were purchased from SIGMA-ALDRICH (Saint Quentin-Fallavier, France).

2.3. Larvicidal Bioassay

Second (L2), third (L3) and fourth (L4) instar larvae were separately introduced into vials containing 100 mL of

BM solutions at the concentration of 0, 1, 5, 10 and 20 mL/L in water containing 10 mg TetraMin® Baby fish food. Six replicates were made for each concentration and the whole experiment was independently repeated three times. Mortality rates were recorded after 24 h of exposure. Larvae were considered as dead if they failed to move after probing with a needle on the siphon or cervical region.

2.4. Larvae Sample Preparation and Protein Extraction

The different enzymes were assayed in fourth instar larvae exposed at each BM concentration because they were less susceptible and surviving individuals were obtained even at 20 mL/L. Fourth instar larvae ($n = 50-60$) were ground in a buffer containing 10 mM NaCl, 1% Triton X-100, 40 mM sodium phosphate pH 7.4 and 2 mg/ml antipain, leupeptin and pepstatin A, 25 units/ml aprotinin and 0.1 mg/ml soybean trypsin inhibitor as protease inhibitors [28] to obtain 10% (w/v) tissue extracts. Larvae were homogenized at 4°C with a high speed homogenizer Tissue-Lyser II (Qiagen®) for three periods of 30 sec, at 30-sec intervals. The tissue homogenates were centrifuged (Eppendorf® Centrifuge 5415 R) at 15,000 g for 20 min at 4°C . The supernatant was used for enzyme and protein assays using Tecan Infinite F 500 UV-visible spectrophotometer.

2.5. Enzyme Assays

All enzyme assays were performed in triplicate at 25°C . All enzyme activities were expressed as variations of absorbance units per min (AU/min).

Glutathione-S-transferase activity was determined in a reaction medium containing 1 mM EDTA, 2.5 mM reduced glutathione (GSH) and 1 mM 1-chloro-2,4-dinitrobenzene (DNCB) and 100 mM potassium phosphate pH 7.4 [29]. The conjugation reaction was monitored at 340 nm. Alkaline phosphatase activity was determined in a medium containing 20 μM (MgCl_2), 2 mM *p*-nitrophenol and 100 mM Tris-HCl pH 8.0 [30]. The hydrolysis reaction was monitored at 410 nm. Superoxide dismutase activity was determined in a medium containing 25 μM of NBT, 100 μM of xanthine, 0.833 units/mL xanthine oxidase and 100 mM sodium phosphate/carbonate pH 7.8 [31]. The dismutation reaction was monitored at 560 nm. SOD activity corresponded to the difference in NBT reduction between assays performed in the absence and in the presence of tissue extract. Lactate dehydrogenase activity was determined in a reaction medium containing 5 mM EDTA, 0.2 mM β -NADH, 2 mM sodium pyruvate and 50 mM triethanolamine pH 7.6. The reaction was monitored at 340 nm. Glucose-6-phosphate dehydrogenase activity was determined in a medium containing 1 mM D-glucose-6-phosphate, 0.5 mM β -NADP⁺, 10 mM MgCl_2 and 100 mM Tris-HCl pH 7.4. The reaction was monitored at 340 nm.

2.6. Protein Assay

Protein concentrations were estimated using the method developed by Bradford [32] with bovine serum albumin (BSA) as the standard.

2.7. Data Analysis

The dose-response relationships obtained from bioassays were subjected to a probit analysis for calculating LC_{50} and

LC₉₀ at 95% confidence interval for upper and lower limits, according to Finney [33], using PoloPlus version 1.0 (LeOra Software). Statistical analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). Values were analyzed by one-way analysis of variance (one-way ANOVA) and a Tukey's multiple comparison was used as a post test. A p -value < 0.05 was considered statistically significant. Data were expressed as means \pm standard deviations (SD).

3. RESULTS

3.1. Larvicidal Activity of Biostop Moustiques[®]

The larvicidal activity of BM was studied on second (L2), third (L3) and fourth (L4) instar larvae. In L2 (Fig. 1), the lowest concentration of 5 mL/L induced $22.2 \pm 2.6\%$ and $13.0\% \pm 0.0$ mortality in Kisumu and Acerkis strains, respectively. At 10 mL/L, $52.8 \pm 1.9\%$ and $23.9 \pm 1.0\%$ mortality were recorded in Kisumu and Acerkis strains, respectively. At these two concentrations, mortalities were significantly higher in Kisumu than in Acerkis ($F_{(9,29)} = 2.26.10^3$, $p < 0.001$). At 20 mL/L, 100% mortality was observed in Kisumu strain and no significant difference was registered with Acerkis strain ($p = 0.181$).

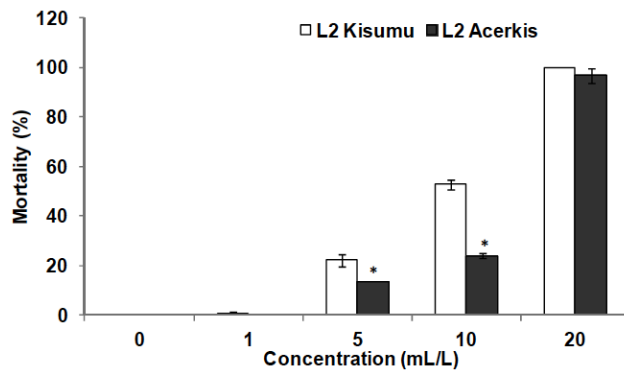


Fig. (1). Toxicity of Biostop Moustiques[®] to third second larvae of *An. gambiae* s.s. Second instar larvae were exposed for 24 h to BM at different concentrations. After 24 h of exposure, mortality was recorded. Asterisks indicate statistically significant differences between Kisumu and Acerkis strains for a same concentration (* $p < 0.05$).

In L3 (Fig. 2), $11.7 \pm 1.7\%$ and $7.2 \pm 1.0\%$ mortality were respectively observed in Kisumu and Acerkis strains at 5 mL/L ($F_{(9,29)} = 3.5. 10^3$, $p = 0.009$). At 10 mL/L, $73.3 \pm 2.9\%$ and $53.9 \pm 1.9\%$ mortality were observed in Kisumu and Acerkis strains, respectively ($F_{(9,29)} = 3.5. 10^3$, $p < 0.001$). At 20 mL/L, 100% mortality was induced in both strains.

In L4 (Fig. 3), no difference was observed between strains at each concentration ($F_{(9,29)} = 247.35$, $p = 1.0$) (Fig. 3). The highest effects were elicited at 20 mL/L and were $91.0 \pm 6.5\%$ and $91.0 \pm 1.7\%$ mortality in Kisumu and Acerkis strains, respectively.

The LC₅₀ values were assessed in larvae of the three developmental stages (Table 1). In Kisumu strain, LC₅₀ values were 9.8, 7.82 and 8.13 mL/L for L2, L3 and L4, respectively. In Acerkis strain, LC₅₀ values were 13.9, 9.15 and 8.08 mL/L, for L2, L3 and L4, respectively. In Kisumu

strain, LC₉₀ values were 17.82, 12.46 and 20.34 mL/L for L2, L3 and L4, respectively. In Acerkis strain, LC₉₀ values were 19.3, 14.74 and 20.02 mL/L for L2, L3 and L4, respectively. It is noteworthy that the dead larvae showed necrosis of tissues.

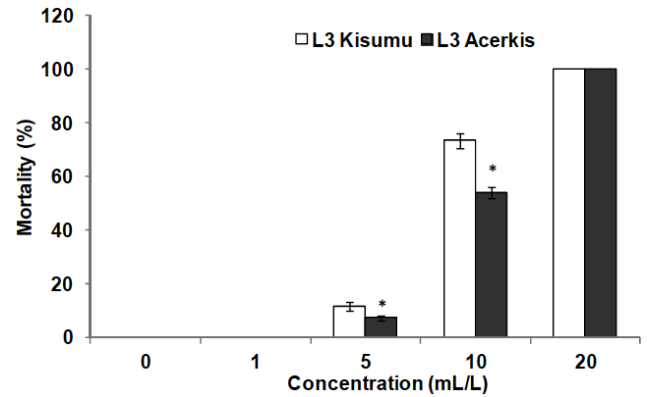


Fig. (2). Toxicity of Biostop Moustiques[®] to third instar larvae of *An. gambiae* s.s. Third instar larvae were exposed for 24 h to BM at different concentrations. After 24 h of exposure, mortality was recorded. Asterisks indicate statistically significant differences between Kisumu and Acerkis strains for a same concentration (* $p < 0.05$).

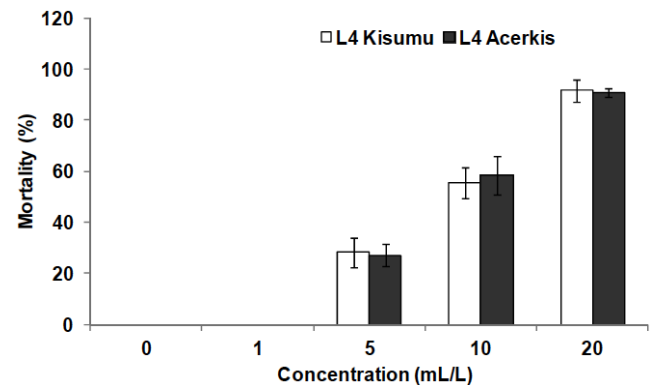


Fig. (3). Toxicity of Biostop Moustiques[®] to fourth instar larvae of *An. gambiae* s.s. Fourth instar larvae were exposed for 24 h to BM at different concentrations. After 24 h of exposure, mortality was recorded. No statistically significant differences were observed between Kisumu and Acerkis strains for a same concentration (* $p < 0.05$).

3.2. Physiological Effects of Biostop Moustiques[®]

In Kisumu, GST activity increased at 10 and 20 mL/L. It was 47.43 ± 3.92 AU/min/mg of tissue for controls, and 50.17 ± 3.57 , 65.61 ± 5.71 and 72.40 ± 10.84 AU/min/mg of tissue at 5, 10 and 20 mL/L, respectively (Fig. 4A). ALP activity increased at all doses. It was 13.77 ± 0.95 AU/min/mg of tissue for controls, and 17.20 ± 0.70 , 20.28 ± 1.55 , 20.13 ± 2.38 and 20.68 ± 2.29 AU/min/mg of tissue at 1, 5, 10 and 20 mL/L, respectively (Fig. 4B). SOD activity did not change at 1 and 5 mL/L but completely disappeared at 10 and 20 mL/L. LDH increased only at 5 mL/L with an activity of 124.18 ± 9.69 AU/min/mg of tissue (Fig. 4D). G6PDH activity increased at all concentrations except at the highest. It was 3.67 ± 0.86 AU/min/mg of tissue for controls

Table 1. Larval toxicity of Biostop moustiques® to *Anopheles gambiae* s.s.

Strain	Larval stage	LC50 / LC90 (mL/L)	95% Confidence interval		Chi-square (χ^2)
			LC50	LC90	
Kisumu	L2	9.8 / 17.82	-	-	-
	L3	7.82 / 12.46	7.40 - 8.26	11.53 - 13.73	1.26
	L4	8.13 / 20.34	5.58 - 11.11	14.01 - 53.43	4.89
Acerkis	L2	13.9 / 19.3	-	-	-
	L3	9.15 / 14.73	6.98 - 12.19	11.32 - 31.16	7.05
	L4	8.08 / 20.02	7.39 - 8.78	17.53 - 23.70	1.99

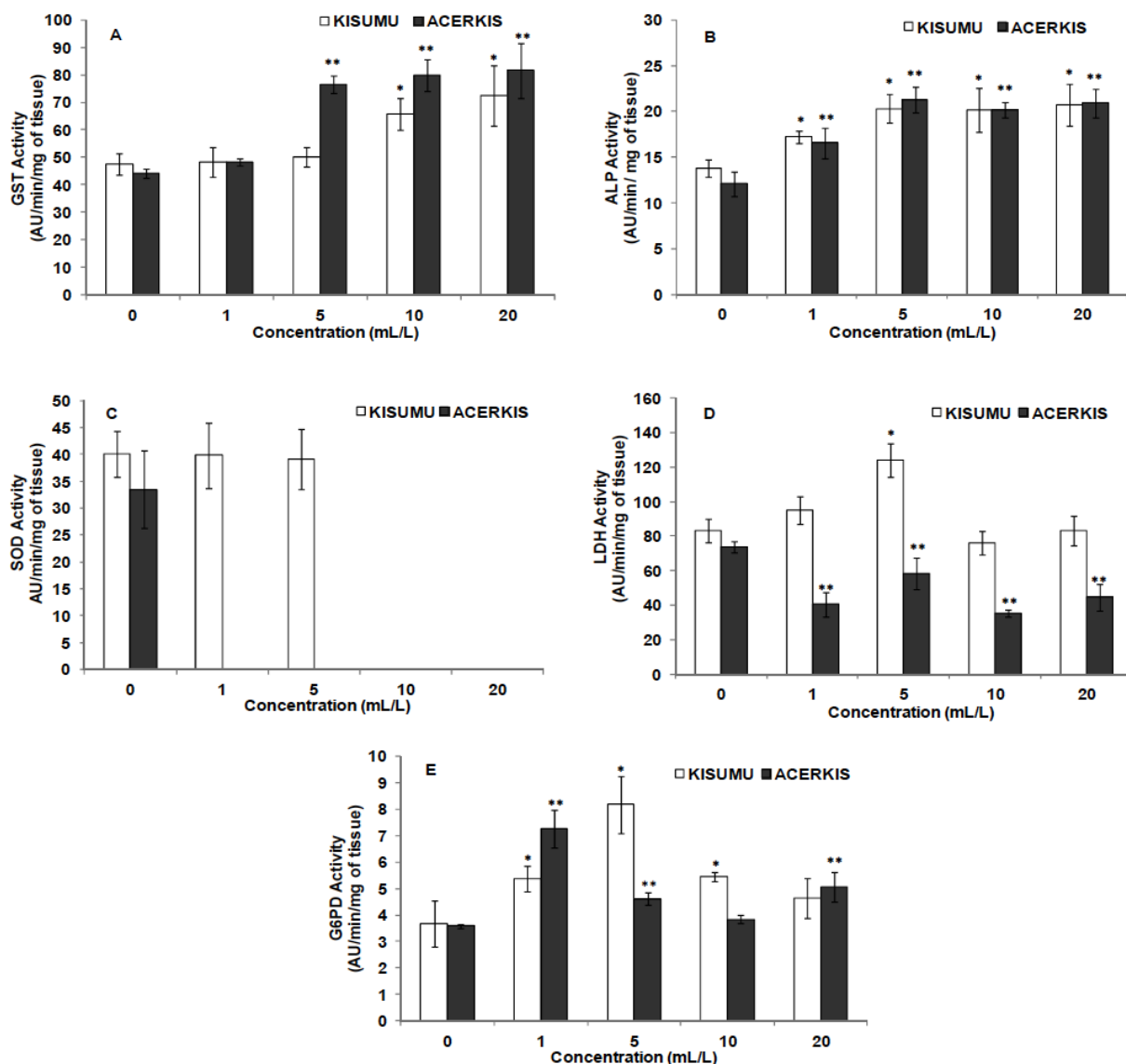


Fig. (4). Physiological changes induced by Biostop Moustiques®. Fourth instar larvae of susceptible and resistant strains of *An. gambiae* s.s. were exposed for 24 h to BM. After 24 h, surviving larvae were sampled and physiological modulations were explored by investigating tissue activities of GST (A), ALP (B), SOD (C), LDH (D) and G6PDH (E). Data corresponded to means \pm SD of tissue activities from three repetitions performed in hexaplicates. Asterisks indicate statistically significant differences between control and exposed larvae: [* Kisumu, $p < 0.001$ for all the enzymes except for GST: $p = 0.001$ at 10 mL/L, G6PDH: $p = 0.005$ at 1 mL/L and 0.003 at 10 mL/L, (** Acerkis, $p < 0.001$ for all enzymes except for LDH: $p = 0.003$ at 5 mL/L, G6PDH: $p = 0.003$ at 5 mL/L].

and 5.35 ± 0.48 , 8.17 ± 1.09 , 5.45 ± 0.18 and 4.64 ± 0.75 AU/min/mg of tissue at 1, 5, 10 and 20 mL/L, respectively (Fig. 4E).

In *Acerkis*, GST and ALP exhibited profiles close to those observed in Kisumu. GST activity increased at 5, 10 and 20 mL/L. It was 44.24 ± 1.77 AU/min/mg of tissue for controls, and 76.55 ± 3.05 , 79.97 ± 5.77 and 81.67 ± 10 AU/min/mg of tissue at 5, 10 and 20 mL/L, respectively (Fig. 4A). ALP activity increased at all concentrations. It was 12.07 ± 1.35 AU/min/mg of tissue for controls, and 16.53 ± 1.63 , 21.21 ± 1.40 , 20.11 ± 0.84 and 20.89 ± 1.56 AU/min/mg of tissue at 1, 5, 10 and 20 mL/L, respectively (Fig. 4B). Exposure to BM elicited the complete repression of SOD at all concentrations (Fig. 4C). LDH was 73.57 ± 3.21 AU/min/mg of tissue for controls and decreased at all concentrations. It was 40.39 ± 7.07 , 58.27 ± 9.04 , 35.21 ± 1.88 and 44.64 ± 7.69 AU/min/mg of tissue at 1, 5, 10 and 20 mL/L, respectively (Fig. 4D). G6PDH activity globally increased with an effect particularly marked at 1 mL/L. It was 3.58 ± 0.09 AU/min/mg of tissue for controls, and 7.26 ± 0.72 , 4.61 ± 0.23 , 3.84 ± 0.17 and 5.07 ± 0.56 AU/min/mg of tissue at 1, 5, 10 and 20 mL/L, respectively (Fig. 4E).

In both strains, the tissue protein contents increased at 5, 10 and 20 mL/L ($F_{(9,59)} = 46.21$, $p < 0.001$) (Fig. 5). Compared to the respective controls, this increase was more important in *Acerkis* than in Kisumu ($p < 0.001$). This general increase of protein contents resulted in modulation profiles of specific activity that were very similar to those of tissue activity (Fig. 6).

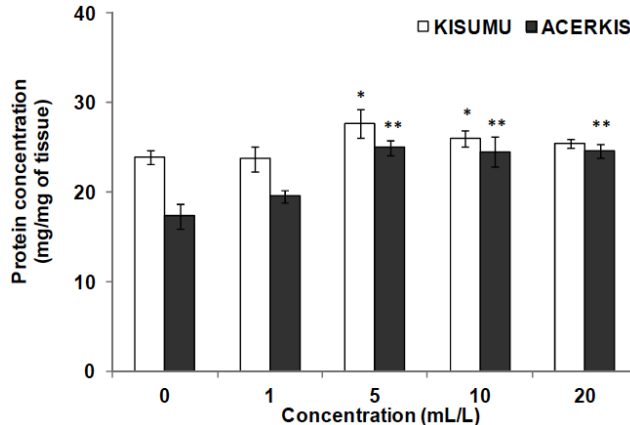


Fig. (5). Effect of Biostop Moustiques® on protein concentration. Fourth instar larvae of susceptible and resistant strains of *An. gambiae* s.s. were exposed for 24 h to BM. After 24 h, surviving larvae were sampled and analysed for protein contents. Data corresponded to means \pm SD of protein concentrations standardized to tissue mass from three repetitions performed in hexaplicates. Asterisks (* and **) indicate statistically significant differences ($p < 0.001$) between control and exposed (* and **: $p < 0.001$) except for Kisumu, $p = 0.027$ at 10 mL/L and *Acerkis* $p = 0.021$ at 1 mL/L.

4. DISCUSSION AND CONCLUSION

4.1. Larvicidal Activity of Biostop Moustiques®

In this study, the BM oil showed a larvicidal effect against different stages of *Anopheles gambiae*. It exhibits the same effect on L2, L3 L4 at 20 mL/L both in susceptible and

resistant strains of the malaria vector *An. gambiae*. At dose < 20 mL/L, it induces different effects in the two strains with a higher toxicity in Kisumu strains. In a preliminary bioassay, more than 90% mortality in fourth instar larvae of a wild population of *An. gambiae* s.l was elicited at BM concentration of 20 mL/L. BM presents different toxicity profiles in L2 and L3, with a higher toxicity at low concentrations in Kisumu. In L4, BM presents similar toxicity profiles and the LC50 obtained confirmed that L4 of both strains are similarly susceptible to BM. Besides, microscopic observation of the dead larvae showed necrosis of tissues. Some plant extracts that have insecticidal effect on mosquitoes are even used for water purification, as it is the case for *Moringa oleifera* [34]. The insecticidal activity of different plant extracts could have important implications in mosquito larvae control. However, further researches are needed to state on the effectiveness of the biocide on natural breeding mosquito strains as far as this study was only done on laboratory susceptible/resistant strains of *An. gambiae*. BM derived from coconut oil can be supposed to have non-toxic effect on human being and then need to be considered in search of alternatives to conventional insecticides.

Plants extracts contain secondary metabolites that have insecticidal, antifeedant or repellent activity in pests of agricultural and medical importance [7, 35, 36]. However, extracts from certain plants such as *Persea americana* or *Azadirachta indica* can elicit cell and tissue lysis and impairment of hormonal secretion in mosquitoes [37, 38].

Some studies seem to indicate a low toxicity of plant extracts to non-target species. It has been demonstrated that some plant extracts exhibit larvicidal properties but an absence of toxicity to vertebrates such as fishes or mice [7, 39].

4.2. Physiological Effects

Generally, insect resistant to insecticides often exhibits elevated GST activity regardless of the class of insecticides [40, 41]. GST has been revealed as a useful biomarker to detect exposures to metals, organic pollutants [18] and pesticides [42]. In this study, BM induces an increase of GST activity in both strains, which could be interpreted as a biological response to detoxify BM components. GST is also involved in the detoxification of lipid peroxides caused by oxidative stress [17]. However, exposure to BM results in a complete repression of SOD in both strains although SOD, with catalase, represents primary defenses against reactive oxygen species [43, 44]. Thus, an increase of GST could be a response against oxidative damages induced by BM components and increased by SOD repression elicited by BM.

Plant extracts may cause disorganization of midgut epithelium [37]. The insecticidal lectins from *Galanthus nivalis* and *Canavalia ensiformis* significantly increased the level of alkaline phosphatase activity in *Lacania oleracea* larvae [45]. In the present study, BM increases ALP activity in both mosquito strains regardless of the concentration, which could be a protective physiological response against the action of BM. However, this contrasts with the decrease elicited by azadirachtin in the larvae of two insect pests, *Cnaphalocrocis medinalis* and *Spodoptera litura* [46, 47]. Thus, the modulation of ALP could be interpreted rather in terms of a specific response towards a given stressor than in terms of a non-specific protective response.

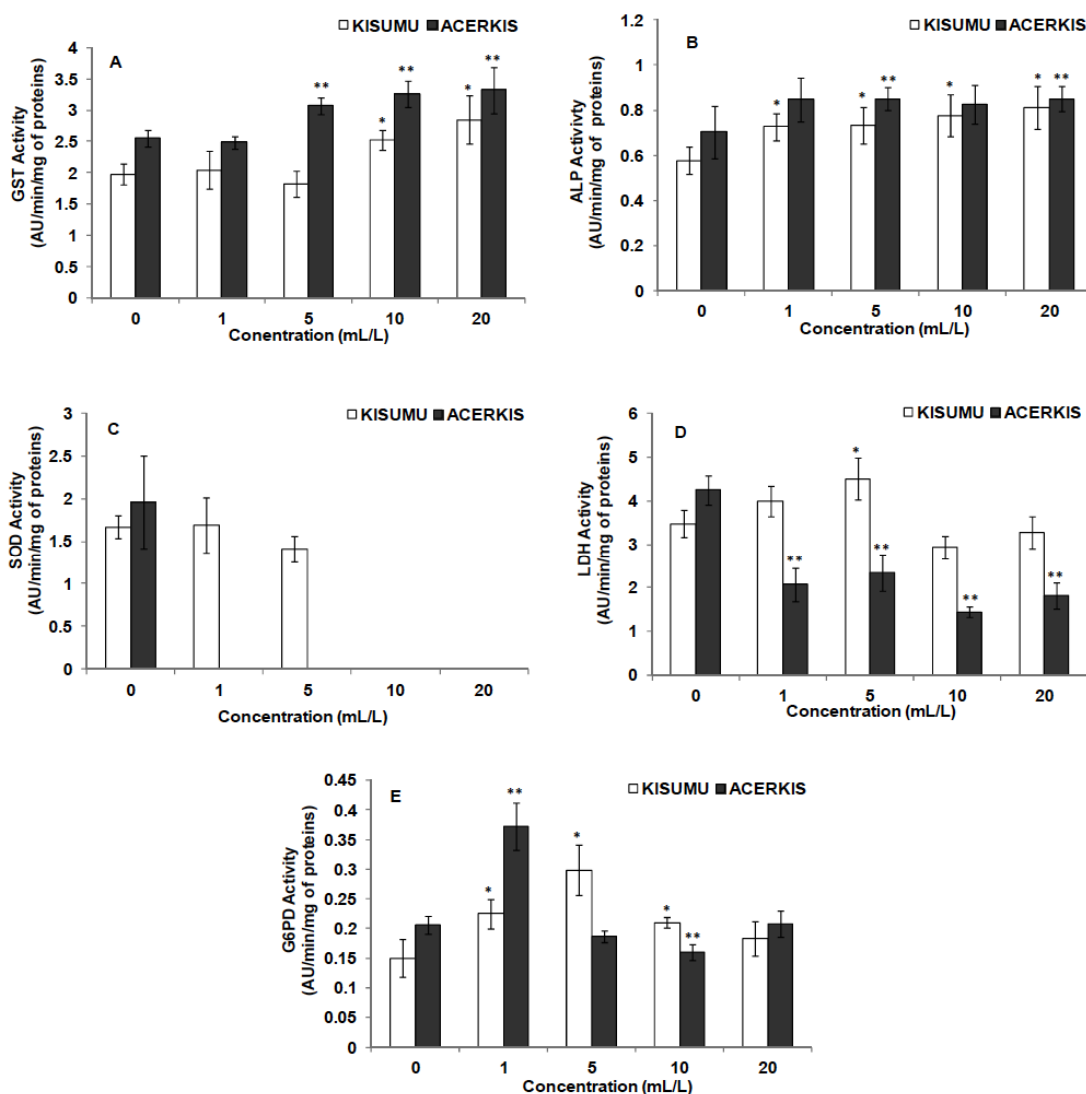


Fig. (6). Physiological changes induced by Biostop Moustiques®. Fourth instar larvae of susceptible and resistant strains of *An. gambiae* s.s. were exposed for 24 h to BM. After 24 h, surviving larvae were sampled and physiological modulations were explored by investigating specific activities of GST (A), ALP (B), SOD (C), LDH (D) and G6PDH (E). Data corresponded to means \pm SD of specific activities from three repetitions performed in hexaplicates. Asterisks indicate statistically significant differences between control and exposed larvae: [($*$ Kisumu, $p < 0.001$ for all the enzymes, except for GST: $p = 0.01$ at 10 mL/L, ALP: $p = 0.024$ at 1 mL/L, 0.016 at 5 mL/L and 0.002 at 10 mL/L, G6PDH: $p = 0.002$ at 1 mL/L and 0.002 at 10 mL/L), ($**$ Acerkis, $p < 0.001$ for all the enzymes, except for GST: $p = 0.003$ at 5 mL/L, ALP: $p = 0.43$ at 5 mL/L and 0.047 at 20 mL/L, G6PDH: $p = 0.012$ at 10 mL/L)].

LDH is an important glycolytic enzyme present in animal tissues [48]. LDH is widely used in toxicology and in clinical chemistry to diagnose cell, tissue and organ damages [49]. Its activity in insects treated with insecticides, either conventional or botanical, has been widely studied [50-52]. In the present study, LDH activity only increases at 5 mL/L of BM and does not change at the other concentrations in Kisumu strain. In Acerkis strain, LDH strongly decreases at all concentrations. This result is consistent with the large LDH decrease observed in *Schistocerca gregaria* exposed to neem limonoids, such as azadirachtin [49]. Because LDH is involved in energy production, the high decrease of LDH activity after treatment with BM could explain tissue necrosis observed in dead larvae.

The involvement of G6PDH in defense against oxidative stress has been investigated in mammals [53-55]. G6PDH is

involved in the production of the reducer/antioxidant coenzyme NADPH by catalyzing the transformation of glucose-6-phosphate into 6-phosphogluconolactone in the pentose phosphate pathway [56]. This reaction is increased in oxidative conditions by an inhibition of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) by reactive oxygen species [57]. This results in the shift of glucose-6-phosphate from glycolysis towards the pentose phosphates pathways. Thus, the increase of G6PDH is consistent with an oxidative stress induced by BM because the complete repression of SOD deprives larvae from one of the most efficient antioxidant defense.

BM elicits tissue necrosis and more than 90% mortality in larvae of susceptible and resistant strains of *An. gambiae*. BM also induces drastic physiological changes that sign, among other phenomena, an oxidative stress. The induction

of an oxidative stress is particularly significant in *Acerkis* resistant strain because this latter is supposed to be better equipped to fight against oxidative conditions.

In this study, we have tested BM, a coconut oil derived biocide, and have used only laboratory strains of *An. gambiae*, a malaria vector. Thus, taking into account the very encouraging results, testing BM with wild strains appears to be particularly relevant.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Fabrice Chandre (IRD, Montpellier, France) for providing mosquito eggs, Marianne Cousin and the technical staff of the *Laboratoire de Toxicologie Environnementale* (INRA, Avignon, France). We also thank the technical staff of the *Unité de Recherche en Ecotoxicologie* (URET/UL, Lomé, Togo). The first author is grateful to the *Service de Coopération et d'Action Culturelle* of the French Embassy in Lomé (Togo) for its financial support for the doctoral training scholarship granted to him.

REFERENCES

- [1] World Health Organization. The World Malaria Report 2010. Geneva: World Health Organization 2010.
- [2] PNL. Rapport d'activité du Programme National de Lutte contre le Paludisme (Togo), 2009; p. 60.
- [3] Roberts DR, Andre RG. Insecticide resistance issues in vector-borne disease control. *Am J Trop Med Hyg* 1994; 50 (suppl): 21-34.
- [4] Nauen R. Insecticide resistance in disease vectors of public health importance. *Pest Manag Sci* 2007; 63: 628-33.
- [5] Mullai K, Jebanesan A. Larvicidal, ovicidal and repellent activities of leaf extract of two cucurbitaceous plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Trop Biomed* 2006; 24:1-6.
- [6] Ferreira PMP, Carvalho AFU, Farias DF, et al. Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. *Ann Brazilian Acad Sci* 2009; 81: 207-16.
- [7] Shivakumar MS, Kataria R. Comparative efficacy of Azadirachtin on larval population of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae) in Gujarat, India. *Int J Pharm Bio Sci* 2011; 2: 41-7.
- [8] Obamanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI. Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus*. *Afr J Biotechnol* 2006; 5: 761-5.
- [9] Senthilkumar N, Varma P, Gurusubramanian G. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* Liston. *Parasitol Res* 2009; 1042: 237-44.
- [10] Sharma NN, Qadry JS, Subramaniam B, et al. Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Pharm Biol* 1998; 36: 3-7.
- [11] Ojewole JAO, Rahim S, Shode FO. Mosquito larvicidal properties of aqueous extract of *Senna didymobotrya*. *Nig J Nat Prod Med* 2000; 4: 46-7.
- [12] Sosan MB, Adewoyin FB, Adewunmi CO. Larvicidal properties of three indigenous plant oils on the mosquito *Aedes aegypti*. *Nig J Natl Prod Med* 2001; 5: 30-3.
- [13] Jaiswar SP, Sachan R, Singh RK, Agarwal M. Free radicals in female infertility. *J Obstet Gynecol India* 2006; 56: 64-7.
- [14] Hayes JD, Wolf CR. Role of glutathione transferase in drug resistance. In: Sies H, Ketterer B, Eds. *Glutathione conjugation: mechanisms and biological significance*: Academic Press London 1988; pp. 315-55.
- [15] Mannervik B, Danielson UH. Glutathione transferases: structure and catalytic activity. *CRC Crit Rev Biochem* 1988; 23: 283-37.
- [16] Pickett CB, Lu AY. Glutathione S-transferases: gene structure, regulation, and biological function. *Annu Rev Biochem* 1989; 58: 743-64.
- [17] Leaver MJ, George SG. A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation. *Mar Environ Res* 1998; 46: 71-4.
- [18] Yang Y, Cheng JZ, Singhal SS, et al. Role of glutathione S-transferases in protection against lipid peroxidation: overexpression of hGST A2-2 IN K562 cells protects against hydrogen peroxide-induced apoptosis and inhibits Jnk and caspase 3 activation. *J Biol Chem* 2001; 276: 19220-30.
- [19] Coleman J. Structure and mechanism of alkaline phosphatase. *Annu Rev Biophys Biomol Struct* 1992; 21: 441-83.
- [20] Vlahovic M, Lazarevic J, Peric-Mataruga V, Ilijin L, Mrdakovic M. Plastic responses of larval mass and alkaline phosphatase to cadmium in the gypsy moth larvae. *Ecotoxicol Environ Saf* 2009; 72: 1148-55.
- [21] McComb RB, Bower GG, Posen S. *Alkaline phosphatases*. New York: Plenum Press. 1979.
- [22] Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; 35: 1147-50.
- [23] Taylor M, Lamb DC, Cannell RJP, Dawson MJ, Kelly SL. Cofactor recycling with immobilized heterologous cytochrome P450 105D1 (CYP105D1). *Biochem Biophys Res Commun* 2000; 279: 708-11.
- [24] Wu RSS, Lam PKS. Glucose-6-phosphate dehydrogenase and lactate dehydrogenase in the green-lipped mussel (*Perna viridis*). Possible biomarker for hypoxia in the marine environment. *Water Res* 1997; 31: 2797-801.
- [25] Sorge F, Tamburro M, de Pertat T, les membres du Groupe de pédiatrie tropicale. Usages et effets des insectifuges cutanés chez les nourrissons lors de l'épidémie de chikungunya à La Réunion en 2005-2006: étude INR 2009. 2011; *BEH* 6/22 février 2011.
- [26] Alout H, Djogbénou L, Berticat C, Chandre F, Weill M. Comparison of *Anopheles gambiae* and *Culex pipiens* acetylcholinesterase 1 biochemical properties. *Comp Biochem Physiol B* 2008; 150: 271-7.
- [27] Djogbénou L, Weill M, Hougard JM, Raymond M, Akogbeto M, Chandre F. Characterization of insensitive acetylcholinesterase (*ace-IR*) in *Anopheles gambiae* (Diptera: Culicidae): resistance levels and dominance. *J Med Entomol* 2007; 44: 805-810.
- [28] Belzunces LP, Toutant JP, Bounias M. Acetylcholinesterase from *Apis mellifera* head. Evidence for amphiphilic and hydrophilic forms characterized by TritonX-114 phase separation. *Biochem J* 1988, 255: 463-470.
- [29] Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. *J Biol Chem* 1974; 249: 7130-9.
- [30] Bounias M, Kruk I, Nectoux M, Popeskovic D. Toxicology of cupric salts on honeybees. V. Gluconate and sulfate action on gut alkaline and acid phosphatases. *Ecotoxicol Environ Saf* 1996; 35: 67-76.
- [31] McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythro-cuprein (hemocuprein). *J Biol Chem* 1969; 244: 6049-55.
- [32] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1976; 72: 248-54.
- [33] Finney DJ. *Probit Analysis*. 3rd ed. London: Cambridge University Press 1971.
- [34] Schwarz D. Water clarification using *Moringa oleifera*. Eschborn: JDWH Information Service 2000, [Online] Available from: <http://www.gtz.de/gate/gateid.afp>
- [35] Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol Res* 2008; 103: 1383-90.
- [36] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; 1: 124-9.

- [37] Koua HK, Han SH, d'Almeida M-A. Histopathology of *Anopheles gambiae* s.l. Giles, 1902 (Diptera, Culicidae) subjected to the larvicidal activity of the aqueous extract of *Persea americana* Miller, 1768 (Lauraceae). Bull Soc Pathol Exot 1998; 91: 252-6.
- [38] Seye F, Ndione RD, Ndiaye M. Etude comparative de deux produits de neem (huile et poudre) sur les stades préimaginaux du moustique *Culex quinquefasciatus* (Diptera: Culicidae). Afr Sci 2006; 2: 212-25.
- [39] Promsiri S, Naksathit A, Kruatrachue M, Thavaara U. Evaluation of larvicidal activity of medicinal plants extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on non-target fish. Insect Sci 2006; 13: 179-88.
- [40] Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW, Sun CN. Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamond back moth, *Plutella xylostella*. Insect Biochem Mol Biol 1998; 28: 651-8.
- [41] Vontas JG, Small GJ, Hemingway J. Glutathione S-transferases as antioxidant defence agents confere pyrethroid resistance in *Nilaparvata lugens*. Biochem J 2001; 357: 65-72.
- [42] Taysse L, Chambras C, Marionnet D, Bosgiraud C, Deshaux P. Basal level and induction of cytochrome P450, EROD, UDPGT and GST activities in carp (*Cyprinus carpio*) immune organs (spleen and kidney). Bull Environ Contam Toxicol 1998; 6: 300-5.
- [43] Munday R, Winterboume CC. Reduced glutathione in combination with superoxide dismutase as an important biological antioxidant defense mechanism. Biochem Pharmacol 1989; 38: 4349-52.
- [44] Sies H. Oxidative stress: from basic research to clinical application. Am J Med 1991; 91: 313-23.
- [45] Fitches E, Gatehouse JA. A comparison of the short and long term effects of insecticidal lectins on the activities of soluble and brush border enzymes of tomato moth larvae (*Lacanobia oleracea*). J Insect Physiol 1998; 44: 1213-24.
- [46] Nathan SS, Kalaivani K, Murugan K, Chung PG. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leafhopper. Pestic Biochem Physiol 2005a; 81: 113-22.
- [47] Nathan SS, Kalaivani K, Chung PG. The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pestic Biochem Physiol 2005b, 83: 46-57.
- [48] Kaplan LA, Pesce AJ. Clinical chemistry: theory, analysis, correlation, 3rd ed. Mosby-Year Book Inc: St. Louis 1996; pp. 609-10.
- [49] Ribeiro S, Guilhermino L, Sousa JP, Soares AMVM. Novel bioassay based on acetylcholinesterase and lactate dehydrogenase activities to evaluate the toxicity of chemicals to soil isopods. Ecotoxicol Environ Saf 1999; 44: 287-93.
- [50] Azmi MA, Ahmad I, Navqi SNH, Akhtar K. Level of lactate dehydrogenase (LDH) in resistant and susceptible strains of culicine mosquitoes of the Karachi region after treatment with DDT, malathion and cyfluthrin. Turk J Zool 2002; 26: 97-100.
- [51] Senthil Nathan S, Kalaivani K, Chung PG, Murugan K. Effect of neem limonoids on lactate dehydrogenase (LDH) of the rice leafhopper, *Cnaphalocrocis medinalis* (Guenée) (Insecta: Lepidoptera: Pyralidae). Chemosphere 2006; 62: 1388-93.
- [52] Hamadah KhSh, Biasiouny AL, Ghoneim KS. Alterations in the lactate dehydrogenase activity of the desert locust *Schistocerca gregaria* by the wild plant *Fagonia bruguieri* (Zygophyllaceae). Egypt Acad J Biol Sci 2010; 3: 1-9.
- [53] Ozmen I, Ercisli S, Hizarci Y, Orhan E. Investigation of antioxidant enzyme activities and lipid peroxidation of *Rosa canina* and *Rosa dumalis* fruits. Acta Hort 2005; 690: 245-8.
- [54] Sasmaz S, Arican O, Kurutas EB. Oxidative stress in patients with nongenital warts. Mediat Inflamm 2005; 4: 233-6.
- [55] Özbek YS, Özdemir F, Ozmen I, Büyükoğlu TI, Konak S, Özçelik H, Genç H. Effects of extracts obtained from *Nepeta italica* L. and *Nepeta cilicia* Boiss. Apud Bentham on antioxidant enzymes. Hacettepe J Biol Chem 2012; 40: 149-54.
- [56] Grant C. Metabolic reconfiguration is a regulated response to oxidative stress. J Biol 2008; 7: p. 1.
- [57] Chuang DM, Hough C, Senatorov V. Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. Annu Rev Pharmacol Toxicol 2005; 45: 269-90.

Received: December 19, 2014

Revised: January 04, 2015

Accepted: January 05, 2015

© Ahadji-Dabla *et al.*; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.