GALLS OF *Guiera senegalensis* J.F. Gmel (Combretaceae) AS POTENTIAL SOURCE OF INSECTICIDES AGAINST THE MAIN COWPEA PEST *Callosobruchus maculatus* (FAB.) (Coleoptera: Bruchidae) DETOXIFICATION ENZYMES

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**ABSTRACT**

The cowpea weevil, *Callosobruchus maculatus* (F.), is a major pest of cowpea (*Vigna unguiculata* (L.) Walp.) in storage. A laboratory study was conducted to investigate the inhibition potential of *Guiera senegalensis* galls on *C. maculatus* detoxification enzymes. Methanol extract from galls of *G. senegalensis* exhibited strong inhibitory effects on the activity of *C. maculatus* acetylcholinesterase (AChE) and mixed-functional oxidase enzymes. The chemical composition of the extracted oils was examined by gas chromatograph (GC)–flame ionisation detectors (FID) and mass spectrometry. Twelve components were identified, namely 2-Hydroxy-4-methoxybenzophenone, n-Pentadecane, Octadecane, 1,2-Benzenedicarboxylic acid, dibutyl ester, Palmitic Acid, Hexadecane, Cis-vaccenic acid, Oleyl alcohol, stenol, n-Eicosane, n-Heneicosane and vincubine. This plant could be used for improving the efficacy to various insecticide classes against *C. maculatus* and as an alternative to synthetic insecticides.

**Key Words:** *Callosobruchus maculatus*, enzymatic detoxification system, galls, *Guiera senegalensis*

**RÉSUMÉ**

*Callosobruchus maculatus* (F.) est un ravageur majeur des graines du niébé (*Vigna unguiculata* (L.) Walp) en stock. Une étude de laboratoire a été conduite pour investiguer le potentiel d’inhibition des galles de *Guiera senegalensis* sur le système de détoxification enzymatique de *C. maculatus*. L’extrait méthanolique de *G. senegalensis* a montré de fort effet inhibiteur sur l’activité enzymatique de l’acétylecholinestérase (AChE) et des oxydases à fonction mixte. La composition chimique de l’huile extraite a été examinée par chromatographie en phase gazeuse couplée à un détecteur à ionisation de flamme (GC-FID) et par spectrométrie de masse. Douze (12) composés ont été identifiés, à savoir 2-Hydroxy-4-methoxybenzophenone, n-Pentadécane, Octadécane.
benzène-1,2-dicarboxylate de dibutyle, Acide Palmitique, Hexadécane, Acide Cis-vaccinique, Alcool oléylque, sténol, n-Eicosane, n-Hénicosane et la vincubine. Cette plante pourrait être utilisée pour améliorer l’efficacité de diverses classes d’insecticides contre C. maculatus et comme alternative aux insecticides de synthèse

Mots Clés: Callosobruchus maculatus, système de détoxification enzymatique, galles, Guiera senegalensis

INTRODUCTION

Cowpea [Vigna unguiculata (L.) Walp.] is one of the most nutritious grain legumes for human consumption in many parts of the world, especially in tropical and subtropical regions (Tiroesele et al., 2014; Nenaah et al., 2015; Lü et al., 2017). Insect damage is the major constraint to cowpea grain production. Cowpea weevil, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), is the main post harvest pest of cowpea, which causes damage to the seeds and poses a great challenge to the process of assuring food security in developing tropical countries (Kolawole et al., 2014; Adelani et al., 2016). Over 90% of the insect damage to cowpea seeds is caused by C. maculatus. The pest can cause up to 100% loss of stored cowpea seeds in a few months in West Africa (Nenaah et al., 2015).

Infestation of cowpea by this insect begins in the field before harvesting (Mbata and Payton, 2013). This bruchid reduces seed weight, market value and germination viability of the seeds (Kolawole et al., 2014; Tiroesele et al., 2014). Synthetic pesticides are used against C. maculatus infestation of seeds and management of cowpea seed storage pests. Their excessive use can promote emergence of resistant insects, residues in food grains and in the environment (Gatehouse and Boulter, 1983).

The resistance of insects to synthetic pesticides results from three main mechanisms: (i) reduction of insecticide penetration, (ii) enhanced detoxification of insecticides before they reach their target-site by esterases (ESTs), glutathione S-transferases (GSTs) and cytochrome P450 monooxygenases (P450s) or mixed function oxidases (MFO), and (iii) modification of sensitivity of target enzymes to insecticides (Kim and Boo, 2004; Malathi et al., 2017).

The diverse negative impacts of synthetic pesticides on the environment, humans and non-target organisms have necessitated the search for better alternatives (Tiroesele et al., 2014; Adelani et al., 2016). Several traditional methods such as ash, sand, dry pepper and botanical extracts are employed by resource-poor farmers in Africa for protecting harvested cowpea (Mbata and Payton, 2013; Tiroesele et al., 2014). The natural insecticides can be used in pest control because plants are rich sources of secondary substances, which may be bioactive against a large number of insects (Dutra et al., 2016). Osman et al. (2014) showed that both petroleum ether and ethanol extracts of the leaves of G. senegalensis are promising biocontrol candidates as acaricidal agents against Hyalomma anatolicum. Sombié et al. (2013) also earlier showed that the galls of Guiera senegalensis possessed inhibitory potential of acetylcholinesterase, enzyme involved in insect neurotoxicity.

The objective of this study was to determine the effect of G. senegalensis galls extract on C. maculatus enzymatic detoxification systems and also to investigate the methanol extract bioactive compounds.

MATERIALS AND METHODS

Plant material and extraction. The plant material was constituted by galls from G. senegalensis. The galls were collected from Ouagadougou, Burkina Faso and were air-dried in the laboratory and then grounded into powder. Twenty five grammes of this powder was mixed with 250 ml of methanol, and soaked for 24 hours. The methanol was then
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evaporated from the supernatant to obtain the methanol extract.

**Insect material.** For enzymes extraction, the insects *C. maculatus* were freshly provided by the Laboratory of Fundamental and Applied Entomology, University of Ouaga I Professor Joseph Ki-Zerbo. The insects were collected from different locations of Burkina Faso (Ouagadougou, Bama, Ouahigouya, Nouna) and in others countries (Niger and Senegal).

**Enzymes extraction methods**

**Esterase and GST extraction.** For each strain, fifty adult insects were kept in a refrigerator (4°C) for 15 minutes, and then ground and homogenised in 2 ml of homogenisation buffer (0.1 M sodium phosphate buffer, pH 7, 5) at 4 °C. The homogenate was centrifuged at 5000 rpm at 24 °C for 20 minutes; the supernatant was collected to constitute the source of enzyme. The enzyme solution was divided into 100 µ l aliquots and stored at -70 °C until use (Scharf *et al*., 1998).

**Acetylcholinesterase extraction.** For each strain, fifty adult insects were kept in a refrigerator (4°C) for 15 minutes and homogenised at 4 °C in 2 ml of ice-cold sodium phosphate buffer (0.1M, pH7.5) containing 1mL.L⁻¹ of Triton X-100. The homogenate was centrifuged at 5000 rpm at 24°C for 20 minutes; the supernatant was collected to constitute the source of the acetylcholinesterase enzyme. Supernatants from centrifugations were divided into 100 µ l aliquots and stored at -70 °C until use (Roditakis *et al*., 2009).

**Oxidase extraction.** For each strain, fifty adult insects were introduced in a refrigerator (4 °C) for 15 minutes and homogenised in 2 ml of water. The homogenate was then centrifuged at 5000 rpm at 24°C for 20 minutes; the supernatant was carefully collected to constitute the source of oxidase enzyme. Supernatants from centrifugations were divided into 100 µ l aliquots, and stored at -70 °C until use (Carletto *et al*., 2010).

**Enzymes inhibition assay.** Enzyme inhibition assay was performed to estimate the inhibition percentage of enzymes by the methanol extract of *G. senegalensis* galls. All assays were replicated three times.

**PNPA esterase inhibition assay.** The assay mixture consisted of 20 µl of supernatant (enzyme source), 156 µl PNPA assay buffer and 20 µl of extract solution at final concentration of 100 µg.ml⁻¹ in a 96 wells microplate. Four microliters of the substrate PNPA stock solution was added and the reaction was measured for 2 min at 405 nm at 30 °C in a microplate reader. A blank without the substrate PNPA was prepared for background subtraction. Each assay was made in triplicate (Scharf *et al*., 1998).

**CDNB glutathione S-transferase inhibition assay.** Within a 96 wells microplate, 5 µl of supernatant was added to 265 µl of CDNB assay buffer and 20 µl of extract solution at final concentration of 100 µg ml⁻¹. Ten microliters of CDNB stock solution was added and the reaction was measured for 2 min at 334 nm at 30°C in a microplate reader. A blank was read without CDNB stock. Each assay was made in triplicate (Scharf *et al*., 1998).

**Oxidase inhibition assay.** In a 96 wells microplate, potassium phosphate buffer (60 µl, 62.5 mM, pH 7.2) was mixed with 10 µl of enzyme source, 100 µl of the solution A and 20 µl of extract solution at final concentration of 100 µg ml⁻¹. Then 10 µl of hydrogen peroxide (3% in water) was added. Absorbance in greenish colour was read at 630 nm at 25 °C for 2 minutes in a microplate reader. A blank was made without hydrogen peroxide. Each assay was made in triplicate (Carletto *et al*., 2010).
Acetylcholinesterase inhibition assay.
Fourty microliters of homogenisation buffer (sodium phosphate, 0.1 M pH 7.5) was mixed to 20 µl of supernatant, 100 µl of DTNB (3 mM in 250 mM Tris-HCl (pH 8 buffer, 0.1 M NaCl, 0.02 M MgCl$_2$) and 20 µl of extract solution at final concentration of 100 µg ml$^{-1}$. The reaction was then started by the addition of 20 µl ATCI substrate (4 mM in water) and was run for 5 min at 405 nm. A blank without ATCI was run for background subtraction. Each assay was made in triplicate (Ellman et al., 1961).

Gas Chromatography (GC)–Mass Spectrometer (MS) analysis. For gas chromatography (GC)-flame ionisation detectors (FID) and mass spectrometry analysis, the crude methanol extract was purified by Silica gel column chromatography eluted with hexane-acetone (50:50).

Shimadzu-GC-9A gas chromatograph, FID at 220, N$_2$ at 1.0 ml min$^{-1}$, SPB-5 capillary column (30 m × 0.53 mm ID; 0.3 µmdf), split ratio 1:30 injector temperature 240 °C, column temperature maintained at 50 °C for the first five minutes and then raised to 235 °C (5 °C per minute); followed by five minutes at 235 °C. GC-MS: Hewlett-Packard 5890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer, with source at 270 °C at 70 eV. Injector was set at 270 °C with splitting ratio 1:30. The analysis was performed on the aforementioned programme on equivalent column HP-5 (25 m × 0.22 mm and 0.25 µmdf). The compounds in the purified methanol extract were identified by comparison of their mass spectra with the spectra from the library using the NIST mass spectral search programme 1998.

Statistical analysis. The results are presented as mean ± SD for triplicate analysis and were subjected to one-way analysis of variation ANOVA with Turkey’s Significant Difference test. P < 0.05 was considered significant. The Pearson Correlation test was used to study the origin effect of insect strains on inhibition of *C. maculatus* enzymatic detoxification systems. The statistical analysis was performed using XLSTAT Version 7.5.2 (Addinsoft, FRANCE).

RESULTS AND DISCUSSION

Enzyme inhibition potential. Table 1 shows methanol extract from galls of *G. senegalensis* inhibitory potential on *C. maculatus* glutathion s-transferase, acetylcholinesterase, total esterase and oxidase enzymes from potential insecticides susceptible or tolerant *callosobruchus maculatus* populations collected in different locations in Burkina Faso (Ouagadougou, Bama, Ouahigouya, Nouna) and in Niger and Senegal. There was significant difference (P < 0.05) in inhibition activities of the four enzymes between the strains of *C. maculatus* from the different locations.

TABLE 1. Inhibition potential of *G. senegalensis* on *C. maculatus* enzymatic detoxification systems

<table>
<thead>
<tr>
<th>Localities</th>
<th>Glutathion S-transferase (%)</th>
<th>Acetylcholinesterase (%)</th>
<th>Esterase (%)</th>
<th>Oxidase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bama</td>
<td>4.32 ± 0.51$^a$</td>
<td>45.07 ± 3.65$^b$</td>
<td>36.28 ± 1.54$^c$</td>
<td>91.9 ± 5.3$^a$</td>
</tr>
<tr>
<td>Ouagadougou</td>
<td>7.06 ± 0.75$^d$</td>
<td>16.17 ± 0.47$^d$</td>
<td>-19.9 ± 11.35$^d$</td>
<td>96.95 ± 3.57$^a$</td>
</tr>
<tr>
<td>Nouna</td>
<td>9.24 ± 1.00$^c,d$</td>
<td>61.19 ± 2.93$^d$</td>
<td>62.43 ±10.8$^b$</td>
<td>96.15 ± 2.9$^a$</td>
</tr>
<tr>
<td>Ouahigouya</td>
<td>10.44 ± 0.66$^b$</td>
<td>31.34 ± 1.19$^c$</td>
<td>28.31 ± 1.84$^c$</td>
<td>96.67 ± 0.88$^a$</td>
</tr>
<tr>
<td>Niger</td>
<td>22.50 ± 1.62$^a$</td>
<td>35.4 ± 0.831$^c$</td>
<td>35.71 ± 0.64$^c$</td>
<td>92.37 ± 2.33$^a$</td>
</tr>
<tr>
<td>Senegal</td>
<td>12.56 ± 0.78$^b$</td>
<td>50.41 ± 4.82$^b$</td>
<td>89.95 ± 0.92$^a$</td>
<td>94.87 ± 5.17$^a$</td>
</tr>
</tbody>
</table>

Different letters in the same column represent significant differences (P < 0.05)
Galls of Guiera senegalensis J.F. GMEL (Combretaceae) as potential source of insecticides

At the concentration of 100 µg mL⁻¹, methanol extract decreased AChE, total Esterase, GST and Mixed Function oxidase activities. The inhibition of glutathione S-transferase enzyme was moderate along all the different locations; and ranged from 4.32 ± 0.51 to 22.50 ± 1.62%. Among the locations, Niger strain C. maculatus was the most susceptible to the G. senegalensis galls extract, since it exhibited the highest inhibition on glutathione S-transferase. Glutathione transferases (GSTs; EC 2.5.1.18), members of a superfamily of multifunctional dimeric proteins is an important function in the detoxification of many endogenous and exogenous toxic substances, including allelochemicals from plants (Kolawole et al., 2011; Zhou et al., 2016). Insect GSTs contribute significantly to organophosphate, organochlorine, cycloidiene, and pyrethroid resistance (Wang et al., 2014). The weak inhibition potential of G. senegalensis on GST suggests that the insecticide agents in this plant may act by other mechanisms. The insecticides of plant origin act by many mechanisms of action. The proposed mechanisms of their action include (i) inhibition of acetylcholinesterase (AChE) and/or agonist/antagonist of cholinergic acetylcholine nicotinic receptor, (ii) blockage of the GABA-gated chloride channel (iii) inhibiting the mitochondrial system, (iv) blocking octopamine receptors by working through tyramine receptors cascade and disruption of hormonal balance (Rattan, 2010).

The methanol extract of G. senegalensis showed a notable inhibition potential for the AChE enzyme extracted from C. maculatus strains from Nouna, Senegal and Bama, respectively (Table 1). The C. maculatus strain from Ouagadougou was the most resistant to galls extract by showing the lowest inhibition on the AChE enzyme.

Acetylcholinesterase (AChE; EC 3.1.1.7), widely found in insect nervous systems plays a major role in the activity of the central nervous system (CNS) and peripheral nervous system (PNS), and is the target of organophosphate and carbamate insecticides (Rajashekar et al., 2014). Callosobruchus maculatus AChE inhibition by the methanol extract of G. senegalensis could be one of the toxicity mechanisms of this plant against insects (Cespedes et al., 2013).

Esterase inhibition activity was found to be significantly higher (P < 0.05) in Senegal insects strains (89.95 ± 0.92) than strains in others locations; whereas no Esterase inhibition activity was recorded in C. maculatus strain collected from Ouagadougou laboratory (-19.9 ± 11.35). Ouagadougou laboratory strain could be tolerant to the toxic effect of G. senegalensis by increasing the activity of acetylcholinesterase and esterase enzymes. Malathi et al. (2017) showed that the increased levels of esterases are responsible for the resistance to organophosphates, carbamates and pyrethroids in many insects.

Oxidase inhibition activity was also observed to be higher, but not significantly different among strains collected in Ouagadougou, Ouahigouya, Nouna, respectively (Table 1). A similar phenomenon was observed across Senegal, Niger and Bama insect strains, respectively (Table 1). In all the different locations, the oxidase enzymes from the different strains of C. maculatus showed strong susceptibility to the toxic effect of the compounds in the methanol extract of galls from G. senegalensis. Elevated level of mixed function oxidases has been involved in imparting resistance to organochlorines, pyrethroids, and neonicotinoids in many insect species (Malathi et al., 2017). The mixed-function oxidase activities are involved in the respiratory electron transport chain (Wu et al., 2014). The insecticide agents in plant may act by inhibiting the mitochondrial electron transport (Rattan, 2010). The strong inhibition potential of G. senegalensis in mixed function oxidase at low concentration suggests that G. senegalensis extract directly act on the nervous and respiratory systems of the C. maculatus.
Pearson correlation matrix between the effect of location on all the enzymes inhibition is presented in Table 2. Significant positive correlations were found between the inhibition activity of extract on strain from Manga with the inhibitions activities of strains from Niger, Nouna and Ouahigouya (Table 2). The Pearson correlation coefficient analysis also revealed significant relationship between the inhibition activities of extract on strain from Niger with strain of Ouahigouya ($r = 0.998$). The strains of *callosobruchus maculatus* from Bama might have the same susceptibility with the strains from Nouna and Ouahigouya. We also observed that the strains from Niger and Ouahigouya might have also the same susceptibilities (Table 2). Mohan and Gujar (2003) showed wide range of susceptibility in the field populations of *Plutella xylostella* (Linnaeus) to some insecticides on their detoxification enzymes.

**Gas chromatography-mass spectrometry analysis.** The results of GC-FID and GC-MS analysis of the column fraction from methanol galls extract of *G. senegalensis* are presented in Table 3. Twelve compounds have been identified for column fraction from methanol extract with spectral match factor at least 90%. Figures 1 and 2 show the chromatograms obtained by GC-FID and by GC/MS. n-Pentadecane and n-Eicosane found in methanol extract were identified respectively in hydroacetonic extract and

<table>
<thead>
<tr>
<th>Locations</th>
<th>Bama</th>
<th>Ouaga</th>
<th>Niger</th>
<th>Nouna</th>
<th>Ouahigouya</th>
<th>Senegal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bama</td>
<td>1</td>
<td>0.823</td>
<td>0.951*</td>
<td>0.958*</td>
<td>0.966*</td>
<td>0.792</td>
</tr>
<tr>
<td>Ouaga</td>
<td>1</td>
<td>0.916</td>
<td>0.627</td>
<td>0.913</td>
<td>0.361</td>
<td>0.361</td>
</tr>
<tr>
<td>Niger</td>
<td>1</td>
<td>0.845</td>
<td>0.998*</td>
<td>0.906</td>
<td>0.906</td>
<td>0.906</td>
</tr>
<tr>
<td>Nouna</td>
<td>1</td>
<td>0.868</td>
<td>0.913</td>
<td>0.700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouahigouya</td>
<td>1</td>
<td>0.868</td>
<td>0.913</td>
<td>0.906</td>
<td>0.710</td>
<td></td>
</tr>
<tr>
<td>Senegal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* show significative value ($P < 0.05$)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Formula</th>
<th>m/z</th>
<th>Number of insaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxy-4-methoxybenzophenone</td>
<td>C$<em>{14}$H$</em>{12}$O$_{3}$</td>
<td>227.4</td>
<td>7</td>
</tr>
<tr>
<td>n-Pentadecane</td>
<td>C$<em>{15}$H$</em>{32}$</td>
<td>57.4</td>
<td>0</td>
</tr>
<tr>
<td>Octadecane</td>
<td>C$<em>{16}$H$</em>{34}$</td>
<td>57.4</td>
<td>0</td>
</tr>
<tr>
<td>1,2-Benzenedicarboxylic acid, dibutyl ester</td>
<td>C$<em>{16}$H$</em>{32}$O$_{4}$</td>
<td>149.3</td>
<td>5</td>
</tr>
<tr>
<td>Hexadecanoic acid or Palmitic Acid</td>
<td>C$<em>{16}$H$</em>{32}$O$_{2}$</td>
<td>73.3</td>
<td>1</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>C$<em>{16}$H$</em>{34}$</td>
<td>57.4</td>
<td>0</td>
</tr>
<tr>
<td>11-octadecenoic acid or Cis-vaccenic acid</td>
<td>C$<em>{18}$H$</em>{36}$O$_{2}$</td>
<td>55.4</td>
<td>2</td>
</tr>
<tr>
<td>cis-9-octadecen-1-ol or Oleyl alcohol</td>
<td>C$<em>{18}$H$</em>{36}$O</td>
<td>82.4</td>
<td>1</td>
</tr>
<tr>
<td>1-Octadecanol or sipol or stenol</td>
<td>C$<em>{18}$H$</em>{36}$O</td>
<td>83.4</td>
<td>0</td>
</tr>
<tr>
<td>n-Eicosane or icosane</td>
<td>C$<em>{20}$H$</em>{42}$</td>
<td>57.4</td>
<td>0</td>
</tr>
<tr>
<td>n-Heneicosane</td>
<td>C$<em>{22}$H$</em>{44}$</td>
<td>57.4</td>
<td>0</td>
</tr>
<tr>
<td>2, 2, 6,6-Tetramethyl-4-piperidone or vincubine</td>
<td>C$<em>{9}$H$</em>{17}$NO</td>
<td>92.98</td>
<td>1</td>
</tr>
</tbody>
</table>
Galls of *Guiera senegalensis* J.F. GMEL (Combretaceae) as potential source of insecticides

Figure 1. GC-FID Chromatogram of *G. senegalensis* methanol galls extract.

Figure 2. GC-MS Chromatogram of *G. senegalensis* methanol galls extract.
aqueous decoction extract (Sombié et al., 2013). Hexadecane present in methanol extract was also already identified in hydroacetonic and aqueous decoction extracts (Sombié et al., 2013). Monoterpenes have been well documented as active fumigants, repellents, and insecticides toward stored-product insects (Khani and Asghari, 2012).

CONCLUSION

Results of this study suggest that the inhibition of mixed function oxidase enzyme in mitochondrial may be the principal target of G. senegalensis against the adult C. maculatus. The inhibition potential of G. senegalensis on the activities of MFO, esterase and AChE showed that this plant can be used for improving the efficacy to various insecticide classes against C. maculatus. Results of this study suggest a significant potential for this plant as a possible source of natural insecticide that could be used as an alternative to synthetic insecticides.

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