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Research Article

Toxicity and biochemical mechanisms underlying the insecticidal efficacy of two plant extracts on *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) infesting cowpea seeds

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Abstract: The quest for new sources of cheap and eco-friendly insecticide for insect pest management remains a major challenge facing cowpea farmers in many developing countries. In this study, the toxicity and biochemical mechanisms associated with the insecticidal efficacy of acetone and *n*-hexane extracts of *Xylopia aethiopica* (Dunal) and *Senna occidentalis* (L.) on *Callosobruchus chinensis* (L.) were investigated. The insecticidal efficacy varied with concentration, exposure time and extracts type. Acetone extract of *X. aethiopica* was less toxic (LD_{50} = 2.47%) than its *n*-hexane extract (LD_{50} = 1.39%) but with *S. occidentalis*, acetone extract was more toxic (LD_{50} = 0.73%) than *n*-hexane extract (LD_{50} = 1.37%). Acetone extract of both plants evoked a significant reduction in egg-laying and eclosion ability of adult *C. chinensis* compared to *n*-hexane extract except on cowpea pre-treated with acetone extract of *S. occidentalis*. Only the extracts of *X. aethiopica* reduced protein concentration compared to control. The activity of glutathione reductase and glutathione peroxidase was significantly reduced by both extracts of *S. occidentalis* while only *n*-hexane extract of *S. occidentalis* elicited a significant reduction in the activity of glutathione S-transferase and trehalase compared to other treatment levels. GC-MS analysis depicted Diisoocotyl phthalate (50.37%) and isomers of Aromandendrene (19.22%) as the main compounds in *S. occidentalis* and *X. aethiopica* respectively. Both botanical extracts also contained other insecticidal and insectifuge compounds in differential amounts. Overall, the findings demonstrated the potential of both botanical extracts, particularly acetone extract of *S. occidentalis* as alternatives to synthetic insecticide for controlling adult *C. chinensis*.

Keywords: *Xylopia aethiopica*, *Senna occidentalis*, Glutathione reductase, Glutathione peroxidase, Insectifuge

Introduction

Cowpea *Vigna unguiculata* (L.) Walp is a grain legume which plays a major nutritional role in the attainment of food security, predominantly in many developing countries. It is regarded as an important source of cheap protein and vitamins to millions of rural families which constitute a large part of the populace in most developing countries (Langyintuo *et al.*, 2003). The crop is mostly grown in semi-arid tropics covering Africa, Asia, Europe, United States as well as Central and Southern America (Ba *et al.*, 2004; Tan *et al.*, 2012). Its production has been increasing in the last three decades with West and Central Africa accounting for over 72% of the world-wide production. Nigeria, the largest producer and consumer of cowpea, accounts for

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61% of production in Africa and 58% worldwide (FAOSTAT, 2016). Protection of cowpea grains from insect pest infestation, especially during storage, is one of the greatest challenges confronting its production in Africa.

The pulse beetle, *Callosobruchus chinensis* (L.) has been recognized as the major cosmopolitan pest of several leguminous seeds including cowpea in most tropical and sub-tropical regions of the world (Lale and Kabeh, 2004). It is capable of eliciting up to 98% loss if left unchecked for several months on stored cowpea seeds (Hossain *et al.,* 2014). Losses of this magnitude are capable of threatening food security at both household and national levels in many developing countries where cowpea provides cheap source of dietary protein. Both the larvae and pupae develop within the cowpea seed with the larval stage being the main destructive stage due to their voracious feeding activity. Adult exit holes are usually obvious on infested cowpea seeds leading to reduction in weight, nutritional quality and market value of the affected seeds (Prakash *et al.,* 2013; Mainali *et al.,* 2015). In an effort aimed at curtailing the destructive activity of this pest, post-harvest handlers have relied heavily on the use of synthetic insecticides, such as dichlorvos (dichlorovinyl dimethyl phosphate-DDVP), malathion, cypermethrin, aluminium phosphide, pirimiphos-methyl, deltamethrin and carbaryl, among others, especially in large scale storage (Gbaye *et al*., 2012; Perveen and Khan, 2014; Gbaye *et al.,* 2016;). However, the recent ban imposed on the country by the European Union due to high pesticide residue on exported cowpea coupled with many other limitations associated with most chemicals have necessitated the need to opt for a safer and more sustainable means of protecting cowpea seeds during storage.

Insecticides derived from plant origin are ecologically safer means of managing several insect pests attacking stored cowpea. In fact, research has shown that botanical insecticides could have a substantial role in boosting cowpea production in sub-Saharan Africa, where they are believed to be in high abundance (Akinkurolere *et al.,* 2006; Singh, 2011; Adebiyi and Tedela, 2012; Oyeniyi *et al.,* 2015). Consequently, several researchers have screened different botanicals for insect pest management. The effectiveness of botanicals as an insecticide however differs, depending on many factors including the nature of solvent used for extraction, mode of extraction, plant species, insect pest species and the bio-active chemical compounds present in the plant (Tuetun *et al.,* 2004; Tehri and Singh, 2014). *Xylopia aethiopica* (Dunal) and *Senna occidentalis* (Linnaeus) are examples of native plants that their chemical substances have exhibited insecticidal effects against several stored product insect pests (Ngamo *et al.,* 2001; Jirovetz *et al.,* 2005; Kouninki *et al.,* 2005; Habiba *et al.,* 2010; Adesina *et al* 2011; Ratnasekera and Rajapakse, 2012; Silva *et al.,* 2013; Edwin and Jacob, 2017). In spite of several studies on the insecticidal efficacy of both plants, no comprehensive research detailing the insecticidal active compounds from *X. aethiopica* and *S. occidentalis* on *C. chinensis* has been published.

Also, botanicals are known to alter the internal metabolism of insects by influencing the quantity of various biochemical and physiological components in their body, resulting in reduced activity or mortality (Vijayaraghavan *et al.,* 2010). The influence of various botanical pesticides on the activities of detoxifying enzymes in some stored product insect pests has been investigated by various researchers (Ebadollahi *et al.,* 2013; Mojarab-Mahboubkar *et al.,* 2015; Adesina *et al* 2018). But, there is still a dearth of information on the impact of *X. aethiopica* and *S. occidentalis* on the activities of various enzymes involved in the defense system of *C. chinensis* in spite of several studies conducted on both botanicals. This study therefore sought to explore the insecticidal efficacy of chemically characterized extracts of *X. aethiopica* and *S. occidentalis* as well as their effect on the various biochemical parameters of *C. chinensis* infesting cowpea seeds.

Materials and Methods

Chemicals and reagents for biochemical analysis

Acetone, *n*-hexane, dihydrogen potassium phosphate (KH_2PO_4), reduced glutathione

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(GSH), oxidized glutathione (GSSG), reduced nicotinamide adenine dinucleotide phosphate (NADPH), were purchased from BDH chemicals (2039 Center Square Road, Philadelphia, USA.). All reagents used were of analytical grade.

Procurement and preparation of plant materials

Fruits of *Xylopia aethiopica* were purchased from Oba market in Akure, Ondo state while the seeds of *Senna aethiopica* were collected from Igbatoro village in Akure, Ondo State, Nigeria. The fruits of *X. aethiopica* and the seeds of *S. occidentalis* were sorted to remove impurities and immature fruits and seeds, before being air-dried on a laboratory bench. Dried plant part from each flora was later pulverized into fine powder using an electric blender (USHA 500-Watt Motor Power). The powders were sieved using 16 mesh size sieve. Three hundred grams of each plant powder was separately weighed into a glass jar and 600ml of different solvents, *n*-hexane and acetone was poured into each jar separately and allowed to soak for 72h while stirring intermittently. Each preparation was sieved using muslin cloth and concentrated using rotary evaporator at 40 °C and rotary speed of 6 rpm. The extracts were later air-dried on the laboratory bench to ensure that no traces of the solvents were present. The crude extracts from each plant were diluted to obtain concentrations of 1-5% (v/w) with the respective solvents (*n*-hexane and acetone).

Cowpea cultivar and insect culture

Ife-brown cowpea cultivar was used in this study due to its high susceptibility to *C. chinensis* (Gbaye *et al*., 2011). The cowpea cultivar was obtained from Institute of Agricultural Research and Training (IAR & T) Ibadan, Nigeria. The parent stock of *C. chinensis* was obtained from established laboratory culture reared on disinfested cowpea seeds at ambient temperature (27 ± 2 °C) and relative humidity (75 ± 2%). The food medium, Ife-brown cowpea was disinfested in a deep freezer (Haier Thermocool HTF-429H) for 72 h prior to insect rearing.

Bioassays

Toxicity test using n-Hexane and Acetone Extracts of *X. aethiopica* and *S. occidentalis*

Twenty grams of cowpea seeds were weighed into separate 170ml plastic containers (8.7cm in diameter) using Melter Beam PB 3002 weighing balance and thoroughly mixed with 1ml of 0.0% (control), 1, 2, 3, 4 and 5.0% solution of extract of acetone, and *n*-hexane extract of *X. aethiopica*. Ten pairs of newly emerged *C. chinensis* were introduced into each treatment container and covered. Each treatment concentration was replicated four times. The toxic effect of the plant extract was assessed daily for 4 days by counting the number of dead insect. The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen. The procedure for *X. aethiopica* was repeated for *S. occidentalis*, at concentrations of 0.0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% w/v.

Oviposition deterrence and inhibition of adult emergence tests

Four (4) days after mortality test, eggs laid were counted and dead and live adult insects were discarded from the container. The eggs laid were allowed to develop at ambient temperature (27 ± 2 °C) and relative humidity (75 ± 2%) inside insect cage. Observation for adult emergence commenced 24 days after introduction of adult insects and was recorded for 12 days.

Biochemical assay

Preparation of Crude Enzyme

One gram (1g) of insects pre-treated with each extract of plant material was homogenized in 3ml of ice-cold of 10mM phosphate buffer pH 7.0 using pre-chilled pestle and mortar and immediately centrifuged at 10,000 rpm for 30min at 4 °C. The supernatant which served as an enzyme source was collected and stored at -20 °C until used.

Estimation of Protein Concentration

The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as the standard protein. A 0.2mg/ml BSA stock

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solution was freshly prepared and a series of lower concentration (2-10 µg/ml) were prepared into sterile test tubes by serial dilution. This was done by pipetting 2, 4, 6, 8, 10µl of the BSA stock solution into the test tubes, then bringing up the volume in each tube up to 800µl with appropriate volume of distilled water. Also, 10µl of the unknown enzyme solution was pipetted into clean and clearly labeled test tubes and made up to 800µl with appropriate volume of distilled water. To each test tube, 200µl of Bradford reagent was added and allowed to incubate at room temperature for 20min before taking the absorbance of each solution at a wavelength of 595nm against the blank. The blank contained 800µl of distilled water and 200µl of Bradford reagent. The graph of absorbance against concentration was plotted and the concentrations of the unknown protein solutions were extrapolated from the standard calibration curve.

Assay of Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was determined by the method described by Rotruck *et al.* (1973). This method depends on determination of the rate of glutathione oxidation by H_2O_2 as catalyzed by the GPx present in the supernatant. The color developed is read against a reagent blank at 412nm. The activity of GPx was expressed in terms of nanomole of GSH oxidized/min/mg protein.

Assay of Glutathione Transferase

The enzyme activity of GST was determined according to the method of Habig *et al.* (1974). 1-chloro-2, 4-dinitrobenzene (CDNB) and 1, 2-dichloro-4-nitrobenzene (DCNB) were used as substrates. The whole body was homogenised in 100µl phosphate buffer (pH 7.0, 20mM), and then centrifuged at 12 000 rpm at 4 °C for 12min. Ten microliter (10µl) of supernatant was transferred into a cuvette and mixed with 110µl of phosphate buffer (pH 7.0, 20mM), 80µl of substrates CDNB (100mM), and 100µl of GSH (10mM). The absorbance of the mixture was read in a spectrophotometer at 430nm.

Assay of Glutathione Reductase

Glutathione reductase activity assay was carried out according to the method of Saydam *et al.* (1997). The assay mixture (1ml) in final concentration consisted of 700µl of 100mM potassium phosphate buffer (pH 7.4), 1mM EDTA, 100µl of 1mM GSSG, 100µl of 0.16mM NADPH and 100µl of the crude enzyme solution. NADPH oxidation was monitored at 340nm for 3min at 25 °C and the enzyme activity was calculated and expressed as micromoles of NADPH consumed per minute, using an extinction coefficient of 6.220 $M^{-1}cm^{-1}$.

Assay of Trehalase Activity

Trehalase activity assay was carried out according to the method of Jin and Zheng (2009). The reaction mixture containing 0.1ml enzyme solution and 0.1ml of 2% trehalose was incubated at 45 °C for 15min in a thermostatic water bath. Then, 0.1ml of DNSA solution was added and boiled for 5min and allowed to cool. Also, 1ml of distilled water was added to the mixture. The blank (control) contained 0.1ml distilled water instead of enzyme solution. The absorbance of the mixture was read in a spectrophotometer at 540nm. One unit of trehalase activity was defined as the amount of enzyme required to produce 1µmol of glucose per minute under the specified assay condition.

Analysis of plant extracts

Gas chromatographic and mass spectral analysis (GC-MS) of the extract obtained from *X. aethiopica* and *S. occidentalis* was used to determine the different compounds in each extract. The analysis was done on a GC Agilent Technologies 7890A system. One microliter (1µl) of the prepared extract from each botanical was separately injected into GC column (Dimension: HP-5MS, 30m length, 0.32mm internal diameter, 0.25µm thickness). The oven temperature was programmed from 80 °C (hold for 2mins) to 240 °C (hold for 6mins) at the rate of 10 °C/min. Injector used was Agilent Technologies 5975C VLMSD. Temperature was 230 °C. Helium was used as the carrier gas at the rate of 1ml/min. Area normalization was used for determination of

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composition percentage. Tentative identification of the compounds in each extract was done by comparing their spectra with mass spectral databases of National Institute of Standards and Technology (NIST, 2005). The compounds were further confirmed by peak enhancement on GC using authentic compounds.

Statistical Analysis

All data obtained on adult mortality were subjected to analysis of variance (ANOVA) and treatment means were separated using Tukey's HSD at $\alpha = 0.05$. Mortality data and concentration of each botanical extract were also subjected to probit and log transformation respectively to determine the concentration of each extract lethal to 50% of *C. chinensis* (Finney, 1971). All analyses were done using SPSS 22.0 at 0.05 level of significance.

Results

Toxicity of extracts of *X. aethiopica* and *S. occidentalis* to *C. chinensis*

Generally, the insect mortality increased with increasing concentration and exposure time irrespective of the extract (Tables 1 and 2). Both extracts of *X. aethiopica* also elicited significantly higher ($p < 0.05$) mortality with the highest dose 5.0% v/w compared to control except for acetone extract at 24 hours post-treatment (Table 1). At the highest experimental duration (96 hours), higher mortality (48.25-86.50%) was observed with *n*-hexane extract of *X. aethiopica* compared to acetone extract (17.50-71.25%) (Table 1). However, no mortality was observed in bruchid treated with 0.2, 0.4 and 0.6% v/w of *n*-hexane extract of *S. occidentalis* at 24 hours exposure (Table 2). Also, regardless of the extract, there was no significant difference ($p = 0.063$) between the percentage death of insect at all treatment levels of *S. occidentalis* at 24 and 48 hours post-treatment (Table 2). Both extracts of *S. occidentalis* exhibited significantly higher ($p < 0.05$) mortality in bruchids exposed to 5% v/w compared to control at 48 and 96 hours exposure. Acetone extract evoked higher mortality in *C. chinensis* exposed to 0.6, 0.8 and 1.0% v/w of *S. occidentalis* compared to their counterpart exposed to n-hexane extracts at the highest duration (96 hours) (Table 2).

The concentration of *X. aethiopica* and *S. occidentalis* extracts needed to kill 50% of adult *C. chinensis* at 96 hours exposure is shown in Table 3. Acetone extract of *X. aethiopica* was less effective ($LD_{50} = 2.47\%$) against *C. chinensis* compared to n-hexane extract of *X. aethiopica* ($LD_{50} = 1.39\%$). On the contrary, acetone extract of *S. occidentalis* was more potent ($LD_{50} = 0.73\%$) against *C. chinensis* compared to n-hexane extract of *S. occidentalis* ($LD_{50} = 1.37\%$).

Table 1 Percentage cumulative mortality (Mean ± SE) of *Callosobruchus chinensis* on cowpea treated with *n*-hexane and acetone extracts of *Xylopia aethiopica*.

Solvent	Concentration (%)	Exposure time (h)			
		24	48	72	96
n-Hexane	0.0	7.50 ± 2.50^{a}	7.50 ± 2.89^{a}	7.50 ± 4.78^{a}	12.50 ± 4.78^{a}
	1.0	7.55 ± 4.84^{a}	23.50 ± 2.22^{cd}	24.25 ± 2.22^{a}	48.25 ± 5.60^{b}
	2.0	7.65 ± 2.50^{a}	10.25 ± 4.09^{abc}	25.75 ± 8.84^{a}	51.75 ± 5.30^{bc}
	3.0	7.75 ± 4.84^{a}	15.25 ± 6.40^{abc}	32.25 ± 8.40^{a}	62.50 ± 4.41^{bc}
	4.0	7.75 ± 4.84^{a}	20.75 ± 3.90^{bcd}	34.00 ± 7.82^{ab}	72.00 ± 5.52^{cd}
	5.0	16.75 ± 2.92^{b}	31.25 ± 3.72^{d}	61.75 ± 4.44^{b}	86.50 ± 5.00^{d}
Acetone	0.0	0.00 ± 0.00^{a}	2.50 ± 2.50^{a}	12.50 ± 2.50^{a}	17.50 ± 4.49^{a}
	1.0	0.00 ± 0.00^{a}	2.50 ± 2.50^{a}	22.75 ± 4.55^{ab}	30.50 ± 6.49^{ab}
	2.0	2.50 ± 2.50^{a}	7.75 ± 2.59^{a}	16.50 ± 7.10^{a}	32.25 ± 3.34^{ab}
	3.0	5.00 ± 2.90^{a}	15.00 ± 6.46^{ab}	28.50 ± 9.256^{ab}	57.00 ± 4.42^{bc}
	4.0	7.50 ± 2.50^{a}	20.50 ± 0.50^{b}	48.00 ± 2.87^{b}	66.25 ± 8.34^{c}
	5.0	12.50 ± 7.50^{a}	40.75 ± 3.50^{c}	45.25 ± 7.32^{b}	71.25 ± 9.89^{c}

Means having the same alphabet (s) within each column are not significantly different ($p \leq 0.05$) using ANOVA and Tukey's HSD (Honest Significant Difference) at $\alpha = 0.05$.

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Table 2 Percentage cumulative mortality (Mean ± SE) of *Callosobruchus chinensis* on cowpea treated with *n*-hexane and acetone extracts of *Senna occidentalis*.

Solvent	Concentration (%)	Exposure time (h)			
		24	48	72	96
n-Hexane	0.0	2.50 ± 2.50^{a}	2.50 ± 2.50^{a}	5.00 ± 2.89^{a}	5.00 ± 2.89^{a}
	0.2	0.00 ± 2.50^{a}	2.50 ± 2.50^{a}	12.00 ± 0.00^{a}	15.50 ± 6.60^{abc}
	0.4	0.00 ± 2.50^{a}	5.50 ± 2.89^{a}	15.25 ± 6.39^{ab}	23.25 ± 4.50^{abcd}
	0.6	0.00 ± 2.89^{a}	5.00 ± 2.89^{a}	13.00 ± 2.35^{ab}	31.25 ± 3.73^{bcd}
	0.8	2.50 ± 2.89^{a}	5.00 ± 2.89^{a}	23.25 ± 6.07^{ab}	39.00 ± 6.03^{cd}
	1.0	7.75 ± 2.50^{a}	10.25 ± 6.49^{a}	31.00 ± 9.68^{b}	44.00 ± 6.67^{d}
Acetone	0.0	0.00 ± 0.00^{a}	2.50 ± 2.50^{a}	5.00 ± 2.89^{a}	12.50 ± 2.50^{a}
	0.2	2.50 ± 2.50^{a}	5.00 ± 2.89^{a}	7.75 ± 2.59^{a}	11.00 ± 4.49^{a}
	0.4	2.50 ± 2.50^{a}	2.50 ± 2.50^{a}	7.75 ± 2.59^{a}	20.00 ± 3.08^{ab}
	0.6	5.00 ± 2.89^{a}	20.75 ± 4.72^{a}	26.00 ± 6.50^{ab}	37.25 ± 4.25^{bc}
	0.8	5.00 ± 2.89^{a}	10.00 ± 5.77^{a}	46.75 ± 5.65^{b}	54.75 ± 3.95^{cd}
	1.0	7.50 ± 2.50^{a}	18.00 ± 6.38^{a}	46.75 ± 11.95^{b}	68.75 ± 5.53^{d}

Means having the same alphabet (s) within each column are not significantly different ($p \leq 0.05$) using ANOVA and Tukey's HSD (Honest Significant Difference) at $\alpha = 0.05$.

Table 3 Lethal concentration (LC_{50}) of extracts of *Xylopia aethiopica* and *Senna occidentalis* on adult *Callosobruchus chinensis* at 96 hours post-treatment.

Extract	LC_{50} (%)	Lower limits (%)	Upper limits (%)	df	χ^2
n-hexane *X. aethiopica*	1.39	0.08	1.84	18	113.777
Acetone *X. aethiopica*	2.47	1.69	3.44	18	193.691
n-hexane *S. occidentalis*	1.37	0.94	3.80	18	104.609
Acetone *S. occidentalis*	0.73	0.64	0.86	18	93.334

χ^2 = Chi-square; df: degree of freedom.

Effect of *X. aethiopica* and *S. occidentalis* extracts on oviposition and adult emergence of *C. chinensis*

The number of egg laid and adult emergence of *C. chinensis* decreased with increasing concentration of both botanical extracts (Fig. 1). The highest number of egg and adult were observed in untreated cowpea seeds. Higher number of eggs (95) and emerged adults (43.95%) were observed on cowpea pre-treated with 5% v/w of *n*-hexane extract of *X. aethiopica* (Fig. 1A) compared to those observed on cowpea pre-treated with similar concentration of acetone extract of *X. aethiopica* (eggs laid: 80.75; emerged adults: 39.63) (Fig. 1B). Likewise, higher number of eggs (32.75) were observed on cowpea pre-treated with 5% v/w of *n*-hexane extract of *S. occidentalis* (Fig. 1C) compared to the number (12.75) observed on cowpea pre-treated with similar concentration of acetone extract (Fig. 1D). But, more adults (43.14%) emerged from cowpea pre-treated with 5% v/w of acetone extract of *S. occidentalis* (Fig. 1D) compared to percentage adults (31.30%) that emerged on cowpea pre-treated with similar concentration of *n*-hexane extract of *S. occidentalis* (Fig. 1C).

Effect of *X. aethiopica* and *S. occidentalis* extracts on the biochemical profile of *C. chinensis*

Effects of plant extracts on the protein quantity of adult *C. chinensis*

Protein quantity of adult *C. chinensis* treated with acetone (5.80 mg/ml) and n-hexane (5.35 mg/ml) extracts of *X. aethiopica* were lower than that of untreated adult *C. chinensis* (6.034 mg/ml) (Fig. 2). However, higher amount of protein was observed in the body of adult insects treated with acetone (6.24 mg/ml) and n-hexane (6.48 mg/ml) extracts of *S. occidentalis* compared to that of untreated control insects.

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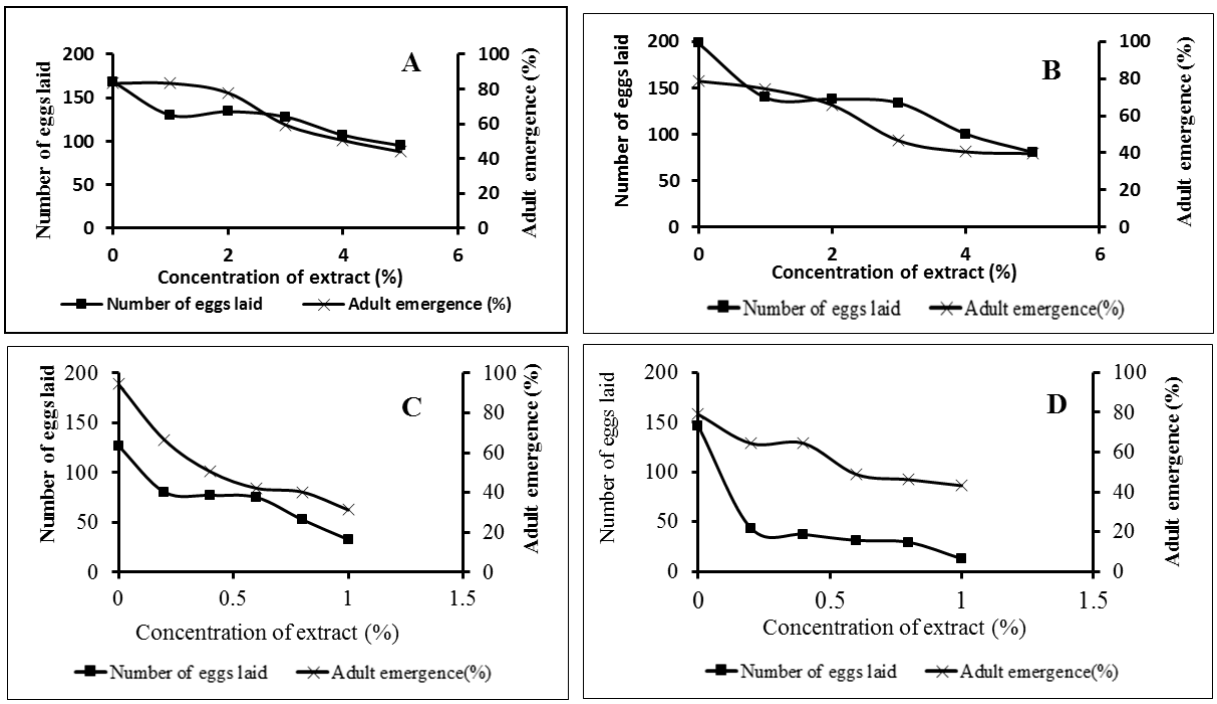

Figure 1 Number of eggs laid and adults of *Callosobruchus chinensis* that emerged from cowpeas pre-treated with **(A)** *n*-hexane *Xylopia aethiopica*, **(B)** acetone *X. aethiopica*, **(C)** *n*-hexane *Senna occidentalis* and **(D)** acetone *S. occidentalis* extracts.

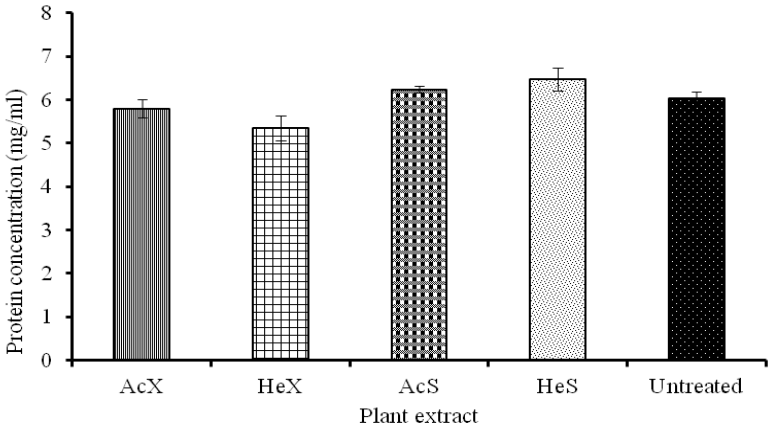

Figure 2 Protein content in adult *Callosobruchus chinensis* exposed to extracts of *Xylopia aethiopica* and *Senna occidentalis*. Each value is a mean of three replicates ± SEM. Note: AcX = Acetone extract of *X. aethiopica;* HeX = *n*-Hexane extract of *X. aethiopica*; AcS = Acetone extract of *S. occidentalis;* HeS = *n*-Hexane extract of *S. occidentalis*.

Effects of plant extracts on the activity of GST, Trehalase, GR and GPx in adult *C. chinensis*

There was a significant reduction ($p < 0.0001$) in the activity of GST ($F_{4, 10} = 1399$) (Fig. 3A) and trehalase ($F_{4, 10} = 21.42$) (Fig. 3B) of adult insects pre-treated with n-hexane extract of *S. occidentalis* compared to the other botanical treatment. Likewise, acetone and n-hexane extract of *S. occidentalis* significantly reduced ($p < 0.05$) the activity of GR and GPx respectively compared to all

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other treatments. Significantly higher ($p < 0.05$) activity of trehalase (3.60mM/min) and GR (49.25µmol/min/ml) was also observed in adult insects pre-treated with n-hexane extract of *X. aethiopica* compared to untreated insects. The activity of GST (112.27µmol/min/ml) and GPx (357.53µmol/min/ml) was significantly higher ($p < 0.05$) in adult insects pre-treated with acetone extract of *X. aethiopica* compared to the activity in untreated insects.

Chemical composition of acetone extract of *X. aethiopica* and *S. occidentalis*

A total of 25 (Table 4) and 14 (Table 5) compounds were identified by GC-MS analysis of the acetone extract of *X. aethiopica* and *S. occidentalis* respectively. The main compounds identified in *X. aethiopica* extract include the isomers of Aromandendrene (19.22%), Germacrene D (18.20%), 9,12-octadecadienoic acid (9.83%), Isospathulenol (9.32%), Bicyclo (4.3.0) nonane (6.48%) and Hexahydronaphthalen-2-2yl methanol (5.11%); while the remaining compounds ranged from 0.81 to 3.85% (Table 4). However, the major compound identified in the acetone extract of *S. occidentalis* is Diisoocotyl phthalate (50.37%) which is a plastic contaminant. Other important compounds include (E)-9-octadecenoic acid ethyl ester (11.89%), Hexadecanoic acid, ethyl ester (7.36%) and Linoleic acid, ethyl ester (6.74%) (Table 5). The chromatograms of acetone extract of *X. aethiopica* and *S. occidentalis* showing the various peak of different compounds identified in each extract are presented in Figures 4 and 5 respectively.

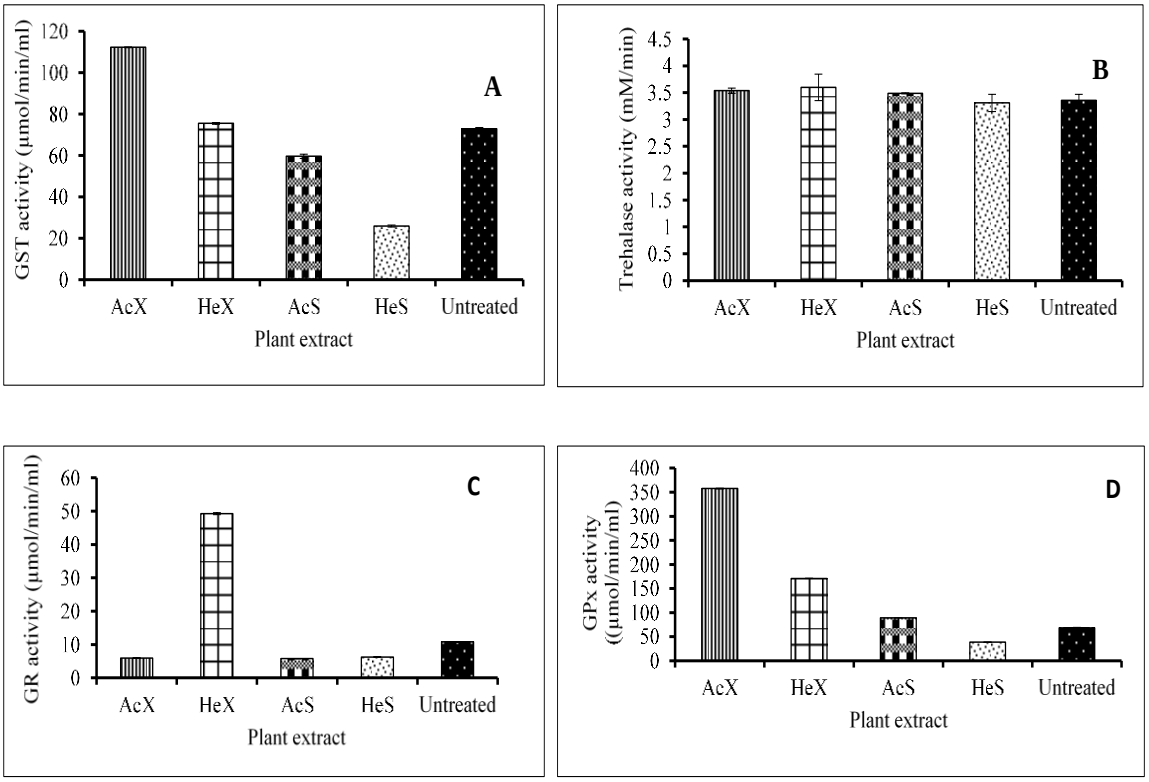

Figure 3 Impact of *n*-hexane and acetone extracts of *Xylopia aethiopica* and *Senna occidentalis* on the activity of (A) Glutathione S transferase (GST), (B) Trehalase, (C) Glutathione reductase (GR) and (D) Glutathione peroxidase (GP). Note: AcX = Acetone extract of *X. aethiopica;* HeX = *n*-Hexane extract of *X. aethiopica*; AcS = Acetone extract of *S. occidentalis;* HeS = *n*-Hexane extract of *S. occidentalis.*

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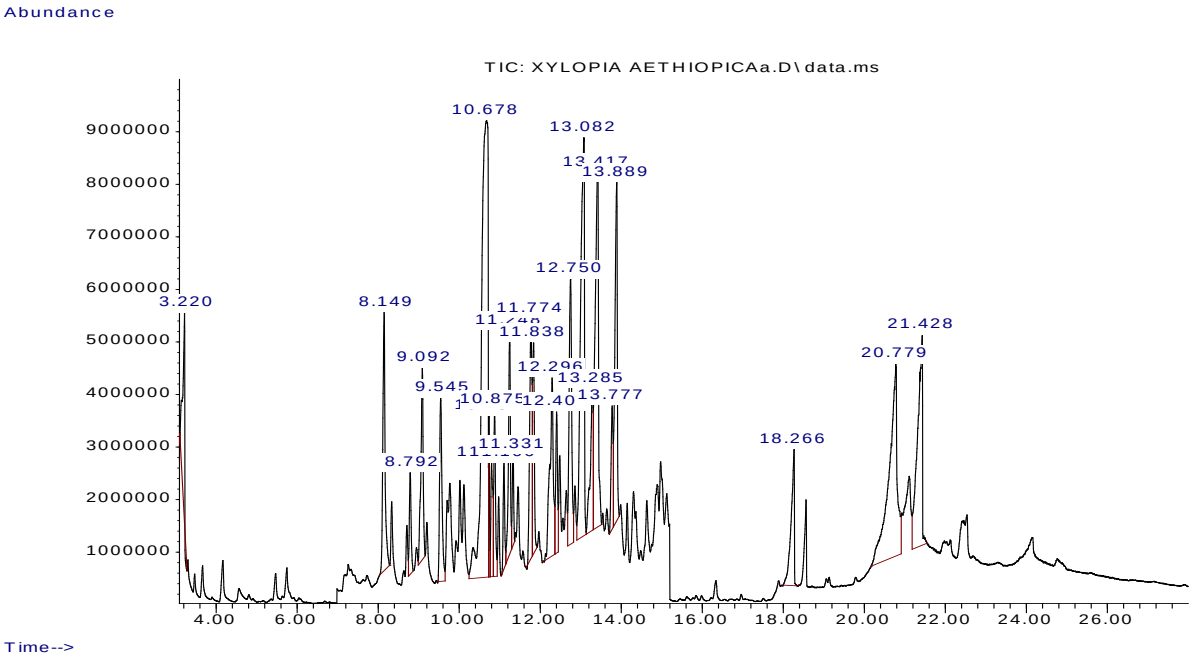

Figure 4 Chromatogram showing the chemical constituents of acetone extract of *Xylopia aethiopica*.

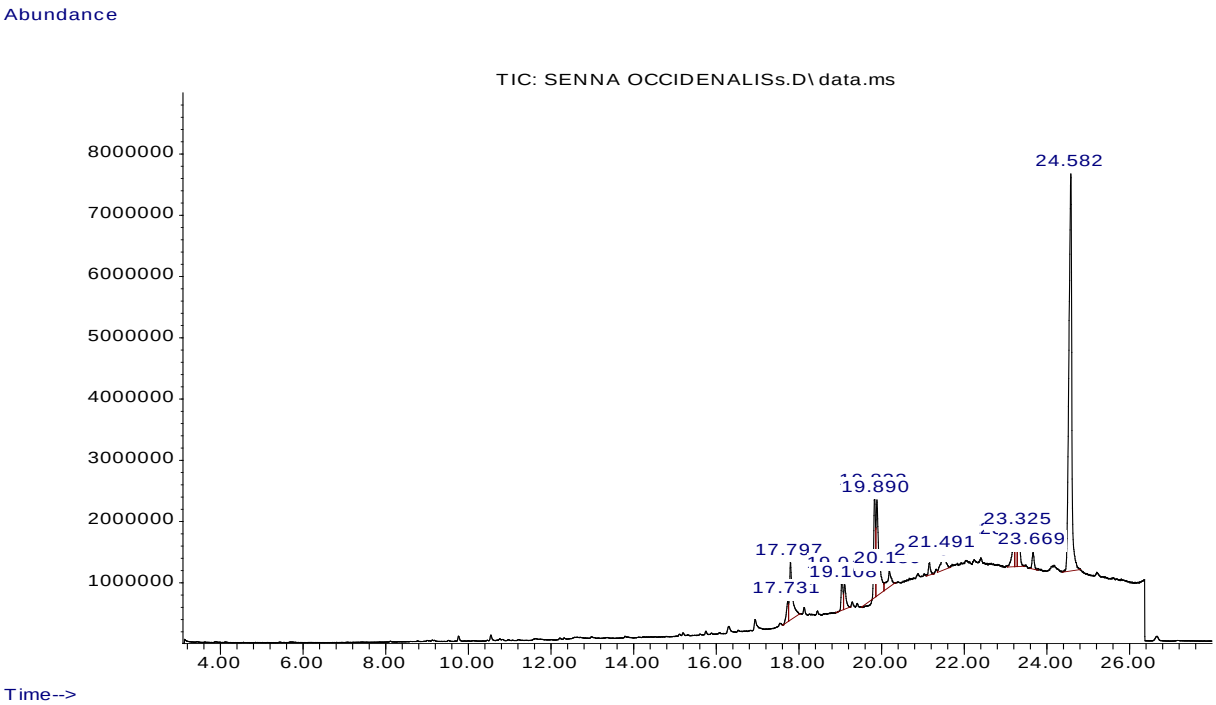

Figure 5 Chromatogram showing the chemical constituents of acetone extract of *Senna occidentalis*.

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Table 4 Chemical constituents of acetone extract of *Xylopia aethiopica.*

SN	RT	Name of compounds	CAS NO	% of total
1	3.220	Trans-beta-ocimene	003779-61-1	2.476
2	8.149	Cyclohexane	020307-84-0	3.625
3	8.792	Copaene	003856-25-5	1.133
4	9.092	Cyclohexane	000515-13-9	2.770
5	9.545	Beta-ylange	1000374-19-1	2.793
6	10.678	Germacrene D	023986-74-5	18.203
7	10.737	Naphthalene	017066-67-0	0.813
8	10.801	Octa hydronapthalene	006980-46-7	1.381
9	10.875	Naphthalene	000483-75-0	1.800
10	11.106	Naphthalene	039029-41-9	0.980
11	11.248	Hexahydronaphthalene	016729-01-4	2.690
12	11.331	Azulene	003691-11-0	0.823
13	11.774	Aromandendrene	000489-39-4	3.405
14	11.838	Aristolene epoxide	1000159-36-6	2.374
15	12.296	Aromandendrene	000489-39-4	3.853
16	12.407	1H-pyrrole,1-butyl	000589-33-3	1.629
17	12.750	Aromandendrene	000489-39-4	4.289
18	13.082	Isospathulenol	088395-46-4	9.317
19	13.285	1H-Cyclopropa(a)naphthalene	020671-49-2	2.417
20	13.417	Aromandendrene	000489-39-4	7.676
21	13.777	Eudesma-4(15),7-dien-1.beta.-ol	119120-23-9	1.439
22	13.889	Hexahydronaphthalen-2-2yl methanol	135118-51-3	5.108
23	18.266	(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalene-1-yl)pent-2-en-1-ol	021738-29-4	2.711
24	20.779	9,12-octadecadienoic acid	000060-33-3	9.826
25	21.428	Bicyclo (4.3.0) nonane	1000156-11-9	6.480

Table 5 Chemical constituents of acetone extract of *Senna occidentalis.*

SN	RT	Name of compounds	CAS NO	% of total
1	17.731	n-Hexadecanoic acid	000057-10-3	1.731
2	17.797	Hexadecanoic acid, ethyl ester	000628-97-7	7.355
3	19.045	8,11-octadecadienoic acid,methyl ester	056599-58-7	2.527
4	19.108	Cis-13-octadecanoic acid, methyl ester	1000333-58-3	2.359
5	19.833	Linoleic acid, ethyl ester	000544-35-4	6.744
6	19.890	(E)-9-octadecenoic acid ethyl ester	006114-18-7	11.887
7	20.189	Oleic acid	000112-80-1	2.846
8	21.162	Alloaromadendrene oxide-(1)	100156-12-8	1.101
9	21.491	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl	004602-84-0	3.111
10	23.211	9-Tricosene, (Z)	027519-02-4	2.772
11	23.231	1-octadecene	000112-32-2	2.099
12	23.325	9-Tricosene, (Z)	027519-02-4	3.580
13	23.669	1-Choloeicosane	042217-02-7	1.518
14	24.582	Diisoocotyl phthalate	000131-20-4	50.368

Discussion

The significant mortality observed in the numbers of adult *C. chinensis* exposed to pre-treated cowpea seeds coupled with the decrease in the oviposition and adult emergence, especially at the highest experimental concentration and exposure time, demonstrated the protective properties of both botanical extracts on cowpea against adult *C. chinensis*. This corroborates previous findings by other researchers where the insecticidal efficacy of both botanicals against other insect pest has been established (Habiba *et al.,* 2010; Adesina

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et al., 2011 Ratnasekera and Rajapakse, 2012; Silva *et al.*, 2013). Considerable death of adult *C. chinensis* on cowpea seeds pre-treated with each extract may be ascribed to the pungent smell emanating from both botanicals. This could have upset the normal respiratory activities of adult bruchids, resulting in asphyxiation and subsequent death (Ashamo *et al.*, 2013; Akinneye and Oyeniyi, 2016). Acetone extract of *X. aethiopica* was however less toxic to adult *C. chinensis* than its n-hexane extract but with *S. occidentalis*, acetone extract was more toxic to the bruchid. The variations in toxicity suggest a differential solubility of insecticidal active compounds in each botanical extract due to the solvent used for extraction.

Regardless of the nature of extract, lower LC_{50} values were observed for *S. occidentalis* than *X. aethiopica*. This shows that the former was more toxic to *C. chinensis* than the latter. This could be attributed to the presence of compounds such as n-hexadecanoic acid, oleic acid, and linoleic acid in *S. occidentalis* which are completely absent in *X. aethiopica*. These compounds identified in considerable amount in *S. occidentalis* have been established to be insecticidal and insectifuge (Kiran and Devi, 2007; Devi *et al.*, 2014). On the contrary, lower toxicity of extracts of *X. aethiopica* to *C. chinensis* may be attributed to the lower concentration of compounds such as cyclohexane and naphthalene that have been established to be toxic to man and insect (Frank, 2000; Lew, 2017). The higher proportion of non-insecticidal compounds could have reduced the potency of *X. aethiopica* extract to *C. chinensis* when compared to that of *S. occidentalis*.

Similarly, acetone extract of each botanical was more effective at reducing the egg-laying and adult emergence of *C. chinensis* compared to *n*-hexane extract. An exception was cowpea seeds pre-treated with acetone extract of *S. occidentalis* where more adult bruchids emerged than those pre-treated with *n*-hexane extract. Insectifuge compounds (n-hexadecanoic acid, oleic acid, and linoleic acid) identified in the acetone extract of both extracts could be responsible for considerable reduction observed in the laid eggs of bruchid. These compounds might have retarded movement and mating activities of adult *C. chinensis* and thus, reduced the number of eggs laid. Likewise, the reduction in the adult emergence from cowpea pre-treated with extracts of both botanicals, especially acetone extract, compared to control shows that they possess an obvious effect on post-embryonic development of *C. chinensis*. This could be linked to the penetration of toxic substance from the extract into the egg through the chorion which might have suppressed further embryonic development. For instance, a larvicidal agent known as Oleic acid (Devi *et al.*, 2014) was identified in *S. occidentalis* extract and this could be responsible for higher reduction in adult emergence observed in this study. El-Wakeil (2013) has earlier reported that oleic acid, linoleic acid and their alkyl esters are the dominant compounds in ethanol extract of *Cassia fistula* which inhibit oviposition and adult emergence.

Both extracts of *X. aethiopica* evoked a decrease in protein concentration compared to control but higher concentration of protein was observed with *S. occidentalis*. Proteins are essential biochemical components which play a major role in the development and growth of insects. The ability of both extracts of *X. aethiopica* to induce a considerable decrease in the level of protein suggested the possibility of extract-induced stress in the beetle exposed to this botanical extract. Nath *et al.* (1997) and Mojarab-Mahboubkar *et al.* (2015) had earlier opined that insect break down proteins into amino acids which enter the tricarboxylic acid (TCA) cycle as a keto acid, and supply energy for insect survival. Consequently, the reduction of tissue protein under insecticidal stress as observed in this study could serve as a coping mechanism needed to supply energy for bruchid survival (Rawi *et al.*, 1995). On the contrary, the increase in protein level in bruchids exposed to *S. occidentalis* compared to control could be a signal of developing tolerance to the extracts, considering the higher toxicity observed with this extract compared to *X. aethiopica*.

Glutathione S-transferases (GST), Glutathione peroxidase (GPx) and Glutathione reductase (GR)

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are essential groups of enzymes found in most organisms including insects which play an integral role in detoxification of toxins (Ebadollahi, 2011; Mojarab-Mahboubkar *et al.*, 2015). Extracts of *S. occidentalis* elicited significant reduction in the activity of GST, GPx and GR compared to their *X. aethiopica* counterpart. This indicates a higher decline in the detoxifying ability of *C. chinensis* with *S. occidentalis* than *X. aethiopica*, corroborating our earlier result that *S. occidentalis* was more toxic to *C. chinensis* than *X. aethiopica*. The decrease in the detoxifying ability of *C. chinensis* with *S. occidentalis* is in compliance with previous findings that botanical extracts could reduce the activity of detoxifying enzymes (Coruh *et al.*, 2007). However, the rate of activity of the three detoxifying enzymes studied in this study varied with the type of solvent used for extraction. Highest reduction in GST and GPx activities were achieved with *n*-hexane extract of *S. occidentalis* compared to all other treatment levels while that of GR was achieved with acetone extract of *S. occidentalis*. The activity of GST and GPx however increased significantly with acetone extract of *X. aethiopica* compared to all other treatment levels while that of GR increased with n-hexane extract. It could therefore be inferred that GST, GPx and GR in *C. chinensis* do not play a major role in the detoxification of acetone and n-hexane extracts of *X. aethiopica* (Mojarab-Mahboubkar *et al.*, 2015).

Trehalose is the main haemolymph sugar found in larvae, pupae and adult of insects while trehalase is an enzyme which catabolizes one mole of trehalose to two moles of glucose, thus providing energy for most physiological activities of insects (Tompson, 2003; Klowden, 2007; Ge *et al.*, 2011). Lower activity of trehalase was observed in bruchids exposed to extracts of *S. occidentalis* compared to those exposed to *X. aethiopica*. The reduction in trehalase activity in bruchids exposed to *S. occidentalis* could have reduced energy needed for flight, movement and reproduction in adult bruchids, thus reducing the number of eggs laid and adult emergence. The enhanced trehalase activity observed with extracts of *X. aethiopica* suggest that more moles of glucose might have been produced by adult bruchids through catabolism, thus providing insects with sufficient energy to recover from physiological stress created by *X. aethiopica* extracts. This could be responsible for lower toxicity of *X. aethiopica* to adult *C. chinensis* when compared to *S. occidentalis*.

In summary, results obtained from this study show that n-hexane and acetone extracts of *X. aethiopica* and *S. occidentalis* possess: insecticidal, oviposition-deterring and eclosion-inhibiting properties. The rate of effectiveness of each botanical extract varied with the solvent used for extraction. The internal metabolism of adult *C. chinensis* was affected by both botanical extracts through changes in the amount of proteins, GST, GPx, GR and trehalase in the body of *C. chinensis* compared to untreated insects. *S. occidentalis* was however observed to be more toxic and evoked higher decline in the detoxifying ability of *C. chinensis* than *X. aethiopica*. A significantly higher decrease in protein concentration was also observed with *X. aethiopica* extracts compared to control. Extracts of both botanicals were found to contain differential amounts of insecticidal and insectifuge compounds. Due to differential solubility of insecticidal active compounds in each botanical extract, it would be interesting to investigate if mixing of both botanical extracts will augment or reduce the quantity of these compounds as well as their effectiveness. This is being further investigated in our laboratory.

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اثرات سمّی و سازوکارهای بیوشیمیایی دو عصاره گیاهی روی سوسک چینی حبوبات *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) در دانه‌های لوبیا چشم‌بلبلی

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چکیده: تلاش برای یافتن منابع جدید حشره‌کش ارزان و سازگار با محیط‌زیست برای مدیریت آفات هم‌چنان یک چالش بزرگ برای کشاورزان لوبیاکار در بسیاری از کشورهای در حال توسعه است. در این مطالعه، اثرات سمی و سازوکارهای بیوشیمیایی عصاره‌های استونی و اهگزانی دو گونه گیاهی تحت نام‌های *Xylopia aethiopica* (Dunal) و *Senna occidentalis* (L.)، روی سوسک چینی حبوبات *Callosobruchus chinensis* (L.) بررسی شدند. کارایی حشره‌کش با غلظت، مدت زمان در معرض قرار گرفتن و نوع عصاره‌ها متفاوت بود. سمّیت عصاره استونی *X. aethiopica* (LD_{50} = 2.47%) کم‌تر از عصاره هگزانی (LD_{50} = 1.39%) بود، اما در مورد گونه گیاهی *S. occidentalis*، سمّیت عصاره استونی (LD_{50} = 0.73%) بیش‌تر از عصاره هگزانی (LD_{50} = 1.37%) بود. عصاره استونی از هر دو گیاه موجب کاهش معنی‌داری در تخم‌گذاری و خروج حشرات کامل شدند. تنها عصاره *X. aethiopica* غلظت پروتئین را نسبت به شاهد کاهش می‌دهد. فعالیت گلوتاتیون ردوکتاز و گلوتاتیون پراکسیداز توسط هر دو عصاره از گیاه *S. occidentalis* به‌طور معنی‌داری کاهش یافت، این درحالی است که تنها عصاره هگزانی *S. occidentalis* باعث کاهش معنی‌داری در فعالیت گلوتاتیون S- ترانسفراز و ترهالاز در مقایسه با سایر تیمارها شد. آنالیز GC-MS، وجود دی ایزوکوتیل فتالات (۵۰/۳۷ درصد) و ایزومرهای آرومان دندرن (۱۹/۲۲ درصد) به‌ترتیب به‌عنوان ترکیب اصلی در *S. occidentalis* و *X. aethiopica* را نشان داد. هم‌چنین هر دو عصاره گیاهی حاوی ترکیبات دیگر حشره‌کش در مقادیر مختلف بودند. به‌طور کلی یافته‌ها نشان می‌دهد که عصاره‌های گیاهی، به‌ویژه عصاره استونی گیاه *S. occidentalis* می‌توانند به‌عنوان جایگزینی به‌جای حشره‌کش‌های مصنوعی برای کنترل حشرات کامل سوسک چینی حبوبات به‌حساب آیند.

واژگان کلیدی: *Xylopia aethiopica*، *Senna occidentalis*، گلوتاتیون ردوکتاز، گلوتاتیون پراکسیداز، حشره‌کش

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