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*In-vitro*Pesticidal effects of Water hyacinth leaf and Cashew nut shell extracts against *Acanthoscelides obtectus*and *Zonocerus variegatus*

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***Abstract*:**
Background: *The study investigated the effects of Water hyacinth methanol extract (WHME) and Cashew nut shell extract (CNSE) on in-vitro activities of selected enzymes in Acanthoscelides obtectus and Zonocerus variegatus*
.***Materials and Methods****: Water hyacinth leaves and cashew nut shells were macerated and subjected to Soxhlet extraction to obtain WHME and CNSE, respectively.Acanthoscelides obtectus and Zonocerus variegatus were divided into two portions each. One portion was homogenized with phosphate buffer (pH 7.4), while the other portion was homogenized with Tris-HCl buffer (pH 7.8), and then centrifuged. Total proteinand activities of superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST), acetylcholinesterase (AChE) and carboxylesterases (CES)ofthe homogenates were spectrophotometrically assayed, at varying concentrations of WHME and CNSE, using Cypermethrin (CYP) and chlorpyrifos (CPF) as positive controls.*
Results*: The WHME, CNSE, CPF and CYP significantly ($p < 0.05$) reducedcatalase and SODactivities in A. obtectus, while the activities were increased by WHME and reduced by CPF in Z. variegatus. In-vitro GST was increased by WHME and CNSE in A. obstectus and Z. variegatus, but reduced by CYP and CPF in A. obstectus.The AChE activities in A. obstectus and Z. variegatus were reduced by WHME and CNSE compared with positive controls.Activity of CES was reduced by CNSE in A. obstectus and Z. variegatus similar to CYP.*
Conclusion*: Water hyacinth leaf and cashew nut shellextracts exhibitedin-vitro insecticidal effects against Acanthoscelides obstectus and Zonocerus variegatus, via antioxidant imbalance and cholinergic disruption.*
Key words: *Water hyacinth; cashew nut shell;Acanthoscelides obtectus; Zonocerus variegatus; biopesticides.*

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I. Introduction

The field and store activities of many insect pests have generally inflicted serious agricultural sabotage, withgreat economic losses to crop farmers, locally and at the global level[1]. Among such pests, *Zonocerus variegatus*[2] and *Acanthoscelides obstectus* (Say)[3] have been well associated to great losses to corn and beans farming, which are staple foods, all over the world.

Acanthoscelides obstectus (Say) (Coleoptera: Bruchinae), commonly called Bean weevil, is both a field and store pest of leguumenous crops including,*Phaseolus vulgaris*, *Phaseoluslunatus* and many others[3]. The wide distribution of this insect has been reported in many parts of the world such the Europe [4,5], America[6], Australia[7], the Mediterranean region[8] and Africa[9]. The presence of the Bruchid insect family across the globe has been suggested to be due to the adaptive ability of the insects, as they are distributed across the temperate and tropical regions of the world[10]. Under suitable conditions, *A. obstectus* exhibits a very high growth rate, the life cycle being 3-4 weeks, and within one year, it can produce several generations of itself [11,12]. On infestation by *A. obstectus*, the female insect lays an egg beside a single bean seed, which later enters the seed to develop first into a larva, and then a pupa (first instar larva). At the final instar larval stage, the larva feeds on the bean seed to make small openings at the base of the seed[13]. The damaging effect of the insect also raises the temperature and water content of the bean seed, thereby compromising the physiological integrity and germination potential of the bean seed[14].

Zonocerus variegatus is a polyphagus insect, whose destructive activity has been well prevalent in the western and central parts of African continent. Grasshopper exhibits incomplete metamorphosis, involving the egg, nymph (imago) and adult stages. Although, the nymph resembles the adult, the former lacks reproductive

capacity. A study by Anandet al[15] noticed that *Zonocerus variegatus* is very rich in both calories and proteins, thereby making the insect useful as grasshopper meal[16], and as a replacement for fish meal used as growers broiler diet[17].An *in-vitro* study carried out by Adeleke et al[18]showed the potential of the seed kernel extract of *Ricinuscommunis* (castor) to disrupt both the antioxidant and cholinergic systems of *Zonocerus variegatus* (grasshopper) nymph, whereas, the adult insect was not significantly affected.

Pest control and management has been largely undertaken by the applications of synthetic insecticides belonging to pyrethroids and organophosphates[4]. Such synthetic agents have been reported to undergo resistance in the targeted insects, and also exert off-target toxicities affecting beneficial organisms, through environmental contamination[19,20]. The need for plant-based insecticides therefore becomes imperative, as alternative strategies, since phytochemicals are biodegradable and eco-friendly [21,22].

Eichhornia crassipes (Water hyacinth) is an invasive perennial aquatic plant belonging to the Pontideraceae family[23]. It is widely distributed to many parts of the world [24,25], although, its original home was the Brazilian Amazonia[26]. Water hyacinth has been reported to possess antitumour[27], antioxidant[28], antimicrobial and anti-inflammatory[29] properties. In addition, this plant was reported to possess insecticidal activities, affecting developmental stages in female *Culexquinquefasciatus* (mosquitoes)[30], *Corcyra chevalonica* (rice moth)[31]and *Spodopteralittoralis*(cotton leaf worm)[32].

Anacardium occidentalis (Cashew), on the other hands, belongs to the plant family of Anacardiceae majorly localized to the tropical regions of the world[33]. A nut of cashew consists of an outer and inner shell enclosing a kernel. The space in-between the outer and inner shells contains a viscous dark brown coloured liquid referred to as cashew nut shell liquid (CNSL)[34]. The CNSL is reported to contain anacardic acid, cardol, cardanol and many other organic compounds[35]. Studies have shown that the components of CNSL could exhibit antibacterial, antifungal[36], antioxidant[37] and anti-inflammatory[38] properties. Furthermore, the CNSL has been reported to show insecticidal activity against termite[39], rice weevil[35], as well as cockroach[40].

The objective of the study was to investigate*in-vitro* effects of the extracts of Water hyacinth (*Eichhornia crassipes*) leaves and Cashew (*Anacardium occidentalis*) nut shell on activities of selected antioxidant enzymes and esterases in *Acanthoscelides obstectus* and *Zonocerus variegatus*, and their possible biopesticidal properties against these insect pests.

II. Materials and Methods

Chemicals and Reagents

Acetylthiocholine, methanol, Acetone, Paranitrophenylacetate, paranitrophenol, Triton-X-100, Dithionitrobenzoic acid (DTNB), acetylthiocholine iodide and Adrenaline were purchased from Sigma Chemical Company, and Hopkins and Williams company, United Kingdom. All other chemicals were of good analytical grades.

Collection and Extraction of *Eichhornia crassipes* (water hyacinth) leaves

The leaves of *E. crassipes* (water hyacinth) were collected at Olubere River, Aroje, Ogbomoso, Oyo state, Nigeria in May, 2019. The leaves were air dried at the room temperature for about three weeks and then pulverizedusing electric grinder to obtain a coarse powder. The powder was subjected to Soxhlet extraction (dissolving 25 g of pulverized nut shell in 250ml of methanol), followed by concentration using a rotary evaporator. The extract concentrate was driedin an electric ovenat 40^{o}C to obtain a dry powder used as the Water hyacinth methanol extract (WHME).

Collection and Extraction of *Anacardium occidentalis*(Cashew) nut shell

Cashew Nuts were bought from WAZO market, Ogbomosho, Oyo state, Nigeria in May, 2019. The nuts were de-shelled, and the shells were air-dried for three weeks at the room temperature. The shells were then pulverized using electric grinder, and then subjected to Soxhlet extraction (dissolving 25 g of pulverized nut shell in 250ml of n-hexane). The extract obtained was concentrated using rotary evaporator and then subjected to oven drying at 40^{o}C, to obtain oily Cashew nut shell extract (CNSE).

Collection, culturing and homogenization of *Acanthosclides obstectus* (Bean weevils)

*Acanthoscelides obtectus*was collected from the infested bean seeds (*Phaseolus vulgaris*) from the Research and Demonstration farm, belonging to the Faculty of Agriculture, Ladoke Akintola University of Technology, Ogbomoso, Nigeria in July, 2019. The insects were confirmed by Dr. Olayioye A. in the Department of Crop and Environmental Protection from the same Faculty. In the Laboratory, the insects were kept on the bean seeds at about 25^{0}C and 65 % relative humidity, and exposed to 16 hours of light and 8 hours of darkness[41]. The insects were collected from the culture system, and divided into two categories. One category was homogenized using phosphate buffer (pH 7.4), while the second category was homogenized with Tris-HCl

buffer (pH 7.8). The insect homogenates were centrifuged using a refrigerated centrifuge at 10,000*xg* for 5 minutes. The supernatants were kept at 4^0C until used for analysis.

Collection and homogenization of adult *Zonocerus variegatus* (Grasshopper)

Thirty adult grasshoppers (*Zonocerus variegatus*)were collected using a sweeping net on a cassava farmland in Ogbomoso, Oyo State, Nigeria, in June, 2019. The insects were confirmed by Dr. Olayioye A. in the Department of Crop and Environmental Protection, Faculty of Agricultural Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The insects were divided into two categories (fifteen insects each) and then de-winged. One category of the insects was homogenized in phosphate buffer (pH 7.4) while, the second category was homogenized with Tris-HCl buffer (pH 7.8). The insect homogenates were centrifuged using a refrigerated centrifuge (HITACHI model) at 10,000 *x g* for 5 minutes. The supernatants were kept at 4^0C until used for analysis.

Determination of Total Protein in insect Homogenates

The total protein of homogenatesof *Acanthosclides obstectus* and adult *Zonocerus variegatus*was determined spectrophotometrically using the Biuret method described by Lowry *et al*[42].

Determination of Catalase activity

Catalase activities in the insect homogenates were estimated according to the method of Aebi[43], with some modifications. The reaction mixture contained 4.0 ml of hydrogen peroxide solution (0.2 M), 5.0 ml of Phosphate buffer (0.01 M, pH 7.0) and 1.0 ml of properly diluted insect homogenates(the two insects treated separately). An aliquot of 0.3 ml each of WHME and CNSE at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml was added to the reaction mixture at the room temperature. Each reaction mixture was also treated with similar concentrations of Cypermethrin and Chlorpyrifos, separately, in place of the extracts (as positive controls). The control mixture contained neither the extracts nor the commercial insecticides. An aliquot of 1.0ml of the reaction mixture was blown into 2 ml of dichromate/acetic acid reagent at 60 seconds intervals.Change in absorbance was monitored at 240nm for 180 seconds at an interval of 60 seconds, and the enzyme activity was expressed as units/mg protein.

Determination of Superoxide dismutase activity

The superoxide dismutase (SOD) activities in insect homogenates were estimated according to the method described by Misra and Fridovich[44], with some modification. Briefly, a mixture of 0.2 ml diluted insect homogenate (the two insects treated separately) and 2.5 ml carbonate buffer (0.05M, pH 10.2) was allowed to equilibrate in the spectrophotometer. Each mixture was treated with 0.2 ml of WHME and CNSE separately at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml, followed by addition of 0.3ml of freshly prepared adrenaline (0.3M) as substrate. Each mixture was also treated with similar concentrations of Cypermethrin and Chlorpyrifos, separately, in place of the extracts (as positive controls). The control mixture contained neither the extracts nor the commercial insecticides. The absorbance was taken spectrophotometrically at 480nm every 30 seconds for 150 seconds, and enzyme activity was expressed as Units/mg protein.

Determination of Glutathione-S-transferase activity

Glutathione-S-transferase (GST) activity of insect homogenates was assayed according to the method of Habiget al[45] with modification. The reaction mixture contained 30 µl of GSH (0.1 M), 150 µl of CDNB (20 mM), 2.79 ml of phosphate buffer (0.1 M, pH 6.5) and 30 µl of insect homogenates added sequentially. This is followed by addition of 0.3 ml each of WHME and CNSE, at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml, to the mixture. Each mixture was also treated with similar concentrations of Cypermethrin and Chlorpyrifos, separately, in place of the extracts (as positive controls). The control mixture contained neither the extracts nor the commercial insecticides.The blank contained 30 µl of GSH, 150 µl of CDNB and 2.82 ml of phosphate buffer (pH 6.5). The reaction was allowed to run for 60 seconds at 31^0C before the absorbance was read spectrophotometrically against the blank at 340nm. GST activity was calculated using the formula below:

The extinction coefficient of CDNB = $9.6 mM^{-1} cm^{-1}$

$$\text{GST specific activity} = \text{OD/min} \times \frac{1}{9.6} \times \frac{1}{0.03\text{ml/ mg protein}}$$

GST specific activity = µM Conjugate/min/mg protein

Determination of acetylcholinesterase Activity

The activity of acetylcholinesterase (AChE)enzyme in insect homogenates was estimated according to the methods described by Ellman[46], andNachmansohn and Neumann[47], with modification. The reaction mixture (the two insects treated separately) contained 2.6 ml phosphate buffer (0.1M, pH 7.4), 0.1 ml Dithionitrobenzoic acid (DTNB) and 0.4 ml insect homogenate. This is followed by addition of 0.3 ml each of WHME and CNSE, at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml, to the mixture. Then 0.1 ml of Acetylthiocholine iodide solution was added, as substrate, to initiate the reaction. Each reaction mixture was also treated with similar concentrations of Cypermethrin and Chlorpyrifos, separately, in place of the extracts (as positive controls). The control mixture contained neither the extracts nor the commercial insecticides. The rate of acetylcholinesterase activity was measured spectrophotometrically by following the increase in intensity of the yellow product at 412nm for 10 mins at an interval of 2 minutes. Acetylcholinesterase activity was calculated using the formula below with the molar extinction of 1.361x $mmol^{-1}$ xmm^{-1}:

$$\text{Acetylcholinesterase activity} = \frac{\text{Change in absorbance x Total reaction volume}}{\text{Time x sample volume x molar extinction}}$$

AChE activity = U/mg protein

Determination of carboxylesterase Activity

Carboxylesterase (CE) activities in insect homogenates were determined using the method described by Clement and Erhardt[48], with modification, using paranitrophenyl acetate as a substrate for the enzyme. Each of *A. obstectus* (bean weevil) and *Z. variegatus* (grasshopper) was homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.8 with 1 % Triton X-100 at 25^0C) using a tissue homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 *x g* for 5 minutes at 4 ^{0}C to obtain supernatant. An aliquot of 0.5 ml of diluted supernatant (1:10 dilution) of each insect was added to 2 ml of the working buffer (0.1 M Tris-HCl, pH 7.8, containing 2 mM EDTA at 25 ^{0}C), followed by 0.3 ml each of WHME and CNSE, at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml. The mixture was incubated at 37 ^{0}C for 10minutes and the reaction was initiated by adding 0.2 ml of paranitrophenyl acetate (50 mM)(prepared in acetone) as substrate. Each reaction mixture was also treated with similar concentrations of Cypermethrin and Chlorpyrifos, separately, in place of the extracts (as positive controls). The control mixture contained neither the extracts nor the commercial insecticides. The change in absorbance was read spectrophotometrically at 405nm, for 5 minutes at an interval of 1 min. The blank reagent contained 2.0 ml of working buffer and 0.2 ml paranitrophenyl acetate. Paranitrophenol standard curve was prepared to calculate the activity of Carboxylesterase enzyme, expressed as mM/min/ml protein.

Statistical Analysis

Data were expressed as Mean ± Standard deviation. Student T-test and One-way analysis of variance (ANOVA) test were used for statistical comparison of treatments, taken significant values at $p < 0.05$. Data analysis was done using the Statistical Package for Social Sciences (SPSS) softwarefor Windows version 10.0 (USA).

III. Results

Catalase activity

The result in table 1 showsthat WHME, CNSE, CPF and CYP significantly ($p < 0.05$) reduced the catalase activity in *Acanthoscelides obtectus* (bean weevil) across the concentrations (10- 80 µg/ml), compared with the negative control. The activity of catalase enzyme in adult *Zonocerus variegatus* (grasshopper) was found to be significantly ($p < 0.05$) increased by both WHME and CNSE, while the activity was found to be significantly reduced by both CPF and CYP across the concentrations (Table 2).

Superoxide dismutase activity

The results in table 3 shows that WHME, CNSE, CPF and CYP all significantly ($p < 0.05$) reduced the SOD activity in *A. obstectus* across all the concentrations (10- 80 µg/ml)used in the experiment compared with the control. Table 4 shows that WHME significantly ($p < 0.05$) elevated the activity of SOD in adult *Z. variegatus* (grasshopper), while CNSE and CPF significantly ($p < 0.05$) reduced the activity of the enzyme across the concentrations under study. However, treatment with CYP has no significant effect ($p > 0.05$) on the enzyme activity in adult*Z. variegatus*, across the concentrations.

Table 1: Effects of WHME, CNSE, CPF and CYP on in-vitro Catalase activity in *Acanthoscelides obstectus*

Conc. (µg/ml)	Catalase activity $x10^{-3}$ (U/mg protein)			
	WHME	CNSE	CPF	CYP
10	0.31± 0.02[a]	0.31± 0.01[a]	0.34±0.01[a]	0.32± 0.01[a]
20	0.64± 0.02[a]	0.20± 0.01[a]	0.72±0.08[a]	3.55± 0.35[b]
30	0.97 ± 0.01[a]	0.35± 0.07[a]	0.46± 0.64[a]	3.95± 0.07[b]
40	1.30 ± 0.04	0.35± 0.02[a]	0.69± 0.04[a]	0.72± 0.08[a]
50	0.60 ± 0.01[a]	0.54± 0.23[a]	0.34± 0.01[a]	2.61± 0.43[b]
60	0.40 ± 0.00[a]	0.36± 0.08[a]	0.21± 0.29[a]	0.37± 0.06[a]
70	0.76 ± 0.01[a]	0.83± 0.04[a]	0.34± 0.01[a]	1.28± 0.04
80	0.96 ± 0.02[a]	1.04± 0.06[a]	0.66± 0.01[a]	0.71± 0.07[a]
Control		1.95± 0.06		

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

Table 2: Effects of WHME, CNSE, CPF and CYP on in-vitro Catalase activity in adult *Zonocerus variegatus*

Conc. (µg/ml)	Catalase activity $x10^{-3}$ (U/mg protein)			
	WHME	CNSE	CPF	CYP
10	22.5 ± 0.636[b]	31.85 ± 0.64[b]	0.61 ± 0.01[a]	1.4 ± 0.14[a]
20	6.69 ± 0.127[a]	39.75 ± 0.78[b]	8.08 ± 0.11	1.68 ± 0.11[a]
30	15.95 ± 0.064[b]	36.05 ± 0.70[b]	2.4 ± 0.14[a]	1.28 ± 0.03[a]
40	29.75 ± 0.354[b]	34.4 ± 0.14[b]	0.66 ± 0.06[a]	0.35 ± 0.07[a]
50	45.96 ± 0.063[b]	31.9 ± 0.49[b]	0.33 ± 0.03[a]	11.7 ± 0.14[b]
60	20.05 ± 0.064[b]	24.5 ± 0.71[b]	0.32 ± 0.03[a]	0.66 ± 0.08[a]
70	52.97 ± 0.042[b]	25.6 ± 0.99[b]	1.65 ± 0.07[a]	1.06 ± 0.08[a]
80	22.96 ± 0.0636[b]	23.55 ± 0.78[b]	0.31 ± 0.01[a]	2.35 ± 0.07[a]
Control	8.6±0.28			

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

Table 3: Effects of WHME, CNSE, CPF and CYP on in-vitro Superoxide dismutase activity in *Acanthoscelides obtectus*

Conc. (µg/ml)	Superoxide dismutase activity $x10^{-3}$ (U/mg protein)			
	WHME	CNSE	CPF	CYP
10	2.01 ± 0.01[a]	2.06 ± 0.08[a]	2.00 ± 0.01[a]	3.11 ± 0.15[a]
20	2.01 ± 0.01[a]	1.01 ± 0.01[a]	6.00 ± 0.04	4.02 ± 0.02[a]
30	3.01 ± 0.01[a]	2.45 ± 0.64[a]	1.00 ± 0.01[a]	4.03 ± 0.04[a]
40	8.00 ± 0.01[b]	2.51 ± 0.56[a]	2.00 ± 0.02[a]	6.08 ± 0.11
50	2.01 ± 0.01[a]	1.86 ± 0.06[a]	9.00 ± 0.01[b]	3.11 ± 0.15[a]
60	4.06 ± 1.00[a]	1.26 ± 0.36[a]	3.00 ± 0.01[a]	1.00 ± 0.01[a]
70	2.01 ± 0.01[a]	1.96 ± 0.06[a]	3.01 ± 0.01[a]	2.00 ± 0.01[a]
80	6.00 ± 0.01	2.02 ± 0.02[a]	2.01 ± 0.01[a]	2.00 ± 0.01[a]

Control	5.77 ± 0.01

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control (p< 0.05)
[b]- Activity significantly higher compared with control (p< 0.05)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

Table 4: Effects of WHME, CNSE, CPF and CYP on in-vitro Superoxide dismutase activity in adult *Zonocerus variegatus*

Conc. (µg/ml)	Superoxide dismutase activity $x10^{-3}$ (U/mg protein)			
	WHME	CNSE	CPF	CYP
10	18.9 6± 0.64[b]	2.98 ± 0.03[a]	7.41 ± 0.73[a]	10.05 ± 0.78
20	17.57 ± 0.08[b]	2.00 ± 0.07[a]	6.03 ± 0.35[a]	10.01 ± 0.48
30	15.81 ± 0.27[b]	4.03 ± 0.03[a]	2.00 ± 0.37[a]	12.05 ± 0.71
40	14.59 ± 0.58	6.98 ± 0.39[a]	4.75 ± 0.54[a]	13.57 ± 0.68
50	16.56 ± 0.43[b]	5.03 ± 0.03[a]	5.75 ± 0.36[a]	9.70 ± 0.44
60	13.01 ± 0.35	7.04 ± 0.05[a]	9.53 ± 0.65[a]	14.95 ± 0.71
70	15.06 ± 0.78[b]	5.06 ± 0.08[a]	8.55 ± 0.36	9.45 ± 0.36
80	15.96 ± 0.64[b]	7.01 ± 0.02[a]	7.4 ± 0.78[a]	9.56 ± 0.29
Control	12.22 ± 0.29			

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control (p< 0.05)
[b]- Activity significantly higher compared with control (p< 0.05)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

Glutathione-S-transferase (GST) activity

The result in figure 1 shows that the *in-vitro* activity of GST enzyme in *A. obstectus* (bean weevils) was significantly elevated by WHME only at most of the concentrations, while CNSE significantly ($p < 0.05$) elevated it at all the concentrations. However the activity was found to be significantly reduced ($p < 0.05$) by both CPF and CYP across all the concentrations compared with the negative control. In figure 2, the *in-vitro* GST activity in *Z. variegatus* (grasshopper) was significantly ($p < 0.05$) increased by WHME, CNSE and CYP at most of the concentrations, while CPF showed no significant ($p > 0.05$) effect compared with negative control.

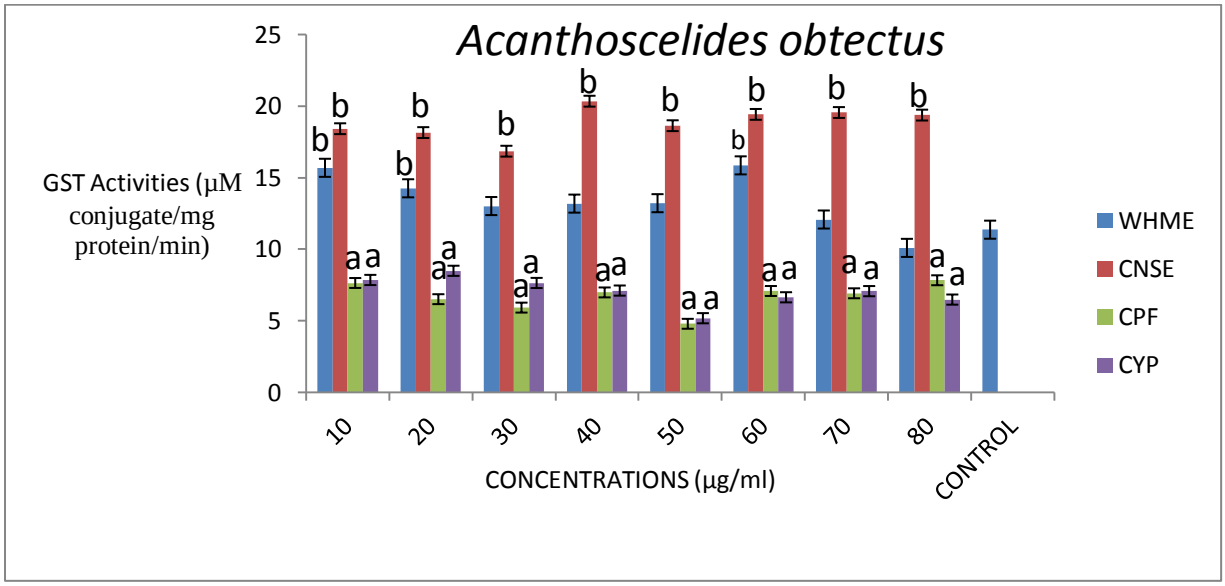

Fig 1:Effects of WHME, CNSE, CPF and CYP on Glutathione-S-transferase (GST) enzyme activities in *Acanthoscelides obtectus* (Bean weevil)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

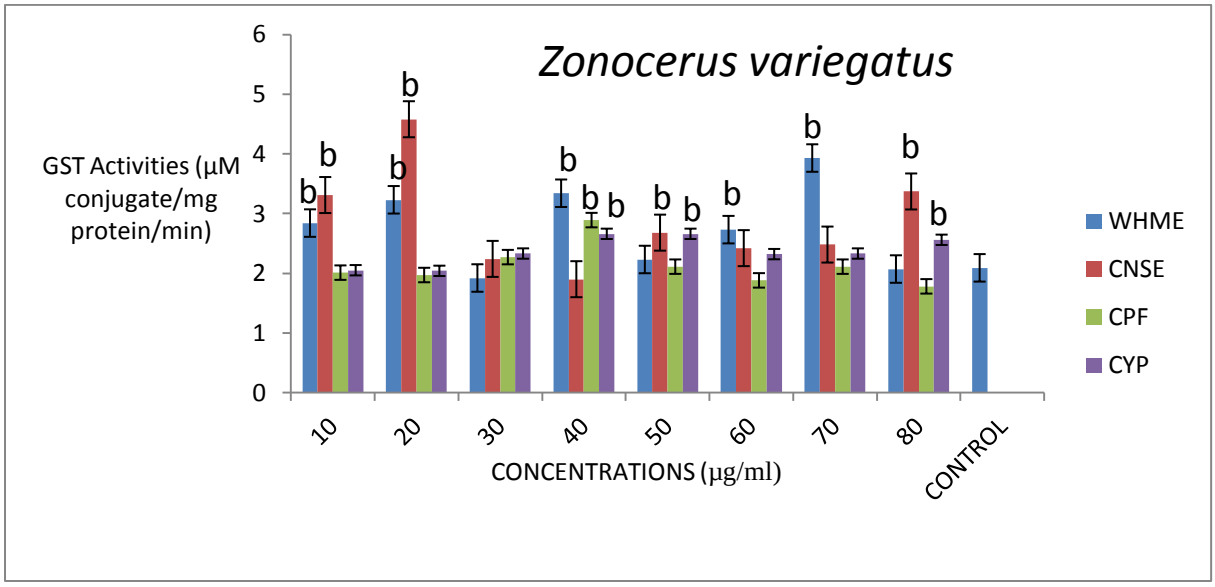

Fig. 2: Effects of WHME, CNSE, CPF and CYP on Glutathione-S-transferase (GST) enzyme activities in adult *Zonocerus variegatus* (Grasshopper)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

Acetylcholinesterase (AChE) activity

The *in-vitro* activity of AChE in *A. obstectus* was significantly ($p < 0.05$) lowered by WHME, CNSE and CYP at nearly all concentrations used in the study, compared with negative control (Figure 3). However, CPF was found to increase the activity at high concentrations 50 – 80 µg/ml (Figure 3). The result in figure 4 shows that WHME and CNSE significantly ($p < 0.05$) reduced the *in-vitro* activity of AChE at all concentrations in *Z. variegatus*, compared with negative control. However, CPF was found to significantly reduce the activity, while CYP elevated it at most of the concentrations.

Carboxylesterase (CES) activity

In figure 5, WHME and CPF were observed to significantly ($p < 0.05$) increase the carboxylesterase (CES) *in-vitro* activity, while both CNSE and CYP significantly reduced it at most concentrations in *A. obstectus*, compared with negative control. However, in *Z. variegatus*, WHME, CNSE, CPF and CYP significantly ($p < 0.05$) reduced the CES activity, as shown figure 6.

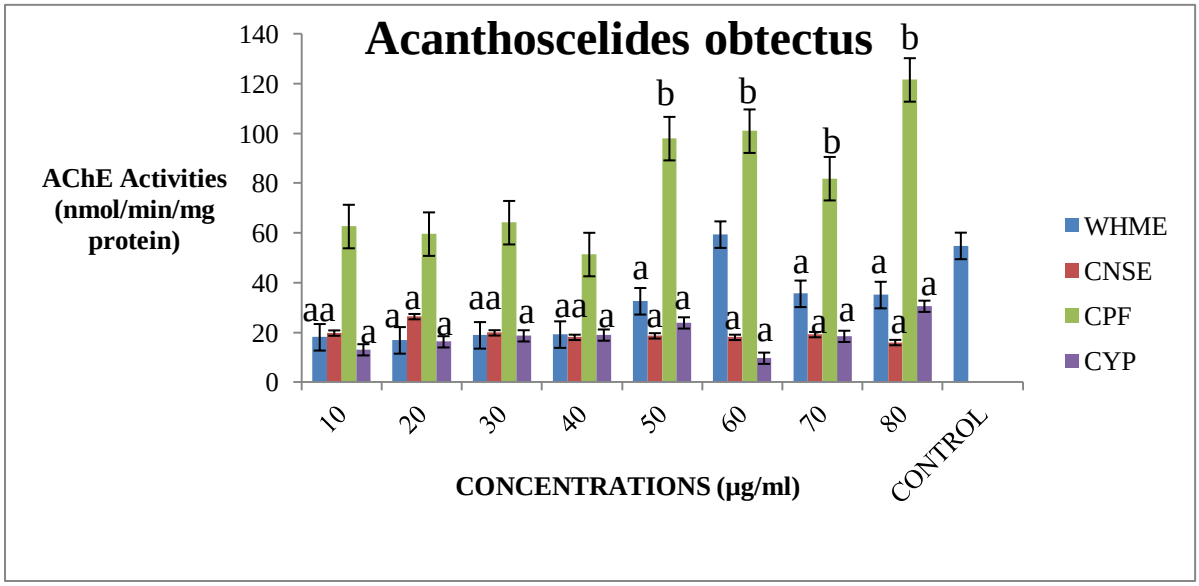

Fig. 3: Effects of WHME, CNSE, CPF and CYP on Acetylcholinesterase(AChE) enzyme activities in *Acanthoscelides obtectus* (Bean weevil)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

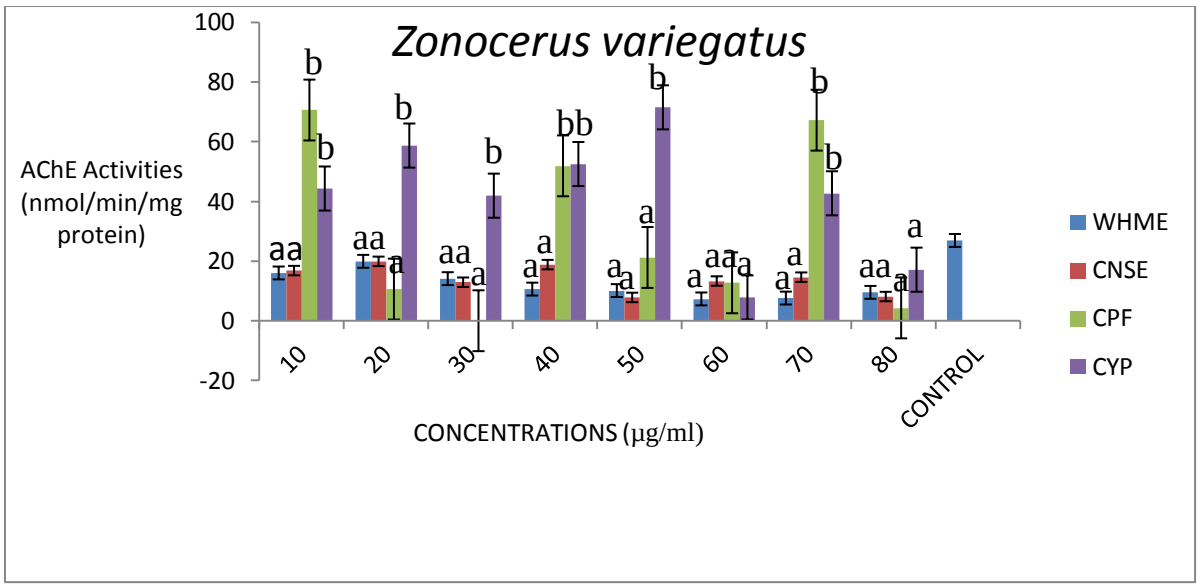

Fig. 4: Effects of WHME, CNSE, CPF and CYP on Acetylcholinesterase(AChE) enzyme activities in adult *Zonocerus variegatus* (Grasshopper)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

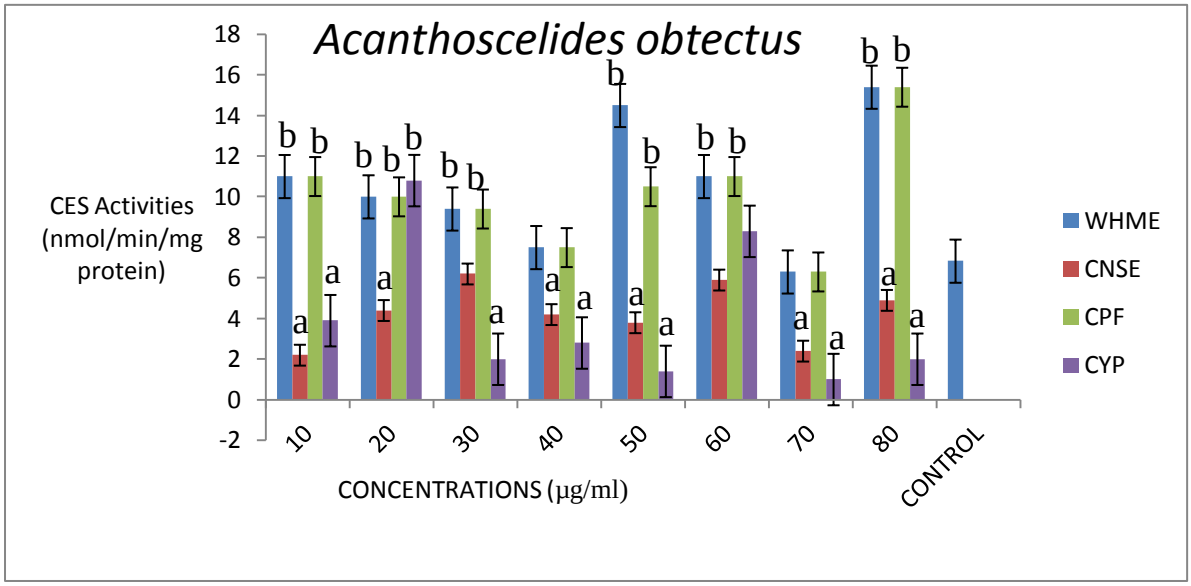

Fig. 5: Effects of WHME, CNSE, CPF and CYP on Carboxylesterase(CES) enzyme activities in *Acanthoscelides obtectus* (Bean weevil)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

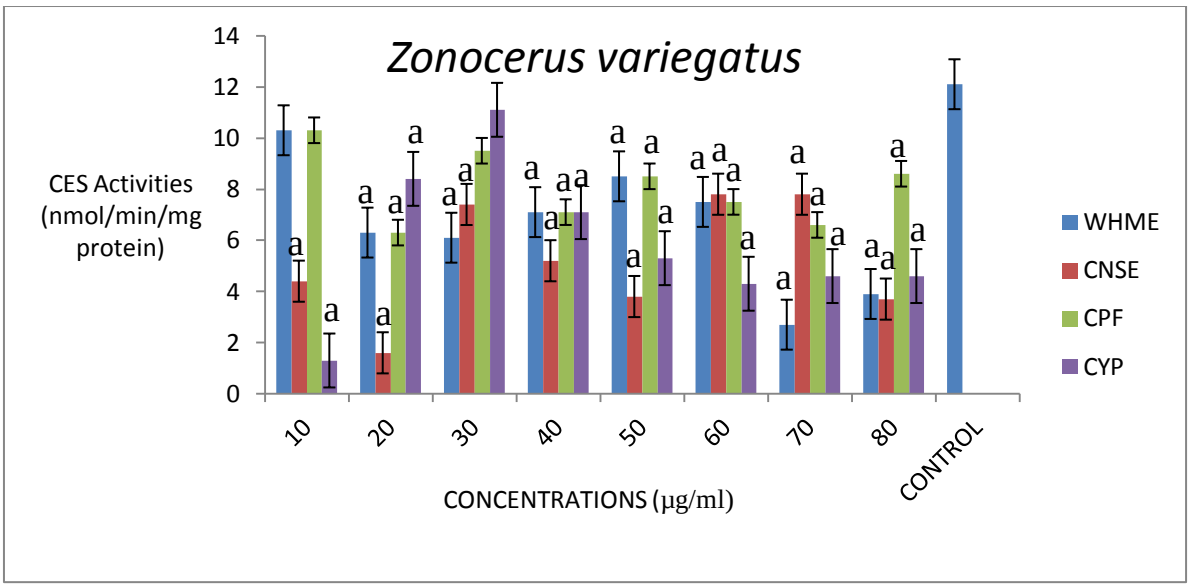

Fig. 6: Effects of WHME, CNSE, CPF and CYP on Carboxylesterase(CES) enzyme activities in adult *Zonocerus variegatus* (Grasshopper)

Values expressed as mean ± standard deviation
[a]- Activity significantly lower compared with control ($p< 0.05$)
[b]- Activity significantly higher compared with control ($p< 0.05$)
WHME-Water hyacinth methanol extract, CNSE-Cashew nut shell extract, CPF- Chlorpyrifos, CYP-Cypermethrin

IV. Discussion

In the recent times, plant-based pesticides have been highly preferred to the conventional synthetic pesticides, since the former are quite biodegradable and constitute no environmental pollution[21,22]. The present study investigated the in-vitro activities of Water hyacinth methanol extract (WHME) and Cashew nut shell

extract (CNSE) on some of the antioxidant enzymes and esterases in *Acanthoscelides obstectus* (bean weevils) and *Zonocerus variegatus* (grasshopper), using chlorpyrifos and cypermethrin as positive controls.

A complex antioxidant system has been documented in different insects[49,50]. Superoxide dismutase and catalase are two major antioxidant enzymes responsible for detoxifying superoxide anions. While SOD enzyme converts superoxide anion into hydrogen peroxide, catalase detoxifies the hydrogen peroxide to form water and molecular oxygen[51,52]. The present study reveals that, in-vitro activity of catalasewas inhibited by WHME, CNSE, CPF and CYP in *A. obtectus* across the concentrations used. However, treatment with WHME and CNSE induced catalase activity in adult *Z. variegatus*, while CPF and CYP inhibited the activity across the concentrations. In *A. obtectus*, in-vitro SOD activity was inhibited by WHME, CNSE, CPF and CYP across the concentrations used in the study. In adult *Z. variegatus*, the activity of the enzyme was elevated by WHME, while CNSE and CPF reduced it across the concentrations used in the study. Insecticides, such as organophosphates have been reported to induce oxidative stress and alter antioxidant status through in-vitro and in-vivo studies[53].The present study has shown that WHME, CNSE and the two positive controls demonstrated inhibitory effects on catalase and superoxide dismutase in *A. obtectus*. This inhibition indicates possible insecticidal effectsof these agents via induction of oxidative stress in the insect. On the other hand,adult *Z. variegatus*showed high activities of catalase and SOD against WHME and CNSE, indicating the ability of the insect to detoxify superoxide radicals, thereby conferring resistance to oxidative stress in the insect.Polyphagous grasshoppers such as *Melanoplus sanguinipes*[54], *Romalea microptera*[55] and*Z. variegatus*[18]are less susceptible to changes in antioxidant status, due to exposure to a wide variety of plant chemicals.A study by Rodriguez-Gonzalez et al[41] has shown that essential oils from *Ocimumbasilicum* and *Cymbopogonwinterianus* could exhibit insecticidal activities on*A. obtectus* (bean weevil).

In insects, GST enzymes are responsible for detoxification of insecticides and plant allelochemicals, as well as mediation of oxidative stress responses induced by these chemicals agents[56-58]. Among the GST isoenzymes, the delta and epsilon classes are specific to insects, and they play significant roles in the adaptation of these animals to environment agents[59]. The detoxification of organophosphate insecticides by GSTs has been proposed to occur through O-dealkylation and O-dearylation. The O-dealkylation involves conjugation of glutathione to the alkyl group of the insecticide, while O-dearylation occurs via interaction between glutathione and the leaving group of the insecticide[60]. The present study has shownthat in-vitro GST enzyme activities in both *A. obstectus* and adult *Z. variegatus* were elevated by CNSE and WHME.However, the GST activity was reduced by both CYP and CPF in the bean weevils, but elevated in adult *Z. variegatus.*Several studies have shown that the major classes of insecticides are associated with increased activity of GSTs due to elevated gene transcription and amplification[56,57], thereby inducing a protective mechanism in insects[60].The elevated activity of GST observed in the present study thus suggests the ability of this enzyme to induce resistance in both *A. obstectus* and adult *Z. variegatus* on exposure to WHME and CNSE. Studies have shown that insecticides may become incapacitated due to the defense mechanisms inherently present in insects[61].A recent study on the molecular basis of resistance of *Anopheles funestus*to insecticides such as DDT, permethrin and dieldrin revealed the involvement of GST and carboxylesterase enzymes[62].

Acetylcholinesterase (AChE) is a hydrolytic enzyme catalyzing the breakdown of acetylcholine into acetate and choline at the neuromuscular junction[63,64]. An inhibition of the AChE could therefore result in persistent accumulation of acetylcholine at the neuromuscular junction, leading to cholinergic disruption[65]. The present study has shownthat WHME and CNSEsignificantly reduced the in-vitro activity of AChE enzyme in both *A. obstectus*and*Z. variegatus*, as against the two commercial insecticides used as positive controls.Studies by Stasiuket al[66]and Almeida et al[67]revealed that cashew nut shell liquid (CNSL) could potentially inhibit acetylcholinesterase activity. The ability of the two extracts used in this study to substantially reduce AChE activity in both *A. obstectus* and *Z. variegatus* is an indication that, the extracts could exert insecticidal potential throughneurological damagein the insects.

The activities of carboxylesterases (CES) in both *A. obstectus* and *Z. variegatus* were significantly inhibitedby CNSE, similar to CYP. However, WHME inhibitedthe activity of the enzymein adult *Z. variegatus*, and elevated it in *A. obstectus*, similar to CPF. Carboxylesterases hydrolyze carboxyl esters through reversible acylation of a serine residue in the active site of this enzyme, to yield the alcohol moiety of the carboxyl ester and the acylated CES intermediate. The acylated CES intermediate is finally decomposed via a nucleophilic addition of waterto form the free CES enzyme and a corresponding carboxylic acid[68]. Through the activities of CES, insects and mammals have developed resistance against several carboxyl ester-containing exogenous compounds[69,70].In a recent in-vitro study, it was noticed that CYP, CPF and castor seed kernel extract substantially reduced the activity of CES in*Podagrica sjosdteti*, a species of flea beetles[71]. The reduction in the activities of CES in both *A. obstectus* and *Z. variegatus*, as obtained in the present study, is an indication of susceptibilityto CNSE and CYP toxicities, andpossible insecticidal effects in these insects. Furthermore, the fact thatWHME and CPF inhibited in-vitro CES activity in *Z. variegatus*, and induced it in *A. obstectus*, may indicate species differences in response of the enzyme to these two agents. Carboxylesterase enzymes have been

documented to show species differences[72] and multiple ligand-binding sites[73] in their functions and responses to inhibitors.

V. Conclusion

The data obtained from the present study has revealed that water hyacinth methanol extract and cashew nut shell extract could exhibit insecticidal effects against*Acanthoscelides obtectus*and *Zonocerus variegatus*, via antioxidant imbalance and cholinergic disruption.Water hyacinth methanol extract and cashew nut shell extract could therefore be used as biopesticides alternative to conventional pesticides, cypermethrin and chlorpyrifos, in combating *A. obstectus*and *Z. variegatus.*

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