

CHEMICAL COMPOSITION AND INSECTICIDAL EFFECTS OF *AZADIRACHTA INDICA* AND *HELIANTHUS ANNUUS* LEAVES EXTRACTS ON ANTIOXIDANT AND CHOLINERGIC ENZYMES IN WEEVILS

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ABSTRACT

Azadirachta indica and *Helianthus annuus* are reported to possess insecticidal potentials due to presence of a myriad of chemical compounds in them. Synthetic insecticides are not eco-friendly and could induce resistance in target insects. Certain botanical agents are capable of insecticidal actions, with no harm to the ecosystem. Fresh leaves of *Azadirachta indica* and *Helianthus annuus* were collected air-dried, pulverized and subjected to Soxhlet extraction using methanol and rotary evaporation to obtain *A. indica* leaf methanol extract (AILME) and *Helianthus annuus* leaf methanol extract (HALME). The extracts were chemically characterized using High-Performance Liquid Chromatography (HPLC). Enzyme assays were carried with the extracts against the superoxide dismutase (SOD), catalase and acetylcholinesterase of maize and bean weevils. The HPLC Chromatogram of AILME alone was found to contain β – caryophyllene, trans- β – farnes, α – ionone, phytol, ascaridol, phyllene, quercetin, azadirachtol, azadirachnol, Azadirachta A, myricetin and α – funebren. However, the chromatogram of a mixture of AILME and HALME showed presence of β – caryophyllene, trans- β – farnes, furostan, α – ionone, phytol, ascaridol, caffeic acid, α – amyrin, β – amyrin, α – tocopherol, quercetin, azadirachtol, Azadirachta A, avanasterol, myricetin, α – funebren, phytic acid, lecithin and cephalin. The mixture of AILME and HALME reduced the activities of SOD, catalase and acetylcholinesterase enzymes in the maize weevil and bean weevil comparable to Dichlorvos and Phostoxin. *Azadirachta indica* and *Helianthus annuus* leaves contain compounds which could induce insecticidal actions in maize weevils and bean weevils, via oxidative and anti-cholinergic mechanisms.

Keywords: *Azadirachta indica*, *Helianthus annuus*, insecticidal action, phytochemicals, Weevils

INTRODUCTION

In the recent time, pesticides from botanical sources have been well-studied for their potential for ecofriendly pest management and sustainable agriculture (Hikal *et al.*, 2017). *Azadirachta indica* has been mostly studied among the promising plants for biopesticidal development (Aribi *et al.*, 2020). Neem (*Azadirachta indica*) is a fast-growing evergreen plant widely distributed in the Indian subcontinent, America and Africa (Islas *et al.*, 2020). The tree can attain a height of about 20 meters, with branches, compound leaves and fragrant white flowers. The fruits are small and oval-shaped drupes, with one seed (Adusei and Azupo, 2022). Neem trees are highly valued for their ecological importance, including improvement of soil fertility, prevention of erosion and serving as windbreaks in the arid

areas (Prasad and Prasad, 2018). The presence of the several chemical constituents in neem has been responsible for its several biological properties, making the plant useful in control and management of many diseases (Alzohairy, 2016).

Although, all the parts of the neem tree, including leaf, bark, flower, twig and seed kernel are rich in biologically active compounds, a study carried out by Kumar *et al.* (2016) noticed the maximum of activity with the seed kernel. Soni *et al.* (2012) quantified the azadirachtin content of neem leaves methanol extract as being up to 73.62%. A study carried out by Adeleke *et al.* (2021) using GC-FID technique on *Azadirachta indica* seed kernel extract revealed the presence of maliacin, nimbin, nimbidin, nimbolide, quercetin, salannin, saladucin, azadirachtin and

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azadiradione. Among these compounds, Azadirachtin has been widely studied for its biological activities significant to the control of agricultural pests (Kilani-Morakchi *et al.*, 2021). More than 130 of the neem compounds are limonoid-based triterpenoids with high potentials as medicinal and insecticidal agents (Chen *et al.*, 2018). Neem has been reported to have both herbicidal and pesticidal potentials due to the activities of its compounds on specific physiological processes in weeds and insect pests. This makes the plant suitable for sustainable agriculture, and as a source of an environmentally friendly alternative to synthetic pesticides (Oulhaci *et al.*, 2018).

The sunflower is an annual plant with rough-hairy stems attaining between 100 and 300 cm in height. It is a flowering plant, in which the disk flowers are reddish-brown, and the ray flowers are yellow in colour (Al-Snafi, 2018). Etievant *et al.* (1984) documented the presence of phenols, alcohols, terpene hydrocarbons, esters and oxygenated compounds in sunflower using Gas chromatographic technique. *H. annuus* was shown to possess high DPPH [1, 1-diphenyl-2-picrylhydrazyl] radical scavenging activity in a concentration-dependent manner (Subashini and Rakshitha, 2012). Extracts of the plant were reported to be potential against *Plasmodium falciparum* K1 strain (Mohamed *et al.*, 2014) and *Plasmodium berghei* infected Swiss albino mice (Ejabe *et al.*, 2011). The ethanol extract of *H. annuus* stem was demonstrated to possess antimicrobial effect against several organisms, including *Candida albicans*, *Aspergillus niger* and *Staphylococcus aureus* (Adetunji *et al.*, 2014).

The present study was design to chemically characterize the hydroethanolic extracts of *Azadirachta indica* and *Helianthus annuus* leaves, and investigate the effects on some antioxidant enzymes and acetylcholinesterase in maize weevils and bean weevils.

Materials and Methods

Collection and extraction of *Azadirachta indica* and *Helianthus annuus* leaves. Fresh leaves of *Azadirachta indica* and *Helianthus annuus* were collected from the premises the Department of Biochemistry, Ladoke Akintola University of technology Ogbomoso (8.1700° N and 4.2636° E), Oyo State, Nigeria, in January 2025. After air-drying, at the room temperature for about two weeks, 250 g each of the leaves was separately pulverized with a mechanical blender, and

subjected to Soxhlet extraction using methanol, and rotary evaporation at 65°C, followed by Oven-drying at 45 °C, to obtain *A. indica* leaf methanol extract (AILME) and *Helianthus annuus* leaf methanol extract (HALME), which were kept in an air-tight amber-coloured bottled and stored at 4°C.

High performance liquid chromatography (HPLC) analysis

The AILME and a mixture of AILME and HALME (1:1) were subjected to analysis by HPLC machine (Agilent) at a flow rate of 0.5 mL/min with an injection volume of 20 mL. A mixture of acetonitrile and methanol (80:20, v/v) was used as the mobile phase. The C18 (4.5 x 250 mm, 5µm) column was run at the room temperature for 8 minutes and the eluent was detected at 210nm.

2.3 Collection and homogenization of insects

Maize weevils and bean weevils were collected from the Department of Crop and Environmental Protection, Faculty of Agricultural Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The two groups of insects were separately homogenized in phosphate buffer (pH 7.4) and centrifuged at 10000 x g for 10 minutes. The supernatant was kept at 4°C until use.

Protein determination

The total protein levels of the insect homogenates were determined using a commercial kit according to the method of Lowry *et al.* (1951)

Determination of Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity in insect homogenates was determined by the method of Misra and Fridovich (1975), with modifications. Briefly, an aliquot of 0.2 ml of the diluted insect homogenate was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. Then, 0.2 ml aqueous solution of AILME + HALME (15, 30, 45, 60, 75 and 90 µg/ml) was added to the reaction mixture, followed by 0.3ml of freshly prepared 0.3M epinephrine. Commercial insecticides (Dichlorvos and Phostoxin) were used in place of the extract at similar concentrations as standards. Absorbance was spectrophotometrically taken at 480nm for 150 seconds at an interval of 30 seconds. Enzyme activity was expressed as Units/mg protein.

Determination of Catalase activity

Catalase activity of the insect homogenates was determined according to Aebi (1984) with modifications. The reaction mixture contained 4

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ml of hydrogen peroxide solution (0.2 M) and 5 ml of Phosphate buffer (0.01 M, pH 7.0) in a 10ml flat bottom flask. The homogenate containing crude enzyme (1.0 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion and 0.3 ml aqueous solution of AILME + HALME (15, 30, 45, 60, 75 and 90 µg/ml) was added in separate test tubes at the room temperature. Commercial insecticides (Dichlorvos and Phostoxin) were used in place of the extract at similar concentrations as standards. An aliquot of the reaction mixture (1 ml) was blown into 2 ml of dichromate acetic acid reagent at 60 seconds intervals, monitoring the change in absorbance at 240nm at an interval of 60 seconds, for 180 seconds. The enzyme activity was expressed as Units/mg protein.

Acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity in insect homogenates was determined following the methods described by Ellman, (1961), and Nachmansohn and Neumann, (1975) with modifications. The reaction mixture contained 2.6 ml phosphate buffer (0.1M, pH 7.4), 0.1 ml Ellman's reagent (DTNB), 0.4 ml insect homogenate and 0.3 ml aqueous solution of AILME + HALME (15, 30, 45, 60, 75 and 90 µg/ml). This was followed by addition of 0.1 ml of acetylthiocholine iodide solution (substrate) to the reaction mixture, to initiate the reaction. Similar concentrations of commercial Chlorpyrifos and Cypermethrin were used in place of extract. The control mixture contained neither a combination of AILME and HALME nor the commercial pesticides. The rate of acetylcholinesterase activity was monitored spectrophotometrically by measuring the absorbance of the product at 412nm at an interval of 2 minutes for 10 minutes. Acetylcholinesterase activity was calculated using the formula below, taking the molar extinction to be $1.361 \times \text{mmol}^{-1} \times \text{cm}^{-1}$:

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

$$\text{AChE activity} = \text{U/mg protein}$$

Results and Discussions

Figure 1 shows the HPLC Chromatogram of *A. indica* leaf methanol extract (AILME). The compounds (and the percentage abundances) present in the extract include β – caryophyllene (31.88%), trans- β – farnes (9.16%), α – ionone (10.49%) phytol (0.97%), ascaridol (0.65%), phyllene (0.63%), quercetin (11.71%), azadirachtol (31.09%), azadirachnol (0.76%), Azadirachta A (0.94%), myricetin (0.925) and α – funebren (0.82%). The HPLC of a mixture of AILME and HALME has identified compounds

including β – caryophyllene (27.36%), trans- β – farnes (11.00%), furostan (0.59%), α – ionone (9.69%), phytol (1.52%), ascaridol (0.65%), caffeic acid (0.75%), α – amyryn (2.21%), β – amyryn (1.89%), α – tochopherol (0.78%), quercetin (7.55%), azadirachtol (24.66%), Azadirachta A (1.14%), avanasterol (1.50%), myricetin (0.89%), α – funebren (1.68%), phytic acid (2.135), lecithin (0.90%) and cephalin (0.77%) (Table 2). The *in-vitro* SOD activities were significantly ($p < 0.05$) lowered by the mixture of AILME and HALME, Dichlorvos and Phostoxin in bean weevil and maize weevil relative to control, at all the concentrations applied as shown in table 1. The results in table 2 also reveal that the activities of catalase in both bean weevil and maize weevil were significantly ($p < 0.05$) reduced by the mixture of AILME and HALME, Dichlorvos and Phostoxin compared with control. The *in-vitro* activities of acetylcholinesterase enzyme in the two insects were similarly found to be significantly ($p < 0.05$) reduced by treatments with a mixture of AILME and HALME, Dichlorvos and Phostoxin (Table 3).

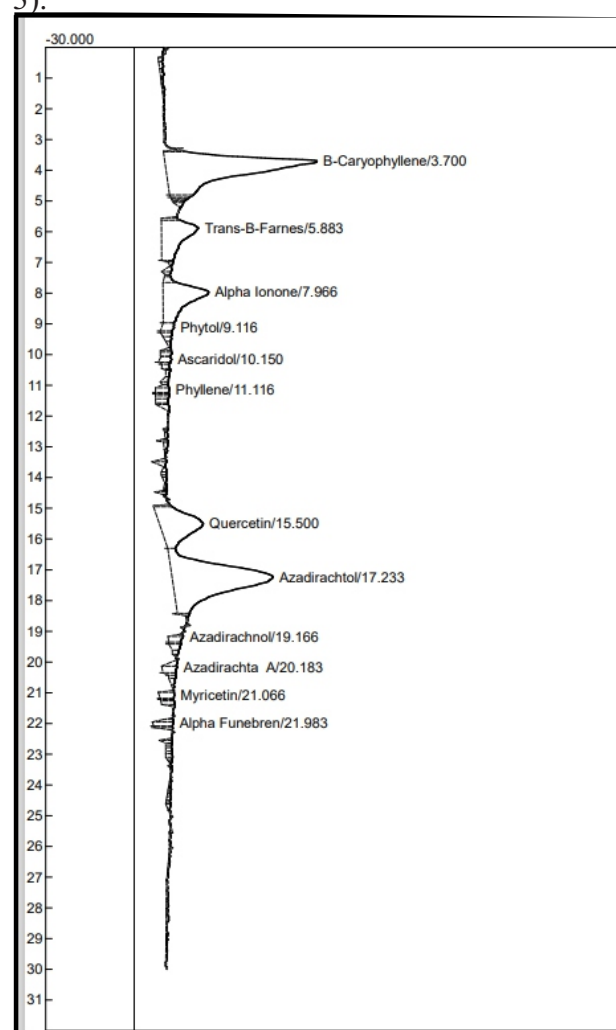


Fig.1. High-Performance Liquid Chromatogram of *A. indica* leaf methanol extract (AILME)

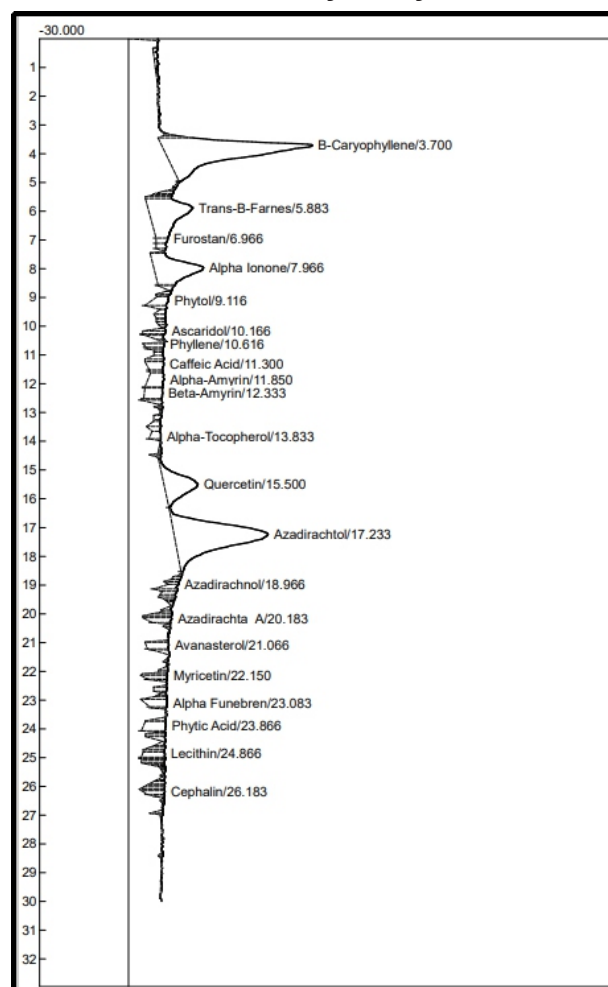


Fig.2. High-Performance Liquid Chromatogram of mixture of *A. indica* leaf methanol extract and *Helianthus annuus* leaf methanol extract

Table 1: In-vitro effects of a mixture of *A. indica* and *Helianthus annuus* leaves methanol extract, Dichlorvos and Phostoxin on Superoxide dismutase activity in bean weevil and maize weevil

Concentration (µg/ml)	SOD activity ×10 (U/mg protein)				
	Bean weevil homogenate		Maize weevil homogenate		
	AILME + HALME	Dichlorvos	Phostoxin	AILME + HALME	Dichlorvos Phostoxin
15	2.10 ± 0.02*	4.30 ± 0.02	1.97 ± 0.21*	1.25 ± 0.10*	3.11 ± 0.04* 2.12 ± 0.01*
30	2.13 ± 0.21*	3.11 ± 0.91*	2.42 ± 0.10*	1.43 ± 0.10*	3.60 ± 0.15* 2.33 ± 0.11*
45	3.01 ± 0.06*	1.99 ± 0.54*	3.21 ± 0.11*	3.75 ± 0.02*	3.99 ± 0.03* 2.09 ± 0.04*
60	2.71 ± 0.00*	3.51 ± 0.20*	3.13 ± 0.25*	2.62 ± 0.21*	2.72 ± 0.00* 3.02 ± 0.03*
75	2.66 ± 0.11*	1.78 ± 0.0*	2.77 ± 0.07*	2.34 ± 0.36*	3.55 ± 0.13* 2.61 ± 0.10*
90	3.14 ± 0.10*	3.22 ± 0.20*	2.82 ± 0.03*	3.53 ± 0.44	3.42 ± 0.12* 3.28 ± 0.08*
Control		4.03 ± 0.31			3.96 ± 0.21

Data expressed as Mean ± Standard deviation
*- Significantly lower compared to control (p < 0.05)
AILME - *A. indica* leaf methanol extract
HALME - *Helianthus annuus* leaf methanol extract

Table 2: In-vitro effects of a mixture of *A. indica* and *Helianthus annuus* leaves methanol extracts, Dichlorvos and Phostoxin on catalase activity in

Bean weevil and maize weevil

Concentration (µg/ml)	Catalase activity ×10 ³ (U/mg protein)				
	Bean weevil homogenate			Maize weevil homogenate	
	AILME + HALME	Dichlorvos	Phostoxin	AILME + HALME	Dichlorvos Phostoxin
15	1.057 ± 0.01*	0.803 ± 0.01*	2.101 ± 0.01*	1.418 ± 0.03*	1.027 ± 0.11* 3.110 ± 0.00*
30	1.212 ± 0.16*	1.106 ± 0.02*	1.924 ± 0.16*	0.990 ± 0.12*	1.903 ± 0.10* 2.762 ± 0.13*
45	0.091 ± 0.10*	2.22 ± 0.11*	2.110 ± 0.12*	1.902 ± 0.31*	2.702 ± 0.15* 2.963 ± 0.50*
60	0.436 ± 0.00*	1.914 ± 0.04*	1.611 ± 0.32*	3.021 ± 0.18*	2.002 ± 0.19* 3.403 ± 0.33*
75	1.041 ± 0.01*	0.935 ± 0.10*	3.205 ± 0.02*	2.306 ± 0.13*	2.210 ± 0.21* 2.910 ± 0.20*
90	0.933 ± 0.17*	1.505 ± 0.028*	2.310 ± 0.23*	2.911 ± 0.29*	1.852 ± 0.82* 3.042 ± 0.15*
Control		4.201 ± 0.31			3.867 ± 0.43

Data expressed as Mean ± Standard deviation
*- Significantly lower compared to control (p < 0.05)
AILME - *A. indica* leaf methanol extract
HALME - *Helianthus annuus* leaf methanol extract

Table 3: In-vitro effects of a mixture of *A. indica* and *Helianthus annuus* leaves methanol extract, Dichlorvos and Phostoxin on Acetylcholinesterase activity in bean weevil and maize weevil

Concentration (µg/ml)	Acetylcholinesterase activity ×10 ³ (U/mg protein)				
	Bean weevil homogenate			Maize weevil homogenate	
	AILME + HALME	Dichlorvos	Phostoxin	AILME + HALME	Dichlorvos Phostoxin
15	0.518 ± 0.01*	0.271 ± 0.20*	1.003 ± 0.05*	0.591 ± 0.01*	0.179 ± 0.05* 0.276 ± 0.12*
30	0.705 ± 0.19*	0.520 ± 0.13*	1.232 ± 0.09*	0.514 ± 0.03*	0.196 ± 0.01* 0.393 ± 0.18*
45	1.062 ± 0.30*	0.519 ± 0.21*	0.792 ± 0.15*	0.537 ± 0.08*	0.283 ± 0.10* 0.377 ± 0.00*
60	0.492 ± 0.04*	0.493 ± 0.11*	1.301 ± 0.18*	0.698 ± 0.11*	0.288 ± 0.12* 0.463 ± 0.13*
75	0.521 ± 0.18*	1.302 ± 0.03*	0.800 ± 0.29*	0.847 ± 0.14	0.311 ± 0.04* 0.390 ± 0.11*
90	0.669 ± 0.11*	0.703 ± 0.40*	0.622 ± 0.21*	0.741 ± 0.21*	0.295 ± 0.10* 0.532 ± 0.12*
Control		2.255 ± 0.22			0.805 ± 0.21

Data expressed as Mean ± Standard deviation
*- Significantly lower compared to control (p < 0.05)

AILME - *A. indica* leaf methanol extract
HALME - *Helianthus annuus* leaf methanol extract

Discussions

Natural molecules as insecticides have been found to be ecofriendly, and as alternatives to synthetic counterparts (Bohoun et al., 2021). The present study examined the phytochemicals in the hydroethanolic extracts of *Azardiracta indica* and *Helianthus annuus* leaves, and the effects on some antioxidant enzymes and acetylcholinesterase in maize weevils and bean weevils.

The HPLC of *Azardiracta indica* seed hydroethanolic extract showed the presence of β – caryophyllene, trans – β – farnes, α – ionone, azadirachtol and quercetin as the prominent compounds. Salannin (about 47.0 %) has been reported to be the most prominent phytochemical

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in the seed kernel of neem, using GC-FID analysis as documented by Adeleke et al. (2021). However, a quantitative HPLC study by Soni et al. (2012) noticed that azadirachtin level was about 73.62% in a methanol extract of neem leaves. The HPLC of the mixture of AILME and HALME identified furostan, caffeic acid, α – amyryn, α – tocopherol, avanasterol and lecithin, in addition to those identified in the chromatogram of AILME. Caryophyllene oxide was reported to be present in *H. annuus* (Ceccarini et al., 2004). However, a study by Chohan et al. (2022) revealed that β – caryophyllene and α -pinene showed insecticidal action against *Myzus persicae*. Alpha- amyryn has been demonstrated to induce low fecundity, pupation and adult emergence through inhibition of the juvenile hormone in a malarial vector, *Anopheles stephensi* (Kuppusamy et al., 2009). Adusei and Azupo (2022), using caterpillar, aphids and whiteflies, noticed that azadirachtin could be compound responsible for the bio-pesticidal potential of neem, through prevention of feeding, leading to impairments of growth and reproduction. Chauddhary (2017) suggested the implication of azadirachtin in disruption of mechanisms involved in the life cycles of insects susceptible to neem extract. Furthermore, azadirachtin has been reported to inhibit the synthesis and functions of 20-hydroxy (20E) ecdysone and juvenile hormone (JH), both of which trigger molting and metamorphosis in insects. Azadirachtin blocks 20-hydroxy (20E) ecdysone receptors and interferes with larval development, leading to incomplete molting and death in insects (Bensebaa et al., 2015; Alzohairy, 2016). The compound also inhibits the productions of the morphogenetic peptide hormone and allatotropins in the *corpus cardiacum* complex, resulting in reduction of hemolymph levels of ecdysteroid and juvenile hormone. The insect therefore has reduced pupation, malformation or a failure of adult emergence, which compromise the survival of the insect (Bezzar-Bendjazia et al., 2017). The compound also disrupts the endocrine functions by inducing structural changes in the nuclei of the insect glands (Mordue et al., 2010). Azadirachtin has demonstrated inhibition of the excitatory cholinergic transmission and calcium ion channel according to Qiao et al. (2014). Furthermore, Boulahbel et al., (2015) documented the interference of Azadirachtin with the synthesis and uptake of yolk protein in to the oocytes, possibly responsibly resulting in low

fecundity and fertility as observed in *Helicoverpa armigera* (Ahmad et al., 2015) and *Drosophila melanogaster* (Oulhaci et al., 2018). In addition, azadirachtin could potentially interfere with neuroendocrine signaling pathway, feeding process, reproductive behaviour, protein synthesis and energy metabolism in insects (Gupta et al., 2017).

Oxidative stress has been described as a condition in the level of cellular oxidant species outweighs the antioxidant system (Kramer et al., 2021). In this study, the enzyme assay has shown the *in-vitro* potential of the mixture of AILME and HALME to inhibit the SOD and catalase activities in bean weevil and maize weevil comparable to treatment with Dichlorvos and Phostoxin. Different studies have investigated the activity of catalase in cockroach exposed to insecticides (Jankowska et al., 2023) and bees exposed to pesticides (Wang et al., 2023), and noticed decrease in the activity of the enzyme. Fumigation with phosphine could moderately induce SOD activity, and reduce the activities of catalase and glutathione peroxidase (GPx). This results in conversion of superoxide anion to hydrogen peroxide, without detoxification to water by either catalase or GPx. Phosphine has also been documented to react with hydrogen peroxide to form hydroxyl radical, which is even more reactive than reactive oxygen species (Quistad et al., 2000).

Acetylcholinesterase (AChE) catalyzes the acetylcholine degradation in the synaptic cleft to choline and acetate (Dvir et al., 2010). Insecticides such as Organophosphates and Carbamates irreversibly inhibit AChE, causing continuous muscular contraction in mammals, leading to flooding of the synapse with acetylcholine (Cochran, 2011). However, in insects, the activity of this enzyme has been reported to be affected by certain phytochemicals and synthetic insecticides (Adeleke et al., 2019, 2021). The present *in-vitro* study has revealed that a mixture of AILME and HALME reduced the acetylcholinesterase activity in both maize weevil and bean weevil similar to Dichlorvos and Phostoxin. This finding is in accordance with the report of Porter et al. (1993), which documented that phosphine attenuated the activity of acetylcholinesterase, causing elevation in the level of acetylcholine in the synapse. Through *in-vitro* and *in-vivo* studies using insects, it has been shown that phosphine causes hyperactivity, and in extreme cases, excitotoxicity as reported by (Al-Hakkak et al., 1989).

Conclusion

This study has demonstrated that *Azadirachta indica* and *Helianthus annuus* leaves are rich in several compounds, which are insecticidal against maize weevils and bean weevils, via oxidative and anti-cholinergic mechanisms. The two plants are therefore promising sources of bio-pesticides against store insect pests.

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