Suleiman M and Sani I, Archiv Zool Stud 2018, 1: 004
DOI: 10.24966/AZS-7779/100004

HSOA Archives of Zoological Studies

Research Article

Insecticidal Activity of Ethanolic Leaf Extracts against the Maize Weevil, *Sitophilus Zeamais* Motsch (Coleoptera: Curculionidae)

***Suleiman M and Sani I**

Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria

Abstract

Laboratory studies were conducted in order to evaluate the toxicity of ethanolic leaf extracts of *Euphorbia balsamifera* Aiton, *Lawsonia inermis L.*, *Mitracarpus hirtus (L.)* DC and *Senna obtusifolia L.* against *sitophilus zeamais* Motsch. Ten 7 day old *S. zeamais* were released in 20g of disinfested sorghum grains treated with ethanolic extracts of the botanicals in plastic bottles at different concentrations of 2.5, 5.0 and 10.0x10^4 ppm at 30°C and 70% RH. Adult mortalities and lethal concentrations that killed 50% (LC_{50}) of the weevils' population at 24 Hours After Treatment (HAT) were determined. Concentration of body protein as well as inhibition rate of Acetylcholinesterase (AChE) activity in *S. zeamais* exposed to sorghum treated with the botanical extracts were also investigated. Results showed that the adult mortality ranged from 82.50 ± 2.50 to 100.00 ± 0.00% within 3 Days After Treatment (DAT). *L. inermis* had the highest LC_{50} value (1.72 x 10^4 ppm), while *E. balsamifera* had the lowest (1.12 x 10^4 ppm). Biochemical tests showed that the botanicals reduced body protein and inhibited the activity of Acetylcholinesterase (AChE) in *S. zeamais*. The botanicals were found to be toxic to adult *S. zeamais* and therefore could be utilized to reduce the weevils' infestations in sorghum grains.

Keywords: Acetylcholinesterase; Body protein; Botanicals; LC_{50}; Mortality; *Sitophilus zeamais*

***Corresponding author:** Suleiman M, Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria, Tel: +234 8067195818; E-mail: mohammed.suleiman@umyu.edu.ng

Citation: Suleiman M, Sani I (2018) Insecticidal Activity of Ethanolic Leaf Extracts against the Maize Weevil, *Sitophilus Zeamais* Motsch (Coleoptera: Curculionidae). Archiv Zool Stud 1: 004.

Received: September 01, 2018; **Accepted:** October 26, 2018; **Published:** November 09, 2018

Introduction

Synthetic insecticides have been commonly used for controlling pests in stored products [1-4]. However, this has resulted in problems such as pest resurgence and increase in costs of application arising from the development of resistance to insecticides [5,6]. This has led to the search of alternative, eco-friendly methods of controlling insect pests of storage such as the use of botanicals [7,8]. In this regard the toxicity of many plant materials has been evaluated against *S. zeamais* in stored grains with recorded success [7,9-12].

One of the ways used to evaluate the toxicity of insecticides is by measuring their mortality effects on insects. Ethanolic, methanolic and acetonic extracts of various plant materials have been reported to cause high mortality of *S. zeamais* [9,12-14]. It was reported that some medicinal plants such as *olax subscorpioidea* Oliver (Santalales: Olacaceae), *aframomum melegueta k. schum* (Zingiberales: Zingiberaceae), and *zingiber officinale* Rosce (Zingiberales: Zingiberaceae) caused adult mortality of *s. zeamais* which ranged from 18.35 to 88.35% after 72 hours of exposure [15]. Tilahun and Daniel reported the toxicity effects of seed powder of *Azadirachta indica a. Juss* (Sapindales: Meliaceea) against *s. zeamais* and recorded 23.00 to 28.67% adult mortality after 45 days of treatment [16].

Another way of assessing toxicity of insecticides is by evaluating their effects on biochemical parameters of insects [17]. Kumar and Michael noted that the carbohydrates, proteins and lipids play an important role in the biochemical process underlying growth and development of insects [18]. Fox, et al., reported that the reproductive capacity of adult female insects is affected largely by their body protein content [19]. Piri, et al., noted that proteins are important for individual-level fitness-associated traits such as body size, growth rate and fecundity [20]. At high levels of organization, proteins have been linked to population dynamics, life cycles and even biological diversification [20]. Some of the biochemical composition of *acanthoscenlides obtectus* Say, *S. oryzae, S. granarius* and *T. confusum* have been investigated [17-19,21,22]. However, little is known with regards to toxicity of botanicals against body protein of *S. zeamais*.

In addition to body protein, enzymes such as Acetycholinesterase (AChE) play a vital role in insect survival. AChE is a key enzyme of the cholinergic system regulating the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine [23]. Some botanicals were reported to inhibit AChE activity in some insect pests of storage such as *S. oryzae*, *S. granarius* and *T. castaneum*, but little is known on effects of botanicals in inhibiting AChE activity in *S. zeamais* [24-28]. This study was carried out to investigate the ability of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* to cause adult mortality, reduce concentration of body protein and inhibit AChE activity in *S. zeamais*.

Materials and Methods

Mass rearing of *S. zeamais*

One hundred adults of *S. zeamais* were obtained from infested sorghum grains at Katsina Central Market and then introduced into 500ml rearing bottles containing 250g of the disinfested sorghum grains. The bottles were covered with muslin cloth and secured with rubber bands. They were then kept in an incubator for oviposition at 30°C and 70% RH for seven days, after which the parents were removed. The bottles were maintained in the incubator under the same condition for adult emergence. Progeny of 7 days old were sieved and used for the laboratory experiments.

Preparation of the botanicals

Fresh leaves of *E. balsamifera*, *L. inermis, M. hirtus* and *S. obtusifolia* were shade-dried and ground into powder using a laboratory blender. One hundred grams of each plant powder was dissolved in 400ml of ethanol in conical flasks. Mouths of the conical flasks were properly corked and kept in a refrigerator for 48 hours. The extract was separated using muslin cloth and filtered with Whatman No.1 filter papers as described by Khaliq, et al., [29]. The filtrate was separately concentrated by evaporating excess solvents using rotary evaporator with rotary speed of 3 to 6 rpm for 8 hours. The resulting extract was air-dried to remove traces of the solvent and stored in refrigerator at 4°C [30].

Adult mortality tests of *S. zeamais* exposed to sorghum grains treated with ethanolic leaf extracts

The method of de Oliveira, et al., was adopted to assess adult mortality of *S. zeamais* in sorghum grains treated with ethanolic leaf extracts of the test plants [31]. Crude extracts were diluted with ethanol to make different concentrations of 2.5, 5.0 and 10.0 x 10^4 ppm**.** Twenty grams of sorghum grains were weighed and put into 250 ml plastic bottles. The grains were impregnated with 2ml of the ethanolic extracts at three concentrations, while the control contained the grains. Ten weevils were released into each of the bottles after complete evaporation of the solvent. The grain mass was mixed thoroughly with the aid of glass rod and air-dried until complete solvent evaporation. All treatments were arranged in a Completely Randomized Design (CRD) in the incubator and replicated four times. The set-ups were inspected daily and dead weevils in each treatment were removed and recorded daily for three days.

Percent adult mortality was assessed as;

$$\%\,\text{Molarity} = \left(\frac{\text{Number of Dead Weevils}}{\text{Total}}\right) \times 100$$

Determination of Lethal Concentrations (LC_{50}) of ethanolic leaf extracts

To evaluate LC_{50} of the ethanolic extracts of the botanicals against *S. zeamais*, methods of Ebadollahi and Mahboubi were employed [32]. The number of dead weevils at the end of 24 hour exposure to the extracts was used to determine the LC_{50} of the botanicals by using probit analysis with SPSS (version 16.0) software package [33].

Determination of body protein in *S. zeamais* treated with ethanolic leaf extracts

Samples for protein assays were prepared following Askar, et al., [17]. Adult weevils exposed to LC_{50} of ethanolic extracts of the botanicals for 7 days were freeze killed at - 20°C. A sample of 0.5g of the insects was taken from each bottle and homogenized in 10 times (5mL) volumes (w/v) of phosphate buffer (pH 7.2) using a glass homogenizer under ice. The total homogenates were centrifuged at 4000 rpm for 10min at 4°C using a centrifuge. The supernatant was transferred to new eppendorfs tubes and preserved at - 20°C for spectrophotometry.

The body protein was estimated following the method of Lowry, et al., in triplicates with Bovine Serum Albumin (BSA) as the standard [34]. The absorbance was measured spectrophotometrically after 30mins at 750nm against the blank for both test samples and the standard solutions. The protein concentration was calculated and expressed as µg/ml wet tissue using the formula:

$$\text{Concentration of Body Protein } X = \frac{y}{m}$$

Where:

x = The concentration

y = Absorbance of the solution

m = Slope of the standard curve

Determination of AChE activity inhibition in *S. zeamais* exposed to ethanolic leaf extracts

S. zeamais adults were fumigated with LC_{50} of ethanolic extracts of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* as fumigant toxicity assay. After seven days of fumigation, the weevils were homogenized in phosphate buffer saline (50mM, pH 8) using a Teflon glass tissue homogenizer. The homogenate was centrifuged at 10,000 rpm for 20min at 4°C. The supernatant was filtered and used as the AChE preparation. Methods of Ellman, et al., and Chaubey were adopted for the assay and AChE activity was determined spectrophotometrically [28,35]. Absorbance in the treated samples was evaluated at 412nm for 6min and compared to that of the control. All measurements were made in triplicate [25,26,28].

AChE activity inhibition was calculated as follows [26]:

$$\%\,\text{AChE Activity Inhibition} = \frac{A_{\text{Control}} - A_{\text{Treatment}}}{A_{\text{Control}}} \times 100$$

Where:

$A_{Control}$ = The absorbance of the untreated control

$A_{Treatment}$ = The absorbance of the treated samples

Data analysis

Data generated were tested for normality using Shapiro-Wilk and Jacque-Bera normality tests [36-38]. Data from adult mortality tests were subjected to two-way ANOVA using Graph Pad Prism (version 7.03). Probit analysis was employed to calculate LC_{50} of the extracts with the significant differences determined by X^2 goodness of fit using SPSS (version 16.0). One-way ANOVA was used to test if there were significant differences in the body protein and AChE activity inhibition in adults of *S. zeamais* exposed to LC_{50} of the extracts by using PAST (version 2.17). Significantly different means were separated by Bonferroni's and Tukey's multiple comparisons tests [38]. All analyses were performed as statistically significant $p<0.05$.

Results

Adult mortality of *S. zeamais* in sorghum grains treated with ethanolic leaf extracts

Ethanolic leaf extracts of the selected botanicals applied at different concentrations of 2.5, 5.0 and 10.0x10^4 ppm exhibited varying percentage mortalities of *S. zeamais* within 1, 2 and 3 days after treatment (Table 1).

Treatments	Conc.(x 10^4 ppm)	Mean Adult Mortality (% ± S. E.)		
		Days After Treatment (DAT)		
		1	2	3
E. balsamifera	2.5	65.00 ± 2.89^{bc}	90.00 ± 4.08^{ab}	100.00 ± 0.00^{a}
	5	75.00 ± 2.89^{ab}	95.00 ± 2.89^{a}	100.00 ± 0.00^{a}
	10	85.00 ± 2.89^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
L. inermis	2.5	55.00 ± 2.89^{c}	77.50 ± 4.79^{b}	97.50 ± 2.50^{a}
	5	72.50 ± 2.50^{b}	92.50 ± 2.50^{ab}	100.00 ± 0.00^{a}
	10	77.50 ± 2.50^{ab}	95.00 ± 2.89^{a}	100.00 ± 0.00^{a}
M. hirtus	2.5	67.50 ± 4.89^{bc}	80.00 ± 4.08^{ab}	95.00 ± 2.89^{a}
	5	70.00 ± 4.08^{bc}	92.50 ± 4.89^{ab}	100.00 ± 0.00^{a}
	10	75.00 ± 2.8^{9ab}	95.00 ± 2.89^{a}	100.00 ± 0.00^{a}
S. obtusifolia	2.5	55.00 ± 2.89^{c}	72.50 ± 2.50^{b}	82.50 ± 2.50^{b}
	5	67.50 ± 4.79^{bc}	85.00 ± 2.89^{ab}	95.00 ± 2.89^{a}
	10	70.00 ± 4.08^{bc}	90.00 ± 4.08^{ab}	100.00 ± 0.00^{a}
Control	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Table 1: Mortality of adult *S. zeamais* exposed to 2.5, 5.0 and 10.0 x 10^4 ppm of ethanolic botanical extracts in three days after treatment.

Conc = Concentration

Means in the same column followed by different letter superscript are significantly different at p<0.05 by the Bonferroni's Multiple Comparisons Test.

Analysis of variance indicated that the difference in adult mortalities of *S. zeamais* was highly significant among the botanicals (F (4, 45) = 2947.00, p<0.0001) as well as within the concentrations (F (2, 45) = 18.00, p<0.0001). Bonferroni's multiple comparisons test revealed that at 2.5 x 10^4 ppm, *E. balsamifera*, *L. inermis* and *M. hirtus* caused statistically similar mortality which was higher than that in *S. obtusifolia* at 3 DAT. At 5.0 and 10.0 x 10^4 ppm, all the botanicals had the same effect on the weevils' mortality within 3 DAT.

Lethal Concentration (LC_{50}) of ethanolic leaf extracts against *S. zeamais*

Table 2 shows that *L. inermis* had the highest LC_{50} value as 1.72 x 10^4 ppm, while *E. balsamifera* had the lowest as 1.12 x 10^4 ppm at 24 HAT. The probit equations were y = 1.074x - 0.053, y = 1.065x - 0.251, y = 0.365x + 0.296 and y = 0.669x - 0.100 for *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia*, respectively (Table 2). The slopes varied between 0.365 ± 0.723 and 1.074 ± 0.739. X^2 goodness of fit (0.005 - 0.092) reveals that the LC_{50} of ethanolic extracts against the weevils was not significantly different (p>0.05) among concentrations of each of the botanicals.

Concentration of body protein in *S. zeamais* exposed to ethanolic leaf extracts

The protein in *S. zeamais* exposed to ethanolic extracts of the botanicals appeared to follow the order: *E. balsamifera*<*L. inermis*<*M. hirtus*<*S. obtusifolia*<control (Figure 1). Significant difference (F (4, 10) = 2861.00, p < 0.0001) existed in protein content among the weevils exposed to sorghum grains treated with LC_{50} of ethanolic extracts of different test botanicals. Further, Tukey's multiple comparisons test revealed that all treatments differed from each other with insects exposed to *E. balsamifera* having the least body protein content.

Botanicals	LC_{50} (x 10^4 ppm)	95% CI	Slope ± S.E.	X^2	p value
E. balsamifera	1.12	0.97 - 3.11	1.074 ± 0.739	0.005	0.946
L. inermis	1.72	0.88 - 3.01	1.065 ± 0.712	0.092	0.762
M. hirtus	1.15	1.07 - 2.30	0.365 ± 0.723	0.006	0.938
S. obtusifolia	1.41	1.21 - 2.55	0.669 ± 0.701	0.065	0.798

Table 2: LC_{50} of ethanolic leaf extracts against adult *S. zeamais* at 24 HAT.

HAT = Hours After Treatment; CI = Confidence Interval; X^2 = Chi-square.

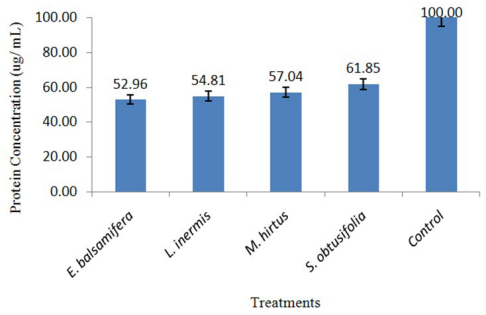

Figure 1: Concentration of body protein in *S. zeamais* exposed to LC_{50} of ethanolic leaf extracts.

Inhibition of AChE activity in *S. zeamais* by ethanolic leaf extracts

The inhibition of AChE activity in *S. zeamais* by ethanolic extracts was 79.61, 77.65, 74.90 and 70.98 for *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia*, while none was recorded in weevils exposed to the untreated grains (Figure 2). One-way ANOVA revealed that inhibition of the AChE activity was significantly different (F (4, 10) = 4190.00, p<0.0001) among the botanical treatments. Tukey's pairwise comparisons showed that the inhibition activity was statistically similar in weevils exposed to *E. balsamifera* and *L. inermis* but higher than the rest.

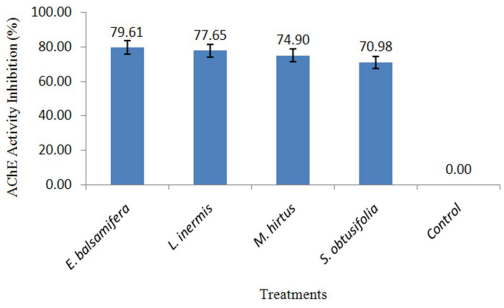

Figure 2: AChE activity inhibition in *S. zeamais* exposed to LC_{50} of ethanolic leaf extracts.

Discussion

Effect of ethanolic extracts on adult mortality of *S. zeamais*

Ethanolic extracts of the selected botanicals were found to be highly effective by resulting in total mortality of adults of *S. zeamais* in sorghum grains at all concentrations of *E. balsamifera* and higher rates of *L. inermis*, *M. hirtus* and *S. obtusifolia*. The efficacy of the test botanicals is in line with earlier findings in which ethanolic extracts of some plant species caused adult mortality of maize weevils [10,13]. Ajayi reported that ethanolic extract of *D. regia* seeds applied at 2.0mg/g maize grains resulted in 12.50% adult mortality of *S. zeamais* after five days of exposure [10]. Similarly, findings of Ibrahim, et al., showed that application of ethanolic leaf extract of *C. odorata* at 10ml / 50g maize caused 14.00% adult mortality of *S. zeamais* at 7 DAT [13]. The observed increase in mortality of *S. zeamais* caused by ethanolic extracts with increase in post treatment period in this study agrees with previous findings [10,12,13].

Ethanolic leaf extracts of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* were generally found toxic to adult *S. zeamais* and suppressed their survival in sorghum grains by causing considerably high mortality. All the selected botanicals were effective against the weevil even at lower concentrations. This was possible because plant species contain secondary metabolites which are vast storehouse of compounds such as the steroids, phenolic compounds and tannins with wide range of biological activity reported to have great impact on insecticidal activities [39,40]. Other bioactive compounds such as terpenoids, flavonoids, alkaloids, saponins and glycosides were found in the leaf extracts of *E. balsamifera*, *L. inermis* and *S. obtusifolia* by others and reported to be pesticidal in nature [40-42]. Ileke, et al., reported the presence of alkaloids, flavonoids, saponins and tannins, in the powders and methanolic extracts of *M. fragrans* and *A. melegueta* and concluded their insecticidal activity against *S. zeamais* [14]. The characteristic smell of the study botanicals might have also contributed to their insecticidal activity by repelling the insects away from the grains as suggested by Ileke, et al., [14].

Lethal Concentration (LC_{50}) of ethanolic leaf extracts against *S. zeamais*

The present study investigated LC_{50} of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* against *S. zeamais* in sorghum grains due to lack of information on this aspect in previous studies. However, lethal concentrations of other botanicals used to control insect pests of storage were determined [43-45].

LC_{50} of the plants showed that the ethanolic extracts had great efficiency by causing 50% adult mortality of *S. zeamais* in sorghum even at a concentration below the lowest amount applied within 24 HAE. *E. balsamifera* was more effective than the other botanicals followed by *M. hirtus*. The effectiveness the selected botanicals in killing adult weevils at lower concentration within short period is in conformity with Biswas, et al., who reported high efficiency of *L. inermis* against *T. castaneum* [39].

Low LC_{50} values of ethanolic extracts of the test botanicals concur with Rani and Devanand, who reported that among methanolic extracts of the plants tested *Momordica charantia L* [43]. was the most effective with LC_{50} of 2.82mg / 20g maize grains against *S. zeamais*.

Effect of ethanolic leaf extracts on body protein of *S. zeamais*

There was a decrease in body protein of the maize weevils exposed to lethal concentrations of all the botanicals compared to the control. The reduction in body protein agrees with Askar et al., who reported that both clove oil and diatomaceous earth reduced body protein of *S. zeamais* and *S. oryzae* from 0.10mg/ml in the control to 0.09 and 0.06mg/ ml, respectively [17]. It was earlier reported by Piri, et al., that sub-lethal concentrations of spinosad resulted in a significant decrease in protein content of *Glyphodes pyloalis* Walker [20]. This decrease in body protein could be attributed to low feeding efficiency of the insects due to antifeedant effect of many other insecticides and hence a decrease in protein concentration [40,46]. Another reason might be as a result of protein break down into amino acids which help in energy supply to the insect [47].

It was found that application of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* in the form of powders and extracts significantly affected body protein of *S. zeamais* which could probably affect the aforementioned biological aspects of the weevil. The reduction in concentration of the body protein might have contributed to decline in the insect's population and hence decreased infestations in the sorghum grains. This could also be attributed to decrease in vitellogenin, a precursor of yolk component, present in the body protein of vitellogenic females, which its amount reflects the insect reproductive performance [48].

Effect of ethanolic leaf extracts on the activity of AChE in *S. zeamais*

AChE is a hydrolytic enzyme in insect body that influences the nervous system [27]. In the present study the LC_{50} of ethanolic extracts of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* significantly inhibited the activity of AChE in *S. zeamais* agreeing with Li, et al., who reported that methyl alcohol, ethyl acetate and petroleum ether fruit extracts of *Illicium verum hook. F* [27], inhibited the activity of AChE in *S. zeamais*. Similarly, the LC_{50} of essential oils of *Pimpinella anisum* and *Ocimum basilicum* have demonstrated their interference with AChE activity in *S. granarius* [25]. Recently, methanolic extract of *Urginea maritime* at a concentration of 1000µg/ml was found to result in 73.37% inhibition of the activity of AChE in *S. oryzae* [26]. Also, another finding has confirmed the inhibitory effect of botanicals on the activity of AChE where Chaubey reported that LC_{50} of *Cuminum cyminum* essential oil reduced AChE activity in *S. zeamais* to 66.90% compared to 31.59% of the control [28].

The inhibitory effect on AChE activity in *S. zeamais* exposed to lethal concentrations of all the botanicals has shown their possible potentiality as biopesticides. This is because it has been reported that inhibition of AChE in cholinergic synapses of the nervous system is the primary mechanism of acute toxicity of insecticides [25]. Therefore, the botanicals probably have neurotoxic effects on *S. zeamais* by interfering with the passage of impulses in the insect nervous system leading to inability of AChE to hydrolyze acetylcholine which builds up in the synapse and results in excessive neuro excitation [24]. The mortality effects of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* against *S. zeamais* recorded in this study may also be attributed to their ability to inhibit the activity of AChE as suggested by Rajashekar, et al., [24]. Thus, the neurotransmitting system of the weevils represents a target for insect control.

Conclusion

It was found that application of ethanolic extracts of the selected botanicals resulted in high adult mortality of *S. zeamais* even at the lower concentration of 2.5 x 10^4 ppm after three days of treatment with *E. balsamifera* as the most effective followed by *L. inermis* and therefore could be used to reduce *S. zeamais* infestations in sorghum grains.

The LC_{50} of the botanicals were found to be either lower than or within the range of recommended dose/concentration (1-5%) of botanicals in stored commodity. Findings of this study have revealed that application of *E. balsamifera*, *L. inermis*, *M. hirtus* and *S. obtusifolia* at the lowest concentration of 2.5 x 10^4 ppm could cause the death of 50% *S. zeamais* within one to three days. Lethal concentrations of all the botanicals reduced body protein of *S. zeamais* and have the ability to interfere with neurotransmitting system of the weevil by inhibiting the activity of AChE.

The toxicity of the test botanicals against adult *S. zeamais* infers that they could be utilized to reduce the weevils' infestations in stored sorghum. However, further investigations on their toxicity on mammals and other insect pests are hereby recommended.

References

1. Nguemtchouin MGM, Ngassoum MB, Chalier P, Kamga R, Ngamo LST, et al. (2013) *Ocimum gratissimum* eseential oil and modified montmorillonite clay, a means of controlling insect pests of stored products. J Stored Prod Res 52: 57-62.
2. Baributsa D, Baoua IB, Bakoye ON, Amadou L, Murdock LL (2017) PICS bags safely store unshelled and shelled groundnuts in Niger. J Stored Prod Res 72: 54-58.
3. Nayak MK, Daglish GJ (2017) Base-line susceptibility of field populations of *Rhyzopertha dominica (F.)* to spinosad in Australia. J Stored Prod Res 70: 1-6.
4. Paudyal S, Opit GP, Osekre EA, Arthur FH, Bingham GV, et al. (2017) Field evaluation of the long-lasting treated storage bag, deltamethrin incorporated, (ZeroFly® Storage Bag) as a barrier to insect pest infestation. J Stored Prod Res 70: 44-52.
5. Liu ZL, Zhao NN, Liu CM, Zhou L, Du SS (2012) Identification of insecticidal constituents of the essential oil of *Curcuma wenyujin* rhizomes active against *Liposcelis bostrychophila* badonnel. Molecules 17: 12049-12060.
6. Cosmas P, John CT, Agathar K, Ronald M, Kufa M, et al. (2018) Use of botanical pesticides in controlling *Sitophilus zeamais* (Maize Weevil) on stored *Zea mays* (Maize) grain. Modern Concepts and Developments in Agronomy 1: 1-4.
7. Suleiman M (2014) Efficacy of some spices as sorghum grain protectants against *Sitophilus zeamais* Motschulsky [*Coleoptera: Curculionidae*]. African Journal of Agricultural Research 9: 841-845.
8. Suleiman M, Rugumamu CP (2017) Management of insect pests of stored sorghum using botanicals in Nigerian traditional stores. Journal of Stored Products and Postharvest Research 8: 93-102.
9. Adedire CO, Akinkurolere RO, Obembe OM (2012). Efficacy of cashew kernel extracts in the control of the maize weevil, *Sitophilus zeamais* (*Coleoptera: Curculionidae*). Archives of Phytopathology and Plant Protection 45: 831-839.
10. Ajayi OE (2013) Insecticidal activity of powder and extracts of *Delonix regia* seed against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). FUTA Journal of Research in Sciences 1: 54-62.
11. Wini GJ, Nukenine EC, Ndjonka D, Suh C, Adler C, et al. (2015) Efficacy of diatomaceous earth and wood ash for the control of *Sitophilus zeamais* in stored maize. Journal of Entomology and Zoology Studies 3: 390-397.
12. Suleiman M, Rugumamu CP, Ibrahim ND (2018) Insecticidal toxicity of some botanicals against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in stored sorghum grains in Nigeria. Journal of Entomology and Zoology Studies 6: 1280-1287.
13. Ibrahim YJ, Abugri AD, Afun JVK (2016) Efficacy of ethanolic leaf extract of Chromolaena odurata in controlling *Sitophilus zeamais* in stored maize. Journal of Experimental Agriculture International 14: 1-10.
14. Ileke KD, Adesina JM, Obajulaye EO (2016) Synergic effects of two botanical entomocides as pest-protectants in maize grains. Journal of Biological Research 89: 34-39.
15. Oni MO, Ogungbite OC (2015) Entomotoxicant potential of powders and oil extracts of three medicinal plants in the control of *Sitophilus zeamais* infesting stored maize. Journal of Plant and Pest Science 2: 8-17.
16. Tilahun FE, Daniel DH (2016) Effect of neem leaf and seed powders against adult maize weevil (*Sitophilus zeamais* Motschulsky) mortality. International Journal of Agricultural Research 11: 90-94.
17. Askar SI, Al-Assaal MS, Nassar AMK (2016) Efficiency of some essential oils and insecticides in the control of some *Sitophilus* insects (Coleoptera: Curculionidae). Egyp J Plant Pro Res 4: 39-55.
18. Kumar MDA, Michael AS (2012) Changes in the activities of proteins and carbohydrates in haemolymph of silkworm, *bombyx mori* treated with greenleaf. International Journal of Environmental Sciences 3: 119-125.
19. Fox CW, Stillwell RC, Wallin WG, Hitchcock LJ (2006) Temperature and host species affect nuptial gift size in a seed-feeding beetle. Funct Ecol 20: 1003-1011.
20. Piri F, Sahragard A, Ghadamyari M (2014) Sublethal Effects of Spinosad on Some Biochemical and Biological Parameters of *Glyphodes pyloalis* Walker (*Lepidoptera: Pyralidae*). Plant Protect Sci 50: 135-144.
21. Ali NS, Ali SS, Shakoori A (2011) Effects of sublethal doses of talstar on biochemical components of melathion-resistant and -susceptible adults of *Rhyzopertha dominica*. Pakistan J Zool 43: 879-887.
22. Lingampally V, Solanki VR, Kaur A, Raja SS (2013) Effect of andrographolide on the protein content of *Tribolium Confusum* (Duval). International Journal of Pure and Applied Zoology 1: 70-79.
23. Praveena A, Sanjayan KP (2011) Inhibition of acetylcholinesterase in three insects of economic importance by linalool, a monoterpene phytochemical. Insect Pest Management, A Current Scenario 340-345.
24. Rajashekar Y, Raghavendra A, Bakthavatsalam N (2014) Acetylcholinesterase inhibition by biofumigant (Coumaran) from leaves of Lantana camara in stored grain and household insect pests. Biomed Res Int 2014: 1-6.
25. Abo El Makarem H, El Kholy SE, Abdel-Latif A, Seif AI (2015) Physiological and biochemical effects of some essential oils on the granary weevil, *sitophilus granartius* (*L.*) (*coleoptera: curculionidae*). Egypt J Exp Biol (Zoo.) 11: 117-123.
26. Maazoun AM, Hlel TB, Hamdi SH, Belhadj F, Jemâa JMB, et al. (2017) Screening for insecticidal potential and acetylcholinesterase activity inhibition of *urginea maritima* bulbs extract for the control of *Sitophilus oryzae (L.)*. J Asia-Pac Entomol 20: 752-760.
27. Li J, Guo Q, Han S, Jiang L, Liang G (2013) Types, morphologies and distribution of antennal sensilla of *Quadrastichus erythrinae* (*Hymenoptera: Eulophidae*). Fla Entomol 96: 1288-1297.
28. Chaubey MK (2017) Evaluation of insecticidal properties of *Cuminum cyminum* and *Piper nigrum* essential oils against *Sitophilus zeamais*. Journal of Entomology 14:148-154.

29. Khaliq A, Nawas A, Ahmad MH, Sagheer M (2014) Assessment of insecticidal potential of medicinal plant extracts for control of maize weevil, *Sitophilus zeamais* Motschulsky (*Coleoptera: Curculionidae*). Basic Research Journal of Agricultural Science and Review 3: 100-104.

30. Abou-Elnaga, Zeinab Sh (2015) Efficacy of extracts of some Egyptian plants against economically important stored grains pest *Sitophilus oryzae* L. Journal of Entomology and Zoology Studies 3: 87-91.

31. de Oliveira TA, Ronche-Teles B, da Fonseca CRV, da Silva SLR, Santos PA, et al. (2012) Insecticidal activity of *Vitex cymosa* (*Lamiaceae*) and *Eschwelera pedicellata* (*Lecythidaceae*) extracts against *Sitophilus zeamais* adults (*Curculionidae*). Emir J Food Agr 24: 49-56.

32. Ebadollahi A, Mahboubi M (2011) Insecticidal activity of the essential oil isolated from *Azilia eryngioides* (PAU) hedge et lamond against beetle pests. Chil J Agr Res 71: 406-411.

33. SAS Institute (1999) SAS/STAT User's Guide, Version 8, Volume 2. SAS Institute, North Carolina, USA.

34. Lowry OH, Passonneau JV, Rock MK (1961). The stability of pyridine nucleotides. J Biol Chem 236: 2756-2759.

35. Ellman GL, Courtney KD, Andres V Jr., Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95.

36. Sokal RR, Rohlf FJ (1995) Biometry (3rdedn): A Series of books in biology. WH Freeman and Company, New York, USA. Pg no: 887.

37. Jolicoeur P (1999) Introduction to Biometry (1stedn). Springer+Business Media LLC, New York, USA. Pg no: 516.

38. Le CT (2003) Introductory Biostatistics. John Wiley & Sons Inc., New Jersey, USA, Pg no: 553.

39. Biswas KK, Sharmin N, Rabbi MA (2016) Evaluation of the insecticidal activity of *Lawsonia inermis* Linn. against the red flour beetle, *Tribolium castaneum* (Herbst). Natural Products 12: 8-11.

40. Hikal WM, Baeshen RS, Said-Al Ahl HAH (2017) Botanical insecticides as simple extractives for pest control. Cogent Biology 3: 1-16.

41. Doughary JH, El-mahmood AM, Tyoyina I (2008) Antimicrobial activity of leaf extracts of *Senna obtusifolia (L.)*. Afr J Pharm Pharmaco 2: 007-013.

42. Wagini NH, Soliman AS, Abbas MS, Hanafy YA, Badawy EM (2014) Phytochemical analysis of Nigerian and Egyptian henna (*Lawsonia inermis L.*) leaves using TLC, FTIR and GCMS. Plant 2: 27-32.

43. Rani PU, Devanand P (2011) Efficiency of different plant foliar extracts on grain protection and seed germination in maize. Research Journal of Seed Science 4: 1-14.

44. Naeem M, Anjum SI, Ismail M, Khan J, Bibi S, et al. (2015) Laboratory assessment of different botanical extracts and cypermethrin against insect pests. Journal of Entomology and Zoology Studies 3: 84-88.

45. Jayakumar M, Arivoli S, Raveen R, Tennyson S (2017) Repellent activity and fumigant toxicity of a few plant oils against the adult rice weevil *Sitophilus oryzae* Linneaus 1763 (*Coleoptera: Curculionidae*). Journal of Entomology and Zoology Studies 5: 324-335.

46. Etebari K, Mirhoseini SZ, Matindoost L (2005) A study on interaspecific diversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers. Insect Sci 12: 87-94.

47. Nath BS, Suresh A, Varma BM, Kumar RP (1997) Changes in protein metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (*Lepidoptera: Bombycidae*) in response to organophosphorus insecticides toxicity. Ecotoxicol Environ Saf 36: 169-173.

48. Rugumamu CP (2006) Varietal role in the management of the larger grain borer, *Prostephanus truncatus* (horn) in stored maize. Tanzania Journal of Science 32: 13-22.

Journal of Anesthesia & Clinical Care

Journal of Addiction & Addictive Disorders

Advances in Microbiology Research

Advances in Industrial Biotechnology

Journal of Agronomy & Agricultural Science

Journal of AIDS Clinical Research & STDs

Journal of Alcoholism, Drug Abuse & Substance Dependence

Journal of Allergy Disorders & Therapy

Journal of Alternative, Complementary & Integrative Medicine

Journal of Alzheimer's & Neurodegenerative Diseases

Journal of Angiology & Vascular Surgery

Journal of Animal Research & Veterinary Science

Archives of Zoological Studies

Archives of Urology

Journal of Atmospheric & Earth-Sciences

Journal of Aquaculture & Fisheries

Journal of Biotech Research & Biochemistry

Journal of Brain & Neuroscience Research

Journal of Cancer Biology & Treatment

Journal of Cardiology & Neurocardiovascular Diseases

Journal of Cell Biology & Cell Metabolism

Journal of Clinical Dermatology & Therapy

Journal of Clinical Immunology & Immunotherapy

Journal of Clinical Studies & Medical Case Reports

Journal of Community Medicine & Public Health Care

Current Trends: Medical & Biological Engineering

Journal of Cytology & Tissue Biology

Journal of Dentistry: Oral Health & Cosmesis

Journal of Diabetes & Metabolic Disorders

Journal of Dairy Research & Technology

Journal of Emergency Medicine Trauma & Surgical Care

Journal of Environmental Science: Current Research

Journal of Food Science & Nutrition

Journal of Forensic, Legal & Investigative Sciences

Journal of Gastroenterology & Hepatology Research

Journal of Gerontology & Geriatric Medicine

Journal of Genetics & Genomic Sciences

Journal of Hematology, Blood Transfusion & Disorders

Journal of Human Endocrinology

Journal of Hospice & Palliative Medical Care

Journal of Internal Medicine & Primary Healthcare

Journal of Infectious & Non Infectious Diseases

Journal of Light & Laser: Current Trends

Journal of Modern Chemical Sciences

Journal of Medicine: Study & Research

Journal of Nanotechnology: Nanomedicine & Nanobiotechnology

Journal of Neonatology & Clinical Pediatrics

Journal of Nephrology & Renal Therapy

Journal of Non Invasive Vascular Investigation

Journal of Nuclear Medicine, Radiology & Radiation Therapy

Journal of Obesity & Weight Loss

Journal of Orthopedic Research & Physiotherapy

Journal of Otolaryngology, Head & Neck Surgery

Journal of Protein Research & Bioinformatics

Journal of Pathology Clinical & Medical Research

Journal of Pharmacology, Pharmaceutics & Pharmacovigilance

Journal of Physical Medicine, Rehabilitation & Disabilities

Journal of Plant Science: Current Research

Journal of Psychiatry, Depression & Anxiety

Journal of Pulmonary Medicine & Respiratory Research

Journal of Practical & Professional Nursing

Journal of Reproductive Medicine, Gynaecology & Obstetrics

Journal of Stem Cells Research, Development & Therapy

Journal of Surgery: Current Trends & Innovations

Journal of Toxicology: Current Research

Journal of Translational Science and Research

Trends in Anatomy & Physiology

Journal of Vaccines Research & Vaccination

Journal of Virology & Antivirals

Submit Your Manuscript: http://www.heraldopenaccess.us/Online-Submission.php

Herald Scholarly Open Access, 2561 Cornelia Rd, #205, Herndon, VA 20171, USA.
Tel: +1-646-661-6626; E-mail: info@heraldsopenaccess.us
http://www.heraldopenaccess.us/