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Original Article

Insecticidal Activities of *Azadirachta indica* A.J (Meliaceae) leaf and Seed on Stored Grain Weevils and Toxicological Studies of Pesticide-protected Stored Grains in Nigeria

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Abstract

Introduction: *Azadirachta indica* has been used in Nigeria for decades in traditional medicine for managing various diseases notably are fever, aches, common cold, as well as urinary tract infections.
Objectives: In this study we determined the effects of *A. indica* seed and leaf extracts on developmental stages of stored grain weevils (*Acanthoscelides obtestus and Sitophilus spp.*) and evaluate the toxicity of pesticide-protected stored grains which had lasted for at least six months in stores in Nigeria.
Methods: *A. indica* leaves and seeds were prepared after air drying into powder forms (PF), aqueous extract (AE) and ethanol extract (EE). Percentage Mortality of weevils, as well as oviposition deterrence was determined in each bag and for each preparation. The results were compared with that of a standard pesticide dichloropropane-dichloropropene force (DD force). Acute toxicity studies were carried on some of the most common pesticide-protected grains; maize and bean stored for at least six months and sold in local markets for consumption in Nigeria.
Results: The result showed that AE of leaf and seed exhibited 100% insecticidal and oviposition deterrence on *Acanthoscelides obtestus* (bean weevil) than maize and guinea corn weevil (*Sitophilus spp.*). An insecticidal activity increases with increased storage time. These results were comparable to the standard pesticide DD force at $p \leq 0.05$ (ANOVA). Acute toxicity determination showed that LD_{50} value were 363.10 mg/Kg and 977.10 mg/Kg for beans and maize respectively.
Conclusion: The study showed that *Azadirachta indica* aqueous leaf and seed extracts exhibited great oviposition deterrence and insecticidal activities on stored grain weevils, and can serve as an alternate cheap pesticide for stored grains with zero level of toxicity to humans.

1. Introduction

Azadirachta indica is a tree in the mahogany family *Meliaceae*. It is native to India, Myanmar, Bangladesh, Malaysia and Pakistan [1], and grows in tropical legume. *Azadirachta indica* (Neem) is known by many different names including: Neem (Punjabi), Nees (Arabic), Vemba (Tamil), Paraiso (Spanish), Dogo-yaro (Hausa). Products made from *Azadirachta indica* trees have been used in India for over two Millennium for their medicinal properties. Neem products are believed by Ayurvedic precisely to be anthelminthic, antifungal, anti-diabetic, anti-bacteria and antiviral [2]. *Azadirachta indica* (Neem) leaves are dried in Indian and placed in cap boards to prevent insects eating the clothes and also while storing rice in tins [2].

The Neem tree is noted for its drought resistance normally it trims in area with sub-amid and to sub-humid condition with an annual rainfall 400-1,200 millimeters (16-471in). It can grow in regions with an annual rainfall below 400 mm but in such cases it depends largely on ground water. Neem can grow in different types of soil but is thrives best on well drained deep and sandy soil [3]. Neem (*Azadirachta indica*) are dried in India and placed in cupboards to prevent insect eating the clothes, and also while storing rice in tins. Neem leaves are dried and burnt in the tropical regions to keep away mosquitoes [4]. *Azadirachta indica* oil is also used for healthy hair improve liver function, detoxify the blood and balance blood sugar level[5]. Neem leaves have also been used to treat skin diseases like eczema, psoriasis etc. The oil can cause some forms of tropic encephalopathy and ophthalmopathy if consumed in large quantities. It also is used for preparing cosmetic such as soap, shampoo, balms and creams as well as tooth paste[6].

Azadirachta indica is a key ingredient in non-pesticidal management (NPM) providing a natural alternative to synthetic pesticides. It acts as an anti-feedant, repellent and egg laying. It also suppresses the hatching of pest insect from their eggs [7]. Safety assessment of various 'Neem' derived preparation were made and compared with the ingestion of residue on food treated with them as insecticides. It was concluded that the use of Neem- derived insecticides should not be discourage, if applied with care [8]. Many compounds have been isolated from Neem, and are mainly flavonoids with insecticidal properties[9].

Pesticide residues in Nigeria are analyzed and monitored in an accredited dedicated laboratory at National Food Directorate and Control [10]. Many of these pesticide residues build up to harmful levels in the body as well as in the environment, which is deliberately applied to a crop, and this differentiates residue from pesticide contamination which is always unintentional. WHO sees pesticide residue as any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products and impurities that are considered to be of toxicological significance; pesticide residue definitions are established for maximum residue limits enforcement purposes and for products exposure assessment[11].

Strong evidence also exist other negative outcome from pesticides exposure including neurological birth defect, fatal death

and neurodevelopment disorder [12]. The American Medical Association recommended limiting exposure to pesticides using safer alternatives regarding the long effect of low dose pesticides exposure. Even through consuming organic food these chemicals can have negative health effect. The Nigeria Environmental Protecting Agencies limit the amount of each pesticide that can be present in the food item or stored foods, because member of different chemicals may form one of many dangerous interactions and have an unmeasured synergistic effect [13].

Therefore, the aim of this present study is to determine the effects of *A. indica* seed and leaf extracts on developmental stages of stored grain weevils (*Acanthoscelides obtestus and Sitophilus spp*.) as well as to evaluate the toxicity of pesticide-protected stored grains which had lasted for at least six months in stores sold in Nigeria local markets for human consumptions.

2. Materials and Methods

2.1 Plant collection and identification

Leaves and seeds of *Azadirachta indica* was collected at Daniya, Bali Local Government of Taraba State, Nigeria in July, 2014. It was identified and authenticated in the Department of Science Laboratory Technology, Federal Polytechnic Bali with a voucher number *FEDPOBAL2014MEL001* deposited in the department.

2.2 Preparation of Plant Materials

The collected leaves and seeds of *A. indica* were dried under shed (room temperature) inside the laboratory to avoid decomposition of chemical components by sunlight for two weeks. Dried sample was pulverized into powder with electronic blender. The seed and leaf powder were weighed and stored in a sterile rubber container for future use.

2.2.1 Preparation of macerated aqueous (AE) and ethanol extract (EE) of *A. indica*

Powdered leaves (2000 g) and seeds of *A. indica* were each extracted for 24h using in 1.5L absolute ethanol in a 500mL separating funnel under a room temperature and concentrated using rotary evaporator. Similarly, fresh leaves and seeds were separately squeezed in a basin containing 1.5L water and filtered, with the shaft discarded.

2.3 Experimental Design for the Extracts

Our experimental design measures the effects of the following formulations: **1**. *A. indica* Leaf and Seed Powder **2.** Macerated aqueous extract **3.** Ethanol extracts on stored maize, guinea corn and bean infested weevils and un-infested grains (control group).

The experiment was conducted in five groups using the leaves and seed powder (LSP), aqueous extract (AE) and ethanol extract (EE) on the stored grains (maize, beans and guinea corn) infested with weevils. And also the control was set-up using the leaves and seed powder, aqueous extract and ethanol extract with un-infested grains.

***Group 1: Azadirachta indica seed and leaf powders*:** The leaf and seed powders were mixed differently on different sack containing maize, guinea corn, bean infested weevil to investigate the oviposition deterrence activities.

***Group 2: A. indica seed and leaf macerated aqueous extracts*:** 40 mL of *Azadirachta indica* seed and leaf extract was mixed separately in different sack containing maize, bean and guinea corn infested with weevils.

***Group 3: A. indica seed and leaf ethanol extracts*:** Leaf and seed ethanol extracts were molded into balls differently and form a pellet. Three pellets were mixed in each different sack containing maize, guinea corn and bean infested weevils.

Group 4: A. indica seed and leaf powders, macerated aqueous and ethanol extracts on stored grains un-infested with weevil (control): *Azadirachta indica* seed and leaf powders were mixed with stored grains (maize, guinea corn, beans) without weevils separately in a sack (bag) to evaluate pest repellant action (insecticidal) of the plant. Similarly, the *A. indica* seed and leaf ethanol extracts and the pellets were also mixed the grains separately in a different sack to see whether the grains will be infested with weevils.

Group 5: Dichloropropane-dichloropropene force (DD force) effects on grains infested with weevils (chemical control): 100 mL DD force was mixed with the grains each infested with weevils to compare the oviposition deterrence and insecticidal action with that of *A. indica*.

2.4 Toxicological Studies of Pesticide-protected Stored Grains in Nigeria

2.4.1 Experimental animals

Twenty six white male albino mice were obtained from the Department of Pharmacology and Clinical Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on diet specially prepared from chick grower's mash (Pfizer Company, Nigeria) and were given water *ad libitum* throughout the study period. Animals' weights ranged from 20 - 25 g .

2.4.2 Experimental design for acute toxicity Study

Acute toxicity study was conducted in accordance with Lorke's method[14]. The study was conducted in two phases using a total of twenty six albino male mice. In the first phase, nine mice were divided into 3groups of 3 mice each. Groups 1, 2 and 3 animals were given 10,100 and 1000 mg/kg body weight (b.w.) of the extract, respectively, to possibly establish the range of doses producing any toxic effect. Each mouse was given a single dose after at least 5 days of adaptation. In addition, a fourth group of three rats was setup as control group and animals in the group were not given the extract. In the second phase, further specific doses (1600, 2900 and 5000mg/kg b.w.) of the extract were administered (i.p) to three mice (one mice per dose) to further determine the correct LD_{50} value. The extract was dissolved in phosphate buffered saline (PBS) solution and given via intraperitoneal route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity whereas LD_{50} was calculated as geometrical mean of least tolerant dose multiply by highest toxic dose[15]. At the end of 14 days, all surviving mice were sacrificed following US-FDA regulation of inhuman treatment of animals [15], and then autopsied at the Department of Science laboratory Technology Federal Polytechnic, Bali. Internal organs were examined microscopically for pathological changes and compared to the control group. The weights of these organs were also taken and the mean organ-body weight ratios calculated and compared with those of the control group.

2.4.3 Histopathological studies in mice

Histological examination was done by fixing the organs (liver) in 4% formaldehyde. They were subsequently processed and embedded in paraffin wax. Tissue blocks were sectioned 5 µm thick and stained with haematoxylin and eosin (H & E) for detailed observation [16].

3. Results

Table 1: Effects of powdered parts of *Azadirachta indica* on weevil infested grains

Test sample(g)	Weevil death / % Oviposition deterrence			
(Grain+ powder)	Week 1	Week 2	Week 3	Week 4
IM + PAI Leaf	9/20	12 /21	17/23	32/40
IM + PAI Seed	7/18	19/25	22/28	37/45
IB + PAI Leaf	7/18	9/20	27/30	**
IB + PAI Seed	6/17	11/20	32/40	**
IG + PAI Leaf	11/20	21/28	40/65	56/72
IG + PAI Seed	9/20	15/19	24/30	36/44

•IM (infested maize), IB (infested bean), IG(infested guinea corn), PAI (powder of *Azadirachta indica* leaf) ** (100% mortality).

Table 2: Effect of *A. indica* leaf and seed ethanol extracts on weevil infested grains

Test sample(g)	Weevil death / % Oviposition deterrence			
(Grain/Extract)	Week 1	Week 2	Week 3	Week 4
IM + AEAI Leaf	**11/20**	**18/24**	29/32	**48/68**
IM + AEAI Seed	5/17	16/20	**27/30**	41/66
IM + AEAI Seed	11/20	21/28	37/60	**
IB + AEAI Seed	6/19	18/24	58/79	**
IG + AEAI Leaf	8/20	21/28	19/25	21/28
IG + AEAI Seed	13/24	20/27	36/58	40/66

•IG (infested guinea corn), IB (Infested bean), IM (infested maize), AEAI (maceration aqueous extract of *A. indica* leaf), ** (100% oviposition deterrence).

Table3: Effect of *A. indica* leaf and seed ethanol extract pellets on weevil infested grains

Test sample(g)	Weevil death / % Oviposition deterrence			
(Grain/pellets)	Week 1	Week 2	Week 3	Week 4
IM + AIP Leaf	4 /12	7 /18	11/20	13/22
IM + AIP Seed	2/10	6/18	8/19	11/20
IB + AIP leaf	10/20	12/22	14/24	19/28
IB + AIP Seed	7/18	9/20	12/22	20/30
IG + AIP Leaf	6/18	10/20	13/22	17/27
IG + AIP Seed	9/20	12/22	15/24	6/18
UIGs+LSP(controls)	**	**	**	**
DD force (pesticide)	**	**	**	**

•IM (Infested maize), IB (Infested beans), IG (Infested Guinea corn) AIP(*Azadirachta indica* pellets), UIGs(un-infested grains), LSP(leaf and seed powders mixed), **(100% insect repellant; insecticidal and oviposition deterrence), DD force(dichloropropane- dichloropropene force, India).

Figure 1: Dead weevils killed by A. indica formulations on stored grains (see the arrows).

Table 4: LD_{50} determination of aqueous extracts of Bean, Maize and Guinea corn Grains in Mice

Group Doses	Animal died/Animal survived		
(mg/kg) (i.p)	Bean	Maize	Guinea corn
10	0/3	0/3	0/3
100	0/3	0/3	0/3
1000	3/0*	0/3	0/3
1600	1/0	1/0[tm]	0/1
2900	1/0	1/0[tm]	0/1
5000	1/0*	1/0[tm]	0/1

• LD_{50} > 5000mg/kg (guinea corn grains), n=3 in phase I, n=1 in phase II, tm (toxicity for maize grains) LD_{50} =790.5mg/Kg, * LD_{50} = 3162.3mg/Kg (for bean).

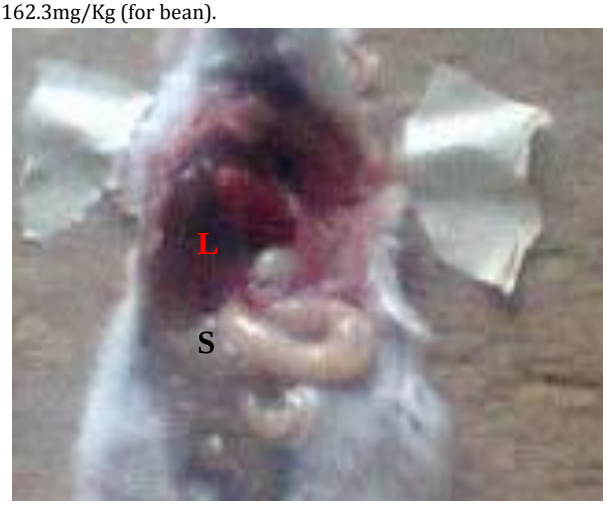
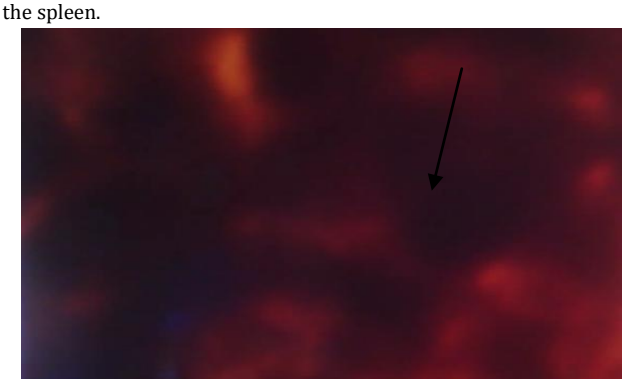
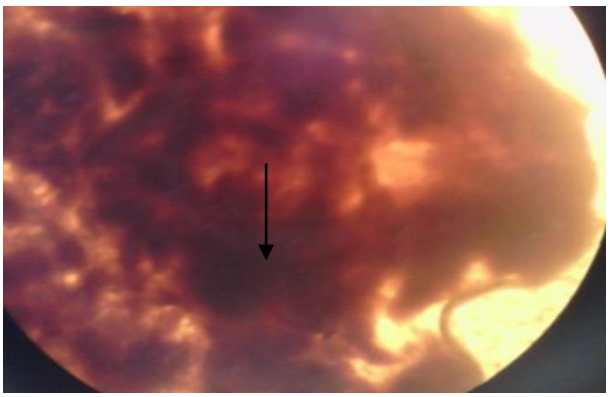


Figure 2: Showing the congestion of liver and the moderate enlargement of spleen of the rate cause by the administration maize extract to mice; **L =** congestion of liver, **S** = moderate enlargement of the spleen.



a.



b.

Figure 3a and b: Showing the coagulated necrosis caused by the administration of the maize extracts to the mice 40x

4. Discussion

Azadirachta indica resources are useful alternatives to synthetic chemical pesticide in the control of insect pest. It constitutes rich resources of bioactive molecules. Rapid degradation of the active ingredient makes plant derived substances more acceptable as they reduce the risk of residue contamination on grain because they are biosynthetic. Most of the *Azadirachta indica* derived substances have lethal action on insects and are more selective to insect but less harmful to beneficial organism because of their specificity [17].

The results obtained in this present studies showed targeted insecticidal activities of *A. indica* extracts on grain-infested weevils. % Oviposition deterrence properties increased with increased storage periods of grains [tables 1,2 and 3]. Mechanism of

pesticidal activities of *A. indica* formulations was by osmotic shock, as seen in the behavior of weevils killed by the powder formulations. This was supported by the fact that many pesticides exhibit insecticidal action by osmotic shock of the CNS of the insects, thereby disorienting their nervous connectivity [18],[Fig.2]. From the result obtained in table 3, *A. indica* seed and leaf powder were most effective in oviposition deterrence as well as insecticidal actions on stored grains weevils, as seen in the 100% oviposition deterrence [table 3]. These results were comparable to the control pesticide DD force which had exhibited lethal effect on both targeted and non-targeted species (table 3).

But the use of DD force as pesticide for stored grains in Nigeria, had been shown to be toxic even after six months of storage [table 4]. Liver necroses, congested liver, abnormality in spleen as well as ocular deformation were noticed in mice [Figure 3a and b]. Our result showed that bean and maize grains stored for at least six months and sold in Nigeria local markets were toxic at doses investigated upon, which the main cause of histological aberrations was observed in the animals after two weeks of bean and maize extracts administration (i.p). An incidence of bean poison was witnessed in Nigerian North-Central State Kogi; where boarding students died of this[19].

LD_{50} value of the extracts were determined to be 3162.3mg/Kg and 790.5mg/Kg of bean and maize extracts (table 4).Toxicological studies also showed that most of animals tolerated doses of 10mg/Kg b.w and 100 mg/Kg b.w of the grain extracts, but were killed as the dosage increased in phase two of the experiment for bean and maize extracts. These grains undoubtedly, are the most consumed grains in most Nigerian homes, and results of this study portrays danger signals for the National Agency for Food Drug Administration and Control(NAFDAC) to swing into action to prevent a re-occurrence of food poison in Nigeria. The studies further revealed that there were progressive weight losses in all the animal groups as the extracts were administered. These studies then showed that the use of DD force and the likes as pesticide for stored grains produced lethal effects on both beneficial and non-beneficial organisms (pests) [20], and possess latent effects on stored grains when consumed by humans as was observed in *in vivo* mice model.

5. Conclusion

The result obtained from this study indicated that *Azadirachta indica* leaf and seed extracts can be used as an alternative pesticide for stored grains, this safe cost, prevent grain poison, and safe time as well as easy accessible from natural source. However, we recommend that grains sold in local markets in Nigeria and other countries of the world should protected from pest infestation by use of pesticides from natural sources like *A. indica* so as to prevent any toxic effects. Measures should also be put in place by food regulating agencies to detoxify stored grains and their products before pushing them to local markets for consumption.

Acknowledgement

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Ethical Issues

The animals used in this study were according to animal research ethic of Ahmadu Bello University, Zaria, Nigeria.

Conflict of interest

We declare no conflict of interest.

References

[1] Krishan N, Suetanes P, Jans P, and Gaw R. A draft of the genome and four transcripts of a medicinal and pesticidal angiosperm *Azadirachta indica* 2012.

[2] Zillur R and Shamin J (2013). Neem research and development society of pesticide science India, edited by N.S Randhawa and B.S Parmer 5th revised edition: 500.

[3] German G.S, Rotundo G and Christopher, D.A.J. Stored Product Pest, 2007; 43: 229-223.

[4] Denloya A.A, Tesilm, H and Makanjuala W.A. Assessment of the efficiency of actellic and sumithion in protection grains from insect infestation during storage". *Journal of entomology* 2008; 5 (1):24-30.

[5] Alade, G.O, Akanmu, M.A, Obuotor, E.M, Osasan, S.A, Omobuwajo, O.R. Acute and oral subacute toxicity of methanolic extract of *Bauhinia monandra* leaf in rats. *Afr. J.Pharm. Pharmacol*. 2009; 3: 354-358.

[6] Abdullaev, A and Sadiq, M. Toxicology *in vitro*. *International journal of farming and Allied Sciences*. 2003: 1363-1368.

[7] Akunyilli, D and Ivbijaro, M.F.A. Pesticides regulation and their implementation in Nigeria; Entomological Society of American (ESA) Annual Meeting 2006.

[8] Banjo, A. D, Aina, S. A, and Rije, O. I. Farmers` Knowledge and Perception towards Herbicides and Pesticides Usage in Fadama Area of Okun-Owa, Ogun State of Nigeria", *African Journal of Basic and Applied Science*, 2010; 2(5&6): 188-194.

[9] Chikwe, A. NAFDAC Axes 20 fast food Outlets. Task Operators on Good Hygienic Practices. Tuesday, July 6, 2010. *www.nigerianbestforum.com*

[10] Cooper, J and Dobson, H. The benefits of pesticide mankind and the environment", *Crop Protection*, 2007; 26: 1337-1348.

[11] Dua, R, Sunkaria, A, Kumar, V, Gill, K.D. Impaired mitochondrial energy metabolism and kinetic properties of cytochrome oxidase following acute aluminium phosphide exposure in rat liver. *Food and Chemical Toxicology*; 2010; 48(1):53–60.

[12] Eskenazi, B, Marks, A.R, Bradman, A, Fenster, L, Johnson, C, Barr, D.B. *In- utero* exposure to dichlorodiphenyltrichloroethane (DDT) and (DDE) and neurodevelopment among young Mexican American children. *Paediatrics*; 2006; 118(1):233–41.

[13] Shrestha, P, Koirala, P and Tamraka, A. S. Knowledge, practice and use of pesticide among commercial vegetable growers of Dhading district, Nepal. *The journal of Agriculture and Environment;* 2010; 11: 95 -100.

[14] Lorke, D. A New Approach to Practical Acute toxicity Testing. *Achieves of Toxicology*; 1983; 54: 275 – 287.

[15] Mada, D.A and Hussein, I.D. Investigation into the effect of pesticide application on soil and environment with crop protection machine in Southern Adamawa State, Nigeria. *The intentional journal of Engineering and science* (JES); 2013; 2 (12): 71- 76.

[16] Ogunjimi, S. I and Farinde, A. S. Farmers` knowledge level of precautionary measures in agro-chemical usage on cocoa production in Osun and Edo States, Nigeria". *International Journal of Agriculture and Forestry*, 2012; 2(4):186-194.

[17] Otitodun, G. O, Opita, G and Okonkwo, E. O. Efficacy of Nigeria – derived diatomaceous earth, botanical insecticides, and riverbed sand against *Sitophilus oryzea* (Coleoptera: Curculionide) and *Rhyzopertha dominica* (Coleoptera: Bostrichdea) on wheat". *The Concept of Withholding Period and Pesticide;* 2012; 603:353- 373.

[18] Simon, E (2008). Beans sold to the public contained gammalin 20. Vanguard (Nigeria) August 12. Available from: http://allafrica.com/stor ies/200808120734.html

[19] Olabode, O. S, Adeshina, G. O and Olapeju, T. R. A Survey of Agricultural Chemicals Available to Farmers in South Western Nigeria", *International Journal of Agricultural Economic and Rural Development*, 2011; 4(1):12-14.

[20] Keri, H. J. Nigeria`s Status on Pesticide Registration and Maximum Residue Levels (MRLs) held in Alexandra Egypt. 30th March - 2nd April: 10pp 2009.

