American Journal of Biomedical and Life Sciences
2019; 7(2): 31-35
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20190702.11
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

The Insecticidal Activity of Neem (*Azadirachataindica*) Against Weevils in Stored Bambara Nuts (*Vignasubterranea*) and Beans (*Phaseolus vulgaris*)

Mbah-Omeje Kelechi Nkechinyere

Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Agbani, Nigeria

Email address:
kelechimbahomeje@gmail.com

To cite this article:
Mbah-Omeje Kelechi Nkechinyere. The Insecticidal Activity of Neem (*Azadirachataindica*) Against Weevils in Stored Bambara Nuts (*Vignasubterranea*) and Beans (*Phaseolus vulgaris*). *American Journal of Biomedical and Life Sciences.* Vol. 7, No. 2, 2019, pp. 31-35.
doi: 10.11648/j.ajbls.20190702.11

Received: November 23, 2018; **Accepted**: March 18, 2019; **Published**: May 6, 2019

Abstract: This study was undertaken to examine the insecticidal properties of Neem plant. Powder and aqueous extracts of Neem, *Azadirachata indica* leaves were evaluated as grain protectants against the bambara nut weevil (*Callosobruchusmaculatus*) and bean seed weevil (*Acanthoscelidesobtectus*), in the laboratory at 1.5, 2.0, and 2.5 (%v/w) concentrations per 20g of bambara nut and bean seeds respectively. Aqueous and powder extracts of *Azadirachataindica* leaves were applied to the bambara nut and beans seed using the contact method of application in the laboratory. Results revealed that 2.0%v/w ($p=0.014$) and 2.5% v/w ($p=0.008$) had significant increase in adult mortality of *C. maculatus* and *A. obtectus* in aqueous treatment of *A. indica* on beans and bambara nut after 72h at $p<0.05$. Similar results were obtained for beans and bambara nuts using powdered *A. indica* treatment. Percentage grain weight damage in bambara nut powder treatment of *A. indica* decreased significantly ($p=0.002$) at ($P<0.05$) in a proportionate, dose dependent manner and there was no adult emergence for either of bambara nut weevil or bean weevil. The 1.5 (%v/w) concentration was the least effective when compared with other concentrations. There was significant difference between *A. indica* treated grains and the control. There was no observed discoloration of the treated seeds. *A. indica* (aqueous and powder) extracts effectively reduced the weight loss of the treated bambara nut and seeds with 2.5% concentration being the most effective after 168hours. Phytochemical analyses of the extracts revealed presence of alkaloids, tannins, phenols, terpenes, saponins, cardiac glycosides, steroids while reducing sugars, carbohydrates and anthraquinones were absent. This study shows the insecticidal effects of *A. indica* against crop pests and at such there is need to further exploit Neem in order to maximize the potential. Farmers in developing countries can use *A. indica* as an alternative to chemical pesticide in rural grain storage.

Keywords: Azadirachataindica, Cowpea, Callosobruchusmaculatus, Acanthoscelidesobtectus and Bambara Nuts

1. Introduction

Pests are a major constraint which reduces yield and quality of harvested products both on the field and in storage [15]. *Acanthoscelidesobtectus* (bean weevil)is one of the most common weevil and grows exponentially on treated grain and can account up to 100% damage. Also in the tropics, *Callosobruchusmaculatus* heavily infest cowpea in storage [14]. In Nigeria, the dry weight loss due to infestation by *C. maculatus* can cause grain yield loss of up to 75% particularly in those parts of Nigeria where effective techniques of production are limiting [14]. The nutritional advantages associated with cowpea over other arable crops render all parts of the plants susceptible to insect pests. Cowpea is attacked by pests from the seedling stage up to maturity [11]. While synthetic pesticides kill pests they cause harm to other beneficial living organisms and create serious health and environmental risk. Studies indicate that a wide range of health problems are closely related to the widespread use of toxic chemicals on crops. These problems include birth defects, hormonal problems and nervous system damage [10]. Synthetic pesticides often contaminate water supplies and drain nutrients from the soil [7]. Synthetic pesticides are of increasing concern as they accumulate in the

environment, so much of attention has been diverted towards the natural compounds that could replace the synthetic ones [2]. Biopesticides are biodegradable, cheaper and more accessible in less developed countries [7]. Neem plant has been known for three decades for its potential against insect pests [2]. *Azadiractaindica* also known as Indian neem is an ever green, hard tree which is native to sub-continent. Both leaves and fruit of neem have bitter taste having fungicidal, insecticidal and nematicidal properties [2]. The principle component that has insecticidal activity in neem extracts is a limonoid, azadirachtin. It has been evaluated as the most promising insecticide of botanical origin, used against more than 400 species of insects [16]. The possible mode of action of azadirachtin is anti-feedancy which affects the acdysteroid and juvenile hormones and it directly affects tissues which are believed to be controlled by developmental hormones. Fungi, viruses and protozoa have also been reported sensitive to azadirachtin, apart from its action against insects [2]. It has been shown that azadirachtin acts on the mitotic cells and blocks the microtubule polymerization [3]. The anti-proliferating effect of azadirachtin is due to blocking of cell cycle and induction of apoptosis [1]. It has been suggested that azadirachtin is highly reactive and have many cellular molecules as target in cytoplasm as well as in nucleus [16]. Certain activites of genes and proteins are also altered byazadirachtin [3]. Another neem derived compound is the saponin which is known to have insecticidal activity [17]. Saponins are the surface-active glycosides that naturally occur in plants, animals and microganisms. Many saponins are found to have antimicrobial activity that inhibit moulds and protect plants from insect attack [17]. Neem plant is a potential source of biopesticides that has been appraised against many important insect species. Neem plant is found growing wild in Nigeria making it readily available to farmers at no cost. The use of neem could offer an alternative to synthetic pesticides which are highly persistent, broad spectrum and carcinogenic.

The aim and objective of the study is to determine the effectiveness of *Azadirachta indica* leaf powder and aqueous extract on *Callosobruchusmaculatus* and *Acanthoscelidesobtectus* in stored bambara groundnuts and cowpea respectively.

2. Materials and Method

2.1. Collection of Samples

The bambaranut (Vigniasubterranea) and black eyed cowpea seed (bean seed) were used for the study. They were purchased from Eke Agbani market in Enugu state and authenticated at the Agricultural department of Enugu state university of science and technology. The plant materials used were leaves of *Azadirachta indica* (neem) and were collected from Agbani area in Enugu state. Pirimiphos – methyl was used as a standard check. A culture of *C. maculates* and *A. obtectus* were maintained in the laboratory at room temperature was used for the study.

2.2. Preparation of Aqueous Extract

Processing of aqueous extract was prepared with modifications as described by Uddin *et al.,* (2013). Dry leaves of *A. indica* were ground into powder with sterile mortal. The powders were then sieved through a 0.02 sieve and prepared into extracts using distilled water. The extracts were prepared into three (3) concentrations of 1.5g, 2.0g and 2.5g. A control using only distilled water was also included. 1.5g, 2.0g and 2.5g of pulverized leaves of *A. indica* were dissolved in 100ml (giving 1.5%, 2.0% and 2.5% w/v) each of sterile distilled water, stirred vigorously and left for 24h. The mixtures were filtered using whatman's filter paper no. 1 to obtain solution free of solids. Contact method of application was used.

2.3. Seed Treatment with Aqueous A. Indica Leaf Extract

Test organisms were inhibited according to method by Uddin*et al*., (2013). Clean whole bambara nuts and bean seeds (20g) each were steeped in the filtrate of the different concentrations (1.5, 2.0 and 2.5) for thirty seconds and quickly removed and air dried for 5hours. They were placed in transparent 300ml plastic containers. Bambara nuts and bean seeds steeped in water served as control while pirimiphos – methyl (0.5% w/w) were included as standard check. 6 newly emerged adults of *C. maculates* and *Acanthoscelidesobtectus* each (3 males and 3 females) were added into each plastic container. There were 4 replicates for each treatment in a completely randomized design.

2.4. Seed Treatment with Powdered A. Indica Leaf Extract

A 20g sample of clean whole bambara nuts and bean seeds each were placed in 300ml transparent plastic bottles. Different concentrations of 1.5g, 2.0g and 2.5g of *A. indica* extract were added into each bottle respectively. They were agitated vigorously to allow uniform spread on the seeds. 20g of untreated bambara nuts and bean seeds served as the control while pirimiphos-methyl protected bambara nuts and beans served as standard check. 6 newly emerged adults of *C. maculates* and *Acanthoscelidesobtectus* (3 males and 3 females) were added into each plastic container. There were three replicates for each treatment in completely randomized design. Adult mortality, oviposition and F1 progeny emergence were assessed. Evaluation of damaged seed, weight loss and germination were assessed.

2.5. Preparation of Plant Extract for Phytochemical Screening

The leaves of the identified plant were collected and air dried, the dried sample was milled into fine powder by pounding.

2.6. Extraction of Methanol Extract

200g of the air dried powdered *A. indica* was weighed and mixed in 1L of methanol. The mixture was left at room temperature for 7 days for maceration, with daily intermittent

shaking. After 7 days, the solution was filtered using mushin cloth and the filtrates was evaporated to dryness using a rotary evaporator at 40^0C. They were stored at 20^0C until use.

2.7. Phytochemical Analysis of the Plant Extract

The methanol extracts of the plant was subjected to phytochemical screening tests to determine their chemical constituents using standard methods of [19-20].

2.8. Data Analysis

The data collected were transformed using square root transformation and then subjected to analysis of variance. The means were separated using the least significant difference (LSD).

3. Results

3.1. Insecticidal Effect of A. Indica Leaves on C. Maculatusin Stored Bambara Nuts

At 168 hours, total mortality of *C. maculatus* were recorded for both aqueous and powdered treatments of stored bambara nuts (Table 1).

Table 1. *Effects of Neem leaf (Powder and Aqueous) on the Mortality of C. maculatus in stored Bambara nut.*

Botanical	Conc (%)	Mortality Aqueous Treatment					Mortality Powdered Treatment				
		24	48	72	96	168	24	48	72	96	168
Azadrichtaindica	Control	0.00^a	0.00^a	0.00^a	0.00^a	0.67^a	0.00^a	0.00^a	0.00^a	0.00^a	1.00^a
	1.5	2.67^b	4.33^b	5.00^b	5.00^b	6.00^b	0.00^a	1.33^b	3.00^b	4.00^b	6.00^b
	2.0	2.00^b	4.33^b	6.00^b	6.00^b	6.00^b	1.33^b	2.67^c	3.67^b	4.67^b	6.00^b
	2.5	2.00^b	3.67^b	3.67^c	3.67^c	6.00^b	1.33^b	2.33^c	3.67^b	5.00^b	6.00^b

Note: Means values with different alphabetic superscripts differ significantlybetween concentrations within exposure (in a column) duration based on LSD analysis at $p < 0.05$.

3.2. Insecticidial Effect of A. Indica Leaves on A. Obtectus in Stored BEANS

At 168 hours, total mortality of *A. obtectus* was observed only in the aqueous treatment. The mean death rates of the weevils varied in the aqueous and powdered treatment of stored beans.

Table 2. *Effects of Neem leaf (Powder and Aqueous) on the Mortality ofA. Obtectus in stored Beans.*

Botanical	Conc (%)	Mortality Aqueous Treatment					Mortality Powdered Treatment				
		24	48	72	96	168	24	48	72	96	168
Azadrichtaindica	Control	0.00^a	0.00^a	0.00^a	0.00^a	0.67^a	0.00^a	0.00^a	0.00^a	0.00^a	0.67^a
	1.5	1.00^a	2.00^a	2.50^b	3.67^b	5.00^b	0.00^a	1.67^b	2.33^b	3.00^b	3.00^b
	2.0	0.67^a	2.00^a	2.67^b	4.00^b	4.67^b	0.00^a	1.33^b	2.67^b	4.00^c	4.00^c
	2.5	0.67^a	1.58^a	2.00^b	2.67^b	6.00^c	0.00^a	1.33^b	2.67^b	4.00^c	4.08^c

Note: Means values with different alphabetic superscripts differ significantly between concentrations within exposure (in a column) duration based on LSD analysis at $p < 0.05$.

3.3. Effect of A. Indicaleaves on Reducing Weight Loss in Stored Bambara Nuts

The percentage weight loss of treated stored bambara nuts is shown in Table 3. At 2.5% aqueous treatment of A. indica completely prevented weight loss in stored bambara nuts. There was significant difference ($P<0.005$) between aqueous treatment and powdered treatments of bambara nuts at all concentrations.

Table 3. *Percentage Grain Weight Loss of Treated Bambara nuts.*

Botanical	Conc.	Initial Weight (g) (Aqueous and Powder)	Final Weight (g)Aqueous Treatment	Final Weight (g)Powder Treatment	% Weight Loss Aqueous	% Weight Loss Powder
Azadrichtaindica	Control	20	17.28^a	16.95^a	13.60	15.25
	1.5	20	17.42^a	17.16^b	12.90	14.20
	2.0	20	18.37^b	17.63^c	8.15	11.85
	2.5	20	19.39^c	17.84^d	3.05	10.80

Note: Means values with different alphabetic superscripts differ significantly between concentrations within exposure (in a column) duration based on LSD analysis at $p < 0.05$.

3.4. Effect of A. Indica Leaves on Reducing Weight Loss in Stored Beanseeds

The percentage weight loss of treated stored bean grains is shown in Table 4. There was significant difference ($P<0.005$) between aqueous treatment and powdered treatments of bambara nuts at 2.5% and the aqueous treatments were more effective in preventing weight loss at all concentrations than the powdered treatments.

Table 4. *Percentage Grain Weight Loss of Treated Bean Seeds.*

Botanical	Conc.	Initial Weight (g) (Aqueous and Powder)	Final Weight (g)Aqueous Treatment	Final Weight (g)Powder Treatment	% Weight Loss Aqueous	% Weight Loss Powder
Azadirachtaindica	Control	20	17.09a	16.94a	14.55	15.30
	1.5	20	17.30a	17.10b	13.50	14.50
	2.0	20	17.70b	17.71c	11.50	11.45
	2.5	20	18.18c	17.53d	9.10	12.35

Note: Means that are significantly different between treatments based on LSD analysis at $p < 0.05$ are labelled with different letters.

3.5. Phytochemical Analysis of Azadirachta Indica

The methanol extract of *Azadirachtaindica*leaves showed presence of some important phytochemicals like alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids. These phytoconstituents have important pharmacological activities like anti mutagenic, anti-inflammatory, antibacterial, antiprotozoal, and antioxidant properties (Table 5).

Table 5. *Phytochemical Analysis of Methanol Extract of Azadirachta indica leaves.*

Phytochemical	Test	Observation	Inference
Alkaloids	Wagner's test	Red precipitate	+
Tannins and Phenolic compounds	Lead test	Green colour	+
Terpenoids and Phytosterols	Salkowaski's test	Reddish-brown colour	+
Saponins	Foam test	Presence of emulsion	+
Flavonoids	Ferric chloride test	White precipitate	+
Glycosides	Brown ring	-	+
Carbohydrates	Fehling's test	-	-

4. Discussion

Recently the use of synthetic pesticides such as carbamates, organophophates has reduced and interest has been shifted toward environmental friendly and less toxic pesticides of plant origin [17]. In the present study, the aqueous suspensions expressed more inhibitory effect on the weevils than the powder for all the test samples (Table 1 and Table 2). Tables 1 and 2, show the effect of the various concentrations of neem leaf on weevils, in stored bambara nuts and beans respectively. The results of these, though varied in terms of magnitudes, took a similar trend. In all the cases, it was detected that the maximum effectiveness of these preparations were mostly attained at 2.5% concentration of the neem leaf, 168 hours after their application. This is similar to the works of Uddin *et al.,* (2013) who reported that at 2.5% concentration *A.indica*was effective against *C. maculatus.* The study is also in line with the work of Schmutterer (1995) who reported that both the leaves and fruits of the neem plant have fungicidal, insecticidal and nematicidal properties.

From the study, the toxicity rate of both the powder and aqueous extracts ranged from 11-100%, on six weevils (*C. maculatus* and *A.obtectus)* each starting from the first 24 hours till the last 168 hours. The control (distilled water) was not effective (0%). Neem extracts in the aqueous forms gave better effects compared to the powdered forms. This supports the findings of Addor (1995) who observed that aqueous solution of *A.indica* readily forms a complete coating around the seeds which immobilizes the insects consequently causing their deaths. This study also is in agreement with the study of Uddin and Abdulazeez (2013) who found out that the aqueous extracts evaluated were more efficient for the first two daysin causing mortality than the powder. The results of this study also showed that the death rates per time for the weevils were increased with respect to concentration levels used (Tables 1 and 2). The higher the concentration of the neem, the faster it's killing effect on the weevils. Concentrations of 1.5% and above for aqueous treatments were seen to be effective after 24 hours since they were able to kill half or more of the total number of the weevils, while for powdered treatments were effective after 48 hours. Both powdered and aqueous treatments of *A.indica*killed all weevils after 168 hours for all concentration in stored cowpea but that was not the case for stored bean seeds. Thus neem leaf extracts are more effective in killing weevils in stored cowpea than in stored bean.

From Table 2, the test carried out for a period of 24 hours on stored bean had fewer numbers of recorded deaths of the weevil compared to the same time in stored bambara nuts. The number of death of the weevils increased as the concentration for the neem increased. For the 1.5% concentration of the powder neem treatment in stored cowpea, a drop in the number of deaths for the weevils was recorded after 168 hours. This could be attributed to some factors such as mutagenic species. There were significant differences in adult mortality ($P < 0.05$) between the powder and aqueous treatments at 24, 48, 72 and 168 hours after infestation for all the treatments. This observed mortality may be due to the azadirachtin contained in the neem. Achio*et al.* (2012) reported that neem serve as an insect repellent in rice paddies, as well as being effective on soil-borne fungal pathogens and plant parasitic nematodes.

Based on relatively better insecticidal effects of the neem leaf in stopping weevil from destroying stored grains and reducing their weights, hence the initial and final weights of the stored bambara nuts and bean seeds were determined and the final weight loss converted to percentages. The findings

are illustrated in Table 3 and 4. In the aqueous treatments, the concentration of 2.5% followed by 2.0% was the most effective in preventing weight loss in bambara nut and bean seeds. This supports the work of Boeke*et al*. (2004), who reported that powder and aqueous extracts of *A.indica, J. curcas*and*Vernoniaamygdalina* significantly reduced the number of progeny emergence. Emosairue and Ubana (1998) found *A. indica* to be very effective in reducing progeny emergence and in turn reducing grain damage or weight loss. Uddin and Abdulazeez (2013) also reported that at 2.5% *A. indica*effectively prevented weight loss in cowpea seeds. From the study, the preliminary phytochemical analysis of methanol extract of *A. indica*leaves showed the presence of alkaloids, tannins, phenols, terpenes, saponins, cardiac glycosides, steroids while reducing sugars, carbohydrates and anthraquinones were absent. This is in line with the study of Madaki*et al*. (2016) who also reported the presence of alkaloids, tannins, phenols, terpenes, saponins, cardiac glycosides, steroids in neem leaves. Results from this study contradict the findings of Tiwari and Rao (2002) who did not find most of the phytochemical constituents obtained. The biopesticidal property of the plant is also attributed to saponins which have been reported to possess biopesticidal activity [18-19]. Schmutterer (1995) also reported that neem plant is a potential source of drugs and eco loving pesticides.

5. Conclusion

This study reveals that the powder and aqueous extract of *Azadirachtaindica* leaf has proven to have insecticidal activities on *Callosobruchusmaculatus* and *Acanthoscelidesobtectus* and was effective in reducing the weight loss in stored bambara nuts and bean seeds. It also reveals that neem leaves contain phytochemical constituents that enables it elicit medicinal and biopesticidal properties. This study however, justify the scientific use of this plant as biopesticide against many pests of agricultural importance.

References

[1] F. M. Abou-Tarboush, H. M. El-Ashmaoui, H. I. Hussein, D. Al-Rajhy and M. Al-Assiry. Effect of azadirachtin of neem x-4.5m SWR/Juice. Saudi *J BiolSci*; 16 (2009) 69-76.

[2] R. B. Raizada, M. K. Srivastava, R. A. Kaushal and R. P. Suigh. Azadirachtin , a neembiopesticide: sub chronic toxicity assessment in rats. *Food chem.* 39 (2001) 477-83.

[3] A. J. Mordue (Luntz), E. D. Morgan and A. J. Nisbet. Azadirachtin, a natural product in insect control In: Gilbert, L. I. Latrou, K. J. Gill, S. S. (Eds), comprehensive molecular science. Elsevier, Oxford; 6 (2005) 117-135.

[4] P. Sinimons and H. D. Nelson. Insects on Dried fruits 1975. Agriculture Handbook. USD. Agricultural Research Service (1975).

[5] J. P. Morrissey and A. E. Osbourn. Fungal Resistance to plant antibiotics as a mechanism of pathogenesis. *MicrobiolMolBiol Rev*; 63 (1999) 708-24.

[6] S. Gus-Mayer, H. Brynner, H. A. Schneider-poetsh and W. Ruidiger, Avenscosidase from oat: purification, sequence analysis and biochemical characteristization of a new member of the BGA family of beta-glucosides. *Plant MolBiol;* 26 (1994) 909-21.

[7] T. Hooper. "Push-pull" Using Plants as natural pesticides," The naked Scientists: Science Interview (2008).

[8] Neem: A tree of solving Global Problems. Board of Science and Technology for International Development at the Natural Research Council. Washington D. C.: Natural Academy of Sciences (1992).

[9] A. A. Bajwa and A. Ahmed. Potential applications of Neem based products as biopesticides. The Health; 3 (4) (2012) 116 – 120.

[10] R. O. Uddin, II. andR. W. Abdulazeez. Comparative efficacy of neem, false sesame, Endl and the physic nut in the protection of stored cowpea. (Vignaunguiculata) L. walp against the seed beetle *Callosobruchusmaculatus* (F); (6) (2003) 827-834.

[11] S. O. Emosairue and U. B. Ubana. Field Evaluation of Neem for the Control of some Cowpea Insect Pests in Southern Nigeria. Global *Journal of Pure and Applied Science*, 4 (1998) 237-241.

[12] G. Ntoukam, L. L. Murdock, R. E. Shade, L. W. Kitch, C. Endondo, B. Ousmane and J. Wolfson. Storageof Bean/Cowpea, Midcourse 2000 Research Meeting, April 9-14, (2000). Senegal. P 3-4.

[13] Addor, R. W. Insecticides In: Agrochemicals from Natural Products. Godfrey, C. R. A (Ed). Marcel Dekker, Inc. (1995) Pp1-62.

[14] Profit, M. Bruchid Research at Royal Holloway, University of London. (1997) Pp 1-3.www.rhul.ac.uk/biosci/credland/bruchid.htm.

[15] Singh, B. Improving the Production and Utilization of Cowpea as Food and Fodder. Field Crops Research., 84 (2003) 149- 150.

[16] Schmutterer, H. The Neem Tree, Azadirachtaindica A. Juss and Other Meliaceous Plants: Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. VCH, Weinheim, Germany (1995).

[17] A. A Bajwa and A. Ahmad. Potntial applications of Neem based Products as Biopesticides. the Health; 3(4) (2012)116-120.

[18] A. K. Tiwari and J. M. Rao. Diabetic mellitus and multiple therapeutic approaches of phytochemicals. Present status and future prospects. Current Science. 83 (1) (2002) 30-31.

[19] W. C. Evans and G. E. Trease. Pharmacognosy (15th edition) W. B. Saunders company Ltd. London. (2002) 191-393.

[20] G. E. Trease and W. C. Evans. Pharmacognosy. 4th edition, W. B. Saunders company, USA (1996) 243-283.

[21] A. Sofowara. Medicinal plants and traditional medicine in Africa Rep. Spectrum books Ltd. Ibadan. (2006) 150.

[22] F. M. Madaki, A. Y. Kairu, M. T. Bakare-Odunola, S. C. Msailafiya, R. U. Hamzah and Janet Edward. Phytochemical and proximate analysis of methanol leaf extract of neemAzadirachtaindica. European Journal of Medicinal plants 15 (2) (2016) 1-6.