The Effect of Neem (*Azadirachta Indica*) Bark Extracts on Maize Weevil (*Sitophilus Zeamais*) in Stored Maize (*Zea Mays*) in Lokoja, Kogi State

Pelumi Olusanmi (pelumiolusanmi@gmail.com)
Federal University Lokoja

Research Article

Keywords: Integrated Pest Control, Natural Products, Bio-pesticides, Post-harvest, Bioactivity, Mortality, Efficacy, Biological Pest Control

Posted Date: August 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3283513/v1

Abstract

Background

This study investigated the effect of crude aqueous and ethanolic extracts of Azadirachta indica against Sitophilus zeamais (Maize weevil) on stored Zea mays (Maize). The pesticidal activity of Azadirachta indica extracts on Sitophilus zeamais was evaluated at concentrations of 500, 1000, and 1500mg/ml. The cold maceration method was employed in this study for the preparation of extracts. The mortality rate of the weevils was monitored every 24 hours for 72 hours consecutively. Measured and recorded mortality were presented in tabular format, indicating the concentrations and total number of death counts. The data obtained were analyzed using SPSS (Version 21). Proportions of the mortality rate of Sitophilus zeamais in relation to concentrations of aqueous and ethanolic extracts were compared using ANOVA.

Results

Inferences drawn from this study showed that both the crude aqueous and ethanolic extracts were effective at the maximum concentration, but the ethanolic extract showed a higher rate of effectiveness than the crude aqueous extract.

Conclusion

To further assess their potential as an insecticide against pests in storage seeds, it is also advised to extract Azadirachta indica using a different solvent. Although these extracts have an impact on maize weevil populations, biosafety research should be done to determine their toxicity to humans. To evaluate the practical application of botanical insecticides, field tests should be conducted.

BACKGROUND

The plant *Zea mays* is a member of the Poaceae or Gramineae family. It is a type of cereal grass related to wheat, rice, oats, and barley, coming in second place after wheat in terms of global grain production, with rice coming in third (Ogunsina et al., 2011).

During storage and transportation, corn grain may get infested with cereal pests. *Sitophilus zeamais* Motschulsky's maize weevil, a 14-inch-long, reddish brown to black snout weevil, is one of them (Casey, 2006). Attacks may begin in mature crops of maize or sorghum when the grain's moisture content (MC) drops to 18–20%.

Subsequent infestations in storage occur as a result of the transfer of infested grain into storage or as a result of the pest flying into storage facilities, most likely attracted by the odor of the stored grain (Casey, 2006). A high infestation of this pest in stored maize can result in weight losses of up to 30–40%;

however, losses are usually about 4–5%. (Casey, 2006). The insect's chewing damage causes increased respiration in the grain (hot spots), which encourages the generation of heat and moisture and, in turn, creates a suitable living environment for molds, leading to the production of aflatoxin (Dahiya 2005).

The result is that bacterial development is encouraged at extremely high moisture levels, which eventually leads to depreciation and then utter loss (Dahiya, 2005). Demissie et al. (2008) claim that when grains have been harmed, their market value, germination rate, weight, and nutritional value will all decrease. As a result, to lessen the harm caused by *Sitophilus zeamais* and, consequently, to lessen food insecurity, effective and affordable measures must be implemented throughout Africa (Radha, 2014).

Effective pest control is no longer achieved by using large amounts of pesticides, in part *due* to the rising cost of petroleum-derived products but primarily because using too many pesticides encourages the rapid evolution of pest resistance, destroys natural enemies, transforms formerly harmless species into pests, harms other non-target species, and contaminates food (Golob *et al.*,1980). Degradable control agents that are less hazardous to humans are therefore urgently needed. Among these is the use of natural pesticides, especially in storage, that have low mammalian toxicity and are efficient at preventing and/or suppressing insect infestations (Golob *et al.*,1980).

Several specific benefits make the use of botanical pesticides to defend plants against pests particularly promising. Compared to commonly used synthetic pesticides, pesticidal plants are typically significantly safer (Saxena et al., 2011). Pesticidal plants have existed in nature as a part of it for millions of years without causing harm or having a negative impact on the ecology. Additionally, plant-based pesticides are more affordable and environmentally friendly (Saxena *et al.*,2011). Additionally, several plants have multiple chemicals acting as active principles, giving rise to their diverse biological characteristics.

Since azadirachtin is renowned for its low toxicity against beneficial insects, using natural and quickly biodegradable crop protection inputs like it can be a helpful component to manage pests (Koona *et al.*, 2004). In the nations where neem is located, almost every part of the tree—including the base, trunk, bark, leaves, blossoms, fruits, and seeds—is known to have some purpose (Radwaski, 1981).

Also examined were neem's insecticidal qualities by Tanzubil (1991), Becker (1994), and many others. Neem has demonstrated some toxic effects against some insects in addition to other insecticidal activities (feeding and ovipositional deterrent, insect growth regulator, and repellent); however, all of these effects are dose-dependent, differ from insect to insect, and affect the same insect at different stages in different ways.

Neem oil, when used in amounts ranging from 5 to 20% and applied to packing bags, has a potent insecticidal effect on stored grain beetles, according to Muhammad et al. (2005). Therefore, the pesticidal potential of *Azadirachta indica*'s crude aqueous and ethanolic extracts against maize weevil (*Sitophilus zeamais*) has been examined in this work

The present methods for controlling stored grain pests rely mainly on synthetic pesticides, such as carbon disulfide, phosphine, or malathion; carbaryl; pirimiphos-methyl; or permethrin fumigation or dusting (Ileke and Oni, 2011).

Their widespread and indiscriminate usage has resulted in the emergence of resistant strains as well as the accumulation of harmful residues on food grains consumed by humans (Sharma and Meshram, 2006). In addition to direct toxicity to users, many synthetic pesticides have high persistence, poor application knowledge, rising application costs, pest resurgence, insect genetic resistance, and deadly impacts on non-target animals (Oni and Ileke, 2008; Akinkurolele et al., 2006). Because of these issues, a more comprehensive and less destructive strategy for maize weevil control is being used, which is why botanicals are being used.

Due to issues including pesticide resistance, the resurgence of pest species, environmental pollution, and hazardous risks to humans and non-target organisms, the consequences of chemical-based insecticides have attracted considerable public concern. Due to these issues, it is now necessary to investigate and develop alternative solutions employing plant-based materials that are both environmentally friendly and biodegradable, are not poisonous to organisms other than the target species, and help reduce the chance of new resistant species arising. Although numerous plant species have been employed for this purpose, maize weevil destruction still necessitates the availability of a greater variety of plant materials.

This study intends to create a biopesticide that is efficient against the storage pest *Sitophilus zeamais* instead of synthetic pesticides that are harmful to humans and the environment.

This study aims to evaluate the effect of Neem (Azadirachta *indica*) bark extracts on maize weevil. With specific objectives: To determine the mortality of *Sitophilus. zeamais* exposed to different concentrations of the extracts of *Azadirachta indica* bark and to compare the degree of effectiveness of both aqueous and ethanolic extracts of *Azadirachta indica* bark on *Sitophilus zeamais*

METHODS

Study Area

The research took place in Lokoja, Kogi State. Lokoja is the capital of Kogi State and is located at the confluence of the Niger and Benue rivers. Lokoja is a commercial center covering an area of about 3180 km2. It is located at 7.8023°N latitude and 6.7333°E longitude. The town is located in Nigeria's tropical wet and dry savannah climate zone, where the weather is hot all year. Guinea savannah with long grasses and a few trees down is the main vegetation type of Lokoja. Yams, cassava, corn, sorghum beans, fish, palm produce, shea, nuts, and cotton are the major agricultural products that are traded in the city. The wet season in Lokoja is oppressive and overcast. The average annual temperature is 66°F, with temperatures rarely falling below 69°F or rising over 101°F.

Site of Experimentation

The research took place at the Federal University Lokoja Biological Science Laboratory in Kogi State.

Collection of Insects for Research

The insects were obtained from naturally infested, stored maize grain bought from the international market in Lokoja, Kogi State. The insects were identified based on their morphological features (Haistead, 1963).

Collection and Disinfestations of Maize Grain

The maize grains used for the study were purchased from the international market in Lokoja, Kogi State, Nigeria. Before the experiment, the grains were disinfested in the oven at 40°C to eliminate any form of insect infestation and allowed to cool to room temperature before use (Bakele *et al.*,1996)

Plant Sample Collection

Fresh samples of *Azadirachta indica* (neem) bark were collected in the Adankolo residential area and transported to the herbarium in a tightly sealed plastic bag for identification. The sample was taken to the laboratory after it had been identified.

Plant Sample Preparation

The sample was delivered to the lab and properly washed three times with distilled water to remove dust and soil particles. The sample was dried in the open air at room temperature, at a temperature of 28°C + 2°C. Samples were evaluated regularly, and when they were dry enough to break, they were pulverized into a fine powder with a mortar and pestle and stored in a tightly sealed container to preserve the active ingredient.

Preparation of Plant Extracts

300g of the powdered form of the bark was soaked in 1000 ml of 70% ethanol, and then the setup was allowed to sit for 24 hours. After 24 hours, the 300g of powdered bark that was soaked was sieved with muslin cloth into a 250 ml beaker, placed in a water bath, and allowed to evaporate to dryness. The Slurry obtained from the dried extract of Azadirachta indica was weighed separately as follows: 0 g (control), 5.0g, 10.0g, and 15.0g into bottles labeled A, B, and C, respectively, according to the measured weight in grams. An equal volume of 10 ml of 70% ethanol was poured into each bottle to yield 500g/L, 1000 g/L, and 1500g/L concentrations, respectively. Similarly, an extract of the botanical was prepared following the same procedure described for ethanolic extraction above before being used in the bioassay.

Bioassay

The bioassay was conducted according to the procedure described by Ntonifer et al. (2011), with slight modifications. 10g of disinfested maize grain were weighed into separate beakers, and different concentrations of the aqueous and ethanolic extracts were separately inoculated into separate beakers containing 10g of disinfested maize. All the beakers were shaken vigorously to ensure proper mixing of the maize grains with the extracts. The maize grains were air-dried on filter paper and poured back into the beaker. Ten adults of the insects were inoculated into each beaker (Mulungu et al., 2007). All the beakers were labeled according to the concentration used and covered with a muslin cloth to permit aeration (Akinwumi et al., 2007). A control experiment was also set up with the same amount of cowpea seeds and weevils but no plant extracts. A mortality count for *Sitophilus zeamais* was carried out daily post-treatment. Data on the mortality of weevils was recorded by counting the number of weevils that did not respond to the pin probe and dead weevils were removed from the sample in each reading.

Data Analysis

Measured and recorded mortality were presented in tabular format, indicating the concentrations and total number of death counts. The collected data were examined using SPSS (Version 21). Proportions of the mortality rate of *S. zeamais* in relation to concentrations of aqueous and ethanolic extracts were compared using ANOVA. It was also used to compare the proportions of the mortality rate of *Sitophilus zeamais* between extracts for each concentration. Paired t-test was used to calculate the 95% limits of the upper confidence limit and lower confidence limit. Results with P-values < 0.05 were considered to be statistically significant

RESULTS

The effect of *Azadirachta indica* aqueous extract on the mortality of *Sitophilus zeamais* was recorded for each concentration for a period of 72 hours. The control had no dead weevil at 24 hours, 48 hours, and 72 hours of the bioassay, whereas the aqueous extract treatments showed varying levels of mortality across concentrations No deaths were recorded for the first 48 hours of the first concentration, nor for the first 24 hours of the second and third concentrations of the treatment. The 1500 mg/L concentration recorded the highest mortality rate of *Sitophilus zeamais*, followed by 1000 mg/L and 500 mg/L.

There was no significant difference in mortality of *Sitophilus zeamais* across the concentrations of aqueous extract of *Azadirachta indica*; $F(2, 6) = 1.938$, $P = .224$ Nevertheless, There was a significant difference in the mortality rate of *Sitophilus zeamais* across hourly exposure to aqueous extract of *Azadirachta indica*: $F(2, 6) = 6.125$, $P = .036$

The posthoc comparisons using the Scheffe test indicated that the mean score for the first 24 hours of exposure ($M = .00$, $SD = 0.000$) was significantly different from 72 hours ($M = 2.67$, $SD = .577$, $P = .037$). There was no statistically significant difference in mean scores between 24 hours and 48 hours ($P = .177$). Also, the mean score of 48 hours of exposure is not significantly different from that of 72 hours (.475).

Table 1

Mortality Effect of Aqueous Extract of *Azadirachta indica* on *Sitophilus zeamais*

Hours of Treatment	**Mortality Rate of Neem Extract Concentrations**			
	C_1	C_2	C_3	Control
24 hours	ND	ND	ND	ND
48 hours	ND	2	3	ND
72 hours	2	3	3	ND
Total	2	5	6	0
Key: ND = No Death				

Table 2

One-Way ANOVA Result for Variation in Mortality rate of *Sitophilus zeamais* across the Concentration of Aqueous Extract of *Azadirachta indica*

ANOVA					
Mortality Rate					
	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	6.889	2	3.444	1.938	.224
Within Groups	10.667	6	1.778		
Total	17.556	8			

Table 3

One-Way ANOVA Result for Variation in Mortality rate of *S. zeamais* Across Exposure Hours to Aqueous Extracts of *Azadirachta indica*

ANOVA					
Mortality Rate					
	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	10.889	2	5.444	6.125	.036
Within Groups	5.333	6	.889		
Total	16.222	8			

Table 4
Descriptive Statistics of One-Way ANOVA Result for Variation in Mortality rate of *S. zeamais* across Exposure Hours to Aqueous Extracts of *Azadirachta indica*

Descriptive ANOVA Analysis								
Mortality Rate								
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
24 Hours	3	.00	.000	.000	.00	.00	0	0
48 Hours	3	1.67	1.528	.882	-2.13	5.46	0	3
72 Hours	3	2.67	.577	.333	1.23	4.10	2	3
Total	9	1.44	1.424	.475	.35	2.54	0	3

Table 5
Post-Hoc Results for Hourly Variation in Mortality Rate of *S. zeamais*

Multiple Comparisons						
Dependent Variable: Mortality Rate						
Scheffe						
(I) Hours	(J) Hours	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
24 Hours	48 Hours	-1.667	.770	.177	-4.14	.80
	72 Hours	-2.667*	.770	.037	-5.14	− .20
48 Hours	24 Hours	1.667	.770	.177	− .80	4.14
	72 Hours	-1.000	.770	.475	-3.47	1.47
72 Hours	24 Hours	2.667*	.770	.037	.20	5.14
	48 Hours	1.000	.770	.475	-1.47	3.47
* A 0.05 significance level indicates a significant difference in the mean.						

Table 6 reveals the mortality rate of an ethanolic extract of neem on maize weevil. The total number of deaths was recorded for each concentration for a period of 72 hours. The control had no dead weevil at

24 hours, 48 hours, and 72 hours of the bioassay, whereas the ethanolic extract treatments showed varying levels of mortality across concentrations.

The first concentration had a record of 1 death after 24 hours of treatment; the second concentration recorded no death; and the third concentration had 2 deaths recorded. On the 48-hour mark, the first concentration had a zero death record; three deaths were recorded for the concentration, while the third concentration recorded two deaths. A mortality rate of 3, 2, and 3 was recorded for concentrations 1, 2, and 3, respectively, after 72 hours of treatment.

The 1500 mg/L concentration recorded the highest mortality rate of *S. zeamais*, followed by 1000 mg/L and 500 mg/L. There was no significant difference in mortality of *S. zeamais* across the concentrations of ethanolic extract of *Azadirachta indica*; F (2, 6) = .467, P = .648 (Table 7). There was also no significant difference in the mortality rate of *S. zeamais* across hourly exposure to aqueous extract of *Azadirachta indica* (Table 8)

Table 6
Mortality Effect of Ethanolic Extract of *Azadirachta indica* on *S. zeamais*

Hours of Treatment	**Mortality Rate of Neem Extract Concentrations**			
	C1	C2	C3	Control
24 hours	1	ND	2	ND
48 hours	ND	3	2	ND
72 hours	3	2	3	ND
Total	4	5	7	0
Key: ND = No Death				

Table 7
One-Way ANOVA Result for Variation in the Mortality rate of *S. zeamais* across the Concentration of Ethanolic Extract of *Azadirachta indica*

ANOVA					
Mortality Rate					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1.556	2	.778	.467	.648
Within Groups	10.000	6	1.667		
Total	11.556	8			

Table 8
One-Way ANOVA Result for Variation in Mortality rate of *S. zeamais* across Exposure Hours to Ethanolic Extract of *Azadirachta indica*

ANOVA					
Mortality Rate					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	4.222	2	2.111	1.727	.256
Within Groups	7.333	6	1.222		
Total	11.556	8			

The mortality rate of *S. zeamais* was higher in all concentrations of ethanolic extract than in aqueous extract, with the exception of 1000 mg/L and 1500mg/L concentrations at 72 and 48 hours, respectively, in which aqueous extract had a higher mortality rate (Table 1 and Table 6). There was no significant difference (P = .347) in the mortality rate of *S. zeamais* between extracts of *Azadirachta indica* for each concentration (Table 9). Mortality of weevils in aqueous and ethanolic concentrates of *Azadirachta indica* was very strongly positively correlated (r = .722, p < .028) (Table 10). The aqueous concentrate (M = 1.44, SD = 1.424) was less than the ethanolic concentrate of 1.78 (Table 11). There was no statistical significant difference (95% CI (-1.102 to − .435), t(8) = -1.000, P = .347) (Table 9).

Table 9
Result of Paired Sample Test for the Mortality of *S. zeamais* in aqueous and ethanolic concentrates of *Azadirachta indica*

Paired Samples Test									
		Paired Differences					**T**	**df**	**Sig. (2-tailed)**
		Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
					Lower	**Upper**			
Pair 1	Aqueous Mortality – Ethanolic Mortality	− .333	1.000	.333	-1.102	.435	-1.000	8	.347

Table 10
Correlation of Mortality rate of *S. zeamais* in aqueous and ethanolic concentrates of *Azadirachta indica*

Paired Samples Correlations				
		N	**Correlation**	**Sig.**
Pair 1	Aqueous Mortality & Ethanolic Mortality	9	0.722	0.028

Table 11
Descriptive Statistics of Paired T-Test of Mortality Rate of *S. zeamais* in aqueous and ethanolic concentrates of *Azadirachta indica*

Paired Samples Statistics					
		Mean	**N**	**Std. Deviation**	**Std. Error Mean**
Pair 1	Aqueous Mortality	1.44	9	1.424	0.475
	Ethanolic Mortality	1.78	9	1.202	0.401

DISCUSSIONS

In Africa's smallholder farms, it is standard practice to use plant materials as a form of product protection. Recent research efforts have concentrated on the utilization of naturally occurring substances derived from plants as effective substitutes for the typical synthetic pesticides used to safeguard goods that are being kept (Abebe, 2006; Taddese, 2003). The results of this study's analysis support the notion that Neem products provide a cutting-edge method for managing pests in stored goods that has been tried and true.

The bioassay investigation revealed that the aqueous and ethanolic extracts of *A. indica* showed promising pesticidal activity against *S. zeamais*. This is in agreement with the study by Okolo and Iledun (2019), which tested the pesticidal activity of *Azadirachta indica* extracts against several cowpea pests, including Aphids.

The Neem (*Azadirachta indica)* extracts exhibited a direct toxic effect on the red flour beetle and other arthropods, according to a 2009 study by Mamum *et al*. Neem extracts are employed as foliar sprays because of their insecticidal qualities (Subbalakshmi,

No mortality was recorded in the first 24 hours of exposure of *S. zeamais* to the treatment, even as the concentration increased.

After 48 hours, no death was recorded in the first treatment, but mortality was recorded in both the second and third treatments, albeit with varying degrees of success.

After 72 hours, deaths were recorded in all the treatments, with the second and third treatments having the highest number of deaths. Total insect mortality increased with an increase in the mass of extract used and the treatment duration, as shown in Table 1, which is in conformity with the work of Abdullahi et al. (2014), in which the highest mortality of *S. zeamais* was recorded at the highest treatment concentration and hours

At 24 hours of exposure of *S. zeamais* to treatment, death was recorded in the first concentration and the third concentration only (Table 6).

After 48 hours, no death was recorded in the first treatment, but mortality was recorded in both the second and third treatments, albeit with varying degrees of success.

After 72 hours, deaths were recorded in all the treatments, with the first and third treatments having the highest death counts.

Total Insect mortality increased with an increase in the mass of extract used and the treatment duration, as shown in Table 6, which is in conformity with the work of Kossou (1989) and Awan (2011), who reported that Ethanolic extracts of neem were more toxic than neem powder to *Sitophillus zeamais* confined for 72 hours on treated grains. However, no extract was able to achieve 100% insect mortality after 24 hours.

The mortality of *Sitophilus zeamais* in seeds treated with the extract was significantly different from the weevil mortality in untreated seeds

CONCLUSION

This study demonstrates that neem extracts in aqueous and ethanolic forms are somewhat efficient against maize weevils since the weevil death rate significantly increased with increasing concentration and treatment time. The currently used conventional chemical pesticides will eventually be replaced with bio-pesticides as more is learned about their use. Neem can be developed into a powerful biopesticide with a lot of room for creativity. Neem should be progressively planted and produced worldwide to obtain its active ingredient (azadirachtin), which is responsible for interrupting the life cycle of pests. This is due to the growing trend toward using biofertilizers, insecticides, and pesticides.

It is advised that a similar study be carried out utilizing other components of the test plant, such as their roots, blooms, or even the entire plant, in order to further assess their effectiveness against pests like the maize weevil and other significant pests of stored goods. In order to further assess their potential as an insecticide against pests in storage seeds, it is also advised to extract those plants using a different solvent.

Although these extracts have an impact on maize weevil populations, biosafety research should be done to determine their toxicity to humans. To evaluate the practical application of botanical insecticides, field tests should be conducted.

List Of Abbreviations

A. indica - *Azadirachta indica*

ANOVA- -Analysis Of Variance

CI- Confidence Interval

Df- Degrees of freedom

F- Variation between samples

M- Mean

N- number

r - Pearson's correlation coefficient

Sig- Significance

S. zeamais - *Sitophilus zeamais*

SPSS- Statistical Package For social sciences

STD- Standard

t - Time

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests

FUNDING

No funding was received

AUTHOR'S CONTRIBUTIONS

Not Applicable

ACKNOWLEDGEMENTS

My profound gratitude goes to Almighty God, the God of all possibilities, for making the study and its processes successful.

I will love to thank my supervisor, **Mr. Yakop Dakum,** for sacrificing his time to supervise my work despite his busy schedule and for the encouragement, fatherly role played, and love shown to me during this study. I appreciate the Head of the Department of Biological Sciences, **Professor Adang Lucas,** and the entire staff of the department, whose encouragement made it possible to achieve the goal, I appreciate you all sincerely.

I also wish to express my profound gratitude to my parents, Mr. Timothy Olusanmi and Mrs. Oluwakemi Mary Olusanmi, and my siblings; Pamilerin Kehinde Olusanmi, Olumide Kingsley Olusanmi, and Favour Enitan Olusanmi, for their support and encouragement.

Also to my Uncle, Dr. Isaac Tolawase Atomode, I say a huge thanks to you, sir, and may the Lord continue to uplift you.

May the Almighty God reward you all.

References

1. Abdullahi, M., A.A. Baba, and J. M. Auta (2014) Comparative efficacy of the bark and root powders of *Acacia nilotica* against the maize weevil *Sitophilus zeamais* (Motsculsky) (Coleoptera: Curculionidae) in Kano State, Nigeria, *Journal of Entomological Research* 38(2):123–129
2. Abebe, F. (2006). Evaluation of botanicals and varietal resistance against maize weevil (*Sitophilus zeamais* Motsch) (Coleoptera: Crculionidae) in stored maize grain, M.Sc. Thesis, Haramaya University, Ethiopia
3. Akinkurolele, R.O., C.O. Adedire, and O.O. Odeyemi (2006) Laboratory evaluation of the toxic properties of forest anchomanes, Anhomanesdifformis, against pulse beetles,*Callosobruchus maculatus* (Coleoptera: Bruchidae), *Insect Science,* 13: 25–29
4. Akinwumi F.O., Fasakin E.A., and Adedire C.O. (2007) Toxic and repellence activities of four plant extracts against *D.maculatus* Degeer in Smoked African mudcat fish, Clariasgariepinus Burchell *Journal of Entomology,* 4:149–15
5. Awan, M. U. (2011) Toxicity of neem (Azadirachta indica) extracts against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions *Journal of Biological*

Control 25(3): 185–189

6. Bakele, A.J., Obeng-Ofori, D., and Hassanali, A. (1996). Evaluation of *Ocimum suave* (wild) as a source of repellants, toxicants, and protectants in storage against three stored product pests *International Journal of Pest Management,* 42(2):139–142
7. Becker, H. (1994). Neem oil locks out spores. *Agricultural Research*, 43: 20–22.
8. Casey Sclar D. (2006), Neem: Mode of Action of Compounds Present in Extracts and Formulations of *Azadirachta indica* Seeds and Their Efficacy to Pests of Ornamental Plants and to Non-target Species,
9. Dahiya, S. (2005) Alternatives to Chemical Pesticides Retrieved October 12, 2005, from http://wwwtribuneindia.com/1999/99 Jul 26/agrohtm # 2
10. Demissie G., Tefera T., and Tadesse A. (2008) Efficacy of Silicosec, filter cake, and wood ash against the maize weevil, Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), on three maize genotypes *Journal of Stored Products Research*, 44:227–31
11. Halstead, D.G.H. (1963): External sex differences in stored-product Coleoptera Bulletin of Entomological Research, 54(1), 119–134
12. Ileke and M. O. Oni (2011) "Toxicity of some plant powders to maize weevil, Sitophilus zeamais (motschulsky) [Coleoptera: Curculiondae] on stored wheat grains (Triticum aestivum),", *African Journal of Agricultural Research*, Vol. 6, Issue 13, pp.
13. Koona, P. and Njoya, J. (2004), Effectiveness of Soybean Oil and Powder from Leaves of *Lantana camara* Linn. (Verbenaceae) as Protectants of Stored Maize against Infestation by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), Asia Network for Scientific Information
14. Mamun, M. S. A., Shahjahan, M., and Ahmad, M. (2009). Laboratory evaluation of some indigenous plant extracts as toxicants against the red flour beetle, T*ribolium castaneumJournal of Bangladesh Agriculture University,* 7(1): 1–5.
15. Muhammad, S., Khan, M., & Saleem, M. (2005). Efficacy of neem oil against stored grain pests Pakistan *Journal of Biological Sciences*, 8(1), 115–118.
16. Mulungu, L.S., Lupenza, G., S.O.W., and R.W. (2007) Evaluation of botanical products as Stored grain protectants against maize weevil *S. zeamais Journal of Entomology, 4:258–262.*
17. Ntonifer, N.W., Farbanka, D.N., and Mbuh, J.V. (2011) Potency of *Chenopodium ambrioses* powder and its combination with wood ash on *S. zeamais* in stored maize *Journal of Entomology, 8:375–383.*
18. Ogunsina, O.O., M.O. Oladimeji, and L. Lajide (2011), Insecticidal action of hexane extracts of three plants against bean weevil, Callosobruchus maculatus (F.), and maize weevil, *Sitophilus zeamais* motsch *Journal of Ecology and the Natural Environment,* 3: 23–28
19. Okolo, E. T., and Iledun, O.C. (2019). Insecticidal effect of neem (*Azadirachta indica*) extracts obtained from leaves and seeds on pests of cowpea (Vigna unguiculata). Sumerianz *Journal of Agriculture and veterinary sciences* (4): 20–28

20. Oni, M.O., and K.D. Ileke (2008) Fumigant toxicity of four botanical plant oils on the survival, egg laying, and progeny development of the dried yam beetle, *Dinoderus porcellus* (Coleoptera: Bostrichidae) Ibadan. *Journal of Agricultural Research,* 4: 31–36.
21. Radha, R. (2014). Toxicity of three plant extracts against bean weevil, Callosobruchus maculatus (F.), and maize weevil, *Sitophilus zeamais* motsch *International Journal : Current Research*, 6: 6105–6109
22. Radwaski, S.A. (1981), Multiple Land Utilization in the Tropics: An Integrated Approach with a Proposal for an International Neem Tree Research and Development Program. In Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss), Schmutter, H., K.R.S. Ascher, and H. Rembold (Eds.), Agency for Technological Co-Operation, Berlin, Germany, pp. 267–278,
23. Saxena, R.C., Jilani G., and Kareem A.A. (2011), Effects of Neem on Stored Grain Insects, in Jacobson (ed.), Forum on Phytochemical Pesticides. Volume I. The neem tree Boca Raton, Florida: CRC Press.
24. Sharma, K., and Meshram, N. M. (2006) Bioactivity of essential oils from Acorus calamus against *Sitophilus oryzae* Advances in plant Biopesticides, 10, 25–32
25. Subbalakshmi Lokanadhan, P. Muthukrishnan, and S. Jeyaraman (2012), Neem Products and Their Agricultural Applications, *Journal of Biopesticides* 5(2):72-76
26. Taddese, A. (2003). Studies on some non-chemical insect pest management options for farm-stored maize in Ethiopia Ph.D. Thesis, University of Giessen, Germany
27. Tanzubil, P.B. (1991). Control of some insect pests of cowpea (*Vigna unguiculuta)* with neem (Azadirachta indica A. Juss.) in Northern Ghana *Tropical Pest Management*, 37: 216-217.