International Journal of Organic agricultural Research & Development
Volume 20 (2) (2025)

BIOACTIVE CHARACTERIZATION AND EFFICACY OF NEEM SEED LOTION AGAINST STORED PRODUCT INSECTS OF MAIZE (*ZEA MAYS* L.), RICE (*ORYZA SATIVA* L.) AND COWPEA (*VIGNA UNGUICULATA* L. WALP)

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Acknowledgement:
This research work was sponsored by Tertiary Education Trust Fund (TetFund), Abuja through the 2024 Institutional Based Research (IBR) Intervention project. The authors are very grateful to TetFund for the provision of the fund, equipment and tools for this research.

ABSTRACT

Insects pest of stored products are responsible for the significant losses of grain crops in storage and the best approach is the application of insecticides. Synthetic insecticides have immediate effects on the target insects; however, associated with adverse health implications. In view of this fact, there is a need for alternative to conventional chemicals. Thus, this study was conducted to assess the efficacy of the neem seed lotion against stored product insects; *Sitophilus zeamais, Sitophilus oryzae, Rhyzoper thadominica,* and *Tribolium castaneum*. Chemical compositions of neem seed were determined using HPLC analysis while evaluation of the bioactive chemical constituents from formulated neem seed lotions was done with six concentrations (100, 80, 60, 40 20 and 0% v/v) at Crop and Environmental Protection department, LAUTECH, Ogbomoso, Nigeria. and each treatment was arranged in complete randomized design with four replications. The results revealed different bioactive compounds and the major bioactive compounds from quantitative analysis is Azadirachtin A which is the real insecticidal active compound while qualitative analysis revealed Saponins, Glycosides, Steroids and Anthraquinones strongly present in the neem seed formulation. The tested neem seed lotion exhibited insecticidal action against the studied insects within 24 hours of exposure and response of the studied insects to neem seed lotions was the same and the efficacy of this bio-insecticide was not dose-dependent. Therefore, this neem seed lotion can be incorporated into the management program of stored product insects as an alternative to synthetic insecticides.

***Key words**: Neem seed lotion, Maize, Cowpea, Rice, Weevils, Azadirachtin A, Saponin, Glycosides, Steroids and Anthraquinones*

INTRODUCTION

There is no doubt that Maize, rice and cowpea are dominant crops among farmers in African countries especially in Southern part of this country. These crops have been the major sources of income to both peasant and commercial farmers (Sheahean and Barret, 2017). As a result of this, there is a need for every resource farmer to embark on the strategies of pest control in the store just to achieve a reasonable profit (Starthers*et al*., 2013; Sheahean and Barret, 2017).
One of the major obstacles in farming practice is the post-harvest loss largely due to stored products insects. Studies have shown that 15-30% loss experienced by the farmers in the store is due to insect pests attack aside from other devasting problems. Loss due to the storage, also reduces market price as well as nutritional contents of the harvested products. Ogunwolu and Idowu, (1994)
The major use of insecticides is in agriculture, but they are also used in home and garden settings, industrial buildings, for vector control, and control of insect parasites. Synthetic insecticides like organochlorides, organophosphate, carbamates and diamides have popularly been used in the past, but due to their detrimental effects on man, animals and the environment, there is a need to develop eco-friendly and less hazardous insecticides Ogunwolu and Idowu, (1994).
Pesticide resistance is one of the factors responsible for changing rapidly our approach to antibiotics and insecticides. Due to this change, plant-derived natural products are considered valuable candidates to reverse this negative trend

(Benelli, *et al.,* 2017). The European Commission defines biopesticides as "a form of pesticide based on micro-organisms or natural products" (European Commission DG ENV., 2008). The United States Environmental Protection Agency defines bio-pesticides as "certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals" United States (Environmental Protection Agency, 2023). Bio-pesticides are regarded by the US and European authorities as posing fewer risks of environmental and mammalian toxicity (Environmental Protection Agency, 2023). Bio-pesticides are more than 10 x (often 100 x) cheaper and 3 x faster to register than synthetic pesticides (Marrone, 2024). The bio-insecticide market is growing more that 10% yearly, which is a higher growth than the total insecticide market, mainly due to the increase in organic farming and integrated pest management and also due to benevolent government policies (Marrone, 2024). They are easier, faster and cheaper to register, usually with lower mammalian toxicity. They are more specific, and thus preserve beneficial insects and biodiversity in general. This makes them compatible with IPM regimes. They degrade rapidly cause less impact on the environment. They have a shorter withholding period. Neem seed-based bio-pesticides have been used in Nigeria to control pests and increase crop yields. Neem is a plant that contains bioactive compounds that can repel insects, inhibit their growth, and deter oviposition. Research has shown that neem application to crops in the field or under storage conditions affect insect pests in many ways. Among these it causes feeding deterrence, oviposition deterrence, moulting /metamorphosis inhibition and insect repellence (Ferdenache *et al*. 2019; Kraiss and Cullen, 2008). Antifeedant can also mean feeding deterrence. Antifeedant as chemicals in plants/products that inhibit feeding, although do not kill the insect directly (Takulder, 2006). Neem bioactive constituents, Azadirachtin and AZ-containing extracts show distinct antifeedant activity, primarily by chemoreception (primary antifeedancy), but also through reduction in food intake due to toxic effects after consumption (secondary antifeedant) in lethal quantities (Mulla and Su, 1999). The chemicals turn the product less palatable causing the insect to starve and die of hunger. The odour in neem that is almost similar to that of garlic/sulphur repels insects away. NRC (1992) attributed antifeedant of neem due to meliantriol and selanin constituents. Oviposition deterrence is another effect of neem on insect pests where female insects are disallowed from laying eggs on a given substrate. Insects' life cycle begins with the laying of eggs, however, when eggs were not laid, the metamorphosis process is affected. Another way neem can control insect pest is by metamorphosis inhibition. The changes that occur in the development of an insect from egg to adult are referred to as metamorphosis. The number of stages however differed among insects with 3 and 4 depending on the insect type. Neem can cause disruption in the development of eggs, larvae or pupae, blocking moulting of larvae or nymph (NRC, 1992). This action was by inhibiting the release of morphogenetic peptide, prothoracicotropic hormone (PTTH) and allatotropins from the brain-corpus cardiac complex (Ascher 1993: Mondal and Chakraborty, 2016). Jacobson (1987) reported life cycle of yellow mealworm, Tenebrior molitor (L.) being inhibited when the larva was treated with 0.1μg azadirachtin. The larvae maintained juvenile characteristics but failed to moult. Adults of Antestia bugs, Antestiopsis ortitalis Bechuacina observed metamorphosis defects when the 5th nymphal stage of the insect was treated with methanolic extract of neem kernel extract. Insect repellence is an effect of neem. Insects are known to study the environment around them with their antennae. They are tactile organs, acting as olfactory organs and a few cases as organs of hearing (Dike, 2014). Any change in the environment, the insect responded by going towards the stimulus if it is food source or run away if it is danger. Studies carried out by past researchers (Ahmed *et al*.,2009; Rahman and Talukder, 2006 and Muhammad *et al*., 2018) reported significant lower population of insect on neem treated plots compared with the controls. The mechanism of repellence could be attributed to repulsive neem odour. Insects Affected by Neem Products Research conducted by Sokame*et al*. (2015), Ekeh *et al*. (2013), Suleiman and Yusuf (2011), Oparaeke *et al*. (2005), Ogunwolu and Idowu (1997) had shown that neem and its products were identified to cause toxicity to many insects in addition to other pest organisms such as mites and nematodes. NRC (1992) and Neem Foundation (2014) reported that about 200 insect species, mites and nematodes are controlled using neem. These insect pests fall within various insect orders widely believed to be injurious to crops. Such orders include but not limited to, Coleoptera e.g red flour beetle (Herbst), Tribolium confusum (Coleoptera: Tenebrionidae); cowpea weevil, *Callosobruchus maculatus* Fab. (Coleoptera: Chryso milidae) and colorado potato beetle, Leptinotersa decemlineata Thomas (Coleoptera: Chryso milidae), and hosts of others. Therefore, this research was conducted to determine the efficacy of formulated neem seed lotion on common weevils of maize, rice and cowpea.

MATERIALS AND METHODS

Study site: This study was carried out at Crop and Environmental Protection Department Insectarium. Ladoke Akintola University of Technology Ogbomoso, Nigeria. The temperature of the insectarium ranges from 26^{0}C - 28^{0}C with relative humidity of 65%

Insect Culture

The studied weevils (*S. zeamais, S. oryzae, R. dominica* and *T. castaneum*) were obtained from highly infested maize, rice and cowpea grains at a local market in Ogbomoso, Oyo state and were used to raise new progenies and this was done following the procedures described by Gyabeng *et al*., (2024). Newly emerged unsexed adult of *S. zeamais, S. oryzae, R. dominica* and *T. castaneum* (100) were picked carefully with insect brush and introduced into a transparent plastic jar() covered at the top with muslin cloth.

Neem seed collection and production of neem lotion

Fully matured neem seeds were harvested from the neem tree. The seeds were de-pulp and later air-dried on the laboratory benches for 12 weeks under ambient room temperature, 27^{o}C – 32^{o}C. The properly dried seeds, 13 – 15% moisture content, were crushed using attrition mill into powder which was later soaked into water: ethanol (95%) at ratio of 50:50 for three days. Adjuvants were added to the neem lotion for storability and spray effectiveness and enhancement. The content was sieved using muslin cloth and stored in plastic container (50 L size) for two weeks before application.
HP LC analysis of Neem lotion: 200 ml neem lotion was taken to Central Research laboratory, Federal University of Technology, Akure, Ondo State, Nigeria for bioactive constituents' analysis.

Method of High-performance liquid chromatography (HPLC) analysis

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

Phyto-chemical analysis of neem seed: Fully matured neem seeds were harvested from the neem tree. The seeds were de-pulp and later air-dried on the laboratory benches for 12 weeks under ambient room temperature, 27^{o}C – 32^{o}C. The properly dried seeds, 13 – 15% moisture content, were crushed using attrition mill into powder which was later soaked into water: ethanol (95%) at ratio of 50:50 for three days. The content (neem lotion) was sieved using muslin cloth and stored in plastic container (50 L size) for phyto-chemical analysis. The Phyto-chemical screening involves identifying the bioactive compounds in neem seed at the Pure and Applied Chemistry Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Test for Alkaloids; Mayer's and Wagner's reagent test: To 2 mL of neem lotion in different tube. 2 mL of 2 % hydrochloric acid was added. Then 3drops of Mayer's and Wagner's reagent were added. Presence of a creamy white/yellow precipitate was observed for Mayer's and a brown/reddish precipitate for Wagner's signifies the presence of alkaloids.

Test for Tannins: Braymer's test: To 2 mL of neem lotion, 2 mL of water was added with 3 drops of (10%) ferric chloride. The formation of Blue-green color precipitate indicates the presence of tannins.

Test for Saponins: Frothing Test: To 2 mL of neem lotion, 2 mL of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1em layer of foam is observed which shows the presence of saponin.

Test for Flavonoids: Alkaline reagent test: To 2 mL of neem lotion, 2 ml of 2% sodium hydroxide was added and few drops of dilute hydrochloric acid. An intense yellow color, becomes colorless on addition of diluted acid which shows the presence of flavonoids.

Test for Quinones: Sulphuric acid test: To 2 mL of neem lotion, 2 mL of isopropy alcohol was added and 2 ml, concentrated sulphuric acid. A red color was formed and this indicates the presence of quinones.

Test for Glyeosides: Sulphuric acid test: To 2 mL of neem lotion, 3 mL of chloroform and 10 % ammonia solution was added. There was no colour changes which indicates the presence of glycosides.

Test for Cardiac Glycosides: To 0.5 mL of neem lotion, 2 mL of glacial acetic acid and few drops of 5 % ferric chloride were added, then was under layer with 1 mL of concentrated sulphuric acid. There information of brown ring at the interface which indicate that cardiac glycosides is present.

Test for Terpenoids: To 0.5 mL of neem lotion, 2 mL of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

Test for Phenols: Ferric chloride test: To 2mL of neem lotion, 2 mL of distilled water followed by few drops of 10% ferric chloride was added. Formation of Dark green/bluish black color indicates the presence of phenols.

Test for Coumarin: Sodium hydroxide test: To 2 mL of neem lotion, 2 mL of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarin.

Test for Steroids: Salkowski test: 2 mL of neem lotion was mixed with 2 mL of chloroform, followed by the addition of 10drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The emergence of a red-rose color indicates the presence of steroids.

Test for Anthocyanins: HCI test*:* To 2 mL of neem lotion, 2 mL of 2N HCI and 3 drops of ammonia solution was added Pink-red sol. which turns blue-violet after addition of ammonia indicates the presence of anthocyanin.

Test for Anthraquinones: Borntrager's test: To 2mL of neem lotion 2 mL drops of 10% ammonia solution was added then shake vigorously for 30 seconds. A pink, violet, or red colored solution indicates the absence of anthraquinone.

Bioassay

Each of the Unsexed adult insects (100) namely; *S. zeamais,S. oryzae, R.dominica*and*T. castaneum* was introduced to the designated small transparent jar0.78 m^3 0.78 m^3(4.5 cm X 5.5 cm) and arranged in a complete randomized design with four replications. Medicated was used to measure 20 ml of the following 100% neem lotion, 80% neem lotion + 20% distilled water, 60%neem lotion + 40% distilled water, 40% neem lotion + 60 distilled water, 20% neem lotion + 80% distilled water, and distilled water only, this concentration was equivalent to 100, 80, 60, 40, 20 and 0% respectively and this was sprayed on each of the studied insect separately. The number of mortalities was assessed after 24 hours of exposure.

RESULTS AND DISCUSSION

Use of secondary plant metabolites as an environmentally friendly approach alternative to synthetic pesticides has been widely advocated, in view of the fact that conventional chemicals constitute as one of the environment pollutants and inability of our local farmers to avoid high cost of these synthetic agrochemicals due to high exchange rate. Thus, natural toxicants had been tested against insect pests and found to be effective against the observed insects (Tembo et al., 2018; Okagu et al., 2023). However, stored product insects cause high economic damage to the stored products thereby reduce the profit margins of farmers. As a result of the above-facts, natural insecticide was developed from neem seed oil and tested against major insect pests of common crops in South Western part of Nigeria, namely; rice (*Tribolium castaneum and Sitophilus oryzae*), cowpea (*Callosobruchus maculatus*), and maize (*Rhyzoper thadominica* and *Sitophilus zeamais*) and these insects were used as index in this study. Both quantitative and qualitative of phytochemical of the Neem seed were determined and GC-MC was used in the analysis of the chemical constituents of the neem seeds.

The result of the quantitative analysis of the neem seed revealed 13 major chemical compounds; as stated in the table 1, four out of the isolated chemical compounds were strongly present (Saponins, Glycosides, Steroids and Anthraquinones) while four chemicals were moderately present (Alkaloids, Tannins, Quinones and Phenols) meanwhile, the rest secondary metabolites were slightly present. However, HPLC analysis revealed different chemical active compounds namely; β – caryophylene (31.88%), trans- β – farnes (9.16%), α – ionone (10.49%) phytol (0.97%), ascaridol (0.65%), phyllene (0.63%), quercetin (11.71%), azadirachtol (31.09%), azadirachnol (0.76%), Azadirachta A (0.94%), myricetin (0.925) and α – funebren (0.82%) (Fig 1). The HPLC Chromatogram of a mixture of *Azardiracta indica* seed and *Helianthus annuus*hydroethanolic extracts has been shown in figure 2. The identified compounds include β – caryophylene (27.36%), trans- β – farnes (11.00%), furostan (0.59%), α – ionone (9.69%), phytol (1.52%), ascaridol (0.65%), caffeic acid (0.75%), α – amyrin (2.21%), β – amyrin (1.89%), α – tochopherol (0.78%), quercetin (7.55%), azadirachtol (24.66%), Azadirachta A (1.14%), avanasterol (1.50%), myricetin (0.89%), α – funebren (1.68%), phytic acid (2.135), lecithin (0.90%) and cephalin (0.77%). Several studies have established insecticidal and nematicidal potentials of these chemicals such as quercetin, Azadirachta A, and phytol (Fig. 2)

S/N	Phytochemicals	Neem seed
1	Alkaloids	++
2	Tannins	++

3	Saponins	+++
4	Flavonoids	-
5	Quinones	++
6	Glycosides	+++
7	Cardiac Glycosides	+
8	Terpenoids	+
9	Phenols	++
10	Coumarin	+
11	Steroids	+++
12	Anthocyanins	+
13	Anthraquinones	+++

Key: +slightly present, ++ moderately present,+++ Highly present and – absent,

Fig.1. High-Performance Liquid Chromatogram of *Azardirachta indica*seed hydroethanolic extract

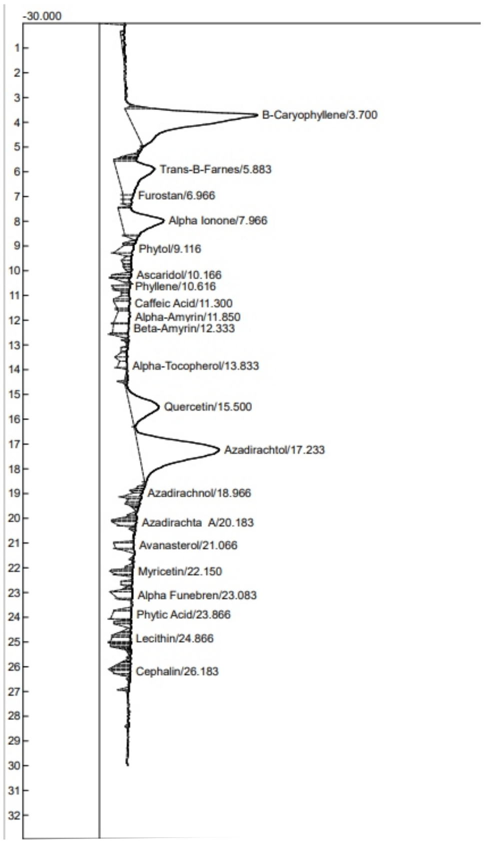

Fig.2. Superimposed High-Performance Liquid Chromatogram of *Azardiracta indica* seed and *Helianthus annuus*hydro ethanolic extracts

This study demonstrated insecticidal effects of the neem seed against the studied insects and 100% mortality of the insects was observed within 24 hours of exposure which implies that the four studied insects significantly responded the same to the formulated neem seed oil. However, no mortality was observed from the untreated insects throughout the experimental trials. The insecticidal potential of this biopesticide can be attributed to the observed quantitative chemicals such as Saponins, Glycosides, Steroids and Anthraquinones which are strongly present in addition to; Alkaloids, Tannins, Quinones and Phenols. This aligns with the previous report by Kishore et al., (2014) who had established insecticidal potential of Phenol isolated from plant extracts against arthropod larvae. Alkaloid, flavonoids, triterpenoids and phytol contributed to insecticidal potential of plants used as botanical insecticides (Lee et al.,2024; Akintan *et al.,*2020; Alao et al; 2025)while saponin from the class of steroidal or triterpenoidal has been proved to be insecticide and nematicide against various pests(Lee et al., 2000; Samuel et al., 2016). Anthraquinones and other bioactive compounds from different plant extracts had been reported as Mosquitocidal (Kambou *et al*., 2008; Samuel *et al.,* 2024).

Furthermore, bioactive compound from qualitative analysis is another contributing factor in the insecticidal action of this neem oil formulations. Studies have shown that, Azadiracthin A detected from HPLC analysis is a major natural chemical from neem seed which had been reported against wide range of insects(Sheahan *et al.,* 2017; Benuzzi and Ladurner, 2018). Other chemical insecticidal active components revealed by HPLC such as Phytol, Quercetin and β – caryophyllene (Hamat *et al.,* 2016; Akintan *et al.,*2020).

However, the earlier reports have shown that the observed chemical compounds from both quantitative and qualitative analyses can act as repellents, and antifeedants likewise these bioactive compounds can easily penetrate the cuticles of the studied insects, consequently interfere with DNA function of the insects(Senthil *et al*., 2021; Mohsin *et al*., 2023).

The data suggest that the effectiveness of this neem oil formulations did not depend on the dosage applied. For instance, the same number of mortalities were observed after 24 hours. This observation contradicts the earlier report that insecticidal efficacy of the tested biopesticide formulations is a function of the concentrations used (Aminu *et al*., 2022; Pariyasi, 2022). This suggests that the least concentration should be

used in the control of *S. zeamais, S. oryzae, R. dominica* and *T. castaneum.*
Table 3: Effect of neem lotion of the storage insect pest of maize, cowpea and rice

Table 3: Effect of neem lotion of the storage insect pest of maize, cowpea and rice

Name of insect	Concentration of Neem lotion	Number of insects exposed to neem lotion	Number of insects found dead after exposure
Maize weevil (*Sitophilus zeamais*)	100%	100	100
	80%	100	100
	60%	100	100
	40%	100	100
	20%	100	100
	10%	100	100
	0%	100	0
Rice weevil (*Sitophilus oryzae*)	100%	100	100
	80%	100	100
	60%	100	100
	40%	100	100
	20%	100	100
	10%	100	100
	0%	100	0
Lesser grain borer (*Rhyzoperthadominica*)	100%	100	100
	80%	100	100
	60%	100	100
	40%	100	100
	20%	100	100
	10%	100	100
	0%	100	0
Red flower beetle (*Tribolium castaneum*)	100%	100	100
	80%	100	100
	60%	100	100
	40%	100	100
	20%	100	100
	10%	100	100
	0%	100	0
Cowpea weevil (*Callosobruchus maculatus*)	100%	100	100
	80%	100	100
	60%	100	100
	40%	100	100
	20%	100	100
	10%	100	100
	0%	100	0

Conclusion and Recommendation

Based on this study, formulated neem seed lotion effectively controlled stored product insects of maize, cowpea, and rice. These are the major cultivated crops in western part of this country. Also, the quantity of dosage to be used is very minimal due to the fact that no significant difference was detected among the dosage tested which implies that the least concentration will not be expensive as higher dosage. This tested insecticide from neem seed is environmentally friendly when compare with the conventional chemicals. However, further study will be conducted on this formulation against termites, mosquito, spider, housefly and ants to confirm if this neem seed lotion is broad spectrum activity. Therefore, this neem seed lotion formulations can be used as perfect alternative to synthetic insecticides against stored product insects. In addition to this, at least 10% v/v of this formulated insecticide should be applied against studied insects.

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