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MORTALITY OF MAIZE PESTS (SITOPHILUS ZEAMAIZE) AND BEAN PESTS (COLOSOBROCUS MACULATUS) EXPOSED TO EXTRACTS OF NEEM (AZADIRACHTA INDICA) BARK, ROOT AND ONION BULB.

By

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Abstract

This study was carried out to evaluate Mortality of maize pests (sitophilus zeamaize) and bean pests (colosobrocus maculatus) exposed to extracts of neem (azadirachta indica) bark, root and onion bulb against. S. zeamais and C. maculatus were cultured and maintained at a temperature of 26-27°C and relative humidity of 65-70%. There were fourteen (14) treatments in the first experiment which was replicated three times and arranged in completely randomized design (CRD). Mortality of Pest, Number of holes/ punctures and Weight loss were assayed for four weeks. The results showed that at 4 weeks of treatment, there was a significant difference (P>0.005) between the neem onion bulb powder, neem leaf and bark treatments and the control. The highest mortality rate was recorded in neem bark powder (3.59%) followed by the neem root powder (2.71%). During 8 weeks of the experiment, neem bark powder recorded the highest mean mortality (10.8%) against C. maculatus and C. zeamais. Followed by neem root powder (8.33%) while the biopesticidal effects was recorded from onion bulb powder (3.22%); Similarly, at 12 weeks, neem bark powder had the highest mortality against the weevils with a value of 26.2% followed by the neem root powder (10.7%) while the control treatment recorded no mortality against the weevils. At 20 and 24 weeks of the experiment, there was a marked increase in mortality rates for both weevils used for the study. The 50% lethal dosage of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean were: Neem bark powder (100g) 1.94(1.49-2.00); Neem root powder (100g) 2.73(2.68-2.84); and Onion bulb powder (100g) 3.27(3.12-3.40) respectively. The study revealed that some of the plant materials (onion bulb) despite not giving very high mortality of Sitophilus zeamais and Colosobrocus maculatus, were effective in performance than the untreated control.

Keywords: *mortality, maize pests (Sitophilus zeamaize), bean pests (Colosobrocus maculatus) neem (Azadirachta indica) bark, root, and onion bulb.*

INTRODUCTION

In Nigeria, as in other African countries, grains are protected from insects by the use of chemicals which may be synthetic or natural Products such as oils, powders and extracts from plants and non-chemical methods such as mechanical, physical, biological and cultural methods (Stephen, & Samuel, 2023), are employed in grain preservation. The wide scale use of synthetic chemical insecticides such as organophosphates, pyrethroids, carbamates and others are justified by the report that they possess quick action and are thus effective in preventing or reducing damage by insects (Daramola, 2018). The superiority of chemical methods in their efficacy over non-chemical methods has led to over reliance and misuse of these chemicals. It has however been demonstrated that continuous reliance on pesticide technology has its limitations, such as pest resurgence, secondary pest out breaks (Ogbalu, et al., 2023), development of resistance and environmental hazards, including human poisoning and toxicity to other non-target organisms (Umeh, et al., 2022), mutagenicity and carcinogenicity in humans (Fasunwon, & Banjo, 2018). In addition, non-selective insecticides kill beneficial insects thereby causing an imbalance in the ecosystem The persistence of synthetic insecticides in the environment and in treated foods is a major constraint to their use (Nwachukwu et al., 2018) and for these reasons relatively few chemicals are registered for use on grains and in storage. There are already caution to the two widely used fumigants (methyl bromide and phosphine) because of its ozone depletion potential (Anene, 2018) which has led to the progressive restriction in the use of phosphine. The subsistence nature of agriculture in developing countries coupled with the high cost of synthetic insecticides, poor information and inconsistent supply of the synthetic pesticides has been given as the reasons for farmer's reluctance in using synthetic pesticides (Nwachukwu et al., 2022). These limitations and the concern of the public on the problems associated with the continuous use of conventional insecticides have necessitated the search for possible alternatives that may be effective against target pests or organisms, but safer to non-target organisms, man and his environment.

Over the decades, pesticides have been widely applied as a standard practice to control agricultural pests in the field (Anene, 2018). Their constant use has caused selection of resistances in agricultural pests, environmental pollution with negative side effects on human health and on non-target arthropods (Adedire, Obembeom, Akinkurolere & Oduleye, 2019). The demand for food increases with an ever-growing population. Thus, it is necessary to protect crops and stored grains from pests. The multivoltine pests, maize weevil (S. zeamias) and cowpea weevil (Callosobruchus maculatus Fab. (Coleoptera: Bruchidae), causes significant damage to stored pulses. It has been reported that C. maculatus alone can cause as much as 90% damage during the three to six months of storage (Agunloye, 2022). Due to their potencies to cause lethality in most life stages of a range of pests, synthetic pesticides are frequently used to protect both crop plants and stored grains (Agour, Mssillou, Mechchate, Es-Safi, Allali, Barnossi, Kamaly, Alshawwa, Moussaoui, & Bari, 2022). Synthetic pesticides can adversely affect non-target organisms, including humans, accumulate in the environment, pollute soil and ground water (Alamu et al., 2023). Some of the synthetic pesticides are also carcinogenic. The overuse of synthetic pesticides for insect control poses risks to wildlife and even humans. Toxic potency of synthetic pesticides and their potential effects have stirred interest from the public and regulatory agencies in alternative options for pest management.

Among the diverse environmental-friendly and safe strategies for pest management, biopesticides represent one of the best alternatives to chemicals (Nwachukwu et al., 2018). These compounds have various physiological and behavioral effects on insect pests (Batiha, Hussein, Algammal, George, Jeandet, Al-Snafi, Tiwari, Pagnossa, Lima, & Thorat, 2021). The insecticidal activity of botanicals is well known for their use for thousands of years worldwide in all the agricultural regions. Botanical insecticides are generally less harmful to the environment and their use avoids the development of insect resistance (Aktar, & Isman, 2022). Active substances in botanical insecticides degrade easily and rapidly through natural degradation processes (Ahmad, Beg, & Joshua, 2020).

The use of botanicals in the protection of grains against insect infestation has been an age-long practice among small-scale farmers in Africa (Nwachukwu et al., 2020). Botanical insecticides comprise only a very small portion of the total volume of insecticides used annually; nonetheless, they remain important in insect pest management because they are believed to provide the most effective control against insect pests that have become resistant to other insecticides (Agwu et al., 2022). Plant-derived insecticides are short-lived in the environment, thus pose less risk to non-target organisms and are accepted by organic certification programs and certain consumer groups because they are naturally occurring (Adedire et al., 2019). Botanical insecticides are believed to possess certain attributes which put them at a higher advantage over conventional insecticides. These include low mammalian toxicity although not always true, less persistence in the environment, selectivity towards target pests and nonphytotoxicity (Odewole et al., 2023). These have led to the belief that plant-derived insecticides are safer than synthetic products. However, the rapid degradation of phytochemical insecticides on exposure to light may lead to production of less toxic or nontoxic compounds rendering them unstable and less persistent (Olaniran, et al., 2021), hence necessitating repeated applications.

Materials and methods

Description of experimental site

This research work was carried out at the Biology laboratory, School of Biological Sciences, Federal University of Technology, Owerri. The Federal University of Technology Owerri lies between latitude 05° 21' and 05° 42'North and longitude 07° 48' and 06° 53'East. Owerri consists of tropical rainforest zone with average annual rainfall distribution of 2,250-2800mm. This region produces many agricultural products (Nwachukwu, et al., 2020).

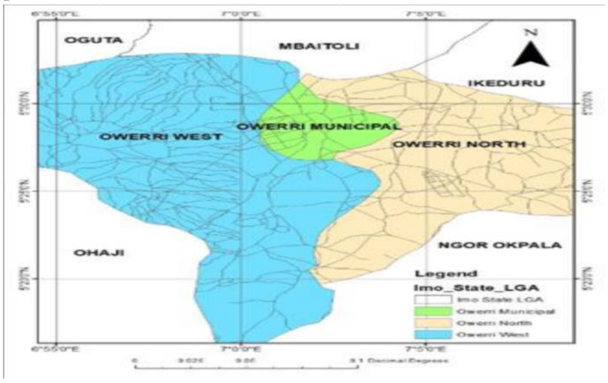

Figure 1: Map Owerri metropolis

Sources: Ibeh et al., 2021.

Collection of Maize and bean grain used for the Experiments

Clean and well sieved grains of maize (*Zea mays*) and bean seed (*V. unguiculata* Walp) was used for the experiments. The maize and bean grains was procured from Ekeonunwa market in Owerri. The seeds were frozen at -4°C for four days, to sterilize it. The essence of this is to kill any live insect(s) that may be present. The grains was sorted manually and only large grains was used for the study.

Collection and preparation of botanicals; Neem plant (Azadirachta indica) Bark, Root and Onion Bulb (Nwachukwu et al., 2018)

Fresh and matured Neem plant were used for the experiment (*Azadirachta indica* A. Juss). The Bark and Root of Neem Plant and Onion Bulb was air dried at room temperature until they are totally dried. The Bark and Root of Neem Plant and Onion Bulb was grinded using a blender and sieved through a 0.25 mm mesh to obtain uniform particle size which is similar with the procedures followed by Araya (2018) and Nnodim, et al (2003). The resulting powders was kept separately in containers, Labeled and stored at room temperature until when needed.

Fresh onion bulbs were purchased from the Ekeonunwa market. The cloves were peeled and surface sterilized using ethanol (99.9%). The onion bulbs were dried in the open shaded area and grinded. The resultant powder were labeled and stored until the time of spraying.

Rearing of S. zeamais and C. maculatus

S. zeamais and *C. maculatus* were cultured and maintained at a temperature of 26-27°C and relative humidity of 65-70%. Infested maize and bean grains were procured from the market and the adult *S. zeamais* and *C. maculatus* was allowed to oviposite in a container of clean and uninfested maize and bean grains. The parent adults were be removed after 20 days by sieving the grains with a 2.0mm sieve. The beetles that subsequently emerged was used for the bioassays (Nwachukwu et al., 2018).

Treatments and experimental design

There were fourteen (14) treatments in the first experiment which was replicated three times and arranged in completely randomized design (CRD). There were controls in the experiment (the untreated and the standard/synthetic check using permethrin) for comparison. Treatments were in powdered form (for the botanicals). Each treatment was measured and introduced (mixed with maize and bean grains) in appropriate ratios in each jar. The adult insects reared were introduced to each replicate.

Bioassay procedures in Experiment

Neem extracts (bark and root) of the following concentrations 100g, 200g, 300g and control (0g) which was weighed and each added to healthy uninfested maize and bean seeds in one-litre plastic jars. After introduction of the predetermined adult insects into each experimental jar; the following data was collected; the onion bulb bioinsecticides was applied using a hand sprayer at the rate of 100g, 200g, 300g and 0g respectively.

Parameters measured

Mortality of Pest

The number of dead weevil in each treatment was regularly checked per day to ascertain the mortality following the method of Julián et al., (2023) The number of dead weevil was counted and recorded using the formula:

Percentage mortality = Number of dead insects x 100
Total number of insects 1

Number of holes/ punctures

In accordance with the method of Nwachukwu et al., (2020), the grains was thoroughly examined to check for exit holes or puncture on each of the beans in each treatment replicates using a portable hand lens and holes observed was counted.

Weight loss

The grains was examined to determine if there is any weight lost due to infestation by the weevils. This was done by weighing the whole grain in each replicate using a weighing balance as described by Ileke & Oni (2011), thus

(%) loss in Weight = (Initial weight-Final weight) × 100
Initial weight 1

Weevil perforation index (WPI) according to the method of Parugrug & Roxas, (2018)

The weevil perforation index was calculated using the method of Gever & Echezona (2023):

Total number of treated grains perforated X 100
Total number of infected grains perforated 1

From the formula above, a weevil perforation index that is greater than 50% indicates the enhancement of infestation by the weevil or a negative ability of the plant material or insecticide(s) tested; while a weevil perforation index that is less than 50% implies a positive protection of the seeds by the protectant (plant material).

Phytochemical analysis of Neem (Azadirachta Indica) Bark, Root and Onion Bulb

Phytochemical analysis of Neem (Azadirachta Indica) Bark, Root and Onion Bulb powder for alkaloids, tannins, flavonoids, saponins, terpenoids and cardiac glycosides was carried out according to the procedures of Jwuh et al., (2020); Nwachukwu et al. (2018).

Alkaloid

A few ml of leaf powder was prepared; two drops of Mayer's reagent was added along the side of the test tubes. 1.0ml portion was treated similarly with Dragenduff's reagent. Appearance of white creamy precipitate indicates the presence of alkaloids (Iwuh et al., 2019).

Tannins

0.5g of powered sample of the leaf was boiled in 20ml of distilled water in a test tube and filtered 0.1% $FeCl3$ was added to the filtered sample and observed for brownish green or a blue- black colouration which shows the presence of tannins (Iwuh et al., 2019).

Flavonoids

Exactly 20mg of neem leaf powder was dissolved in 1ml of distilled water. Precisely 0.5ml of dilute ammonia solution was added to it and concentrated sulphuric acid was added

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later. A yellow colour indicates the presence of flavonoids. The yellow colour disappeared on allowing the solution to stand following the method of Abbasi et al., (2020).

Saponins

In line with the method of Mansour et al, (2021), closely 2g each of powered sample leaf was boiled together with 20ml of distilled water in a water bath and filtered 10ml of the filtered sample was mixed with 5ml of distilled water in test tubes and shaken vigorously to obtain a stable persistent forth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicated the presence of saponins.

Terpenoids

Precisely 20mg of leaf powder was dissolved in 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added to it. A reddish brown discolouration at the interface shows the presence of terpenoids following the method of Abbasi et al., (2020).

Cardiac Glycosides

Exactly 20ml of the leaf powder was dissolved in Iml of glacial acetic acid and 1-2 drops of ferric chloride solution will be added. Closely 0.5ml of concentrated sulphuric acid will be slowly added along the side of the test tube. A brown ring at the interface indicated a deoxy-sugar characteristic of cardenolides, or cardiac glycoside constituent following the method of Abbasi et al., (2020).

Phlobatannins

This was carried out using the method of Parugrug & Roxas (2018). An aqueous extract of the leaf samples of neem was boiled with 1% aqueous hydrochloric and deposition of a red precipitate was taken as evidence for the presence of phlobatannin.

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) and significant mean differences were separated by Duncan Multiple Range Test (DMRT) using SAS (2010) package.

Results and discussion

Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean seeds

Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean seeds within the experimental periods is shown in Figures 4.1 to 4.6. The results showed that at 4 weeks of treatment, there was a significant difference (P>0.005) between the neem onion bulb powder, neem leaf and bark treatments and the control. The highest mortality rate was recorded in neem bark powder (3.59%) followed by the neem root powder (2.71%). It was observed that no mortality was recorded for onion bulb powder at 4 weeks and control respectively (Figure 4.1).

During 8 weeks of the experiment, neem bark powder recorded the highest mean mortality (10.8%) against *C. maculatus* and *C. zeamais*. Followed by neem root powder (8.33%) while the biopesticidal effects was recorded from onion bulb powder (3.22%); no insect death was observed from the control at 8 weeks (Figure 4.2).

Similarly, at 12 weeks, neem bark powder had the highest mortality against the weevils with a value of 26.2% followed by the neem root powder (10.7%) while the control treatment recorded no mortality against the weevils (Figure 4.3). at 16 weeks, a high mortality rate was recorded in neem bark powder (39.8%) followed by neem root powder (33.8%) while onion bulb ranked the least with a value of 30.6% (Figure 4.4).

At 20 weeks of the experiment, there was a marked increase in mortality rates for both weevils used for the study. The highest mortality rate was observed from neem bark powder (66.54%) followed by neem root powder (48.4%) and onion bulb (32.2%)

At 24 weeks of the experiment, there was an increase in mortality rates for both weevils used for *C. maculatus* and *C. zeamais*. The highest mortality rate was observed from neem bark powder (88.14%) followed by neem root powder (78.4%) and onion bulb (59.4%). Figure 4.7 reveals that Seed damage and insect perforation index on maize and bean seeds was highest in control (50.71% and 48.3%) while the least perforation index was observed from neem bark powder (10.6% and 9.6%).

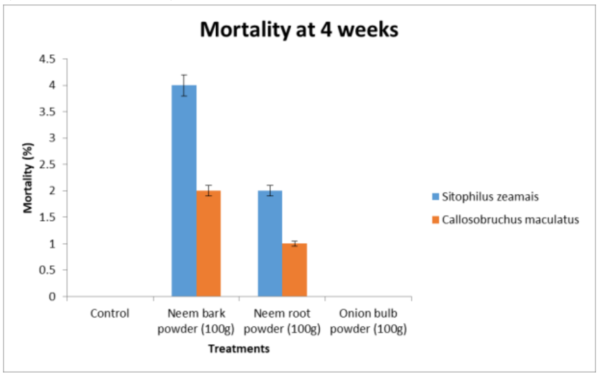

Figure 4.1: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 4 weeks

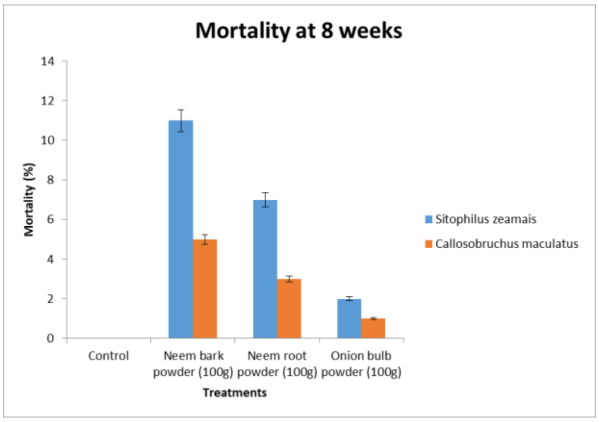

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Figure 4.2: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 8 weeks

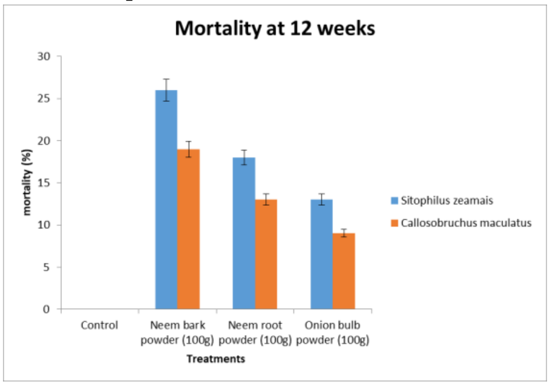

Figure 4.3: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 12 weeks

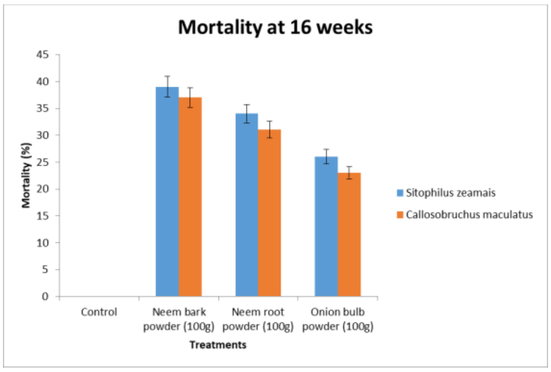

Figure 4.4: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 16 week

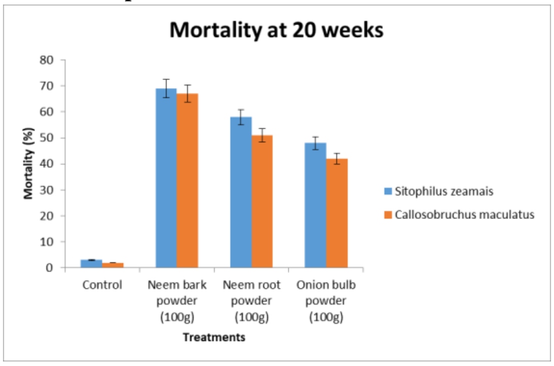

Figure 4.5: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 20 weeks

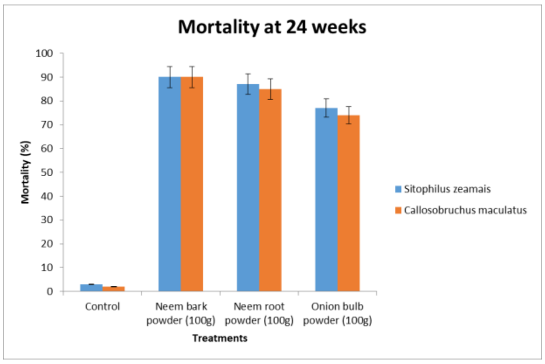

Figure 4.6: Comparative effect of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean at 24 weeks

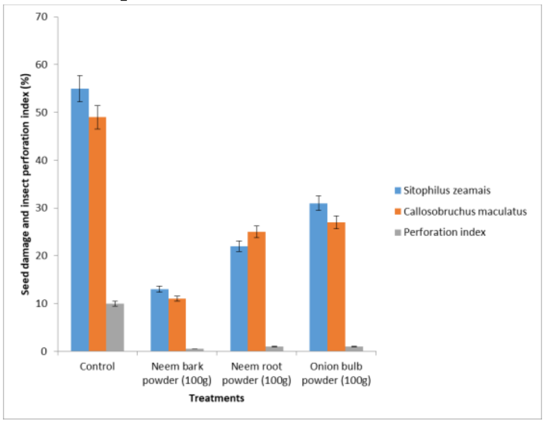

Figure 4.7: Seed damage and insect perforation index on maize and bean seeds

4.1.5 Lethal Dosage (LD_{50}) of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean.

The lethal dosage (LD_{50}) of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean.shown in Table 4.5. The 50% lethal dosage of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean were: Neem bark powder (100g) 1.94(1.49-2.00); Neem root powder (100g) 2.73(2.68-2.84); and Onion bulb powder (100g) 3.27(3.12-3.40) respectively.

Table 4.5: Lethal Dosage (LD_{50}) of neem leaf powder, neem bark powder and onion bulb powder on mortality of insect species of maize and bean.

Treatments	**LD_{50} at 95% Confidence interval (Lower limit – Upper limit)**
Control (0g)	0.0 (0.00-0.00)
Neem bark powder (100g)	1.94(1.49-2.00)
Neem root powder (100g)	2.73(2.68-2.84)
Onion bulb	3.27(3.12-3.40)

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powder (100g)	

Discussion

The result of the study on effect neem (Azadirachta indica) bark, root and onion bulb plant powders on the mortality of *Sitophilus zeamais* and *Colosobrocus maculatus* on stored maize and bean grains showed that were significant differences (P>0.05) in insect mortality on the plant powders evaluated. Mortality increased with increase in study period (DAT).The study revealed that some of the plant materials (onion bulb) despite not giving very high mortality of *Sitophilus zeamais* and *Colosobrocus maculatus*, were effective in performance than the untreated control. This finding agrees with the report of (Owusu-Akyaw, 1991) who reported that some local plants and plant parts do not only exhibit insecticidal properties, but also antifeedant properties that inhibit the activities of *Sitophilus zeamais* and *Colosobrocus maculatus*. According to some early research work done on similar botanical plant materials, it was reported that varying degrees of successes were recorded on the insecticidal, repellent and antifeedant properties of the botanicals on *S. zeamais* (Obeng-Ofori et al, 2020; Udo 2021; Arannilewa et al, 2019 and Assawalam et al, 2022).

However, neem bark gave the highest performance at the concentration level of 100g as it caused the second highest mortality of the beetles next to the root powders recorded total mortality of the test insect after 24 DAT. It performed better when compared to onion bulb. This finding supports earlier report by (Lale, 2002) that *A. indica* possesses contact stomach and respiratory poisoning properties attributed to the active constituent nicotine. Similar effects of plant materials as crop seed protectants have been observed in the treatment of cowpea and maize grains against maize beetles (Asawalam et al, 2007) It has been observed and reported that the insecticidal property of any plant material, would depend on the active constituents of the plant material (Ofuya & Dawodu, 2020).

However, the results obtained from the study, disagreed with (Danjumma et al, 2009) who reported that 100 g of *N. tabacum* applied in 50g of maize grains resulted in 100% mortality of S. zeamais, as findings from this study revealed that the best performance of *N. tobacum* of 6.0 g/150 kg of maize seeds indicated 43.3% adult mortality at 24 DAT.

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