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INSECTICIDAL ACTIVITY OF AZADIRACHTA INDICA AGAINST CALLOSOBRUCHUS MACULATUS ON STORED BEANS IN NASARAWA STATE, NIGERIA

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Abstract

Callosobruchus maculatus is a major storage pest of beans causing significant losses in quantity and nutritional quality. The use of synthetic insecticides is associated with health and environmental risks, hence the need for botanical alternatives. This study evaluated the insecticidal efficacy of neem Azadirachta indica leaf, seed and bark extracts against C. maculatus and determined their effects on the proximate and mineral composition of beans. Crude ethanolic extracts of neem leaf, seed and bark were prepared and tested at 10, 20, 30, 40 and 50g/ml. Twenty adults of C. maculatus were introduced to 400g of pests-free beans. Mortality was recorded at five weeks after treatment. Proximate and mineral compositions of uninfested and infested beans and phytochemical composition of neem parts were determined using standard AOAC methods. Mortality increased with increasing concentration for all extracts. Leaf and seed extracts were equally potent, both recording 100% mortality at 40 g/ml. Bark extract recorded 78.0% mortality at 40 g/ml and 100% at 50g/ml. Infestation significantly reduced the nutritional quality of beans. Protein and carbohydrate decreased from 10.50% to 8.13% and 58.80% to 51.70% respectively. Potassium, calcium, iron, copper and zinc decreased from 619.0, 128.3, 49.0, 0.90 and 7.08 mg/100g to 430.0, 112.2, 29.0, 0.54 and 4.05 mg/100g respectively. Ash, crude fibre and moisture contents increased after infestation.

Keywords: *Callosobruchus maculatus*, Beans, *Azadirachta indica*, mortality, proximate composition, mineral composition, phytochemicals, botanical insecticide

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Phytochemical analysis revealed that neem leaves contained 11.35% alkaloids, 13.13% triterpenoids and 3.80% flavonoids; seeds had 10.50% alkaloids and 26.50% carbohydrate; bark had 10.82% tannins and 9.03% glycosides.

Introduction

Stored grains are heavily damaged by insect pests. These pests cause damage to stored grains resulting in both qualitative and quantitative losses (Taddese *et al.*, 2024). The main reason behind the occurrence of stored grain pests is the presence of favorable climates for their growth and survival. At various processing stages of grains, i.e., during the process of development and maturation of seeds, processing in threshing yards, during transmission of seeds, or storage large number of insect pests gain access to stored grains. Some pests start damaging the seeds at the ripening stage and continue during storage. Old bags, storage structures, old containers are the major source of infestation (Singh, *et al*., 2018).

The dispersal and distribution of stored grain pests are caused by the movement of grains from one area to another either by a passive or active flight of pests as some a dult insects possess strong flight. Almost one thousand species are stored grain pests of different stored products all around the world. The majority of stored grain pests belong to two orders, i.e., Coleoptera and Lepidoptera *(*Ahmad *et al*., 2021).

Stored grain pests possess a serious threat to dried, stored, durable and, perishable agricultural products and non-food derivatives of agricultural products worldwide. Stored grain pests cause serious post-harvest losses, almost 9% in developed countries to almost 20% or more in developing countries (Pei *et al*., 2018), besides, they also cause contamination of food products by the presence of various live insects, insect products like chemical excretions or silk, dead insects or some other storage structures. Almost 8–10%, that is, 13 million tons of grains lost due to insects and 100 million tons due to failure to store properly is estimated in stored food products all around the world. Pests such as various insects, pathogens, mites possess serious threats and cause severe damage to grains by producing certain enterotoxins and mycotoxins (Islam *et al*., 2024)

Study Area

The study was carried out in Nasarawa State Nigeria located in North Central Region. It lies between latitude 7°. 45' and 9°. 25' North of the equator and between longitude 7° and 9° 37' East of the Greenwich Meridian. Nasarawa state has a total land area of 27,137.8sqkm and is bounded to the east by the States of Taraba and Plateau, to the north by Kaduna State, to the south by the states of Kogi and Benue and to the West by the Federal Capital territory (Nasarawa Geographic Information Service, 2026).

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Sample Collection

Samples of infested beans grains sold in Masaka market, Karu Local Government Area of Nasarawa state were procured randomly from selected traders. Ten kilograms of beans grain were obtained within the sampling period in labeled polythene bag, and were taken to zoology laboratory Department, Nasarawa State University, Keffi. .Grain samples were sieved so as to recover all emerged insect pests, thereafter, species of *Callosobruchus maculatus* were carefully harvested for the purpose of incubation in order to obtain a pure breed for later reinfestation. Beans grain for the incubation was obtained from the stock samples procured. 400g of beans were introduced separately in 75cl capacity kilner jar to which 10 pairs of adult *Callosobruchus maculatus* were introduced for oviposition for one week, and then removed to allow the eggs hatch. The samples were observed daily until emergence of F1 progenies. The newly emerged insects at maturity were later reintroduced into the pest-free beans, and were divided into fifteen portions of 500g, and the neem extracts, the leaves, seeds and the bark pellets were applied at 10g/ml, 20g/ml, 30g/ml, 40g/ml and 50g/ml respectively. The experimental conditions were at 60 ± 10% RH, 26 ± 2 °C, and 12:12 L: D.

Plant Collection

This plant depends on the ethnobotanical knowledge. Neem tree with low seeds bearing branches were drawn, the seeds released into the sheets, fresh leaves were cut in a vertical slit from top to bottom, and bark were cut deep into the wood trunk using knife/cutlass and was taken for analysis as described by (Santos *et al*., 2018).

Phytochemical screening of neem Etracts using Thin Layer Chromatography (TLC).

The three parts of neem Extracts (leaves, seeds and bark) was screened for Alkaloids, Phenols, Flavonoids, Carbohydrates, Tannin, Terpenoid, Triterpenoid, Glycosides, Resins and Phlobatannin. This was done by dissolving the organic and aqueous extracts in chloroform and methanol (1:1) using capillary tubes, spots of the dissolved extracts will be made on the base line of the aluminum TLC plates. The plates will be put in a chamber containing appropriate chromatographic solvent system (mobile phases) specific for the determination of the presence of a given class of phytochemical constituents (Santos *et al*., 2018).

Pelletization of the neem extracts

The plant extracts powder were sieved at 0.0007mm, and weight 19g at 2:1 binder for seeds, 27g at 1:1 binder for leaves and 20g at 1.05g binder for bark, and was thoroughly mixed with excipient and binders to create a uniform blend, the blended mixture is then granulated using a wet granulation process. The extrusion of the granulation were forced

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through an extrusion screen to form cylindrical extrudates, which are further broken up and rounded into pellets using a spheronizer, and then dried using a fluid bed dryer.

Proximate Sample preparation

Sample preparation was conducted based on the procedure reported by Akhila and Rani (1999).

Determination of mineral compositions

The wet oxidation method was employed in the digestion of the samples (Saidu *et al.*, 2013).

During analysis, 2g of each sample and 100 mL concentrated nitric acid (HNO_3) were placed in a 400 mL beaker, swirled, and allowed to react for 10 min, then evaporated on a hot plate and allowed to cool. Then, 50 mL conc. HNO_3 and 10 mL of per-chloric acid ($HCLO_4$) was added and evaporation continued until the sample was completely ash (grey in color). The digest was then transferred to a 100 mL volumetric flask and diluted to the mark with deionized water. Buck Scientific Model Atomic Absorption Spectrophotometer (AAS, Model 210VGP) was used for the analysis of Fe, Cu, Zn, Ca, and K.

Quantitative and Qualitative phytochemical Analysis of Neem etracts

The phytochemical screening of *Azadirachta indica*_ revealed the presence of several bioactive compounds in both leaf and bark that are responsible for insecticidal activity.Neem leaves contained 11.35% alkaloids, 22.90% carbohydrate, 22.90% flavonoids, 3.62% phenols 3.12% terpenoids, and 13.13% triterpenoids. The high percentage of alkaloids and triterpenoids, especially azadirachtin which is a triterpenoid, explains the strong antifeedant and growth regulatory effects observed against *Sitophilus. zeamais* and *Callosobruchus. maculatus*. Phenols and flavonoids also contribute antioxidant and repellent properties. This agrees with Akhila and Rani (1999) who reported alkaloids, flavonoids and terpenoids as major bioactive constituents of neem leaf. Neem bark had 3.80% flavonoids 10.82% tannins, and 9.03%. glycosides. The relatively high tannin content suggests strong astringent and antinutritional properties that can interfere with insect digestion and enzyme activity. Glycosides are also known to be toxic to storage pests. Similar results were reported by (Maina *et al.*, 2021) who found tannins and glycosides in high concentration in neem bark.5.5580 Phlobatannins, and 26.50% carbohydrate were also detected in the leaf. Phlobatannins have been associated with antimicrobial activity which can reduce secondary fungal infection in stored grains.

Table 1; Quantittative and Qualitative phytochemical Analysis of neem extracts

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Parameters	Seeds	Qltive	Qntive	Leaves	Qltive	Qntive	Bark	Qltive	Qntive
Alkaloid	+	10.50%		+	11.35%		-		
Carbohydrate	+	26.50%		+	22.9%		-		
Flavonoid	-			+	3.80%		+	3.80%	
Phenol	+	3.62%		+	3.62%		-		
Tannin	-			-			+	10.82%	
Terpenoid	-			+	3.12%		-		
Triterpenoid	-			+	13.13%		-		
Resins	-			-			-		
Phlobatannin	+	5.5580ppm		-			-		
Glycosides	-			-			+	9.03%	

Mean mortality rate of Callosobruchus maculatus exposed to neem leaves extract for five weeks.

Table 2 shows that *Callosobruchu maculatus* displayed high mortality at high doses of neem leaves extract, at 50g/ml and 40g/ml concentrations mortality rate was at hundred percent from week one to five, greater than 30g/ml to 20g/ml, and 10g/ml with zero mortality. In a comparable manner, mortality rate increased from week one to five with decreasing survival rate of the weevils. This findings aligned with, (Gever *et al*., 2023) who recorded high mortality rate of *Callosobruchus maculatus* on neem powder. In a related study, (Souza *et al.,* 2020) reported that mortality of *Callosobruchus maculatus* was higher in bioactive compounds than the synthetic insecticides.

Table 2: Mean Mortality rate of *Callosobruchus maculatus* exposed to neem leaves extract for five weeks

Concentration (g/ml)	No of weevils	Mortality wk 1	wk 2	wk 3	wk 4	wk 5	Total	% Mortality
10	20	20	20	20	20	20	100	25.0
20	20	20	19	18	15	13	85	39.0
30	20	14	11	7	3	00	35	95.0
40	20	00	00	00	00	00	00.0	100
50	20	00	00	00	00	00	00.0	100
Total	100	54(24.5)	50(22.7)	45(20.5)	38(17.3)	33(15.0)		220

Table 3 shows meen mortality of *Callosobruchus maculatus* exposed to neem seeds extract for five weeks. It was observed that *Callosobruchus maculatus* displayed knockdown at much higher concentration of neem seeds extract, at 50g/ml, mortality was significantly greater than 40g/ml, and the trend continued to 10g/ml concentration.

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This is consistent with (Gever *et al.*, 2023; Agbo *et al.*, 2019) who reported a similar knockdown rate of *Callosobruchus maculatus* against neem seeds to be less than that of leaves. These findings highlight the variation in *Callosobruchus maculatus* mortality rate among these neem extracts. Again, *Callosobruchus maculatus* families avoid ovipositing on seeds treated with leaf extract more strongly than with untreated seed extract which lead to fewer eggs laid and higher adult mortality.

Table 3: Mean Mortality rate of *Callosobruchus maculatus* exposed to neem seeds extract for five weeks

Concentration (g/ml)	No. of weevils	Mortality wk 1	wk 2	wk 3	wk 4	wk 5	Total	% Mortality
10	20	20	20	20	20	20	100	20.0
20	20	20	20	20	19	17	96	36.0
30	20	11	9	8	6	3	37	90.0
40	20	9	4	00	00	00	13	100
50	20	00	00	00	00	00	00	100
Total	100	60(24.4)	53(21.5)	48(19.5)	45(18.3)	40(16.3)	246	

The neem bark extract also recorded mortality of *Callosobruchus maculatusat* at the highest concentration compared to leaves and seeds extracts. This can associated with the low azadirachtin composition found in the neem bark extract which are contact toxins that are antifeedants and growth disruptor in neem bark. This study agreed with wide findings of (Quintero *et al.*, 2025; Manneh *et al.*, 2025) who also found out that leaves and seeds extracts are more protectant than bark extract.

Table 4: Mean Mortality rate of *Callosobruchus maculatus* exposed to neem Bark extract for five weeks

Concentration (g/ml)	No. of weevils	Mortality wk 1	wk 2	wk 3	wk 4	wk 5	Total	% Mortality
10	20	20	20	20	20	20	100	0.00
20	20	20	20	20	20	20	100	31.0
30	20	15	12	10	9	8	54	45.0
40	20	10	7	5	2	00	24	78.0
50	20	00	00	00	00	00	00.0	100
Total	100	65(23.4)	59(21.2)	55(19.8)	51(18.3)	48(17.3)	278	

Proximate composition (%) recorded in Beans before and after infetatio

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Ash content increased from 2.00 ± 0.23% to 2.80 ± 0.11% after infestation. The rise is likely due to the accumulation of insect excreta, exuviae, and body fragments in the damaged seeds, which contribute to higher mineral residue on ashing. Similar increases were reported by(Yankah *et al.,* 2020) in beans infested by bruchids.. Crude protein decreased from 10.50 ± 0.16% to 8.13± 0.72%. This reduction can be attributed to the feeding activities of larvae and adults which utilize seed proteins for growth and development. The finding agrees with Rajkumar and Selvalasingam (2021).who recorded significant protein loss in *C. maculatus*- infested beans.. Crude fibre also increased from 2.20 ± 0.31% to 2.90 ± 0.31%. This may be due to damage to the cotyledon and exposure of more fibrous seed coat materials during feeding. A similar trend was observed by (Abolaji *et al* 2020) in infested pulses.. Moisture content rose from 9.20 ± 0.11% to 10.10 ± 0.09%. The increase is attributed to metabolic water released during insect respiration and feeding. This corroborates the report of Akhila and Rani (1999) who noted higher moisture in bruchid-damaged cowpea, which further predisposes grains to fungal attack.Carbohydrate content showed a marked decrease from 58.80 ± 0.06% to 51.70 ± 0.13\%. Carbohydrates serve as the primary energy source for *C. maculatus* during development. The observed depletion is consistent with Hailu Kasseg, (2018), who reported significant carbohydrate loss in beans after bruchid infestation.

Table 5; Proximate composition (%) recorded in Beans before and after infestation

Beans	Ash Content	Crude Protein	Crude Fibre	Moisture	Carbohydrate
Before infestation	2.0±0.23	10.5±0.16	2.2±0.31	9.2±0.11	58.8±0.06
After infestation	2.8±0.11	8.13±0.72	2.9±0.31	10.1±0.09	51.7±0.13

Mineral composition (mg/100g) recorded in Beans before and after infestation

Potassium had the highest decline, from 619.00 ± 0.09 and reduced to 430.00 ± 0.03. Potassium is abundant in the cotyledon which is the main tissue consumed by bruchid larvae. The extensive feeding and tunnelling by *Callosobruchus. maculatus* leads to direct removal of potassium-rich tissues. This is in agreement with Guerrien and Caralleto (2018) who reported similar K losses in bruchid-infested beans . Calcium decreased from 128.30 ± 0.04 and reduced to 112.20 ± 0.01. Calcium is important for seed coat integrity and enzyme activation. The reduction may be due to utilization by the insect for cuticle formation and to the physical removal of damaged seed parts. Similar Calcium depletion was observed by Rosentraler and Evers. (2019) in *Callosobruchus. maculatus*_damaged legumes. Iron content dropped from 47.00 ± 0.01 to 29.00 ± 0.06}. Iron is crucial for

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haemoglobin synthesis in insects. The significant loss indicates that developing larvae sequester Iron from the seed for growth. This corroborates the findings of (Singh *et al.*, 2018) who recorded lower Fe in infested pulses. Copper declined from 0.90 ± 0.03 to 0.54 ± 0.08 and Zinc from 7.08 ± 0.09 to 4.05 ± 0.02. Both Cu and Zn are trace elements required for insect enzyme systems. Their reduction suggests bioaccumulation by the pest and loss through damaged seed tissues. This trend supports the report of (Adeyemi *et al.*, 2022) who noted depletion of micro minerals in stored beans due to bruchid attack.

Table 6; Mineral composition (mg/100g) recorded in Beans before and after infestation

Maize	Potassium	Calcium	Iron	Copper	Zinc
Before infestation	619.0±0.09	128.3±0.04	47.0±0.01	0.9±0.003	7.08±0.009
After infestation	430.0±0.03	112.2±0.01	29.0±0.06	0.54±0.08	4.05±0.02

CONCLUSION

Neem leaf and seed extracts exhibited strong insecticidal activity against *C. maculatus*, with 100% mortality achieved at 40g/ml. Infestation caused significant losses in protein, carbohydrate and essential minerals of beans. Application of neem leaf extract at 40 g/ml is recommended for the protection of stored beans to preserve yield and nutritional quality. The presence of alkaloids, terpenoids, triterpenoids, flavonoids, phenols and tannins in neem leaf, seed and bark supports their traditional use as grain protectants. *Callosobruchus. maculatus* infestation led to a decline in the nutritional quality of beans, with significant losses in protein and carbohydrate, and increases in ash, fibre, and moisture. This deterioration reduces the food and market value of the grain, and highlights the importance of protective treatments such as neem-based botanicals.

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