

Efficacy of *Azadirachta indica* A. Juss Leaf Powder at Varying Concentrations Against *Callosobruchus maculatus* (Fab.) Infestation in Two Varieties of Stored Cowpea (*Vigna unguiculata* L. Walp.) in Makurdi, Nigeria

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Research Article

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Abstract

Post-harvest losses of cowpea (*Vigna unguiculata* L. Walp.) due to infestation by the cowpea weevil *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) remain a critical constraint to smallholder food security across sub-Saharan Africa. This study evaluated the efficacy of *Azadirachta indica* A. Juss leaf powder applied at three concentration levels (0%, 10%, and 20% of grain weight) against *C. maculatus* infestation in two varieties of cowpea (white and brown) stored over four weeks in Makurdi, Benue State, Nigeria. A 2×3 factorial experiment in a Completely Randomised Design (CRD) with three replicates was used, giving 18 experimental units. Parameters assessed included seed weight (g), number of seeds with holes, insect mortality, and seed germination (out of 10 seeds). Results showed that 20% *A. indica* leaf powder significantly ($P < 0.05$) preserved seed weight, reduced the number of seeds with holes, increased insect mortality, and maintained higher germination counts compared to the 10% treatment and the untreated control across both varieties. The white variety recorded comparatively lower weight loss than the brown variety. Phytochemical screening of the leaf powder confirmed the presence of nine bioactive compounds including alkaloids, tannins, saponins, terpenoids, flavonoids, anthraquinones, cardiac glycosides, phenolic compounds, and steroids, which collectively account for the observed antifeedant, repellent, and insecticidal activity. These findings support the adoption of *A. indica* leaf powder at 20% concentration as a practical, chemical-free grain protectant accessible to smallholder farmers in north-central Nigeria.

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is the most important grain legume in sub-Saharan Africa, valued for its high protein content of 23 to 25%, tolerance to drought and sandy soils, and critical contribution to household food security among smallholder farming communities (Singh et al., 2018; Fatokun et al., 2014). Nigeria is the world's largest producer of cowpea, accounting for approximately 50% of total production in Africa, with an annual output exceeding 5.3 million metric tonnes as of 2018 (FAO, 2019). Despite this dominance in production, post-harvest losses of cowpea in smallholder storage systems remain a persistent and severe constraint, with estimates of grain loss ranging from 20 to 80% within the first three months of storage under traditional conditions (FAO, 2011; Baributsa et al., 2010).

The cowpea weevil, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae), is the principal storage pest responsible for these losses. The insect deposits eggs on grain surfaces and the larvae develop entirely within individual seeds, consuming the cotyledon and germ before emerging as adults. A single infested grain shows characteristic circular emergence holes that render it unfit for human consumption or replanting. Under ambient storage conditions typical of the Guinea Savanna zone of north-central Nigeria — temperatures of 21.7°C to 40°C and moderate to high relative humidity — *C. maculatus* populations can complete a full life cycle within 25 to 30 days, resulting in rapid and severe grain deterioration (Liamngee et al., 2020).

Synthetic insecticides including organophosphates and pyrethroid formulations have been widely used for stored cowpea protection in Nigeria. However, their high cost, inaccessibility to resource-limited farmers, risks to human health from grain residues, and adverse effects on non-target organisms and soil ecology have intensified the search for safe, affordable, and locally accessible botanical alternatives (Liamngee et al., 2020; Ilesanmi and Gungula, 2016). Among botanical protectants evaluated for stored grain management, *Azadirachta indica* A. Juss (neem) has been the most extensively studied, demonstrating antifeedant, repellent, oviposition-deterrent, insect growth-regulatory, and direct insecticidal properties against a broad range of storage pests (Ogbuewu et al., 2011; Lale and Mustapha, 2000).

While neem seed kernel extract and seed oil formulations have received substantial attention, neem leaf powder presents a particular advantage for smallholder application: leaves are abundantly available on neem trees which grow widely across the Guinea Savanna zone of Nigeria, and the preparation of leaf powder requires no specialised equipment or purchased inputs. Previous work by Liamngee et al. (2020) demonstrated the efficacy of neem leaf powder against *C. maculatus* in stored cowpea, but comparative evaluation of leaf powder concentration effects across cowpea varieties under the specific conditions of Makurdi, Benue State remains limited in the published literature.

This study was therefore conducted to evaluate the efficacy of *A. indica* leaf powder at two concentration levels (10% and 20% of grain weight) against *C. maculatus* infestation in white and brown varieties of stored cowpea over four weeks in Makurdi, Nigeria, and to document the phytochemical constituents of the leaf powder responsible for its observed biological activity.

2. Materials and Methods

2.1 Study Location

The study was conducted in the Zoology Laboratory of Benue State University, Makurdi, Benue State, Nigeria. Makurdi is located in north-central Nigeria (latitude 07°43'N, longitude 08°35'E; altitude 104 m above sea level) within the Southern Guinea Savanna agroecological zone. Ambient temperature during the study period ranged from 21.7°C to 40°C, representative of typical storage conditions for smallholder farmers in the region.

2.2 Source of Cowpea Seeds

Cowpea seeds of two varieties — white and brown — were obtained from the seed bank of Joseph Saawuan Tarkaa University, Makurdi. Seeds were visually screened and only those showing no evidence of prior *C. maculatus* infestation (no emergence holes, no visible eggs, no adult weevils) were selected. Selected seeds were stored at 4°C in airtight containers until use, then sun-dried prior to the experiment to remove residual moisture and any dormant insects.

2.3 Preparation of Neem Leaf Powder

Fresh leaves of *Azadirachta indica* were collected from mature trees in Makurdi. Leaves were rinsed with running water to remove surface debris and air-dried for 10 days. Dried leaves were ground using a pestle and mortar and the resulting powder stored in a sealed, dust-free container at room temperature until use.

2.4 Preparation of Treatment Concentrations

Leaf powder concentrations were calculated as a percentage of the grain weight, following the method of Liamngee et al. (2020). Each experimental unit contained 200 g of cowpea grain. Concentrations applied were 0% (control, 0 g), 10% (20 g neem leaf powder per 200 g grain), and 20% (40 g neem leaf powder per 200 g grain). Each dose was thoroughly mixed with the grain to ensure uniform surface coverage.

2.5 Experimental Design and Setup

The experiment was a 2×3 factorial in a Completely Randomised Design (CRD), with two factors: cowpea variety (white, brown) and neem leaf powder concentration (0%, 10%, 20%). Three replicates were used per treatment combination, giving 18 experimental units in total. Each unit consisted of 200 g of cowpea grain in a plastic container infested with five pairs (5 male and 5 female) of adult *C. maculatus* at the start of the experiment. Containers were covered with perforated lids to allow aeration while preventing insect escape, and maintained under ambient laboratory conditions for four weeks. Observations and data collection were conducted at weekly intervals (Weeks 1, 2, 3, and 4).

Table 1
Experimental design: 2×3 factorial CRD with three replicates.

Factor	Level	Treatment Description	Replicates
Variety	White	White cowpea, clean	3
	Brown	Brown cowpea, clean	3
Concentration	0% (Control)	0 g neem powder per 200 g grain	3
	10%	20 g neem powder per 200 g grain	3
	20%	40 g neem powder per 200 g grain	3
Total units	18		

2.6 Parameters Measured

Seed weight (g): One hundred seeds per experimental unit were weighed on a balance at each weekly assessment.

Seeds with holes (n): Total number of seeds showing visible emergence holes or feeding cracks per experimental unit was counted weekly.

Insect mortality (n): The number of adult weevils showing no visible movement or irritability response was counted weekly in each container.

Seed germination (n): Ten seeds were randomly selected from each unit at each weekly assessment, placed on moistened absorbent paper in petri dishes, and germination counted at 72 hours as seeds showing emergence of the seed leaf (radicle). Results expressed as count out of 10.

2.7 Phytochemical Screening

An aqueous extract of *A. indica* leaf powder was prepared and subjected to qualitative phytochemical screening for alkaloids (Dragendorff's test), tannins (ferric chloride test), anthraquinones (Bornträger's test), saponins (froth test), terpenoids (Salkowski test), cardiac glycosides (Keller-Killani test), flavonoids (lead acetate test), phenolic compounds (ferric chloride test), and steroids (Salkowski test), following the methods described by Kumar et al. (2011).

2.8 Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA). Treatment means were separated using Fisher's Least Significant Difference (F-LSD) at 5% level of significance ($P \leq 0.05$). All analyses were conducted using SPSS software.

3. Results

3.1 Effect of Neem Leaf Powder Concentration on Seed Weight

Seed weight declined progressively across all treatments over the four-week storage period, reflecting the combined effects of grain moisture loss and weevil feeding activity. The 20% neem leaf powder concentration consistently recorded the highest mean seed weight at all weekly assessments in both varieties, while the untreated control (0%) showed the most rapid and severe weight decline (Table 2). The main effect of concentration on seed weight was non-significant at Week 1 ($P > 0.05$) but was significant at Weeks 2, 3, and 4 ($P < 0.05$), confirming that the protective effect of neem leaf powder becomes progressively pronounced over the storage period.

The white variety recorded substantially higher mean seed weight than the brown variety at all timepoints (F-LSD significant, $P < 0.05$). This difference is attributed to the inherent seed morphology and the comparatively lower susceptibility of the white variety to *C. maculatus* feeding activity under the conditions of this study.

Table 2
Main effect of neem leaf powder concentration and cowpea variety on seed weight (g) during four weeks of storage. NS = not significant.

Treatment	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
Variety				
White	33.41	31.04	28.10	25.23
Brown	16.72	15.32	14.01	12.54
F-LSD (0.05)	2.11	1.70	1.04	1.01
Concentration				
0% (Control)	23.97	21.23	16.15	10.95
10%	24.50	24.12	23.33	22.03
20%	24.73	24.20	23.68	23.08
F-LSD (0.05)	NS	2.08	1.15	0.95

3.2 Effect on Number of Seeds with Holes

No seeds with holes were recorded in any treatment at Week 1, reflecting the absence of *C. maculatus* progeny emergence in the first week of infestation. From Week 2 onwards, the number of seeds with holes increased significantly in the untreated control relative to neem-treated seeds ($P < 0.05$). The 20% concentration consistently produced the lowest number of seeds with holes across both varieties throughout the storage period (Table 3). The brown variety recorded a higher number of seeds with holes than the white variety at Weeks 2, 3, and 4 ($P < 0.05$), consistent with its higher susceptibility to weevil feeding activity.

Table 3
Main effect of neem leaf powder concentration and cowpea variety on number of seeds with holes during four weeks of storage. NS = not significant.

Treatment	Week 1 (n)	Week 2 (n)	Week 3 (n)	Week 4 (n)
Variety				
Brown	0.00	6.87	13.33	20.56
White	0.00	4.44	7.98	13.33
F-LSD (0.05)	NS	1.17	1.65	1.89
Concentration				
0% (Control)	0.00	8.50	17.00	26.50
10%	0.00	5.83	9.00	14.33
20%	0.00	2.50	5.83	10.00
F-LSD (0.05)	NS	1.44	2.01	2.32

3.3 Effect on Insect Mortality

No adult weevil mortality was recorded in any treatment at Week 1. From Week 2, insect mortality increased across all treatments, with the 20% neem leaf powder concentration recording significantly higher cumulative mortality than both 10% and the untreated control at Weeks 2, 3, and 4 ($P < 0.05$) (Table 4). No significant difference in mortality was observed between the white and brown varieties ($P > 0.05$), indicating that variety did not influence the insecticidal effect of the treatment. The untreated control (0%) recorded the lowest cumulative mortality throughout the storage period, consistent with uninhibited weevil population growth in the absence of any botanical protectant.

Cumulative insect mortality at 20% concentration reached 16.67 per experimental unit at Week 4 (Table 4). As the initial infestation was 10 insect pairs (20 individuals) per unit, this represents mortality of approximately 83% of the introduced population at the highest dose, which is notably higher than what was observed at 10% (45%) and the control (29%). This finding indicates that the 20% concentration exerts a direct insecticidal effect beyond the antifeedant activity observed at lower doses, consistent with the dose-dependent azadirachtin concentration in the leaf powder.

Table 4
Main effect of neem leaf powder concentration and cowpea variety on insect mortality during four weeks of storage. NS = not significant.

Treatment	Week 1 (n)	Week 2 (n)	Week 3 (n)	Week 4 (n)
Variety				
White	0.00	3.00	6.56	10.44
Brown	0.00	3.01	6.33	10.56
F-LSD (0.05)	NS	NS	NS	NS
Concentration				
0% (Control)	0.00	1.33	3.83	5.83
10%	0.00	1.67	5.00	9.00
20%	0.00	5.67	10.50	16.67
F-LSD (0.05)	NS	1.19	1.86	2.82

3.4 Effect on Seed Germination

Germination counts were uniformly high (10 out of 10 seeds) across all treatments at Week 1, with significant differences emerging from Week 2 onwards ($P < 0.05$). Seeds treated with 20% neem leaf powder maintained the highest germination counts at Weeks 2, 3, and 4, while the untreated control recorded the lowest germination, declining from 9.33 at Week 2 to 6.67 at Week 4 (Table 5). No significant difference in germination was observed between the white and brown varieties ($P > 0.05$), indicating that variety did not interact with treatment concentration in determining germination outcome. The preservation of germination capacity in neem-treated seeds reflects the antifeedant protection of the embryo and cotyledon from *C. maculatus* larval feeding, rather than any direct stimulatory effect of neem on seed physiology.

Table 5
Main effect of neem leaf powder concentration and cowpea variety on seed germination (out of 10 seeds) during four weeks of storage. NS = not significant.

Treatment	Week 1 (n)	Week 2 (n)	Week 3 (n)	Week 4 (n)
Variety				
White	10.00	9.56	8.22	7.67
Brown	10.00	10.00	7.33	7.22
F-LSD (0.05)	NS	NS	NS	NS
Concentration				
0% (Control)	10.00	9.33	6.50	6.67
10%	10.00	10.00	7.67	7.00
20%	10.00	10.00	9.17	8.67
F-LSD (0.05)	NS	0.61	1.14	0.65

3.5 Phytochemical Composition of *Azadirachta indica* Leaf Powder

Qualitative phytochemical screening of *A. indica* leaf powder confirmed the presence of nine bioactive compound classes (Table 6). All tested compounds yielded positive results, indicating a broad-spectrum bioactive profile consistent with the multiple modes of action observed against *C. maculatus* in this study.

Table 6
Qualitative phytochemical screening of *A. indica* leaf powder. + = positive result.

Phytochemical	Test Method	Observation	Result
Alkaloids	Dragendorff's Test	Creamish/pale yellow precipitate	+
Tannins	Ferric Chloride Test	Brownish green/blue-black colouration	+
Anthraquinones	Bornträger's Test	Rose pink colouration	+
Saponins	Froth Test	Honeycomb froth	+
Terpenoids	Salkowski Test	Reddish brown colouration	+
Cardiac Glycosides	Keller-Killani Test	Brown ring at interface	+
Flavonoids	Lead Acetate Test	Intense yellow colour	+
Phenolic Compounds	Ferric Chloride Test	Bluish black colour	+
Steroids	Salkowski Test	Reddish brown ring at interface	+

4. Discussion

The results of this study demonstrate that *Azadirachta indica* leaf powder applied at 20% of grain weight provides the most effective protection among the concentrations evaluated against *Callosobruchus maculatus* infestation in stored cowpea, significantly reducing grain damage, preserving seed weight, increasing insect mortality, and maintaining germination capacity over a four-week storage period. These findings are consistent with the broader literature on neem-based botanical protectants for stored cowpea in Nigeria and West Africa (Liamngee et al., 2020; Lale and Mustapha, 2000; Okolo and Iledun, 2019).

The dose-dependent protective effect observed across all parameters – from 0% through 10% to 20% concentration – reflects the increasing concentration of bioactive compounds at the grain surface with higher doses of neem leaf powder. The phytochemical profile confirmed in this study – including alkaloids, tannins, saponins, flavonoids, terpenoids, and cardiac glycosides – corresponds to the class of secondary metabolites collectively responsible for neem's antifeedant, growth-inhibitory, and insecticidal activity against storage insects (Ogbuewu et al., 2011). Azadirachtin, the principal bioactive limonoid in neem tissue, acts primarily by inhibiting feeding behaviour, disrupting moulting hormone pathways, and reducing oviposition rates in *C. maculatus* (Schmutterer, 1990). The additional bioactive compounds identified – particularly the saponins, tannins, and phenolic compounds – contribute synergistically to the observed insecticidal and antifeedant effects (Lale, 2002).

The significantly higher seed weight preserved at 20% concentration compared to the control is directly attributable to the reduction in feeding activity by adult weevils and the suppression of larval development within seeds. Insects feeding on the endosperm result in progressive loss of seed dry matter, and the weight preservation observed in neem-treated seeds indicates that the powder effectively reduced the rate and extent of this feeding activity. The superior performance of the white variety in terms of weight retention and lower number of seeds with holes is consistent with reports of varietal differences in susceptibility to *C. maculatus* in cowpea, attributed to differences in seed coat composition, tannin content, and seed hardness (Liamngee et al., 2020).

The insect mortality data from this study are of particular practical significance. At 20% concentration, cumulative mortality by Week 4 was approximately 83% of the introduced weevil population (16.67 out of 20 individuals), substantially exceeding the 45% mortality recorded at 10% and 29% in the control. This level of mortality at 20% indicates a direct insecticidal effect operating in addition to the antifeedant and grain-protective effects observed at lower doses. This finding is consistent with the report of Liamngee et al. (2020) that higher doses of neem leaf powder significantly increase weevil mortality, and with the observation of Isinkaye and Ode (2018) that dosage level has a significant effect on weevil population under storage conditions. The toxic action of azadirachtin on *C. maculatus* through cuticular penetration, as reported by Ilesanmi and Gungula (2010), likely contributes to this insecticidal effect at higher powder concentrations.

The preservation of seed germination in neem-treated cowpea confirms that *A. indica* leaf powder at the concentrations evaluated does not exert phytotoxic effects that compromise seed viability. Germination counts at 20% concentration (8.67 out of 10 seeds at Week 4) were significantly higher than those in the untreated control (6.67 out of 10), a difference attributable entirely to the differential protection of the seed germ from *C. maculatus* larval feeding. This compatibility with seed germination is an important practical attribute for smallholder farmers who retain stored cowpea as both food and planting material for the subsequent season.

The accessibility of *A. indica* leaf powder as a grain protectant within the Guinea Savanna zone of north-central Nigeria requires emphasis for extension and policy purposes. Neem trees are widely distributed across Benue State and the broader region, and leaf collection, air drying, and grinding to powder require no specialised equipment, purchased chemicals, or training beyond what can be communicated in a single farmer workshop. The 20% concentration recommended by this study represents 40 g of neem leaf powder per 200 g of cowpea grain – a quantity that is practical for smallholder-scale application and that imposes minimal additional handling burden compared to current informal storage practices.

5. Conclusion

Azadirachta indica leaf powder applied at 20% of grain weight (40 g per 200 g cowpea) significantly preserved seed weight, reduced grain damage, increased insect mortality, and maintained seed germination capacity against *Callosobruchus maculatus* infestation in stored cowpea over four weeks under ambient conditions in Makurdi, Nigeria. The 20% concentration was superior to the 10% treatment and the untreated control across all parameters evaluated in both white and brown varieties. Phytochemical screening confirmed nine bioactive compound classes responsible for the observed antifeedant, repellent, and insecticidal activity. The treatment is locally available, zero-cost in preparation, and compatible with seed viability, making it a practically deployable alternative to synthetic insecticides for smallholder post-harvest cowpea management.

The following recommendations are made based on the results of this study:

1. (i) Smallholder farmers in north-central Nigeria and comparable agroecological zones should be encouraged to adopt *A. indica* leaf powder at a minimum concentration of 20% for post-harvest cowpea protection.
2. (ii) Agricultural extension workers should be equipped with practical demonstration materials on leaf powder preparation, concentration calculation, and application, to enable effective farmer-level uptake.
3. (iii) Further studies should investigate extended storage periods beyond four weeks, the combined efficacy of neem leaf powder with other locally available botanicals such as *Piper guineense* and *Eugenia aromatica*, and the effects of varying storage humidity conditions on the longevity of neem powder bioactivity.

Declarations

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