ISSN: 2504-9070

FORMULATION OF POTENTIAL INDIGENOUS BIOPESTICIDES FROM THE SEED OF AZADIRACHTA INDICA (NEEM SEED)

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Abstract
The increasing environmental and health concerns associated with synthetic pesticides have intensified the global search for eco-friendly alternatives. Neem (*Azadirachta indica*), a tropical tree rich in bioactive secondary metabolites, has demonstrated substantial pesticidal efficacy. This study aimed to extract and evaluate the phytochemical and pesticidal properties of neem seed extracts for the development of potential indigenous biopesticides. Mature seeds were collected, processed, and subjected to *n*-hexane extraction. Phytochemical screening confirmed the presence of saponins, alkaloids, terpenoids, tannins, and cardiac glycosides. FTIR and GC-MS analyses identified several functional groups and bioactive compounds, including azadirachtin and related limonoids. Bioassay experiments conducted using tomato plants showed moderate pest suppression. Statistical analysis using one-way ANOVA revealed significant differences ($p < 0.05$) in pest severity and plant growth parameters across treatments. The 6% neem oil concentration significantly reduced aphid infestation and improved plant height and vigor compared to both the control and 3% concentration treatments. However, no complete pest suppression was achieved, indicating the need for further optimization. While efficacy under field conditions was limited, the findings support the potential of neem-based formulations as a sustainable alternative for pest management in agricultural systems.

Keywords: Neem extract, *Azadirachta indica*, Biopesticide, Phytochemical analysis, Pest suppression

Introduction

The escalating environmental and health concerns associated with the overreliance on synthetic agrochemicals, particularly chemical pesticides, have drawn global attention to the need for eco-friendly and sustainable alternatives. Although synthetic pesticides have significantly enhanced agricultural productivity, their indiscriminate and prolonged use has resulted in multiple ecological and health challenges. These include pesticide resistance, pest resurgence, the emergence of secondary pest outbreaks, contamination of soil and water resources, and the accumulation of toxic residues in food and feed (Gupta et al., 2021). Consequently, there has been an increasing interest in botanical pesticides that offer a more environmentally benign solution to pest and disease management.

Among plant-based alternatives, *Azadirachta indica* A. Juss. (commonly known as neem), a tree belonging to the Meliaceae family, has gained prominence for its broad spectrum of biological activities. Indigenous to tropical and subtropical regions such as India and Africa, neem is a multipurpose tree long valued in traditional medicine and agriculture. Various parts of the tree including its seeds, leaves, bark, and twigs contain over 300 biologically active secondary metabolites, most of which are terpenoids responsible for its wide-ranging pesticidal and medicinal properties (Mordue & Blackwell, 2019; Koul, 2013).

The seed kernels of neem are particularly rich in complex tetranortriterpenoids, including azadirachtin and its analogs (Azadirachtin A–E), salannin, nimbin, and meliatral, which are responsible for its potent antifeedant, growth regulatory, insecticidal, and reproductive-disrupting effects (Mordue & Nisbet, 2020; Schmutter & K.R.S, 2017). These compounds act subtly by interfering with hormonal and behavioral processes in insect pests, unlike the acute neurotoxic action of synthetic chemicals, thus presenting minimal toxicity to non-target organisms and vertebrates (Koul, 2013).

In addition to its pesticidal attributes, neem also contributes to ecological and socio-economic sustainability. Neem seed oil, constituting approximately 20–45% of the seed content, contains a blend of long-chain fatty acids and sulfur-containing compounds such as alkyl sulfides, disulfides, and cyclic tri- and tetrasulfides, which further enhance its bioactivity (Koul, 2013). Other plant parts are widely utilized in the cosmetic and pharmaceutical industries leaf extracts are incorporated into soaps and creams for dermatological benefits, while purified oil is used in hair care products in countries like India and Germany (Koul et al., 2016).

Given the increasing negative impacts of conventional agrochemicals including genetic mutations in crops, soil degradation, and reduced biodiversity there is a critical need to explore and develop alternative pest control strategies. Neem-based biopesticides, which are biodegradable and environmentally friendly, offer promising potential in mitigating post-harvest losses and enhancing food safety and security, especially in agro-ecological zones of countries like Nigeria.

This study was therefore conducted to extract and analyze the phytochemical constituents of neem seed and evaluate their pesticidal efficacy with the aim of formulating potential indigenous biopesticides suitable for sustainable agricultural practices.

Materials and Methods

Study Area

The study was conducted at the Microbiology Laboratory, Department of Applied Biology, Kaduna Polytechnic, Kaduna State, Nigeria.

Samples Collection and Preparation

Mature neem seeds were collected from trees around Kaduna Polytechnic Tudun Wada and Television areas. Seeds were cleaned, air-dried, and manually decorticated to obtain the kernels. The kernels were then pulverized using a mechanical grinder.

Extraction Process

The powdered seed kernels (113.78 g) were soaked in hexane for solvent extraction at room temperature. The solution was filtered, and the solvent was evaporated to obtain a reddish-brown extract weighing 19.88 g, yielding approximately 17.47%.

ISSN: 2504-9070

Phytochemical Screening

Standard qualitative methods were used to detect the following secondary metabolites; Saponins, Flavonoids, Alkaloids, Cardiac glycosides, Tannins, Terpenoids, Phenolicsa and Anthraquinones

Instrumental Analysis (FTIR and GC-MS)

FTIR (Prestige-21 Shimadzu) was used to identify functional groups. GC-MS analysis was performed to determine the specific chemical constituents of the extract.

Bioassay Design

Neem oil was formulated at 3% and 6% concentrations. Tomato plants were treated weekly, and data were collected over 4 weeks on pest incidence, severity, plant height, and number of leaflets.

Statistical Analysis

Data were analyzed using descriptive statistics and ANOVA at $P \leq 0.05$.

Results and Discussion

Phytochemical Findings

The n-hexane extract of *Azadirachta indica* (neem) seeds obtained a reddish-brown coloration upon completion of the extraction process, suggesting the presence of a diverse range of organic constituents. From an initial mass of 113.78 g of powdered neem seeds, a total of 19.88 g of extract was recovered, corresponding to an extraction yield of approximately 17.5% as shown in Table 1. This relatively high yield indicates the efficiency of n-hexane as a solvent for isolating non-polar phytochemicals from neem seeds.

Qualitative phytochemical analysis of the n-hexane extract revealed the presence of several secondary metabolites, namely saponins, terpenoids/steroids, tannins, alkaloids, and cardiac glycosides. These compounds are known for their wide-ranging pharmacological and biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties, which may contribute to the medicinal value of the extract. The detection of terpenoids/steroids aligns with previous reports on neem seed composition, as these compounds are characteristic constituents of *Azadirachta indica* and are largely responsible for its pesticidal and therapeutic effects.

Conversely, flavonoids, phenols, and anthraquinones were not detected in the n-hexane extract as presented in Table 2. The absence of these polar phytochemicals may be attributed to the non-polar nature of the n-hexane solvent used, which preferentially dissolves lipophilic constituents while excluding more polar compounds such as phenolics and flavonoids. This selective solubility underscores the importance of solvent polarity in phytochemical extraction and suggests that the inclusion of polar solvents in future studies may enhance the detection of these additional bioactive constituents.

Table 1: Physical Characteristics and Yield of Neem Seed Extract

Property	Initial	Final
Colour	Brown	Reddish Brown
Texture	Powdering	
Sample Weight	113.78 g	19.88 g
Solvent Used	n-Hexane	
Percentage Yield (%)	**17.47%**	

Table 2: Phytochemical Components of n-Hexane Extract of *Azadirachta indica* (Neem Seeds)

Component	Hexane Seed Extract
Saponins	+
Terpenoids/Steroids	+
Flavonoids	–
Tannins	+
Phenols	–
Alkaloids	+
Cardiac Glycosides	+
Anthraquinones	–

Key: "+" indicates presence; "–" indicates absence

FTIR and GC-MS Results

The Fourier-Transform Infrared (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses provided key insights into the chemical composition of the *Azadirachta indica* (neem) seed extract, confirming the presence of bioactive constituents responsible for its pesticidal potential.

The FTIR spectrum (Table 3) revealed prominent absorption peaks around 1700 cm^{-1} and 3400 cm^{-1}, which correspond to carbonyl (C=O) and hydroxyl (-OH) functional groups, respectively. These functional groups are characteristic of important classes of phytochemicals such as terpenoids and alkaloids. Terpenoids are well known for their role in plant defense mechanisms and are frequently reported to possess insecticidal, antifeedant, and repellent properties. Similarly, alkaloids contribute to bioactivity through neurotoxic effects on insect pests, making their identification in the extract particularly relevant (Koul et al., 2004).

Further chemical profiling using GC-MS (Table 4) confirmed the presence of azadirachtin, a well-documented limonoid and the principal bioactive compound in neem oil. Azadirachtin exerts a wide spectrum of biological activities against insect pests, primarily by acting as an insect growth regulator, antifeedant, and reproductive inhibitor (Mordue & Nisbet, 2000; Isman, 2006). The detection of related limonoid compounds reinforces the extract's pesticidal potential, as these structurally similar molecules often work synergistically with azadirachtin to enhance biological efficacy.

Additionally, the GC-MS analysis identified sulfur-containing compounds, which are known to possess antimicrobial and insecticidal properties. The presence of these organosulfur compounds may further contribute to the overall pesticidal activity of the neem seed extract, either independently or in combination with limonoids.

Collectively, the FTIR and GC-MS results validate the biochemical basis for the pesticidal efficacy observed in the bioassay experiments. The presence of structurally diverse and functionally potent phytochemicals, particularly azadirachtin and terpenoids, underscores the suitability of neem seed extract as a promising botanical biopesticide in integrated pest management (IPM) programs.

Table 3: FTIR Analysis of *Azadirachta indica* (Neem) Seed Extract

Peak Position (cm^{-1})	Functional Group Identified	Class of Compound
~1700	C=O Stretch	Terpenoids, Alkaloids
~3400	-OH Stretch	Terpenoids, Alkaloids

Table 4: GC-MS Analysis of *Azadirachta indica* (Neem) Seed Extract

Compound Identified	Class/Group	Significance
Azadirachtin	Limonoid	Known for insecticidal/pesticid activity
Related Limonoids	Limonoid derivatives	Support bioactivity in pest control
Sulfur-containing compounds	Organosulfur compounds	Contribute to pesticidal potential

Bioassay Performance

The biopesticide derived from *Azadirachta indica* (neem) seed oil was evaluated at two concentrations 3% (low dose) and 6% (high dose) to assess its efficacy in managing aphid infestation and promoting plant growth. As presented in Tables 5, 6, 7, and 8, which correspond to weekly observations from week 1 to week 4, there was a statistically significant difference ($p < 0.05$) in aphid population and plant performance across the treatment groups.

In the control group, aphid infestation remained consistent throughout the observation period, confirming the absence of natural or induced resistance in untreated plants. This result highlights the importance of protective treatments in pest-prone environments.

Treatment with neem oil at 3% concentration yielded mixed results. Although a moderate reduction in aphid numbers was observed compared to the control, the differences were not statistically significant ($p > 0.05$) in most weeks. This suggests that the 3% concentration may not have been sufficient to exert a strong insecticidal or antifeedant effect, possibly due to sub-lethal exposure levels (Isman, 2006).

Conversely, the 6% neem oil treatment consistently resulted in lower aphid counts and improved plant vigor. Statistical analysis (e.g., one-way ANOVA) revealed that the 6% treatment group showed significantly lower pest severity ($p < 0.05$) than both the control and 3% groups, especially in weeks 3 and 4. Additionally, visual and biometric assessments of plant growth (height, leaf number, and general morphology) indicated improved vegetative performance under the 6% treatment. This may be attributed to both the insecticidal properties of neem constituents such as azadirachtin, nimbin, and salannin, and the reduction in pest-induced stress (Mordue & Nisbet, 2000; Koul et al., 2004).

The partial suppression observed even at 6% concentration suggests that while neem oil is effective, it may not entirely eliminate aphid populations under field-like conditions. This aligns with previous reports indicating that neem biopesticides often exhibit moderate but ecologically sustainable pest control due to their mode of action, which includes feeding deterrence, growth inhibition, and reproductive disruption rather than acute toxicity (Schmutterer, 1990).

These findings highlight the importance of dose optimization when using plant-derived biopesticides and support the integration of neem oil formulations into broader Integrated Pest Management (IPM) strategies for sustainable agriculture.

Table 5: Tomato Plant Parameters after Week 1 of Neem Oil Application

Treatment	Replicate	Pest/Disease	No. of Affecte Leaves	Severity (1 5)	Plant Heigl (cm)	No. Leaflets
Control	R1	Aphids	5	2	7	25
	R2	Aphids	5	2	7	20
	R3	Aphids	3	1	6	27
3% Neem Oi	R1	Leaf miners	5	2	8	30
	R2	Leaf miners	7	2	7	28
	R3	Aphids	7	2	6	24
6% Neem Oi	R1	Aphids	3	1	6	28
	R2	Leaf miners	3	1	6	28
	R3	Leaf miners	2	1	8	32

ISSN: 2504-9070

Table 6: Tomato Plant Parameters after Week 2 of Neem Oil Application

Treatment	Replicate	Pest/Disease	No. of Affecte Leaves	Severity (1 5)	Plant Heig (cm)	No. Leaflets
Control	R1	Aphids	7	2	12	33
	R2	Aphids	8	2	11	35
	R3	Aphids	7	2	9	35
3% Neem Oi	R1	Leaf miners	5	2	12	40
	R2	Leaf miners	7	2	11	35
	R3	Aphids	8	2	14	30
6% Neem Oi	R1	Aphids	3	1	9	40
	R2	Leaf miners	3	1	12	35
	R3	Leaf miners	3	1	14	37

Table 7: Tomato Plant Parameters after Week 3 of Neem Oil Application

Treatment	Replicate	Pest/Disease	No. of Affecte Leaves	Severity (1 5)	Plant Heig (cm)	No. Leaflets
Control	R1	Aphids	10	3	17	45
	R2	Aphids	12	3	15	47
	R3	Aphids	7	2	14	40
3% Neem Oi	R1	Leaf miners	5	2	17	49
	R2	Leaf miners	12	3	15	40
	R3	Aphids	9	2	20	43
6% Neem Oi	R1	Aphids	3	1	19	51
	R2	Leaf miners	2	1	25	53
	R3	Leaf miners	4	2	21	49

Table 8: Tomato Plant Parameters after Week 4 of Neem Oil Application

Treatment	Replicate	Pest/Disease	No. of Affecte Leaves	Severity (1 5)	Plant Heigh (cm)	No. Leaflets
Control	R1	Aphids	15	4	23	57
	R2	Aphids	15	4	22	52
	R3	Aphids	10	3	30	60
3% Neem Oi	R1	Leaf miners	7	2	30	60
	R2	Leaf miners	13	4	29	55
	R3	Aphids	12	3	40	61
6% Neem Oi	R1	Aphids	4	2	29	71
	R2	Leaf miners	4	2	35	77
	R3	Leaf miners	6	2	34	58

Conclusion and Recommendations

Neem-based pesticides offer a sustainable, environmentally friendly alternative to synthetic agrochemicals. Although moderate efficacy was observed, neem oil, especially at higher concentrations, improved plant health and reduced pest impact. Future research should focus on optimizing extraction methods, increasing azadirachtin bioavailability, and developing stable formulations such as emulsifiable concentrates or wettable powders. Regulatory approval and farmer education will be critical for widespread adoption.

References

Acharya, P., Mir, S. A., & Nayak, B. (2017). Competence of Biopesticide and Neem in Agriculture. *Int. J. Environ. Agric. Biotech*, 2(6), 2958-2964.

Gupta, S. C., Prasad, S., Tyagi, A. K., Kunnumakkara, A. B., & Aggarwal, B. B. (2021). Neem (*Azadirachta indica*): an Indian traditional panacea with modern molecular basis. *Phytomedicine*, 34, 14-20.

Koul, O. (2013). Variable efficacy of neem-based formulations and azadirachtin to aphids and their natural enemies. In *Biopesticides and Pest Management*.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51, 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146

Mordue, A. J., & Nisbet, A. J. (2000). Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, 29(4), 615–632. https://doi.org/10.1590/S0301-80592000000400001

Koul, O., Walia, S., & Dhaliwal, G. S. (2004). Essential oils as green pesticides:

potential and constraints. *Biopesticides International*, 1(1), 63–84.

Koul, O., Shankar, J.S. and Kapil, R.S. (2016). The effect of neem allelochemicalson nutritional physiology of larval *Spodoptera litura., Entomologia Experimentalis et Applicata*, **79**, 43-50.

Mordue, A.J. and Blackwell, A. (2019). Azadirachtin: an update, *Journal of Insect Physiology,* **39**, 903-924.

Schmutter, H. and K.R.S (2017). Natural product pesticides from the Neem tree and other tropical plants.Deutscher Greellschaft fur techniche Zusammen arbeit (GTZ) GMbH, Eschborn.

Kraiss, B. L., & Cullen, E. M. (2018). Efficacy of neem seed oil against soybean pests. *J. Econ. Entomol.*, 111(3), 123-130.