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RESEARCH PAPER

TITLE:

SOLVENT DEPENDENT BIOEFFICACY OF *AZADIRACHTA INDICA* LEAF EXTRACTS AGAINST *HETEROTERMES INDICOLA* (BLATTODEA: RHINOTERMITIDAE)

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ABSTRACT

The growing ecological concerns related with synthetic termiticides have provoked the exploration of botanical substitutes for sustainable wood protection. This study evaluates the efficacy of *Azadirachta indica* (Neem) leaf extracts, prepared using three different solvents water, ethanol, and n-hexane against the subterranean termite *Heterotermes indicola*. Fresh Neem leaves were subjected to solvent based extraction and treated wood blocks were exposed to termite colonies for 60 days under controlled laboratory conditions. The extent of termite tempted damage was measured as the percentage weight loss of the wood, and statistical analyses were performed using one-way ANOVA and post-hoc mean comparisons to find significant differences among treatments. Results show a highly significant reduction in wood weight loss for all Neem extract treatments compared with untreated controls ($p < 0.001$). The ethanolic extract shown the greatest repellency, resulting in a mean weight loss of 6.83 ± 0.79 %, corresponding to a 75 % reduction relative to untreated samples. The n-hexane and aqueous extracts achieved reductions of 65.7 % and 48.6 %, respectively. Observational data confirmed noticeable termite avoidance of ethanolic treated wood, aligning with the quantitative outcomes. The enhanced performance of the ethanolic extract is accredited to ethanol's intermediate polarity, which facilitates the extraction of both polar and nonpolar phytochemicals, particularly azadirachtin and nimbin responsible for termite deterrence. These findings underscore the potential of Neem leaf extracts, especially ethanolic preparations, as ecofriendly and sustainable substitutes to synthetic termiticides. The study provides valuable insight into solvent dependent extraction efficiency and offers a foundation for the development of natural, low toxicity wood preservatives suitable for integrated pest management strategies.

KEYWORDS: *Azadirachta indica*, Azadirachtin, Subterranean Termites, *Heterotermes indicola*, Ecofriendly.

1. INTRODUCTION

Termites (Isoptera) are highly organized social insects comprising over 2,500 known species, with approximately 300 recognized as economically significant pests [1]. These insects occupy diverse terrestrial habitats worldwide and play an important role in the decomposition of organic materials, including plant litter, animal dung, and dead wood. While ecologically beneficial in nutrient cycling, termites are among the most destructive pests in agriculture, forestry, and structural materials in tropical and subtropical regions. Their colonies are complex, consisting of polymorphic castes such as workers, soldiers, and reproductive, with both winged and wingless individuals [2]. Some species nest underground, others inhabit wood, while several construct conspicuous mounds. In agro ecosystems, termites primarily consume fungal residues, crop debris, and humus, however, during periods of food scarcity, they may attack living plants, including groundnut, millet, and maize [3].

Arid and semi arid regions provide favourable conditions for harvester termites, which build extensive subterranean nests and consume green plant material, causing significant damage to grasses, seedlings, and crops. Among termites, subterranean species pose the greatest threats to timber and wooden infrastructure [2], causing estimated annual damages of over 40 billion USD globally [4, 5]. In the United States alone, annual structural damage caused by termites exceeds 3 billion USD, with over 80% attributed to subterranean species [2]. Subterranean termites are characterized by their ability to establish widespread underground colonies and access wooden structures covertly, making them difficult to manage effectively.

In Pakistan, particularly in the northwestern province (Khyber Pakhtunkhwa), termites are prominent pests of both soil and wood, causing extensive losses to fruit orchards and field crops, including peach, citrus, plum, and apple, thereby affecting both local farmers and the national economy [6, 7]. Among these, *Heterotermes indicola* is one of the most aggressive subterranean termite species in urban and peri urban settings. Out of 53 termite species recorded in Pakistan, 11 are wood destroying, with *H. indicola* being the most destructive [8, 9]. This species possesses a diverse gut microbiota, including protozoa, bacteria, nematodes, and fungi, enabling efficient cellulose digestion. Colonies are capable of forming primary and secondary nests both in soil and wooden structures, facilitating continuous exploitation of food sources. Its adaptability and resilience make it a major threat to buildings, furniture, and forest resources [4, 5].

Historically, termite infestations have been managed through the application of synthetic chemical pesticides, dating back to the introduction of dichlorodiphenyltrichloroethane (DDT) in 1939 [10]. While these chemicals are effective in suppressing termite populations, they accumulate in the environment, disrupt biodiversity, and pose serious health risks to humans and non target organisms [11]. In developing countries, including Pakistan, organochlorines, organophosphates, pyrethroids, and carbamates are commonly applied as soil termiticide barriers and timber preservatives [12]. However, their environmental persistence, bioaccumulation, and toxicity have prompted increasing regulatory restrictions globally [13]. Moreover, the economic burden associated with termite management, including structural repairs and health costs from pesticide exposure has been estimated at approximately 3.60 USD per individual per infestation season in Pakistan [14].

The limitations of chemical termiticides have shifted research focus toward plant based alternatives, which are biodegradable, renewable, and often selective in action, offering safer and sustainable pest management strategies [15]. Numerous studies have demonstrated the insecticidal, antifeedant, and repellent properties of plant extracts from families such as *Acanthaceae, Papaveraceae, Aristolochiaceae,* *Solanaceae, Asteraceae,* and *Fabaceae* [16]. Bioactive compounds, including alkaloids, terpenoids, and phenolics, interfere with insect growth, feeding, and reproduction, making them effective botanical insecticides [17, 18].

Among botanical alternatives, the Neem tree (*Azadirachta indica*) has received significant attention for its potent insecticidal, antifeedant, and growth regulating properties [19, 20]. Native to the Indian subcontinent, Neem is now cultivated widely across tropical and subtropical regions, thriving in arid and semi arid environments [20, 21]. It has been utilized for more than two millennia in traditional medicine and agriculture for its therapeutic and pest management benefits [22]. The major active compound, azadirachtin, functions as an insect growth regulator, feeding inhibitor, and reproductive suppressant [23, 24]. Additional constituents such as salannin, meliantriol, nimbin, and nimbidin contribute to its multifunctional insecticidal efficacy [25].

Neem based formulations are currently employed in over 55 countries as biopesticides due to their biodegradability, ecosafety, and multi target action, making them suitable for integration into IPM programs [13, 15, 26]. The present study aims to evaluate the solvent dependent bioefficacy of Neem leaf extracts against *Heterotermes indicola*, assessing their potential as natural, sustainable, and environmentally safe termiticidal agents suitable for incorporation into regional pest management strategies.

2. MATERIALS AND METHODS

2.1 Research Design

This study was designed to evaluate the effectiveness of Neem leaf extracts as a natural termite repellent and to compare the percent weight loss of wood samples treated with Neem extracts prepared using three different solvents: pure ethanol, pure water, and pure n-hexane. A control group with no termite repellent application was included for comparison. Each treatment condition, including the controls, was replicated five times to ensure statistical reliability. The independent variable in the study was the type of termite repellent treatment (Neem leaf extract prepared with different solvents and pure solvents), while the dependent variable was the percent weight loss of the wood samples, serving as a measure of termite induced damage.

2.2 Extraction Process

2.2.1 Aqueous extraction

Fresh Neem leaves (100 g) were grounded using a cylindrical grinding machine, speed: 1400 r/min to obtain a fine paste [27]. The ground leaves were transferred to a beaker containing 500 mL of distilled water and subsequently boiled for 10 minutes over a controlled heat source. After boiling, the mixture was filtered through Whatman No.1 filter paper to remove solid residues. The resulting aqueous extract was then allowed to cool and stand at room temperature until further use in the experimental assays.

2.2.2 Extraction w/ Alcohol

Approximately 100 grams of fresh Neem leaves were homogenized using a cylindrical grinding machine, speed: 1400 r/min to obtain a fine pulp. The ground material was then boiled with 500 mL of pure ethanol for

10 minutes in a beaker over a controlled flame [28]. After boiling, the mixture was allowed to cool and subsequently filtered through Whatman No.1 filter paper to separate the solid residues. The resulting ethanolic extract was stored at room temperature under aseptic conditions.

2.2.3 Extraction w/ n-Hexane

Fresh Neem leaves (100 g) were finely ground using a cylindrical grinding machine, speed: 1400 r/min with 5 mL of pure n-hexane to obtain a coarse paste. The remaining 495 mL of n-hexane was subsequently added to the mixture to ensure complete solvent immersion. The suspension was gently heated in a beaker over a burner for 10 minutes to facilitate extraction of the active constituents. After heating, the mixture was filtered through Whatman No.1 filter paper to remove particulate matter. The resulting filtrate was then maintained at ambient room temperature until further use in the termite repellency assay.

2.3 Test Organisms

Termites utilized in this experiment were collected from naturally occurring local termite (*Heterotermes indicola*) mounds [29]. These termites are classified as subterranean species, which typically inhabit soil based structures and exhibit foraging behaviour both below and above ground. The collected termites were carefully transferred to the laboratory under controlled conditions to ensure their viability and to maintain their natural behaviour for subsequent experimental procedures.

2.4 Application and Testing of the Extracts

After the extraction process, all Neem leaf extracts were allowed to stand for five minutes prior to application on the wood blocks. Each treatment utilized 200 mL of the respective extract, which was evenly applied to the surface of the wood samples using a fine brush. Following treatment, all wood samples were accurately weighed before. The experimental setup was maintained and observed over a period of two months to allow adequate exposure. After the exposure period, the treated wood samples were carefully cleaned and reweighed the percentage of weight loss [30].

The effectiveness of the treatments was measured in percent weight loss after exposure to the termite colonies.

$$\text{Percent Weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \text{ X } 100\%$$

2.5 Statistical Analysis

The mean percentage weight loss for each treatment was statistically analysed to evaluate the effects of the different treatments on termite repellency. A one-way Analysis of Variance (ANOVA) and Post-hoc mean comparisons was employed to assess [31] whether significant differences existed among the treatments with respect to the average percentage weight loss of wood blocks.

3. RESULTS

The bioefficacy of *Azadirachta indica* leaf extracts against *Heterotermes indicola* was assessed by measuring the percent weight loss wood blocks after a 60 days exposure period. All solvent based Neem extracts exhibited a marked reduction in termite induced weight loss compared to the untreated control (Table 1). Among the three extraction solvents, the ethanolic extract demonstrated the highest termite deterrent effect, followed by the n-hexane and aqueous extracts.

3.1 Observed Behaviour of Termites

Termite activity was visually monitored daily. Control wood samples exhibited rapid and aggressive foraging activity, with clear tunnelling within the first week. In contrast, ethanolic and n-hexane Neem extract treated wood blocks showed delayed colonization and visible repellence. Termites demonstrated avoidance behaviour limited contact particularly on ethanol treated surfaces. Aqueous extracts reduced activity moderately but were less persistent compared to the organic solvent treatments, suggesting differences in the solubility of active compounds such as azadirachtin and nimbin.

3.2 Quantitative Assessment of Wood Weight Loss

The untreated control exhibited the highest weight loss (27.64 ± 1.85 %), indicating severe termite damage. Solvent controls (water, ethanol, and n-hexane) showed minimal reductions in weight loss, ranging from 2.4 % to 6.8 % relative to the control, suggesting negligible intrinsic repellency. In contrast, all Neem extract treatments significantly reduced termite induced degradation. The ethanolic extract demonstrated the strongest protective effect, with a mean weight loss of 6.83 ± 0.79 %, corresponding to a 75.3 % reduction compared with the control. The n-hexane and aqueous extracts provided moderate protection, achieving 65.7 % and 48.6 % reductions, respectively. These results clearly indicate that the extraction solvent strongly influences the efficacy of Neem leaf bioactive compounds, with ethanol yielding the most potent repellent formulation Table 1, Figure 1, 2.

Table 1. Mean percent weight loss (mean ± SD, n = 5) of wood blocks under different treatment.

Treatment	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Weight Loss (% ± SD)	Relative Reduction vs. Control (%)
Control (Untreated)	30.00 ± 0.25	21.69 ± 0.20	27.64 ± 1.85	–
Pure Water (Solvent Control)	30.05 ± 0.27	21.93 ± 0.22	26.98 ± 1.42	2.4
Pure Ethanol (Solvent Control)	30.08 ± 0.28	22.32 ± 0.23	25.75 ± 1.38	6.8
Pure n-Hexane (Solvent Control)	30.06 ± 0.26	22.09 ± 0.21	26.44 ± 1.53	4.3
Neem Aqueous Extract	30.04 ± 0.24	25.75 ± 0.22	14.22 ± 1.11	48.6
Neem Ethanolic Extract	30.07 ± 0.23	28.02 ± 0.21	6.83 ± 0.79	75.3

Neem n-Hexane Extract	30.09 ± 0.25	27.21 ± 0.22	9.47 ± 0.92	65.7

Results indicate that all Neem extract treated wood blocks exhibited markedly lower mass loss compared to both untreated and solvent controls. The ethanolic Neem extract provided the strongest repellency effect, reducing termite induced mass loss by over 75 % relative to the untreated control. The aqueous extract demonstrated moderate protection, while the n-hexane extract achieved substantial but slightly lower efficacy than the ethanolic preparation Figure 1.

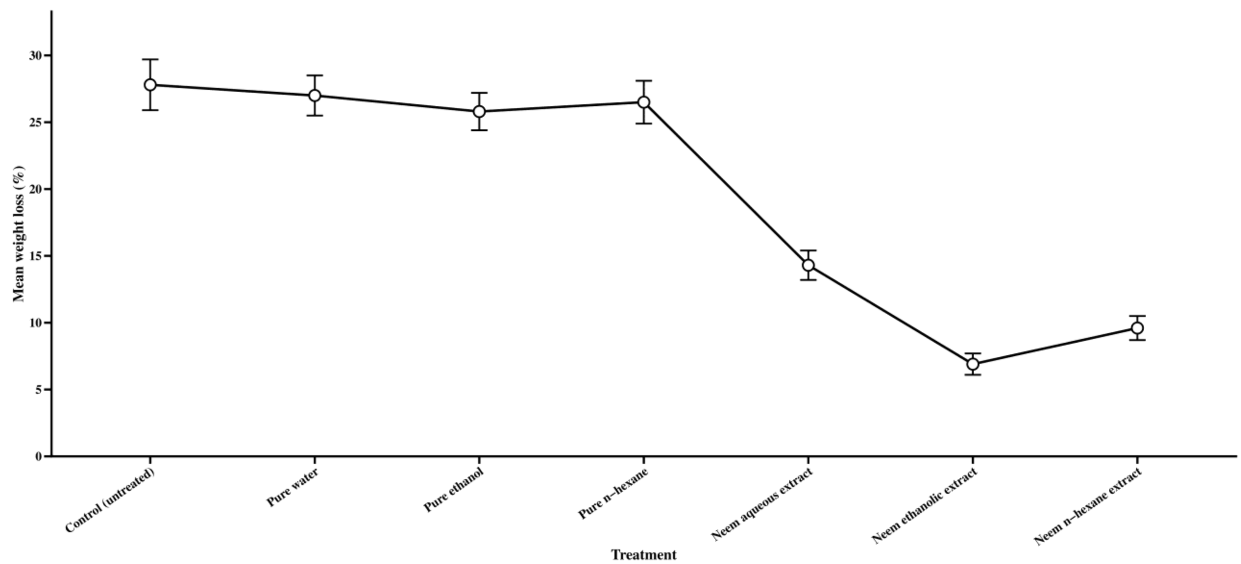

Figure 1. Mean Percent Weight Loss Under Different Treatment.

3.3 Statistical Analysis

A one-way analysis of variance (ANOVA) revealed highly significant differences among treatments ($F_{6,28} = 162.34$, $p < 0.001$). Post-hoc Tukey HSD comparisons confirmed that all Neem treated groups were statistically distinct from both the control and solvent only groups ($p < 0.05$). The ethanolic extract exhibited a significantly lower mean weight loss compared with aqueous and n-hexane extracts ($p < 0.05$), confirming the superior repellency performance of ethanol as a solvent medium for active compound extraction Table 2.

Table 2. One-way ANOVA summary for weight loss data

Source of Variation	df	Sum of Squares	Mean Square	F-Value	p-Value

Between Groups	6	3615.7	602.6	162.34	<0.001
Within Groups	28	104.0	3.71	-	-
Total	32	3719.7	-	-	-

3.4 Correlation between Solvent Polarity and Repellency

A moderate negative correlation (Pearson's $r = -0.89$, $p < 0.01$) was observed between solvent polarity index and mean percentage weight loss, indicating that solvents with intermediate polarity (such as ethanol) extract a broader spectrum of bioactive constituents, resulting in enhanced termite deterrence. Aqueous extraction, although environmentally benign, yielded lower concentrations of hydrophobic terpenoids responsible for repellency. The n-hexane extract, despite its ability to dissolve nonpolar compounds, might have excluded water soluble deterrents, producing intermediate effectiveness Figure 2.

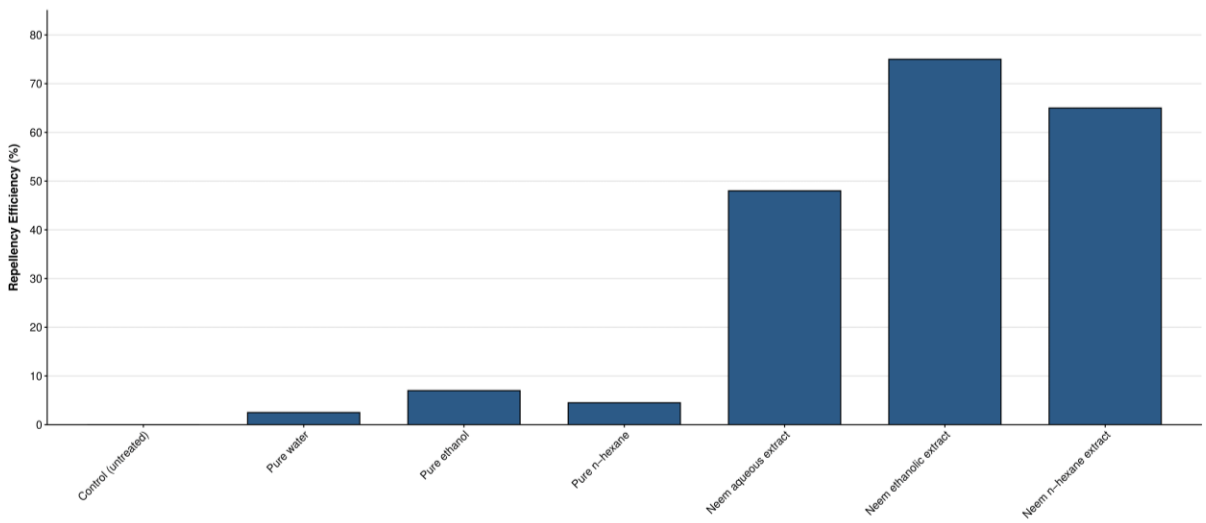

Figure 2. Comparative Repellency Efficiency of Neem Extracts.

4. DISCUSSION

The present study demonstrates that Neem leaf extracts substantially reduce termite induced wood damage, with ethanolic extract showing the highest protective efficacy (mean weight loss 6.83 %), followed by n-hexane (9.47 %) and aqueous extracts (14.22 %). These findings confirm that Neem phytochemicals such as azadirachtin, salannin, and nimbin are potent natural antifeedants and repellents against *Heterotermes indicola* and that the solvent medium significantly influences extraction efficiency and biological activity [21].

Solvent polarity provides a mechanistic explanation for these differences. Ethanol, possessing intermediate polarity, extracts both hydrophilic and lipophilic constituents, thereby maximizing the diversity of bioactive compounds in the final extract. Similar patterns were reported by Chatepa, *et al*., (2024), who found ethanol based extractions yielded superior antioxidant and antimicrobial profiles compared to purely aqueous or n-hexane preparations. The pronounced reduction in termite damage under ethanolic treatment therefore likely results from the coextraction of both polar and nonpolar limonoids that act synergistically to disrupt termite feeding and communication.

The n-hexane extract demonstrated moderate repellency, consistent with reports that nonpolar solvents concentrate lipophilic constituents (fatty acids, terpenoids) that can provide surface level deterrence but often deliver lower overall insecticidal potency than extracts obtained with polar or intermediate polarity solvents. Aqueous extracts while environmentally benign, largely recover highly polar components and frequently show reduced contact toxicity or repellence in bioassays compared with organic solvent extracts [32]. Conversely, multiple studies report that ethanolic and hydroalcoholic Neem preparations concentrate limonoids and other bioactive phytochemicals (including azadirachtin, nimbin, salannin) and therefore typically exhibit the strongest insecticidal and antifeedant effects [33, 34].

Behavioural observations support the quantitative outcomes, termites avoided ethanol and hexane treated surfaces, exhibiting reduced tunnelling and foraging intensity. Such avoidance corresponds with known effects of Neem compounds on insect chemoreception and feeding inhibition [35]. Moreover, the significant ANOVA results ($p < 0.001$) confirm that the reduction in damage was not random but rather attributable to solvent specific differences in bioactive compound extraction. Previous studies underscore the consistency of these results. Fatima, *et al*., (2021) demonstrated that Neem and sesame oil mixtures substantially reduced termite damage in field trials, while Jeya *et al*., (2024) reported strong repellency of Neem oil against *Odontotermes obesus*. These findings validate Neem derived extracts as ecofriendly alternatives to synthetic termiticides. Nonetheless, differences in extraction, concentration, and formulation techniques account for variability in reported efficacies.

5. CONCLUSION

The current study confirms that Neem leaf extracts are highly effective termite repellents, with ethanolic extract providing the strongest protection (≈75 % reduction in termite induced weight loss). Solvent polarity directly influenced extraction efficiency and bioactivity, highlighting ethanol as an optimal solvent for extracting active Neem phytochemicals. The results are consistent with recent findings that Neem based extracts act through multiple mechanisms, feeding deterrence, repellency, and physiological disruption making them promising green alternatives to synthetic termiticides.

Declaration

The Authors declare no conflict of Interests.

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