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Research Article

Toxic effect of *Acacia nilotica* and *Bauhinia alba* leaf extracts on the gut fauna of *Heterotermes indicola* (Wasmann)

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ABSTRACT

The South Asian subterranean termite, *Heterotermes indicola* is a serious hazard to agriculture due to its destructive feeding habits and resistance to conventional pesticides. Toxic effects of *Acacia nilotica* and *Bauhinia alba* leaf extracts were investigated on the termite mortality and their impact on termite gut microbiota. Extracts were evaluated for their ability to influence protozoa counts and colony-forming unit (CFU) densities within *H. indicola*, indicating digestive disruption. Findings revealed that both extracts exhibit dose-dependent mortality effects on *H. indicola* with LC_{50} values of 153.61% and 65.27% respectively. Furthermore, higher concentrations of these extracts correlated with reduced protozoa counts and altered CFU densities, suggesting significant alterations in termite gut microbiota. The study underscores the biopesticidal potentials of *A. nilotica* and *B. alba* and highlights their ecological implications in termite management.

Keywords: *Heterotermes indicola*, *Acacia nilotica*, *Bauhinia alba*, leaf extracts.

INTRODUCTION

Termites, belonging to the order Isoptera, are renowned for their ability to digest cellulose, making them not only crucial decomposers and formidable pests of agriculture as well as urban settings (Arumugam et al., 2018). *Heterotermes indicola* is one of the termite species that stands out as a significant damaging species due to its subterranean habits and extensive feeding on cellulose-rich materials of crops and wooden structures (Korb and Thorne, 2017). Digestive capabilities of termites are facilitated by symbiotic relationships with gut microorganisms, including bacteria and protozoa, which aid in cellulose breakdown (Brune and Ohkuma, 2011). Disrupting these symbiotic relationships through targeted control measures represents a promising avenue for termite management. Because of their antibacterial and anti-inflammatory qualities, *Acacia nilotica* and *Bauhinia alba*, which are well-known for their therapeutic uses in traditional medicine, have become viable options for alternative to synthetic insecticides (Ahmed et al., 2012).

Toxic effect of ethanolic leaf extracts of *Piper nigrum* and *Tamarindus indica* were evaluated against *H. indicola*, and the significant outcomes were revealed (Tabassum and Aihetasham, 2024). Similar studies reported that the *Calotropis gigantea* extract also had the insecticidal impact against *H. indicola* (Tabassum et al., 2023). In a study by Tabassum et al. (2024), *Ficus religiosa* and *Curcuma longa* leaf extracts also showed insecticidal potentials against *H. indicola.*Currently, the toxicity of *A. nilotica* and *B. alba* leaf extracts was evaluated against *H. indicola*, particularly the impacts on termite mortality and its gut microbiota. Understanding how these plant extracts influence termite physiology and digestive processes is critical for developing environmentally sustainable pest management strategies.

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By elucidating the mechanisms underlying their efficacy, this research aims to contribute to the development of novel approaches for termite control that minimize environmental impact.

MATERIALS AND METHODS

Collection of Termites

Misbah-Ul-Haq et al. (2015) protocol was adopted to collect worker castes of *H. indicola* from old wood logs of the botanical garden, University of the Punjab, Lahore, Pakistan.Termites were maintained in a laboratory environment at 26±2ºC and 80% relative humidity. Healthy, active termite workers were chosen for the experiment and placed in Petri dishes containing moist filter paper soaked in distilled water for subsequent testing (Aihetasham et al., 2018). The specimen was identified at the Entomology Lab, Institute of Zoology, University of the Punjab, Lahore (Akhtar, 1974).

Extraction of Botanicals

Acacia nilotica and *B. alba* leaves were obtained from the University of the Punjab, Lahore. Samples for taxonomic identification were sent to the Institute of Botany. The leaves were further cleaned, air-dried in a dehumidified chamber at 70°C, and then used in powdered form. Ethanolic extracts were prepared with Soxhlet's apparatus. Powdered leaves weighing 20 grams from the species *A. nilotica* and *B. alba* were used in the extraction process. Extraction was carried out using 200 ml of 100% ethanol through 8 cycles until the solvent became colorless, which indicated completeness. Four dilutions of 25%, 50%, 75%, and 100% of the leaf extracts were prepared using distilled water to obtain the respective desired concentrations. The extraction was performed based on Vogel et al. (1964) procedure.

Anti-Termite Bioassay

The effectiveness of plant extracts against termites was assessed using bioassays following Smith's (1979) technique. After a thorough cleaning, the Petri plates were left in an oven to dry for 24 hours. The filter paper was used to cut out circles the size of Petri plates, which were then soaked in 1 milliliter of chosen extract solutions and allowed to dry at room temperature. The termites were separated into two groups: 1) Distilled water was used to impregnate the control group, and 2) varying concentrations of extracts were administered to the experimental groups. 100 termite workers were released in each Petri plate. In all Petri plates, the humidity level was maintained by inserting a cotton plug saturated in water. Until 100% mortality was achieved, mortality rates were monitored every 24 hours.

$$Mortality\ (\%) = \frac{Dead\ termites\ (number)}{Total\ number\ of\ termites} \times 100$$

Termite Gut Isolation and Protozoan Count

The total gut protozoan population was counted following the Lewis and Forschler (2004) reported methodology. Termite guts were isolated using sterilized needles and forceps, homogenized with 70% saline solution following Peterson et al., (2015) and gut contents were analyzed using a hemocytometer. Four random squares per sample were counted, and the mean protozoa count per ml of gut suspension was calculated. Additionally, the following formula was used to calculate the total number of protozoa per milliliter of intestinal suspension:

$$Total\ number\ of\ protozoa\ per\ ml = \frac{(No. of\ cells\ counted \div 4)}{Area\ \times Width}$$

Bacteria Count

Nutrient agar and nutrient broth were prepared and autoclaved. Gut contents from termites treated with *A. nilotica* and *B. alba* extracts were inoculated into nutrient broth and incubated at 37°C for 24 hours. Dilutions were prepared and spread on nutrient agar plates. Colonies were counted after incubation to determine bacterial CFU per ml.

$$CFU\ per\ ml = \frac{(Number\ of\ colonies\ \times Total\ dilution\ factor)}{Volume\ of\ culture\ plated\ (ml)}$$

Statistical Analysis

Comparison of mortality (%) induced by treatments in *H. indicola* was evaluated using one-way ANOVA (P<0.05). LC_{50} was calculated by 'probit analysis' through Minitab (ver. 21).

RESULTS AND DISCUSSION

The effectiveness of *B. alba* and *A. nilotica* ethanolic leaf extracts against the subterranean termite *H. indicola* was assessed in this study. The LC_{50} values for *B. alba* and *A. nilotica* in *H. indicola* were 153.61% and 65.27%, respectively, indicating concentration-dependent death. The mortality rate was highest at 100% concentration, progressively reduced at 75% and 50% concentration, and was lowest at 25% concentration. The findings showed that within eight hours of exposure, 100% of *H. indicola* workers died as a result of exposure to the ethanolic extracts of *A.*

nilotica. Mortality rates at 75%, 50%, and 25% concentration were 100%, 88%, and 74.6% respectively after 10, 12 and 14 hours of treatment application. Similarly, the mortality (%) by *B. alba* at 100%,75%,50% and 25% was 100%, 89%, 80% and 70.6% respectively (Figure 1 & Table 1**).** All plants were active significantly against *H. indicola* with $F(1, 3) = 5.771$; $P<0.957$**.** All concentrations were found to have a significant effect on termite survival as compared to control $F(1.000, 1.000) = 25.55$; $P<0.1243$ (Table 2**)**. Both plants used, showed a significant ($P<0.05$) decrease in termite population. Qurat ul Ain and Aihetasham (2024) studied the efficacy of ethanolic extract of *A. nilotica* and *E. camaldulensis* against *C. heimi* and found both plants effective however greater mortality was observed at 30% concentration of both plants. *A. nilotica* demonstrated faster and more effective termite control than *B. alba* across all concentrations tested, highlighting its potential as a potent biopesticide (Hassan et al., 2019). Baeshen and Baz (2023) evaluated the efficacy of hexane and acetone leaf extracts of *A. nilotica* against *Culex pipiens* and *Aedes aegypti* and concluded that both extracts cause mortality in mosquito larvae and reduced female fecundity.

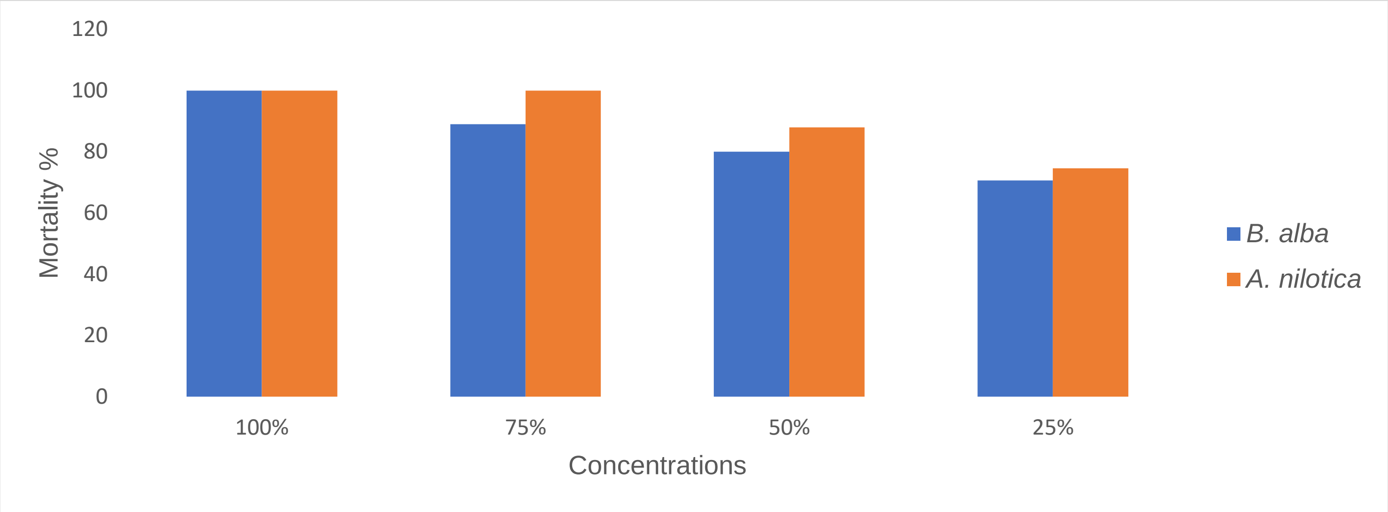

Figure 1. Mortality (%) of *H. indicola* after exposure to leaf (*B. alba* & *A. nilotica)* extracts.

Table 1. LC_{50} values of *leaf* extracts against *H. indicola*

Extracts	LC_{50} %	Standard error	C.I lower value	C.I upper level
B. alba	153.61	126.549	95.475	2675.16
A. nilotica	65.27	12.673	46.167	114.58

Table 2. One-way ANOVA for *H. indicola* mortality.

ANOVA	SS	DF	MS	F(DFn,DFd)	P value
Concentration	878.4	3	292.8	F(1.000,1.000)=25.55	P=0.1243
Plant type	66.13	1	66.13	F(1, 3)= 5.771	P=0.0957
	34.38	3	11.46		
	978.9	7			

Extracts Efficacy on Gut Protozoans of *H. indicola*

At the end of the experiment, the gut of *H. indicola* was separated by dissection. Protozoans were identified by microscope OLYMPUS U-TV0.5XC-3 model number 7G09372 JAPAN at 10×, hemocytometer (4×4) was used to calculate the number of protozoans. The total population of gut protozoans and their reduction after feeding on *A. nilotica* and *B. alba* leaf extract-treated filter papers was 87.9% and 80.3% respectively. Response of aggregate protozoan densities was considerably dose-dependent. The greatest reduction in protozoans was noted at the highest extractive concentration (100%) concentration which also coincided with the highest termite mortality. Both *A. nilotica* and *B. alba* extracts showed concentration-dependent effects on termite gut protozoa (Azizi-Shotorkhoft et al., 2016). *B. alba* exhibited stronger inhibitory effects compared to *A. nilotica* across all concentrations tested (Ahmed et al., 2012). At 600 ×103 cells/mL, the control group had the highest protozoa count, serving as a reference point for comparison. Protozoans that were present in termite gut after exposure of extracts of *A. nilotica* and *B alba* at different concentrations are described (Table 3).

Table 3. Presence of specific protozoans in *H. indicola* workers after treatment with plant extracts.

Extracts	Concentration	*Trichonympha*	*Holomastigotides*	*Pseudotrichonympha*	*Spirotrichonympha*
alba	100	✔	………..	✔	………..
	75	✔	✔	✔	…………
	50	✔	✔	✔	✔
	25	✔	✔	✔	✔
nilotica	100	✔	…………	…………..	…………
	75	✔	✔	………..	………..
	50	✔	✔	✔	✔
	25	✔	✔	✔	✔
Control		✔	✔	✔	✔

* "✔" = present

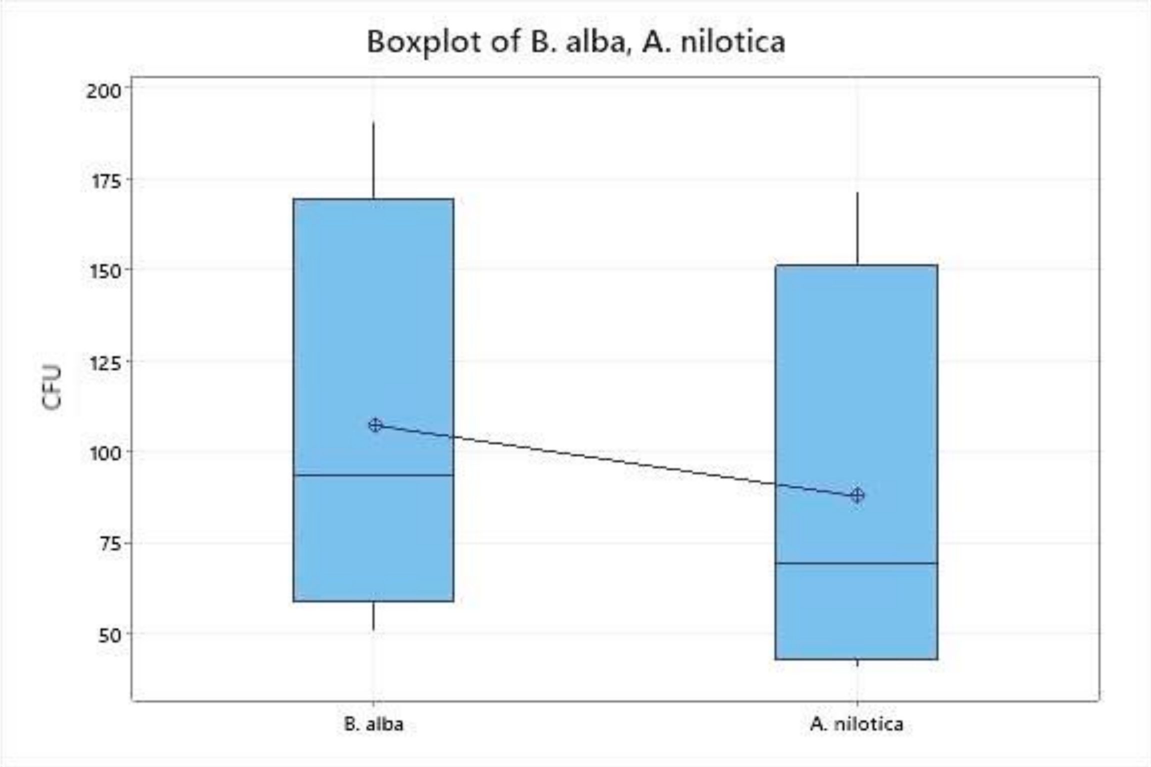

Figure 2. Boxplot of CFU for *A. nilotica, B-alba.*

Table. 3. T-tests for bacterial assays, indicating the impact of plant extracts on gut microbial communities.

Plant extracts	T-Value	DF	P-Value
A. nilotica	1.98	5	0.104
B. alba	0.46	5	0.665

Effects of Plant Extracts on Gut Bacteria

The colony-forming units (CFU) per Petri plate showed varying impacts on bacterial growth when the results of the two tests using extracts from *A. nilotica* and *B. alba* were compared. At a 100% extract concentration, the CFU for *A.*

nilotica varied between 39 and 42. The CFU levels ranged from 45 to 52 at 50%, which was higher. According to the results provided by Afzal et al. (2022) the CFU values at the 75% concentration ranged from 89 to 91, while the values at the 25% concentration ranged from 168 to 174. The CFU for *B. alba*, on the other hand, was 50.6 at 100% concentration, 82.6 at 75%, 104.6% at 50% concentration, and 190.6 at 25% dilution. These results are equivalent to those of Govindarajan et al. (2016). In summary, both of these plant extracts affect bacterial growth, but the *A. nilotica* shows a pattern in which with the increase in concentration, it raises CFU, however in *B. alba*, the values of CFU were varying for respective concentrations. It all depends upon plant extract and its concentration for the exact impact on bacterial growth. Fig. 2 displays the CFU boxplot for *A. nilotica* and *B. alba*. While *B. alba* had a range of effects on bacterial growth, *A. nilotica* demonstrated a trend of increased CFU with higher doses (Eutick et al., 1978). T-test was applied for bacterial assay, it gives P value and T value of 0.104, 1.98 for *A. nilotica*, and 0.46, 0.665 for *B. alba* respectively as shown in Table 3. T-tests for bacterial assays, indicating the impact of plant extracts on gut microbial communities (Gomathi et al., 2004).

CONCLUSION

At comparable doses, *A. nilotica* demonstrated a quicker and more effective termite control than the ethanolic extract of *B. alba*. Particularly, at both 100% and 75% concentrations, *A. nilotica* induce 100% mortality in *H. indicola*. In comparison, the *B. alba* extract showed somewhat less effectiveness at the same concentrations. With many researchers taking interest in environmentally preferable solutions, issues of termite infestation, both in urban and rural fields, might be addressed through environmentally friendly approaches with proper research and development in this area. These findings of this investigation significantly enrich the ongoing discourse on sustainable pest management methodologies.

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