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Effect of Leaf Extract of *Syzgium cumini* and *Curcuma longa* on Gut Fauna of *Heterotermes indicola*

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Abstract

Termites cause substantial economic losses globally, and their symbiotic gut protozoa play a key role in cellulose digestion and survival. Plant-based termiticides offer an eco-friendly alternative to synthetic chemicals. This study aimed to evaluate the termiticidal and protozoa-suppressing effects of ethanolic leaf extracts of Syzygium cumini and Curcuma longa on Heterotermes indicola. Leaves of both plants were extracted in ethanol using a Soxhlet apparatus. Three extract concentrations (10%, 5%, and 3% v/v) were tested in a no-choice bioassay. Mortality rates were recorded over 72 hours, and gut protozoal counts were quantified using hemocytometry. Both extracts exhibited concentration-dependent activity. S. cumini showed the highest mortality (95.33%), followed by C. longa (90.33%) at 10% concentration. Similarly, gut protozoal counts were reduced to 120 × 10³/mL with S. cumini and 190 × 10³/mL with C. longa at 10% concentration, compared to 520 × 10³/mL in the control. The ethanolic leaf extracts of S. cumini and C. longa are promising candidates for the development of botanical termiticides. Their dual effect on termite mortality and gut protozoa highlights their potential for sustainable termite control strategies.

1. Introduction

Termites (in the infraorder Isoptera) contain about 3,000 species worldwide (Chand *et al*., 2018). H. *indicola* poses a serious risk to various structures, including public areas, dykes and dams, agri-horticultural projects, and timber-in-service, which can result in financial loss (Manzoor *et al*., 2016; Chand *et al*., 2018; Mahapatro and Chatterjee, 2018; Shinde and Pardeshi, 2025). Termite damage is predicted to cost billions of dollars each year (Duquesne & Fournier, 2024). Bacteria, protists (only found in lower termites), a few fungi, and bacteriophages are found in one to multiple compartments that make up the termites' gut. These organisms live in mutualistic relationships and provide food by breaking down the tough plant biomass (Kumar *et al*., 2020). Termite colonies rely on the symbiotic gut protozoa to break down cellulose in their workers' stomachs, and the gut bacterial ecosystem is thought to give termites vital nutrients (Tikhe, 2017). These microbes transform cellulose and hemicellulose into long-chain fatty acids, which are further transformed into short-chain fatty acids that termites eventually consume. In termites' guts, digestive enzymes such as chitinases, peroxidases, proteases, lysozymes, and carbohydrate active enzymes (CAZymes) are crucial for turning cellulose and other plant materials into nutrition (Kumar *et al*., 2020).

There are several chemical anti-termite remedies that can contaminate the environment and pose a risk to human health (Manzoor *et al*., 2016). All this concerns the eco-

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friendly sustainable management of termites with reference to the already existing and emerging eco-technologies (Mahapatro and Chatterjee, 2018). It is also important to find another termiticide composed of natural components because the impact of environmental pollution is more concerning in the aspect of termite than the termiticide resistance (Andina *et al*., 2022). The process of treating termites with plant-based extracts has the potential to be an alternative to alleviate the environmental burden that these types of pesticides continuously cause, provided that this field is thoroughly researched (Joshua *et al*., 2023). Different species of plants like Solanum xanthocarpum (Shinde and Pardeshi, 2025), clove essential oils (Andina *et al*., 2022), and other herbal termitoxicants can kill termites (Mishra *et al*., 2021).

Turmeric, a kitchen spice (*Curcuma longa*), possesses medicinal properties; it is against the microbes and against the fungus (Cruz *et al*., 2024). Solvent extracts of *Curcuma longa* have extraordinary insecticidal activity (Abdelgaleil *et al*., 2019). The smooth, well-known *Syzygium cumini* tree possesses antibacterial, anti-inflammatory, and antioxidant qualities, among others (Kumar and Singh, 2021). The selected two plants of *Syzygium cumini* and *Curcuma longa* were high in their reported bioactive phytochemicals /insecticidal and known /antimicrobial character. S. *cumini* contains flavonoids, tannins and phenolic compounds with antibacterial and antifungal activities as aforementioned whereas C. *longa* (turmeric) comprises bioactive compounds like curcumin and related compounds that have strong insecticidal, antifungal and antimicrobial activities respectively (Cruz *et al.*, 2024).

Considering this, the current study is designed to assess the effectiveness of ethanolic leaf extracts of C. *longa* and S. *cumini* against H. *indicola*, with a particular emphasis on two important parameters: the reduction of intestinal protozoa (novelty in the study) and termite mortality. In order to find more environmentally friendly termite control options for urban and agricultural settings, our method sheds light on the deadly and microbiome-disrupting impacts of plant extracts.

2. Materials and Methods

2.1. Materials

The Healthy workers and soldiers of H. *indicola* (Wasmann), and leaves of *Syzygium cumini* (Jamun) were collected from the Botanical Garden of Punjab University, New Campus, Lahore. The leaves of *Curcuma longa* (Turmeric) were collected from Division Kasur, Punjab. Ethanol was purchased from Sigma-Aldrich. The Soxhlet apparatus was bought from a local equipment supplier. A lab microscope was used for counting flagellates. A Neubauer improved hemocytometer was used for protozoa counting. All the materials used in the experiment were sanitized thoroughly prior to use.

2.2 Termite Colony Maintenance

There were workers and soldiers of Heterotermes *indicola* that were obtained on infested *Populus euramericana* trees in Lahore, Pakistan. Sterile forceps were then used to remove termites carefully along the galleries and placed on a petri dish filled with enclosed moisture to avoid desiccation during shipping in the wet plastic containers lined with wet filter papers. Termites were acclimatized in the laboratory at least one week prior to experiments. About 50-100 workers per sterile Petri-dish (9 cm diameter) were maintained which contained 5 g of oven-dried, pesticide-free soil and a disc filter paper of about 1 cm diameter against the diameter of the Petri-dish immersed in a drop of distilled water as a food source. A cotton plug which had been saturated in distilled water was inserted into each Petri -dish and re-moistened after every 24 hours to ensure there was humidity. Colonies were maintained under regulated environmental conditions in an incubator at 28±2°C, 75-80 percent relative humidity, and total darkness which is similar to the natural subterranean living environment of the termites. The Petri dishes were examined on daily basis, to eliminate dead individuals and to add moisture enabling the healthy and active colonies of the future bioassays (Tabassum *et al*., 2024).

2.3 Plant Material Processing and Extraction

After two weeks of air drying at room temperature, the leaves of C. *longa* (Turmeric) and S. *cumini* (Jamun) were kept apart in polyethylene bags until they were needed. The dried leaves were subsequently powdered in a mechanical grinder and sieved in order to obtain a finely ground powder with a uniform particle size. 20 grams of leaf powder were extracted with 200 millilitres of ethanol in the Soxhlet extraction method by putting the sample in a thimble lined with filter paper. The Soxhlet apparatus consisted of a round-bottom flask containing ethanol, a condenser that continuously circulated distilled water, and an isomantle apparatus to control the heating at a constant temperature of boiling ethanol. After six hours of extraction, the ethanol extract was allowed to cool and then used (Banerjee and Narendhirakannan, 2011). Plant extract was diluted to 10, 5, and 3 percent (v/v) with the use of ethanol as the solvent. These three concentrations were used, because they were proven to reproduce termiticidal or insecticidal properties sufficiently. All the dilutions were prepared fresh before experimentation.

2.4 Anti-termite Assay

The efficiency of the plant extracts against H. *indicola* was determined using a no-choice bioassay. Dry soil free of pesticides was obtained from the lawn of the Institute of Zoology located at the University of the Punjab. The stones and pebbles in the soil were removed, and the soil sterilized by 24 h of incubation at 70 °C. To dry the Petri plates, which are to be used in an anti-termite test, they were thoroughly washed and dried in an oven by placing the plates with their covers open at 70oC heat for 24 hours. Each Petri plate was filled with 5 g of dried soil, and a circular piece of filter paper was placed on top of the soil surface. Termites were separated into two groups: experimental and control. The experimental group's filter papers were individually treated with 10%, 5%, or 3% (v/v) leaf extract of each plant and left to air-dry, whilst the control group's filter sheets were treated with distilled water. In each Petri plate, about fifty worker termites were added. A cotton plug soaked in water was inserted into each plate, which was then stored at 26°C in the dark to maintain humidity levels between 75 and 80%. For eight hours, mortality was recorded hourly. The purpose behind recording hourly mortality was to assess the initial toxic effects of plants on termite mortality. The following formula was then used to make additional observations at 24-hour intervals:

$$\text{MR\%}=\left(\text{No. of dead termites} / \text{Total termites}\right) \times 100$$

After 72 hours of treatment exposure, both mortality and protozoan suppression were assessed.

2.5 Dissection of Termite

Healthy worker termites were selected for dissection. Termites were momentarily immobilized by chilling and then superficially cleaned with 70% ethanol to remove external contaminants. Dissections were carried out under a stereomicroscope in a sterile environment using sterilized dissection tools. Each termite was placed on a clean glass slide, and the abdomen was gently opened with fine forceps

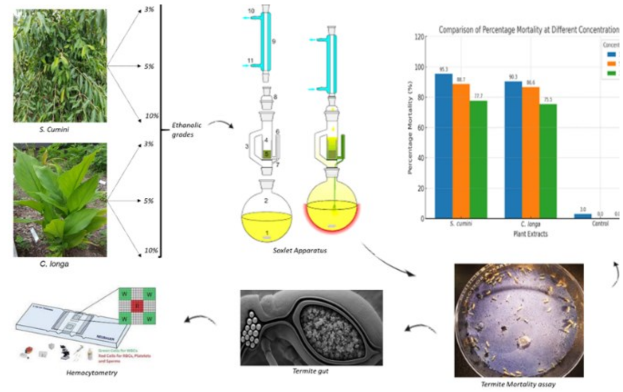

Figure 1. Schematic workflow of the study: (A) Preparation of ethanolic leaf extracts of *Syzygium cumini* and *Curcuma longa*, (B) Bioassay procedure exposing termites to extracts, (C) Quantification of gut protozoan density via hemocytometry, (D) Analysis of dose-response effects on termite mortality and protozoal reduction.

to expose the gut. The entire gut was carefully removed intact and transferred into a drop of 0.2% saline solution on a coverslip for subsequent protozoan counting. All procedures were performed aseptically to minimize contamination (Figure 1)

2.6 Gut protozoa count

A sanitized cover slip was adorned with a drop of 0.2% saline solution. A sterile needle was used to delicately remove the entire gut of a worker termite that had been decapitated and placed close to the drop. After opening the intestines in the saline solution, everything inside was carefully squeezed out. After making sure there was nothing left behind, the empty gut was taken out of the cover slip. The cover slip was rotated in all directions to distribute the drop carrying the stomach contents uniformly. The cover slip was then flipped onto a hemocytometer, and four randomly chosen 1 mm² squares of the hemocytometer grid were used to count the number of flagellates under 10X magnification. The average number of flagellates per square was determined. After measuring the spread smear's diameter, the radius was calculated by dividing it by two. The formula πr^2 was used to compute the smear's total area, and the following calculation was used to determine the smear's total flagellate population:

$$\text{Total flagellates} = \text{Avg. flagellates per unit} \times \text{Total area (mm}^2\text{)}$$

3. Results

3.1 Termiticidal Efficacy of Ethanolic Leaf Extracts

The ethanolic leaf extracts of both *Syzygium cumini* and *Curcuma longa* exhibited significant, concentration-dependent mortality against workers of *Heterotermes indicola* over a 72-hour exposure period (Table 1). Mortality progressed steadily, with pronounced increases between 24 and 72 hours for all active treatments.

At the highest concentration tested (10%), *S. cumini* extract induced the greatest mortality, reaching 95.33 ± 0.58% at 72 hours. The same concentration of *C. longa* extract resulted in 90.33 ± 0.58% mortality. Efficacy decreased with lower extract concentrations. At 5% concentration, mortality was 88.67 ± 2.08% for *S. cumini* and 86.67 ± 2.08% for C. *longa*. The lowest concentration (3%) yielded 77.67 ± 1.15% and 75.50 ± 2.65% mortality for *S. cumini and C. longa*, respectively. In stark contrast, mortality in the control group (distilled water) remained minimal at 1.00 ± 1.00% at 72 hours, confirming the health of the test subjects and the specific activity of the plant extracts.

Heterotermes indicola mortality increased in a concentration-dependent manner in response to the ethanolic extracts of *Syzygium cumini* and *Curcuma longa*. According to Table 1, S. *cumini* produced 95.33% mortality within 72 hours, while *C. longa* caused an average mortality of 90.33% at 10% concentration. The mortality rates for *C. longa* and *S. cumini* dropped to 86.6% and 88.66%, respectively, at 5% concentration. For *C. longa* and *S. cumini*, the lowest concentration (3%) resulted in 75.5% and 77.7% mortality, respectively. On the other hand, only 3% of the control group, which received distilled water treatment, exhibited any mortality after 72 hours due to age factor and potential self-contamination.

Statistical analysis confirmed a highly significant effect of treatment on termite mortality. Post-hoc Tukey's HSD tests revealed that all extract treatments at all concentrations

Table 1. Mean Percentage Mortality (± Standard Deviation) of *Heterotermes indicola* Exposed to Ethanolic Leaf Extracts of Curcuma longa and *Syzygium cumini* Over Time

Treatment	**Concentration**	**n**	**Cumulative Mortality at Key Time Intervals (%)**		
			24 h	48 h	**72 h**
Curcuma longa	10%	3	28.33 ± 2.31	33.33 ± 0.58	**90.33 ± 0.58**
	5%	3	26.67 ± 1.53	35.33 ± 1.53	**86.67 ± 2.08**
	3%	3	22.67 ± 1.53	24.67 ± 1.53	**75.50 ± 2.65**
Syzygium cumini	10%	3	28.67 ± 1.15	33.33 ± 0.00	**95.33 ± 0.58**
	5%	3	27.67 ± 1.15	33.33 ± 0.00	**88.67 ± 2.08**
	3%	3	23.00 ± 1.00	26.33 ± 1.53	**77.67 ± 1.15**
Control (Dist. water)	—	3	0.00 ± 0.00	0.67 ± 1.15	**1.00 ± 1.00**

Note: *Values represent Mean ± Standard Deviation (SD). n = number of biological replicates (each with 50 termites). Concentrations are expressed as % (v/v) of the crude ethanolic extract. Bold indicates the final mortality assessment at 72 hours. The low control mortality (1.0% at 72 h) validates the health of the test organisms.*

resulted in mortality significantly higher than the control. Furthermore, a direct comparison between the two plant extracts at the 10% concentration showed that *S. cumini* was statistically more effective than *C. longa* (Mean Difference = 5.00%) (Table 2).

3.2. Effect on Gut Protozoan Density

The ethanolic extracts also caused a marked reduction in the density of protozoan symbionts within the termite gut, correlating with the termiticidal activity (Table 3). Treatment with 10% S. *cumini* extract resulted in the most substantial decline, reducing protozoan counts to 120 ± 15 x 10^3 cells/mL, representing a 76.9% reduction from the control. The 10% C. *longa* extract reduced counts to 190 ± 20 x 10^3 cells/mL (63.5% reduction).

A clear dose-response relationship was observed. At 5% concentration, protozoan densities were 260 ± 25 x 10^3 cells/mL (S. *cumini*) and 295 ± 30 x 10^3 cells/mL (C. *longa*). At the 3% concentration, counts were 360 ± 20 x 10^3 cells/mL and 480 ± 25 x 10^3 cells/mL, respectively. The control group maintained a high and stable protozoan density of 520 ± 30 x 10^3 cells/mL. The reduction in gut protozoan density for all treatments was statistically significant compared to the control ($p < 0.01$), with the 10% concentrations of both extracts being significantly more effective than their lower concentrations ($p < 0.05$).

4. Discussion

Authors Metabolites such as flavonoids, glycosides, alkaloids, sitosterols, phenols, and tannins were created by plants. These phytochemicals found in plant parts aid in defending the plants against insect pest attacks. However, each plant produces them differently. Additional factors that have been discovered to influence the production of phytochemicals include plant age and plant parts (flower, leaf, fruit, bark, stem, root, and seed) (Prabhu *et al*., 2018). Termites severely damage or destroy agricultural perennial and annual crop plants, historical sites, wildlife, and forest tree plantations, resulting in significant economic losses (Verma *et al*., 2009). It is recognised that biopesticides, mostly made of botanical ingredients, have anti-pest properties (Mishra *et al*., 2021).

In the current study, H. *indicola* reacted to ethanolic leaf extracts of S. *cumini* and *Curcuma longa*. Both plant extracts were toxic to H. *indicola* workers in a dose-dependent way. Their effectiveness also varied according to the exposure period. The ability of ethanolic extracts of the plants under test to regulate termite populations was

Table 2. Statistical Analysis of Mortality Data at 72 Hours (One-Way ANOVA with Tukey's HSD Post-Hoc Test)

Comparison	Mean Difference (%)	Standard Error	Significance
S. cumini 10% vs. Control	94.33	0.67	***
C. longa 10% vs. Control	89.33	0.67	***
S. cumini 10% vs. *C. longa* 10%	5.00	0.67	**
S. cumini 5% vs. Control	87.67	0.67	***
C. longa 5% vs. Control	85.67	0.67	***
S. cumini 3% vs. Control	76.67	0.67	***
C. longa 3% vs. Control	74.50	0.67	***

Note: **** $p < 0.001$; ** $p < 0.01$. All treatments are significantly different from the control. S. cumini 10% is statistically more effective than C. longa 10% ($p = 0.001$).***

determined. H. *indicola* was killed by the two extracts used. Curcumin exhibited insecticidal action on the survival of G. intestinalis (bot fly) larvae at their third instar, which was time- as well as concentration- dependent. In the G. intestinalis larvae, the aqueous extract of curcumin was found to be highly toxic (Attia *et al*., 2022). Tabassum *et al*. only identified that the H. *indicola* died at high concentrations of the extracts. The current study is in line with the above-mentioned citations.

longa boosted the mortality rate of the red flour beetle. The antifedant, toxicant, and growth-regulating properties of garlic (*Allium sativum*) and Turmeric (*Curcuma longa*) leaf extracts were assessed against adults of T. *castaneum* using acetone. These findings indicated that the degree of deaths was directly proportional to the concentration of the extracts. The two plants, namely A. *sativum* and C. *longa*, significantly reduced the amount of weight loss in addition to the larvae, pupae, and adult development. In a study, promising results were obtained in reducing the microbial burdens with the use of Turmeric extracts (Wu *et al*., 2024) and these studies are in accordance with the findings of performed experiments.

Table 3. Density of Gut Protozoan Symbionts in *H. indicola* After Exposure to Ethanolic Leaf Extracts

Treatments	**Concentration**	**Protozoan Density (x 10^3 cells/mL)**	**Reduction vs. Control (%)**
Syzygium cumini	10%	120 ± 15 a	76.9
	5%	260 ± 25 b	50.0
	3%	360 ± 20 c	30.8
Curcuma longa	10%	190 ± 20 a	63.5
	5%	295 ± 30 b	43.3
	3%	480 ± 25 d	7.7
Control (Water)	—	520 ± 30 e	—

Note: *Values are Mean ± SD (n=3). Different lowercase letters within the column indicate statistically significant differences (p < 0.05, One-Way ANOVA with Tukey's HSD).*

Ethanolic extracts of leaf material of *Syzygium cumini* and *Curcuma longa* showed specific dose-dependent reductions in the gut protozoal density of H. *indicola*. Specifically, the greatest decrease in protozoa numbers was recorded at 10 percent S. *cumini* extract. These findings agree with the evaluation of Chetri *et al*. (2022) on the larvicidal potential of *Curcuma longa* against the A. culex larvae. Chander *et al*. (2000) assert that Turmeric (*Curcuma longa*) extract remains repellent to T. *castaneum*, Oryzaephilus surinamensis, Cryptolestes ferrugineus, Sitophilus oryzae and Corcyra cephalonica when it is in a laboratory after three months of exposure (Chander *et al*., 2000), which exactly correlates with the present study. According to Ali *et al*. (2014), the concentration of extracts from A. *sativum* and C.

5. Conclusion

The current research showed that *Syzygium cumini* and Curcuma long a leaf ethanolic extracts are potential treatments of Heterotermes *indicola* with both termiticidal and protozoa-inhibitory actions in a concentration-dependent manner. As presented in the study findings, termites could be successfully managed using plant extracts as opposed to synthetic insecticides. These plants have bioactive phytochemicals that can be refined into a source of preservatives that protect against the effects of termite damage and their attacks. S. *cumini* was more efficacious than C. *longa* in all concentrations, and showed slightly higher termite mortality at all concentrations, with a maximum of 95.33%. These results can confirm the prospect of using these plant extracts as environmentally friendly substitutes to synthetic termiticides. The isolation and characterization of bioactive compounds, formulation optimization, and field trials ought to be the future direction of studies that could help verify the usefulness of the compounds in providing integrated management of termites.

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8. Conflict of Interest

The authors declare no conflict of interest.

9. Authors' Contribution

Amber Zahid conceived and supervised the study, while all co-authors (Tayyaba Saeed, Ayesha Aihetasham, Muhammad Adnan Ali, Javeria Shakir, Gull-e-Tooba, Iqra Tabassum, Iqra Khalil, Maryam Iqbal, Sara Tariq, Zarmeen Ali, Esha Zahid, and Laraib Zahid) contributed to data collection, analysis, manuscript drafting, and final approval of the submitted version.

10. SDGs Addressed

This study supports SDG 15 by promoting biodiversity-friendly pest control and SDG 12 by encouraging sustainable alternatives to chemical pesticides. It also aligns with SDG 3 by reducing health risks linked to synthetic chemicals.

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