

## BioScientific Review (BSR)

Volume 6 Issue 3, 2024

ISSN(P): 2663-4198, ISSN(E): 2663-4201

Homepage: <https://journals.umt.edu.pk/index.php/bsr>



Article QR



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**Author (s):** Rafia Tabassum, and Ayesha Aihetasham

**Affiliation (s):** University of the Punjab, Lahore, Pakistan.

**DOI:** <https://doi.org/10.32350/bsr.63.01>

**History:** Received: January 08, 2024, Revised: June 14, 2024, Accepted: June 30, 2024, Published: July 02, 2024

**Citation:** Tabassum R, Aihetasham A. Bioactivity of medicinal plants piper nigrum and *Tamarindus indica* against *Heterotermes indicola* (Wasmann). *BioSci Rev.* 2024;6(3):01-12. <https://doi.org/10.32350/bsr.63.01>

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**Conflict of Interest:** Author(s) declared no conflict of interest



UMT

A publication of

The Department of Life Sciences, School of Science  
University of Management and Technology, Lahore, Pakistan

# Bioactivity of Medicinal Plants *Piper nigrum* and *Tamarindus indica* against *Heterotermes indicola* (Wasmann)

Rafia Tabassum and Ayesha Aihetasham\*

Institute of Zoology, University of the Punjab, Lahore, Pakistan

## ABSTRACT

**Background.** Termites, notorious for causing significant damage to crops, plantation forests, and buildings, pose a serious threat as pests. Conventional control methods rely on the use of insecticides, which have been reported to be hazardous to various other forms of life as well. In contrast, lower termites host protozoa in their gut, facilitating cellulose digestion through the release of cellulase enzymes.

**Method.** This study explored the efficacy of ethanolic leaf extracts from two medicinal plants namely *Piper nigrum* and *Tamarindus indica* against *Heterotermes indicola* (Wasmann). GC-MS analysis of the plant extracts revealed their distinct chemical compositions.

**Results.** The *T. indica* extract comprised compounds such as Benzene, 1,1'(1-methylethylidene) Bis [4-methoxy, 3-O-Methyl-d-glucose, Benzoic acid, 3,4,5-trihydroxy-, methyl ester, 9,12,15, octadecatyrien-1-ol, (Z, Z, Z), 1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl), Di-n-decylsulfone, and 2R-Acetoxymethyl-1,3,5-trimethyl-4-(3-methyl-2-buten-1-yl)-1-cyclohexanol. On the other hand, *P. nigrum* extract contained Octacosanol, Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-, B-Amyrin, Behenic alcohol, and Humulane-1,6-dien-3-ol. Both plant extracts exhibited repellent properties against *H. indicola*.

**Conclusion.** The LC<sub>50</sub> values for *T. indica* and *P. nigrum* were found to be 14.83% and 12.20%, respectively. While, the LC<sub>90</sub> values for *T. indica* and *P. nigrum* were -4.26% and -1.19%, respectively.

**Keywords:** *Heterotermes indicola*, *Piper nigrum*, protozoan fauna, *Tamarindus indica*

## Highlights

- Both plant extracts were found to be effective against *H. indicola*.
- Highest concentrations were found repellent to *H. indicola*.
- GC-MS analysis was performed for the chemical characterization of leaf extracts.

## 1. INTRODUCTION

Termites are present all over the world and are an important part of the soil ecosystem. They are known to search for food by tunneling through the soil [1]. They

account for 10% of animal biomass in tropical and subtropical environments, therefore, their presence is prominent [2]. They inflict severe harm to plants, as well as to agricultural crops such as barley, rice, sugarcane, and millet [3-5]. They serve as

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\*Corresponding Author: [ayesha.zool@pu.edu.pk](mailto:ayesha.zool@pu.edu.pk)

decomposers, enhancing soil fertility and productivity [6]. They create underground networks for movement and are notorious for causing significant damage to wooden structures [7, 8].

Many plant's oil extracts have been found to be effective anti-termite agents, such as *Pinus roxburghii*, *Eucalyptus camaldulensis*, *Morus alba* [9], *Cymbopogon citratus*, *Eucalyptus citrodora*, *Eucalyptus globules*, *Cedrus atlantica*, *Taiwania cryptomerioides* [10, 11], *Coleus ambionicus* [12], *Calotropis procera* [13], *Justicia adathoda* [2], and Isoborneol [14]. Black pepper or *Piper nigrum* is a member of the family Piperaceae. Its native to southern India where it has been revered for centuries as a symbol of royalty [15]. The word pepper derives from the Sanskrit term Pipali and is recognized by various names in different Indian languages such as Milagu, Kuru Mulagu, Kari Menasu and Kali Mirch. Its major producers are India, China, Vietnam, Sri Lanka, Thailand, Brazil, Malaysia, and Madagascar which contribute significantly to the global market [16]. Widely featured in Asian cuisine, black pepper also holds potential applications in alternative medicine, fragrance production, pesticides formulation, and food preservation [17].

The evergreen *Tamarindus indica* tree may grow to a height of 24 meters and may have a width of 7 meters [18]. It is native to the arid regions of Africa including Madagascar and Mozambique to the south, Senegal to the west, and Ethiopia and Sudan to the east [18, 19]. In Asia, *T. indica* is most commonly found in Bangladesh, Thailand, and Indonesia. In Americas, it is found in Mexico and Cost Rica. Every part of the *T. indica* tree including its roots, leaves, fruit, and bark hold significant industrial and economic value. Moreover, it has a rich nutritional content and a wide

range of medical applications [20]. The fruit is a rich source of eighteen (18) different amino acids, lacking only in tryptophan (82%) [21]. Similarly, its seeds also provide a significant and easily accessible protein source, particularly in regions where protein deficiency is prevalent [22]. A study evaluated the efficacy of *P. gutta* and *P. pinnata* heartwood extracts against *C. gestroi* (Wasmann) and observed that *P. gutta* extracts with MeOH were the most effective and caused 89.93% termite mortality [23].

The current study examines the phytotoxic potential of *T. indica* (imlee) and *P. nigrum* (black pepper) leaf extracts against *H. indicola* (Wasmann). It involves the extraction of selected plant leaves using Soxhlet extractor and the collection of *H. indicola* to test the feeding bioactivity of the extracts using laboratory bioassays. GC-MS analysis was used to characterize the structural properties of chemicals present in leaf extracts. The results may be helpful for the management of termites in houses, gardens, and agricultural fields without any environmental hazards.

## 2. MATERIAL AND METHODS

### 2.1 Collection of *H. indicola*

The workers and soldiers cast of *H. indicola* species were collected from the old trees of *Populous euramericana* in Lahore. The collected specimens were then maintained on water soaked filter paper and 5g of oven dried soil in each petri plate for at least one week.

### 2.2 Leaf Collection

The leaves of selected plants were collected from trees located in the Botanical Garden of the University of the Punjab, Lahore. The leaves were dried for

three (03) days under shade and stored in polythene bags for experimental purposes.

### 2.3 Extract Preparation

The leaves of medicinal plants were ground into fine powder using a grinder. For extraction, 20g of each leaf powder was taken separately with 200ml of ethanol in a Soxhlet extractor. The powdered plant material was placed in the thimble, which was then attached to the Soxhlet apparatus. The solvent was added into a round bottom flask connected to a condenser supplied with continuously flowing distilled water. Then, it was heated in an isomantle with a round flask having a flat bottom. The temperature was maintained at the boiling point of ethanol. When heated, it started to evaporate and move through the condenser. The first cycle was completed when the solvent reached the level of the syphon and was pumped back into the round bottom flask. For each extract to be finished, 6-8 cycles had to be completed. After the extraction process was completed, the flask was removed from the isomantle and allowed to cool. The following concentrations of the extracts were prepared to perform the experiment, that is, 30%, 20%, 10%, and 5%. Extraction was performed by following the protocol of Vogel et al. [24].

### 2.4 Gas Chromatography/ Mass Spectrometry (GC-MS)

For GC-MS analysis, extracts from the Soxhlet extractor underwent hydro distillation to determine their constituent parts. The samples were distilled at a temperature below 200°C and filtered through filter paper with pores of 0.20µm. The GC temperature ranged from 50°C to 250°C at a rate of 4°C per minute, with a 5-minute hold for the solvent. The injector was heated to 250°C. Helium gas, serving as the inert gas, flowed at a rate of 1.0

ml/min in the splitless mode and 2µl of samples were injected [25].

The percentage composition of each sample was computed and a qualitative analysis was performed based on the percentage area of each peak of the sampled chemicals. The mass spectrum of each compound was compared to the mass spectrum from the NIST 98 spectra collection (USA National Institute of Science and Technology software).

### 2.5 Anti-termite Assay

To determine the efficacy of plant extracts against termites, an assay was carried out following the Smith's [26] protocol. Petri dishes were thoroughly cleaned, washed, and then dried in an oven for an entire day. Circles were cut out from filter paper, soaked in 250µl of selected extract solutions, and dried at room temperature. The termites were divided into two sorts of groups. The experimental groups were treated with concentrations of extract solutions, while the control group was impregnated with distilled water. A total of one hundred (100) termite workers were then introduced in each petri plate. Humidity level was maintained in each plate by placing a cotton plug soaked in water. All experimental petri plates were stored in the dark at 26°C and the humidity level was maintained between 75-80%. Mortality rates were recorded after every 24 hours until the completion of the experiment and 100% mortality was obtained.

$$\text{Mortality rate} = \frac{\text{No. of dead termite workers}}{\text{No. of termites used in treatment}} \times 100$$

### 2.6 Repellency Assay

Filter papers of 9cm in diameter were cut into two equal halves for the repellency test. One half of each filter paper was exposed to extracts at concentrations 30%,

20%, 10%, and 5%, respectively. While, the other half was treated with distilled water (untreated). A total of ten (10) termites were released into the central area. The experiment was conducted for two (02) hours and the reading of repellency was taken every fifteen (15) minutes by counting the number of termites on both untreated and treated filter papers. Each concentration of the eight (08) plant extracts was replicated three times. After two (02) hours, the number of termites on both treated and untreated filter paper discs was counted to determine the level of repellency. Treatments were declared repellent when 21 (average of three replicates) of 30 termites were discovered on untreated filter paper for five (05) consecutive readings.

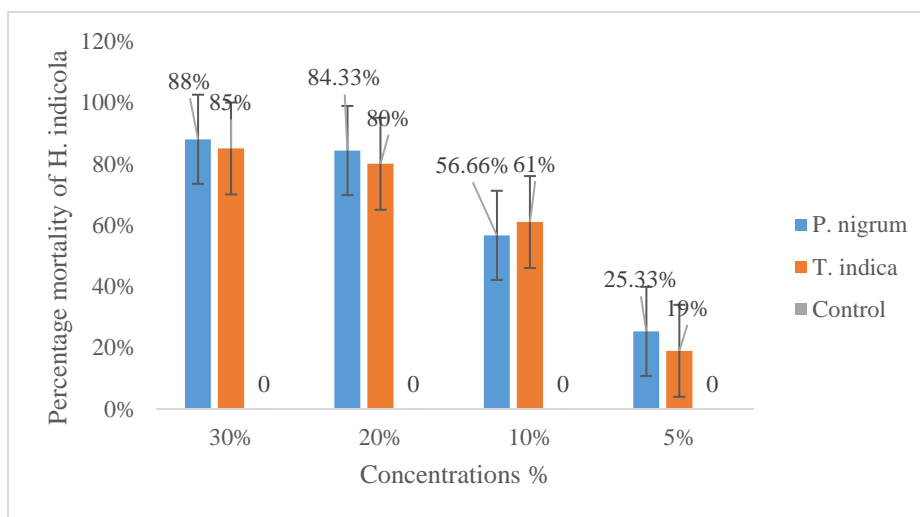
## 2.7. Statistical Analysis

The percentage mortality of termites was measured and evaluated using one-way ANOVA with  $p = 0.05$  considered as statistically significant ( $p < 0.05$ ).  $LC_{50}$  and  $LC_{90}$  were calculated using 'probit analysis' in Minitab version 21.

## 3. RESULTS

### 3.1 Anti-termitic Assay

The ethanolic leaf extracts of *T. indica* and *P. nigrum* were used to determine their efficacy against the subterranean termite *H. indicola* (Wasmann). Both plants showed concentration dependent mortality in *H. indicola* with  $LC_{50}$  value of 12.20% and 14.83%, respectively. The highest mortality was observed at 30% concentration, which decreased at 20% and 10% concentration levels and was the lowest at 5% concentration. The results indicated that the ethanolic extracts of *P. nigrum* caused 88% mortality in worker termites of *H. indicola*. The mortality rate at 20%, 10%, and 5% concentration was 84.33%, 56.66%, and 25.33%, respectively after 10 days of experiment with  $LC_{90}$  value of 25.57%. Similarly, the percentage mortality of *T. indica* at 30%, 20%, 10%, and 5% was 85%, 80%, 61%, and 19% respectively with an  $LC_{90}$  value of 27.64%, as shown in Figure 1 and Table 1.



**Figure 1.** Percentage Mortality of *H. indicola* at Different Leaf Extract Concentrations

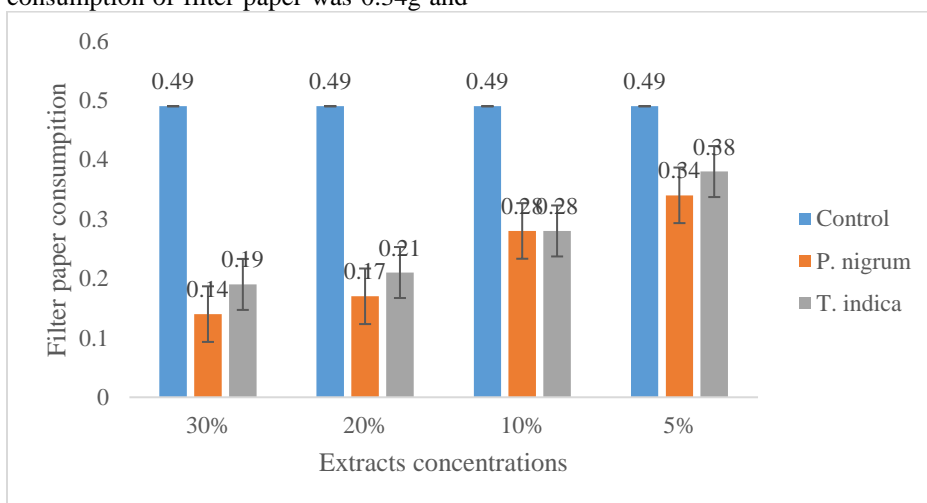
**Table 1.** Median Lethal Concentration (LC<sub>50</sub>) and (LC<sub>90</sub>) of Filter Paper Treated with *P. nigrum* and *T. indica* Leaf Extracts

Sr No.	Plants name	LC <sub>50</sub>	LC <sub>90</sub>	Significance	95% confidence interval
3	<i>T. indica</i>	14.83	-4.26	Significant	-0.9991-0.0846
4	<i>P. nigrum</i>	12.20	-1.19	Significant	-0.9993-0.0880

### 3.2 Filter Paper Consumption

In comparison to filter paper treated with plant extracts at various doses, *H. indicola* consumed significantly more solvent only treated filter paper. In case of *P. nigrum* and *T. indica*, the maximum consumption of filter paper was 0.34g and

0.38g, respectively. Whereas, a minimum consumption of 0.14g and 0.20g was observed at 30% and 5% concentrations, respectively. As the concentrations increased, the consumption of filter paper by *H. indicola* decreased, as shown in Figure 2.

**Figure 2.** Filter paper consumption by *H. indicola* at different leaf extract concentrations

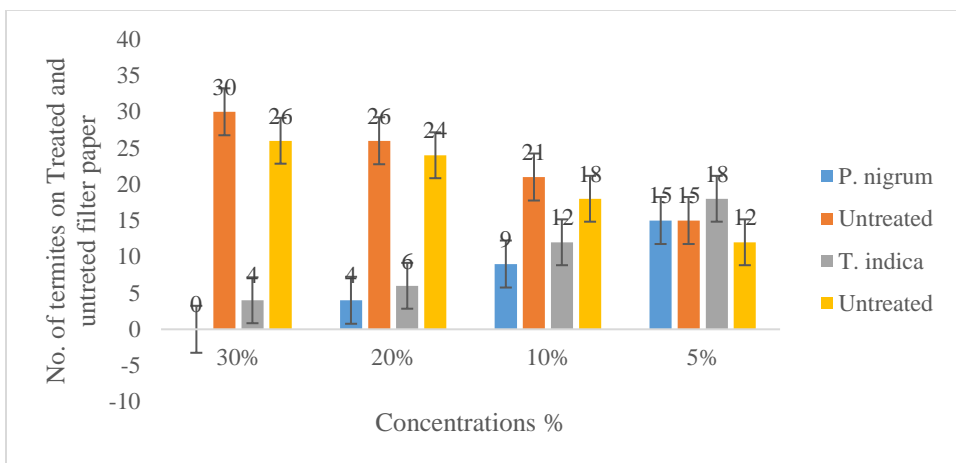
### 3.3 Repellency Assay

Termite workers were exposed to *P. nigrum* concentrations of 30%, 20%, 10%, and 5%, as well as untreated filter paper. Since more than 20 termites were found on untreated filter paper, all concentrations except 5% concentration were determined to be repellent to *H. indicola*. Conversely, *T. indica* at concentrations of 30% and 20% also showed repellency to *H. indicola*, as shown in Figure 3.

### 3.4 GC-MS Analysis and Characterization of Extracts

Table 2 shows the retention time, molecular formula, structural formula (based on the NIST14 library used by GCMS software), and percentage composition of sample for the chemical components identified from each of the tested leaves extract. GC-MS analysis identified a total of 17 chemical compounds from *P. nigrum* and 16 from *T. indica*. Chromatograms from GC-MS analysis of

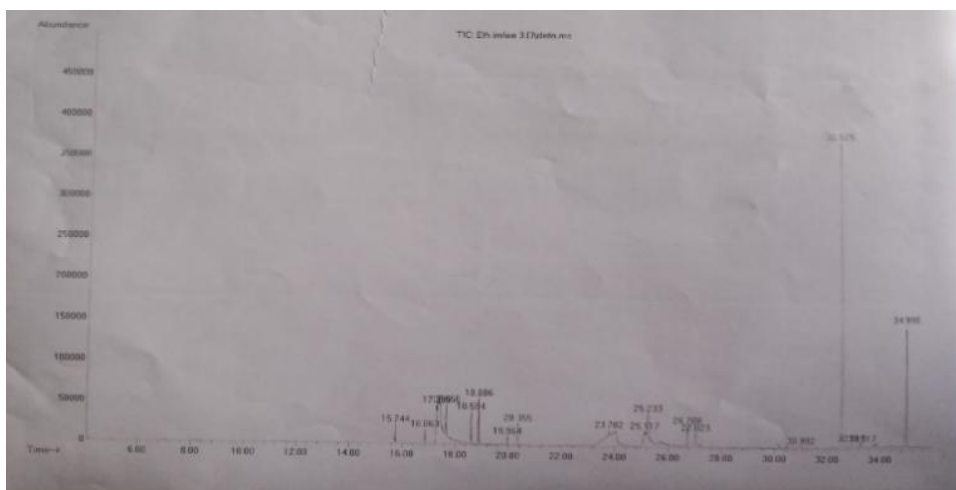
solvent extracted leaves are shown in Figure 4 and Figure 5.



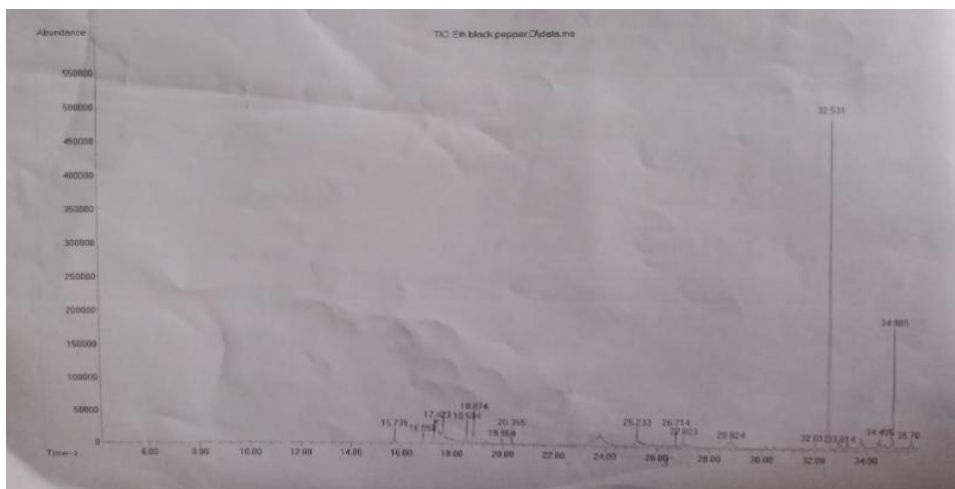
**Figure 3.** Repellency test of *P. nigrum* and *T. indica* against *H. indicola*

**Table 2.** Two-way ANOVA for Mortality of Termites

ANOVA Table	SS (Type III)	df	MS	F (DFn, DFd)	P value	Significance
Plants type	10.86	1	10.86	F(1, 3) = 1.003	0.3903	No
Concentrations	5206	3	1735	F(3, 3) = 160.3	0.0008	Yes
Residual	32.47	3	10.82			



**Figure 4.** GC-MS Chromatogram of Leaf Extract Obtained from *T. indica*



**Fig 5.** GC-MS Chromatogram of Leaf Extract Obtained from *P. nigrum*

#### 4. DISCUSSION

GC-MS analysis revealed the existence of phytochemicals in leaf extracts of *P. nigrum* and *T. indica* which are effective against insects. In GC-MS analysis of selected plants, different compounds were identified. *P. nigrum* extract was mainly composed of 1,2,3-Benzenetriol, Squalene, and Vitamin E. On the other hand, the main components of *T. indica* extract were Squalene, Vitamin E, and 3-O-Methyl-d-glucose. Squalene differed chemically from other components and its active fraction was not determined based on percentage. Squalene comprised the majority of the sample. n-Hexadecanoic acid, commonly known as fatty acid, showed effects against *P. falciparum* and exhibited activity against intracellular amastigotes of *L. major*. Additionally, fatty acids demonstrated antimicrobial action through the destruction of cell membranes, disruption of the electron transport chain, and oxidative phosphorylation of microorganisms [27]. Mary and Giri [28] showed the antimicrobial, anti-inflammatory, and anti-cancer properties of

phytol and squalene. Several studies [29–34] have reported that squalene extracted from plants acts as a termiticide.

Both plant extracts were found to be toxic to *H. indicola* workers in a dose dependent manner and their efficiency varied depending on the exposure time. There was a significant difference in mortality as the concentration increased from 5-30%. It was concluded that ethanolic extracts of the tested plants have the capacity to control termite population and exhibit repellent properties.

In a study, Al Ameri and Al jasaany evaluated the efficacy of alcoholic extract and powder of the black pepper fruit against worker termites, with results showing that all concentrations of the extract caused mortality among *Microtermes diversus*, depending on concentration and the duration of exposure [35]. Tabassum et al. [36] investigated the termiticidal effects of ethanolic leaf extracts from *C. gigantea* and *M. alba* against *H. indicola*, concluding that both plants cause significant mortality in *H. indicola* and exhibit repellent properties.



Alkali and Abdullahi evaluated the insecticidal efficacy of ethanolic leaf extract of *T. indica* on egg viability, oviposition, and emergence of *Callosobruchus maculatus* on treated cowpea seeds. The findings indicated that *T. indica* at high concentrations reduced egg viability, oviposition, and adult emergence of *C. maculatus*, suggesting its potential use by traditional farmers as an alternative to synthetic insecticide [37]. Qurat-ul-Ain et al. [38] evaluated the efficacy of ethanolic extracts from *C. fistula* and *P. pinnata* against *C. heimi* and found that both extracts were toxic.

The mode of action might involve contact with the body wall of insects. The elements present in leaf extracts may enter the insects' body system and obstruct normal growth, leading to termite mortality. The results concur with these findings [39] which imply that extract concentration is a significant contributor to termite death and antifeedancy [40]. The presence of phenolic chemicals which are potent antifeedants and serve as organic shields for living plants [41, 42] is the likely cause.

#### 4.1. Conclusion

Ethanolic extracts of *P. nigrum* and *T. indica* have the potential to be used as termite control. Both extracts were found to be toxic against *H. indicola*. To enhance control and management strategies for termite species, further testing of these extracts is necessary against other termite species found in different ecological zones in Pakistan.

#### CONFLICTS OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

#### DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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