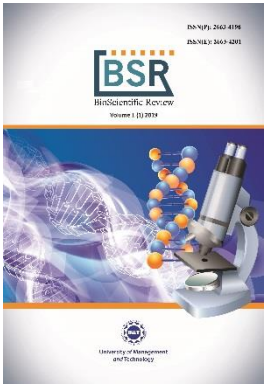


# BioScientific Review (BSR)

Volume 3 Issue 4, December 2021

$ISSN_{(P)}$: 2663-4198 $ISSN_{(E)}$: 2663-4201

Journal DOI: https://doi.org/10.32350/BSR

Issue DOI: https://doi.org/10.32350/BSR.0304

Homepage: https://journals.umt.edu.pk/index.php/BSR

Journal QR Code:


Article: **Efficacy of Ethanolic Plant Extracts of *Zingiber officinale, Raphanus sativus, Rosa indica and Aloe vera* against *Heterotermes indicola***


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Article DOI: https://doi.org/10.32350/BSR.0304.01


Article QR:

Ayesha Aihetasham


Citation: Aihetasham A, Qurat-ul-ain, Ramzan A, Khan MX. Efficacy of Ethanolic Plant Extracts of *Zingiber officinale*, *Raphanus sativus*, *Rosa indica and Aloe vera* against *Heterotermes indicola*. *BioSci Rev.* 2021;3(3):01–12.




Indexing


Estd. 1990

A publication of the
Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

# Efficacy of Ethanolic Plant Extracts of *Zingiber officinale, Raphanus sativus, Rosa indica and Aloe vera* against *Heterotermes indicola*

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**Article Info**

*Received: August 25, 2021*
*Revised: November 4, 2021*
*Accepted: November 4,2021*

**Keywords**

*Aloe vera*, *Heterotermes indicola*, LT50, *Raphanus sativus*, *Zingiber officinale*

**Abstract**

The current study was conducted to evaluate the anti-termitic potential of four common plant species, namely, *Zingiber officinale, Raphanus sativus, Rosa indica, and Aloe vera*. The worker and soldier cast of *Heterotermes indicola* were collected from *Populus euramericana*, old tree, University of the Punjab, Lahore, Pakistan. The extracts of plant species were prepared using Soxhlet Extractor. HR 2118 Philips grinder was used to crush the dried plant material into fine powder. Different concentrations were prepared and 0.5 ml of each concentration was poured on the filter paper and placed in the petri plate (arena). In laboratory bioassay, fifty active workers and five soldiers of *H. indicola* were added into each arena. The biological activity of ethanolic extracts of these plants was evaluated after 24 h and upto 96 h. The ethanol extract of *Z. officinale* caused the highest mortality (100%), while the lowest mortality (68%) was observed in *R. indica* extract. The $LT_{50}$ for *Z. officinale* were 24.34, 40.40 and 64.7 h, for the concentrations of 10%, 5% and 3%, respectively. The findings suggested that these plant extracts can provide environmental friendly management of *H. indicola*. In *A. vera*, significant mortality was observed against *H. indicola*, that is, 94%, 64% and 44% on the exposure of 10%, 5% and 3% concentrations, respectively. While, $LT_{50}$ were 34.66, 65.59, and 100.1 hours against 10%, 5% and 3% concentrations, respectively. In *R. sativus*, the mortality of *H. indicola* was 76%, 50% and 30%, while $LT_{50}$ against them was 59.10, 86.20, and 127.3 h, against the concentrations of 10%, 5% and 3%, respectively. Lowest mortality was observed in *R. indica*, that is, 68%, 44% and 30%, while LT50 were 64.17, 94.58 and 125.5 h at the concentrations of 10%, 5% and 3%, respectively. This study reports that the extracts of *Z. officinale*, *A. vera* and *R. Sativus* have the potential to be used for termite control, especially against *H. indicola*, in order to minimize the damage caused by it.

## 1. Introduction

Termites have been characterized mostly by their colonial behavior. They belong to the insect order Isoptera [1, 2]. They are medium sized, light colored, polymorphic, soft bodied and cellulose eating insects [1-3]. Termites are divided into more than 90 families, distributed in 370 genera, and they are pests of economically important crops [4]. A total of 2650 species of termites have been reported, worldwide. They are an important component of tropical and sub-tropical ecosystems [5, 6].

Subterranean termites (order: Isoptera) are regarded as important pest species to agricultural crops [7, 8]. *Heterotermes indicola* (Isoptera: Heterotermitidae) is a widely distributed termite specie reported from different parts of Pakistan [8, 9]. Termicides that are mostly used include chlordane, dieldrin, heptachlor, sodium acrcinite, depachlor and aposide lindare. The outcome of these termicides is outstanding but they become incorporated in food chain and remain persistent [10-13].

The replacement of synthetic pesticides with biodegradable compounds extracted from living organisms can prevent their adverse effects. To ease environmental burden, we can use plants which have a high degree of diversity and provide a variety of phytometabolites, some of which can be used as insecticides / pesticides [14, 15]. Plant derived products (essential oils) have been investigated for pest control. Plant based insecticides have been evaluated to antagonize the toxic potential of chemical insecticides. Botanical pesticides are, therefore, environmentally safe and friendly. They have numerous advantages or benefits over chemical insecticides, being eco-friendly and biodegradable, and are also effortlessly available [16].

Researchers have reported many plant species which can be used against termites and possess antitermitic attributes, such as Vetiver (*Vetiveria zizaniode)*, lemon grass (*Cymbopogon citratus*) and Cassia leaf (*Cinnamomum cassia*) [17, 18], Cedar wood (*Cedrus atlantica*), Eucalyptus *(Eucalyptus citrodora, Eucalyptus globules*), Clove bud (*Syzgium aromaticum*) [18, 19], *Calotropis procera* [18, 20], *Coleus amboinicus* [20], Isoborneol [18, 21] and *Calotropis procera* [18, 20].

Natural antitermitic compounds act differently on different termite species [22, 23]. A study evaluated the termicidal effect of methanolic and aqueous leaves extracts of *Piper betle* and *Carcica papaya* against *Coptotermes curvignathus* [24]. The toxic potential of *Pinus roxburghii*, *Cedrus deodara*, *Tectona grandis* and *Dalbergia sissoo* was also evaluated against *H. indicola* [22]. The current research was conducted to evaluate the antitermitic potential of the ethanolic extracts of *Z. officinale, R. sativus, R. indica and A. vera* against *H. indicola* (workers and soldiers). These bioinsecticides can be used to control termites and do not pollute the environment, being ecofriendly.

## 2. Methodology

### 2.1. Collection of Termites and Soil

Worker and soldier cast of *H. indicola* were collected from *Populus euramericana*, old tree, University of the Punjab, Lahore, Pakistan. Termites were kept for 1 week in petri-plates along with 5-gram oven dried soil for further experimentation. Soil (sandy loam) was taken from the Zoology Department lawn, University of the Punjab,

Lahore. It was later sieved, sterilized and then placed at 70°C in drying oven for removing any fungal contamination.

### 2.2. Extract Preparation

The leaves and petals of *R. indica* and the leaves of *A. vera* were taken from the Botanical Garden, University of the Punjab, Lahore. However, *Z. officinale* and *R. sativus* were purchased. All of them were thoroughly washed using distilled water and dried under shade for two weeks.

Extracts were prepared using Soxhlet Extractor. HR 2118 Philips grinder was used to crush the dried plant material into fine powder. Powdered plant material, wrapped into a filter paper, was placed into the Soxhlet Extractor with 200 ml of absolute ethanol. Isomantle was used for heating ethanol. Extracts were obtained after the completion of six cycles. It was kept in Reagent bottles from which 10%, 5% and 3% dilutions were prepared.

### 2.3. Antitermitic Assay

Antitermitic assay was performed using [23]. The Whatman 42 filter paper C was placed in each petri-plate and 0.5 ml of each concentration was poured on the filter paper with a micropipette. Afterwards, 50 active workers and five soldiers of *H. indicola* were added to each petri-plate. Readings were taken for the first eight hours. They were taken again after 24 hours and finally after 96 hours.

$$\text{Mortality Rate in Percentage} = \frac{\text{Dead termites}}{\text{Total termites}} \times 100$$

### 2.4. Repellency Assay

Repellency test was performed using filter papers of a diameter of 9 cm that were cut into two. One half of the filter paper was exposed to 10%, 5%, and 3% concentrations respectively and considered as treated (T). The other half was considered as untreated (UT). Ten termites were released into the gap between the filter papers. Three replicates of each concentration were maintained. After 15 minutes, termite number was counted on each half of the filter paper and the experiment was maintained until 120 minutes. To minimize the effect of light, the whole setup was covered with a black cloth. A concentration was considered repellent if 21 or more termites (out of 30) were present on the untreated area.

### 2.5. Statistical Analysis

Probit analysis was performed using the Statistical Software Minitab (version 18) to evaluate the differences in termite mortality.

## 3. Results

Highest mortality within 96 hours was recorded with *Z. officinale* extract against the termite (*H. indicola*). It caused death to 100% population in both 10% and 5% concentrations, while 66% mortality was observed in 3% concentrated extract. In *A. vera* extract, significant mortality was also observed against *H. indicola*, which lead to 94% mortality at 10%, 64% mortality at 5% and 44% mortality at 3% concentration. In *R. sativus* extract, moderate mortality was observed against *H. indicola*, that is, 76%, 50% and 30% mortality at 10%, 5% and 3% concentrations, respectively. On the other hand, *R. indica* extract caused the lowest mortality rate of 68%, 44% and 30% at the given concentrations (Figure 1).

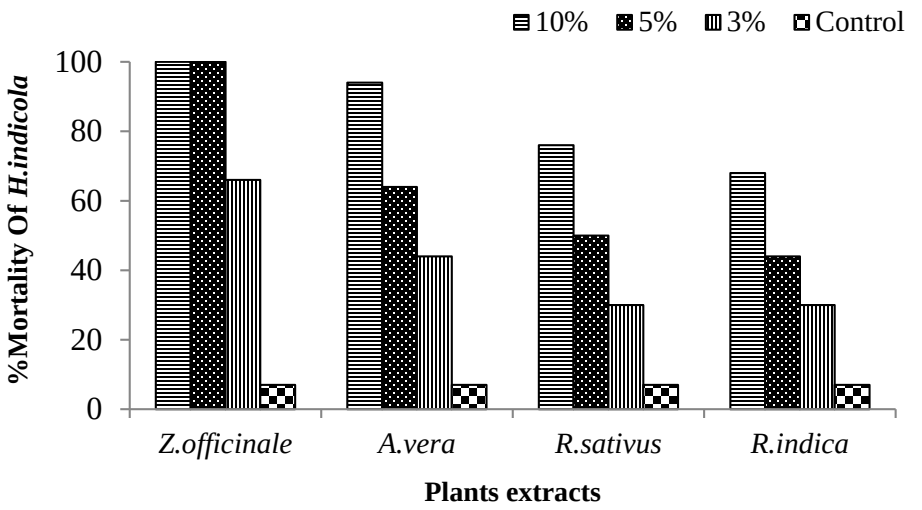


**Figure 1.** Percentage mortality of four different plant extracts against *H. indicola*

$LT_{50}$ were also assessed to estimate the doses with respect to time for all the plant extracts against *H. indicola* (Table 1 and 2). The $LT_{50}$ values for *Z. officinale* were 24.34, 40.40 and 64.7 h for the concentrations of 10%, 5% and 3%, respectively. While $LT_{50\ values}$ were 34.66, 65.59, and 100.1 h, respectively for the same concentrations of *A. vera* (Figure 2). $LT_{50}$ values of *R. sativus* extract were 59.10, 86.20, and 127.3 h for the concentrations of 10%, 5% and 3%, respectively. While 64.17, 94.58 and 125.5 h were observed for *R. indica* at 10%, 5% and 3% concentrations, respectively (Fig 3). Repellence test was also performed to estimate extract efficiency against *H. indicola.* The extract was found to be repellent. Repellence between the different concentrations of each plant extract was non-significant. Various concentrations of *Rosa indica* showed a low level of repellence with respect to the other three plants extracts (Table 3).

**Table 1.** LT50 Values (Hours) of Different Extracts at 10%, 5% and 3% Concentrations

| Sr. No | Extracts | 10% | 5% | 3% |
|---|---|---|---|---|
| 1. | *Zingiber officinale* (Ginger) | 24.34 | 40.40 | 64.7 |
| 2. | *Aloe Vera* (Aloe Vera) | 34.66 | 65.59 | 100.1 |
| 3. | *Raphanus sativus* (Radish) | 59.10 | 86.20 | 127.3 |
| 4. | *Rosa indica* (Rose petals) | 64.17 | 94.58 | 123.2 |
| 5. | Control | 221 | | |

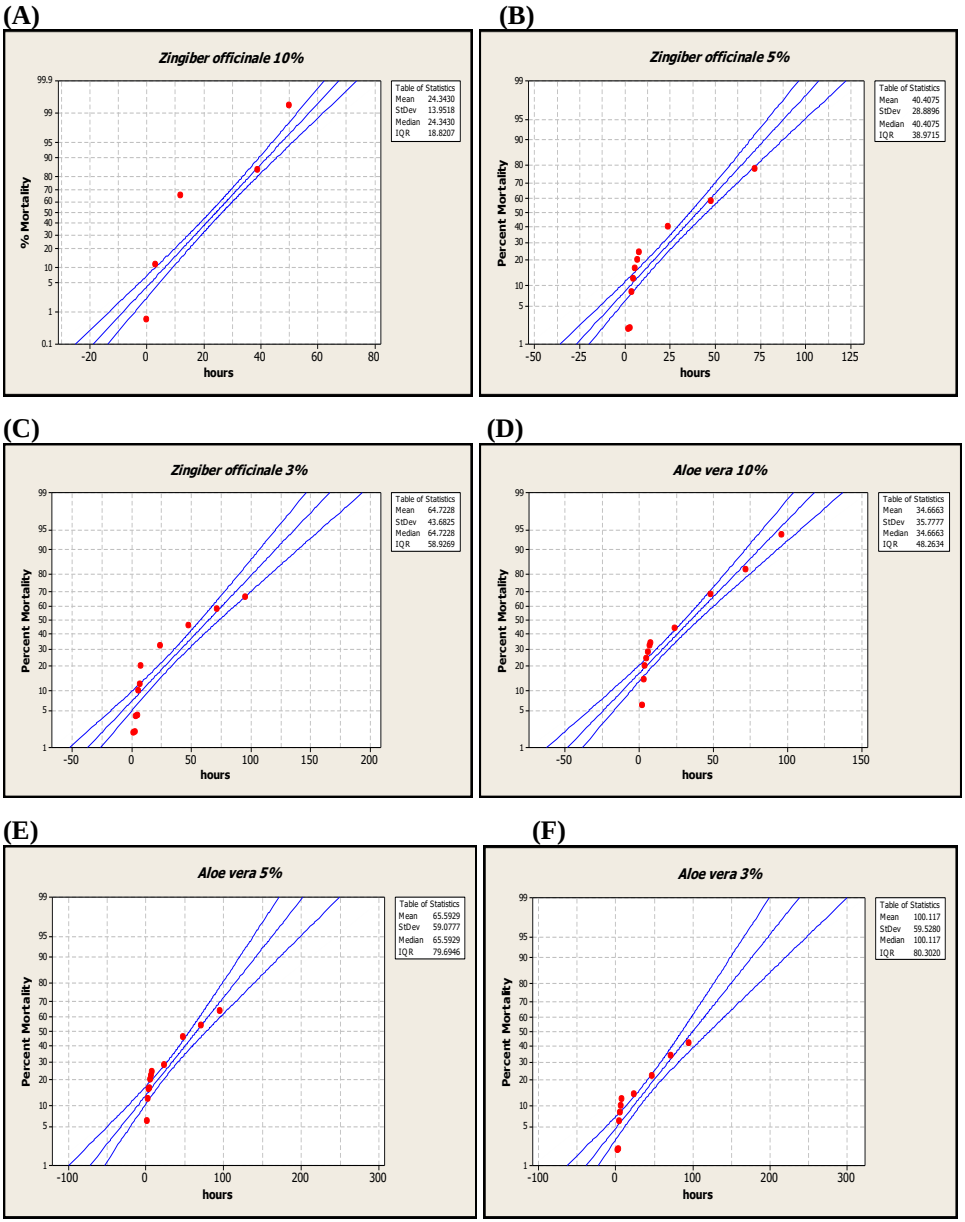


**Figure 2.** (A-C) Probability plot for Regression (R) of *Z. officinale* and (D-F) indicates the probability plot for Regression (R) of *A. vera* at 10%, 5% and 3% concentrations, where S indicates stress time

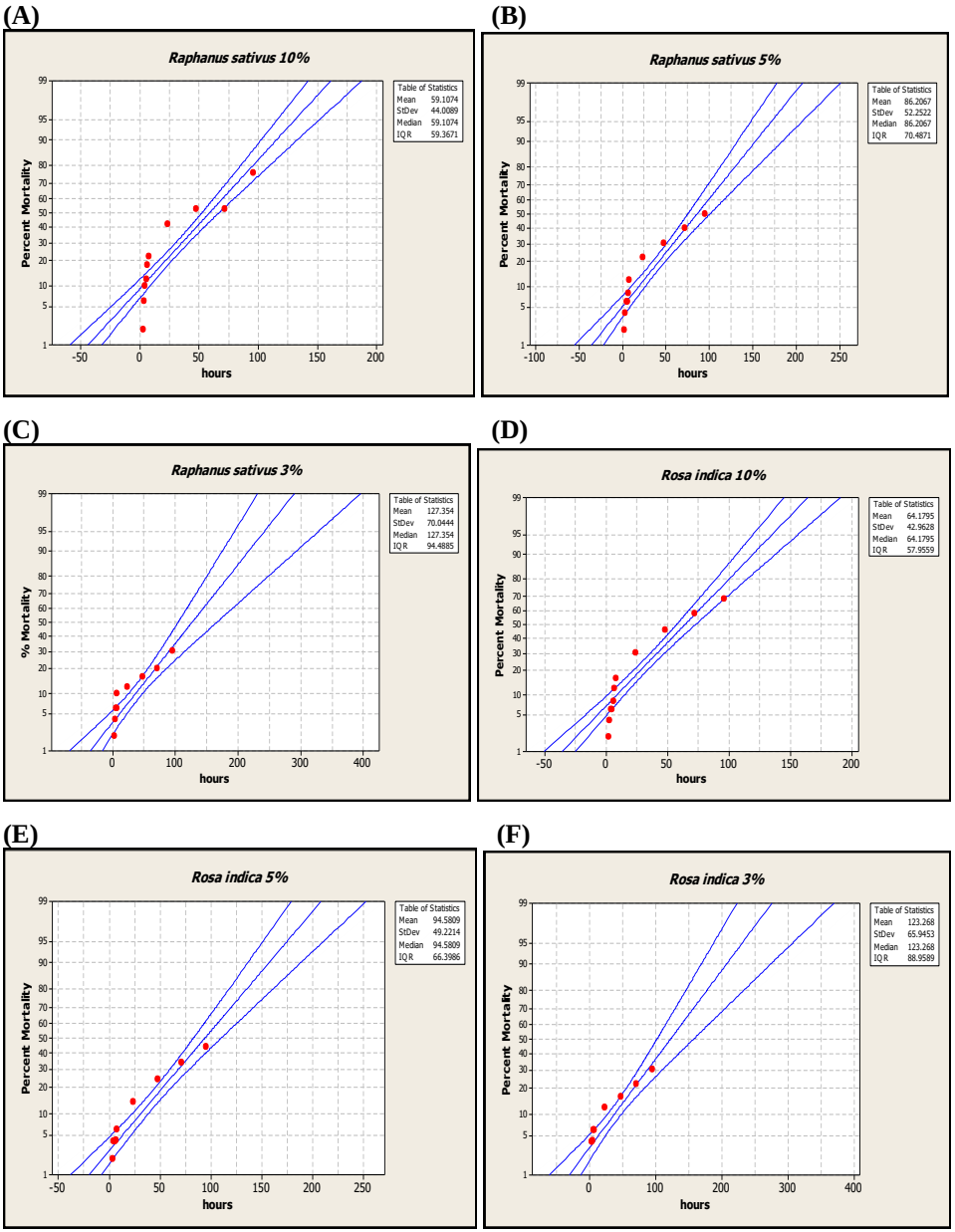


**Figure 3.** (A-C) Probability plot for Regression (R) of *Raphanus sativus* and (D-F) indicates the probability plot for Regression (R) of *R. indica* at 10%, 5% and 3% concentrations, where S indicates stress time

**Table 2.** Analysis of Variance to Measure the Effect of Four Different Plant Extracts on *H. indicola*

| Sr. No | Extracts of | Degree of freedom | Sum of squares | F |
|---|---|---|---|---|
| Within groups | *Zingiber officinale* | 2,6,8 | 387.55 | 249.14 |
| | *Aloe Vera* | 2,6,8 | 794.89 | 223.6 |
| | *Raphanus sativus* | 2,6,8 | 554.00 | 92.33 |
| | *Rosa indica (*petals) | 2,6,8 | 328.22 | 50.93 |
| Between groups | 10% | 4,10,14 | 2351.07 | 191.66 |
| | 5% | 4,10,14 | 751.33 | 134.17 |
| | 3% | 4,10,14 | 510.27 | 83.20 |

**Table 3.** Repellence Test of Four Different Plant Extracts Against *H. indicola*

| Plant Extracts | Concentrations | Treated | Untreated |
|---|---|---|---|
| | | (%) | |
| *Zingiber officinale* | 10 | 23 | 77 |
| | 5 | 16.6 | 83.3 |
| | 3 | 20 | 80 |
| *Aloe vera* | 10 | 16.6 | 83.3 |
| | 5 | 13.3 | 86.6 |
| | 3 | 13.3 | 86.6 |
| *Raphanus sativus* | 10 | 23 | 77 |
| | 5 | 20 | 80 |
| | 3 | 20 | 80 |
| *Rosa indica* | 10 | 36.6 | 63.3 |
| | 5 | 36.6 | 63.3 |
| | 3 | 40 | 60 |

## 4. Discussion

The current study was designed to assess the efficacy of plant extracts against termite (*H. indicola*). *Z. officinale* was found to be the most effective among all plant species in killing termites. These results are supported by the study of [24]. They reported 100% mortality of *H. indicola*, when treated with the aqueous extract of *Z. officinale*. Moreover, our results are further supported by the findings of [25], who studied the effect of *Z. officinale* extract on *Macrotermes bellicosus* and reported significant mortality and high repellency of this extract against *M. Bellicosus.* The repellent effects of *Z. officinale* were also proved against whitefly (*Bemisia argentifolii*) [24, 26].

The effect of *A. vera* extract against termites is reported in this study for the first time. Our findings are further assisted by [27], who tested *A. vera* extract against *Anopheles stephensi* and recorded 81% mortality at 10% concentration against the third instar of *A. stephensi*. Similarly, [28] also tested the effectiveness of *A. vera* at

10% concentration against armyworm larvae (*Spodoptera frugiperda)* and found that almost half of the population was eliminated. Our study concluded that *A. vera*, when used at the same concentration, eliminates 94% of the *H. indicola* population. There is no published work on the application of *R. sativus* extract against termites. However, the plant contains raphanin, which acts as an antibacterial and antifungal force [29, 30]. *R. sativus* extract possesses antimicrobial properties against food spoilage bacteria, such as *Listeria*, *Enterococcus* and *Micrococcus* [30]. Many studies have been conducted to overcome termites using the alcoholic extracts of flowering plants [31-35].

Repellence test was also performed to estimate extract efficiency against *H. indicola.* Similarly, [25] studied the repellence of different plant species against termite (*Macrotermes bellicosus*). The author observed that *Z. officinale* repellency increases with respect to its increase in concentrations. Likewise, when *Z. officinale* combined with *A. sativum*, 81.8% repellency was observed at low concentration (10%) and 88.81% was observed at high concentration (30%). The author in [36] studied the repellence of *A. vera* against the stored grain pest of *Sitophilus oryzae* L. (Coleoptera: Curculionidae). He concluded that out of 45 extracts, 6 samples showed repellence and 4 had the highest repellence, up to 90%. Previously, *A. vera* was used against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogene* [37-39]. The antitermitic activity of *R. indica* is still poorly understood because of the lack of adequate research. The authors in [40] reported the antimicrobial properties of *R. indica* against different pathogens, that is, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, with the zone of inhibition being 26, 27 and 25 mm, respectively. Furthermore, [41] studied different extracts of *R. Sativus* against the mealy bug *Solenopsis tinsley* (Hemiptera: Pseudococcidae) and concluded that ethyl acetate ($LC_{50}$ 421.455 ppm) was the most effective, followed by methylene chloride ($LC_{50}$ 674.960 ppm) and petroleum ether ($LC_{50}$ 875.856 ppm) after 72 h.

## 5. Conclusion

Ethanol extracts of *Z. officinale* and *A. vera* can be used to quickly control the termite species. Similarly, high concentrations of *R. Sativus* and *R. indica* have the potential to control termite as well, as all these extracts were found toxic against *H. indicola*. They are required to be tested further against other termite species found in Pakistan for the cost effective and environment friendly management of termites.

## Conflict of Interest

The author declares no conflict of interest.

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