Efficacy of Ethanic plant extracts of Zingiber officinale, Raphanus sativus, Rosa indica and Aloe vera against Heterotermes indicola

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Abstract

The present study was performed to evaluate the anti-termitic potential of four common plant species of Zingiber officinale, Raphanus sativus, Rosa indica, and Aloe vera. The worker and soldier cast of H.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 dried plant material into fine powder. Different concentrations were made and 0.5 ml of each concentration was poured on filter paper and placed in petri plate (arena). In laboratory bioassay, fifty active workers of H. indicola along with five soldiers were added in each arena. The biological activity of ethanolic extracts of these plants was evaluated after 24 h until 96 h. The ethanol extract of Z. officinale caused the highest mortality (100%) while the least mortality (68%) was observed in R. indica extract. The LT50 for
Present findings suggested that these plant extracts can provide environmental friendly management of *H. indicola*. In *A. vera*, significant mortality was observed against the *H. indicola* i.e. 94%, 64% and 44% on exposure of 10%, 5% and 3% concentrations. While LT\textsubscript{50} were 34.66, 65.59, and 100.1 hours against 10%, 5% and 3% concentrations. In *R. sativus*, mortality of *H. indicola* was 76%, 50% and 30% while LT\textsubscript{50} against was 59.10, 86.20, and 127.3 h, against the concentration of 10%, 5% and 3%. Least mortality was observed in *R. indica* i.e. 68%, 44% and 30% while LT\textsubscript{50} were 64.17, 94.58 and 125.5 h at 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 *Heterotermes indicola* to minimize the damage.

**Keywords:** *Aloe vera*, *Heterotermes indicola*, LT\textsubscript{50}, *R. Sativus*, and *Z. officinale*

1. **Introduction**

Termites have been mostly characterized by their colonial behaviour; belong to insect order Isoptera [1, 2]. They are of medium size, light colour, polymorphic, soft bodied and cellulose eating insects [1, 2, 3]. The termites consist of more than 90 families distributes in 370 genera which was economically important to crops [4]. A total of 2650 species of termites have been reported worldwide. They are an important component of tropical and sub-tropical ecosystem [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 places of Pakistan [8, 9]. Termicides that are mostly used include chlordane, dieldrin, heptachlor, sodium acarcinite, depachlor and aposide lindare. The outcome of these termicides is outstanding but they become incorporated in food chain and remain persistent [10, 11, 12, 13].

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

The researchers reported many plant species which can be used against termites and possessing the antitermitic attributes i.e. 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 (Syzygium aromaticum) [18, 19]. Calotropis procera [18, 20], Coleus amboinicus [20], Isoborneol [18, 21] and Calotropis procera [18, 20].

Natural antitermitic compounds affect differently on different termite species [22, 23]. Another study evaluated the toxic potential/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* [25]. Present research was performed to evaluate the antitermitic potential of ethanolic extract of *Z. officinale*, *R. sativus*, *R. indica* and *A. vera* against *Heterotermes indicola* (workers and soldiers). These bioinsecticides can be used against the control of termites and will not pollute the environment, being ecofriendly.

2. METHODOLOGY

2.1. Collection of termites & 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 Zoology department lawn, University of the Punjab, Lahore. This was later sieved and sterilized. 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 leaves of *A. vera* were taken from Botanical garden, University of the Punjab, Lahore. However *Z. officinale* and *R. sativus* were purchased. Through washing was done using distilled water and were dried under shade for two weeks.
Extracts were prepared using Soxhlet Extractor. HR 2118 Philips grinder was used to crush dried plant material into fine powder. Powdered plant material wrapped into filter paper was placed into Soxhlet Extractor with 200 ml of absolute Ethanol. Isomantle was used for heating Ethanol. Extracts were obtained after completion of six cycles, kept in Reagent bottles from which 10%, 5% and 3% dilutions were prepared.

Anti termitic assay

Anti termitic assay was performed using [26]. The whatman 42 filter paper C were placed in each petri plate. 0.5 ml of each concentration was poured on filter paper with a micropipette. 50 active workers of *H. indicola* along with five soldiers were added in each Petri -plate. Readings were taken for the first eight hours and then readings were taken after 24 hours and finally up to 96 hours.

\[
\text{Mortality rate in percentage} = \frac{\text{Dead termites}}{\text{Total termites}} \times 100
\]

Repellency Assay

Repellency test was performed using filter papers of 9 cm diameter and cutted into two. The one half of filter paper was exposed with 10%, 5%, 3% concentrations and considered as treated (T). The second half was considered as untreated (UT). 10 termites were released into the gap between the filter paper. Three replicates of each concentration were maintained. After 15 minutes, termite’s number was count on each half of filter paper and maintains experiment till 120 minutes. To minimize effect of light, whole setup was covered with black cloth. A concentration was considered repellent if 21 or more termites (out of 30) were present on the untreated area.

Statistical analysis

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

3. Results

Highest mortality within 96 hours was recorded with *Zingiber officinale* extract against the termite (*H. indicola*) and causes death to 100% population in 10% and 5% as well. While 66% mortality was observed in 3% concentrated extract. In *A. vera* extract, significant mortality was also observed against *H. indicola* which leads to 94% mortality at 10%, 64% at 5% and 44% at 3%. In *R. sativus* extract, moderate was observed against the *H. indicola* i.e. 76%, 50% and 30% while *R. indica* extract causes the least mortality of 68%, 44% and 30% (Fig 1).
The LT50 were also assessed to estimate the doses with respect to time for all the plant extracts against *H. indicola* (Table I and II). The LT50 values for *Z. officinale* were 24.34, 40.40 and 64.7 h for concentration of 10%, 5% and 3% while in, *A. vera* LT50 were 34.66, 65.59, and 100.1 h respectively (Fig 2). The LT50 of *R. sativus* extract was 59.10, 86.20, and 127.3 h, while 64.17, 94.58 and 125.5 h were observed in *R. indica* at 10%, 5% and 3% concentrations (Fig 3). The repellence test was also performs to estimate the extract efficiency against the *H. indicola*. The extract was found to be repellent. The repellence between the different concentrations of each plant extracts was non-significant. The repellence between each extracts, *Rosa indica* show low level of repellence with respect to other three plants extracts (Table III).

4. Discussion

The present study was design to assess the plant extracts against the termite (*H. indicola*). The *Z. officinale* was found to be most effective on all plant species. These results are supported by the study of [27]. They reported 100% mortality of *H. indicola* when treated with aqueous extract of *Z. officinale*. Whereas our results recordings are further assisted by the findings of [28], who studied effect of *Z. officinale* extract on *Macrotermes bellicosus* and reported significant mortality and high repellency against *M. Bellicosus*. *Z. officinale* has also proved its repellent effects against whitefly (*Bemisia argentifolii*) [27, 29].

The effect of *A. vera* extract against termites has been reported for the first time. Our recordings are further assisted by [30], who tested *A. vera* extract against *Anopheles stephensi* 81% mortality was recorded at 10% concentration against third instar of *A. stephensi*. [31] also tested the *A. vera* effectiveness at the concentration of 10% against the armyworm larvae (*Spodoptera frugiperda*) and almost half population was eliminated. Our studied concluded that *A. vera* at same 10% eliminates 94% of *H. indicola* population. There is no published work on the application of *R. sativus* extract against termites but plant contains raphanin, which act as antibacterial and antifungal [32, 33]. *R. sativus* extract possess antimicrobial properties against food spoilage bacteria like *Listeria, Enterococcus* and *Micrococcus* [33]. Many studies had been conducted to overcome the termite’s species with the alcoholic extracts of flowering plants [34-38].

The repellence test was also performs to estimate the extract efficiency against the *H. indicola*. [28] studied the repellence of different plants species against termite (*Macrotermes bellicosus*). He observed that *Z. officinale* repellency increases with respect to increase
concentrations. While, when *Z. officinale* combined with *A. sativum*, 81.8 % repellency was observed in low concentration (10%) and 88.81 % at high concentration (30%). [39] 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 which 4 have highest repellence up to 90%. Previously *A. vera* was used against the *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes* [40-42]. The antitermitic activity of *R. indica* was still poorly understood because lack of sufficient work. Although [43] reported the *R. indica* antimicrobial properties against different pathogens i.e. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* with the zone of inhibition 26, 27 and 25 mm respectively. [44] 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. While, high concentrations of *R. Sativus* and *R. indica* have a potential for termite control as well. As all these extracts were found toxic against *H. indicola*, they are further required to be tried against other termite species of Pakistan for cost effective and environmentally friendly management.

Conflict of Interest

The author declare no conflict of interest

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Table I: LT50 values (Hours) of different extracts at 10%, 5% and 3% concentrations

| Sr. No | Extracts                  | 10%   | 5%   | 3%   |
|--------|---------------------------|-------|------|------|
| 1.     | *Zingiber officinale*     | 24.34 | 40.40| 64.7 |
|        | (Ginger)                  |       |      |      |
| 2.     | *Aloe Vera* (*Aloe Vera*) | 34.66 | 65.59| 100.1|
| 3.     | *Raphanus sativus*        | 59.10 | 86.20| 127.3|
|        | (Radish)                  |       |      |      |
Table II: Analysis of Variance for the effect of four different plant extracts on *H. indicola*

| Sr. No | Extracts of           | Degree of freedom | Sum of squares | F     |
|--------|-----------------------|-------------------|----------------|-------|
|        | *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 |
|        | **Within groups**     |                   |                |       |
| 4.     | *Rosa indica* (Rose petals) | 64.17 | 94.58 | 123.2 |
| 5.     | **Control**           |                   | 221            |       |
|        | **Between**           |                   |                |       |
|        | 10%                   | 4,10,14           | 2351.07        | 191.66|
|        | 5%                    | 4,10,14           | 751.33         | 134.17|
Table III: Repellence test of four different plant extracts against *H. indicola*

| Plant Extracts  | Concentrations | Treated (%) | Untreated (%) |
|-----------------|----------------|-------------|---------------|
|                 | 10             | 23          | 77            |
| Zingiber officinale | 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            |
Fig 1. Percentage mortality of four plant extracts against *Heterotermes indicola*

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(A) ![Zingiber officinale 10%](image1)

(B) ![Zingiber officinale 5%](image2)

(C) ![Z. officinale 10% Table of Statistics](image3)

(D) ![Z. officinale 5% Table of Statistics](image4)

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Table of Statistics:

- **Z. officinale 10%**
  - Mean: 24.3430
  - Standard Deviation: 13.9518
  - Median: 24.3430
  - Interquartile Range: 18.8207

- **Z. officinale 5%**
  - Mean: 40.4075
  - Standard Deviation: 28.8896
  - Median: 40.4075
  - Interquartile Range: 38.9715

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10%  5%  3%  Control

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% Mortality of *H. indicola*
Fig. 2: (A-C) Probability plot for Regression (R) of *Zingiber officinale* and (D-F) indicates the probability plot for Regression (R) of *Aloe vera* at 10%, 5% and 3% concentration where S indicates stress time.
Fig. 3: (A-C) Probability plot for Regression (R) of *Raphanus sativus* and (D-F) indicates the probability plot for Regression (R) of *Rosa indica* at 10%, 5% and 3% concentration where S indicates stress time.