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Effects of Three Plant Extracts on the Repellency, Toxicity and Tunneling of Subterranean Termite *Heterotermes Indicola* (Wasmann)

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ABSTRACT: Present study was carried out to determine the anti-termitic activity of three local plants extracts Curcuma longa (Turmeric), Nerium indicum (Oleander) and Melia azedarach (Derek), against subterranean termites Heterotermes indicola (Wasmann). Results revealed that during choice test, all extracts proved to be repellent while in No-choice test extracts proved to be toxic by Direct contact and Indirect exposure test against Heterotermes indicola (Wasmann). In order to find the barrier potential of these extracts, soil barrier test was conducted, at higher concentrations of the extracts barriers were not penetrated by termites, but at lower concentration of Nerium indicum (Oleander) and Melia azedarach (Derek) extracts few tunnels were built at the barrier site. Turmeric extract was found to be more efficient in soil treatments to protect a food substrate against termites as termites did not cross the barrier at both concentrations.

Keywords: *Heterotermes indicola*, mortality, repellency, tunneling, plant extracts.

1.0 INTRODUCTION

Heterotermes indicola (Wasmann) is widely distributed and most notorious subterranean, wood destroying termites in Pakistan. The feeding galleries made by these termites were large; packed with dirty brown color excrements [1]. The intensive use of pesticides produces side effects on many beneficial insects and show acute and chronic effects on living organisms [2]. However, biopesticides utilization is based on the fact that these compounds are plant origin and safer in usage without phototoxic properties [3, 4]. Recently, there has been increasing interest for the development of environment friendly and botanical pesticides, microbial sprays and insect growth regulators [4, 5]. Present study was carried out keeping in view the damages caused by subterranean termites to buildings and agricultural fields of Pakistan and to find out the most effective natural plant extracts as an alternative of chemical pesticides

2.0 MATERIALS AND METHODS

2.1 Test Insect. Subterranean termites *Heterotermes indicola* (Wasmann) were collected by installing traps at termites infestation areas and colony was maintained at 25 ± 1°C and 80 ± 5% RH in plastic container. Colony was fed with moist filter paper serving as a substrate and water resource in laboratory.

2.2 Plants Extracts. Plants extracts were obtained from rhizome of *Curcuma longa* (Turmeric), leaves of *Nerium indicum* (Oleander) and Bark of *Melia azedarach* (Derek) local plants. Each extract was tested for repellent and toxic effects at different concentrations. Ethanol was used as solvent for dilution of each stock solution. Extracts and solvents were applied to filter paper disks or soil 12hrs before testing so that solvents may evapo 107

2.3 Bioassays: A method obtained from litera was adopted with some modifications [6].

2.3.1 Direct choice test: In direct choice test, the test arena consisted of glass Petri plate, divided into two equal parts as treated and untreated portions. Treated portion consisted of extract treated filter paper disk (0.6cm) and untreated part contained ethanol treated filter paper disk (0.6 cm) about 2.5cm apart from each other as shown in Fig1.

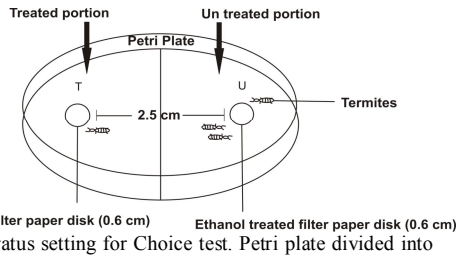

Fig 1: Apparatus setting for Choice test. Petri plate divided into two equal parts as treated and untreated

Different extracts were tested at 100%, 50% and 10% dilutions. Three replicate for each test and control experiments were also conducted. Number of termites in contact with each filter paper was counted for 60 minutes after every 5minutes (n=12 counts).

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2.3.2 Toxicity: In direct exposure test, 0.5% Neutral red dye solution was used as marking dye to determine the digestive toxicity. A moistened, red stained, extract treated cellulose filter paper (3cm diameter) was placed at the bottom of a covered glass Petri plate. 20 termites' workers and soldiers were introduced in test arena as shown in Fig 2 control units consist of blank control contained untreated, red stained moistened, filter paper disk, solvent control consist of red stained solvent (ethanol) treated filter paper disk and starvation control unit containing 3grams of moistened soil covering the bottom of the Petri plate instead of the filter paper. There were three replicates for treated and control units.

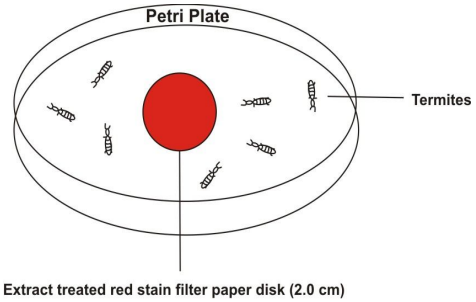


Fig 2: Apparatus setting for Direct exposure test. An Extract treated red stain filter paper present in the center of the Petri plate.

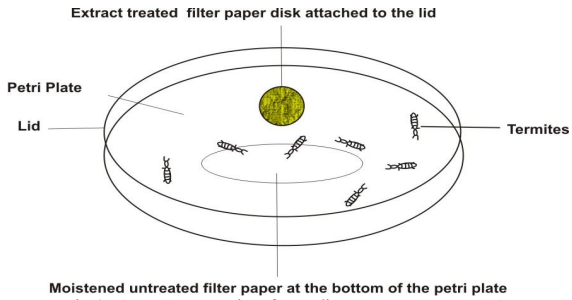


Fig 3: Apparatus setting for Indirect exposure test. An extract treated filter paper disk attached to the Petri plate lid.

During the indirect exposure test, an extract treated filter paper disk (2 cm diameter) was attached to the lid of the Petri plate as shown in Fig 3. Throughout these experiments termites had no direct contact with extract treated filter Paper disk. The bottom contained the moistened, untreated filter paper disk (5 cm diameter) for food and water supply. Twenty (20) termite workers and soldiers were introduced in test arena. Again all extracts were tested in dilutions of 10%, 2% and 1% of the stock solution. Blank and solvent controls were conducted with untreated, unstained moistened filter paper and solvent treated unstained filter paper respectively. Starvation control unit containing 3grams of moistened soil covering the bottom of the Petri plate instead of the filter paper. Three replicates were conducted for treated and control units. This experiment was conducted for about seven days and each day's percentage mortality was calculated per day.

2.3.3 Soil barrier test: The test arena consisted of plexi glass container (10×10 ×10). Each arena was prepared by with a 2cm deep layer of moistened autoclaved soil and two food substrate diagonal to each other 1 cm apart from the wall of the container. Each food substrate consists of 2 cm long cellulose filter paper. This arrangement provides enough space for introducing termites and placing them at the bottom of the arena where tunneling activity can be observed. Each experiment was performed with two different concentrations i.e. 35% and 25% of the stock solution. The soil was divided in to two equal parts with an area in the center devoid of any soil in between the soil partition. In the separate Petri plate, about 5 gram of moist soil was taken and required concentration i.e. 25.0% or 35.0% was mixed in it thoroughly. This extract treated soil was added to the middle portion of the plexi container. In this way a 1.5 cm wide by 2 cm deep horizontal barrier of required concentration of extract treated soil was constructing in the center of the arena as shows in Fig 4.

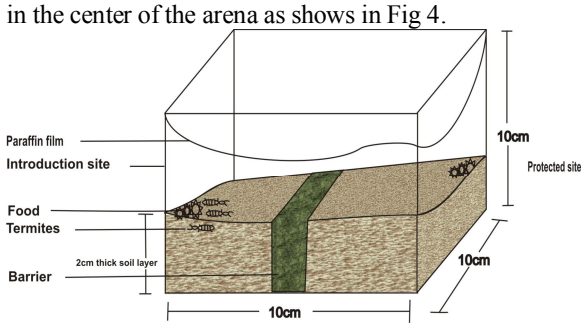


Fig 4: Test arena and barrier arrangement of soil barrier test. A trench of extract treated soil in the center of the test arena divided the untreated soil in to two parts. 50 termites workers and soldiers i.e. *Heterotermes indicola* (Wasmann) were introduced in to food substrate ( ) Termites survival (quantitative) and penetration of treated soil (qualitative) were recorded after 7 days. A second food substrate was located at protected site ( ).

About 50 termites were introduced in the test apparatus. The area where termites were introduced in to food substrate termed as introductory site and while opposite site termed as the protected site. In order to maintain humidity, the whole set up was covered with a paraffin film. All this set up was kept in darkness for the maximum activity of termites for about seven days. Three replicates were conducted and same procedure was repeated with the all stock formulations and for all plant extracts. The control

unit consisted of same procedure but in this case barrier consisted of solvent treated soil barrier in case of solvent control and water treated soil barrier in case of water blank units. After seven days the tunneling habit and infestation of the second food source was observed. Data was compiled by counting number of termites at the introduction site, protective site, at the barrier of the treated soil and number of dead termites.

2.3 Statistic analysis

Mean percentage data were calculated and analyzed by ANOVA. Tukey's standardized range tests were used to compare the difference between treatments using Graph pad prism version 4.00 [7].

3.0 RESULTS AND DISCUSSION

3.1 Results

Results revealed that percentage yield of the extracts for *Curcuma longa*, *Nerium indicum* and *Melia azedarach* were 40.0%, 40.0%, 36.0% respectively. Table 1 shows the mortality of *Heterotermes indicola* (Wasmann) workers and soldiers within 7 days of direct and indirect exposure to extract treated filter paper in the No Choice test. It is evident from the table 1 that each concentration of each extract was sufficient to cause mortality. In control treatments i.e. Starvation control, Blank control (Water) Solvent control (Ethanol), no mortality was observed in direct and indirect exposure tests. Analysis of variance revealed that different concentrations of turmeric, oleander and Derek extracts were significantly different among all treatments as P<0.05 (table1).

Table 1: Mortality of *Heterotermes indicola* (Wasmann) workers and soldiers within 7 days of direct and indirect exposure to extract treated filter paper in no choice test.

	% Mortality[a]			Difference in mortality between direct and indirect exposure test by Unpaired t-test[b]
Experiment	**Direct exposure**	**Dyed termites**	**Indirect exposure**	
Starvation control (Soil)	0.00 ± 0.000[a]	Yes	0.00± 0.000[a]	**No mortality**
Blank control (Water)	0.00 ± 0.000[a]	Yes	0.00± 0.000[a]	**No mortality**
Solvent control (Ethanol)	0.00±0.000[a]	Yes	0.00 ± 0.000[a]	**No mortality**
Turmeric 10.0%	20.00 ± 0.000[b]	No	14.33 ±0.333[b]	**Yes (P < 0.05) P value= 0.0490 df= 4**
Turmeric 2.0%	16.66 ± 0.333[c]	No	12.66± 0.333[c]	
Turmeric 1.0%	14.33± 0.333[d]	No	10.33± 0.333[d]	
Oleander 10.0%	20.00 ± 0.000[b]	No	13.00± 0.000[e]	**Yes (P < 0.05) P value= 0.0456 df= 4**
Oleander 2.0%	15.33 ± 0.333[d]	No	11.66± 0.333[f]	
Oleander 1.0%	13.66± 0.333[e]	No	9.33 ± 0.333[g]	
Derek 10.0%	15.66 ± 0.333[d]	No	11.66± 0.333[f]	**Yes (P < 0.05) P value= 0.0391, df= 4**
Derek 2.0%	12.66 ± 0.333[e]	No	8.66± 0.333[h]	
Derek 1.0%	**11.66± 0.333[f]**	**No**	**7.66 ± 0.333[i]**	

[a]Means followed by similar letters within columns are indicate not-significantly different (P> 0.05, df= 4) and multiple t comparison test. Each mean ± SE is based on 60 termites (3 replicates /20 termites per replicate).
[b]comparison of transformed termites mortality data were made with an Un- paired t- test (Graph pad prism, 2003).
Red stained filter papers were used in these experiments.

Table 2 shows the relative distribution of surviving termites *Heterotermes indicola* (Wasmann) in the test arena having *Curcuma longa, Nerium indicum* and *Melia azedarach* extract treated soil barrier. Barrier was constructed with two different concentrations of the extract i.e. 35.0% and 25.0%. In case of *Curcuma longa* at 35.0% concentration the mean number of termites at the Introduction site was 15.333 ± 0.577, similarly at barrier site 3.333 ± 0.577, while at protected site the mean number of termites was 5.333 ± 0.577. After seven day of the treatment few number of termites were observed making tunnels toward the edge of the arena and reached to the second food source present at the protected site as shows in Fig 6 (C). Termites did not cross the barrier. Large number of the termites died at barrier sites. At 25.0% concentration the mean number of termites at the Introduction site was 14.000 ± 1.000, similarly at barrier site 5.000 ± 1.000, while at protected site the mean number of termites was 7.667 ± 1.155. After seven day of the treatment termites did not cross the barrier in all replicates, but reached to the second food source through the edges of the arena as shows in Fig 6 (D). Analysis of variance also revealed that there was significant difference in the distribution of surviving termites *Heterotermes indicola* at these three sites in test arena treated with turmeric extract as , P= 0.0078, F= 36.48, df= 5. In case of *Nerium*

indicum (Oleander) extract treated soil barrier at 35.0% concentration the mean number of termites at the introduction site was 14.000 ± 0.000, similarly at barrier site 5.333 ± 0.577 while at protected site the mean number of termites was 6.667 ± 1.155. After seven day of the treatment few numbers of termites reached to the second food source present at the protected site termites through edge as shows in Fig 7 (E) effect but did not cross the barrier. Large number of the termites died at barrier sites. At 25.0% concentration the mean number of termites at the introduction site was 12.333 ± 1.155, similarly at barrier site 6.667 ± 1.155 while at protected site the mean number of termites was 9.667 ± 0.577. In two of three replicate, showed tunnels passed through the extract treated soil barrier as shows in Fig 7 (F). Analysis of variance also revealed that there were significant difference in the distribution of surviving termites *Heterotermes indicola* (Wasmann) at these three sites in test arena treated with Oleander extract as F= 18.59, df= 5, P= 0.0204 the relative distribution of surviving termites *Heterotermes indicola* (Wasmann) in the test arena having Derek extract treated soil barrier. At 35.0% concentration the mean number of termites at the Introduction site was 11.667 ± 0.577, similarly at barrier site 7.000 ± 1.000, while at protected site the mean number of termites was 7.333± 0.577. After seven day of the treatment few number of termites made tunnel toward the edge of the arena and reached to the second food source, but did not cross barrier at higher concentration of the extract as shows in Fig 7 (G). At 25.0% concentration the mean number of termites at the Introduction site was 10.667 ± 0.577, similarly at barrier site 6.333 ± 0.577577 while at protected site the mean number of termites was 8.333 ± 0.577. In one of the replicate termites cross the barrier and reached to the seconds food source where these termites infestated the second food source as shows in Fig 7 (H). Analysis of variance also revealed that there was significant difference in the distribution of surviving termites *Heterotermes indicola* (Wasmann) at these three sites in test arena treated with Derek extract as F= 26.777, df= 5, P= 0.0122. In case of both control units relative mean number of termites at all sites was not different. In solvent control and water control units termites were relatively equally distributed in test arena, Protected site and barrier sites highly infestated in test arena after seven day of the treatment in both control units and shows high frequency of tunneling behavior as show in Fig 6 (A and B) .

Table 2: Survival of *Heterotermes indicola* (Wasmann) and distribution of surviving termites after 7 days with different extracts treated soil barrier

plant extracts	Relative distribution of termites in test arena			
	Trials	**Introduction site**	**Barrier**	**Protected site**
Turmeric	**Turmeric 35.0%**	15.33 ± 0.577[a]	3.33 ± 0.577[a]	5.33 ± 0.577[a]
	Turmeric 25.0%	14.00± 1.000[b]	5.00 ± 1.000[b]	7.66± 1.155[b]
	Solvent control (Ethanol)	15.33± 1.528[c]	11.66 ± 1.528[c]	13.00± 1.000[c]
	Blank control (Water)	13.33± 1.528[d]	12.00 ± 2.000[d]	18.00 ± 1.000[d]
Oleander	**Oleander 35.0%**	14.00 ± 0.000[a]	5.33 ± 0.577[a]	6.66 ± 1.155[a]
	Oleander 25.0%	12.33 ± 1.155[b]	6.66 ± 1.155[b]	9.66 ± 0.577[b]
	Blank control (Water)	14.33 ± 1.528[c]	12.33 ± 0.577[c]	15.66 ± 1.155[c]
	Solvent control (Ethanol)	13.33 ± 0.577[d]	11.66 ± 1.528[d]	17.33 ± 0.577[d]
Derek	**Derek 35.0%**	11.66 ± 0.577[a]	7.00 ± 1.000[a]	7.33 ± 0.577[a]
	Derek 25.0%	10.66 ± 0.577[b]	6.33 ± 0.577[b]	8.33 ± 0.577[b]
	Solvent control (Ethanol)	15.00 ± 1.732[c]	13.00 ± 1.000[c]	16.00 ± 1.000[c]
	Blank control (Water)	14.33 ± 1.528[d]	12.33 ± 1.528[d]	17.33 ± 2.082[d]

*Means followed by similar letters within columns are indicate not-significantly different (P> 0.05, df= 5) ANOVA and Tukey's multiple comparison test (Graph pad prism, 2003). Each mean ± SD is based on 150 termites (3 replicates /50 termites per replicate).

3.2 Discussion

Previous study shows that these plants have been used for anti bacterial, antifungal, anti viral and as insect repellent. Turmeric used to repel house hold insects. Oleander and Derek were also used to repel different type of noxious, problematic insects. The repellent and toxic effect of different plant extract on subterranean termites (Isoptera:Rhinotermitidae) has been reported [6]. Toxic effect of leaf extract of *Polgonum hydropiper* and *Pogosteman parviflours* against the termites have also been studied [8]. Extract treated soil barrier was constructed to find

effectiveness of these extract against termites. Termiticidal treated soil barrier have been widely used for the subterranean termites control for the last 50 years [9, 10, 11]. The main objective behind the use of soil barrier or treatment is exclusion of termites from building and other structure in ground contact [12]. The findings of our study are also in coincidence with these studies.

In the soil barrier test, a horizontal barrier of extract was constructed. After seven days of the treatment high mortality rate was observed in all extracts. In the treated soil barrier termites did not penetrate the soil barrier, but only after few days of the treatment tunnels built at the edge of the arena as shown in the Fig 6 and 7. This effect in which tunnels were builds at the edge of the arena called as edge effect. This edge effect is a common feature in the behavior of subterranean termites and can be seen in the field [13]. Tunneling activity reduced in higher concentration of stock solution as compared to lower concentration of the extract. Our results of soil barrier test are in conformity with the results from literature [6]. They reported that untreated and solvent treated control barriers were penetrated by *R. santonensis* termites only after two days. A horizontal barrier with sufficiently high concentration was effective in preventing the subterranean termites from tunneling through extract treated soil barrier and protect the second food sources. According to their experimental work, tunneling activity increased as concentration of stock solution decrease but this statement contradict in case of turmeric extract where in both low and high concentration extract treated barrier was not penetrated as compared to other extracts, but few termites reached to the second food source through edge effect. In control experimental units termites shows high frequency of tunneling activity as compared to treated units, because tunnels were not built and high mortality rate was observed in treated units so we can concluded that these extracts have repellent as well as toxic effects.

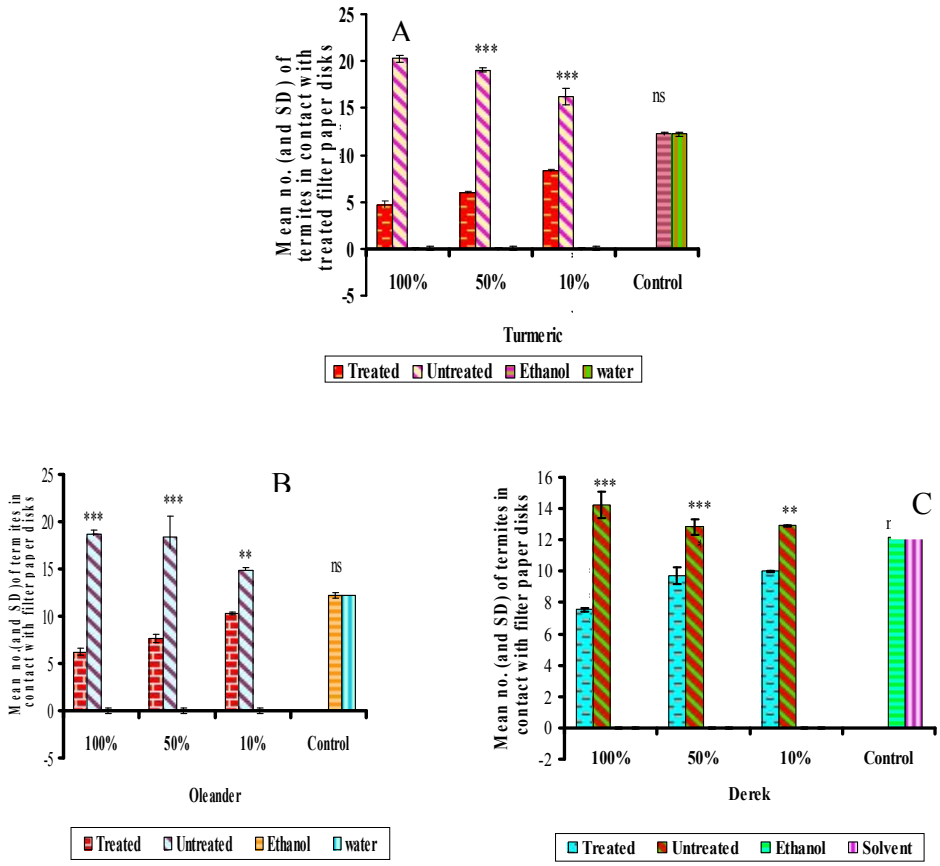


Fig 5: Repellency of *Heterotermes indicola* (Wasmann) for Turmeric (A), Oleander (B) and Derek (C) extract-treated filter paper disks on termite's worker and soldiers in a choice test. Each bar represents the mean ± SD number of termites in contact with a filter paper disks in a 60 minutes period, n=12 counts. Each experiment was evaluated by a paired comparison t-test (*Graph pad prism Version-4*) ***.*P< 0.0001: **. P< 0.001: *. P < 0.01: ns.* no significant difference.

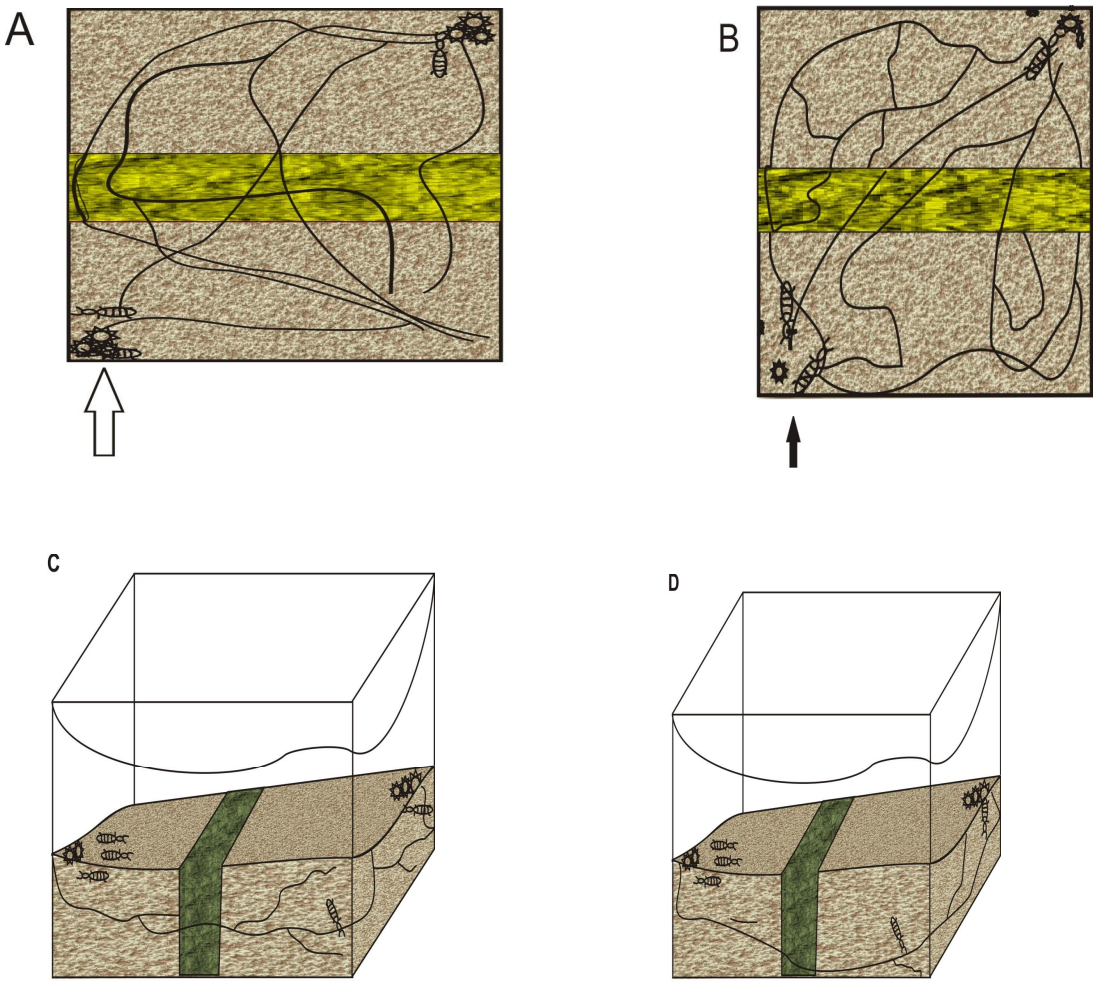
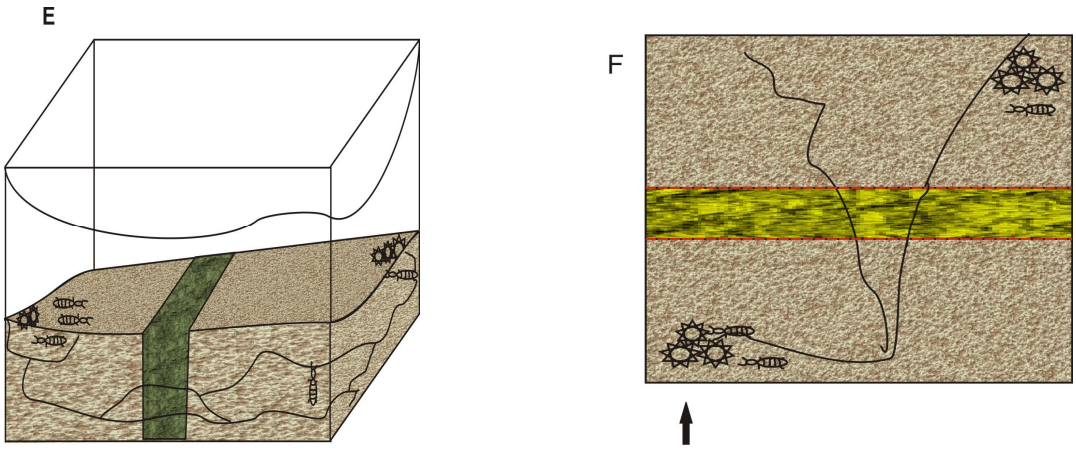


Fig 6: Tunnels built by *Heterotermes indicola* (Wasmann) with in 7 days in test arena with barrier composed of (A) Solvent (Ethanol) treated soil, (B) Blank (Water) treated soil and (C-D) Turmeric extract treated soil at concentration of 35% and 25% respectively. 50 termites worker and soldiers per replicate were introduced in to food substrate ( ) locating in untreated soil.



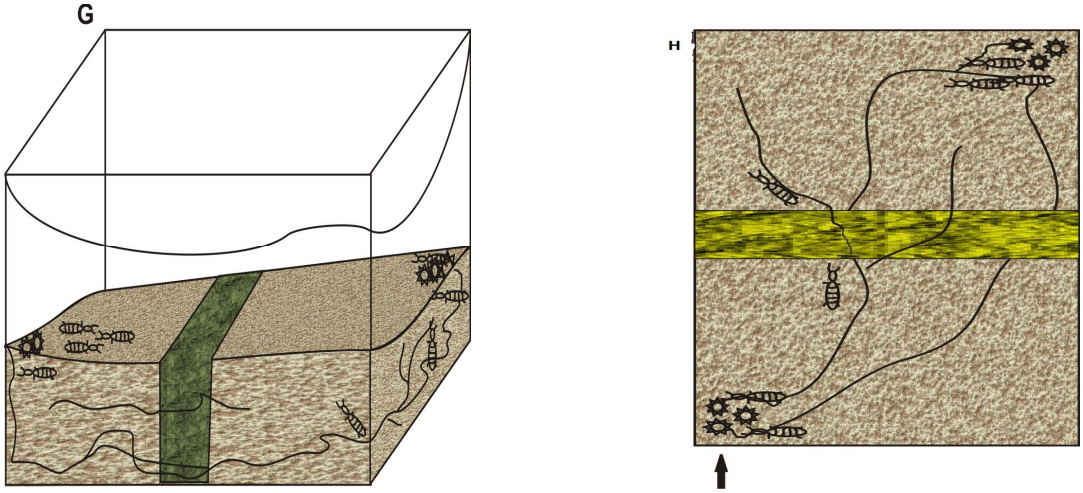


Fig 7: Tunnels built by *Heterotermes indicola* (Wasmann) with in 7 days in test arena with barrier composed of (E-F) Oleander extract treated soil at concentration of 35% and 25% respectively and (G-H) Derek extract treated soil at concentration of 35% and 25% respectively. 50 termites worker and soldiers per replicate were introduced in to food substrate ( ) locating in untreated soil.

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4.0 Conclusion

From the present work, it is concluded that plant extracts of *Curcuma longa*, *Melia azedarach* and *Nerium indicum* in ethanol solvent have termiticidal properties. According to their repellent and toxic effects we can arranged these extracts in the following order

Curcuma longa > *Nerium indicum* > *Melia azedarach*

Such extracts with multiple antitermitic properties will be given priority in future test to isolate antitermitic constituents and determine their mode of action against termites.

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