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Efficacy of single and mixed particles sand size as physical barrier against *Heterotermes indicola* under laboratory conditions

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Abstract
Termites are most important eusocial insect pests known to human beings, spread across the globe and feed on all cellulose containing materials. *Heterotermes indicola* workers cause enormous damages to the host materials. Sand can be a physical barrier to control termites' infestation. *H. indicola* travelled nil distance and made nil number of tunnels in 20 and 30 mesh size particles in 3 cm sand barrier. But in control (60 cm mesh size) it travelled significantly longer distance of 2.13, 3.07 and 3.25 cm, and made significantly more number of tunnels of 1.45 each on day 1, 2 and 3, respectively. *H. indicola* travelled nil distance and made nil number of tunnels in sand mesh sizes of 20 and 30 in 5 cm sand barrier also. But in control (60 cm mesh size), the termite workers travelled significantly longer distance of 1.61, 1.61 and 4.42 cm, while made more number of tunnels of 1 each on day 1, 2 and 3, respectively. The present research findings will devise IPM strategies against *H. indicola* under field conditions.

Keywords: *Heterotermes indicola,* Sand particles, distance travelled, tunnels made.

1. Introduction

Termites are one of the most important eusocial insects [1], known to human beings [2], causing heavy damages by feeding on all cellulose containing materials [3]. Workers are the only damage causing caste [4], having soft body and perform different works of the colony like foraging, cleaning, making of mud tubes, etc. [1, 4, 5]. Their feeding ability depends on the species, size and quality of food located [1]. Subterranean termites have very good searching system consisting of branching gallery system, with architecture [6]. Mud tubes are exploratory in nature and can help in finding the potential termite's infestation [5].

Worldwide, 80% of subterranean termites are considered as a pest [7] and cause severe losses to both structural wood and forests trees. Annually, there is about more than 3 billion dollars damage caused by the termites and about 80% of which is only caused by subterranean termites in U.S [8]. Out of 53, eleven species of termites are of economic importance in Pakistan including *Heterotermes indicola* [9].

H. indicola is a destructive subterranean termite's species which makes galleries or satellite nests to reach the food source [10]. *H. indicola* was a devastating pest when left untreated, causing damage to apricot (5.68%), pear (7%), plum, peach, oranges and leman at Tarnab, Lala kally and The University of Agriculture, Peshawar [11, 12]. To control destructive termites, various kinds of management systems have been developed including chemical and physical control methods [10]. Physically termites can be controlled by using basaltic grains, glass particles, sand, etc. A non-chemical barrier introduced made of graded particles (10 to 16 mesh size sand particle) for the control of termites but due to cheap and easily available termiticides there discovery was neglected until the mid of 80's [13, 14]. The present study aimed to determine efficacy of single and mixed particle sand size as physical barrier against *H. indicola* and to examine its tunneling behavior.

2. Materials and Methods

2.1 *H. indicola* Survey and Sampling

A survey was conducted in search of termite's infestation in Peshawar during 2013-14. Poplar stacks (22 cm) were installed at the infested and visible termite's galleries places with a weekly check for confirmation of infestation. NIFA-TERMAPS were installed at the infested places and checked fortnightly for culture collection [15]. The infested NIFA-TERMAPS along with the debris were brought to the laboratory and inverted in plastic tub (1.5ft diameter).

Several pieces of blotting paper wet with distal water were carefully placed over the termites and debris in the tub, to which live termites were stuck. Then the blotting paper was jerked in another clean tub without transferring any debris and this process was repeated till all termites were transferred to the tub. Then termites were transferred to sterilized Petri dishes, having wet sterilized blotting paper and placed in desiccators at 28 ºC±2 ºC and 80% R.H. after weighing and labeling.

2.2 Determination of single particle size resistant to penetration

Glass tubes of 19 cm were marked in middle for 3 and 5 cm barrier construction. Sieved sand of mesh size 20 (0.85 mm), 30 (0.60 mm), 40 (0.42 mm), 50 (0.30 mm) and a control of 60 (≥ 0.25 mm) (an equal proportional mixture of 60, 70, 80 and pan size sand) was mixed with 18% distilled water followed by the filling of 3 and 5 cm marked glass tubes (19 cm) for making barriers. A single sterilized and weighed 1x3cm blotting paper strip was placed at the one end and termites (50 workers + 2 soldiers) were released at other end of glass tube before sealing the both ends with the aluminum foil and rubber band tightly. Cumulative data of tunneling distance was recorded for three days after 24h. On day 7th the set up was disassembled and data was recorded for % mortality by counting alive termites and weighing the blotting paper consumed. Each mesh size was replicated four times.

2.3 Absence of moisture in single particle size and uniform mixtures of sand

Cotton roll was used at the one end of 5 cm marked glass tube for sealing, which was followed by a 2 cm layer of fine saw dust. Glass tubes were carefully filled with the sterilized single size and uniform mixtures of 0% moisture sand**.** The uniform mixtures of mesh size 20_30 (1:1), 20_30_40 (1:1:1) and 20_30_40_50 (1:1:1:1) were made by mixing single particles prior to filling the glass tubes. Termites (50 workers + 1 soldier) were carefully released after putting glass tubes in the stands through funnel and sealed with aluminum foil and rubber band. Cumulative tunneling data was recorded after every 24h for 3 days. Mortality (%) and blotting paper consumption was noted after disassembling the setup on day 7th. Mesh size 20 was used as a control treatment. Each treatment was replicated five times.

2.4 Statistical analysis

Data was analyzed by using SYSTAT Statistical Package Software, SPSS. Means were calculated by using descriptive statistics and highest significant difference Tukey's HSD was calculated by using one way ANOVA [16].

3. Results and Discussion

3.1 Determination of single particle size resistant to penetration

H. indicola travelled significantly longer mean distance of 2.13, 3.07 and 3.25 cm in 3 cm control barrier on day 1, 2 and 3, respectively (Table 1). *H. indicola* didn't travel any distance (zero distance) in sand mesh size of 20 and 30. The mean number of tunnels made by *H. indicola* in the 3 cm sand was significantly more (1.45 tunnels) in control each on day 1, 2 and 3 while significantly less (zero) tunnels on day 1, 2 and 3 in the sand mesh size 20 and 30.

Table 1: Mean distance travelled (cm) and mean number of tunnels made by *H. indicola* in 3 cm barrier of various sand mesh sizes during 2013-14.

Mesh size	Mean distance ±SE travelled (cm) on			Mean number of tunnels ±SE made on		
	Day1	Day2	Day3	Day1	Day2	Day3
20	0 a	0 a	0 a	0 a	0 a	0 a
30	0 a	0 a	0 a	0 a	0 a	0 a
40	0.24±0.086 b	0.05±0.07 ab	1.00±0.02 b	1±0 b	1±0 b	1±0 b
50	0.65±0.27 bc	1.36±0.20 bc	2.12±0.13 bc	1±0 b	1±0 b	1±0 b
60 (Control)	2.13±0.28 c	3.07±0.27 c	3.25±0.22 c	1.45±0.12 b	1.45±0.12 b	1.45±0.12 b

Means in columns followed by different letters are significantly different at 0.05 level of probability.

The results in table 2 revealed that *H. indicola* travelled significantly longer distance of 1.61, 1.61 and 4.42cm in 5cm control on day 1, 2 and 3 respectively. *H. indicola* didn't travel any distance (zero distance) in sand mesh size of 20 and 30. *H. indicola* made significantly more no. of 1.21 tunnels on day1, 2 and 3 respectively in the 50 mesh size of 5 cm barrier, while significantly less (zero) tunnels were made on day 1, 2 and 3 in the sand mesh size of 20 and 30. The number of tunnels made by *H. indicola* in control was 1 each on day 1, 2 and 3, which was non-significant to mesh size 50.

Table 2: Mean distance (cm) travelled and mean number of tunnels made by *H. indicola* in 5 cm barrier of various sand mesh sizes during 2013-14.

Mesh Size	Mean distance (cm) travelled ±SE on			Mean number of tunnels made ±SE on		
	Day1	Day2	Day3	Day1	Day2	Day3
20	0 a	0 a	0 a	0 a	0 a	0 a
30	0 a	0 a	0 a	0 a	0 a	0 a
40	0.46±0.14b	0.68±0.10 ab	1.16±0.07b	1±0 b	1±0 b	1±0 b
50	0.54±0.18c	0.54±0.18 ab	2.1±0.08 c	1.21±0.11 b	1.21±0.11 b	1.21±0.11 b
60 (Control)	1.61±0.27d	1.61±0.27 c	4.42±0.11d	1±0 b	1±0 b	1±0 b

Means in columns are followed by Standard error and different letters shows significance at 0.05 level of probability.

The present results are first of its kind in testing *H. indicola* at the mentioned particle sizes. The volcanic debris of 1.18 and 2.40 mm sizes used against Philippine subterranean termites and found it effective [17]. Sand particle sizes found effective against *C. vastato* were 1.00 to 2.36, 1.18 to 2.36, and 2.0 to 2.36 mm [18]. Sintered glass particles of size 1.7-2.4 mm were found effective against *C. acinaciformes* and *M. darwiniensis* [19]. Lahar particles of 1.18-2.36 mm diameter were found effective against *C. vastator* penetration [20], while 1.18-1.14 mm to 2.0-2.36 mm of uniform particles prevented *N. luzonicus* and *M. losbanosensis* and 1.70-2.00 mm to 2.00-2.36 mm prevented *M. gilvus* from penetration in Lahar barrier [21]. Basaltic screenings of 1.7-2.4 mm were found effective against two *Coptotermes* species in field and lab bioassays [22]. There was no penetration for smooth sand and crushed particles in mesh size of 8, 10, 12, and 14 (2.8 to 2.36, 2.36 to 2.0, 2.0 to 1.7, and 1.7 to 1.4 mm) respectively, while mesh 7+8 (2.36-3.35 mm) and mesh 16 (1.18-1.4mm) were half tunneled or not, based on particle shape [23]. In field experiments mesh size of 2.00-2.36 and 2.36-2.80 mm were effectively used against *C. formosanus* and *R. flavipes* [24]. It was found that 1.70-2.36 mm and 1.00-2.36 mm particle sizes were less penetrated out of thirteen particle sizes by *C. formosanus* and *R. flavipes* [25].

There was no blotting paper consumption among the treatments as the barriers were not breached. Maximum mortality was caused in all the treatments which can be explained by the fact that termites were dead at other end of tunnel probably because of rupturing there lightly sclerotized body with the particles or cut off or stuck in [22].

3.2 Absence of moisture in single particle size and uniform mixtures of sand

Single particle size of 20, 30, 40, 50 and uniform mixture of 20_30, 20_30_40 and 20_30_40_50 were used with a control of mesh size 20. In all the treatments, 0 cm distance was travelled and 0 mean numbers of tunnels were recorded after 24hr for the first three days. On day 7, 100% mortality and 0 gm blotting paper consumption was recorded. Based on recorded data no statistical analysis was possible because of 0 cm for mean number of tunnels and mean distance travelled with 100% mean mortality and mean 0 gm blotting paper consumption for all treatments.

Various moisture levels (0, 5, 10, 15, 20, and 25 %) used in sand resulted that distribution and wood consumption of *M. crassus* and location preference of *C. gestroi* was effected by changing moisture parameters with no difference on wood consumption [26]. *C. gestroi* workers activity was affected by increase in the moisture [27]. Sands having higher moisture were more tunneled as compared to sand having lower moisture by *C. formosanus* and *R. flavipes* [28].

4. Conclusion

Sand can be used as a physical barrier to control *H. indicola* infestation especially in buildings. The present results revealed that *H. indicola* travelled nil distance and made nil number of tunnels in 20 and 30 mesh size sand particles in 3 cm and 5 cm sand barriers. So, these particle sand sizes can be used in devising IPM strategies against *H. indicola* in buildings and under field conditions.

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