

DETERMINATION OF APPROPRIATE ATTRACTIVE BAIT FOR *HETEROTERMES INDICOLA*

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Received: Jan 26, 2007; Revised: Jun 15, 2007; Accepted: Jun 22, 2007

Abstract

Different constituents including termite body extract, agar and carbohydrates were tested to make bait that is acceptable and palatable for subterranean termite, *Heterotermes indicola*. Extracts of whole termites in different solvents i.e. hexane, acetone and propanol were tested for attractiveness to *H. indicola* using crude body extract and Soxhlet extraction methods. Overall crude body extracts elicited higher attraction response. Among the solvents tested, propanol extract showed a consistent attraction response. Among various carbohydrates tested, 3% glucose increased the *H. indicola* survival. Agar concentration at 3% made the bait more palatable for *H. indicola* than at 2% or 1% agar.

Keywords: Solvents, extract methods, agar, carbohydrates, termite, *Heterotermes indicola*

Introduction

Due to concealed habitat of subterranean termites, insecticidal application that covers all the foraging territories of a colony is very difficult, costly, and hazardous for the non-target soil dwelling fauna. Toxicant baits, instead, provide an efficient way to administer insecticides to the termite colony. Insecticide, even if picked up by a small portion of workers can easily be transferred to the unexposed workers through trophallaxis or social grooming. The insecticide used in this case need to be non-repellent and more importantly, slow-acting enabling its transfer to unexposed nest-mates before it starts inflicting mortality.

Along with the toxicant materials, bait components need to make the bait attractive and

palatable for termite feeding. Cellulose as saw dust is normally the major component used in a holding medium along with other constituents that make the bait attractive. Attraction of bait will largely determine its efficacy. According to Lund (1966), extract of termite bodies is a powerful attractant for termites and has far greater attraction for termites than does water. Matsumura *et al.* (1968, 1969) noted attraction in the whole body extract of *Reticulitermes virginicus* (Banks). He attributed it to a trail pheromone that he isolated later, but its complete chemical structure could not be determined. It was demonstrated that these extracted compounds elicited trail-following behaviour in other species within the genus

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Reticulitermes as well as *Coptotermes formosanus* Shiraki (Matsumura *et al.*, 1972; Ritter *et al.*, 1975, 1977; Howard *et al.*, 1976; Grace 1987; Yamaoka *et al.*, 1987). Since the trail-following substance showed weak species-specificity, it was possible that the chemical was a common trail pheromone among the family Rhinotermitidae.

The presence and utilization of trail pheromone in termites was well documented by several investigators (Stuart, 1961, 1963, 1969; Moore, 1966, 1969). In spite of many research papers on the behavioural aspects of trail tracking, few trail pheromones were identified chemically (Moore, 1966; Hummel and Kurlson, 1968; Kurlson *et al.*, 1968; Birch *et al.*, 1972). Matsumura *et al.* (1968, 1969) mentioned that these trail pheromones could be extracted from the whole body extract using organic solvents and possibly used as termite attractant bait constituents. Some investigators tested certain nutrient compounds such as carbohydrates to improve the bait uptake. Su *et al.* (1982) reported that higher concentrations of agar stimulated gallery building behaviour in termites.

The main objectives of the present studies were to make an attractive termite bait by: (1) evaluating the attraction of termite body extract in different organic solvents (2) testing the survival of termites at different carbohydrates incorporation into the bait and (3) using suitable agar concentrations to get a proper bait consistency.

Materials and Methods

I- Testing Termite Body Extract as an Attractant

Whole body extracts of *H. indicola* were made using crude body extract and soxhlet extraction methods. Crude body extract was attained as indicated Howard *et al.* (1976). Fifty alive termite workers were immersed in each of 2 ml of hexane, acetone and propanol for one minute and then solution was filtered. The other method used was "soxhlet extraction method" as described by (Lund, 1966) where 22.7 g of

alive worker termites were extracted with acetone, hexane and propanol (100 ml each) in soxhlet extractor.

Termite response to the extracts from both methods was studied by using the procedures described by Ahmad *et al.* (1979) with slight modification. A choice chamber (dia. 150 mm) having a filter paper at its bottom was used. Six rings (A, B, C, D, E, and F) with equal size and distance from each other were drawn by using lead pencil toward outer edge of the filter paper in the choice chamber. The central part (20 mm) of rings each was soaked with 0.1 ml of extracted material from crude body or soxhlet extraction method in hexane, acetone or propanol. Experimental units were kept overnight in open air for evaporation of the solvents. On the next day, the central part of each ring was soaked with 0.1 ml distilled water and then one hundred worker termites (4th - 5th instars) of *H. indicola* were introduced into center of the choice chamber.

The data on the number of termites present in each ring in the choice chamber was noted three times daily for 12 days without disturbing termite activity. The experiment was conducted under controlled laboratory conditions at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ R.H. Treatments were arranged as RCB factorial with two factors (methods) each at three solvents groups [Group I (Chloroform, Petroleum benzene, Xylol), Group II (Ethanol, Formaldehyde, Isobutanol) Group III (Acetone, Hexane, Propanol)] and replicated five times. Statistical computation was performed using PROC GLM (SAS Institute, 1990).

II- Screening of More Solvents for Extraction of Termite Crude Body Extracts

Extra trials were made to screen more solvents for the extraction of termite body extract. Nine organic solvents; ethanol, isobutanol, formaldehyde, chloroform, xylol, petroleum, hexane, propanol and acetone were tested. Due to non-availability of proper choice chambers for testing the chemicals simultaneously, these chemicals were randomly divided into three groups. Group 1 was composed of ethanol, isobutanol, and formaldehyde; group 2

comprised of chloroform, xylol, and petroleum benzene while group 3 included hexane, acetone and propanol. These three groups were screened separately and water was used as control in each group. A choice chamber (dia. 150 mm) having a filter paper at its bottom was used. Four rings (A, B, C, and D) with equal size and distance from each other were drawn by using lead pencil toward outer edge of the filter paper in the choice chamber. The central part (20 mm) of rings A, B, and C, were soaked with 0.1 ml of extracted material by crude body extraction method in the test solvent while, the central part of ring D was soaked with the same quantity of distilled water (control). All these experimental units were kept overnight in open air for evaporation of the solvents. On the next day, the central part of each ring was soaked with 0.1 ml distilled water and then one hundred worker termites (4th - 5th instars) of *H. indicola* were introduced into the center of each choice chamber. The data on the number of termites present in each ring in the choice chamber was noted three times daily for 12 days. These three observations were averaged and daily data were used for analysis. The experiment was conducted under controlled laboratory conditions at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ R.H. Treatments were arranged as randomized complete block design with five replicates five. Analysis of variance was performed using PROC GLM (SAS Institute, 1990) and means were compared by using DMR test.

III- Determination of Carbohydrates as Potential Bait Substrates for *H. indicola*

In order to determine the impact of different concentrations of carbohydrates on the survival of *H. indicola*, eight concentrations i.e. 1, 2, 3, 4, 5, 6, 7, and 8% of sucrose, glucose and maltose were prepared in distilled water. 0.8 ml of each of these treatments was pipetted on two filter papers already placed in a glass petri dish (dia 5.5 cm). Water was used as control. Thirty workers of *H. indicola* were introduced into each dish. The experimental units were kept in controlled laboratory conditions at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH. Survivals of the

termites were recorded daily for five weeks. The experiment was arranged as completely randomized factorial with four treatments each at eight levels (concentrations) with control at only one level. The data were analyzed using PROC GLM (SAS Institute, 1990).

IV- Determination of Best Concentration of Agar for Bait Preparation

Different concentrations i.e. 1, 2, and 3 of agar were tested to find the agar proportion that exhibited maximum consumption by *H. indicola*. For bait preparation, sawdust of poplar wood was thoroughly mixed, sieved through 120 mesh sieve and oven dried at 80°C . Sawdust was mixed with termite body extract in propanol (found as best attractant from the previous experiment) in the ratio of 1 g: 0.5 ml respectively. The mixture was left open for 48 h at room temperature for the evaporation of the solvent. On the second day, three concentrations of agar solution i.e. 1, 2, and 3%, were made by mixing of agar with 3% glucose solution in water in a conical flask. The flask containing the mixture was heated on a water bath, and maintained at $60 \pm 2^\circ\text{C}$. During heating, the mixture was frequently stirred until a clear solution was obtained. The feeding baits were prepared by mixing the treated sawdust with the three concentrations (1, 2, and 3%) of agar in glucose solution (1 g:6.7 ml) whereas, in control, the treated-sawdust was soaked in glucose (3%) solution in water. 12.0 g of each of the 4 treatments were leveled in 4 different compartments of a choice chamber (dia. 19.7×3.4 cm high) internally divided into 4 equal compartments by two plastic walls (8 mm high) placed diametrically at right angles (Figure 1). A group of 1,000 worker termites were introduced in the center of the chambers. For evaluation of bait loss due to evaporation etc., another set with the same quantity of the bait was kept without termites at the same time. The experiment was designed as RCB and replicated four times. The experimental units were kept under controlled laboratory conditions at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH. The tunneling activity was recorded daily for 7 days according to the method described by Ahmad *et al.* (1980) by

tracing the galleries on cellophane paper. Termites were removed and counted from each treatment unit after 7 days and percent bait consumption was calculated according to the following formula

% Bait consumption =

$$\frac{(\text{weight of the control sample} - \text{weight of the test sample}) \times 100}{\text{Weight of the control sample}}$$

Statistical computation was performed using PROCANOVA (SAS Institute, 1990).

Results and Discussion

I- Solvent Extracts of Termite Bodies of *H. indicola* Using Crude Body and Soxhlet Method

Percent response of *H. indicola* to body extracts in acetone, hexane and propanol by crude body and soxhlet extraction method is shown in Table 1. In this studies termite extracts by both the methods elicited an attractant behavior in *H. indicola*. In these results, crude body extract of propanol elicited a highest attraction response of termites. One possibility of this attraction may be due to the heating process in the caused the loss soxhlet extraction method, heating may lose the constituents of the pheromone, that are responsible for

termite attraction. The extraction solvents showed a marked difference in termite response in both the methods. A greater termite response toward extract in propanol by crude body extraction method was evident through out the experimental period as compared with acetone and hexane.

Within the soxhlet extracts, hexane showed greater termite response the first two days. After day 5 this trend changed and more number of termites was found attracted to propanol extract. The attraction of the crude body extract in propanopl was consistent throughout the observational period ranging from 67.60% - 80.60%. The response of termites towards propanol extract by soxhlet method ranged between 38% - 47% individuals between day 5 and 8 after which it declined. This study revealed that the crude body extract of termites in propanol was the best attractant for *H. indicola*.

Crude body extracts of different non-alcoholic solvents were reported as attractive by different investigators. Most investigators have tried non-alcoholic solvents for getting termite extract. Thus extracts in acetone and ether (Lund, 1966), hexane (Mutsumura *et al.*, 1968, 1969; Howard 1976; Traniello and Busher 1985; Tokoro *et al.*, 1990), and n-pentane (Farhat and Iqbal 1980, 1981) were studied and found attractive for termites.

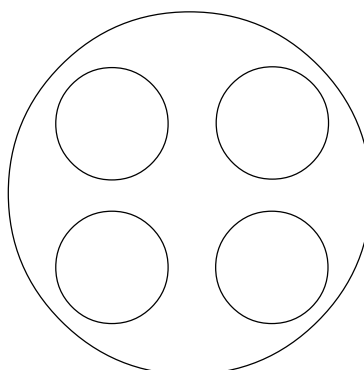


Figure 1. Choice chamber (dia.19.7×3.4 cm high) internally divided into 4 equal compartments placed diametrically at right angles

Attractiveness of propanol extract observed in these studies may partially be attributed to the kinds of solvent as sufficient time for evaporation of the solvent before using in the experiment. The result confirmed that extracts of other alcoholic compounds (ethanol, isobutanol and xylol) were also not attractive.

Screening of Solvents for Extraction of Termite Crude Body Extracts

After initial observation of the fact that crude body extract elicited attraction in *H. indicola*, further experiments were conducted to test other solvents. Nine solvents were tested in 3 groups and using water as a control. Termite response toward the first group (chloroform, petroleum benzene and xylol) was presented in Table 2. A high (74%) termite individuals were recorded on the first day of observation but declined over a time of two days after which termite, preferred water more than chloroform. Number of termites attracted toward xylol kept constantly lower than that of control. For the first few days, Petroleum benzene did not have a significant termite response but

thereafter, a higher and almost constant response was evident. The three extracts (ethanol, formaldehyde and isobutanol) in the second group did not show attraction better than water (Table 3). The results of the extracts of propanol, hexane and acetone were shown in Table 4. Propanol extract showed a constantly higher response throughout the experiment. Tokoro *et al.* (1989, 1990) found that hexane extract of *C. formosanus* and *R. speratus* elicited marvelous trail following activities. This experiment showed no such response for hexane extract by *H. indicola*. It was probably due to the presence of extract of *H. indicola* in propanol that attracted more termites.

Determination of Carbohydrates as Potential Bait Substrates for *H. indicola*

The results on the effect of eight different concentrations of carbohydrates (sucrose, glucose and maltose) on the mean survival percentage of *H. indicola* are presented in Table 5. The data indicated a decrease in survival of the termites on sucrose as compared with control and ranged between 10% - 35% at

Table 1. Percent response of *H. indicola* to its body extracts in different solvents using crude body and soxhlet extraction methods

Days	Extraction methods					
	Crude body extract			Sохhlet		
	Hexane	Propanol	Acetone	Hexane	Propanol	Acetone
1.	1.40 e	70.00 a	0.80 e	31.00 b	19.00 c	7.00 d
2.	2.00 d	71.40 a	2.40 d	52.00 b	13.60 c	3.40 d
3.	1.80 d	72.40 a	0.80 e	51.40 b	18.60 c	7.00 d
4.	1.40 e	72.00 a	0.60 e	52.00 b	13.60 c	6.80 d
5.	0.00 e	74.60 a	0.40 e	24.40 c	38.00 b	19.40 d
6.	0.00 e	68.00 a	11.00 c	5.60 d	61.40 b	12.00 c
7.	0.00 e	67.60 a	18.00 c	8.60 d	49.00 b	7.00 d
8.	0.00 d	75.00 a	6.60 c	7.60 c	47.40 b	11.40 c
9.	0.00 d	75.00 a	6.60 c	7.60 c	47.40 b	11.40 c
10.	0.00 d	75.00 a	2.00 d	7.20 c	17.40 b	11.60 c
11.	0.00 d	78.60 a	2.00 cd	7.20 c	14.00 b	15.00 b
12.	0.00 d	80.60 a	2.00 cd	4.00 c	17.00 b	14.00 b

Means followed by the same letter are not significantly different ($p < 0.05$) DMR test

concentration below 6%. The increasing concentration afterwards drastically reduced the survival reaching 0 % at 8 % concentration Glucose when compared with water did not reduce the survival of *H. indicola* at concentration below 8%, but at 8% survival was lower

as compared with control. Maximum survival (90%) was recorded on 3% glucose. The rest of glucose concentrations were at par with control except at 8% where survival dropped to 41%.

Survival of *H. indicola* did not differ at all the concentrations below 8%. 3% concentration

Table 2. Percent response of *H. indicola* to the crude termite body extract in the first group of the tested solvents

Days	No of individual termites			
	Chloroform	Petroleum benzene	Xylol	Water
1	74.00 a	10.00 b	12.00 b	4.00 c
2	44.40 a	2.40 c	4.80 c	38.80 b
3	28.40 b	0.80 c	1.00 c	41.40 a
4	10.20 b	0.40 c	0.20 c	61.00 a
5	1.00 b	1.00 b	0.00 b	69.00 a
6	1.00 c	75.40 a	0.40 c	7.40 b
7	23.00 b	50.80 a	0.80 d	5.80 c
8	23.80 b	49.00 a	1.80 c	1.80 c
9	21.80 b	36.80 a	0.80 c	0.40 c
10	22.80 b	53.20 a	0.00 c	0.80 c
11	8.20 b	59.80 a	0.00 c	0.80 c
12	0.80 b	59.00 a	0.00 b	0.80 b

Means within each row followed by the same letter are not significantly different ($p < 0.05$) by DMR test

Table 3. Percent response of *H. indicola* to the crude termite body extract in the third group of the tested solvents

Days	No of individual termites			
	Ethanol	Formaldehyde	Isobutanol	Water
1	0.00 b	0.00 d	0.00 c	100.00 a
2	4.40 b	1.80 b	1.00 b	53.40 a
3	9.20 b	1.00 c	1.00 c	43.80 a
4	31.00 a	1.60 b	1.60 b	35.50 a
5	46.00 a	3.00 c	2.00 c	30.00 b
6	30.00 b	4.40 c	6.00 c	37.00 a
7	26.40 a	7.00 c	16.40 b	32.00 a
8	11.80 c	17.80 b	10.80 c	34.00 a
9	7.40 c	15.00 b	8.00 c	37.00 a
10	7.40 c	17.00 b	10.00 c	31.60 a
11	7.00 b	9.00 b	1.60 c	31.60 a
12	8.40 b	8.40 b	2.40 c	36.00 a

Means followed by the same letter are not significantly different ($p < 0.05$) by DMR test

of glucose and maltose increased the survival of termites. It might have been due to the stimulant effect of glucose on termite feeding. Survival rate decreased with increasing concentrations of sugars.

Determination of Best Concentration of Agar for Bait Preparation

The results regarding the bait consumption and response of *H. indicola* to bait with

various concentrations of agar are presented in Table 6. The data on termite activity (tunneling behaviour) showed that when the termites were introduced in the choice chamber, soon all of them were attracted by the bait with 3% agar by the second day of observation, all the termites were in 3% agar chamber busy in making galleries. However, after 4 days the activity increased progressively in 1% and 2% agar also. Overall, maximum activity was observed in the

Table 4. Percent response of *H. indicola* to the crude termite body extract in the second group of the tested solvents

Days	Solvents			
	Acetone	Hexane	Propanol	Water
1	0.80 c	1.40 c	70.00 a	25.00 b
2	2.40 b	2.00 b	71.40 a	2.00 b
3	4.40 b	0.00 b	75.20 a	0.00 b
4	1.40 b	0.00 b	76.00 a	2.80 b
5	0.40 b	0.00 b	74.60 a	0.00 b
6	11.00 b	0.00 c	68.00 a	0.00 c
7	18.00 b	0.00 d	47.60 a	9.00 c
8	6.60 b	0.00 c	75.00 a	2.40 c
9	2.00 b	0.00 b	75.00 a	2.40 b
10	3.40 b	0.00 b	73.40 a	4.80 b
11	3.60 b	0.00 c	77.80 a	0.00 c
12	2.00 b	0.00 b	80.60 a	0.00 b

Means followed by the same letter are not significantly different ($p < 0.05$) by DMR test

Table 5. Effect of different concentration of carbohydrates on the mean percentage survival of *H. indicola*

Concentration (%)	Carbohydrates			Water
	Sucrose	Glucose	Maltose	
1	35.00 c	87.00 a	83.00 ab	78.66 b
2	22.33 b	72.33 a	83.33 a	78.66 a
3	24.33 c	90.00 a	72.33 b	78.66 b
4	37.67 b	86.67 a	82.33 a	78.66 a
5	24.33 b	86.67 a	84.33 a	78.66 a
6	10.00 c	72.00 a	53.33 b	78.66 a
7	1.33 c	86.67 a	82.00 ab	78.66 b
8	0.00 c	41.00 b	43.33 b	78.66 a

Means followed by the same letter are not significantly different ($p < 0.05$) by DMR test within each row.

Table 6. Effect of agar concentrations of on the bait consumption by *H. indicola*

Agar concentration (%)	Bait consumption (%)
1	27.76b
2	22.21b
3	55.78a
Water	8.33c

Means followed by the same letter are not significantly different ($p < 0.05$) by DMR test

bait having 3% agar followed by 2, 1, and 0% (Table 6). Maximum (55.78%) bait consumption was recorded in the bait having 3% concentration of agar, as compared with the control (8.33%) after one week (Table 6). Bait consumption of 1% and 2% agar concentration did not differ from the control. Agar as major components of termite bait, agar has been used by a number of researchers (Spragg and Fox, 1974; Patton and Miller, 1980; Spragg and Paton, 1980; Su *et al.*, 1982; Easey, 1983, 1985; French and Robinson, 1984; Holt and Easey, 1985; Easey and Holt 1988, 1989; Miller, 1990; Su, 1994). We recorded maximum bait consumption and termite tunneling activities in the bait with 3% concentration. This may be due to the fact with the increasing agar concentrations, the bait becomes more solid and termites prefer semi solid medium than soft medium. Su *et al.* (1984) while evaluating insecticides noted that higher concentration of agar stimulated tunneling behaviour in termites.

Conclusion

It is concluded from the results of the experiments that crude body extracts in propanol elicited higher *H. indicola* attraction response. Among various carbohydrates tested, 3% glucose increased the *H. indicola* survival while agar concentration at 3% made the bait more palatable for *H. indicola*.

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