Potential Phagostimulants for the Subterranean Termite, *Microtermes obesi* (Blattodea: Termitidae)

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Abstract

Glucose, yeast, urea, and poplar sawdust extract were tested for their phagostimulant properties on the subterranean termite, *Microtermes obesi* Holmgren (Blattodea: Termitidae). Termites were attracted to all of the compounds tested and they survived for a long period of time. The maximum percent survival was 4% for glucose, 1% for yeast, and 4% for urea. The highest consumption was for a bait having 4% glucose, followed by 3% yeast, 3% urea, and distilled water, respectively. Maximum termite survival was for filter papers soaked in an extract of poplar sawdust that had been boiled for 25 minutes, followed by filter paper soaked in sawdust extract boiled for 20, 15, and 10 minutes respectively. Lower survival of termites was recorded on filter paper that was soaked in sawdust extract that had not been boiled. Maximum bait consumption also was found for filter paper soaked in poplar sawdust extract that had been boiled for 25 minutes; whereas lower consumption was found for sawdust extract that had not been boiled.

Keywords: *Microtermes obesi*, Urea; Yeast; Glucose; Poplar sawdust; Phagostimulant

Introduction

Subterranean termites (Blattodea: Termitidae) cause significant structural damage throughout the world, especially in the tropical and sub-tropical regions [1]. There are both soil-inhabiting and wood-inhabiting termites. In Pakistan, termites cause considerable damage to buildings and wooden structures [2-4], to forests, and to a wide range of agricultural crops [4].

Highly effective chemical treatments have been used to prevent subterranean termite attacks. The frequent use of fast-acting termiticides for control of termites has generated a number of biological and environmental hazards. Interest in the use of slow-acting toxicants to suppress the populations of subterranean termites has been renewed [5,6]. As suggested by Beard [7] the success of slow acting toxicant bait depends upon its attraction, palatability, delayed mortality, and should be introduced into the colony’s gallery system and transferred to unexposed nest-mate by social grooming or to buildings and wooden structures [2-4], to forests, and to a wide range of agricultural crops [4].

To determine the impact of different compounds (urea, yeast and glucose) as potential bait substrates for *Microtermes obesi*...
which were placed in a glass Petri dish (5.5 cm dia). Distilled water was used as the control. Fifty termites (4th-5th instar; 45 workers and 5 soldiers) were introduced into each Petri-dish. Following the procedure of Smith [41], termites were fed for 20 days, and survival was recorded each day. The experimental units were kept in controlled laboratory conditions at 28 ± 2°C and 60 ± 5% R.H. Survival of the termites was recorded daily for twenty days. After twenty days, the experiment was terminated and filter papers were dried in an oven for two hours at 80°C and weighed. Percent bait consumption was calculated by using the following formula:

\[
\text{\% bait consumption} = \frac{(\text{weight of the control sample} - \text{weight of the tested sample})}{\text{weight of the control sample}} \times 100
\]

The experiment was arranged as completely randomized block design (RCB) with four treatments (compounds) at eight levels (concentrations) plus a water control. The data were analyzed by using Co-Stat (CoHort Software, Monterey, CA) at the 5% level of significance. Means were separated by using Turkey’s HSD (honest significant difference) test at the 5% level.

**Preparation of poplar sawdust extract**

Poplar sawdust, the most attractive food for termite species [42], was taken from a saw mill and sterilized at 80°C for 2 hrs in an oven. Then, it was passed through a 30-mesh sieve to obtain very fine particles, which were mixed with distilled water in the ratio of 1:10 (w/v) in a conical glass flasks (i.e., 10 g poplar sawdust was mixed in 100 mL distilled water). The sawdust was boiled for 0, 5, 10, 15, 20, or 25 minutes, and filtered through filter paper (Whatman no. 42) in separate flasks covered with airtight lids. The filtrates were kept in a refrigerator (15°C) until used for experiments.

**Determination of poplar sawdust extract as potential bait substrates for Microtermes obesi**

For this study, we followed the methodology of Grace and Yates [43] with some modifications. Twenty grams of sterilized sand and 3 mL of distilled water were placed into each of 18 clean and sterilized graduated beakers (4 cm dia). Two filter papers (Whatman no. 42, 4.2 cm dia), one soaked in extract and the other in distilled water, were weighed and placed vertically at opposite sides of the beaker in such a way that half of each filter paper was covered in sand. Fifty termites (4th-5th instar; 45 workers and 5 soldiers) were added to each beaker. Daily observations were taken and dead termites were removed with forceps. Survival of the termites was recorded daily for 20 days. After 20 days, the filter papers were separated from sand, washed thoroughly in water, dried in an oven for 2 hrs at 80°C, and weighed. The percent bait consumption was calculated using the formula presented above.

The experiment was arranged as completely randomized block design (RCB) with six treatments (concentrations) and a control. Each treatment was replicated three times. The data were analyzed by using Co-Stat at the 5% level of significance. Means were separated by using Turkey’s HSD test at the 5% level of significance.

**Comparative attractancy tests**

For these experiments, we followed the procedures of Waller et al. [38], with some modifications. We used clean, sterilized choice chambers (dia. 18.4 x 3.3 cm high) that had been internally divided in to five equal compartments by three plastic walls (7 mm high). Twenty grams of sterilized sand (80°C for 24 hrs) with 3 mL distilled water were added to each compartment. Filter papers (Whatman no. 42) were soaked in distilled water, yeast, urea, glucose, or sawdust extract and were placed in the choice chambers in such a way that half of the filter paper was covered in sand. Then, 250 termites (225 workers and 25 soldiers) were added to each choice chamber, and the experiment was replicated three times. Daily observations of the termites were recorded, and after 16 days, the experiment was terminated and the filter papers were re-weighed. The percent bait consumption was determined using the formula given above.

**Results**

**Effect of different compounds on bait consumption and survival of Microtermes obesi**

The urea and yeast treatments significantly reduced *M. obesi* survival at all concentrations, and no insects survived above 5% urea or 6% yeast (Figure 1). Maximum survival was 67.3 ± 1.2% for the 4% urea treatment and 70.0 ± 1.3% for the 1% yeast treatment compared with 75.0% survival in the water control. At all concentrations, glucose either increased (5 concentrations) or had no effect (3 concentrations) on survival of *M. obesi*. The lowest survival was for the 5% glucose treatment (72.7 ± 0.7%), while maximum survival was recorded at 4% glucose (84.0 ± 0.7%).

![Figure 1: Effect of different concentrations of phagostimulants on mean percentage survival of Microtermes obesi.](image.png)

Except for the two highest concentration of urea (6 and 7%), all treatment baits (urea, yeast, and glucose) had increased consumption over the water control (Figure 2). The highest overall bait consumption was for 4% glucose (27.2 ± 0.2%). The highest consumption for yeast was 21.1 ± 0.2% (3%), and for urea it was 15.3 ± 0.5% (3%), compared with the water control 4.5 ± 0.2%. Bait consumption was only 7.6 ± 0.8% for 0.1% glucose.

**Effect of different concentrations of poplar sawdust extract on bait consumption and survival of Microtermes obesi**

Termite survival was significantly higher (P<0.05) for all concentrations of poplar sawdust extract than it was for the water control (Figure 3). Maximum survival (83.3 ± 0.7) was recorded for filter paper that had been boiled for 25 minutes, followed by 82.0 ± 1.2, 80.7 ± 0.7, 77.3 ± 0.7, and 63.3 ± 0.7 for termites fed on filter paper soaked in sawdust extract boiled for 20, 15, 10, and 5 minutes, respectively. Termite survival was 60.0 ± 0.0 for filter paper soaked in
sawdust extract but not boiled and 70.7 ± 0.7% for filter paper and distilled water (control).

Figure 2: Effect of different concentrations of phagostimulants on percent bait consumption by *Microtermes obesi*.

Termites ate significantly more filter paper soaked in sawdust extract than they did filter papers soaked only in water (control) (Figure 3). Average weight loss (i.e., amount consumed by termites) of filter papers soaked in poplar sawdust extract but not boiled was 6.0 ± 0.4 g, which was not significantly different (P>0.05) from the control (5.2 ± 0.3 g). However, weight losses of filter paper soaked in poplar sawdust extracts boiled for 5, 10, 15, 20, and 25 minutes were significantly greater (P<0.005) than the control. The maximum percent bait consumption (20.7 ± 0.9) was for the longest boiling time (Figure 3).

Comparative attractancy test 1: Distilled water, 0.1% urea, poplar sawdust extract, 3% glucose, and 3% yeast

Termites consumed the maximum amount of filter paper (20.9%) that had been soaked in 3% yeast, followed by 18.4%, 14.0%, and 11.7% for filter papers with 3% glucose, poplar sawdust extract, and 0.1% urea, respectively (Figure 4). These treatments all were significantly different (P<0.05) from the control (1.8%).

Figure 4: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust extract, 3% glucose, and 3% yeast.

Comparative attractancy test 2: Distilled water, 0.1% urea, poplar sawdust extract, 3% glucose, and 4% yeast

Termites consumed the maximum amount of filter paper (21.7%) that had been soaked in 3% glucose, followed by 18.1%, 13.2%, and 11.5%, for filter papers with 4% yeast, sawdust extract, and 0.1% urea, respectively (Figure 5). These treatments all were significantly different (P<0.05) from the control (1.8%).

Figure 5: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust, 3% glucose, and 4% yeast.

Comparative attractancy test 3: Distilled water, 0.1% urea, poplar sawdust extract, 4% glucose, and 2% yeast

Termites consumed the maximum amount of filter paper (23.0%) that had been soaked in 4% glucose, followed by 18.8%, 16.7%, and 12.0% for filter papers soaked in 2% yeast, 0.1% urea, and sawdust extract, respectively (Figure 6). These treatments all were significantly different (P<0.05) from the control (1.5%).

Figure 6: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust, 3% glucose, and 4% yeast.
Comparative attractancy test 4: Distilled water, 1% urea, poplar sawdust extract, 2% glucose, and 1% yeast

Termites consumed the maximum amount of filter paper (21.4%) that had been soaked in 1% urea, followed by 20.0%, 18.5%, and 14.3% for filter papers soaked in sawdust extract, 1% yeast, and 2% glucose, respectively (Figure 7). These treatments all were significantly different (P<0.05) from the control (0.8%).

Comparative attractancy test 5: Distilled water, 1% urea, poplar sawdust extract, 1% glucose, and 1% yeast

Termites consumed the maximum amount of filter paper (20.3%) that had been soaked in 1% urea, followed by 18.5%, 17.7%, and 14.1% for filter paper soaked in sawdust extract, 1% yeast, and 1% glucose, respectively (Figure 8). These treatments were all significantly different (P<0.05) from the control (1.1%).

Comparative attractancy test 6: Distilled water, 1% urea, 4% yeast, 4% glucose, and poplar sawdust individually and in different combinations

There were significant (P<0.05) treatment effects among the combinations of treated filter papers bioassayed (Figure 9). The maximum consumption of treated filter papers was for a bait having 4% glucose + 4% yeast + 1% urea + sawdust (22.4%), followed by 17.8, 15.4, 12.6, 10.2, 10.0, 9.3, 9.0, 7.7, 7.2, and 3.4% for filter papers having 4% glucose + 4% yeast + 1% urea, 4% glucose + 1% urea + sawdust, 1% urea + 4% yeast, sawdust extract + 4% yeast, sawdust extract, 4% yeast + 4% glucose, 4% glucose, 4% yeast, 4% urea, and distilled water, respectively (Figure 9). Consumption of the 4% glucose + 4% yeast + 1% urea + sawdust extract was significantly different from the other treatment combinations and control.

Discussion

Our results showed reduced survival of Microtermes obesi at all concentrations of urea and yeast, with zero survival of termites at the highest concentrations of urea (6% and 7%) and yeast (7%). However, glucose did not reduce survival of M. obesi, even at the highest concentration (7%) concentration, and maximum survival for any
treatment was recorded for 4% glucose (84%). At most concentrations, glucose had a stimulant effect on termite feeding. Higher concentrations of phagostimulants have been reported to kill the gut protozoan of termites, which can lead to reduced survival [44].

Termites consumed significantly more filter-paper baits that had been treated with urea, yeast, or glucose than they did filter papers treated with distilled water. The highest bait consumption for each component was 27.2, 21.1, and 15.3 for 4% glucose, 3% yeast, and 3% urea, respectively. This increased consumption might have been due to the phagostimulant effects of these components on termite feeding. Our results agree with those of Reinhard and Kaib [35], who determined that glucose acted as feeding stimulants for *R. santonensis*. Waller and Curtis [15] found that baits treated with the highest concentration of glucose were significantly preferred by subterranean termites in choice evaluations. Waller et al. [38] observed that significantly greater numbers of termites were recruited to yeast and sucrose chambers than they were to the control.

Our results showed that survival of termites feeding on baits with sawdust extract was significantly higher than on untreated baits. Maximum survival of termites was recorded at filter paper boiled for 25 minutes, followed by filter paper soaked in poplar sawdust extract boiled for 20, 15, and 10 minutes, respectively. Termites show a preference for certain species of wood [45,46] and even show higher survivorship on the preferred wood [45,47].

When *M. obesi* were offered choices of filter paper soaked in different components in a five-compartment choice chamber, maximum attraction and bait consumption was found for filter paper soaked in glucose, followed by yeast, poplar sawdust extract, and urea. Minimal attraction and bait consumption was recorded for the control (filter paper soaked in distilled water). It was concluded that glucose, yeast, poplar sawdust extract, and urea are phagostimulants. These results confirm the results obtained for 0.1% urea by Waller [48], poplar sawdust extract [49], 3% yeast [38], and 3% glucose [50]. Subterranean termites are known to regularly consume nitrogen in the form of uric acid when they consume the bodies of nest mates [33,34]. Lysine was identified as a phagostimulant for both *R. santonensis* [35] and *C. formosanus* [51]. Various carbohydrates have been suggested to act as termite phagostimulants [52-54]. Galactose has been reported to significantly increase *Reticulitermes* spp. consumption of paper baits [55]. Populations of *Reticulitermes flavipes* differ in their response to potential phagostimulants [56-59].

Acknowledgments

I am very grateful to Drs. Muhammad Naeem and Ehsan-ul-Haq for their technical help during the investigation. My sincere thanks to Drs. Muhammad Asif and Muhammad Munir for helping me analyze the data. I am very grateful to Drs. Muhammad Tariq and Javeed Khan for their helpful comments. The author is thankful to his parents for their financial support.

References

1. Pearce MJ (1997) Termites-Biology and Pest Management. CAB Internat NY. p. 172.
2. Chhotani OB (1977) Termites of Kanha National Park. Rec Zool Surv India 72: 367-388.
3. Akhtar MS (1980) Some observation on swimming and development of incipient colonies of termites of Pakistan. Pak J Zool 10: 283-290.
4. Dawes-Gromadzki TZ (2005) Termite (Isopeta) fauna of a monsoon rainforest near Darwin, northern Australia. Austral J Entomol 44: 152-157.
5. Su NY, Tamashiro M, Yates JR (1982) Trials on the field control of the Formosan subterranean termite with Amdro bait. The International Research Group on Wood preservation, Document No. IRG/WP/1163, Stockholm, Sweden.
6. Jones SC (1984) Evaluation of two insect growth regulators for the bait-block method of subterranean termite (Isopeta: Rhoitermittidae) control. J Econ Entomol 77: 1086-1091.
7. Beard RL (1974) Termite biology and bait block method of control. Conn.Agric. Exp. Stn. Bull. 748, New Haven, CT.
8. Smythe RV, Carter FL (1970) Feeding responses to sound wood by Coptotermes formosanus, Reticulitermes flavipes and R. virginicus (Isopeta: Rhoitermittidae). Ann Entomol Soc Am 63: 841-847.
9. Waller DA (1988) Host selection in subterranean termites: factors affecting choice (Isopeta: Rhoitermittidae). Sociobiology 14: 5-13.
10. Delaplane KS (1989) Foraging and feeding behaviors of the Formosan subterranean termite. Sociobiology 19: 101-114.
11. Oi FM, Su NY, Koehler PG, Slansky F (1996) Laboratory evaluation of food placement and food types on the feeding preference of Reticulitermes virginicus (Isopeta: Rhoitermittidae). J Econ Entomol 89: 915-921.
12. Doi S, Kurimoto Y, Ohara S, Aoyama M, Yoshimura T (1999) Effects of heat treatments on the feeding behavior of two subterranean termites. Holzforschung 53: 225-229.
13. Botch PS, Brennan CL, Judd TM (2010) Seasonal effects of calcium and phosphate on the feeding preference of the termite Reticulitermes flavipes (Isopeta: Rhoitermittidae). Sociobiology 55: 489-498.
14. Waller DA, Jones CG, La Page JP (1990) Measuring wood preference in termites. Entomol Exp Appl 56: 117-123.
15. Waller DA, Curtis AD (2003) Effects of sugar-treated foods on preference and nitrogen fixation in Reticulitermes flavipes (Kollar) and Reticulitermes virginicus (Banks) (Isopeta: Rhoitermittidae). Ann Entomol Soc Am 96: 81-85.
16. Swoboda LE, Miller DM, Fell RJ, Mullins DE (2004) The effect of nutrient compounds (sugars and amino-acids) on bait consumption by Reticulitermes spp. (Isopeta: Rhoitermittidae). Sociobiology 44: 547-563.
17. Saran RK, Rust MK (2005) Feeding, uptake, and utilization of carbohydrates by western subterranean termite (Isopeta: Rhoitermittidae). J Econ Entomol 98: 1284-1293.
18. Judd TM, Corbin CC (2009) Effect of cellulose concentration on the feeding preferences of the termite Reticulitermes flavipes (Isopeta: Rhoitermittidae). Sociobiology 53: 777-784.
19. Abushama FT, Kambal MA (1977) The role of sugars in the food-selection of termite Microtermes traegardhi (Sjostedt). Z Angew Entomol 84: 250-255.
20. Halfig I, Costa-Leonardo AM, Marchetti FF (2008) Effects of nutrients on feeding activities of the pest termite Heterotermes tenuis (Isopeta: Rhoitermittidae). J Appl Entomol 132: 497-501.
21. Spragg W, Fox RE (1974) The use of radioactive tracer to study the nestbuilding system of Mastotermes darwiniensis Friggat. Insectes Sociaux Paris 21: 309-310.
22. Paton R, Miller DM (1980) Control of Mastotermes darwiniensis Friggat (Isopeta: Mastrotermitidae) with mixex bait. Aust For Res 10: 249-258.
23. Spragg WT, Patton R (1980) Tracing trophallaxis and population measurement of colonies of subterranean termites (Isopeta) using radioactive tracer. Ann Entomol Soc Am 73: 708-714.
24. French JRJ, Robinson PJ (1984) A method for screening termite baits using Coptotermes lacteus mounds. 13th Meeting, Internat. Res. Group Wood Preservation, Sweden, May 28-June 1, 1984. Document No. IRG/WP/1237: 1-6.
