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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 trophallaxis. Studies have shown that subterranean termites prefer foods that contain nutrients [8-12], it is reasonable to suggest that a particular nutrient, or group of nutrients, could be added to a termite bait matrix to enhance its palatability for termites.

Several studies have shown that ions [13], high wood density [14], sugar [15-17], and high levels of cellulose [18] can increase termite's food consumption. Abushama and Kambal [19] reported that *Microtermes traegardhi* Sjöstedt preferred fructose. *Heterotermes tenuis* Hagen responds to trehalose [20]; while *Reticulitermes* spp. showed preference for xylose, ribose, maltose, and fructose [17]. Concentrations of agar and sawdust also have been varied to increase the palatability of termite baits [21-28]. Body extracts of termites in ether, acetone, and hexane have been tested for attractancy against termites [29-32].

Nitrogen compounds are limited in the subterranean termite diet and several amino acids and uric acid have been evaluated to determine their potential as termite feeding stimulants or deterrents [9,33-35]. Henderson et al. [36] reported that urea increased feeding by *Coptotermes formosanus* Shiraki in the laboratory. Akhtar and Raja [37] studied the effect of wood and wood extracts from the Persian silk tree, Albizia procera (Roxb.) (Fabaceae), and the purple orchid tree, Bauhinia variegate L. (Fabaceae), on the survival and feeding response of *Bifiditermes beesoni* (Gardner). Waller et al. [38] reported that yeast was found best phagostimulant for termites, 39 yeast isolates from the hind gut of termites by using RAPD-PCR [39]. Spragg and Fox [21] screened different baits in laboratory and indicated that sawdust from *Eucalyptus tetrodonta* F. Muell. (Myrtaceae) had the highest attraction for *Mastotermes darwiniesis* Froggatt, damp sawdust is preferred food than pine sawdust [40].

The main objective of the present study was to test different compounds (urea, yeast, glucose, and poplar sawdust extract) to find out phagostimulants for management of *Microtermes obesi* Holmgren.

Materials and Methods

Determination of different compounds (urea, yeast, and glucose) as potential bait substrates for *Microtermes obesi*

To determine the impact of different compounds (urea, yeast and glucose) on the survival of *Microtermes obesi*, eight concentrations (0.1, 1, 2, 3, 4, 5, 6, and 7%) of urea, yeast, and glucose were prepared in distilled water. Then, 0.8 mL of each treatment was pipetted onto two previously weighed filter papers (Whatman no. 42, 4.2 cm diam), 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:

$$\%\ bait\ consumption = \frac{(weight\ of\ the\ control\ sample - weight\ of\ the\ tested\ sample)}{weight\ of\ the\ control\ sample} X100$$

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 ± 07%).

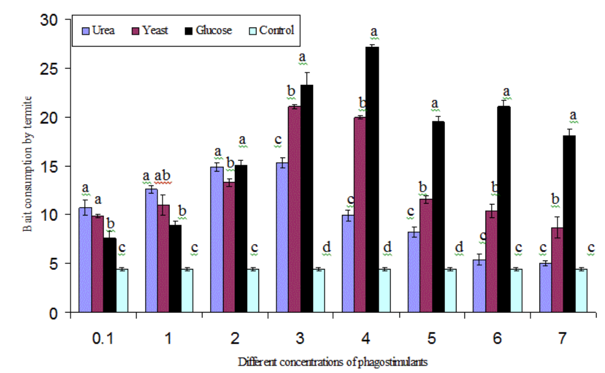

Figure 1: Effect of different concentrations of phagostimulants on mean percentage survival of *Microtermes obesi*.

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

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sawdust extract but not boiled and 70.7 ± 0.7% for filter paper and distilled water (control).

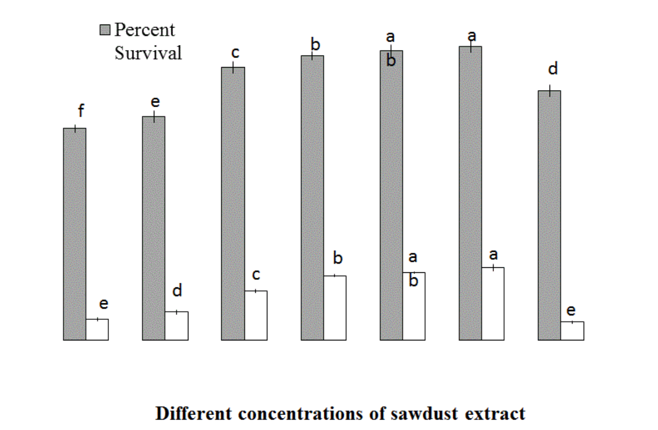

Figure 2: Effect of different concentrations of phagostimulants on percent bait consumption by *Microtermes obesi.*

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).

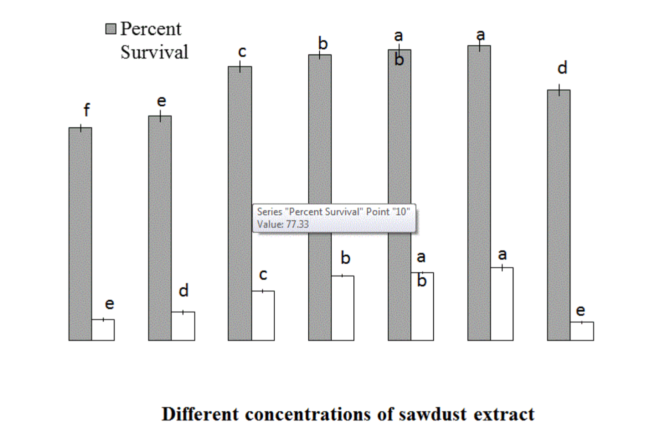

Figure 3: Effect of different concentrations of poplar sawdust extract on mean percentage survival and bait consumption of *Microtermes obesi.*

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%).

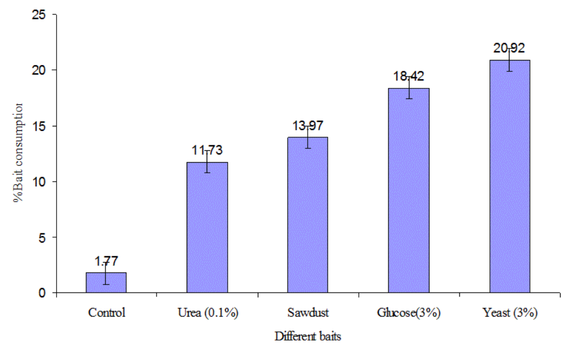

Figure 4: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust extract, 3% glucose, and 3%yeast.

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%).

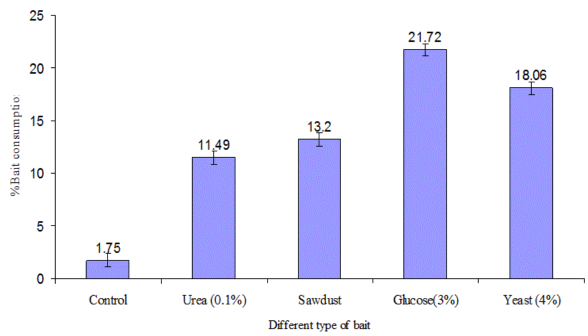

Figure 5: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1%urea, poplar sawdust, 3% glucose, and 4%yeast.

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%).

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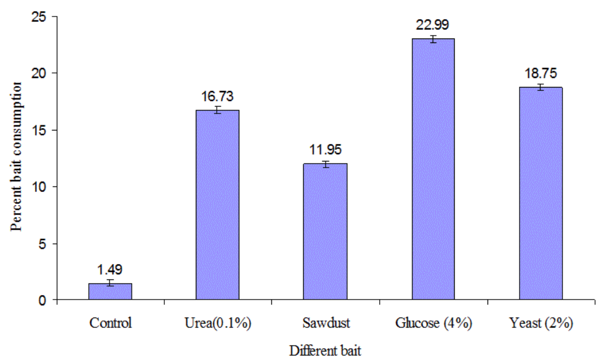

Figure 6: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust, 4% glucose, and 2% yeast.

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%).

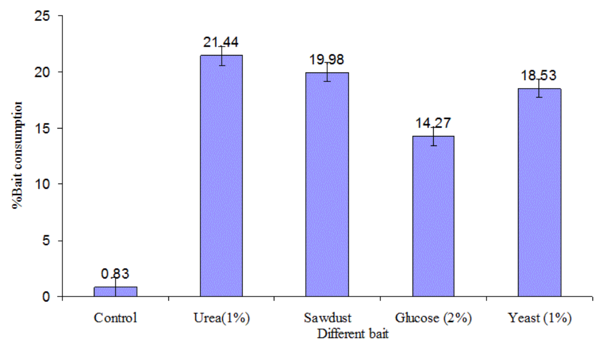

Figure 7: Response of *Microtermes obesi* to filter paper soaked in distilled water, 1% urea, poplar sawdust, 2% glucose, and 1% yeast.

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%).

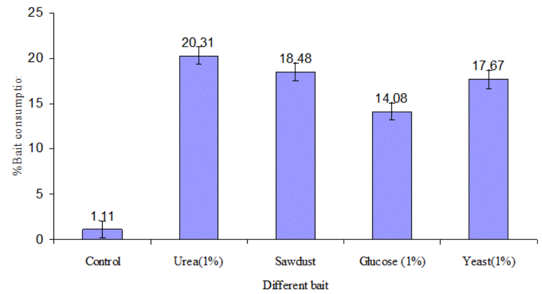

Figure 8: Response of *Microtermes obesi* to filter paper soaked in distilled water, 0.1% urea, poplar sawdust, 1% glucose, and 1% yeast.

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.

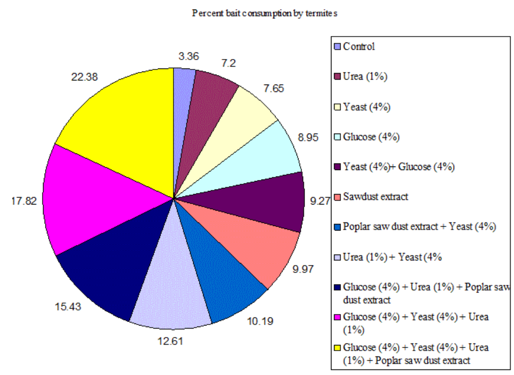

Figure 9: Percent mean bait consumption by *Microtermes obesi* when offered in beaker with distilled water.

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].

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