SCREENING OF CHEMICAL COMPOUNDS FOR SLOW-ACTING TOXICANT CHARACTERISTICS AGAINST SUBTERRANEAN TERMITES

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Abstract

Different compounds viz. boric acid, sodium arsenate, mercuric iodide, molybdophosphoric acid, zinc sulphate, lead acetate and cadmium chloride at 6 different concentration levels, i.e. 0.005, 0.05, 0.1, 0.2, 0.25, and 0.5% prepared in distilled water were tested against *Heterotermes indicola* to screen potential slow-acting toxicants. Among these compounds molybdophosphoric acid, zinc sulphate, lead acetate, and cadmium chloride showed a low mortality rate that did not exceed 76% even at the highest concentration (0.5%) used. The second group of boric acid, sodium arsenate and mercuric iodide exhibited ELT90 within 14 days, in at least concentration. Behavioral observations indicated that among the 3 compounds in the second group, only boric acid (0.1%) when offered in agar medium allowed normal tunneling behavior of the termites.

Keywords*: Heterotermes indicola*, molybdophosphoric acid, zinc sulphate, lead acetate, and cadmium chloride and ELT90

Introduction

For the control of subterranean termites, soil treatment is not an efficient or satisfactory control measure. Flooding of soil with insecticides has many problems associated with it. Huge quantities of insecticide are needed, to cover all the foraging territories of a termite colony, and that can cause ground water pollution. Effects on non-target organisms and cost economics are other major concerns. A safer approach for managing subterranean termites is the use of slow-acting toxicants in baits, which are consumed by the foraging workers and spread to other colony members by trophallaxis, thereby eliminating all or part of the colony. The success of an insecticide used in bait will depend upon its delayed mortality, palatability to termites and on its ability to get transferred among termites in an active form.

Heavy metals, antibiotics, and arsenic and boron compounds have been tested as slow-acting toxicants against subterranean termites in both the laboratory and field (Brill *et al.,* 1987; Yoshimura *et al.,*1987; Watson, 1990). Boric acid and boron salts are effective termiticides (Randall

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et al., 1934; Reirerson, 1966; Williams and Amburgey, 1987; Grace, 1990b; Grace and Abdallay, 1990; Khatoon *et al.,* 1993), although the basis of their toxicity is not well understood (Williams *et al.,* 1990). Sodium and zinc borates are used in wood preservation (Williams and Amburgey, 1987; Barnes *et al.,* 1989) and are currently of interest as soil insecticides (Grace, 1990). According to D'Orazio (1982); Mori (1987); Grace (1990b) and Grace *et al.,* (1990), boron compounds have shown promise as bait toxicants in laboratory tests with subterranean termites *Reticulitermes* spp. and *Coptotermes* spp. Arsenate dust has been injected into termite galleries (Randall and Doody, 1934; Edwards and Mill, 1986; Lin, 1987), and also used to contaminate foraging termite workers collected in traps (Su and Scheffrahn, 1986).

With acute toxicants, the time required to kill termites is similar at high or low concen-trations, but the mortality levels of slow-acting toxicants are often concentration-dependent (Su *et al.,* 1987). The speed of death at various concentrations of an insecticide can be quantified for comparison purposes using the effective lethal time (ELT90), defined as the amount of time required for an insecticide to kill 99% of the treated individuals. Slow-acting toxicants are characterized by a broad range of time, while acute toxicants exhibit a narrow range of ELT90 values (Su *et al.,* 1987). Stringer *et al.* (1964) defined a slow-acting insecticide as one that caused less than 15% mortality after 24 h exposure and more than 89% mortality after 14 days.

The current study describes evaluation of 7 chemical compounds, boric acid, sodium arsenate, mercuric iodide, molybdophosphoric acid, zinc sulphate, lead acetate, and cadmium chloride as slow-acting bait toxicants against subterranean termites, *Heterotermes indicola* and *Microtermes unicolor.*

Materials and Methods

Laboratory Studies

Toxicity Tests Against *H. indicola*

Stock solutions of boric acid (H_3 Bo_3), sodium arsenate (NaH_2 A_3O_4) , Mercuric iodide (HgI_2), molybdophosphoric acid (Mo (H_3 PO_3), zinc sulphate ($ZnSO_4$, $7H_2O$), lead acetate (Pb (CH_3 $COO)_2$, 3H2O), and cadmium chloride ($CdCl_2$) at 6 different concentration levels i.e. 0.005, 0.05, 0.10, 0.20, 0.25, and 0.50% were prepared in distilled water. 0.8 ml of each stock solution was pipetted to 2 filter papers (Whatman No.1, 5.5 cm dia.). Distilled water was used as control. The treated and the control filter papers each were placed separately in a petri dish (dia. 5 × 1.5 cm high) and 30 workers of *H. indicola* were introduced in each petri dish. The experiment was arranged as a completely randomized factorial with 7 factors (chemicals) each at 6 levels (concentration) and replicated 4 times. All experimental units were kept under controlled laboratory conditions at 28 $\pm$ 2^{o}C and 60 $\pm$ 5 % relative humidity (RH). Data on the termites mortality were recorded and dead termites were removed on day 2, 4, 10, and 14. Compounds by concentration interaction were computed over days and interaction means were separated using Duncan's Multiple Range Test (DMRT). Statistical computations were performed using PROC GLM (SAS Institute, 1990).

Behavioural Response of *H. indicola* to Toxicants

Studies on the tunneling responses of *H. indicola* to boric acid, sodium arsenate, mercuric iodide, molybdophosphoric acid, zinc sulphate, lead acetate, and cadmium chloride at 6 different concentration levels i.e. 0.005, 0.05, 0.10, 0.20, 0.25, and 0.50% incorporated in the attractive bait were conducted in accordance with the method described by Springhetti and Sita (1989). For the preparation of toxicant-treated baits, poplar sawdust (120 mesh size) was soaked with termite crude body extract in propanol in the ratio of 1 g : 0.5 ml respectively and kept open for 48 h for evaporation of the propanol. Thereafter the treated saw dust was soaked with the required concentration of the toxicant in the ratio of 1 g saw dust and 3 ml of toxicant solution and kept for 48 h for the evaporation of water. The treated saw dust and hot agar (3%) solution in water was mixed thoroughly in the ratio of 1 g : 6.67 ml respectively. The toxicant-treated baits were tested in choice chambers (7 cm × 3.4 cm), internally divided into 4 equal compartments by 2 plastic walls (8 mm high) placed diametrically at right angles. Twelve grams of toxicant-treated bait of each concentration, and 12 g of the bait without toxicant as the control were spread uniformly in each compartment. Since the choice chamber used had only 4 compartments, 6 concentrations of each compound were tested in 2 groups i.e. 0.005, 0.05, 0.10, and 0.20, 0.25 and 0.50%. The control was included in both the groups. Eight hundred workers of *H. indicola* were introduced into the center of each choice chamber. The experimental units were kept under controlled laboratory conditions at 28 ± 2°C and 60 ± 5% RH. The observations on tunneling behavior were recorded by tracing the tunnels on cellophane paper, according to the method described by Ahmad et al (1980), and also by photographs on days 3, 6, 9, 12, 15, and 18 after the termites, exposure.

Effect of Different Concentrations of Boric acid on *M. unicolor*

After getting promising results with boric acid, 4 different concentrations i.e. 0.10%, 0.20%, 0.25%, and 0.50%, in water were tested against subterranean termites, *M. unicolor*. Two filter papers weighing 500 mg was soaked with 1 ml solution of each concentration and placed in a glass petri dish (dia. 5 × 1.5 cm high). One hundred worker termites obtained from the laboratory-reared stock culture were introduced into each petri dish. The experimental units were kept under controlled laboratory conditions at 28 ± 2°C and 60 ± 5 % RH. The experiment was arranged as completely randomized and replicated 4 times. Data were recorded on mortality 1, 2, 4, 6, 8, 10, 12, and 14 days after the exposure of the termites to boric acid. Statistical computations were performed using PROC GLM (SAS Institute, 1990).

Results and Discussion

Toxicity Tests Against *H. indicola*

The results on the toxicity of different chemical compounds with different concentrations are presented in Table 1. A highly significant ($p < 0.05$) compound by concentration interaction was observed when the data was analyzed by days. Thus simple effects of compounds at each concentration were compared. The data showed that the compounds tested fell into 2 groups. The first group (molybdophosphoric acid, zinc sulphate, lead acetate and cadmium chloride) caused a low mortality rate that did not exceed 76% even at the highest concentrations used. The first group did not cause any mortality until the 4th day. The mortality rate in this group kept low and did not exceed 75% even after 14 days. The ELT90 was not less than 20 days in all the group members. Second group included boric acid, sodium arsenate and mercuric iodide where ELT90 was within 14 days at least 1 concentration. Termiticidal activity of Molybdophosphoric acid against *R. flavipes* has been reported by Brill *et al.* (1987), where 99% mortality was observed after 48 h at a concentration of 1,000 ppm, but Yoshimura *et al.* (1987) observed that sodium molybdate took 5 weeks to induce 100% mortality in *C. formosanus* indicating an undesirably slow kill rate of molybdophosphoric acid against this termite species.

Among the second group, mercuric iodide showed the most acute toxicity 2 days after the start of the experiment and 100% mortality was observed at concentrations exceeding 0.05%. Boric acid caused the lowest mortality when compared with sodium arsenate and mercuric iodide at all the concentrations after 2 days. Four days after feeding, all concentrations of sodium arsenate caused 100% mortality that was higher than the similar concentration of boric acid. After 10 days all the concentrations of boric acid exceeding 0.05% caused 100% mortality and were at par with sodium arsenate. The lowest concentration of sodium arsenate (0.005%) also caused almost 100% mortality after 14 days while concentrations of boric acid lesser than 0.1% caused a much lower mortality.

Thus both sodium arsenate 0.005% and boric acid 0.1% exhibited a delayed mortality effect. Boric acid started with a slower rate as compared with sodium arsenate but the ELT90 value for both the compounds was within 10 days.

Behavioral Response of *H. indicola* to Different Chemical Compounds

The termite activity as shown by the tunneling behavior in the media treated with the 6 different doses of all the 7 compounds indicated that the 3 chemicals i.e. sodium arsenate (Figure 1), lead acetate (Figures 2 and 3) and cadmium chloride (Figures 4 and 5) were completely repellent at all the doses throughout the experimental period as the termites were always found busy in making galleries in the control compartment. Mercuric iodide (0.005 and 0.05%) media indicated the galleries making tendency of termites during the first week (Figure 6). After that all the termites were found dead.

In the zinc sulphate media, all the termites were in the control chamber for the first 3 days, but their activity in the bait treated with 0.005 and 0.05% concentration increased progressively thereafter. The bait treated with zinc sulphate at 0.1% and above were completely repellent throughout the experimental period (Figures 7 and 8). Molybdophosphoric acid showed tunneling activity at concentrations up to 0.10% but no activity was observed at higher concentrations. However, the activity in the control was still progressing even after 32 days (Figures 9 and 10). In the boric acid-treated bait, termites were active and started making galleries within 3 days at 0.005, 0.05, and 0.10% doses and control. These termites were able to live, move and make the galleries actively for 12 days; after 15 days 100% mortality occurred, so, the galleries were found without termites. In the toxicant-treated bait with 0.20, 0.25 and 0.50% boric acid, termites were actively making galleries in the control compartment upto the 3rd day, after which they started tunnels in the

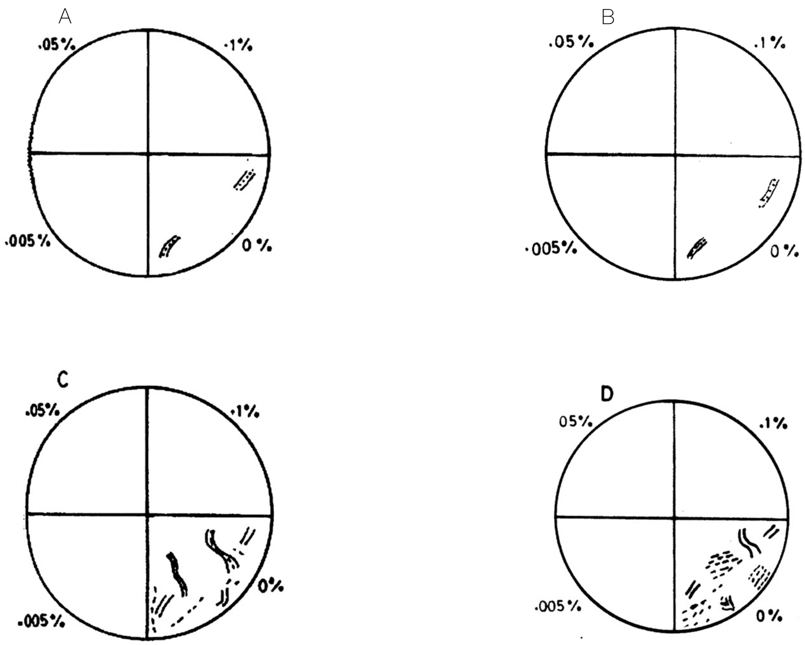

Figure 1. Activity of *H. indicola* in bait treated with 0, 0.005, 0.05, and 1.0% sodium arsenate (NaH_2AsO_4). A, after 3 days; B, after 6 days; C, after 9 days; and D after 12 days.

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treated-bait compartment also (Figures 11 and 12). Although the galleries were not too long in these treatments but the termites were not inhibited by these doses and the galleries increased progressively upto 12 days of experiment. On 14th day, all the termites left the galleries, gathered on the surface of the control compartment and started shivering. It was followed by termite mortality.

Behavioural observations showed termite activity in at least low concentrations of mercuric iodide, zinc sulphate, molybdophosphoric acid, and boric acid. Mercuric iodide showed a slight tunneling activity as indicated by the tunnel size observed but was soon followed by termite mortality. It was indicative of its higher toxicity, which is also confirmed by the force-feeding tests. Zinc sulphate and mlybdophosphoric acid bait were preferred by the termites showing good termite galleries but the termites were active even after 14 days. A similar trend was evident in the force-feeding (toxicity) test where the ELT90 could not be reached even after 14 days. This makes them undesirable as a toxicant.

Boric acid (0.1% and lower concentrations) had good termites activity as indicated by the galleries. It shows the acceptability of boric acid-treated bait by the termites. Both the behavioural observations and force-feeding test showed that the ELT90 value for 0.1% boric acid was within a range of 2 weeks. This makes boric acid a good choice for use as a slow-acting toxicant. Termiticidal activities of boric acid have also been reported by other workers (Randall *et al.*, 1934; Reirerson, 1966; Williams and Amburgey, 1987; Grace, 1990b; Grace and Abdallay, 1990).

Effect of Different Concentrations of Boric Acid on *M. unicolor*

The results on the effect of boric acid-

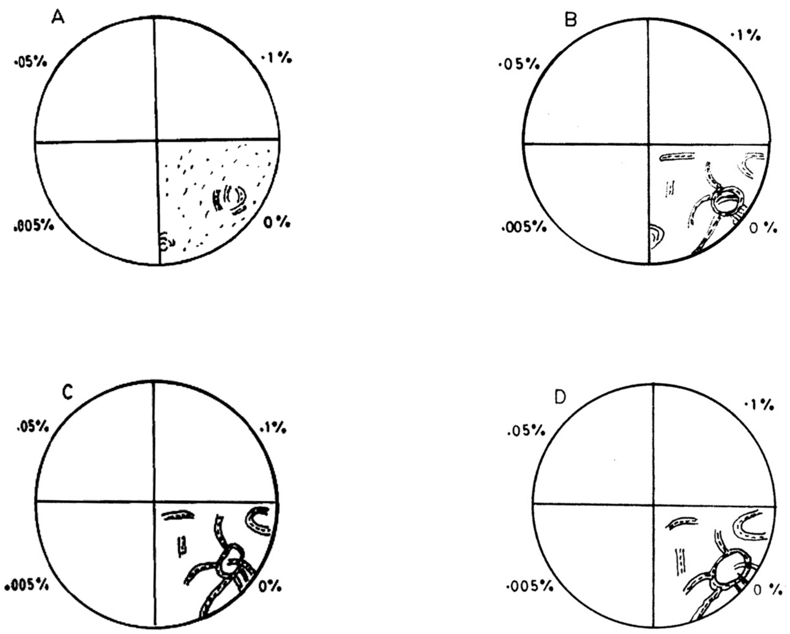

Figure 2. Activity of *H. indicola* in attractive bait treated with 0, 0.005, 0.05 and 1.0% lead acetate {Pb $(DH_3COO)_2$, $3H_2O$}. A, after 9 days; B, after 12 days; C, after 15 days; and D, after 18 days.

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treated bait with different concentrations on the percentage mortality of *M. unicolor* (Table 2) revealed nil mortality at 0.10, 0.20, 0.25, and 0.50% concentrations up to 6, 4, 2, and 1 days respectively. At 0.2% concentration, maximum mortality ($P < 0.05$) of 59.93% occurred on day 14. Going to a still higher concentration (0.25%), there was a sharp increase in mortality after day 6 and 100% mortality was there at day 14. Maximum concentration (0.5%) showed a constant and almost logarithmic increase in the mortality with the passage of time and reached 93.88% by day 8 and 100% by day 10.

Mortality reached 100% by day 14 at 0.25% concentration. Thus 0.25% boric acid seems to be the proper concentration to be used as bait toxicant against *M. unicolor*. The same concentration induced an early mortality in *H. indicola* indicating a higher sensitivity of *H. indicola* toward boric acid.

Ahmad *et al*. (1979) worked on the effect of boric acid and *Bacillus thuringiensis* alone as well as in combination on different species of termites and showed that boric acid used alone

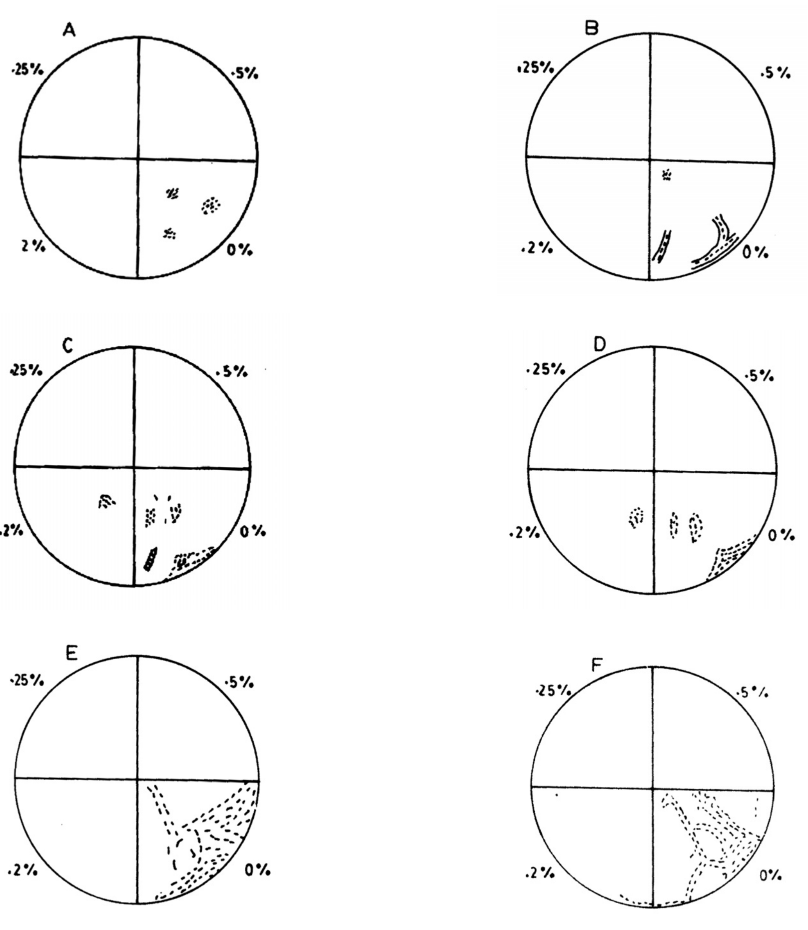

Figure3. Activity of *H. indicola* in attractive bait treated with 0, 0.2, 0.25, and 0.50% lead acetate $\{Pb(DH_3COO)_2, 3H_2O\}$. A, after 6 days; B, after 9 days; C, after 12 days; D, after 15 days; E, after 18 days; and F, after 21 days.

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gave a quicker rate of mortality. Grace and Abdallay (1990) working with boric acid and its salts on *R. flavipes* observed that boric acid caused 100% mortality after 8 days. Grace *et al.* (1990) found similar results to those of Grace and Abdallay (1990) when *C. formosams* was treated with borate dust. He also observed that introduction of treated termites (20% of the group) into groups of untreated termites led to significant mortality indicating toxicant transmission by mutual grooming, trophollaxis behaviour, and possibly by cannibalism. All termites exposed to workers treated with boric acid died within the 15 days test period. According to Su *et al.* (1982); Williams and Amburgey (1987); Smith and Rust, (1990) and Grace *et al.* (1990), there is no avoidance of foraging termites in a borate-treated bait trap or soil, and borate fits the profile of a slow-acting, non repellent toxicant. Khatoon *et al.* (1993) mentioned that boric acid caused 100% mortality after 10 days and did not prohibit the feeding activity of *H. indicola* on treated filter paper even at 2% concentration. Su *et al.* (1994) estimated the oral toxicity of sulfuramid,

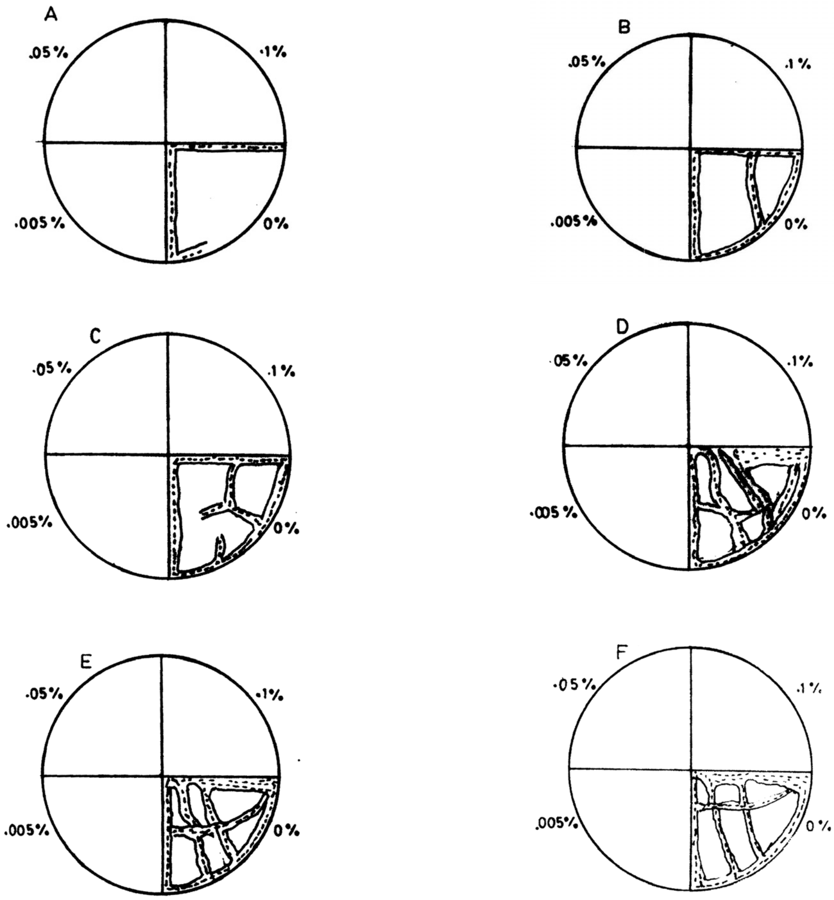

Figure 4. Activity of *H. indicola* in bait treated with 0, 0.005, 0.05 and 0.10% cadmium chloride ($CdCl_2$). A, after 6 days; B, after 9 days; C, after 12 days; D, after 15 days; E, after 18 days; and F, after 21 days.

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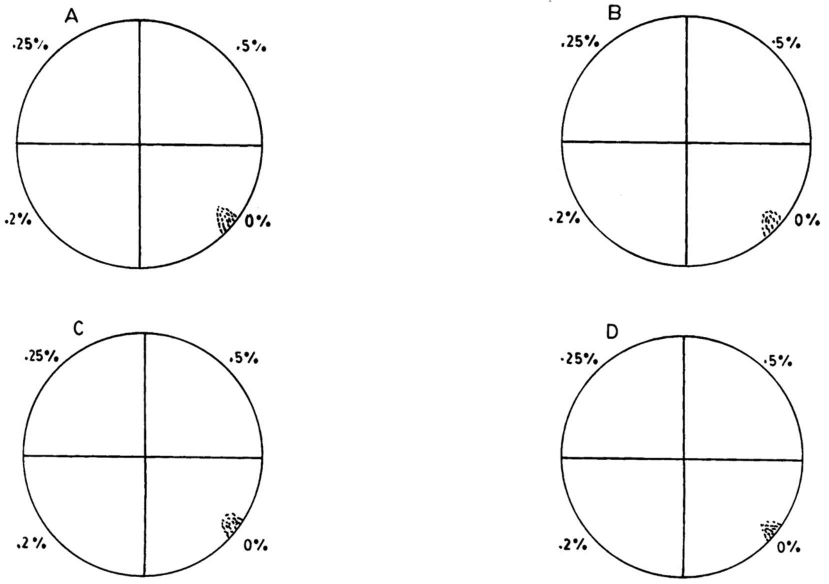

Figure 5. Activity of *H. indicola* in bait treated with 0, 0.20, 0.25, and 0.50% cadmium chloride ($CdCl_2$). A, after 6 days; B, after 9 days; C, after 12 days; and D, after 15 days.
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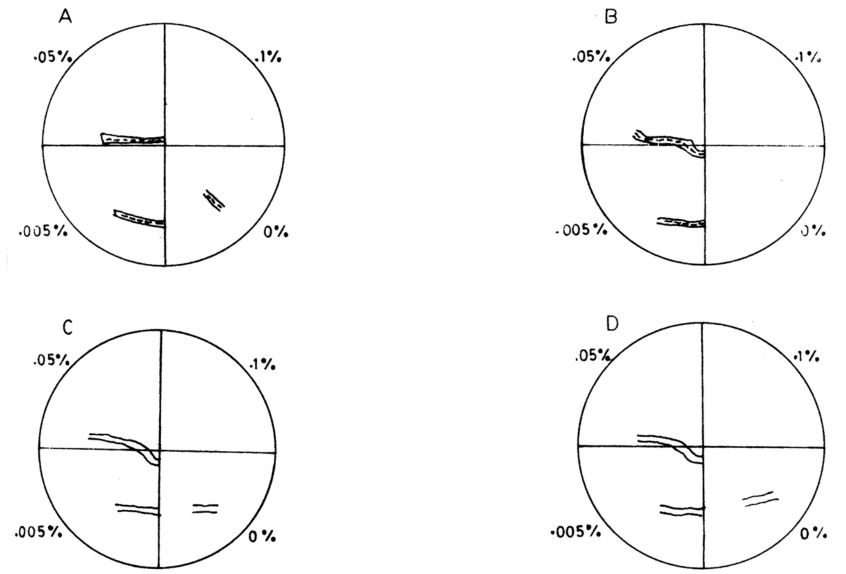

Figure 6. Activity of *H. indicola* in bait treated with 0, 0.2, 0.25, and 0.50% mercuric iodide (HgI_2). A, after 6 days; B, after 9 days; C, after 12 days; and D, after 15 days.
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Table 1. Effect of toxicants and their concentrations on percent mortality of *H. indicola*

Conc.	% mortality						
	Molybdopho -sphoric acid	Zinc sulphate	Lead acetate	Cadmium chloride	Boric acid	Sodium arsenate	Mercuric Iodide
				Day-2			
0.005	0.00	0.00	0.00	7.50	0.00	0.00	0.00
0.050	0.00	0.00	0.00	0.00	5.00	57.50	5.00
0.100	0.00	0.00	0.00	0.00	5.00	80.00	100.00
0.200	0.00	0.00	0.00	0.00	7.50	86.67	100.00
0.250	0.00	0.00	0.00	0.00	17.50	100.00	100.00
0.500	0.00	0.00	0.00	0.00	32.50	100.00	100.00
				Day-4			
0.005	0.00	5.00	10.00	20.00	15.00	32.50	52.50
0.050	0.00	5.00	2.50	2.50	5.00	92.17	22.50
0.100	0.00	2.50	0.00	2.50	7.50	100.00	100.00
0.200	0.00	0.00	4.00	0.00	47.50	97.50	-
0.250	0.00	35.00	10.00	0.00	47.50	100.00	-
0.500	0.00	5.00	0.00	0.00	70.00	-	-
				Day-10			
0.005	5.00	7.50	22.50	22.50	17.50	67.50	100.00
0.050	5.00	17.50	20.00	7.50	10.00	100.00	100.00
0.100	30.00	2.50	15.00	10.00	12.50	-	-
0.200	12.50	2.50	15.00	0.00	100.00	-	-
0.250	2.50	67.50	22.50	12.50	100.00	-	-
0.500	20.00	10.00	7.50	5.00	100.00	-	-
				Day-14			
0.005	37.50	12.50	32.50	22.50	17.50	97.50	100.00
0.050	57.50	22.50	37.50	17.50	25.00	100.00	-
0.100	57.5 1	10.00	62.50	27.50	100.00	-	-
0.200	50.00	2.50	37.50	17.50	-	-	-
0.250	22.50	75.00	27.50	22.50	-	-	-
0.500	42.50	12.50	12.50	20.00	-	-	-

Table 2. Effect of different concentrations of boric acid on the mortality of *M. unicolor*

Days	% concentrations			
	0.10%	**0.20%**	**0.25%**	**0.50%**
1	0.00 a	0.00 a	0.00 a	0.00 a
2	0.00 b	0.00 b	0.00 b	1.00 a
4	0.00 b	0.00 b	1.25 b	17.00 a
6	0.00 c	8.00 b	8.00 b	37.00 a
8	1.76 c	8.65 b	9.66 b	93.88 a
10	0.79 c	7.92 b	8.15 b	100.00 a
12	6.38 d	22.09 c	81.90 b	-
14	17.42 c	59.93 b	100.00 a	-

Means in the same row followed by the same letter are not significantly different at 5% level as determined by DMRT.

dechloran, hydramethylnon and boric acid for C. *formosanus* and *R. flavipes* and mentioned that boric acid was the least toxic of the 4 toxicants tested and that, regardless of doses, 7 - 12 days elapsed before 90% of *R. flavipes* workers were killed by either dechloran or boric acid.

Our studies on toxicity and the behavioral response of *H. indicola* also indicated that boric acid is an effective slow-acting toxicant against subterranean termites.

Conclusions

Behavioral observations showed termite activity in at least low concentrations of mercuric iodide, zinc sulphate, molybdophosphoric acid, and boric acid. Mercuric iodide showed a slight tunneling activity as indicated by the tunnel size observed, but was soon followed by termite mortality. It was indicative of its higher toxicity, which is also confirmed by the force-feeding tests. Zinc sulphate and molybdophosphoric acid

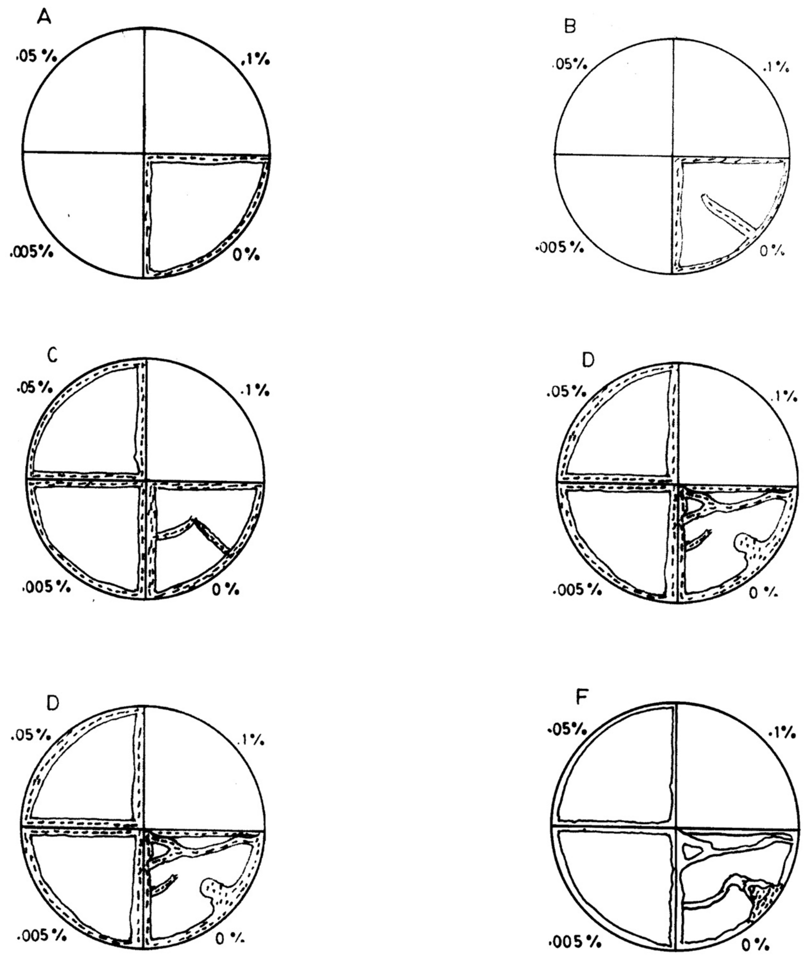

Figure 7. Activity of *H. indicola* in attractive bait treated with 0, 0.005, 0.05, and 0.10% zinc sulphate ($ZnSO_4$, $7H_2O$). A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 15 days; and F, after 18 days

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bait were preferred by the termites showing good termite galleries but the termites were active even after 14 days. A similar trend was evident in the toxicity test where the ELT90 could not be reached even after 14 days. This makes them undesirable as a toxicant. Boric acid (0.1% and lower concentrations) had a good termite activity. It shows the acceptability of boric acid-treated bait by the termites. Both the behavioural observations and force-feeding test showed that the ELT90 value for 0.1% boric acid was within a range of 2 weeks. This makes boric acid a good choice for use as a slow-acting toxicant and 0.25% boric acid is the proper concentration to be used as a bait toxicant against *M. unicolor*. The same concentration induced an earlier mortality in *H. indicola* indicating a higher sensitivity of *H. indicola* toward boric acid.

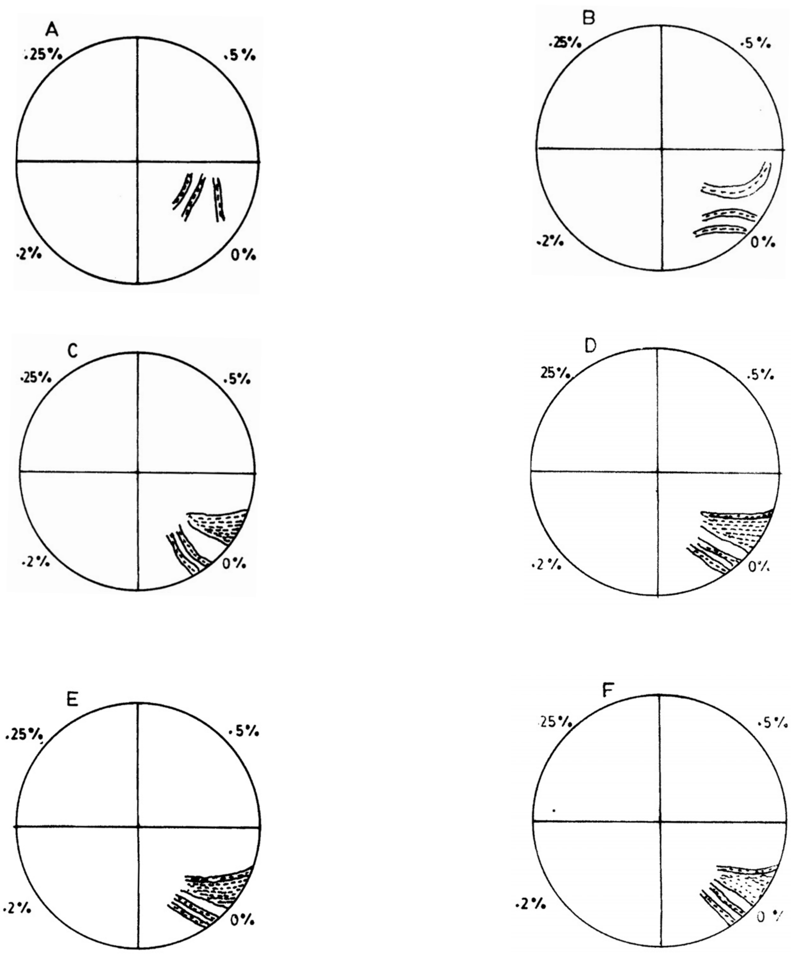

Figure 8. Activity of *H. indicola* in attractive bait treated with 0, 0.2, 0.25, and 0.50% zinc sulphate ($ZnSO_4 7H_2O$). A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 15 days; and F, after 18 days

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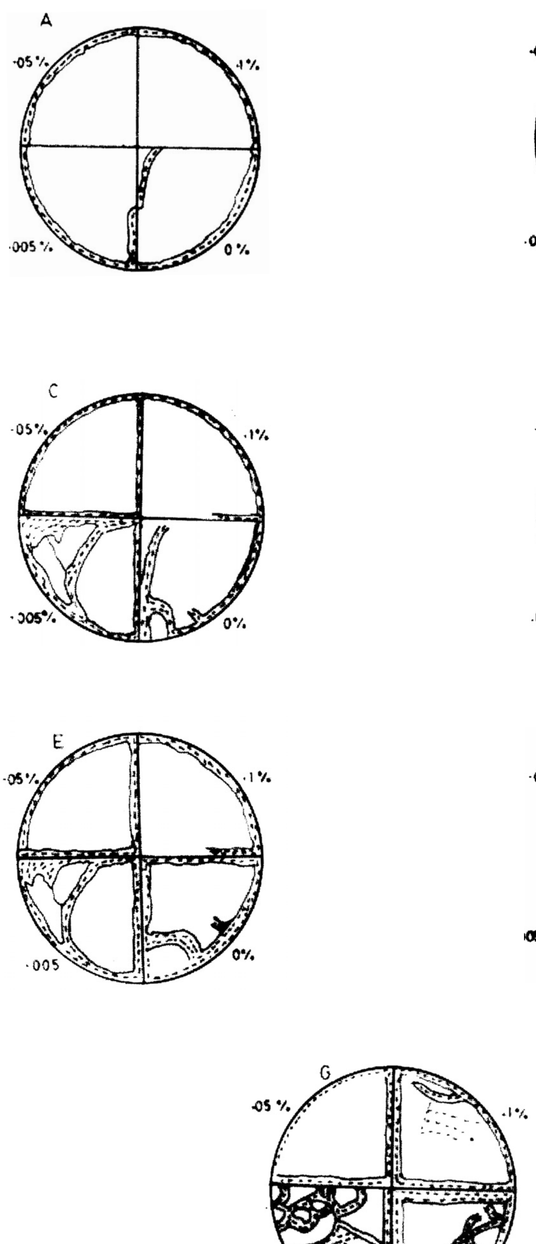

Figure 9. Activity of *H. indicola* in attractive bait treated with 0, 0.005, 0.05, and 0.10% molybdophosphoric acid {Mo(H_3PO_3)}. A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 15 days; and F, after 18 days, and G, after 32 days

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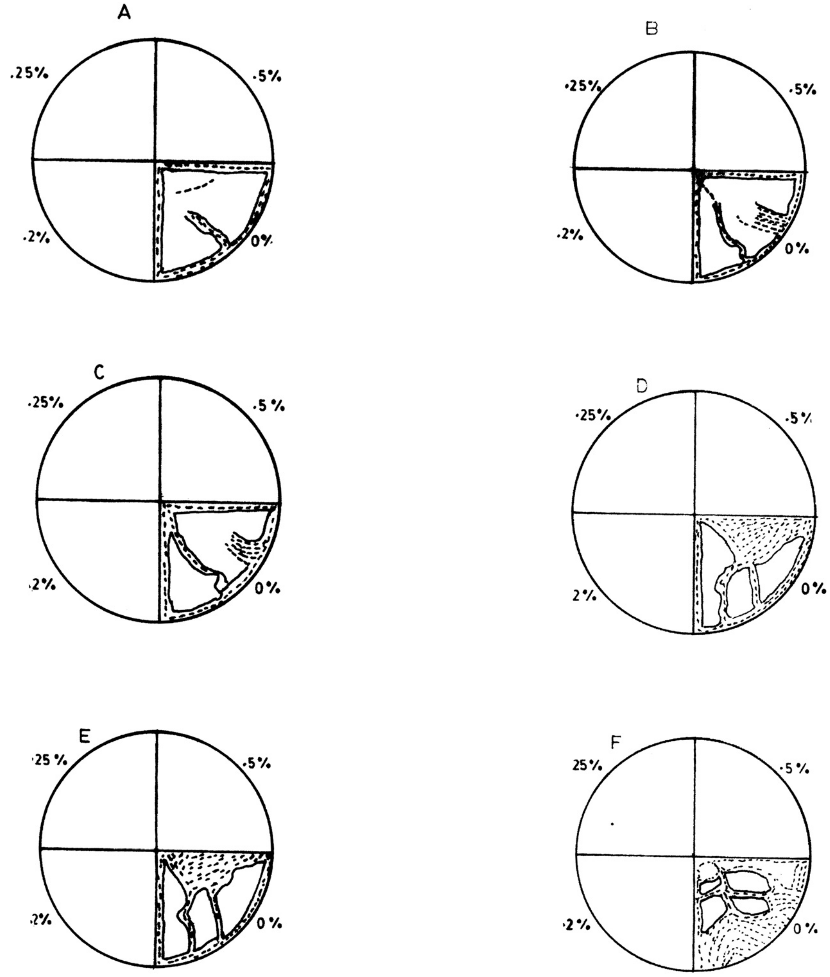

Figure 10. Activity of *H. indicola* in attractive bait treated with 0, 0.20, 0.25, and 0.50% molybdophosphoric acid {$Mo(H_3PO_3)$}. A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 15 days; and F, after 18 days, and G, after 32 days

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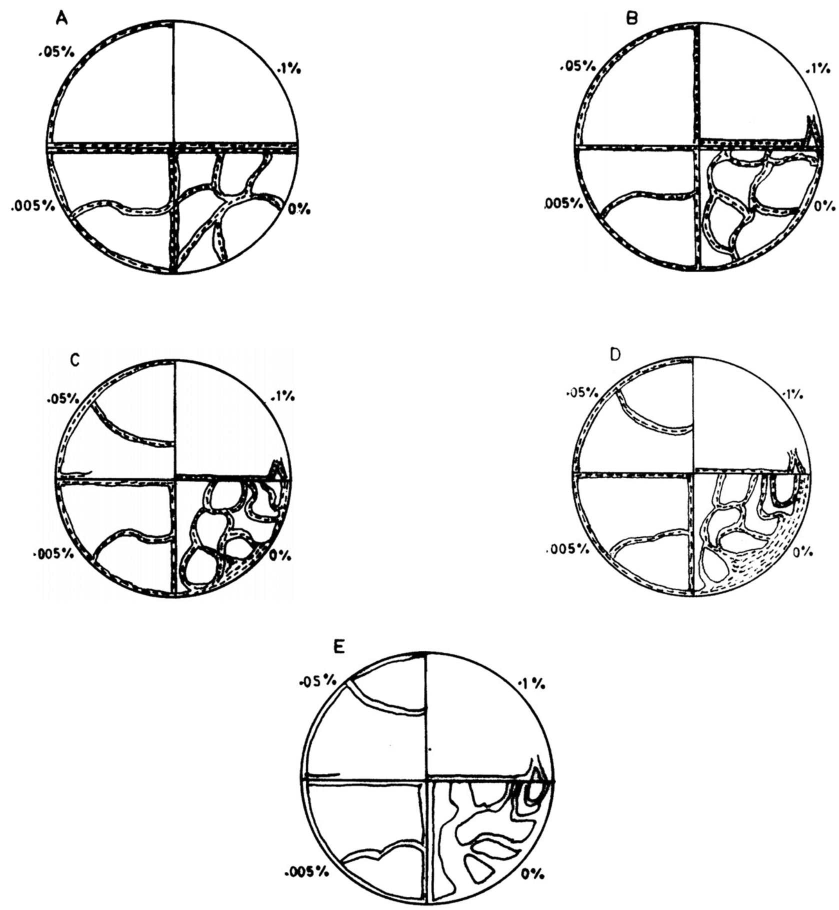

Figure 11. Activity of *H. indicola* in attractive bait treated with 0, 0.005, 0.05, and 0.10% boric acid (H_3BO_3). A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 15 days.

═══ denotes galleries without termites

:·:·: denotes termites

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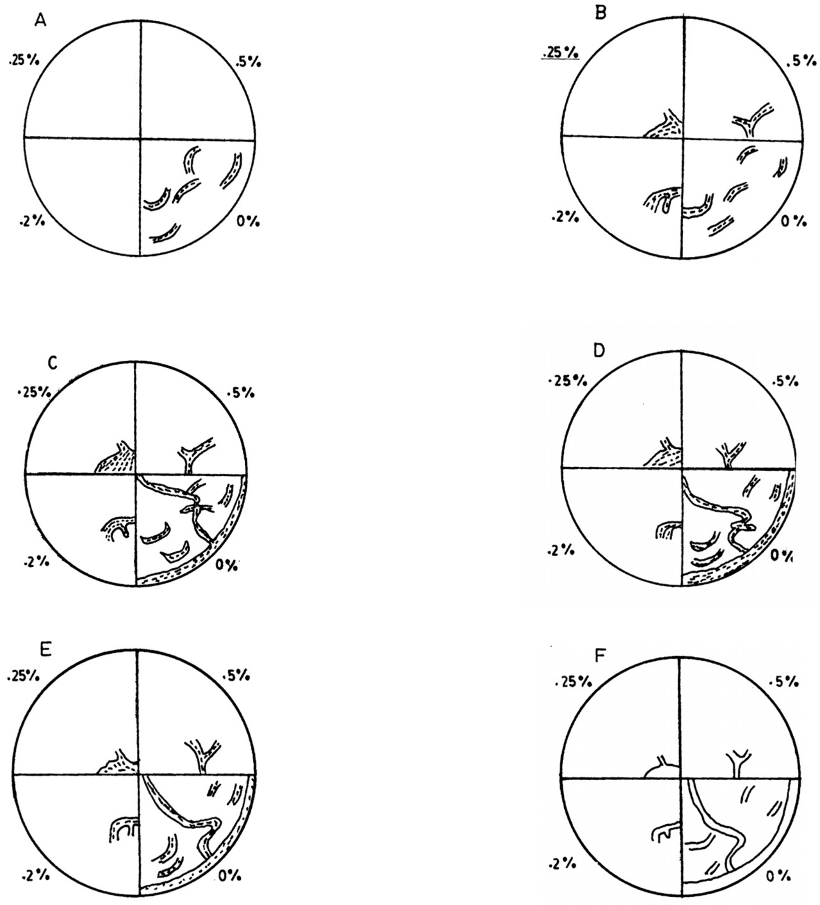

Figure 12. Activity of *H. indicola* in attractive bait treated with 0, 0.20, 0.25, and 0.10% boric acid (H_3BO_3). A, after 3 days; B, after 6 days; C, after 9 days; D, after 12 days; E, after 18 days; and F after 21 days.

═ denotes galleries without termites

:·:·: denotes termites

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