# 3 Results

## 3. 1 The effect of fenoxycarb on *R. santonensis*

In these experiments the effect of fenoxycarb on *R. santonensis* was tested. The optimum doses that are non-repellent or toxic but induce worker to soldier differentiation was determined, as well as the optimal method of application and the ways of fenoxycarb distribution among nest mates. Methods of applications tested were oral, contact and topical in addition to transferability of fenoxycarb by trophallaxis.

### 3.1.1 Oral treatment

#### 3.1.1.1 Mortality

Thirty termite workers were placed in each petri dish. The petri dishes contained one half filter paper treated with different concentrations of fenoxycarb and an untreated half of filter paper. Treatments of 0 (control), 0.32 ppm, 1. 6 ppm, 3.2 ppm, 16 ppm, 32 ppm fenoxycarb illustrated no significant difference in mortality throughout the experimental period. The results showed that fenoxycarb had no toxic effect on *R. santonensis* at the concentrations tested (Table 1, Fig. 8). Although the mortality was always higher at higher concentrations of fenoxycarb

Table 1: Mortality of *R. santonensis* when fed on filter papers treated with different concentrations of fenoxycarb. Results are given in means ± sd; N = 10 replicates; n = 30 workers /replicate.

| Days | Means and standard deviations of mortality at | | | | | | P [(1)] |
|---|---|---|---|---|---|---|---|
| | control | 0.32 ppm | 1.6 ppm | 3.2 ppm | 16 ppm | 32 ppm | |
| 5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 10 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 15 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.3 ± 0.6 | 1.0 ± 0.0 | 1.3 ± 0.6 | 1.0 ± 0.0 | n.s. |
| 20 | 1.0 ± 0.0 | 1.3 ± 0.6 | 1.7 ± 1.2 | 2.0 ± 0.0 | 2.0 ± 0.0 | 1.7 ± 0.6 | n.s. |
| 25 | 2.3 ± 1.2 | 4.3 ± 1.2 | 2.3 ± 2.1 | 4.3 ± 0.6 | 5.0 ± 1.7 | 4.3 ± 0.6 | n.s. |
| 30 | 4.3 ± 1.2 | 6.0 ± 3.0 | 4.3 ± 2.5 | 6.7 ± 0.6 | 8.0 ± 2.0 | 8.3 ± 3.2 | n.s. |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant.

Kruskal-Wallis ANOVA revealed no significant difference ($p > 0.05$) between the treatments at particular sampling days (Table1).

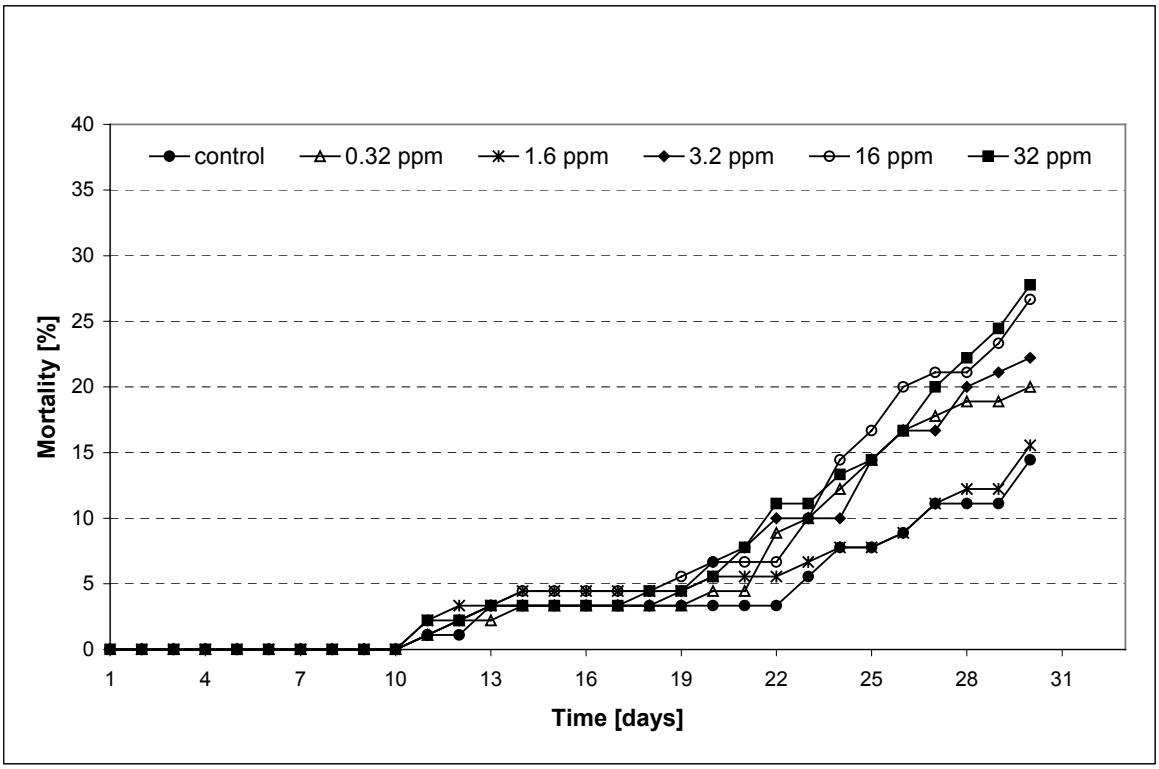



Fig. 8: Mortality [%] of *R. santonensis* workers when fed on filter papers treated with different concentrations of fenoxycarb for 30 days. Accumulative means of 10 replicates (n = 30 workers/replicate).

### 3.1.1.2 Presoldiers production

Differentiation of workers to presoldiers started after the first week of treatment. This only occurred at the higher concentrations of 16 and 32 ppm fenoxycarb. After one week the presoldiers moulted to full soldiers. At the end of the test period, the numbers of presoldiers produced were 19% and 38% at concentrations of 16 and 32 ppm, respectively (Table 2, Fig. 9). No presoldiers appeared at the control. Kruskal-Wallis ANOVA indicated a significant difference of presoldiers production between the treatments tested after day 15.

Statistical analysis (Mann-Whitney U-test) showed a significant difference of pre-soldiers produced at 16 ppm fenoxycarb compared to that produced at 32 ppm at

the end of the experiment. The control was significantly different in relation to treatments 16 ppm and 32 ppm fenoxycarb after day 15.

Table 2: Presoldiers production of *R. santonensis* when fed on filter papers treated with different concentrations of fenoxycarb. Results are given in means ± sd; N = 10 replicates; n = 30 / replicate.

| Days | Means and standard deviations of presoldiers production at | | | P [(1)] |
|---|---|---|---|---|
| | Control | 16 ppm | 32 ppm | |
| 5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 10 | 0.0 ± 0.0 | 1.7 ± 2.1 | 2.0 ± 1.0 | n.s. |
| 15 | 0.0 ± 0.0 | 5. 3 ± 6.7 | 6.0 ± 4.6 | * |
| 20 | 0.0 ± 0.0 | 5.7 ± 3.8 | 8.7 ± 4.6 | * |
| 25 | 0.0 ± 0.0 | 5.7 ± 2.3 | 11.3 ± 6.0 | * |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

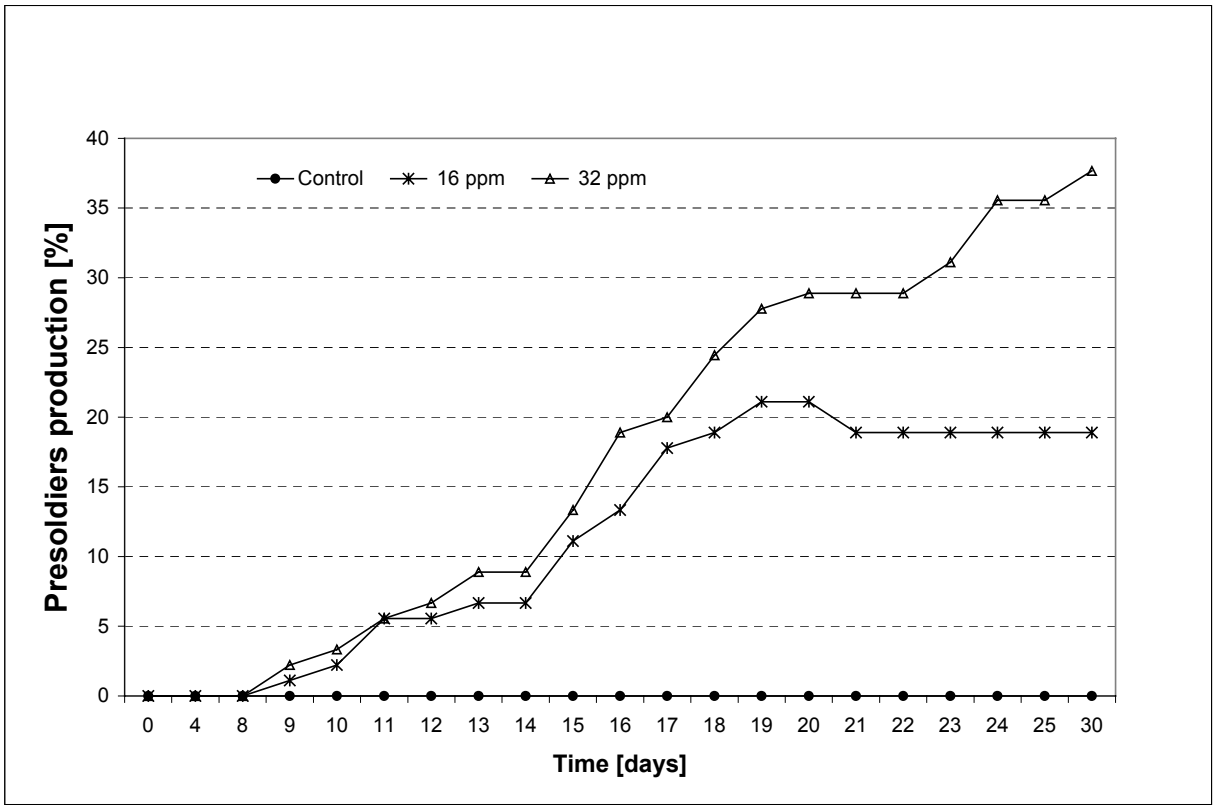



Fig. 9: Presoldiers production [%] of *R. santonensis* when fed on filter papers treated with different concentrations of fenoxycarb for 30 days. Accumulative means of 10 replicates (n = 30/replicate).

### 3.1.1.3 Deterrence test

*R. santonensis* workers were kept in petri dishes containing untreated and fenoxycarb treated halves of filter papers. The results showed that the mean consumption for termites feeding on the treated filter papers was similar to that of the untreated ones (Table 3).

Statistical analysis (Mann-Whitney U-test) showed no significant difference ($p > 0.05$) between the consumed weights when comparing treated and untreated filter papers for each concentration (Table 3 and Fig. 10).

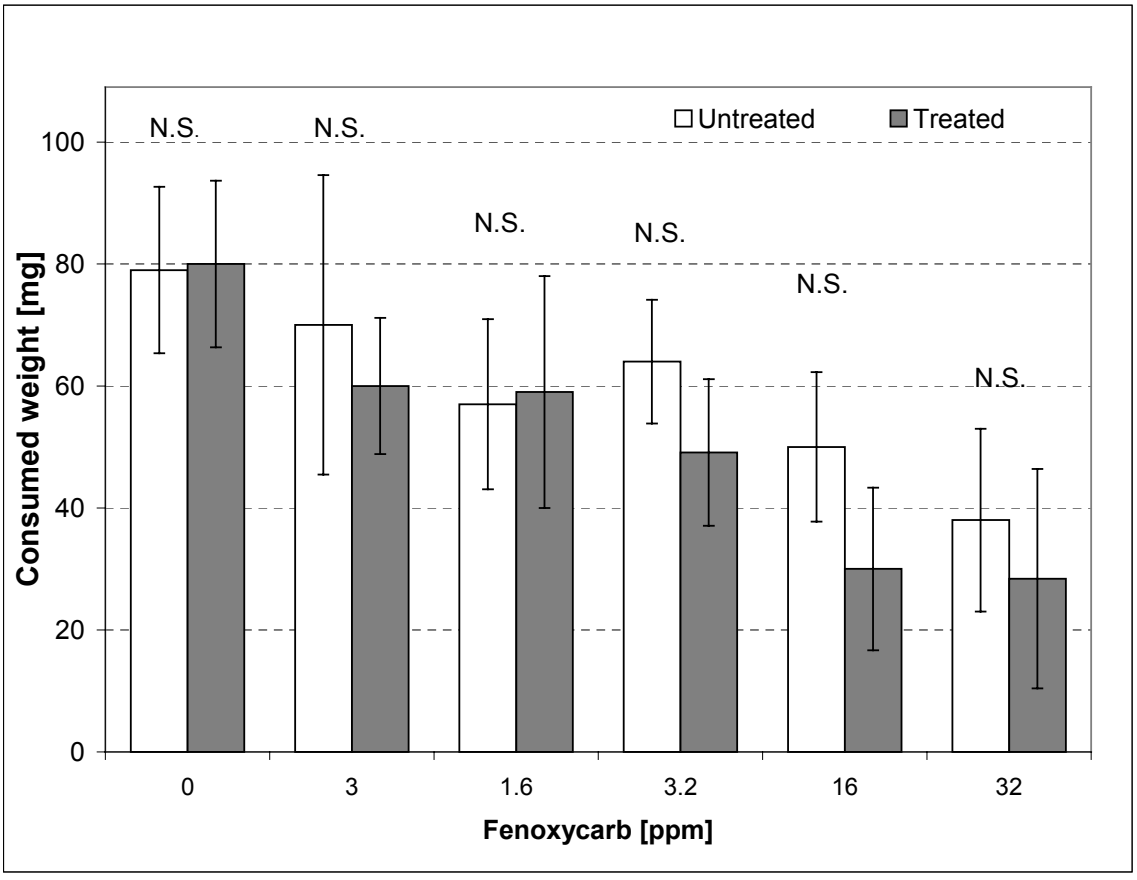



Fig. 10: Consumed weight [mg] of filter papers untreated and treated with different concentrations of fenoxycarb by *R. santonensis* within 30 days; Means ± sd of 10 replicates; (n= 30).

Table 3: Consumed weight of filter papers untreated and treated with different concentrations of fenoxycarb by *R. santonensis* for 30 days. Results are given in means ± sd, N = 10 replicates; n = 30 workers / replicates.

| Fenoxycarb concentration | Means ± sd of consumed weight [mg] | | P (1) |
|---|---|---|---|
| | Treatment | Control | |
| 0 ppm | 79.0 ± 13.6 | 80.0 ± 13.6 | n.s. |
| 0.32 ppm | 70.0 ± 11.2 | 60.0 ± 24.6 | n.s. |
| 1.6 ppm | 57.0 ± 19.0 | 59.0 ± 13.9 | n.s. |
| 3.2 ppm | 64.0 ± 12.0 | 49.1 ± 10.1 | n.s. |
| 16 ppm | 50.0 ± 13.3 | 30.2 ± 12.2 | n.s. |
| 32 ppm | 38.0 ± 18.0 | 28.4 ± 15.0 | n.s. |

(1) Mann-Whitney U-test; n.s. = not significant.

### 3.1. 2 Contact treatment

In this experiment workers of *R. santonensis* were kept in petri dishes, which had previously been treated with different concentrations of fenoxycarb.

#### 3.1. 2.1 Mortality

Mortality in the control increased gradually up to 15% at the end of the experiment (Table 4 and Fig. 11). At the concentration of 32 ppm fenoxycarb, mortality started at day 3 and continued at a similar trend as in the control, reaching 34% at the end of the experiment. At 320 ppm fenoxycarb, mortality started at day 3, reaching a mortality of 9% at day 18. Thereafter, it increased sharply to reach 31% by day 21 and 78% at the end of the experiment.

Kruskal-Wallis ANOVA showed that there was no significant difference ($p > 0.05$) in mortality between the treatments 0, 32 and 320 ppm fenoxycarb until day 18. However, from day 21 up to the end of the experiment, the mortality rate produced by the three treatments developed significantly different ($p < 0.05$) (Table 4). Mann-Whitney U-test revealed that the mortality at the concentration of 320 ppm fenoxycarb was significantly different ($p < 0.05$) in relation to both, 32 ppm fenoxycarb and the control after day 21. However, there was no significant

difference ($p > 0.05$) between the 32 ppm treatment compared to the control throughout the experimental periods.

Table 4: Mortality of *R. santonensis* when exposed to treated petri dishes (contact treatment) with different concentrations of fenoxycarb. Results are given in means ± sd; N = 10; n = 30 workers / replicate.

| Days | Means and standard deviations at | | | P [(1)] |
|---|---|---|---|---|
| | Control | 32 ppm | 320 ppm | |
| 3 | 0.7 ± 0.7 | 0.1 ± 0.3 | 0.5 ± 0.0 | |
| 6 | 1.0 ± 0.8 | 0.2 ± 0.6 | 1.9 ± 1.3 | n.s. |
| 9 | 1.0 ± 0.8 | 0.4 ± 1.3 | 2.0 ± 1.2 | n.s. |
| 12 | 2.7 ± 1.3 | 0.8 ± 1.4 | 2.4 ± 1.2 | n.s. |
| 15 | 3.3 ± 1.3 | 1.6 ± 1.4 | 2.5 ± 1.3 | n.s. |
| 18 | 3.3 ± 1.3 | 1.8 ± 1.7 | 2.8 ± 1.4 | n.s. |
| 21 | 3.4 ± 1.4 | 2.3 ± 1.5 | 9.4 ± 1.9 | * |
| 24 | 3.6 ± 1.4 | 6.3 ± 1.2 | 13.4 ± 2.2 | * |
| 27 | 4.1 ± 1.3 | 7.2 ± 1.0 | 23.0 ± 2.9 | * |
| 30 | 4.4 ± 1.2 | 8.0 ± 0.8 | 23.3 ± 2.5 | * |
| 33 | 4.6 ± 1.5 | 10.2 ±1.6 | 23.4 ± 2.3 | * |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

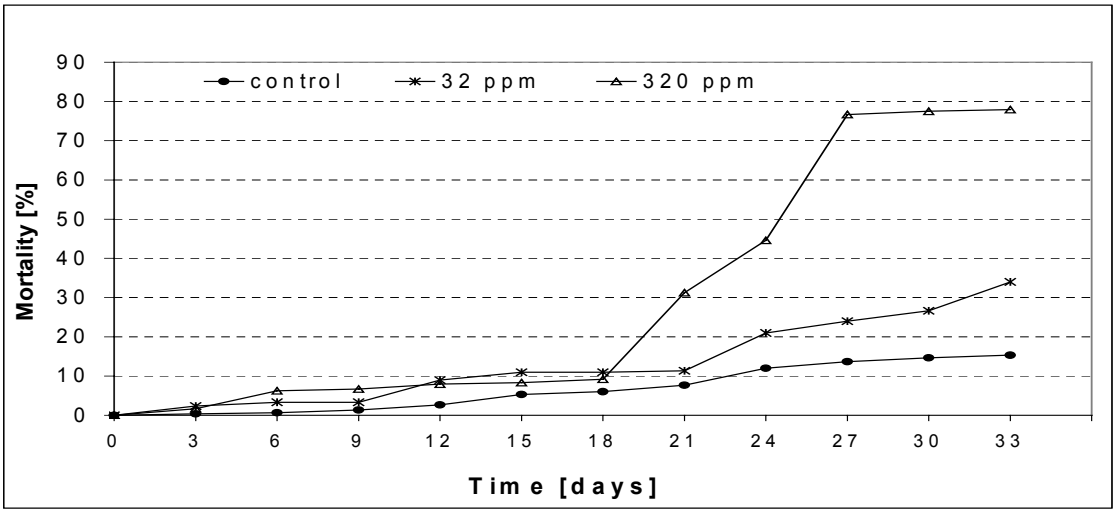



Fig. 11: Mortality [%] of *R. santonensis* when exposed to treated petri dishes (contact treatment) with different concentrations of fenoxycarb for 33 days. Accumulative means of 10 replicates, (n = 30 workers/replicate).

### 3.1.2.2 Presoldiers production

Workers of *R. santonensis* were exposed to fenoxycarb in treated petri dishes. Concentrations used were 320, 32 ppm, and the control. Presoldiers appeared at both fenoxycarb concentrations. No presoldiers appeared in the control (Table 5 and Fig. 12). At a concentration of 32 ppm fenoxycarb differentiation of workers to presoldiers started after day 9. The maximum number of presoldiers produced at this concentration was 44% at the end of the experiment (Fig. 12). At 320 ppm fenoxycarb, the percentage of presoldiers produced was about 6% at day 9 and the maximum number of presoldiers produced was 78%.

Table 5: Presoldiers production of *R. santonensis* when exposed to treated petri dishes with different concentrations of fenoxycarb. Results are given in means ± sd; N = 10; n = 30 workers / replicate.

| Days | Means and standard deviations of presoldiers production at | | | P (1) |
|---|---|---|---|---|
| | Control | 32 ppm | 320 ppm | |
| 3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 9 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.8 ± 0.5 | n.s. |
| 12 | 0.0 ± 0.0 | 2.3 ± 3.1 | 8.0 ± 1.2 | n.s. |
| 15 | 0.0 ± 0.0 | 4.1 ± 4.4 | 9.7 ± 1.2 | * |
| 18 | 0.0 ± 0.0 | 5.5 ± 5.0 | 13.6 ± 1.3 | * |
| 21 | 0.0 ± 0.0 | 7.9 ± 5.2 | 16.0 ± 1.4 | * |
| 24 | 0.0 ± 0.0 | 12.1 ± 8.6 | 19.1 ± 1.6 | * |
| 27 | 0.0 ± 0.0 | 12.3 ± 8.5. | 20.7 ± 12.8 | * |
| 30 | 0.0 ± 0.0 | 12.6 ± 8.1 | 22.0 ± 13.9 | * |
| 33 | 0.0 ± 0.0 | 11.6 ± 7.1 | 22.3 ± 13.0 | * |

(1) Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

Kruskal-Wallis ANOVA revealed that the number of presoldiers produced was not significantly different ($p > 0.05$) between the treatments 0, 32 and 320 ppm fenoxycarb until day 12. Thereafter, a significant difference ($p < 0.05$) between the three treatments occurred (Table 5). Statistical analysis using Mann-Whitney U-test revealed that the presoldiers production at the concentration of 320 ppm

fenoxycarb was significantly higher ($p < 0.05$) to both, 32 ppm fenoxycarb and the control throughout the experimental period after day 15. From day 15 on, a significant difference ($p > 0.05$) also occurred between the 32 ppm treatment and the control.

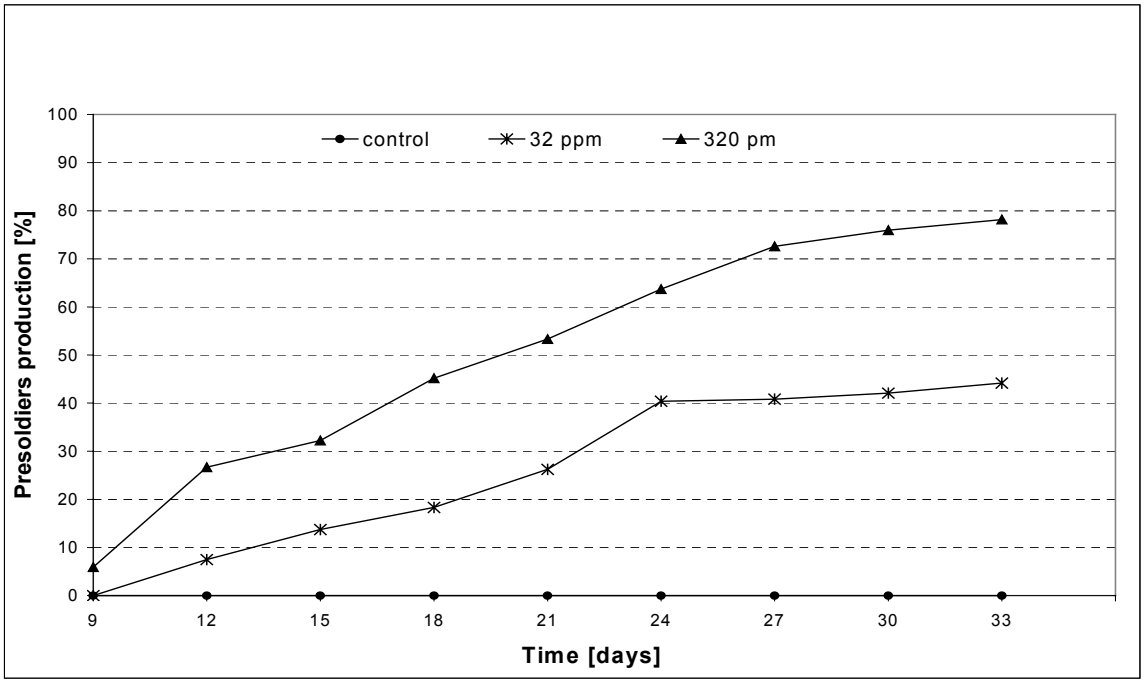



Fig. 12: Presoldiers production [%] of *R. santonensis* when exposed to treated petri dishes with different concentrations of fenoxycarb for 33 days. Accumulative mean of 10 replicates (n = 30 workers/ replicate).

### 3.1.3 Topical treatment

#### 3.1.3.1 Mortality

Topical treatments of *R. santonensis* workers with different concentrations of fenoxycarb revealed the following results. The mortality started at day 6 in the control both at 32 ppm and 320 ppm fenoxycarb and increased gradually reaching about 20% in the control and 27% with 32 ppm fenoxycarb at the end of the experiment (Table 6 and Fig. 13). At 320 ppm fenoxycarb, mortality started at a similar trend as in the other two treatments and increased gradually to reach 40% at day 18. Thereafter, a sharp increase occurred resulting in 100% mortality at day 21.

Statistical analysis (Kruskal-Wallis ANOVA) revealed no significant difference ($p > 0.05$) between the three treatments until day 18 (Table 6). A significant difference occurred at days 21 and 24. Mann-Whitney U-test (Table 6) showed no significant difference ($P > 0.05$) between 32 ppm and the control throughout the experimental period. However a significant difference ($p < 0.05$) at 320 ppm fenoxycarb to both, 32 ppm and the control, occurred at days 21 and 24.

Table 6: Mortality of *R. santonensis* workers when topically treated with different concentrations of fenoxycarb. Results are given in means ± sd; N = 10 replicates; n = 30 workers / replicate.

| Days | Means and standard deviations of mortality at concentrations | | | P [(1)] |
|---|---|---|---|---|
| | Control | 32 ppm | 320 ppm | |
| 3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 9 | 2.4 ± 1.1 | 2.6 ± 1.5 | 3.0 ± 2.0 | n.s. |
| 12 | 3.2 ± 0.8 | 3.4 ± 2.2 | 3.6 ± 2.3 | n.s. |
| 15 | 4.0 ± 1.4 | 5.0 ± 2.3 | 8.2 ± 3.8 | n.s. |
| 18 | 4.8 ± 2.6 | 6.6 ± 3.8 | 12.0 ± 4.6 | n.s. |
| 21 | 5.0 ± 2.5 | 7.2 ± 4.1 | 30 ± 0.0 | * |
| 24 | 5.8 ± 3.8 | 8.0 ± 4.0 | 30 ± 0.0 | * |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

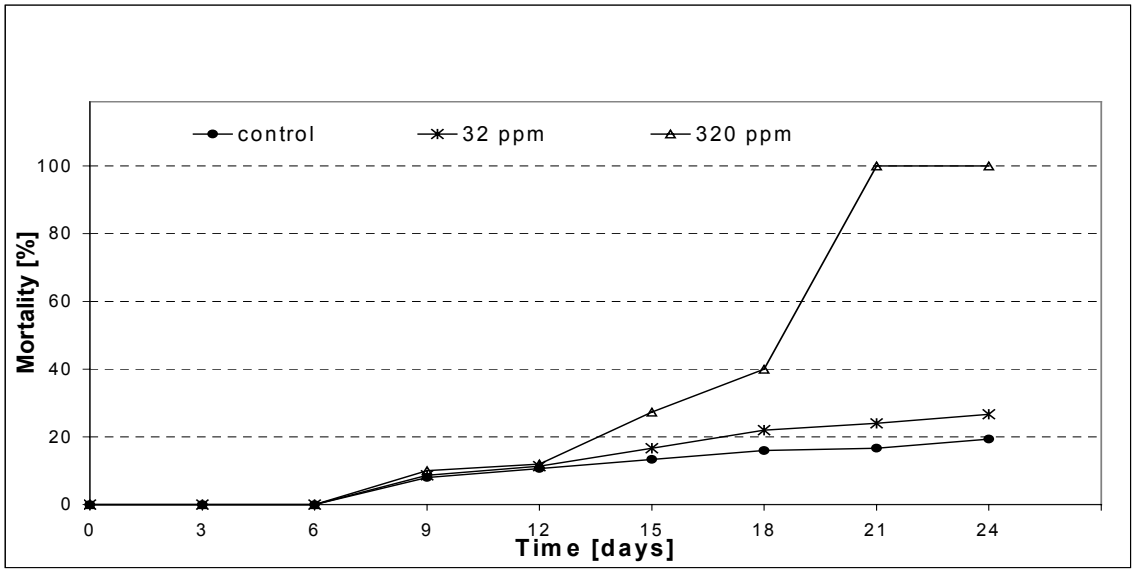



Fig. 13: Mortality [%] of *R. santonensis* when topically treated with different concentrations of fenoxycarb. Accumulative means of 10 replicates (30 workers/replicate).

### 3.1.3.2 Presoldier production

One week after topical application of fenoxycarb to *R. santonensis*, workers started to differentiate to presoldiers at concentrations of 32 ppm and 320 ppm. No presoldiers appeared in the control (Table 7 and Fig. 14). At day 9, the percentage of presoldiers was 6% and 7% at 32 ppm and 320 ppm fenoxycarb, respectively. For 32 ppm fenoxycarb the percentage of presoldiers was 12% at day 12, increasing gradually to reach 27% by the end of the experiment. At a concentration of 320 ppm fenoxycarb the percentage of presoldiers increased sharply up to 59% at day 12 and 64% at day 15.

Statistical analysis (Kruskal-Wallis ANOVA) showed that there was no significant difference ($p > 0.05$) between the three treatments until day 9. However, a significant difference occurred during the following days (Table 7). Mann-Whitney U-test revealed a significant difference ($p < 0.05$) of presoldier differentiation at 32 ppm and 320 ppm fenoxycarb when compared after day 9.

Table 7: Presoldiers production of *R. santonensis* workers when topically treated with different concentrations of fenoxycarb. Results are given in means ± sd; N =10 replicates; n = 30/replicate.

| Days | Means and standard deviations of presoldiers production at concentrations | | | P [(1)] |
|---|---|---|---|---|
| | Control | 32 ppm | 320 ppm | |
| 0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 9 | 0.0 ± 0.0 | 1.8 ± 0.9 | 2.2 ± 1.9 | n.s. |
| 12 | 0.0 ± 0.0 | 3.8 ± 1.3 | 17.8 ± 4.3 | * |
| 15 | 0.0 ± 0.0 | 6.3 ± 1.6 | 19.2 ± 4.1 | * |
| 18 | 0.0 ± 0.0 | 6.8 ± 1.8 | 19.2 ± 4.1 | * |
| 21 | 0.0 ± 0.0 | 8.3 ± 1.9 | 19.2 ± 4.1 | * |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

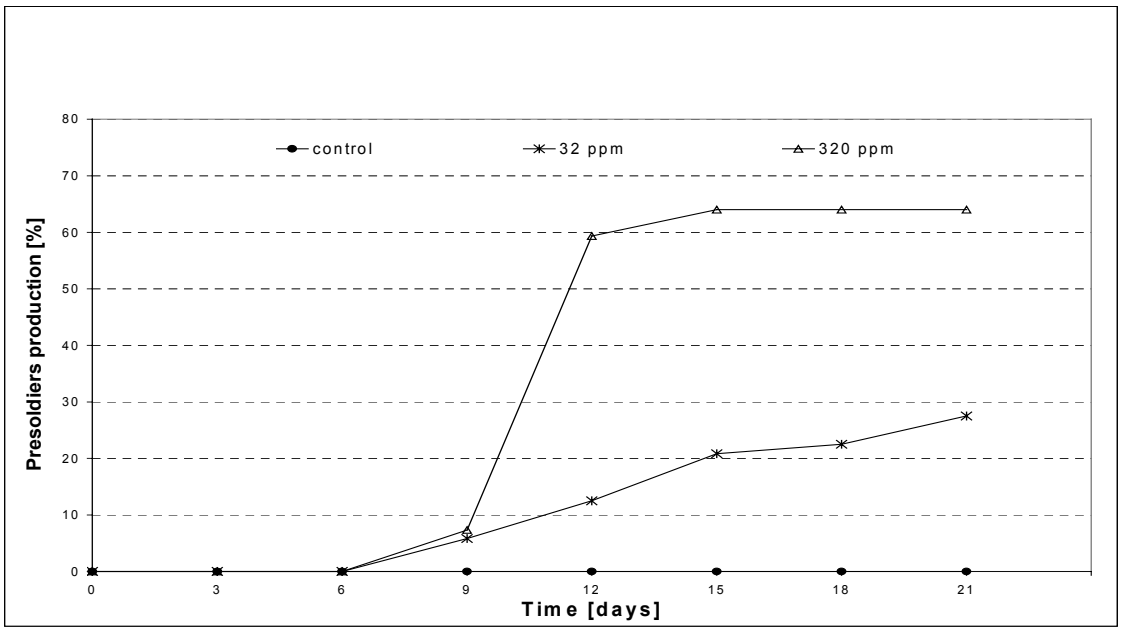



Fig. 14: Presoldiers production [%] of *R. santonensis* workers when topically treated with different concentrations of fenoxycarb. Accumulative means of 10 replicates (n = 30/replicate).

### 3.1.4 Transferability of fenoxycarb among *R. santonensis*

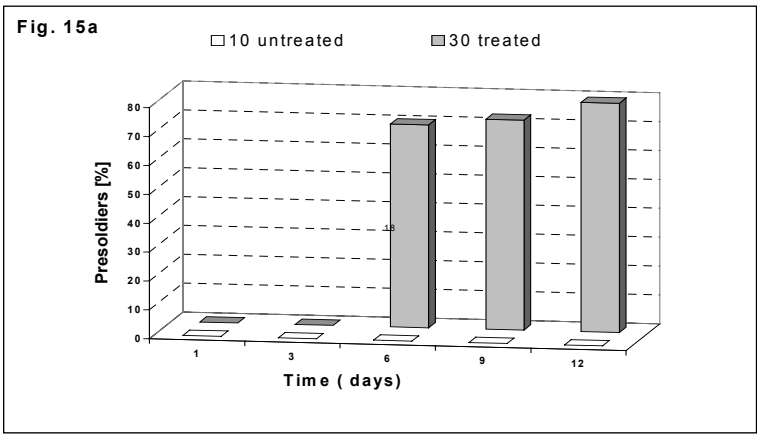
In these experiments the possibility of fenoxycarb distribution by *R. santonensis* through trophallaxis or grooming among the nest mates was studied using either oral or topical treatment of fenoxycarb.

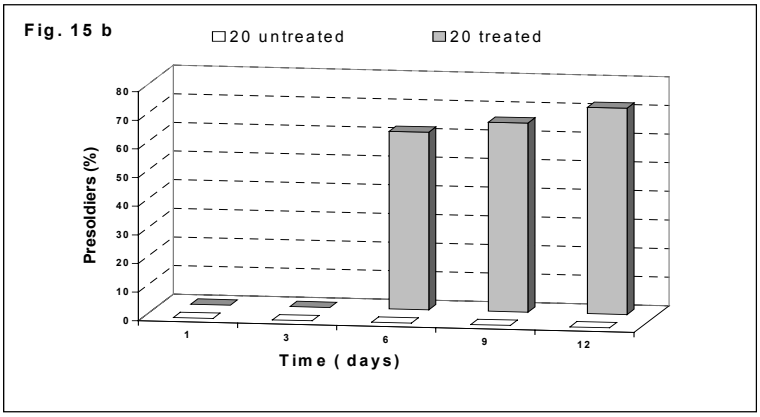
#### 3.1.4.1 Oral treatment

Workers of *R. santonensis* were fed for three days on filter papers treated with either 32 ppm or 320 ppm fenoxycarb and then kept with untreated marked workers (fed on filter papers dyed with 2% w/w neutral red for three days). The untreated groups were starved for 3 days to enhance the feeding process. The ratios between treated and untreated workers were 3:1, 1:1 and 1:3.

In all combinations with 32 ppm fenoxycarb, the treated workers started to differentiate into presoldiers on day 6 after being kept together (Table 8 and Fig. 15 a-c). At day 9 the percentage of presoldiers produced ranged between 66-72% reaching a range of 72-82% at the end of the experiment. None of the untreated workers moulted into presoldiers until the termination of the experiments after 12 days. Results indicated that the amount of fenoxycarb

transferred to the untreated workers was insufficient to induce worker to soldier differentiation.



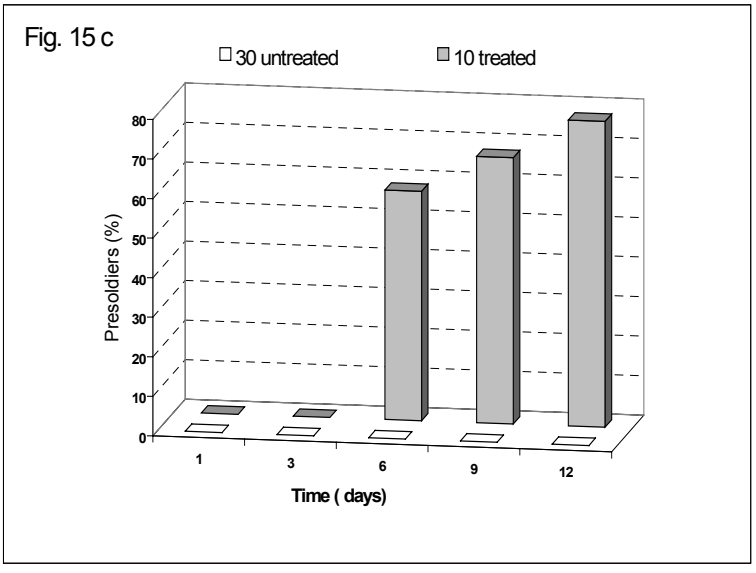




Fig. 15: Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers fed on filter papers treated with 32 ppm fenoxycarb to untreated workers. Three different ratios of treated and untreated were tested: a: 30 treated + 10 untreated; b: 20 treated + 20 untreated; c: 10 treated + 30 untreated. Means of 10 replicates

Table 8: Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers fed on filter papers treated with 32 ppm fenoxycarb to untreated workers. Three different ratios of treated to untreated workers were tested. Results are given in means ± sd; N = 10; n = 40 workers / replicate.

| Days | Means ± standard deviations of presoldiers production [%] (32 ppm fenoxycarb) | | | | | |
|---|---|---|---|---|---|---|
| | 30 / 10[1] | | 20 / 20 | | 10 / 30 | |
| | Treated | Untreated | Treated | Untreated | Treated | Untreated |
| 0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 6 | 70.2 ± 14.7 | 0.0 ± 0.0 | 62 .0 ± 2.6 | 0.0 ± 0.0 | 58 ±13.7 | 0.0 ± 0.0 |
| 9 | 72.7 ± 13.1 | 0.0 ± 0.0 | 66.0 ± 12.0 | 0.0 ± 0.0 | 67.3 ±16.2 | 0.0 ± 0.0 |
| 12 | 79.3 ± 11.5 | 0.0 ± 0.0 | 72.0 ± 14.5 | 0.0 ± 0.0 | 77.3 ±10.3 | 0.0 ± 0.0 |

[1] treated workers / untreated workers

Treated workers (previously fed on filter papers treated with 320 ppm fenoxycarb), were transferred to other petri dishes together with the untreated ones. They started to moult at day 6 after being fed on the treated filter papers. At day 9 the number of presoldiers produced reached 42% and then increased gradually giving 72-80% at the end of the experiment. The untreated workers started to moult at day 6 after being mixed with treated ones (Fig. 16). The number of presoldiers was 20.8% at day 6 and reached 57.1 % at day 18 when the experiment was terminated (Table 9 and Fig. 17).

Fig. 16: Presoldiers that were produced from untreated workers after being kept together with orally treated workers.

Table 9: Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers fed on filter papers treated with 320 ppm fenoxycarb to untreated workers. Ratio of treated to untreated workers was 20 to 20. Results are given in means ± sd; N = 10; n = 40 workers / replicate.

| Days | Means ± SD of presoldiers production | |
|---|---|---|
| | treated | untreated |
| 0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 3 | 1.3 ± 2.98 | 0.0 ± 0.0 |
| 6 | 42.0 ± 6.91 | 20.8 ± 3.1 |
| 9 | 42.0 ± 4.47 | 29.3 ± 5.4 |
| 12 | 53.3 ± 8.16 | 34.5 ± 7.8 |
| 15 | 59.3 ± 8.9 | 41.3 ± 4.5 |
| 18 | 72.7 ± 9.3 | 57.1 ± 6.3 |

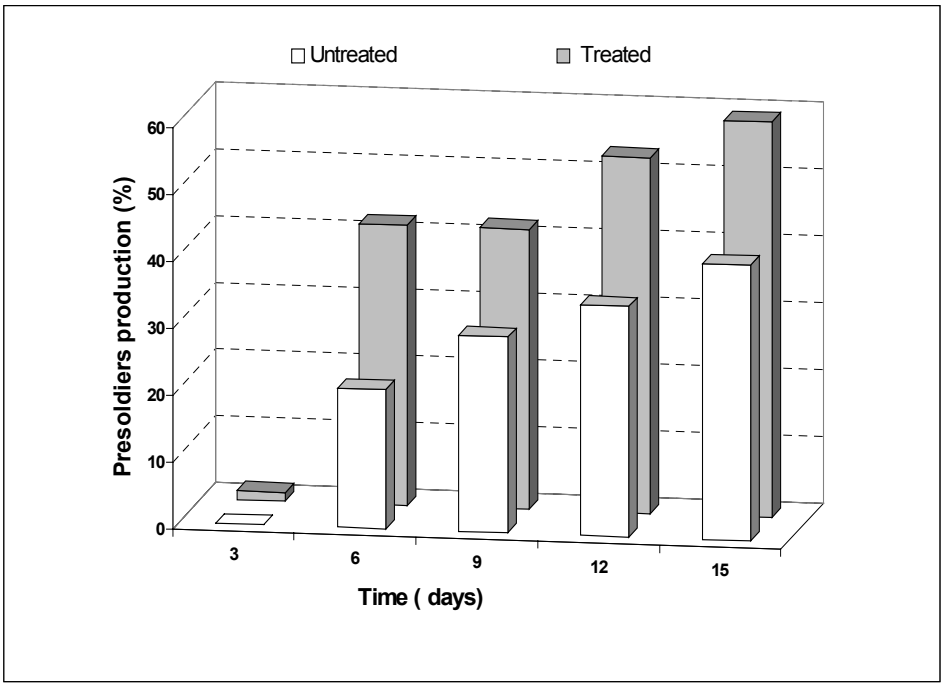



Fig. 17: Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers fed on filter papers treated with 320 ppm fenoxycarb to untreated workers. Ratio of treated to untreated workers was 1:1. Means of 10 replicates; (40 workers/replicate).

### 3.1.4.2 Topical application

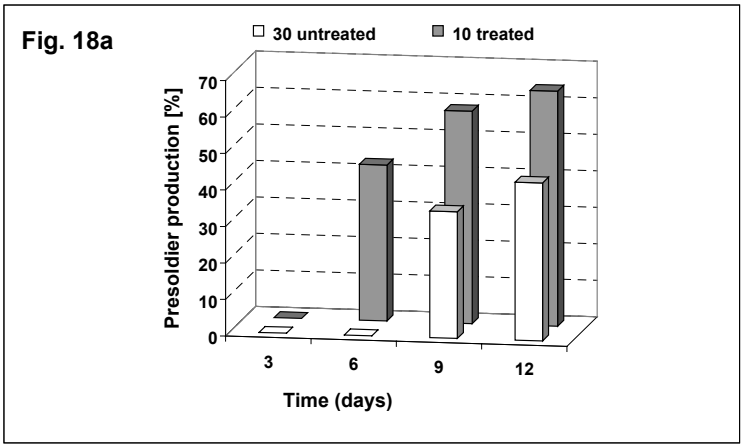
When *R. santonensis* workers were topically treated with 320 ppm fenoxycarb and mixed with untreated workers (marked with neutral red) both groups differentiated to presoldiers (Table 10 and Fig. 18 a-c). The treated workers started moulting to presoldiers on day 5 after being mixed with the untreated group, whereas the untreated group started moulting to presoldiers on day 9. It is apparent from Fig. 18 a-c that the numbers of presoldiers produced from treated workers were always higher than those produced from untreated ones at the different ratios used. The number of presoldiers produced from the untreated workers seems to decrease with decreasing ratios of treated workers.

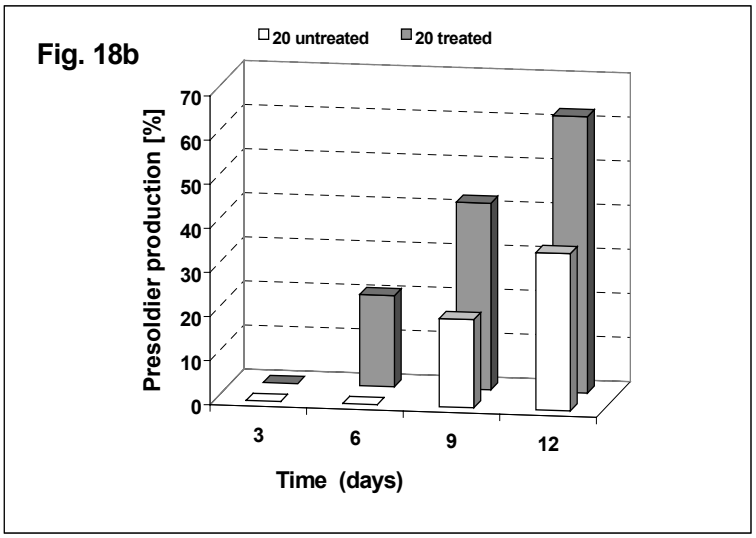
Therefore, it is possible to conclude that fenoxycarb could be transferred between workers and later on nest mates inside the colony by contact or trophallactic behaviour.

Table 10: Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers topically treated with 320 ppm fenoxycarb to untreated workers. Three different ratios of treated to untreated workers were tested. (Mean ± sd; N = 10 replicates, 40 workers/ replicate).

| Days | Means ± standard deviations of presoldiers production [%] (320 ppm fenoxycarb) | | | | | |
|---|---|---|---|---|---|---|
| | 30 / 10[1] | | 20 / 20 | | 10 / 30 | |
| | treated | untreated | treated | untreated | treated | untreated |
| 0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 6 | 42.7 ± 0.0 | 0.0 ± 0.0 | 20.7 ± 0.0 | 0.0 ± 0.0 | 18.7 ± 13.6 | 0.0 ± 0.0 |
| 9 | 58.2 ± 11.3 | 34.7 ± 19.2 | 42.3 ±7.1 | 20.0 ± 14.9 | 39.3 ±19.8 | 16.0 ±11.0 |
| 12 | 64.4 ± 12.9 | 43.3a ± 18.0 | 62.7 ± 5.0 | 35.7b ± 17.9 | 62.0 ±18.6 | 22.0c ± 11.7 |

[1] treated workers / untreated workers, Kruskal-Wallis ANOVA; * means with different letters are significantly at $p \leq 0.05$.



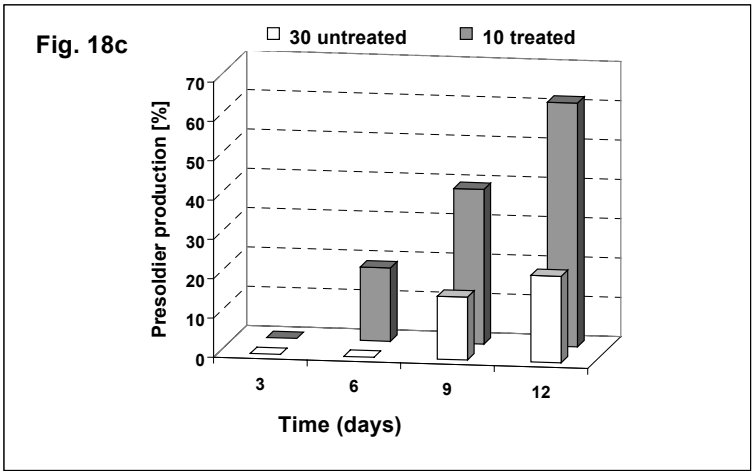




Fig. 18 (a, b, c) :Presoldiers production [%] after transfer of fenoxycarb from *R. santonensis* workers topically treated with 320 ppm fenoxycarb to untreated workers. Three different ratios of treated to untreated workers were tested: Fig. 18a: 30 treated + 10 untreated; Fig. 18b: 20 treated + 20 untreated; Fig. 18c: 10 treated + 30 untreated. Means; N = 10 replicates (40 workers/ replicate).3.2 Quantity of fenoxycarb in workers after different treatments

## 3.2 Quantity of fenoxycarb in workers after different treatments

### 3.2.1 Direct treatments of workers

#### 3.2.1.1 Fenoxycarb in *N. castaneus* workers

Whole body alcoholic extract of 5 workers of *Neotermes castaneus*, were analysed for fenoxycarb as described in chapter 2.2.2.1. The workers were treated with 32 ppm or 320 ppm fenoxycarb by oral treatment or surface treatment.

Oral treatment with filter papers treated with 32 ppm fenoxycarb resulted in the extracted amount of 0.34 ng fenoxycarb per 5 termites, measured at day 5 and decreased to 0.05 ng at 9 day (Table 11, Fig.19). Offering 320 ppm fenoxycarb gave 1.45 ng at day 5 and decreased to 0.75 ng and 0.14 ng after 6 days and 9 days, respectively.

Surface treatment of *N. castaneus* workers with 32 ppm or 320 ppm fenoxycarb resulted in a lower extracted amount of fenoxycarb in the workers. Exposure to 32 ppm fenoxycarb gave an amount of 0.07 ng at day 3 that decreased to 0.03 ng at day 9 (Table 11, Fig.19). The amount measured from a whole body alcoholic extract after 3 days of exposure to 320 ppm fenoxycarb was 0.32 ng which decreased gradually to reach 0.19 ng by day 9.

Statistical analyses using Mann-Whitney U-test were made to compare oral and surface treatment. Results demonstrated that there was a significant difference ($p < 0.05$) in the amount of fenoxycarb measured after the exposure of workers to the two methods of application at both concentrations of 32 and 320 ppm, except at day 9. With oral treatment the extracted amount of fenoxycarb is higher than with contact treatment at both concentrations. The decline of the extracted amount within time probably is due to metabolisation of fenoxycarb, which also leads to the no longer significant different amounts at day 9.

Table 11: Amount of fenoxycarb detected by GC analysis in whole body extracts of *N. castaneus* workers after oral or contact treatment. Results are given in means ± sd; N = 5; n = 5 workers / replicate.

| Time (days) | 32 ppm treatment | | | 320 ppm treatment | | |
|---|---|---|---|---|---|---|
| | Oral | Contact | P | Oral | Contact | P [(1)] |
| 3 | 0.34 ± 0.20 | 0.07 ± 0.04 | * | 1.45 ± 0.84 | 0.32 ± 0.19 | ** |
| 6 | 0.31 ± 0.22 | 0.06 ± 0.04 | * | 0.75 ± 0.43 | 0.25 ± 0.03 | ** |
| 9 | 0.05 ± 0.03 | 0.03 ± 0.02 | n.s. | 0.14 ± 0.08 | 0.19 ± 0.11 | n.s. |

[(1)] Mann-Whitney U-test; n.s: not significant, *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

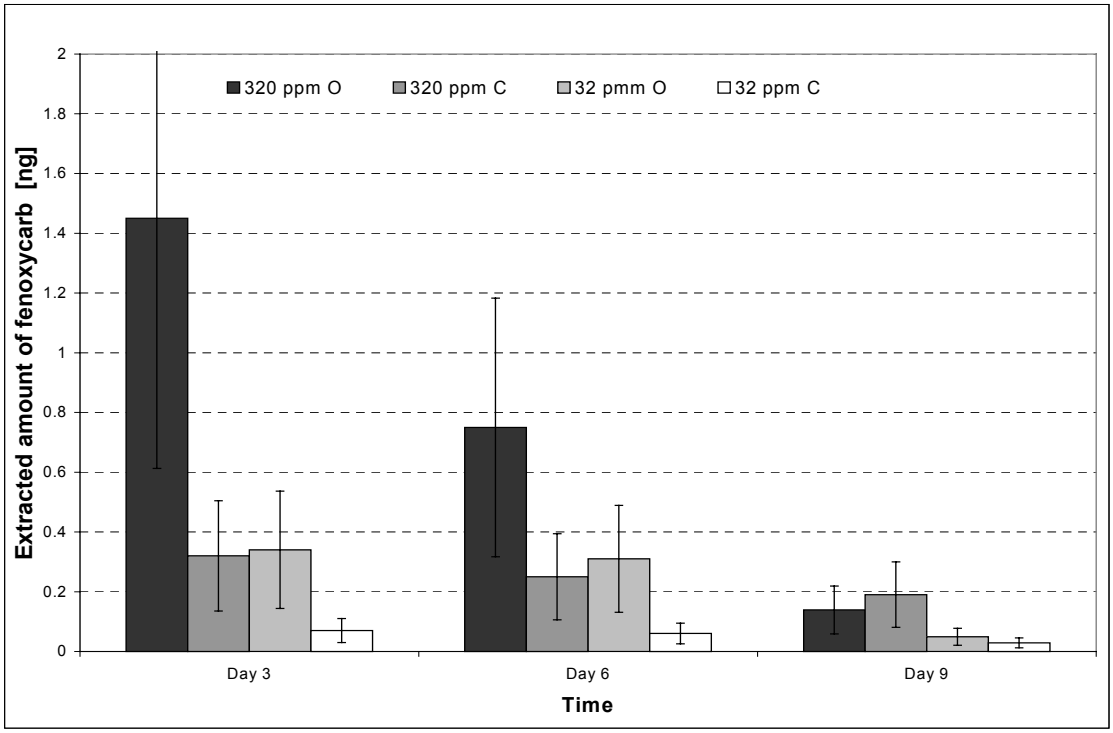



Fig.19: Amount of fenoxycarb [ng] detected by GC analysis in whole body extracts of *N. castaneus* after oral [O] or contact treatment [C]. Results are given in means ± sd; N = 5 replicates; (n = 5 workers / replicate).

### 3.2.1.2 Fenoxycarb in *R. santonensis* workers

Analysis of 20 workers of *R. santonensis* after oral or contact treatment with 320 ppm fenoxycarb revealed no significant difference ($p > 0.05$) between both

treatments at day 3, 6, 9 and 12 (Table 12, Fig. 20). However, a significant difference ($p < 0.05$) of the amount of fenoxycarb was measured at day 15. The amount of fenoxycarb extracted from workers that fed on treated filter papers was 37.7 ng at day 3 and decreased to 23.2 at day 15. At the contact treatment, the amount of fenoxycarb measured was 36.2 ng at day 3, and dropped to 5.8 ng at day 15 (Table 12, Fig. 20).

Table 12: Amount of fenoxycarb detected by GC analysis in whole body extracts of R. santonensis workers after oral or contact treatment with 320 ppm fenoxycarb. Results are given in means ± sd; N = 5 replicates; n = 5workers / replicate.

| Days | Oral treatment [ng] | Contact treatment [ng] | P (1) |
|---|---|---|---|
| 3 | 37.7 ± 18 | 36.2 ± 17.9 | n.s. |
| 6 | 25.5 ± 9.6 | 29.6 ± 10.2 | n.s. |
| 9 | 24.2 ± 11.5 | 28.9 ± 11.0 | n.s. |
| 12 | 24.2 ± 9.5 | 22.0 ± 4.5 | n.s. |
| 15 | 23.2 ± 7.4 | 5.8 ± 3.8 | * |

(1) Mann-Whitney U-test; n.s: not significant, *: significant at P = 0.05.

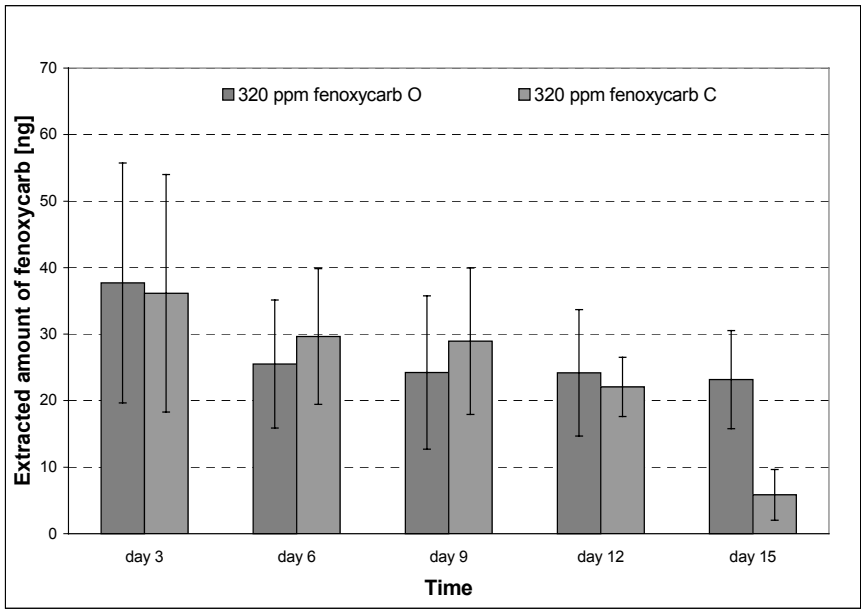



Fig. 20: Amount of fenoxycarb [ng] detected by GC analysis in whole body extracts of R. santonensis after oral [O] or contact treatment [C] with 320 ppm fenoxycarb. Results are given in means ± sd; N = 5; n = 5 workers / replicate.

### 3.2.2. Indirect treatments of workers through transferability of fenoxycarb by trophallaxis.

#### 3.2.2.1. Fenoxycarb in *R. santonensis* workers after oral treatment with 320 ppm

Workers of *R. santonensis* workers were fed on 320 ppm fenoxycarb and then kept together untreated workers. Analysis of extracts from *R. santonensis* after oral treatment revealed the presence of fenoxycarb in treated and untreated workers. *R. santonensis* workers fed on filter papers treated with 320 ppm fenoxycarb resulted in the extraction of 13.2 ng fenoxycarb after 3 days, which dropped to 10.7 ng after 12 days from the beginning of the experiment (Table 13, Fig. 21). Fenoxycarb extracted from untreated groups that have been kept with treated workers was 3.7 ng at day 3 and decreased to 1.1 ng at day 12.

Table 13: Amount of fenoxycarb [fe] [ng] detected by GC in whole body extracts of *R. santonensis* from both workers (fed on treated filter papers with 320 ppm fenoxycarb as donors or untreated workers as recipients. Results are given in means ± sd; N = 5 replicates; n = 20 workers / replicate.

| Time (days) | 320 ppm fenoxycarb | | P |
|---|---|---|---|
| | Donors | Recipients | |
| 3 | 33.2 ± 1.4 | 3.7 ± 0.4 | ** |
| 6 | 22.4 ± 12.2 | 2.2 ± 1.9 | ** |
| 9 | 12.8 ± 7.6 | 1.3 ± 2.2 | ** |
| 12 | 10.7 ± 9.0 | 1.1 ± 1.8 | ** |

(1) Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$.

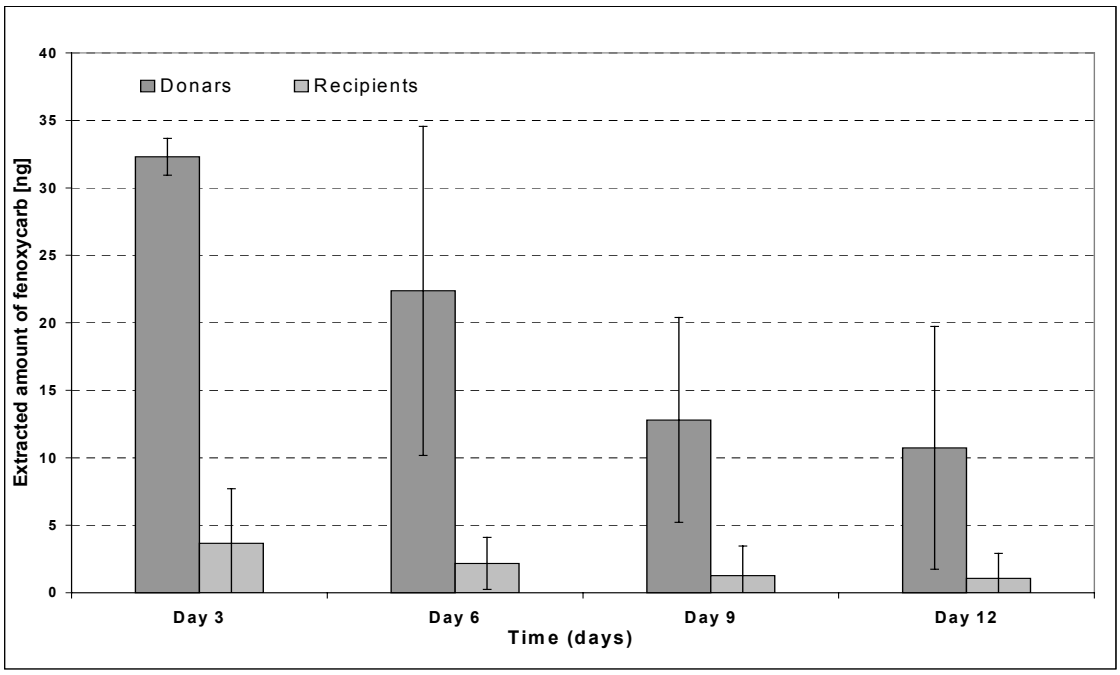



Fig. 21: Amount of fenoxycarb [fe] [ng] detected by GC in whole body extracts of *R. santonensis* from treated workers (fed on treated filter papers with 320 ppm fenoxycarb as donors or untreated workers as recipients. Means ± sd; N = 5 replicates; n = 20 workers / replicate.

### 3.2.2.2. Fenoxycarb in *R. santonensis* workers after topical treatment with 1600 ppm and 3200 ppm.

Workers of *R. santonensis* were topically treated with 1600 ppm or 3200 ppm fenoxycarb and then kept together with untreated workers. Analysis of extracts revealed an amount of 12.5 ng in workers topically treated with 1600 ppm when measured immediately after the treatment. This amount decreased with time reaching 0.9 ng at day 6 (Table 14, Fig. 22). Fenoxycarb extracted from untreated workers was 0.41 ng at day 1 and increased to 1.5 ng at day 3 after being together with treated workers (Table 14, Fig. 22).

In experiments with 3200 ppm fenoxycarb, treated workers had an amount of 28.1 ng when measured immediately after the treatment. This amount decreased with time reaching 13.9 ng after 3 days (Table 14, Fig. 22). Fenoxycarb extracted from untreated workers was 0.95 ng at day 1, and increased to 3.1 ng at day 3 after being together with treated groups (Table 14, Fig. 22).

Table 14: Amount of fenoxycarb [ng] detected by GC analysis in whole body extracts of *R. santonensis* from both topically treated workers as donors and untreated workers (recipients). Two different concentrations were tested: 1600 ppm and 3200 ppm. Results are given in means ± sd; N = 5 replicates; n = 20 workers / replicate.

| Days | 1600 ppm | | P | 3200 ppm | | P |
|---|---|---|---|---|---|---|
| | Donors | Recipients | | Donors | Recipients | |
| 0 | 12.5 ± 3.5 | 0.0 ± 0.0 | ** | 28.1 ± 3.7 | 0.0 ± 0.0 | ** |
| 1 | 9.6 ± 1.34 | 0.41 ± 0.18 | ** | 18.8 ± 2.3 | 0.95 ± 0.3 | ** |
| 2 | 7.0 ± 0.90 | 0.81 ± 0.49 | ** | 14.2 ± 2.2 | 1.84 ± 2.2 | ** |
| 3 | 6.2 ± 0.64 | 1.5 ± 0.67 | ** | 13.9 ± 1.0 | 3.09 ± 1.86 | * |
| 6 | 0.9 ± 0.48 | 0.18 ± 0.04 | n.s. | - | - | |

(1) Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

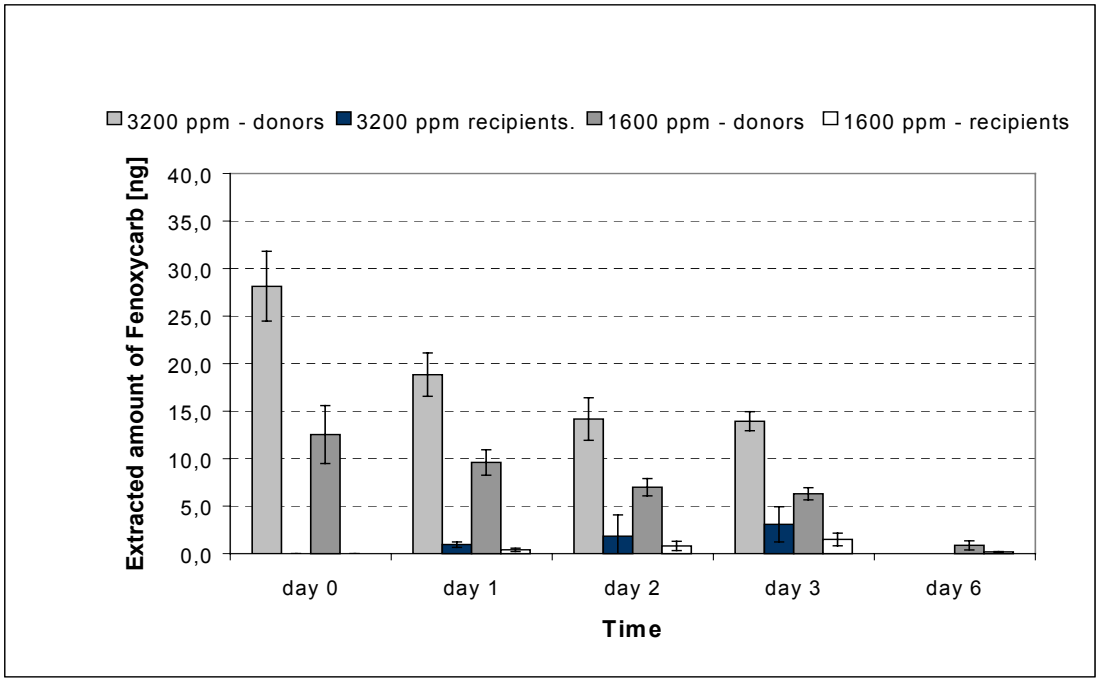



Fig. 22: Amount of fenoxycarb [ng] detected by GC analysis in whole body extracts of *R. santonensis* from both topically treated workers as donors and untreated workers (recipients). Two different concen-trations were tested: 1600 and 3200 ppm. Means ± sd; N = 5; n = 20 workers / replicate.

Statistical analysis using Mann-Whitney U-test revealed significant differences of the extracted amount from donors treated with 1600 ppm or 3200 ppm fenoxycarb when compared to that measured from untreated recipients at different sampling days. For 1600 ppm fenoxycarb there was a significant difference between the measured amount from both treated and untreated groups at day 1, 2 and 3 and no significant difference ($p > 0.05$) at day 6 (Table 14, Fig. 22).

## 3.3 The effect of flufenoxuron on *R. santonensis*

In these experiments the effect of flufenoxuron on *R. santonensis* was tested. The optimum lethal doses that are non-repellent or toxic but influence the formation of chitin during the moulting process had to be detected.

### 3.3.1. Mortality

Workers of *R. santonensis* were fed on filter papers treated with different concentrations of flufenoxuron. After 41 days the control group showed a mean mortality of 10% (Table 15 and Fig. 23). The mortality caused by flufenoxuron was 19% and 26% for 0.2 ppm and 1 ppm, respectively. Treatments of 2, 10, and 20 ppm flufenoxuron resulted in high mortality reaching 45%, 52%, and 65%, respectively. The lethal time to kill 50% ($LT_{50}$) was about 38 days at 10 ppm and 28 days for 20 ppm (Fig. 23). With the lower concentrations $LT_{50}$ was not reached.

Kruskal-Wallis ANOVA showed that there was no significant difference ($p > 0.05$) in mortality between the treatments tested until day 12. However, from day 15 up to the end of the experiment, the mortality was significantly different ($p < 0.05$) between all the treatments (Table 15). Mann-Whitney U-test revealed that the mortality at the concentration of 2 ppm flufenoxuron was not significantly different ($p > 0.05$) from either 10 or 20 ppm flufenoxuron. The mortality caused by 1 ppm flufenoxuron was not significantly different from 0.2 ppm or the control. However, a significant difference ($p < 0.05$) was found when comparing the mortality caused by 2 ppm, 10 ppm and 20 ppm flufenoxuron in relation to 0.2 ppm and 1 ppm flufenoxuron and the control.

Table 15: Mortality of *R. santonensis* when fed on filter papers treated with different concentrations of flufenoxuron. Results are means ± sd; N = 10 replicates; 30 workers/ replicate.

| Days | Means ± sd of mortality at different concentrations of flufenoxuron | | | | | | P (1) |
|---|---|---|---|---|---|---|---|
| | 0.0 ppm | 0.2 ppm | 1.0 ppm | 2.0 ppm | 10.0 ppm | 20.0 ppm | |
| 6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 12 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.3 ± 0.6 | 0.7 ± 0.6 | 0.7 ± 0.6 | n.s |
| 18 | 0.0 ± 0.0 | 1.0 ±1.0 | 1.0 ±1.0 | 5.0 ±1.0 | 4.0 ± 2.0 | 5.0 ± 2.0 | * |
| 24 | 0.7 ± 0.6 | 1.7 ±1.5 | 3.0 ± 1.7 | 6.3 ±1.5 | 5.3 ± 2.5 | 9.7 ± 4.7 | * |
| 30 | 1.7 ± 0.6 | 3.3 ±1.5 | 5.7 ± 2.3 | 9.0 ± 1.0 | 8.0 ± 2.6 | 17.0 ±11.4 | * |
| 36 | 1.7 ± 0.6 | 5.0 ± 3.5 | 7.0 ± 3.6 | 11.7 ± 2.1 | 13.0 ± 4.2 | 18.7 ±10.6 | * |
| 42 | 3.0 ±1.0 | 5.7 ± 4.6 | 8.0 ± 3.0 | 13.7 ± 2.9 | 15.7 ± 4.7 | 19.7 ± 9.6 | * |

(1) Kruskal-Wallis ANOVA; n.s = not significant, * = significant at $p \leq 0.05$.

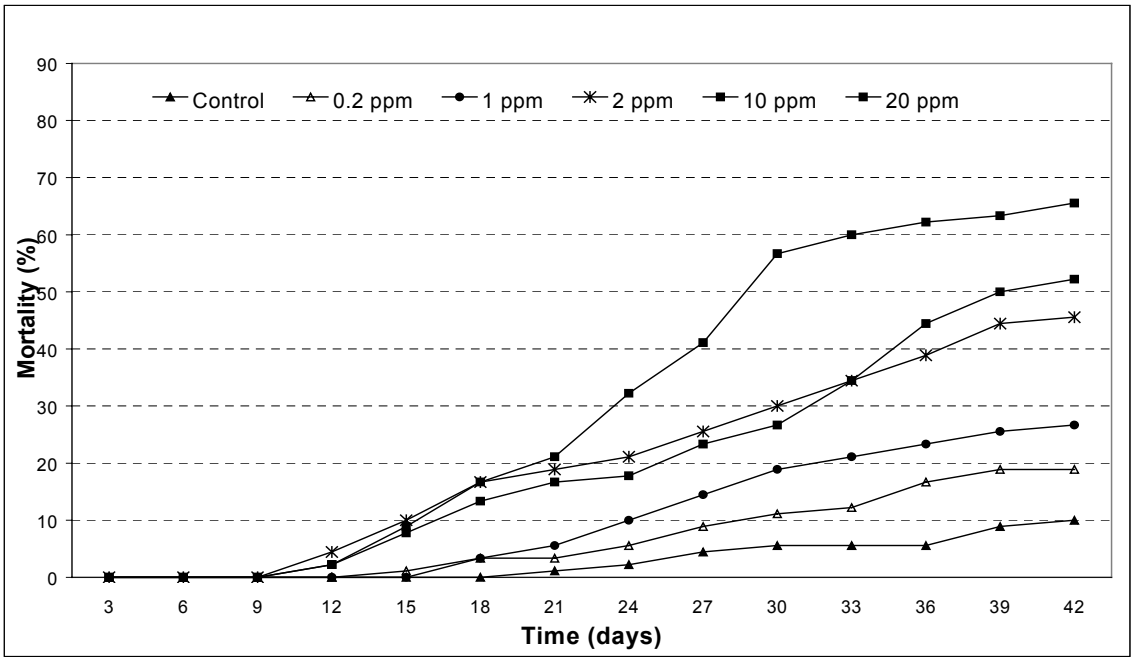



Fig. 23: Mortality [%] of *R. santonensis* workers when fed on filter papers treated with different concentrations of flufenoxuron for 42 days. Accumulative means of 10 replicates (n = 30 workers/replicate).

### 3. 3. 2 Deterrence test

*R. santonensis* workers were kept in petri dishes containing untreated and flufenoxuron treated filter papers. The results showed that workers fed upon both

halves (Table 16). The mean consumption for termites feeding on the treated filter papers was similar to that of the untreated ones. Statistical analysis (Mann Whitney U-test) showed no significant difference ($p > 0.05$) between the consumed weights when comparing treated and untreated filter papers for each concentration (Table 16 and Fig. 24). Comparing the mean weight consumed by termites at different concentration revealed no significant difference ($p > 0.05$) of weight loss at all the concentrations.

Table 16: Consumed weight of filter papers untreated and treated with different concentrations of flufenoxuron by *R. santonensis* for 42 days. Results are given in means ± sd, N=10 replicates; n = 30.

| Flufenoxuron | Means ± sd of consumed weight of treated filter papers | Means ± sd of consumed weight of untreated filter papers | P (1) |
|---|---|---|---|
| 0.2 ppm | 21.94 ± 9.76 | 30.27 ± 8.12 | n.s. |
| 1 ppm | 21.51 ± 4.70 | 24.28 ± 10.65 | n.s. |
| 2 ppm | 18.81 ± 9.76 | 28.14 ± 6.40 | n.s. |
| 10 ppm | 19.17 ± 4.60 | 20.27 ± 4.76 | n.s. |
| 20 ppm | 16.80 ± 6.36 | 17.00 ± 7.30 | n.s. |

(1) Mann Whitney U-test. n.s. = not significant.

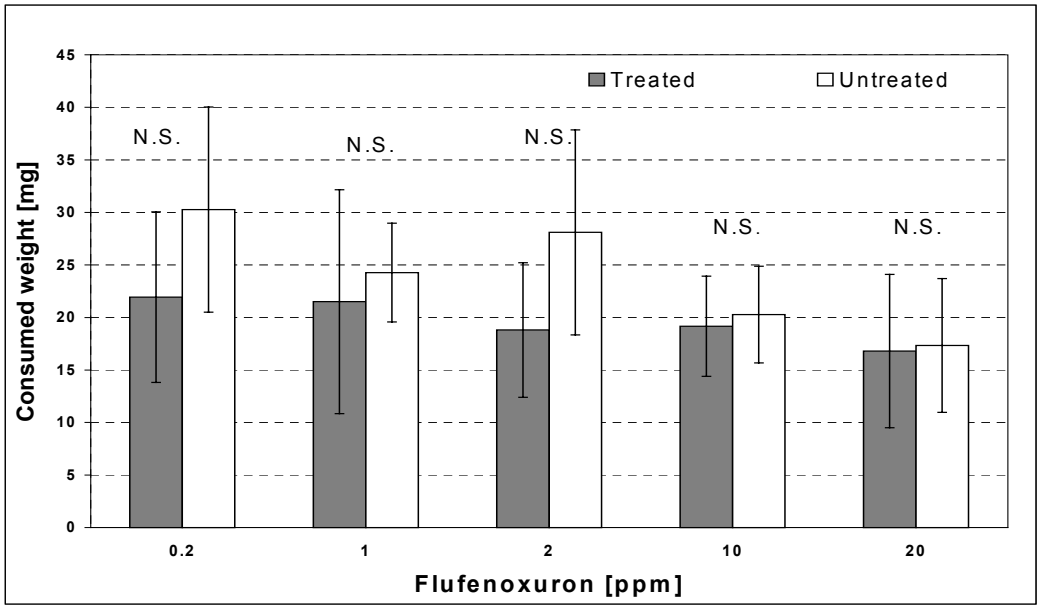



Fig. 24: Consumed weight [mg] of filter papers untreated and treated with different concentrations of flufenoxuron by *R. santonensis* for 42 days. [Means ± sd, N = 10 replicates; n= 30.

## 3.4 Combined treatment of fenoxycarb and flufenoxuron

Results showed that fenoxycarb is a juvenile hormone analogue, which causes presoldiers production. On the other hand, the use of flufenoxuron influences the moulting process causing a significant ecdysis inhibition in termites during moulting. In the following experiments, the two substances were combined to demonstrate the effect of flufenoxuron when the moulting of workers to presoldiers was induced by the use of fenoxycarb.

### 3.4.1 Effect of high doses of fenoxycarb and flufenoxuron

The mortality of *R. santonensis* workers treated with combinations of fenoxycarb and flufenoxuron started after day one, while in the control group dead termites were first observed at day 7. The maximum mortality in the control group was 10%. With the combination of 16 ppm fenoxycarb and 10 ppm flufenoxuron the $LT_{50}$ was reached on day 17, and by day 20 the mortality approached 80% (Table 17, Fig. 25). When 32 ppm fenoxycarb was combined with 20 ppm flufenoxuron, the $LT_{50}$ was reached on day 7 and almost 100% mortality was reached on day 20.Kruskal-Wallis test revealed a significant difference ($p < 0.01$) between the control and both treatments. Using Mann Whitney U-test, a significant difference ($p < 0.05$) between the control and either combinations was found at the end of the experiment at day 20. The differences between 16 ppm fenoxycarb combined with 10 ppm flufenoxuron and 32 ppm fenoxycarb combined with 20 ppm flufenoxuron were also significant ($p < 0.01$) except for day 20 (Table 17).

Table 17:Mortality of *R. santonensis* after oral treatment with filter papers treated with different concentrations of fenoxycarb [fe] and flufenoxuron [fl]. Results are given in means ± sd; N = 10; n = 30 workers / replicate.

| Days | Mean and standard deviation at | | | P (1) |
|---|---|---|---|---|
| | Control | (16 fe +10 fl) ppm | (32 fe+20 fl) ppm | |
| 5 | 0.0 ± 0.0 | 3.0 ± 1.6 | 14.2 ± 5.9 | * |
| 10 | 1.7 ± 0.6 | 9.8 ± 2.8 | 26.6 ± 3.0 | * |
| 15 | 3.3 ± 1.5 | 14.0 ± 3.4 | 28.8 ± 1.8 | * |
| 20 | 4.0 ± 1.0 | 24.0 ± 3.2 | 29.6 ± 0.9 | * |

(1) Kruskal-Wallis ANOVA; n.s : not significant, * : significant at $p \leq 0.05$.

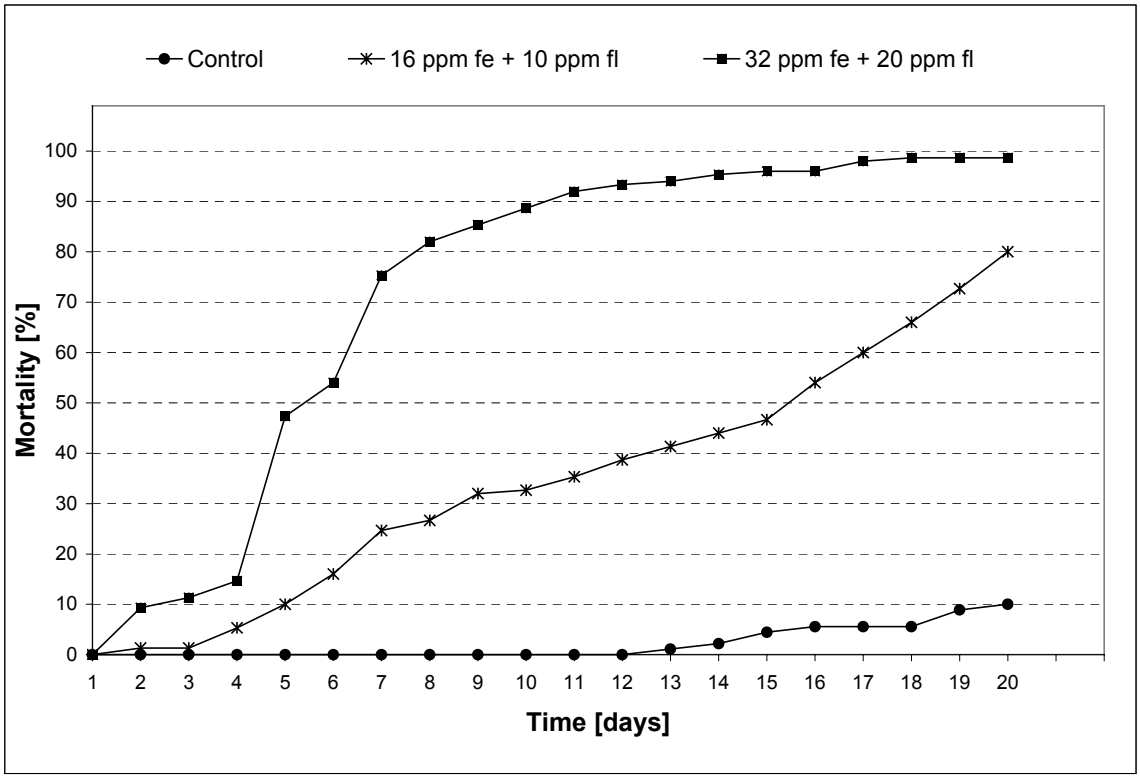



Fig. 25: Mortality [%] of *R. santonensis* after oral treatment with filter papers treated with different concentrations of fenoxycarb [fe] and flufenoxuron [fl] for 20 days. Accumulative means of 10 replicates (n = 30 workers/replicate).

### 3.4.2 Combined treatment of fixed fenoxycarb concentration with varying high concentrations of flufenoxuron

This experiment was carried out using one concentration of fenoxycarb (32 ppm) combined with different concentrations of flufenoxuron. Results showed that 66% mortality of *R. santonensis w*as obtained by a 32 ppm fenoxycarb + 2 ppm flufenoxuron combination on day 31 (Table 18, Fig. 26). The $LT_{50}$ occurred after 22 days of exposure. A combination of 32 ppm fenoxycarb + 0.2 ppm flufenoxuron resulted in 35.5% mortality on day 31 and the $LT_{50}$ was not reached until the end of the experiments. With a combination of 32 ppm fenoxycarb + 0.02 ppm flufenoxuron 12% mortality was obtained at day 31.

Statistical analysis (Kruskal-Wallis ANOVA) showed no differences between the three tested combinations and the control until day 13. A significant difference (p

< 0.01) was found when comparing the mean mortality of all treatments on the following days (Table 18). Using Mann-Whitney U-test, a significant difference ($p < 0.05$) between the mortality caused by the combination 32 ppm fenoxycarb + 2 ppm flufenoxuron and the other combinations as well as the control was found. Also the combination of 32 ppm fenoxycarb + 0.2 ppm flufenoxuron was significantly different to the control. The mortality caused by 32 ppm fenoxycarb + 0.2 ppm flufenoxuron combination was not significantly different from the combination of 32 ppm fenoxycarb + 0.02 ppm flufenoxuron.

Table 18: Mortality of *R. santonensis* after oral treatment with filter papers treated with different concentrations of fenoxycarb [fe] and flufenoxuron [fl]. Results are given in mean ± sd; N = 10 replicates; n = 30 workers/ replicate.

| Days | Mean mortality and standard deviation at | | | | P (1) |
|---|---|---|---|---|---|
| | Control | 32 ppm fe + 0.02 fl | 32 ppm fe + 0.2 ppm fl | 32 ppm fe+2 ppm fl | |
| 4 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.7 ± 2.9 | 0.0 ± 0.0 | n.s. |
| 10 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.7 ± 2.9 | 0.7 ± 0.6 | n.s. |
| 13 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.7 ± 2.9 | 3.3 ± 0.9 | n.s. |
| 16 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.7 ± 2.9 | 7.3 ± 1.2 | * |
| 19 | 0.0 ± 0.0 | 0.7± 0.6 | 5.0 ± 2.7 | 13.3 ± 1.5 | * |
| 22 | 0.3 ± 0.6 | 1.0 ± 1.0 | 6.0 ± 1.4 | 15.3 ± 2.7 | * |
| 25 | 1.0 ± 1.0 | 1.3 ± 1.5 | 7.0 ± 2.1 | 17.3 ± 4.2 | * |
| 28 | 1.7 ± 1.5 | 2.7 ± 3.1 | 10.3 ± 7.0 | 18.3 ± 3.6 | * |
| 31 | 1.7 ± 1.5 | 3.7 ± 4.7 | 10.7 ± 6.8 | 20.0 ± 4.0 | * |

(1) Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $P \leq 0.05$.

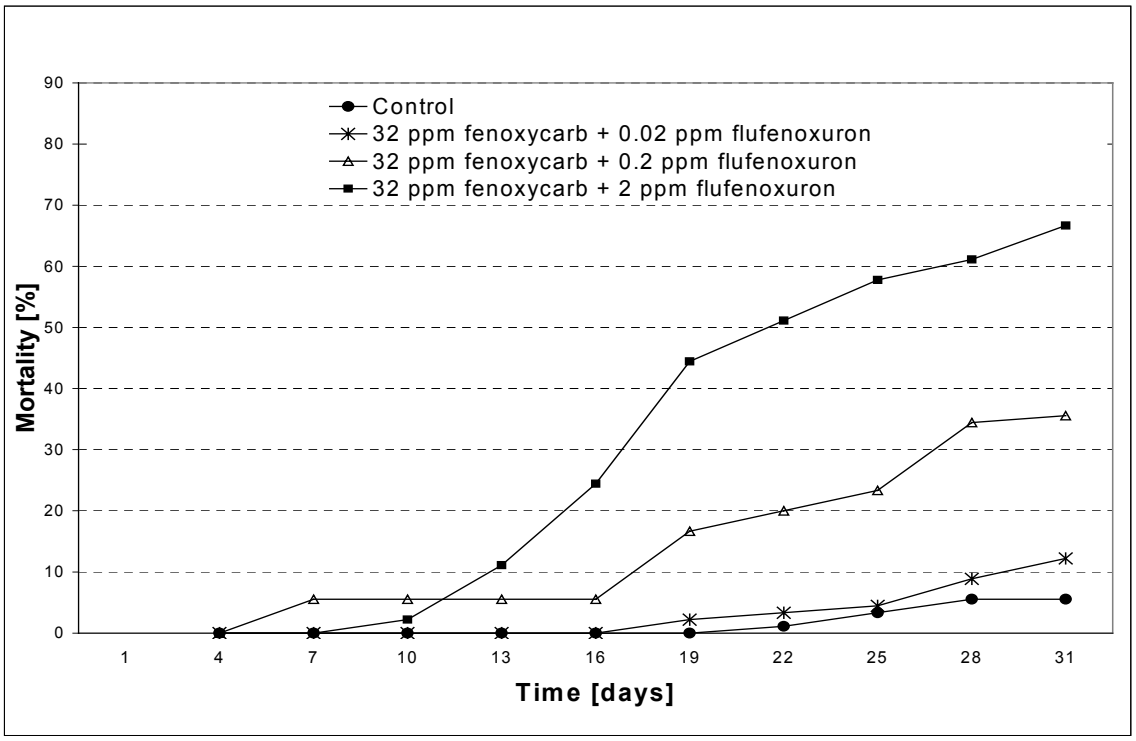



Fig. 26: Mortality [%] of *R. santonensis* after oral treatment with filter papers treated with constant concentration of fenoxycarb and varying flufenoxuron concentrations for 31 days. Accumulative means of 10 replicates (30 workers / replicate).

### 3.4.3 Combined treatment of fixed fenoxycarb concentration and varying lower flufenoxuron concentrations

#### 3.4.3.1 Mortality

Based on the high mortality caused by the combination of 32 ppm fenoxycarb with either 0.2 ppm or 2 ppm flufenoxuron, this experiment was designed using a lower concentration of fenoxycarb together with flufenoxuron. As control, the single substances were tested at the same concentration.

Using the single substances in concentrations of 16 ppm for fenoxycarb and 0.2 ppm for flufenoxuron resulted in a mortality of 11%, which was also found in the

control after 25 days (Table 19, Fig. 27). Combining the two substances at the mentioned concentrations resulted in a mortality of 25%, which is closer to that obtained with 2 ppm of flufenoxuron alone. The addition of 16 ppm fenoxycarb to 2 ppm flufenoxuron increased the mortality up to 40%, which is almost 4 times higher than with 0.2 ppm flufenoxuron alone.

Kruskal-Wallis ANOVA showed that the mortality was significantly different ($p < 0.05$) for all the treatments after day 5 (Table 19). Mann-Whitney U test revealed that the mortality at the concentrations of 16 ppm fenoxycarb and 0.2 ppm flufenoxuron and the control were significantly not different ($p > 0.05$) throughout the experimental period after day 5. Also the mortality caused by a concentration of 2 ppm flufenoxuron alone was not significantly different from the combination of 16 ppm fenoxycarb + 0.2 ppm flufenoxuron. However, a significant difference ($p < 0.05$) was found when comparing the mortality caused by the treatments with 16 ppm fenoxycarb, 0.2 ppm flufenoxuron and the control to 2 ppm flufenoxuron and 16 ppm fenoxycarb + 0.2 ppm flufenoxuron. In addition, the mortality caused by the combination of 16 ppm fenoxycarb + 2 ppm flufenoxuron was significantly different ($p < 0.05$) from all other treatments throughout the experimental period after day 5.

Table 19: Mortality of *R. santonensis* after oral treatment with filter papers treated with fenoxycarb and flufenoxuron. Results are given in means ± sd; N = 20; n = 30 workers / replicate.

| Days | Mean and standard deviation of mortality at concentrations | | | | | | P [(1)] |
|---|---|---|---|---|---|---|---|
| | Control | 16 ppm fe | 0.2 ppm fl | 2 ppm fl | 16 ppm fe + 0.2 ppm fl | 16 ppm fe +2 ppm fl | |
| 5 | 0.0 ± 0.0 | 1.8 ± 0.9 | 1.8 ± 0.4 | 3.9 ±1.2 | 1.1 ± 0.7 | 7.0 ±1.0 | * |
| 10 | 0.0 ± 0.0 | 3.1 ± 0.9 | 3.7 ± 1.1 | 12.6 ± 2.0 | 9.9 ± 3.7 | 23.6 ± 3.0 | * |
| 15 | 3.5 ±1.0 | 4.5 ±1.2 | 6.1 ± 2.1 | 18.1 ± 3.0 | 20.8 ± 2.5 | 30.3 ± 4.1 | * |
| 20 | 5.6 ±1.5 | 7.4 ± 3.1 | 9.1 ± 2.1 | 24.0 ± 3.5 | 23.5 ± 3.7 | 36.5 ± 4.1 | * |
| 25 | 10.0 ± 20 | 10.6 ± 3.1 | 11.8 ± 2.3 | 29.7 ± 3.1 | 24.9 ± 4.0 | 40.4 ± 4.2 | * |

[(1)] Kruskal-Wallis ANOVA; *: significant at $p \leq 0.05$.

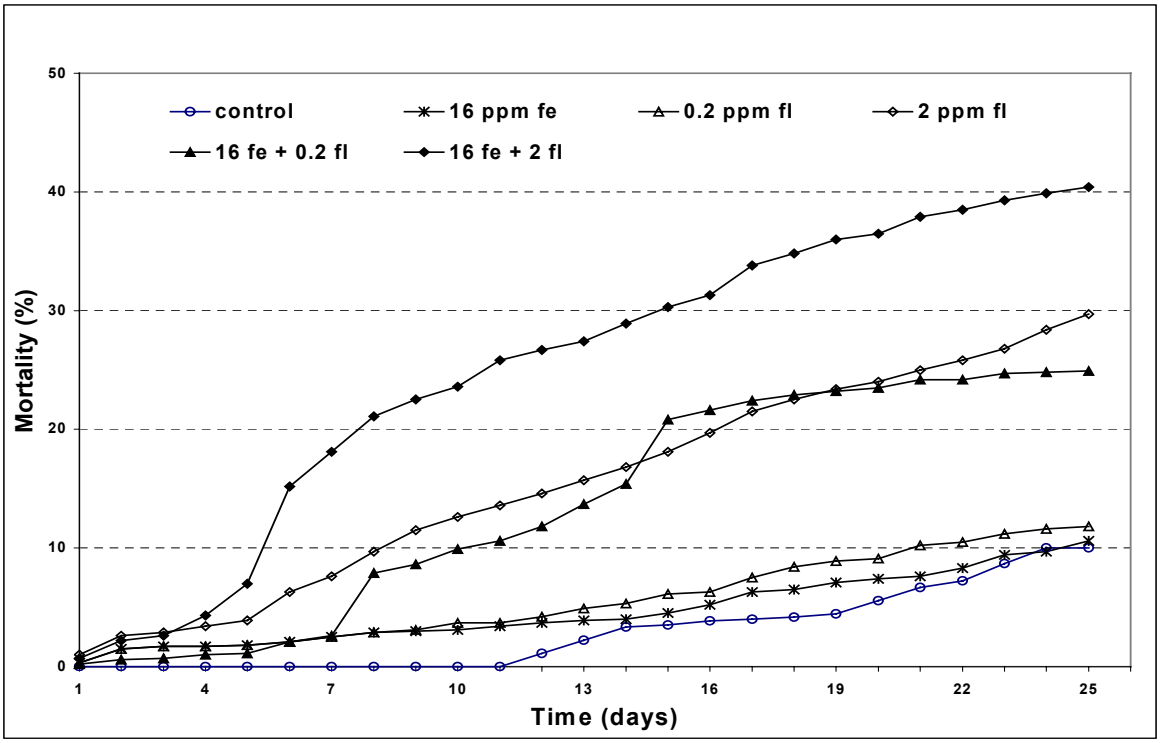



Fig. 27: Mortality [%] of *R. santonensis* after exposure to fenoxycarb [fe] and flufenoxuron [fl] and their combinations for 25 days. Accumulative means of 20 replicates (30 workers / replicate).

### 3.4.3.2 Presoldiers Production

The use of fenoxycarb alone resulted in a high production of presoldiers reaching the maximum (63%) in the third week after the beginning of the experiment (Table 20, Fig. 29). After that it started to decline due to the soldiers inability to survive without workers to feed them. A similar trend was observed for the low concentration of flufenoxuron (0.2 ppm) combined with 16 ppm fenoxycarb, which resulted in a high production of presoldiers reaching 47.6% three weeks after treatment. On the other hand, the higher concentration of 2 ppm flufenoxuron combined with 16 ppm fenoxycarb resulted in a low production of presoldiers. This probably was due to the inhibitory effect of flufenoxuron on the formation of chitin during the moulting process, thus leading to the death of the deformed workers (Fig. 28). The maximum number of presoldiers production at

this treatment was on day 15. The number of presoldiers produced was half of that at treatment of 0.2 ppm flufenoxuron combined with 16 ppm fenoxycarb.

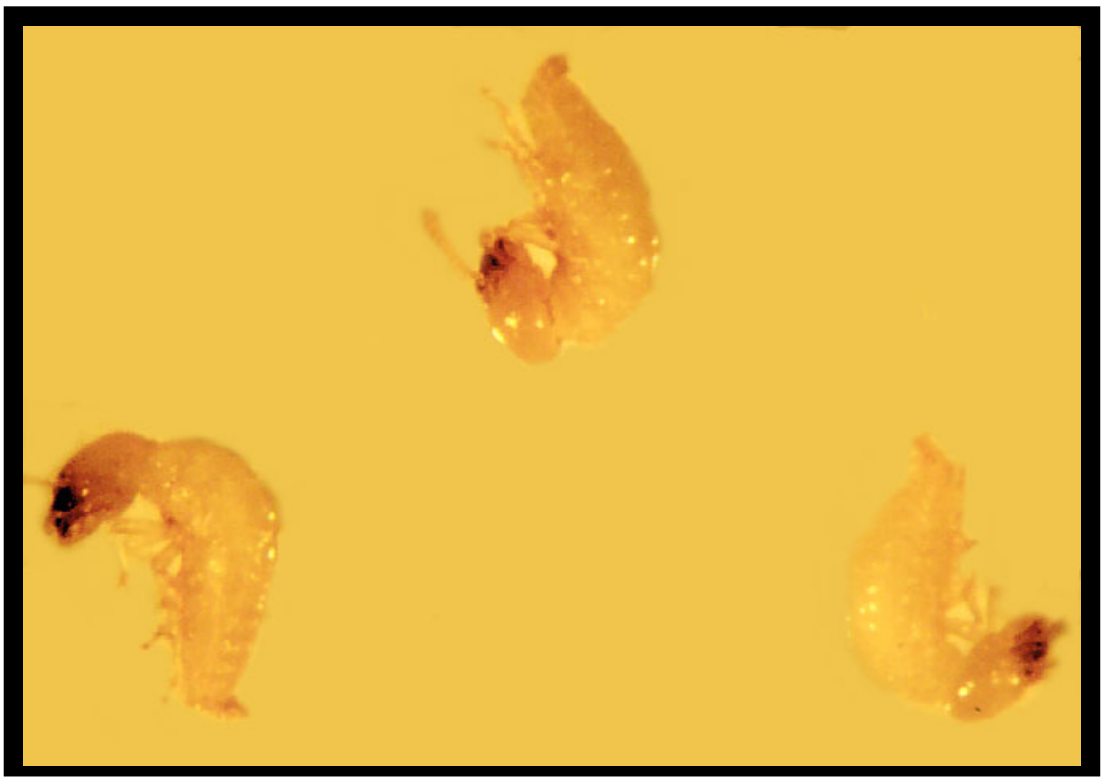


Fig. 28: Workers of *R. santonensis* that failed to complete moulting to presoldiers due to treatment with flufenoxuron.

Statistical analysis (Kruskal-Wallis ANOVA) showed that there was a significant difference ($p < 0.05$) between the three combinations and the control for the selected days, after day 10. Mann-Whitney U test revealed that the number of presoldiers produced at the concentrations of 16 ppm fenoxycarb + 2 ppm flufenoxuron was significantly different ($p < 0.05$) from that produced at 16 ppm fenoxycarb + 0.2 ppm flufenoxuron or 16 ppm fenoxycarb alone throughout the experimental period after day 10. No significant difference ($p > 0.05$) in presoldiers production was found between both treatments of 16 ppm fenoxycarb + 0.2 ppm flufenoxuron and 16 ppm fenoxycarb alone after day 5 until the end of experiment.

Table 20: Presoldiers production [%] of *R. santonensis* when fed on filter papers treated with concentrations of fenoxycarb [fe] and flufenoxuron [fl]. Results are given in means ± sd; N = 20 replicates; n = 30 workers / replicate.

| Days | Mean percentage of presoldiers production ± sd at | | | | P (1) |
|---|---|---|---|---|---|
| | Control | 16 ppm fe + 0.2 ppm fu | 16 ppm fe +2 ppm fl | 16 ppm fe | |
| 5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |
| 10 | 0.0 ± 0.0 | 18.0 ± 6.2 | 5.8 ± 3.1 | 18.5 ± 4.1 | n.s. |
| 15 | 0.0 ± 0.0 | 42.0 ± 8.1 | 13.4 ± 4.1 | 44.4 ± 3.5 | ** |
| 20 | 0.0 ± 0.0 | 47.6 ± 4.3 | 6.4 ± 2.1 | 63.0 ± 8.1 | ** |
| 25 | 0.0 ± 0.0 | 46.6 ± 2.6 | 4.6 ± 1.3 | 59.3 ± 6.7 | ** |

(1) Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$. **: significant at $p \leq 0.01$.

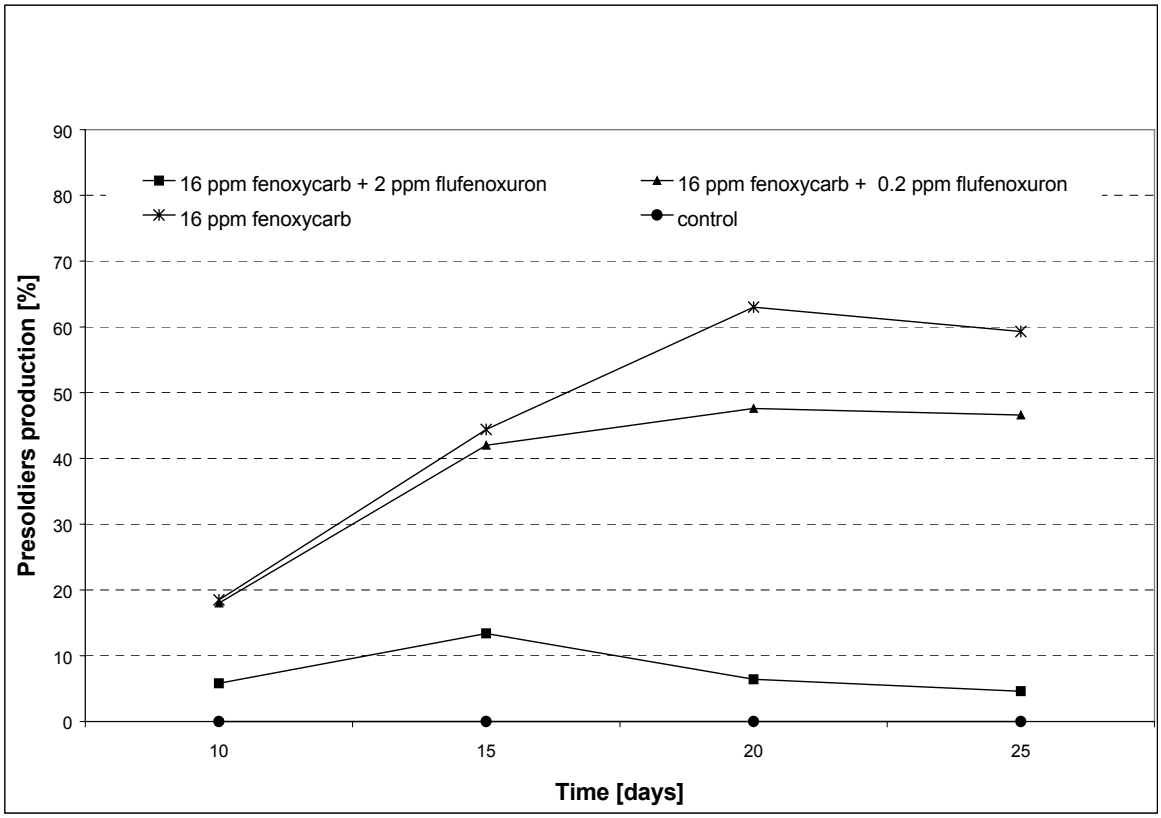



Fig. 29: Presoldiers production [%] of *R. santonensis* when fed on filter papers treated with of fenoxycarb [fe] and flufenoxuron [fl] for 25 days. Accumulative means of 20 replicates (30 workers /replicate).

## 3.5. Effect of fenoxycarb on laboratory colonies

### 3.5.1. Effect of fenoxycarb on *Microtermes sp. Nr. Albopartitus.*

Eighty-five pairs of reproductives of *Microtermes* sp nr. *albopartitus* were collected in Sudan. All alates managed to enter their nest in 1-12 hours. Male and female dug through the soil reaching the bottom of the petri dish where they evacuated the soil and built their royal chamber. It was possible to observe them through the window they had made at the bottom of the dishes. Egg lying started after 3-4 days. After 30 days eggs hatched and the first instar larvae emerged. Fungus combs were built after 40-50 days and fungus was deposited after 0-70 days. Ten weeks later the third instar larvae emerged to the surface foraging for food through built galleries (Fig. 30). From 235 collected colonies, only 25 survived and were established in the laboratory. Treatments were carried out on 12 colonies and 13 were kept as control colonies.

Filter papers treated with 32 ppm fenoxycarb (test) or Methanol (control) were offered to the foragers. The bluish coloration of the hind gut indicated that workers had fed on the treated filter papers and the fenoxycarb was accepted by the foragers and collected to the nest inside the petri dish where it probably was deposited on to the fungus gardens. In test colonies, after three weeks no foragers were out for food anymore. Inside the nest the number of living larvae started to decline, and egg lying decreased (Table 21, Fig. 31). The number of eggs found were less than that in the control colonies (Fig. 32). Later all the larvae died and the fungous contamination followed. In the control egg lying continued and foragers were always foraging with well-established galleries. Many larvae were observed inside the nest.

Table 21: Observable developmental stages of incipient laboratory colonies of *Microtermes sp. nr. albopartitus.* Median of 12 colonies.

| Age of colonies (weeks) | Mean number of eggs | | | Mean number of larvae | | |
|---|---|---|---|---|---|---|
| | Treated | Untreated | P [(1)] | Treated | Untreated | P [(1)] |
| 5 | 5 - 10 | 5 -10 | n.s. | 1-5 | 1 - 5 | n.s. |
| 10 | 10 - 20 | 10 -15 | n.s. | 15 - 20 | 15 - 20 | n.s. |
| 15 | 10 - 20 | 20 - 25 | n.s. | 10 -15 | 20 - 30 | * |
| 20 | 5 – 10 | 20 - 30 | ** | 5 - 10 | 30 - 40 | ** |

[(1)] Mann-Whitney U-test; n.s: not significant, *: significant at $p \leq 0.05$. **: significant at $p \leq 0.01$.

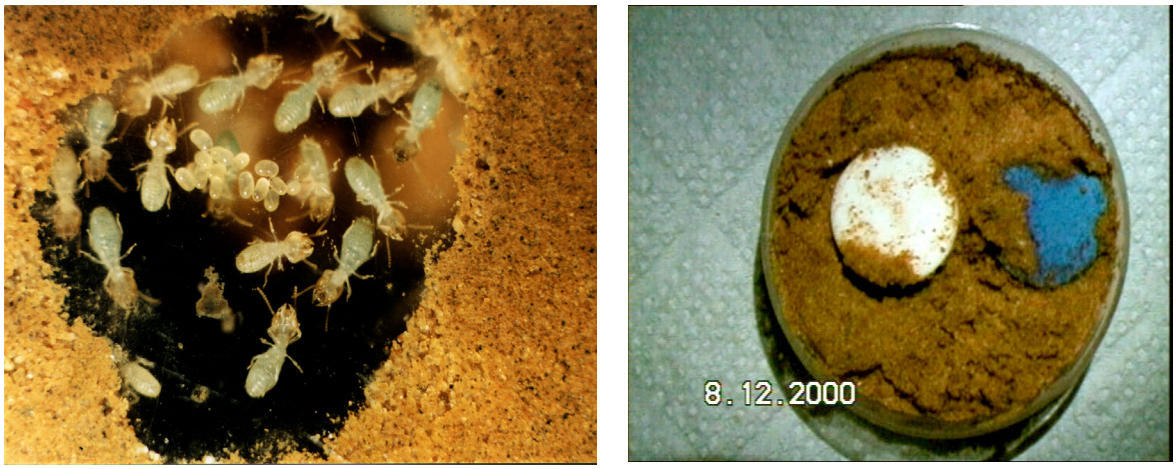



Fig. 30: Left: Termites from colonies fed with filter paper treated with 32 ppm fenoxycarb and blue dye. The blue coloration inside the termites is clearly visible. Right: White control filter paper (left) and filter paper treated with 32 ppm fenoxycarb and blue dye (right), both fed upon by termites and surrounded by foraging galleries.


Fig. 31: Left: Photograph of the window showing the nest at the bottom of a termite colony fed with untreated filter paper for 5 month. Many larvae are visible. Right: Photograph of the window showing the nest at the bottom of a termite colony fed with filter paper treated with 32 ppm fenoxycarb for 5 month. Few larvae are visible.

Fig. 32: Left: Photograph of the window showing the nest with the reproductive pair at the bottom of a termite colony fed with untreated filter paper for 5 months. Many eggs are visible. Right: Photograph of the window showing the nest with the reproductive pair at the bottom of a termite colony fed with filter paper treated with 32 ppm fenoxycarb for 5 months. Few eggs are visible.

### 3.5.2. Effect of fenoxycarb on *Macrotermes bellicosus*

Preliminary tests on a laboratory established colony of *Macrotermes bellicosus* performed in the present study revealed that grass impregnated with fenoxycarb and dyed with 2% neutral red was accepted by foragers for feeding for up to 6 month. When opening the nest after 6 month, no larvae but many presoldiers were observed inside. Furthermore, only few eggs were found in the egg chamber, that normally contains many eggs. Red coloration on the fungus garden clearly indicated that dye-treated food was accepted, collected and deposited into the fungus combs (Fig. 33).

Fig. 33: Fungus comb from *Macrotermes* nest. Red spots showing grass, treated with 320 ppm fenoxycarb, collected by foraging worker, and deposited on the constructed fungus combs.

## 3.6 Acceptance of fenoxycarb and flufenoxuron by *Microtermes i*n the field

Baits treated with fenoxycarb and flufenoxuron were randomly distributed in three plots in Khartoum University campus, namely “Botanical Garden“, “Main Library,“ “National History Museum“. Treated baits were accepted as food source by *Microtermes* species. Monitoring after the third week revealed that consumed weight of the treated baits was comparable to the control for both compounds and the two concentrations tested at the three experimental plots (Fig. 34). After the 3rd week the consumed weight of the treated baits decreased at the Botanical Garden and Natural History Museum and remained almost constant for the rest of the time. At the Main Library a decrease only started after 6th week, after which it also remained constant (Table 22, Fig. 35).

Statistical analysis with Kruskall-Wallis ANOVA and Mann-Whitney U-test showed significant differences in the consumed amount between treatment and control in the 6th, 9th and 12th week at the Botanical Garden, in the 9th and 12th week at the Main Library, and in the 3*rd*, 9th, and 10th week at the Natural History Museum. There were no differences between treatments.

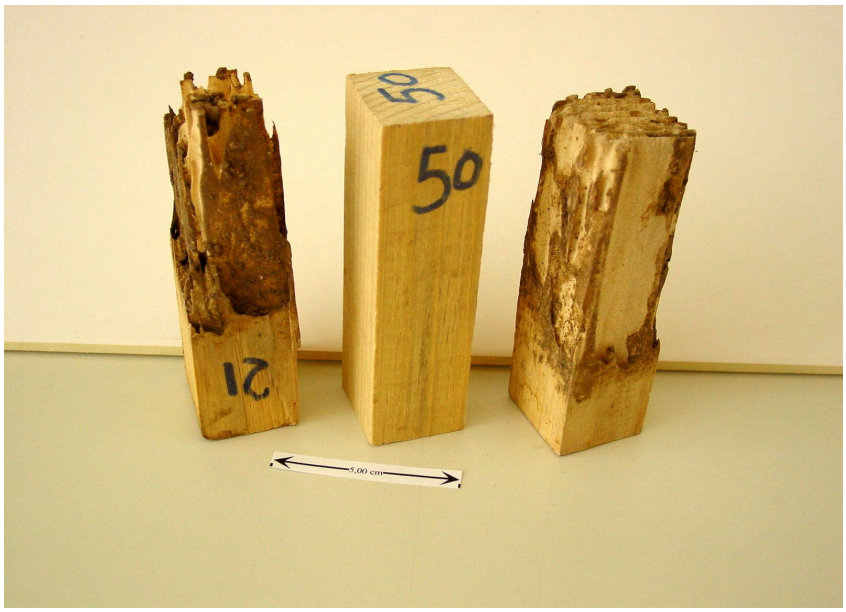



Fig. 34: Baits, before (middle) and after (left and right) being fed on by *Microtermes* sp. in the field.

Table 22: Consumed weight of baits treated with two concentrations of fenoxycarb [fe] and flufenoxuron [fl] and of control baits when offered to *Microtermes spp* in the field at three different locations. Results are given in means ± sd; N = 5 replicates.

| Weeks | 20 ppm [fl] | 200 ppm [fl] | 32 ppm [fe] | 320 ppm [fe] | Control | P [(1)] |
|---|---|---|---|---|---|---|
| | Location Botanical Garden | | | | | |
| | Mean of weight consumed at | | | | | |
| 3 | 5.1 ± 25 | 5.3 ± 4.8 | 3.1 ± 2.1 | 2.9 ± 3.7 | 6.9 ± 4.2 | n.s. |
| 6 | 0.8 ± 1.5 | 0.8 ± 1.6 | 0.6 ± 1.0 | 0.6 ± 0.6 | 5.2 ± 5.6 | * |
| 9 | 0.6 ± 0.7 | 0.5 ± 0.7 | 0.4 ± 0.5 | 0.0 ± 0.0 | 5.5 ± 4.6 | * |
| 12 | 0.1 ± 0.2 | 0.5 ± 0.4 | 0.1 ± 0.3 | 0.0 ± 0.0 | 6.0 ± 3.1 | ** |
| | Location Main Library | | | | | |
| | Mean of weight consumed at | | | | | |
| | 20 ppm [fl] | 200 ppm [fl] | 32 ppm [fe] | 320 ppm ]fe] | Control | |
| 3 | 0.4 ± 0,8 | 2.8 ± 2.4 | 0.1 ± 0.2 | 0.1 ± 0.1 | 1.7 ± 1.5 | n.s. |
| 6 | 0.6 ± 0.9 | 2.3 ± 5.1 | 0.6 ± 0.9 | 0.0 ± 0.0 | 1.0 ± 1.1 | * |
| 9 | 1.4 ±1.5 | 0.4 ± 0.6 | 0.5 ± 0.7 | 0.3 ± 0.1 | 5.4 ± 4.9 | * |
| 12 | 0.4 ± 0.8 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.2 ± 0.1 | 6.6 ± 1.2 | ** |
| | Location Natural History Museum | | | | | |
| | Mean weight of consumed wooden stakes at | | | | | |
| | 20 ppm [fl] | 200 ppm [fl] | 32 ppm [fe] | 320 ppm [fe] | Control | |
| 3 | 1.1 ± 1.0 | 1.3 ± 0.3 | 1.3 ± 0.6 | 0.2 ± 0.2 | 3.8 ± 4.0 | n.s. |
| 6 | 0.4 ± 0.9 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.5 ± 0.8 | * |
| 9 | 0.4 ± 0.8 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.8 ± 3.1 | * |
| 12 | 0.7 ± 0.4 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.7 ± 1.8 | * |

[(1)] Kruskal-Wallis ANOVA; n.s: not significant, *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

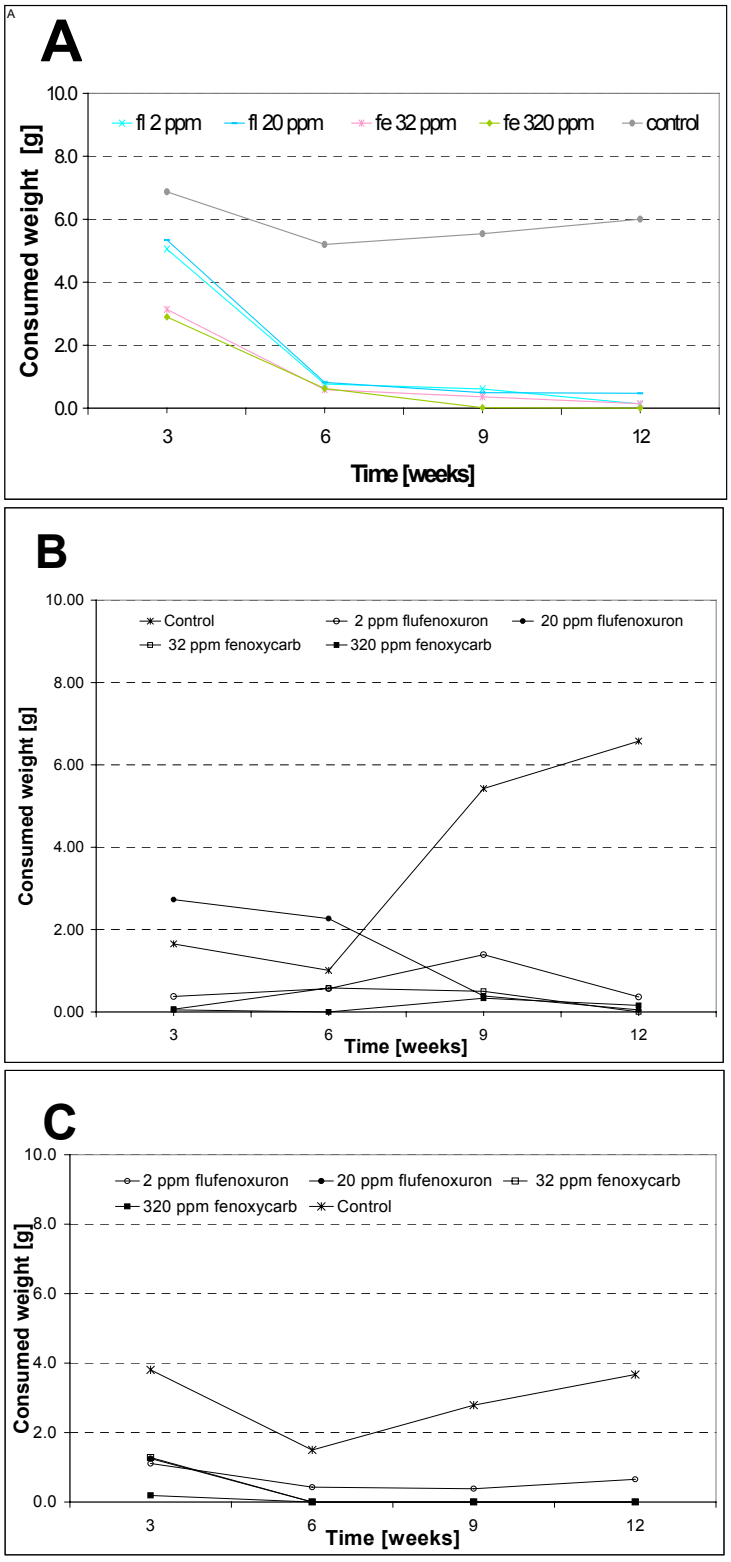



Fig. 35: Consumed weight [g] of baits treated with two concentrations of fenoxycarb [fe] and flufenoxuron [fl] and the control baits when offered to *Microtermes spp* in the field at A: Botanical Garden, B: Main Library, C: Natural History Museum. Means ± sd; N = 5 replicates.