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Study of Trail-Following Behavior of Subterranean Termite, *Heterotermes indicola* (Isoptera: Rhinotermitidae) after Exposure to Various Concentrations of Insect Growth Regulators

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Abstract

Trail following behaviour of subterranean termite, *Heterotermes indicola* (Wasmann) was studied after exposing them to two insect growth regulators, hexaflumuron and lufenuron. Termite workers were exposed to concentrations ranging from 100 to 10,000 ppm of both IGRs and then data were recorded on percent termite workers that reversed or left trail, time taken to complete the trail and speed with which termites travelled on trail. It was observed that both hexaflumuron and lufenuron did not significantly change the trail following behaviour of the exposed termites and may be used effectively in feeding bait for control of *H. indicola*.

Keywords: *Heterotermes indicola*, Trail following, Hexaflumuron, Lufenuron.

Introduction

Trail following behavior is an important character of social insects like termite, ants and honeybees. These all social insects follow the chemical trail laid by their nest fellows which helps them in foraging for food, in coordinative response against any potential threat and in keeping the colony organized^[1]. The subterranean termites are in particular reported to follow the both pheromone trail and non-pheromonal or chemical trail^[2]. Various scientists had used different ways to study the trail following behavior in subterranean termites but the most common method reported is to draw a trail with some synthetic semiochemical or trail pheromone on paper and observe whether termite follow the trail or not^[3]. One of such synthetic chemical is 2-phenoxyethanol which mimics the natural trail following pheromone released by termite workers and often used in inks of some ball point pens, subterranean termites are reported to follow the line drawn by this ink^[4,5].

In present study we also had laid trail by such ink and investigated *H. indicola*'s trail following ability after exposing them to various concentrations of hexaflumuron and lufenuron (insect growth regulators, IGRs). As these two IGRs are often used in termite baits and trail following behavior is an important character of subterranean termites including *H. indicola* which could play an important role in dissemination of these termite baits. Because sometime when toxicants are applied at high doses can cause disorientation, un-coordinated movements and lack of walking ability which can lead to the failure of dissemination of that toxicant^[6]. For example *H. indicola* workers are reported to travel less distance and with reduced speed when they were exposed to different organic extracts at high concentrations^[7]. Similarly it has been reported that workers of western subterranean termite, *R. hesperus* Banks when exposed to high doses of non repellent termiticide showed impaired response to synthetic trail pheromone and after 4 hours 60% termite workers were not able to follow the 10 cm long trail^[8]. Therefore in our present study we investigated any possible impaired response of *H. indicola* against the application of different concentrations of hexaflumuron and lufenuron.

Material and Method

The present study on trail following behavior of *H. indicola* under the influence of hexaflumuron and lufenuron was conducted during 2013 at The University of Agriculture Peshawar and Nuclear Institute for Food and Agriculture, Peshawar, Pakistan.

Termite workers of subterranean termite, *H. indicola* were collected from various infested areas of Peshawar and were exposed to concentrations of 100, 250, 500, 1000, 5000, 10000 ppm of hexaflumuron and lufenuron for 24 hours.

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Stock solutions were prepared and the required concentrations were obtained by dipping blotting papers (9 cm dia.) in insecticidal solution for 5 sec to obtain the required ppm concentrations (weight of blotting paper/ weight of active ingredient). These blotting papers were placed in petri dishes (9cm dia.) which were labeled with permanent marker and sterilized at 127°C for 2 hours prior to the experiment. In total 20 termite workers were exposed in each Petri dish and all the petri dishes were kept in dark place. After 24 hours, exposed termite workers were shifted to other petri dishes provided with same size untreated blotting paper moistened with distilled water.

A 12 cm long trail was drawn on the sterilized strips (15cm long x 5 cm wide) of blotting paper to study the trail following behavior of termite workers. The line was marked at three points i.e. 4, 8, 12 cm. Out of 20 exposed termite workers, 10 were randomly selected and released individually in plastic ring (3 cm diameter × 1 cm high) having small aperture. The ring was placed in such a way that aperture was in-lined with starting point of the trail drawn by that particular ink. The purpose of releasing termite in ring was to give it time to get

settle and follow the trail from one end. For each individual termite worker release, new blotting paper strip and new trail was drawn.

Total number (%) of termites that left the trail, mean travel time and speed with which termites travelled to complete distances of 4, 8 and 12 cm and on trail was determined and subjected to ANOVA followed by Tukey’s HSD test for mean separation. All the experiments were laid in Completely Randomized Design (CRD).

Results

Trail following behavior after exposure to hexaflumuron

Results showed that 20% termite workers reversed or left the trail when treated with concentrations of 500 ppm or less. There was no specific pattern of leaving the trail, some termite workers left before 4 cm, few before 8 cm and others before 12cm. At concentration of 1000 ppm and higher, there was slight increase and total 30% termite workers left the trail before 12 cm but that increase was insignificant. Over all it was observed that hexaflumuron did not affect the trail following behavior at all the tested concentrations (Table 1).

Table 1: Percentage of workers of *H. indicola* treated with various concentrations of indoxacarb which left or reversed on 4, 8 and 12 cm trail

Dose (ppm)	Termites reversed or left the trail between (%)			Total number of termites reversed or left trail (%)
	0 – 4 cm	4 – 8 cm	8 – 12 cm	
0	10	0	10	20% a
100	10	10	0	20% a
250	10	10	0	20% a
500	0	10	10	20% a
1000	30	0	0	30% a
5000	20	10	0	30% a
10000	10	10	10	30% a

Means followed by the same letter are not different at p<0.05 using Tukey’s HSD test.

Mean time taken by the termite workers to complete the distances of 4, 8 and 12 cm on trail were also calculated and It was obvious from the results that time taken was insignificantly different from each other when termites were exposed to various concentrations. Time taken narrowly ranged from 6.15 sec to 8.66 sec, 10.90 sec to 15.50 sec and

16.51 sec to 21.37 sec to complete the distances of 4, 8 and 12 cm respectively when termites were exposed to concentrations ranging from 100 – 10,000 ppm. Although there was slight increase in time taken to complete the trail with the increase in concentrations but that increase was not significant (Table 2)

Table 2: Time taken by termite workers of *H. indicola* treated with different concentrations of hexaflumuron to complete 4, 8 and 12 cm long trail

Dose (ppm)	Time taken to complete (Sec)		
	4 cm trail	8 cm trail	12 cm trail
0	6.15 ± 0.70 a	10.90 ± 0.94 a	16.51 ± 1.73 a
100	6.26 ± 0.77 a	11.01 ± 1.08 a	16.26 ± 1.62 a
250	6.65 ± 0.88 a	11.43 ± 1.21 a	17.21 ± 2.00 a
500	6.70 ± 0.68 a	12.25 ± 1.20 a	18.45 ± 1.85 a
1000	6.87 ± 0.91 a	12.23 ± 1.10 a	18.74 ± 1.40 a
5000	7.88 ± 0.78 a	13.55 ± 1.20 a	19.63 ± 1.28 a
10000	8.66 ± 0.69 a	15.50 ± 1.20 a	21.37 ± 1.76 a

Means followed by the same letter are not different at p<0.05 using Tukey’s HSD test

Mean speeds of termite workers of *H. indicola* to complete the 4, 8 and 12cm trail were also recorded and results revealed that speed of treated termite workers was also not affected by various concentrations of hexaflumuron. When compared with mean speed of untreated termite i.e. 0.71, 0.78, 0.78 cm/sec at 4, 8 and 12 cm respectively, the mean speed of termite workers treated with 100 and 10,000 ppm were not significantly different. Lowest insignificant decrease in speed i.e.

0.59cm/sec recorded was of termite workers which were treated with highest concentration of 10,000 ppm whereas highest speed i.e. 0.78 cm/sec was of untreated workers to complete the distance of 12cm. Difference in speed was insignificantly different at all the tested concentrations as well as there was very little difference in speeds to complete the distances of 4, 8 and 12 cm at some particular concentration (Figure 1).

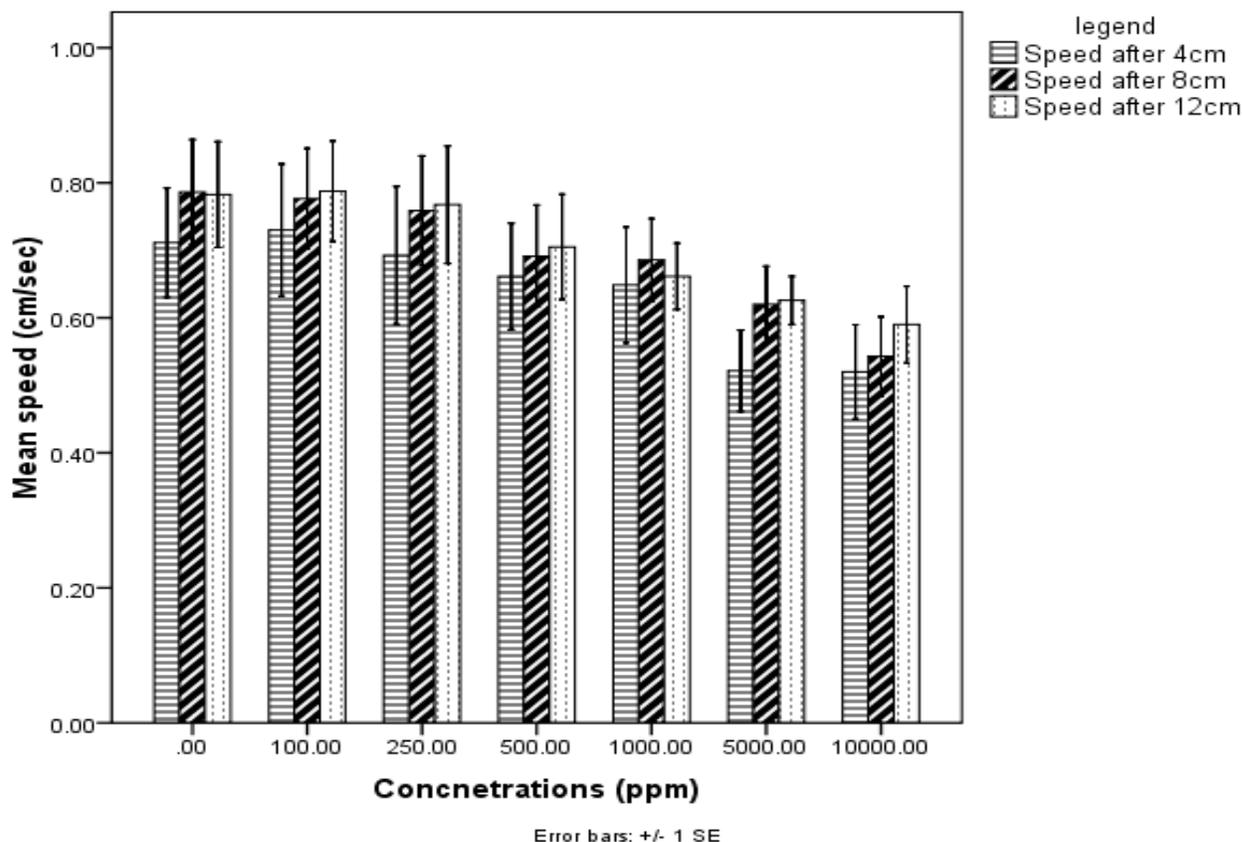


Fig 1: Mean speed of termite workers of *H. indicola* treated with different concentrations of hexaflumuron for completing 4, 8 and 12 cm trail

Trail following behavior after exposure to lufenuron

It was recorded that 20% termite workers reversed or left the trail when treated with concentrations of 1000 ppm or less. At 5000 ppm, 40% termite workers left the trail and at 10,000 ppm, 30% left the trail before 12 cm. There was no specific pattern of leaving the trail, few termite workers left before 4cm, some before 8 cm and others before 12cm. Over all it was observed that lufenuron did not significantly affected the percentage of trail following workers treated with various concentrations (Table 3).

Table 3: Percentages of workers of *H indicola* treated with various concentrations of lufenuron which left or reversed on 4, 8 and 12 cm trail

Dose (ppm)	Termites reversed or left the trail between (%)			Total number of termites reversed or left trail (%)
	0 – 4 cm	4 – 8 cm	8 – 12 cm	
0	10	0	10	20% a
100	10	10	0	20% a
250	0	0	10	10% a
500	20	0	0	20% a
1000	10	10	0	20% a
5000	20	20	0	40% a
10000	10	10	10	30% a

Means followed by the same letter are not different at p=<0.05 using Tukey’s HSD test

It was obvious from the results that time taken to complete distances of 4, 8 and 12 cm was insignificantly different from each other at all the tested concentrations. Time taken narrowly ranged from 6.15 to 7.73 sec, 10.90 to 13.98 sec and 16.51 sec to 19.6 sec to complete the distances of 4, 8 and 12 cm respectively at all the tested concentrations of lufenuron.

Time taken by workers to complete the distance of 12cm of trail was not affected at all by change in concentration of lufenuron (Table 4).

Table 4: Time taken by termite workers of *H. indicola* treated with different concentrations of lufenuron to complete 4, 8 and 12 cm long trail

Dose (ppm)	Time taken to complete (Sec)		
	4 cm trail	8 cm trail	12 cm trail
0	6.15 ± 0.70 a	10.90 ± 0.94 a	16.51 ± 1.73 a
100	6.24 ± 0.97 a	10.5 ± 1.15 a	15.63 ± 1.86 a
250	6.98 ± 0.81 a	12.65 ± 1.01 a	18.19 ± 1.47 a
500	7.73 ± 0.92 a	14.04 ± 1.65 a	19.32 ± 1.70 a
1000	7.18 ± 0.73 a	13.12 ± 1.25 a	19.63 ± 1.66 a
5000	7.32 ± 0.89 a	12.94 ± 1.82 a	18.67 ± 2.51 a
10000	6.93 ± 0.71 a	13.98 ± 1.40 a	19.39 ± 1.77 a

Means followed by the same letter are not different at p=<0.05 using Tukey’s HSD test.

Results revealed that mean speed of treated termite workers was not affected when treated with various concentrations of lufenuron. In comparison with mean speed of untreated termite workers i.e. 0.71, 0.78, 0.78 cm/sec at 4, 8 and 12 cm respectively, the mean speed of termite workers treated with 100 to 10,000 ppm was insignificantly different. Similar height of the bars for 250 to 10,000 ppm in Fig.2 showed that speed of workers remained almost constant after exposure to these concentrations. However the speed of the workers treated with 100 ppm and 0 ppm was slightly greater than all the other tested concentrations. But overall difference in speed was insignificant at all the tested concentrations and also at distances of 4, 8 and 12 cm at some particular concentration.

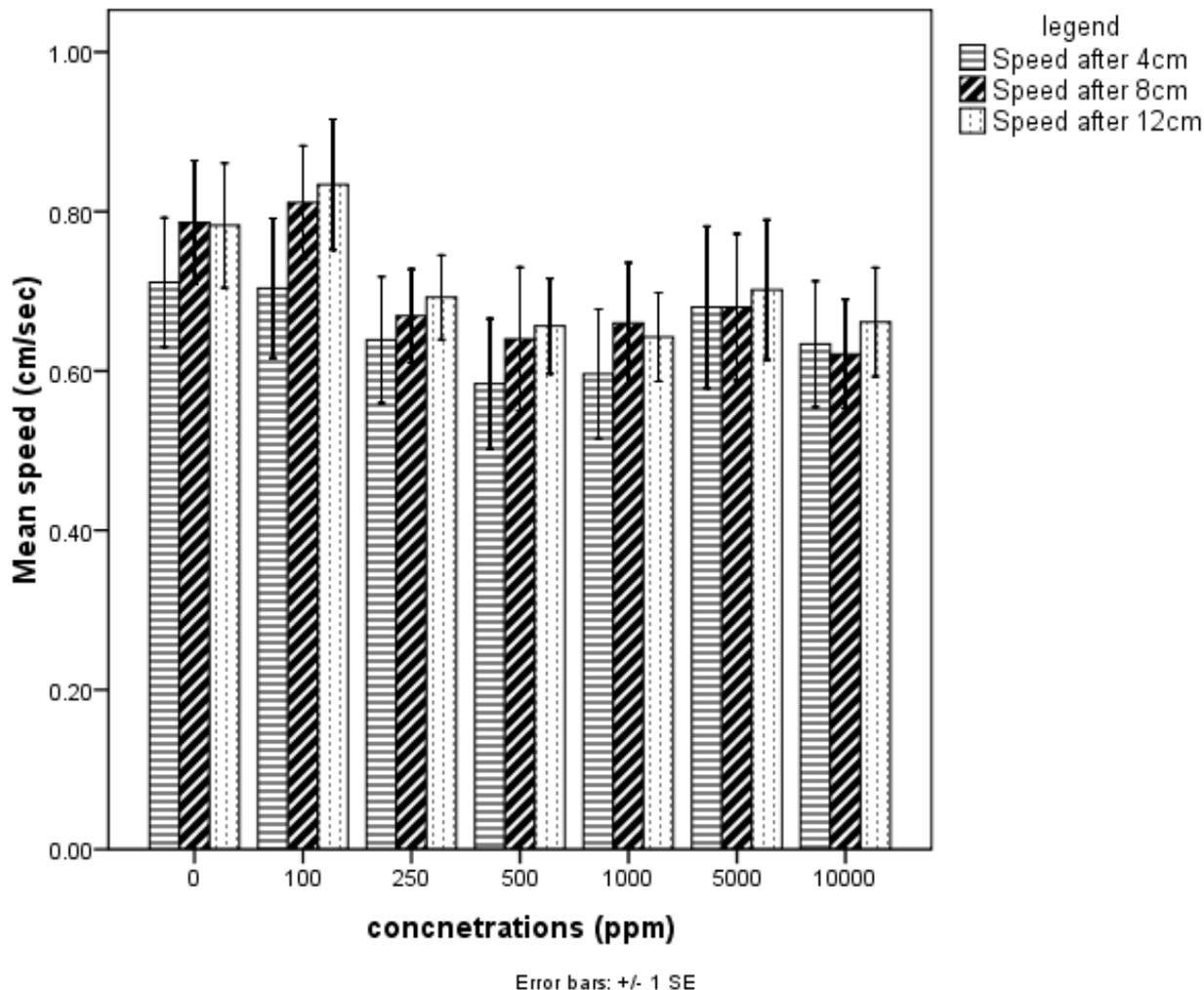


Fig 2: Mean speed of termite workers of *H. indicola* treated with different concentrations of lufenuron for completing 4, 8 and 12 cm trail

Discussion

Results showed that hexaflumuron did not affect the majority of termite workers and they were successfully able to complete the trail of 12 cm and the difference was insignificant between percentages of untreated and treated workers. Hexaflumuron had been tested in termite baits which were applied under ground as well as above ground foraging stations for the control of various subterranean termite species and reported as successful [9-17]. Therefore any bait having hexaflumuron as active ingredient has great potential to be disseminated successfully among the colony member because once bait is located by few foragers; they can recruit more foragers through trail following behaviour. Ibrahim *et al.*, Hu *et al.* [18, 19] and Su 2005b [20] also reported the distance effect of IGRs on subterranean termites and reported all the foragers fed on bait were killed at distance of 50 meters and distances travelled by termites were not affected by IGR. The results were in accordance with our findings that hexaflumuron (IGR) did not affect the movement and trail following of exposed termites because treated termite workers successfully travelled on laid trail. It is obvious from our study that hexaflumuron did not alter trail following behavior of termites. Therefore bait having hexaflumuron as active ingredient once located by few termites in the field could recruit others through trail following behavior. Moreover, the trail laid back to royal chamber by bait-fed termites could allow more workers to reach the bait source and hexaflumuron may successfully be disseminated [21].

Results of trail following studies further revealed that lufenuron also did not significantly affected termite workers and majority of them were able to complete the trail. Only 20% termite workers treated with concentrations less than 1000 ppm left or reversed on trail before completion. A slight insignificant increase i.e. 30 – 40% was recorded in termite workers which were exposed to 5000 and 10,000 ppm treated blotting paper. Time taken to complete the 12 cm trail and speed with which treated termite workers travelled on trail was also insignificantly different from each other. There was slight decrease in speed of termites with the increase in concentrations of lufenuron. As lufenuron is chitin synthesis inhibitor and mostly affects the termite during its molting stage [22]; therefore after 24 hours exposure, most of the termite workers did not get affected and high percentage was able to complete the trail. It has also been reported in earlier studies that chitin synthesis inhibitors like lufenuron when used in bait against subterranean termites usually takes weeks to months to show its effect e.g. Rojas and Morales-Ramos [23] reported 8 – 9 months, Haverty *et al.* [24] reported 10 to 16 months, Sukartana *et al.* [25] reported 6 – 8 weeks, King *et al.* [26] reported 2 weeks and Vahabzadeh *et al.* [27] reported 6 weeks for lufenuron to show its effects. Ibrahim *et al.* and Hu *et al.* [18, 19] also explained that delayed affect caused by any termiticide is an added advantage because termite workers will have more time to disseminate the toxicant though out the colony. We also found that lufenuron did not affect the trail following ability of *H. indicola* workers and also did not affect

its walking ability because there was no significant difference within different treatments.

Communication among biological organism especially termites is an important mechanism for nest mate recognition and colony integration [28]. Pheromones produced by social insects greatly influence their behaviour and development within the colony [29]. Termites also use these pheromones for locating their nesting sites and their nest fellows. These social behaviours towards these stimuli can be manipulated for our best use [30]. Location of feeding sites and recruiting other termite foragers through trail following is an important behaviour of subterranean termites. Usually termite bait are applied on small portion of complex and extensive nesting network of subterranean termites and then this bait is disseminated either by food sharing mechanism (trophallaxis) or by direct feeding by large numbers of foragers [31]. If the toxicant used in the bait does not impact the trail following behaviour of foragers then there is big chance that bait will be directly approach by larger number of termite workers and will more rapidly be disseminated. Therefore on the basis of our results we can recommend hexaflumuron and lufenuron as IGRs that do not alter the trail following behavior of termite workers of *H. indicola* and can successfully be used in termite feeding bait.

Conclusion

We concluded from our studies that both hexaflumuron and lufenuron have potential to be used in the termite feeding bait made for management of *H. indicola* as both did not affect the trail following behavior and termites acted normally after exposure to different concentrations of both IGRs.

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