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Dose Response Relationship of Subterranean Termite, *Heterotermes indicola* (Wasmann) and Two Insect Growth Regulators, Hexaflumuron and Lufenuron

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Abstract
The subterranean termite *Heterotermes indicola* (Wasmann) is one of the most economically important and destructive pest species in Pakistan. It is hard to control with conventional termiticides because of its cryptic foraging behavior and biology. Laboratory studies were conducted at Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan to test various concentrations ranging from 100 – 10,000 ppm (wt/wt) of hexaflumuron and lufenuron to determine dose response relationship. It was concluded that hexaflumuron caused <50% mortality in termites exposed to 100 – 5000 ppm whereas at 10,000 ppm it caused >70% mortality after 25 days and ELT_{90} projected was 74 days. In dose-response study of lufenuron all the concentrations equal or greater than 250 ppm caused > 50% mortality but maximum mortality recorded was >70% which was caused by 10,000 ppm and ELT_{90} recorded was 49.2 days. Both hexaflumuron and lufenuron exhibited characteristics of slow acting toxicants and cause delayed mortalities at all the tested concentrations. Although Lufenuron was found to be relatively more toxic than hexaflumuron but overall mortalities were dose dependent.

Keywords: Insect Growth Regulators, Toxicity, Subterranean termites, *H. indicola*

1. Introduction
Like other congeneric species, *Heterotermes indicola* (Wasmann) is one of the most economically important and destructive subterranean termite pest species in Pakistan [1]. It damages the residential wooden structures as well as the agricultural crops and orchards. It is considered to be one of the most tenacious species since it remains active year round [1]. It has been known to damage fruit orchards and agricultural crops with damage records showing upward of 100% in apricot and pear orchards in northern parts of Pakistan [2]. Its adaptability to dry and hot condition is due to its foraging behavior and biology. A reason for its success in these conditions is its ability to make small foraging tubes which originate from the soil and reach meters above the ground. In addition, termite foragers have relatively small body size which enables them to penetrate narrow cracks, holes and gaps in foundation slabs. These characteristics make it advantageous over other subterranean termites due to its ability to tunnel greater distances to moisture sources because other species do not have ability to exploit these limiting conditions. In addition, due to its ability to make narrow, long and sometimes free hanging foraging tubes it has potential to become a well-established and destructive pest in urban areas [3, 4].
The soil dwelling, cryptic nesting and feeding behavior of subterranean termite such as *Heterotermes spp.* makes them very difficult to understand about their ecology and population dynamics. It is not easy to discover its existence until there are any signs and symptoms of damage [5]. Its infestation can become severe if left untreated. The complex behavioral patterns of subterranean termites along with the hidden nature of their foraging make them tough to control with conventional termiticides. One of the most popular methods of subterranean termite management is the application of repellent soil termiticides beneath a structure which creates a barrier to exclude these insects [6]. Soil corrective treatments using repellent insecticides do not impact the overall population of subterranean termites and they only

prevent the access of termites but the colony remains viable and capable of re-infestation [7]. The surviving colony continues to produce foragers and alates that might infest nearby areas. The termite control industry dependence on repellent soil termiticide barriers has been one of the causative factors for the continuing extension of the subterranean termites [8].

In recent years insect growth regulators (IGRs) and non-repellent termiticides have gained popularity as alternatives to conventional termiticides. These termiticides do not repel foraging termites but inhibit their invasion through lethal contact [9]. Unlike the conventional fast acting repellent soil termiticides, these toxicants impact the colony population of subterranean termite due to their non-repellency and delayed action. Termites exposed to these toxicants appear to be unaware of treated medium and keep on moving and feeding on treated substrate which ultimately results in colony suppression and elimination [10]. In the present study two insect growth regulators, hexaflumuron and lufenuron have been tested against *H. indicola*. Hexaflumuron is basically benzophenyl urea which acts as chitin synthesis inhibitor and interrupts the molting process in insects [11]. It has been reported in literature for controlling numbers of insect pests including subterranean termites [12, 13]. Lufenuron is also a benzoylphenyl urea and chitin synthesis inhibitor which basically known to interfere with sclerotization of insect's cuticle and also reported to disrupt the alimentary tract homeostasis in termites [14].

Research work on laboratory evaluation of IGRs is underdone in Pakistan. Specifically there is no adequate information available on *H. indicola* in relation with hexaflumuron and lufenuron. The present study, therefore, aimed to evaluate these two IGRs as potential active agents in slow acting toxicant baits.

2. Materials and Methods

The present study on dose response relatationship of insect growth regulators and subterranean termite, *Heterotermes indicola* (Wasmann) was conducted at The University of Agriculture Peshawar and Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan during 2013.

3. Collection and maintenance of termite culture

For collection of termites, foraging points were detected by installing Poplar (*Poplus sp.*) wood stakes (4cm wide x 2.5cm thick x 28cm high), a preferred wood by termites. The stakes were hammered 25 cm deep and 2.5 meters apart from each other around the potential infested areas. These stakes were examined every two weeks for the presence of termites. The infested stakes were replaced by underground monitoring stations by digging a cavity in the soil to fit the station so that the upper margin of the station just touched the ground surface. The monitoring station was comprised of a slice bundle surrounded by a 2 mm thick plastic collar (17 cm diameter x 22 cm high). The slice bundle consisted of 5 rectangular wooden poplar slices (15 cm high x 8 cm wide x 1 cm thick) wrapped in a blotting paper, held together by a rubber band and the space between the slices and plastic collar was filled with soil. Exposed end of the PVC pipe was covered with a plastic bag to prevent water in to the trap. Traps were examined every two weeks and infested bundles replaced with new ones. Infested bundles were brought to the laboratory for testing. Termites were separated from soil and debris though sieving. The collected termites were kept in glass Petri dishes (14 cm dia.) containing 2 pieces of moist blotting paper (14 cm dia.) in incubators at 27±2 °C and 80% RH [15].

4. Insect Growth Regulators (IGRs)

Hexaflumuron® Kolfin (50 gm [AI]/L) provided by Burhan Chemicals and Lufenuron® Match (50 gm [AI]/L) provided by Syngenta were tested against *H. indicola.* Formulated termiticides were preferred over technical grad insecticides because they are easy to apply and are also the ones that are actually used in field for the control of termites.

5. Dose-response relationship

Concentrations of 100, 250, 500, 1000, 5000, 10,000 ppm (weight of active ingredient /weight of blotting paper) of both IGRs were obtained through serial dilutions of original formulations. The sterilized circle shaped blotting papers (Millat paper art, Karachi, Pakistan) of 9.0 cm diameter and 0.21 gm in weight were dipped in above mentioned concentrations of hexaflumuron and lufenuron for 5 seconds. The treated blotting papers were then dried at room temperature and were placed in glass petri dishes (9.0 cm dia x 1.5 cm high). After placing in petri dishes all the treated blotting papers were moistened again with 5 ml of distilled water. Each concentration of hexaflumuron and lufenuron was considered as a treatment and was replicated four times. One hundred workers and three soldiers were released in each petri dish for 24 hours having treated blotting paper. After 24 hours exposure the termites were shifted to similar size petri dishes provided with double untreated blotting paper moistened with 5 ml distilled water. All the Petri dishes were kept in incubator as previously mentioned [15]. Mortality data were recorded after every 24 hours.

6. Statistical Design and Analysis

Experiment was designed in Complete Randomized Design (CRD). ELT_{50} (Effective Lethal Time to kill 50% of the population) and ELT_{90} (Effective Lethal Time to kill 90% of the population) were calculated for each concentration by performing Probit analysis. Mortality recorded at the end of experiment was also subjected to one way ANOVA followed by mean separation through Student Newman Keul (SNK) test.

7. Results

7.1. Dose-response relationship of hexaflumuron and workers of *H. indicola*

Hexaflumuron, a chitin synthesis inhibitor, and used as a slow-acting toxicant against different species of subterranean termites was tested against *H. indicola* at concentration rang of 100 to 10,000 ppm. It is evident from mortality trends of treated termite workers over a period of 25 days in actual experiment in response to varying concentrations of hexaflumuron that mortality remained less than 20% in first two weeks regardless of the concentration used. But from there onward mortality gradually started increasing in termite workers treated with concentrations higher than 1000 ppm. Maximum mortality was recorded in termites exposed to highest concentration of 10,000 ppm i.e. 71.6 %. In all the other concentrations, ranging from 100 – 5000 ppm mortality remained less than 50% (Figure 1).

When Mortality rates inflicted by different concentrations of hexaflumuron after 25 days were compared using ANOVA followed by SNK test for means separation, the significantly highest mortality was recorded at 10,000 ppm. Mortality in termites exposed to 1000 to 5000 ppm of hexaflumuron was insignificantly different from each other but it was significantly higher than mortality caused by 100 – 500 ppm and lower than mortality at 10,000 ppm (Table 1).

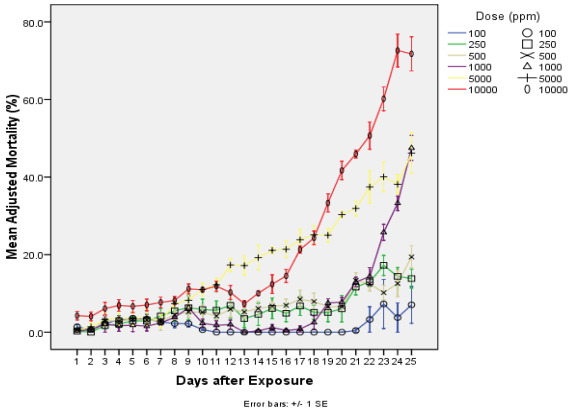

Fig 1: Mean cumulative mortality (adjusted against control) of *H. indicola* after exposure to different concentrations of hexaflumuron

Table 1: Comparison of *H. indicola* mortality 25 days after exposure to various concentrations of hexaflumuron

Dose	Adjusted Mortality after 25 days (%)
100	7.06± 4.7 a
250	13.8± 2.4 a
500	19.4± 2.8 a
1000	47.5± 3.2 b
5000	46.1± 5.2 b
10000	71.6± 4.4 c

Means followed by the same letter are not different at p=<0.05 using SNK test

Estimated lethal time to kill 50% and 90% (ELT_{50} and ELT_{90}) were projected through probit analysis. At lowest tested concentration of 100 ppm of hexaflumuron both ELT_{50} and ELT_{90} were not determined because projected values were too big and were beyond the projection limit of probit analysis. ELT_{50} and ELT_{90} values for concentrations of 250 and 500 ppm were also calculated in years and were too big to consider safe in real. At 1000 and 500 ppm the ELT_{50} projected values were 43 and 31 days respectively whereas ELT_{90} values recorded for both of the concentrations were more than 100 days. Only at 10,000 ppm ELT_{50} was 25 days and ELT_{90} was 74 days which was comparatively lower than all the other ELT values (Table 2).

Table 2: Estimated lethal time (days) required for 50% and 90% mortality (ELT_{50}, ELT_{90} along with 95% Confidence Interval, CI) of *H. indicola* after exposure to various concentrations of hexaflumuron

Dose (ppm)	ELT_{50} (days)	95% CI	ELT_{90} (days)	95% CI	Probit Model
100	ND	ND	ND	ND	ELT = -4.2 + 0.23 ×dose
250	149	89 - 371	1084	421 - 5849	ELT = -5.5 + 2.5 ×dose
500	165	105 - 334	1364	593 - 5074	ELT = -5.3 + 2.3 ×dose
1000	43	33 - 73	104	64 - 308	ELT = -9.1 + 5.6 ×dose
5000	31	29 - 34	108	86 - 131	ELT = -6.2 + 4.1 ×dose
10000	25	22 - 30	74	55 - 119	ELT = -6.4 + 4.6 ×dose

ND= Not Determined

7.2. Dose-response relationship of lufenuron and workers of *H. indicola*

Lufenuron, an insect growth regulator reported to disrupt the molting process in insects was used for studying the response of workers of *H. indicola* towards its different doses. Mortality observed after exposing the termites to a concentration range of 100 - 10,000 ppm over a period of 26 days. It was evident from the mortality trend that mortality remained less than 50% in termite workers exposed to all the concentrations of lufenuron until about three weeks. After that mortality rates got higher and mortality reached about 80% in the termites exposed to the blotting paper treated with highest tested concentration of 10,000 ppm of lufenuron. The mortalities in termites gradually increased over the period of time with the increase of concentrations but overall mortalities for all the concentrations other than 10,000 ppm remained less than 60% (Fig.2).

Mortality after 26 days in actual experiment was as expected recorded minimum at lowest tested concentration of 100 ppm which was significantly less than all the other concentrations of lufenuron. The maximum mortality (75%) was at 10,000 ppm and it was not significantly different from the mortalities that were ranged from 57% to 62% at concentrations ranging from 250 to 5000 ppm (Table 3).

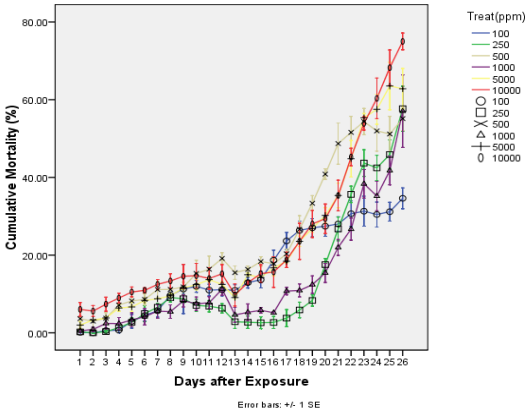

Fig. 2: Mean cumulative mortality (adjusted against control) of *H. indicola* after exposure to different concentrations of lufenuron

Table 3: Comparison of *H. indicola* mortality 26 days after exposure to various concentrations of lufenuron

Dose	Adjusted Mortality after 26 days (%)
100	34.6 ± 2.6 a
250	57.6 ± 5.7 b
500	55.1 ± 3.0 b
1000	57.0 ± 9.3 b
5000	62.8 ± 5.1 b
10000	75.0 ± 2.1 b

Means followed by the same letter are not different at p=<0.05 using SNK test

ELT_{50} narrowly ranged between 24.6 days to 36.3 at all tested concentrations from 100 to 10,000 ppm of lufenuron. It was obvious from the results that 50% population was killed in more than three weeks at all the doses. ELT_{90} values showed much prolonged lethal time of 128 days for 100 ppm whereas again narrow range of values from 45.2 to 64.9 days was projected for concentrations ranging from 250 to 10,000 ppm (Table 4).

Table 4: Estimated lethal time (days) required for 50% and 90% mortality (ELT_{50}, ELT_{90} along with 95% Confidence Interval, CI) of *H. indicola* after exposure to various concentrations of lufenuron

Dose (ppm)	ELT_{50} (days)	95 % CI	ELT_{90} (days)	95 % CI	Probit Model
100	36.3	33.2 - 40.7	128.3	102.4 - 171.6	ELT = -6.2 + 4.0 ×dose
250	27.1	25.7 - 29.3	45.2	39.3 - 55.9	ELT = -14.2+9.9 ×dose
500	24.6	23.6 - 25.9	50.4	43.6 - 62.4	ELT = -9.8+7.0 ×dose
1000	31.1	28.3 - 36.7	64.9	50.3 - 103.1	ELT = -10.3+6.8 ×dose
5000	24.7	23.8 - 25.9	49.4	43.6 - 58.7	ELT = -10.1+7.3 ×dose
10000	24.7	23.4 - 26.4	49.2	42.0 - 62.9	ELT = -10.2+7.3 ×dose

8. Discussion

As it is obvious from the results that for lowest tested concentration of 100 ppm of hexaflumuron both ELT_{50} and ELT_{90} were not determined as they were beyond the projection limit of probit analysis. It means that hexaflumuron was totally ineffective at concentrations equal or less than 100 ppm. Also the projected ELT_{90} for the other concentrations i.e. equal or less than 5000 ppm were more than 100 days therefore they were also considered to be too slow in killing the exposed population of termites. Only 10,000 ppm of hexaflumuron seemed to be effective dose which caused more than 70% mortality in 25 days of actual experiment. The ELT_{50} and ELT_{90} values calculated for 10,000 ppm were 25 and 74 days respectively which seemed to be more appropriate for successful and timely suppression of *H. indicola* colony.

Similarly in case of dose response study of lufenuron results showed that mortality remained less than 50% at all the concentrations until 3 weeks. Although after 3 weeks mortality rates got relatively higher but even then total mortality remained less than 60% at the concentrations up to 5000 ppm and maximum mortality recorded was about 80% at 10,000 ppm after 26 days. ELT_{90} value was much prolonged i.e. 128 days for 100 ppm whereas for concentrations equal or greater than 250 ppm, it ranged from 45.2 – 64.9 days which showed that all these concentrations were insignificantly different in killing 90% of exposed population.

The overall delayed mortality rate at all the tested concentrations confirmed that hexaflumuron and lufenuron both acted as slow acting toxicants against *H. indicola* because slow acting toxicants always take longer time to kill their target insect pests unlike their acute toxicants which kill the target insect pest rapidly both at low and high concentrations [16]. The delayed mortality exhibited by hexaflumuron and lufenuron even at 10,000 ppm can also be explained on the fact that these both are chitin inhibitors and unlike other non-repellent insecticides that attack on nervous or metabolic system, they require molting stage in insects for expressing its mortality [17]. In conformity with our results, Su and Scheffrahn, [18] also reported delayed mortality caused by lufenuron in exposed subterranean termites. Their results showed that lufenuron caused only 50 – 80% mortality after nine weeks. Doppelreiter and Korioth, [19] in accordance with our results reported delayed mortality in workers of *H. indicola* when exposed to various concentrations of another insect growth regulator, diflubenzuron which is known for disrupting the natural growth process. The longer time taken by hexaflumuron and lufenuron to kill the workers of *H. indicola* workers in our study might be advantageous because successful dissemination of toxicant to entire colony though trophallaxes and social grooming requires more time.

It was further observed that mortality was dose dependent for both hexaflumuron and lufenuron. Similar results were reported by Haagsma and Rust, [20] when they evaluated effects of hexaflumuron on mortality of western subterranean termite, *R. Hesperus* Banks. They reported that mortality and transfer of hexaflumuron from donors to recipients increased with the increase in dose and exposure time of workers to treated substrate. Although in some earlier studies hexaflumuron reported to be effective at very low concentrations against various species of subterranean termites [12, 21] but our results of present study revealed that hexaflumuron was effective at much higher dose of 10,000 ppm where ELT_{90} value was significantly lower as compared to other concentrations. The contrary results of our study as compared to above mentioned report might be due to different termite species used in experiments and also the exposure time which was 24 hours in our case which seemed to be less for termites to feed adequately on treated substrate. Mortality caused in our study could only be due to contact of termites with treated blotting paper rather than feeding. So over all higher doses of hexaflumuron were required to cause effective mortality in workers of *H. indicola* especially when they are exposed for limited time of 24 hours to a treated blotting paper.

On the other hand, increase in concentration of lufenuron resulted in slight gradual increase in termite's mortality and overall mortality ranged between 34.6% – 75% at concentrations from 100 – 10,000 ppm after 26 days of actual experiment. Unlike the mortality trends in hexaflumuron, results of dose response study of lufenuron showed that all the concentrations greater than 250 ppm were able to cause more than 50% mortality. But these mortalities were not instant, rather increased gradually over the time span of four weeks. In first 2 - 3 weeks, mortality remained low which might be due the fact that termite workers used in the experiment were of same age and they were not in molting stages. Later on, number of exposed termites entered in molting stage and got killed during ecdysis which was confirmed by higher rate of mortality. The narrow ranged mortality caused by lufenuron at concentrations greater than 100 ppm showed that lufenuron was more toxic than hexaflumuron even at comparatively lower concentrations but the maximum mortality was also recorded at 10,000 ppm just like hexaflumuron. In accordance with our results Vahabzadeh *et al.*, 2007 [11] also reported that lufenuron was comparatively more effective among hexaflumuron, triflumuron and diflubenzuron when they applied against subterranean termite specie *Reticulitermes flavipes*. They observed lufenuron was highly toxic and caused higher mortalities at all the tested concentrations. Similarly Lewis and Forschler, 2010 [14] evaluated commercially available baits having active ingredients as hexaflumuron, diflubenzuron, noviflumuron, novaluron and lufenuron and they also reported lufenuron as highly toxic and suitable for controlling eastern subterranean termite, *R. flavipes*.

9. Conclusion

Over all we concluded from our study that both hexaflumuron and lufenuron were dose dependent and showed characteristics of slow acting toxicant. The concentration of 10,000 ppm seemed to be appropriate with required results of killing termites in effective period of time. The lufenuron was comparatively more toxic than hexaflumuron but both IGRs showed potential to be used in slow acting toxicant bait against *H. indicola*.

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