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Original article (Orijinal araştırma)

Efficacy of indoxacarb and chlorfenapyr against Subterranean termite *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae) in the laboratory

Toprakaltı termiti *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae)'ya karşı indoxacarb ve chlorfenapyr'in laboratuvar koşullarında etkisi

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Summary

Efficacy, feeding deterrence and transfer of indoxacarb and chlorfenapyr by the subterranean termite, *Heterotermes indicola* (Wasmann), were evaluated in laboratory tests at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan during 2013-2014. Chemical concentrations tested ranged from 1 to 100 ppm (wt/wt) of indoxacarb and 1 to 7 ppm (wt/wt) of chlorfenapyr. Observations revealed that indoxacarb caused rapid mortality at doses > 50 ppm. At 10 ppm, mortality was relatively slower and ELT50 and ELT90 (effective lethal exposure times for 50 and 90% mortality) were recorded as 6.7 and 25.3 d, respectively. At concentrations below 10 ppm, it took longer for indoxacarb to cause 100% mortality. In comparison, chlorfenapyr caused rapid mortality at all tested concentrations except the lowest concentration of 1 ppm, and 100% mortality occurred at 9 d, ELT50 and ELT90 calculated as 2.7 and 8.6 d, respectively. Various concentrations of both termiticides ranging from 1 to 100 ppm (wt/wt) were evaluated in feeding deterrence and transfer studies. The results showed that indoxacarb did not deter feeding of *H. indicola* at any concentration, and only consumption of filter paper treated with 100 ppm indoxacarb resulted in 100% mortality. Chlorfenapyr did not deter feeding at concentrations below 100 ppm. Mortality remained low regardless of concentration and did not exceed 60% in the feeding deterrence tests. In transfer studies, indoxacarb was successfully transferred from donors to recipients at concentrations of 70 and 100 ppm. Chlorfenapyr transfer generally caused low recipient mortality and transfer from donors to recipients was only evident at 1 ppm where recipient mortality exceeded 80%.

Keywords: Chlorfenapyr, deterrence, *Heterotermes indicola*, indoxacarb, toxicity, transfer

Özet

Indoxacarb ve chlorfenapyrin'in, Toprakaltı termiti, *Heterotermes indicola* (Wasmann)'da beslenme engelleyici ve bireyler arasında taşınma etkileri 2013-2014 yıllarında Gıda ve Tarım Nükleer Enstitüsü (NIFA) (Peshawar, Pakistan)'nde laboratuvar testleri ile değerlendirilmiştir. Test edilen kimyasal konsantrasyonları indoxacarb için 1-100 ppm (ağırlık/ağırlık) ve chlorfenapyrin 1 ile 7 ppm (ağırlık/ağırlık) arasında değişmiştir. Gözlemler indoxacarb'ın 50 ppm üzerindeki dozlarda hızlı ölüme sebep olduğunu ortaya koymuştur. 10 ppm'de, ölüm nispeten daha yavaş gerçekleşmiş olup, ELT50 ve ELT90 (%50 ve %90 öldürücü etkili maruz kalma süresi) sırasıyla 6.7 ve 25.3 gün olarak kaydedilmiştir. 10 ppm altındaki konsantrasyonlarda, indoxacarb için %100 ölüm daha uzun sürede gerçekleşmiştir. Buna karşılık chlorfenapyrin, 1 ppm'lik en düşük konsantrasyon hariç, test edilen tüm konsantrasyonlarda hızlı bir ölüm oranına sebep olarak %100 ölüm oranı 9 günde saptanmış, ELT50 ve ELT90 ise sırasıyla 2,7 ve 8,6 gün şekilde hesaplanmıştır. Her iki termit öldürücünün 1 ila 100 ppm (ağırlık/ağırlık) aralığındaki çeşitli konsantrasyonları, beslenme engelleyici ve bireyler arasında taşınma çalışmaları için denenmiştir. Sonuçlar, indoxacarb'ın hiçbir konsantrasyonun, *H. indicola'*nın beslenmesini engellemediğini göstermiş ve sadece 100 ppm indoxacarb uygulanmış filtre kağıdının tüketimi %100 ölümle sonuçlanmıştır. Chlorfenapyrin 100 ppm altındaki konsantrasyonlarda beslenme engelleyici özellik göstermemiştir. Ölüm, konsantrasyon ne olursa olsun düşük kalmış ve beslenme engelleme testlerinde %60'ı aşmamıştır. Bireyler arasında taşınma çalışmalarda, indoxacarb taşıyıcılardan alıcılara 70 ve 100 ppm konsantrasyonlarda başarıyla taşınmıştır. Chlorfenapyrin taşınması genellikle düşük alıcı ölümüne sebep olmuş ve taşıyıcılardan alıcılara aktarımı, sadece 1 ppm için %80'in üzerinde olmuştur.

Anahtar sözcükler: Chlorfenapyr, caydırıcılık, *Heterotermes indicola*, indoxacarb, toksisite, transfer

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Introduction

Heterotermes is a genus of subterranean termites which is endemic to most parts of the world and considered as one of the most important economic termite pests (Baker & Carriere, 2011). The genus includes many pest species globally, and includes desert species from the southwest of the USA. These are ranked in the top three major termite pests (Baker & Bellamy, 2006). In Brazil and other parts of South America, *Heterotermes* is reported to cause severe economic loss, damaging forest trees like eucalypts and pines in addition to cash crops like cotton, rice, coffee and cassava. This termite genus is a widely distributed pest in sugarcane crops in Brazil and has been reported to cause crop losses of more than 10 t/ha annually (Batista-Pereira et al., 2004; Jenkins, 2006). In Pakistan, *Heterotermes indicola* (Wasmann) remains active throughout the year as being one of the most persistent subterranean termite species (Manzoor & Mir, 2010). It has been reported as a major pest of crops like sugarcane, plum and apricot in northern Pakistan (Badshah et al., 2004). In addition, this termite has been a significant crop pest in the deserts of India bordering Pakistan (Gera & Kumar, 2011). It has the capacity to destroy standing trees by hollowing them out from the inside, without generating external signs of injury (Balachander et al., 2013).

Current methods of termite control include the application of repellent insecticides, which prevent the entry of termites by creating a continuous chemical barrier (Su, 2005). These repellent insecticides kill only a few termites that come in contact with the chemical and the others are repelled. Thus, there is always a chance of re-infestation since the colony remains viable and active (Su & Scheffrahn, 1988). The surviving colony has the potential to continue to grow and termite problems may increase over time (Su, 2003).

In recent times, the focus of termite research has shifted from work on repellent termiticides to non-repellent termiticides. Unlike repellent termiticides, the non-repellent insecticides do not inhibit termite invasion just by repelling, but rather they rely on termites foraging in treated areas to achieve maximum lethal contact (Su & Scheffrahn, 2000). In general, non-repellent insecticides cause delayed mortality and allow the foraging termites to disseminate the acquired toxicant within the colony through social grooming and trophallaxis. Thus the termite colony is impacted and significantly reduced or eliminated (Thorne & Breisch, 2001). In recent years, novel termiticides are being developed with non-repellent characteristics, for example, chlorfenapyr, indoxacarb, fipronil and imidacloprid (Gahlhoff & Koehler, 2001; Shelton & Grace, 2003; Hu, 2005).

In this study, we evaluated the efficacy of indoxacarb and chlorfenapyr against the subterranean termite *H. indicola*. Indoxacarb belongs to the oxadiazine chemical family, which have low ecotoxicological risks. Indoxacarb acts selectively towards insects, and higher animals quickly degrade it into inactive metabolites. This rapid metabolic degradation is a crucial factor for the safety of higher non-target animals including humans (Wing et al., 2000). In contrast, chlorfenapyr is an aryl-substituted cyanopyrrole with broad-spectrum activity against insects and mites. It is basically a pro-insecticide which becomes activated by the oxidative removal of the N-ethoxymethyl group (Treacy et al., 1994). It also has high binding capacity to soil which is a useful characteristic for a termiticide, resulting in very low leaching rates (Rust & Saran, 2006).

Both insecticides have been under evaluation for the last few years against different subterranean termites. Hu (2005) tested the efficacy and non-repellency of indoxacarb treated soil against, *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki, and Spomer et al. (2011) investigated the penetration of subterranean termite species *R. flavipes* in various kinds of indoxacarb treated soils at different depths. Hu et al. (2005) also investigated transfer of indoxacarb among the workers of *C. formosanus.* Rust & Saran (2006) tested chlorfenapyr for its toxicity, and transfer, against the western subterranean termite, *Reticulitermes hesperus* Banks, and Shelton et al. (2006) investigated the toxicity of chlorfenapyr and its transfer among the workers of *R. flavipes*. Therefore, the main objective of this study was to determine the efficacy of both insecticides against *H. indicola* considering the degree of impact, deterrence and horizontal transfer.

Materials and Methods

Detection and collection of termites

Termites were detected and collected using methods described by Farid et al. (2014). A total of 300 detection stakes (4 × 2.5 × 28 cm) made of poplar wood were placed 2.5 cm apart and 25 cm deep in soil at different locations of Peshawar, Pakistan. Of these, 25 stakes infested by termites were later replaced by same number of NIFA TERMAPS (termite collection traps) made of PVC pipe (17 × 25 cm), containing bundles of five poplar wood slices (15 × 8 × 1 cm) wrapped in strips of filter paper (Whatman® No. 42), held together using a rubber band (Salihah et al., 1993). Traps were examined fortnightly and infested bundles, with significant numbers of termites, were brought to the laboratory. The termites were separated from the trap materials and kept at 27 ± 2°C, 80% RH in glass Petri dishes (14 × 3 cm) containing two pieces of round filter paper, moistened with distilled water. After acclimatization, the termites were used in experiments within 14 d.

Termiticides

Formulated indoxacarb (50 g a.i. per L, Steward®) provided by DuPont® and chlorfenapyr (360 g a.i. per L, Pirate®) provided by BASF® corporation were used to make stock solutions. The tested concentrations were prepared by serial dilutions. Formulated termiticides were used instead of technical grade insecticides because they are easy to mix and apply in soil and they are the actual products used for the control of termites.

Toxicity tests

Concentrations (w/w) ranging from 1 to 100 ppm of indoxacarb and 1 to 7 ppm of chlorfenapyr were prepared in 500 ml glass jars. A suitable range of concentrations was determined by performing preliminary experiments. Round filter paper sheets weighing 0.21 g and measuring 9 cm in diameter were dipped for 5 s in the insecticide solutions, to achieve the required concentration (weight of active ingredient/weight of filter paper). The amount of insecticide solution that 0.21 g filter paper can absorb was determined by Farid et al. (2014). Treated filter papers were dried at room temperature for at least 8 h and two pieces were placed in each glass Petri dish (9.0 × 1.5 cm). In total 100 termite workers and 3 soldiers were transferred to each dish for 24 h, then removed and placed in the same sized dishes containing untreated filter papers. Daily mortality was recorded. Each insecticide concentration was considered a treatment and dishes were replicated four times. Control termites were exposed for 24 h to filter paper dipped in distilled water. Mortality was recorded daily and all experimental units were kept at 27 ± 2°C and 80% RH. Probit analysis was done to calculate the ELT50 and ELT90 (effective lethal exposure times for kill 50 and 90% mortality) of the termites (Su et al., 1987).

Feeding deterrence test

Feeding deterrence caused by indoxacarb and chlorfenapyr was tested using two rectangular pieces (3 × 2 cm) of filter paper held horizontally, 3 cm apart from each other in plastic Petri dishes (9 × 1.5 cm), with bottom papers were roughened with sand paper to facilitate movement of the termites. The dishes were then filled with 25 g of 60 - mesh size sterilized sand, moistened with 20% (w/v) distilled water. One of the two pieces of filter paper was treated by dipping in a prepared termiticide solution. Seven different indoxacarb and eight chlorfenapyr concentrations (weight of active ingredient/weight of filter paper) ranging from 1 to 100 ppm were used, while the second piece of filter paper was left untreated and dipped in distilled water to serve as a control. Dry weights of both the pieces were determined before the experiment by drying them in an oven at 120°C for 6 h. Two hundred workers and 10 soldiers of *H. indicola* were released in each dish, and maintained as described above. Eight dishes were prepared for each concentration (indoxacarb 1, 5, 10, 20, 50, 70 and 100 ppm; chlorfenapyr 1, 3, 5, 7, 10, 25, 50 and 100 ppm) and four dishes disassembled during destructive sampling after 1 and 2 weeks. Used filter papers were cleaned and oven dried as described above. Consumption of both the treated and untreated filter paper for each concentration were determined by subtracting the final weight from initial weight, and compared using paired sample t-test and means were separated by using Tukey's HSD test. Termite mortality was also recorded after 1 and 2 weeks. Termites were considered dead if they showed no movement when probed with a mounted needle (Su & Scheffrahn, 1993).

Transfer test

Toxicant transfer studies used termite workers divided into donors and recipients. The donor termites were exposed to filter paper treated with seven concentrations ranging from 1 to 100 ppm of indoxacarb and eight concentrations ranging from 1 to 100 ppm of chlorfenapyr for 24 h. Recipients were not exposed to treated filter paper, but were fed on filter paper moistened with 0.2 % Nile Blue A (The BDH Ltd, Poole, UK) for 3 d to color them blue to distinguish them from the creamy white donor termites. Donors and recipients were then released together in an equal ratio (1:1) in Petri dishes containing untreated filter paper (9 cm diameter) moistened with 5 ml distilled water. In total, 50 workers were introduced, 25 donors and 25 recipients. Each concentration was analyzed as a treatment and plates replicated four times. The dishes were kept at 27 ± 2°C and 80% RH in desiccators. Live donors and recipients were counted to estimate the respective mortalities after 10 d. Dead termites including those moribund or partially consumed were not removed from the Petri dishes during the experimental period. Percent donor mortality, recipient mortality and dead donors missing (assumed to be consumed by fellow termites) were recorded and subjected to one way ANOVA and means separated using SNK (Student-Newman-Keuls) posterior test. Statistical analysis was performed using SPSS version 16.0 (SPSS, 2007).

Results

Toxicity test

Toxicity tests showed an increase in mortality with increasing insecticide concentrations. At higher concentrations of 50 to 100 ppm of indoxacarb, 100% mortality was achieved within 2 - 3 d, but at lower concentrations of 1 to 20 ppm significant mortality did not occur even after 19 d. However, cumulative percent mortality was higher than control mortality for all concentrations of indoxacarb (Figure 1).

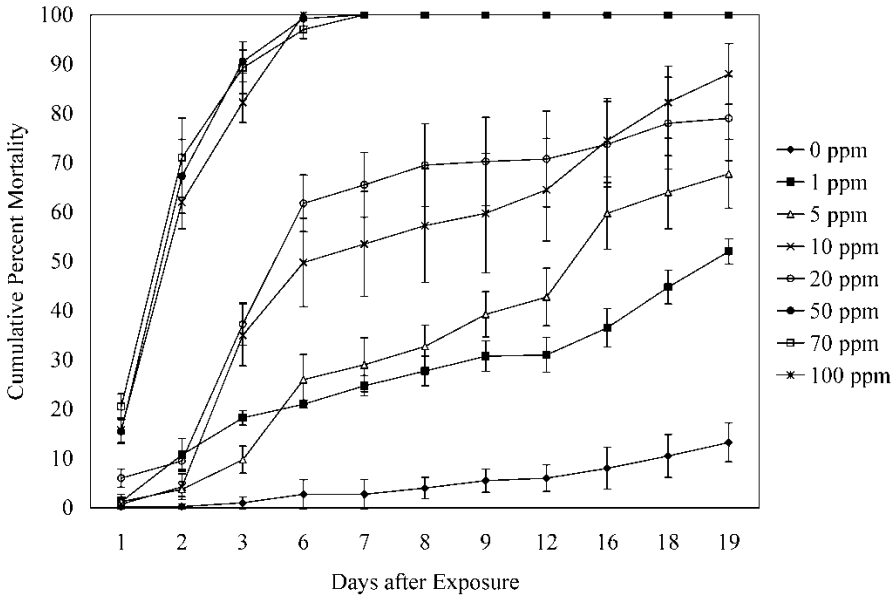

Figure 1. Cumulative percent mortality of *H. indicola* at various intervals after exposure to various concentrations of indoxacarb.

The ELT50 was 23.3 d for 1 ppm and 12.5 d for 5 ppm of indoxacarb. At 10 and 20 ppm of indoxacarb, the same mortality level was achieved in less than 7 and 6 d, respectively. Concentrations greater than 50 ppm was killed 50% of termites in less than 2 d. The ELT90 was projected to be between a few months to over a year (168 to 373 d) for 1 ppm, whereas for 5 and 10 ppm ELT90 was determined as 49.3 and 25.3 d respectively. The higher concentrations of 50, 70 and 100 ppm caused 90% mortality in a much shorter time i.e. 2.8 to 3.5 d (Table 1).

Table 1. Estimated lethal time (d) required for 50 and 90% mortality (ELT50, ELT90), with 95% confidence limits (CI) of *Heterotermes indicola* after exposure to various concentrations of indoxacarb

Dose (ppm)	ELT50 (d)	95% CI	EL 90 (d)	95% CI	Probit Model
1	23.3	23.3 - 27.4	239.0	167.8 - 373.2	ELT = -1.73 + 1.26 × dose
5	12.5	11.8 - 13.2	49.3	43.0 - 57.9	ELT = -2.35 + 2.15 × dose
10	6.7	6.1 - 7.4	25.3	21.3 - 31.6	ELT = -1.85 + 2.23 × dose
20	5.5	4.8 - 6.1	27.4	24.0 - 38.6	ELT = -1.36 + 1.83 × dose
50	1.6	1.5 - 1.7	2.9	2.8 - 3.2	ELT = -1.00 + 4.80 × dose
70	1.5	1.4 - 1.6	3.2	2.9 - 3.4	ELT = -0.74 + 4.03 × dose
100	1.7	1.6 - 1.8	3.3	3.1 - 3.5	ELT = -1.00 + 4.40 × dose

Concentrations of chlorfenapyr (1 to 7 ppm) were assessed in the same manner. At 7 ppm, 100% mortality of exposed workers occurred within 2 to 3 d. At 3 ppm, 100% mortality occurred at 6 d, and the concentration of 1 ppm was the only dose of chlorfenapyr, which took 9 d for 100% mortality to occur (Figure 2).

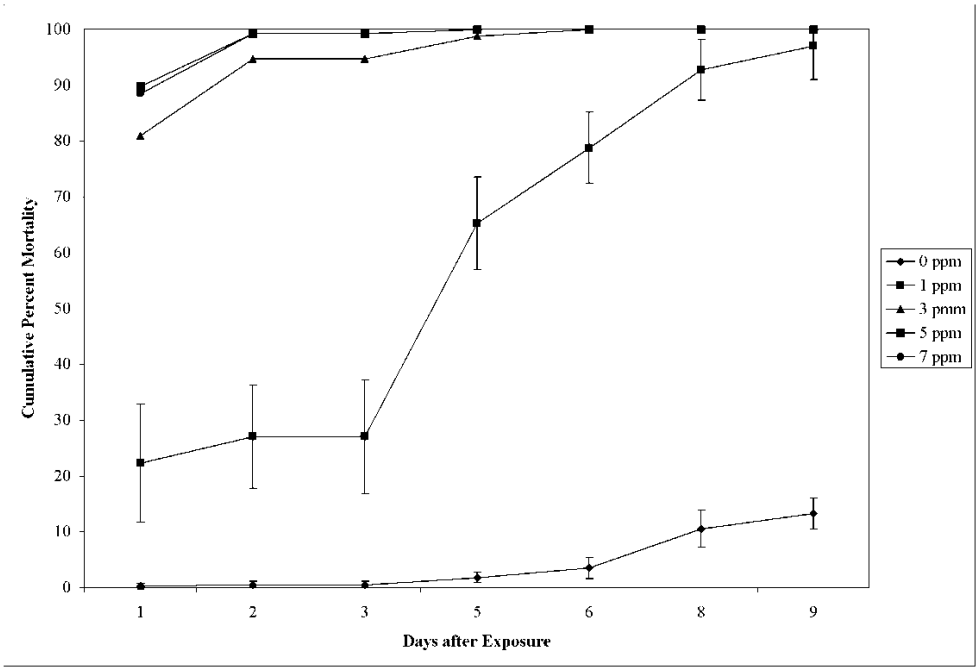

Figure 2. Cumulative percent mortality of *H. indicola* at various intervals after exposure to various concentrations of chlorfenapyr.

The ELT50 and ELT90 recorded was 2.7 and 8.6 d respectively, for 1 pm of chlorfenapyr, whereas for concentrations 3 to 7 ppm, the ELT50 was less than 1 d and ELT90 ranged between 1 to 1.4 d (Table 2).

Table 2. Estimated lethal time (d) required for 50% and 90% mortality (ELT50, ELT90, along with 95% CI) of *Heterotermes indicola* after exposure to various concentrations of chlorfenapyr

Dose (ppm)	ELT50 (d)	95% CI	ELT90 (d)	95% CI	Probit Model
1	2.7	1.9 - 3.4	8.6	6.3 - 14.5	ELT = -1.09 + 2.53 × dose
3	0.4	0.3 - 0.6	1.4	1.2 - 1.7	ELT = 0.87 + 2.40 × dose
5	0.4	0.2 - 05	1.0	0.8 - 1.1	ELT = 1.26 + 3.89 × dose
7	0.5	0.3 - 0.6	1.0	0.9 - 1.1	ELT = 1.20 + 4.11 × dose

Feeding deterrence test

In feeding deterrence tests with indoxacarb, the termites did not distinguish between treated and untreated filter paper at any of the concentrations tested. The consumption of treated and untreated filter paper was not significantly different after one week ($p = 0.06$ to 0.44) or two weeks ($p = 0.11$ to 0.62) for concentrations ranging from 1 to 100 ppm. The consumption of both treated and untreated filter paper was comparatively more at lower concentrations after one or two weeks (Table 3 & 4).

Table 3. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with different concentrations of indoxacarb after one week

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	17.6 ± 0.46a	16.1 ± 0.97a	1.09 (0.38)
5	16.7 ± 0.78ab	14.9 ± 0.43ab	2.93 (0.10)
10	16.2 ± 0.67ab	13.9 ± 0.76abc	3.17 (0.09)
20	14.7 ± 0.29bc	11.1 ± 1.0bcd	3.47 (0.07)
50	12.4 ± 0.61c	10.6 ± 1.3cd	1.40 (0.30)
70	12.5 ± 0.63c	08.4 ± 0.51de	3.83 (0.06)
100	07.2 ± 0.44d	06.2 ± 0.5e	0.94 (0.44)

*Means followed by same letters in a column are not significantly different at $p = 0.05$ using Tukey's HSD test.

Table 4. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of indoxacarb after 2 weeks

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	32.9 ± 0.80a	32.7 ± 0.95a	0.57 (0.62)
5	32.3 ± 0.76a	30.8 ± 0.12a	1.86 (0.20)
10	24.2 ± 0.89b	21.8 ± 0.99b	3.94 (0.59)
20	22.6 ± 0.81bc	18.4 ± 0.80bc	2.74 (0.11)
50	19.3 ± 0.52c	16.5 ± 0.78cd	2.13 (0.16)
70	14.4 ± 0.62d	11.8 ± 0.77de	2.06 (0.17)
100	09.8 ± 0.49e	9.2 ± 0.55e	0.57 (0.62)

*Means followed by same letters in a column are not significant different at $p = 0.05$ using Tukey's HSD test.

When total consumption (treated plus untreated filter paper) and termite mortality were compared for indoxacarb concentrations, it was observed that consumption decreased as the insecticide concentration increased while morality increased. Effective concentrations that resulted in close to 100% *H. indicola* mortality after two weeks in deterrence tests were 70 and 100 ppm. Although total consumption was higher at the lower concentrations of 1 to 50 ppm, mortality did not exceed 40% (even after two weeks). An increase in mortality was observed at both 70 and 100 ppm, over 1 and 2 weeks. After one week, mortality at both concentrations was less than 50% which increased significantly, and reached near 100% by the end of the second week (Figure 3).

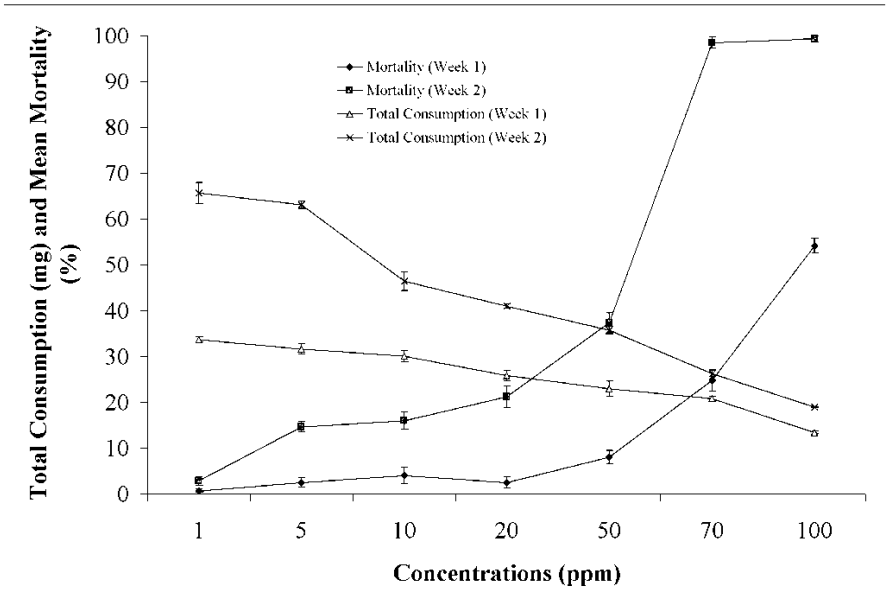

Figure 3. Total consumption of blotting paper (treated + untreated) and percent mortality caused at different concentrations of indoxacarb after week 1 and 2.

Chlorfenapyr proved to be a non-deterrent termiticide at all concentrations for up to one week. At 1 ppm, consumption of untreated filter paper was 22.1 ± 0.83 mg, whereas the consumption of treated filter paper was 20.0 ± 0.94 mg and the difference in consumption was non-significant ($p = 0.28$). The p values calculated were 0.10, 0.40, 0.60, 0.30, 0.46, 0.86 and 0.34 for 3, 5, 7, 10, 25, 50 and 100 ppm of chlorfenapyr, respectively. It is obvious from non-significant p values ($p > 0.05$) that there was no significant difference between the consumption of treated and untreated filter paper at these concentrations. As far as consumption of only treated paper was concerned, it was almost the same at concentrations 3 to 25 ppm after one week. The highest consumption of treated filter paper was recorded at 1 ppm and lowest consumption at 100 ppm (Table 5).

Similarly all the tested concentrations of chlorfenapyr remained non-deterrent ($p = 0.21$ to 0.98) after two weeks, except at 100 ppm where there was significantly ($p < 0.02$) less consumption of treated paper, compared with untreated paper. Highest consumption was recorded at 1 ppm followed by 3 ppm. At concentrations ranging from 5 to 50 ppm consumption was non-significantly different, but higher than 100 ppm and lesser than consumption at 1 and 3 ppm (Table 6).

Table 5. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of chlorfenapyr after one week

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	22.1 ± 0.83a	20.0 ± 0.94a	1.45 (0.28)
3	17.0 ± 0.44bc	18.9 ± 0.72ab	-2.88 (0.10)
5	20.6 ± 0.71ab	18.9 ± 0.86ab	1.04 (0.40)
7	16.9 ± 0.98bc	16.2 ± 0.55bc	0.60 (0.60)
10	14.3 ± 0.96cd	16.5 ± 0.66bc	-1.38 (0.30)
25	14.4 ± 063cd	15.5 ± 0.66bc	-0.9 (0.46)
50	13.4 ± 0.78cd	13.6 ± 0.64cd	-0.18 (0.86)
100	12.9 ± 0.80d	11.4 ± 0.48d	1.22 (0.34)

*Means followed by same letters in column are not significantly different at p = 0.05 using Tukey's HSD test.

Table 6. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of chlorfenapyr after 2 weeks

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	50.9 ± 1.02a	48.2 ± 0.70a	1.79 (0.21)
3	32.0 ± 1.17b	31.3 ± 1.17b	0.33 (0.76)
5	26.3 ± 0.72c	24.1 ± 1.2c	1.32 (0.31)
7	26.3 ± 1.3c	25.0 ± 0.78c	0.70 (0.55)
10	26.2 ± 0.8c	25.5 ±0.69c	0.67 (0.56)
25	20.7 ± 1.29d	21.9 ± 0.78c	-0.58 (0.61)
50	22.0 ± 1.03cd	21.9 ± 0.93c	0.01 (0.98)
100	21.3 ± 1.23cd	13.6 ± 0.29d	5.78 (0.02)

*Means followed by same letters in column are not significantly different at p = 0.05 using Tukey's HSD test.

Mortality recorded in the deterrence tests did not exceed 25 and 60% after one and two weeks respectively, even at the highest tested concentration of 100 ppm chlorfenapyr (Figure 4). Total consumption of untreated and treated filter paper decreased with increasing concentrations. Maximum total consumption (101 mg) occurred at 1 ppm, but mortality was almost negligible even after two weeks. Regardless of consumption, mortality did not exceeded more than 40% for all concentrations up to 50 ppm, even after two weeks.

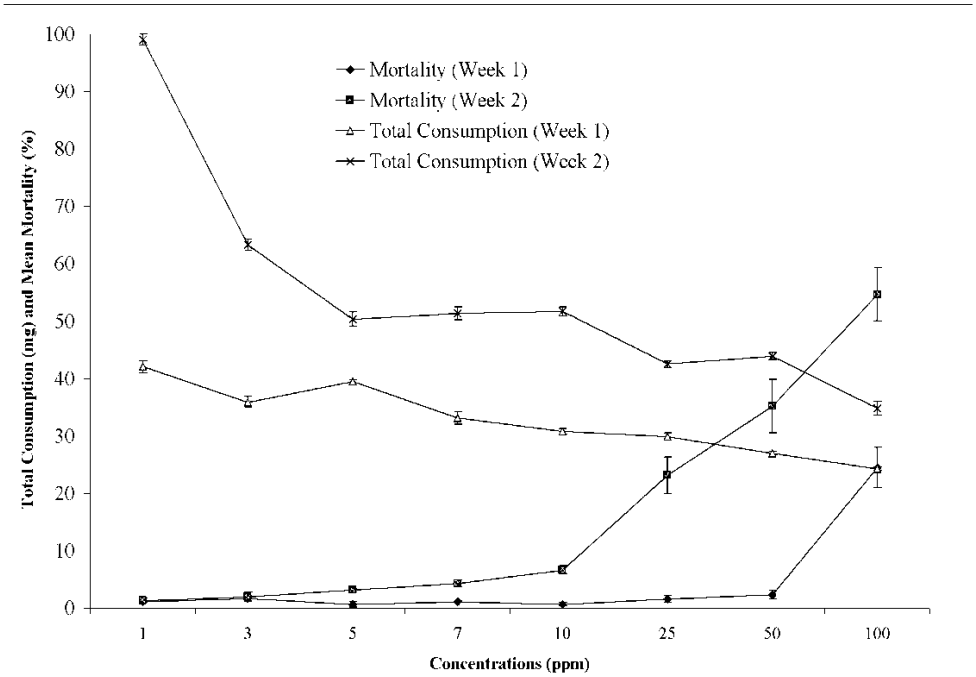

Figure 4. Total consumption of blotting paper (treated + untreated) and percent mortality caused at different concentrations of chlorfenapyr after 1 and 2 weeks.

Transfer test

Transfer tests involving indoxacarb revealed that donor mortality was more than 50% when termites were exposed to concentrations equal to or higher than 5 ppm. At 50 ppm, donor mortality was 97%, whereas 100% mortality was recorded in donor termites exposed to 70 and 100 ppm of indoxacarb. This was significantly higher than the 5% control mortality (0 ppm; $p < 0.0001$). Although high donor mortalities were recorded at most concentrations of indoxacarb, recipient mortality remained less than 50% for all the concentrations equal to or less than 50 ppm. At 70 and 100 ppm recipient mortalities were 93 and 99%, respectively, indicating a significant transfer of indoxacarb from donors to recipients. Whereas, 97% donor mortality at 50 ppm did not result in significant recipient mortality, with only 48% of recipients found dead. At 0 to 20 ppm, the recipient mortality ranged from 5 to 33%, which was significantly lower than mortality at effective concentrations of 70 and 100 ppm of indoxacarb ($p< 0.0001$).

Variability occurred in missing (presumed dead) termite donors, assumed to be consumed by recipient workers. The percentage of missing termite donors significantly decreased with the increase of concentrations ($p < 0.0001$). The lowest number of missing dead donors (14%) was recorded at 100 ppm indoxacarb compared to the highest (75%) within the controls (Table 7).

Results of transfer studies of chlorfenapyr revealed that 100% of donors were killed within 10 d of exposure to concentrations ranging from 3 to 10 ppm. Similarly, at 1 ppm the mortality recorded was 98%, whereas at 0 ppm (control), donor mortality was significantly lower (6%; $p < 0.0001$). In comparison, recipient mortality was low at all but the lowest concentration, indicating a low transfer of chlorfenapyr. At 1 ppm, mortality reached 82% after 10 d, indicating significant transfer of the toxicant. Recipient mortality ranged from 5 to 11% when they were released with the donors exposed to concentrations ranging from 3 to 10 ppm ($p < 0.0001$). Only 1 ppm seemed to be an effective concentration with significant transfer results from donors to recipients.

Table 7. Mean cumulative percent mortality of donors and recipients of *Heterotermes indicola* in 10 d after mixing the donors treated with various concentrations of indoxacarb

Dose (ppm)	Donor mortality ± SE (%)	Recipient mortality ± SE (%)	Dead donor missing (%)
0	6.0 ± 1.1a	5.0 ± 1.0a	75.0 ± 14.4a
1	30.0 ± 4.1b	5.0 ± 2.5a	47.5 ± 4.8b
5	55.0 ± 5.0c	6.0 ± 1.1a	53.2 ± 3.1bc
10	64.0 ± 2.8d	24.0 ± 3.6b	43.3 ±5.1bc
20	76.0 ± 3.6e	33.0 ± 4.4c	45.7± 4.1bc
50	97.0 ± 1.9f	48.0 ± 4.3d	25.7± 1.6cd
70	100.0 ± 0.0f	93.0 ± 3.4e	20.0 ± 1.6d
100	100.0 ± 0.0f	99.0 ± 1.0e	14.0 ± 2.5d

*Means followed by same letters in column are not significantly different at p = 0.05 using SNK test.

The number of missing termite donors, assumed to be consumed by other fellow recipients, was not significantly different (30 to 36%) in all but the lowest concentration of chlorfenapyr. Only at 0 ppm (control) were the number of missing dead donors recorded significantly higher (75 ± 14), than all the other concentrations ($p < 0.001$) (Table 8).

Table 8. Mean cumulative percent donors and recipient *Heterotermes indicola* mortality after 10 d of releasing recipients with donors treated with various concentrations of chlorfenapyr

Dose (ppm)	Donor mortality ± SE (%)	Recipient mortality ± SE (%)	Dead donor missing (%)
0	06.0 ± 1.1a	05.0 ± 1.0a	75.0 ± 14.4a
1	98.0 ± 1.1b	82.0 ± 2.5b	31.6 ± 3.1b
3	100.0 ± 0.0b	11.0 ± 1.9a	30.0 ± 2.5b
5	100.0 ± 0.0b	06.0 ± 2.0a	32.0 ± 3.6b
7	100.0 ± 0.0b	06.0 ± 1.1a	32.0 ± 4.3b
10	100.0 ± 0.0b	7.0 ± 1.9a	36.0 ± 3.6b

*Means followed by same letters in column are not significantly different at p = 0.05 using SNK test.

Discussion

Toxicity test results of indoxacarb showed that concentrations greater than 20 ppm killed 90% of exposed termites rapidly within the span of 2 to 3 d, while lower concentrations of 1 and 5 ppm did not kill the same number of termites even after several weeks. However, at 10 ppm indoxacarb the projected mortality was greater than 90% at 3 weeks. This showed that the contact toxicity of indoxacarb was dose dependent. Exposed termite workers, if not killed instantly, have the potential to disseminate a toxicant to the whole colony through the process of trophallaxis and social grooming (Shelton et al., 2006). In our study, 10 ppm seems to be an appropriate dose at which indoxacarb showed the characteristics of a slow acting toxicant. Whereas concentrations greater than 10 ppm did not provide the opportunity for termites to socialize with other nest mates as they died too rapidly. At the lowest concentrations, toxicant transfer to other unexposed termites was not enough to cause secondary mortality. Hu et al. (2005), also confirmed that indoxacarb was slow acting, and reported that at some concentrations, the required mortality was achieved after about 3 weeks. This would support the concept of colony decline caused by a slower acting termiticide, which is a very important aspect of control. Iqbal & Saeed (2013) evaluated

indoxacarb against *Microtermes mycophagus* (Desneux) and Mao et al. (2011) tested it on *R. flavipes* and *C. formosanus* along with other insecticides. Both studies confirmed a low toxicity and delayed time of action for indoxacarb. Relatively new technologies and most of the non-repellent termiticides are slow acting in nature, and need greater time to cause lethal effects (Su et al., 1987). Based on our findings and supported by previous studies, indoxacarb could be used as a slow acting toxicant, with the potential of transference among conspecific individuals of *H. indicola* through social grooming or physical contact with exposed coworkers, or via a treated medium.

Results of toxicity tests comparing chlorfenapyr and indoxacarb revealed that chlorfenapyr was comparatively more toxic to the termites. All concentrations of chlorfenapyr caused rapid mortality, except 1 ppm, which took more than one week to kill 100% of the workers of *H. indicola*. Mortality of 100% at the low concentration of 1 ppm showed that chlorfenapyr has high contact toxicity against *H. indicola*, and killed termite workers relatively rapidly. Manzoor et al. (2012), similarly reported that chlorfenapyr is highly toxic to *H. indicola* in laboratory bioassays, where 97% mortality was achieved in about 8 h of exposure. Yeoh & Lee (2007) also found that chlorfenapyr and fipronil are highly toxic against the Asian subterranean termite Coptotermes gestroi (Wasmann) even at very low doses. The fast kill rate of chlorfenapyr, along with its non-repellency to *H. indicola* makes it a promising candidate for soil barrier treatments around structures, where quick knockdown of termites is required. Rust & Saran (2006) similarly suggested that chlorfenapyr can be used in soil as an effective chemical barrier because of its non-repellency and high mortality rate. They confirmed that 1 h exposure to 75 ppm chlorfenapyr resulted in 88% mortality of the western subterranean termite, *R. hesperus*. However, in our experiment chlorfenapyr at 1 ppm or less showed relatively slow mortality compared to higher concentrations, and if termites are exposed to concentrations lower than 1 ppm, they die slower and therefore have more time to transfer the toxicant to other nest mates through trophallaxis, social grooming and cannibalism. But ideally, if quick mortality of *H. indicola* is required around a structure, chlorfenapyr could be effective at concentrations more than 1 ppm, applied to media surrounding buildings. Field based research is required to confirm results in the built environment.

Feeding deterrence test results showed that indoxacarb did not deter feeding by *H. indicola* at any of the tested concentrations. This showed that indoxacarb is not only non-repellent but also non-deterrent to termite workers of *H. indicola*. Higher mortalities at 70 and 100 ppm might be due to the combined effect of contact and oral toxicity of indoxacarb. There was an overall decrease in consumption of filter paper at these concentrations, and this could be due to intoxication of termite workers after contact with treated filter paper. It was observed that termites did not avoid contact with treated filter paper and also consumed it regardless of concentration, which indicated the non-repellency and non-deterrence of indoxacarb for *H. indicola*. Yeoh & Lee (2007) confirmed that indoxacarb was a non-deterrent termiticide when tested against *C. gestroi* at the concentrations of 1, 10, 50 and 100 ppm. Spomer et al. (2011) studied the efficacy of indoxacarb and chlorantraniliprole, and also confirmed that subterranean termites showed no deterrence towards indoxacarb. Termites maintained contact and feeding on filter paper treated with 70 to 100 ppm of indoxacarb, which caused high mortalities after 2 weeks, making it a promising candidate to be used as a slow acting toxicant bait against *H. indicola*.

In feeding deterrence tests involving chlorfenapyr, treated filter paper was offered as a food substrate along with untreated filter paper. Termites fed on it during the first week irrespective of the concentration used, showing that chlorfenapyr was a non-deterrent during the first week, but during the second week at 100 ppm a deterrent effect was observed. The consumption of filter paper treated with 100 ppm was 13.6 ± 0.29 mg, which was significantly less than that of untreated filter paper at 21.3 ± 1.23 mg. Termite workers avoiding feeding on 100 ppm treated filter paper, may be due to a learnt behavior after a sublethal exposure according to Su et al. (1995). Termites consuming a sublethal dose during the first week, may have started avoiding the 100-ppm treated filter paper in the second week. Yeoh & Lee (2007) reported that the deterrence properties of chlorfenapyr at concentrations of 1, 10, 50 and 100 ppm (w/w) were mainly concentration dependent, i.e. no deterrence at low concentrations, but at higher concentrations chlorfenapyr became deterrent to termites and consumption was significantly reduced.

Termite mortality due to chlorfenapyr at concentrations of 1 to 50 ppm remained less than 25%, even after 2 weeks. Low mortality at these concentrations suggested that the amount of toxicant acquired by termite workers was not enough to cause high mortality. Even at the highest concentration of 100 ppm, mortality recorded was only 60% after 2 weeks, which was low compared to the mortality caused by indoxacarb at the same dose. This difference in mortality indicated that chlorfenapyr was less effective when fed orally compared to its contact toxicity to *H. indicola*. Low mortality at 100 ppm could be due to lower consumption resulting from avoidance of treated filter paper. Additionally, a high concentration of a non-repellent termiticide acts like a fast-acting termiticide and it has been reported that corpses near treatment areas could repel healthy termites (Su et al., 1995). It is likely that the 60% mortality was due to the combination of contact and feeding toxicity. Shelton et al. (2006) reported rapid contact mortality of *R. flavipes* when exposed to 50, 100, 250 and 500 ppm of chlorfenapyr, all the exposed workers dying within 5 d of treatment, supporting our findings that chlorfenapyr has more contact toxicity than oral toxicity against subterranean termites. However, our results showed that chlorfenapyr became a feeding deterrent at 100 ppm. Rust & Saran (2006) reported that chlorfenapyr did not deter *R. hesperus* even at the higher concentration of 300 ppm. This is likely due to differences in the subterranean termite species tested. Overall our results from feeding deterrence tests suggest that chlorfenapyr was not effective as a feeding toxicant against termite workers of *H. indicola*. At 100 ppm or higher it became a feeding deterrent, while at lower concentrations it did not cause the desired mortality. However, it could be used as a chemical barrier when applied in soil due to its non-repellency and high toxicity to *H. indicola*.

The horizontal transfer of non-repellent insecticides from exposed individuals to unexposed nest mates is a very important process and often considered essential for the successful management of subterranean termites. Our transfer studies on indoxacarb showed more than 50% mortality of donors that were exposed to concentrations equal or greater than 5 ppm, but recipient mortality remained below 50% at most of the concentrations tested except 70 and 100 ppm. The low mortality of recipients at concentrations ranging from 1 to 50 ppm might be because termites failed to acquire sufficient toxicant from donors to cause death. Higher recipient mortalities of 93 and 99% occurred when donors were exposed to 70 and 100 ppm of indoxacarb, respectively. Increased doses and exposure usually causes greater effects on subterranean termites (Hoi, 2007). Hu et al. (2005) investigated horizontal transfer of indoxacarb in subterranean termites *C. formosanus*, and also reported that higher doses caused greater recipient mortality compared to lower doses, and the highest dose of 200 ng/donor resulted in 100% donor and recipient death, in less than 3 weeks.

It was observed that mortality in donors was not instant. Delayed mortality showed the slow acting characteristic of indoxacarb, which ultimately helped in transfer of toxicant from donors to recipients. Neoh et al. (2012) investigated the effectiveness of various non-repellent insecticides including indoxacarb against *C. gestroi* and stated that the amount of toxicant taken up by donor termites and transferred to recipients termites was concentration based. Buczkowski et al. (2012) also studied the horizontal transfer mechanism in subterranean termites, *R. flavipes*. Termite workers were exposed to 5 to 100 ppm of chlorantraniliprole, they found it highly efficient in transferring at concentrations of 25 and 50 ppm. Both concentrations caused 100% mortality in donors and recipients after 3 weeks of releasing them together. High donor mortalities at higher concentrations and subsequent high recipient mortality might be due to their social behavior i.e. grooming, trophallaxis and care of intoxicated (donor) termite workers by toxin-free (recipient) termite workers. The intoxicated or dying termites usually receive extra care and grooming from other active nest mates and they have never been removed or isolated. Healthy termite workers have been observed trying to remove toxicants attached to intoxicated termites (Hu et al., 2006). Another additional effect which could result in higher mortalities, is the missing numbers of dead donors which are assumed to have been eaten by recipients (cannibalism). Kubota et al. (2008) also explained the transfer of toxicant from donors to recipients through trophallaxis, cannibalism and social grooming when they investigated horizontal transmission, and lethal dose of bistrifluron in *C. formosanus*. They confirmed that certain toxicants taken up by donors remained in their body for several weeks, and continued transferring mates through trophallaxis, while some toxicants stuck to the donor bodies and transferred through social grooming.

Results of horizontal transfer of chlorfenapyr among workers of *H. indicola* showed that 98 to 100% of donor termites were killed when exposed to filter paper treated with concentrations ranging from 1 to 10 ppm, but recipient mortality remained at 5 to 11%, except at 1 ppm, when 86% recipient mortality was recorded. The high recipient mortality at 1 ppm was evidence of successful transfer of chlorfenapyr from donors to recipients. The higher recipient mortality at 1 ppm might be due to delayed mortality of donors whereas at other higher concentrations (3 to 10 ppm) donors were killed rapidly before transfer of the toxicant could occur. At 1 ppm the donors were intoxicated and they had sufficient time to acquire an effective amount of toxicant to cause high mortality in untreated workers. Rust & Saran (2006) also reported 100% donor mortality and 96% recipient mortality during their study of horizontal transfer of chlorfenapyr by *R. hesperus*. Hoi (2007) also investigated six insecticides, including chlorfenapyr, for horizontal transfer in *C. gestroi* and reinforced the fact that recipient mortality varied significantly when they were released together with donors exposed to different doses of different insecticides. Recipient mortality mostly depends upon the dose to which donors were exposed. The number of missing dead donors ranged non-significantly between 30 and 36% at concentration ranges of 1 to 10 ppm of chlorfenapyr, indicating that cannibalism was not a factor involved in the transfer of chlorfenapyr. Instead, most of the transfer occurred through social grooming. Shelton et al. (2006), reported only 1 ppm of chlorfenapyr is required per donor to cause high recipient mortality (80%).

Conclusion

Toxicity of both indoxacarb and chlorfenapyr was dose dependent but chlorfenapyr was found comparatively more toxic than indoxacarb. Indoxacarb proved to be non-deterrent to *H. indicola* workers feeding at all concentrations tested, whereas, chlorfenapyr became a feeding deterrent at higher concentrations. In addition, indoxacarb showed potential use as slow acting toxicant bait because at certain doses it caused delayed mortality, while chlorfenapyr caused rapid contact mortality at all concentrations tested. Therefore, chlorfenapyr has potential as a soil barrier application. Indoxacarb and chlorfenapyr were both transferred from donors to recipients, and transfer was dose dependent.

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