Pakistan J. Zool., pp 1-8, 2022. DOI: https://dx.doi.org/10.17582/journal.pjz/20221110111139

In-vitro Toxicity of Synthetic Insecticides against Subterranean Termites, *Coptotermes heimi* (Isoptera: Rhinotermitidae)

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Article Information
Received 10 November 2022
Revised 03 December 2022
Accepted 29 December 2022
Available online 14 April 2023 (early access)

Authors' Contribution
MZM conceived the experiment plan and protocol. MQ, MZS and UA conducted the experiment and analyzed the data; MZM and MA designed and drafted the manuscript. ABMR provided technical assistance and proofread the manuscript. All authors have read and approved the manuscript.

Key words
Subterranean termites, *Coptotermes heimi*, Synthetic insecticides, Laboratory toxicity, Fipronil, Chlorpyrifos

ABSTRACT

Subterranean termites cause significant damage to agricultural crops and wooden infrastructures worldwide. *Coptotermes* and *Odontotermes* were found as the most abundant and damaging genera of subterranean termites in Pakistan. Many conventional synthetic insecticides are being used to combat termite infestations with often unsatisfactory control results. This study assessed the comparative toxicity of some prevailing synthetic insecticides with different modes of action against subterranean termites *Coptotermes heimi* Wasmann (Isoptera: Rhinotermitidae) which was found as a dominant termite species in district Sargodha. Filter paper disc-based bioassays revealed that all insecticides showed a significant impact ($P < 0.001$) on the mortality of *C. heimi* workers and this mortality response was directly proportional to insecticidal concentrations and exposure times. Significantly higher mortality was recorded by chlorpyrifos (100.0%) and fipronil (95.0%) at 72 h post-exposure with minimum LC_{50} values of 1.29 and 2.04%, respectively. Similar trend of effectiveness was exhibited by their LT_{50} values. Minimum mortality of *C. heimi* workers was recorded by the formulations of chlorantraniliprole and abamectin. Based on overall study results, it is concluded that chlorpyrifos and fipronil are effective synthetic termiticides and are recommended to the indigenous farmers for combatting subterranean termite infestations.

INTRODUCTION

Termites belong to order Isoptera with 12 families and 3500 species described so far (Davies *et al.*, 2021). These invertebrates constitute a major insect fauna of humid and temperate regions and contribute to the ecosystem in terms of both beneficial and harmful aspects (Brauman *et al.*, 2015). These invertebrates play a key role in ecological processes such as biodegradation of plant based organic matter and nutrients cycles (Majeed, 2012). However, many termite species are economic pests of agricultural crops, forest trees and other wooden structures (Rouland-Lefèvre, 2010). In Pakistan, about 53 species of termites have been identified in various ecological zones that are damaging many agricultural crops and wooden infrastructures (Manzoor and Mir, 2010).

Coptotermes, *Microtermes* and *Odontotermes* are the most prevalent genera of pest termites in Indo-Pak region (Rajagopal, 2002). Among 80 subterranean termite species, 38 belong to genus *Coptotermes* (Rust and Su, 2012; Krishna *et al.*, 2013). These termites are highly destructive pests of a wide array of agricultural crops and wooden household structures (Ahmed and Qasim, 2011; Manzoor *et al.*, 2011). Their infestation has been challenging in both agricultural crops and urban areas (Katsumata *et al.*, 2007; Gazal *et al.*, 2014).

Synthetic insecticides have been the prime option to combat subterranean termite infestation worldwide. Various synthetic insecticides are used against subterranean termites including carbosulfan, deltamethrin, DDT,

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0030-9923/2022/0001-0001 $ 9.00/0

Abbreviations
ANOVA, analysis of variance; CRD, completely randomized design; GABA, gamma-aminobutyric acid; HSD, honestly significant difference; LC_{50}, median lethal concentration; LT_{50}, median lethal time.

chlorpyrifos and triazophos etc. (Ahmed *et al.*, 2006; Manzoor *et al.*, 2012; Paul *et al.*, 2018). These traditional synthetic insecticides remain highly persistent with long-term residual effects causing the eradication of beneficial organisms and other soil microbes, and secondary pests' outbreak is another concern (Desneux *et al.*, 2007; Paul *et al.*, 2018). With extensive use of synthetic chemicals, resistance against these insecticides has developed in various insects including termites (Zhu *et al.*, 2016). Novel insecticides with different chemistry and modes of action have always been a viable source of protection against different pests including termites in various insecticide resistance management programs (Iqbal and Saeed, 2013; Paul *et al.*, 2018). Thus, there is a need to explore some differential chemistry insecticides that should be environment friendly and effective to control termite population. Instead of traditional methods, novel chemistry insecticides have become promising tools for mitigating the problems of pest resistance and environmental contamination. These insecticides are not only target-specific but also safer for non-target fauna, *i.e.,* predators and parasitoids. Previous studies have shown that novel chemical insecticides are effective against different species of termites (Mao *et al.*, 2011; Rashid *et al.*, 2012; Iqbal and Saeed, 2013; Akbar *et al.*, 2019).

The pesticide resistance and environmental pollution caused by traditional insecticides with limited modes of action necessitate screening out currently available synthetic insecticides with different modes of action against indigenous subterranean termite species. We therefore evaluated some selected synthetic insecticides having different modes of action *viz;* abamectin, chlorantraniliprole, chlorpyrifos, deltamethrin, emamectin benzoate, fipronil, lambda-cyhalothrin, lufenuron, and profenofos against subterranean termites *Coptotermes heimi* (Isoptera: Rhinotermitidae) which is a dominant wood infesting subterranean termite species in Pakistan. Some of these selected insecticides are registered against termites such as chlorpyrifos, fipronil.

MATERIAL AND METHODS

Collection and maintenance of termites

First of all, an extensive survey was conducted in different localities of district Sargodha (Punjab, Pakistan) in order to determine the prevailing status of termite infestation in study area. For this purpose, small land-hold farmers, and rural and urban dwellings were surveyed randomly and samples of termite infested materials (crop stubbles and wooden infrastructures) were collected and brought to the laboratory of Entomology, University of Sargodha for identification. Termite soldier individuals were observed under an inverted trinocular microscope (XDS-3, Optika SRL, Italy). *Coptotermes* and *Odontotermes* were found as the most abundant and damaging genera of subterranean termites.

For *in-vitro* evaluation of different synthetic insecticides as detailed in Table I, *C. heimi* (Wasmann) (Isoptera: Rhinotermitidae) was used as model species because it was the most abundant species among the collected termite samples. Intact portions of *C. heimi* colony along with the termite individuals were collected from a fallen infested log of sheshum (*Dalbergia sissoo* DC.) and were maintained for few days in a rearing glass box (30 × 30 × 30 cm) under controlled conditions in dark (25±2 °C and 65±5% RH).

Table I. Synthetic insecticides used in this study.

Insecticide	IRAC Group	Mode of Action	Brand name	Company	Label dose (mL acre^{-1})
Abamectin	6 (Avermectins)	Glutamate-gated chloride channel allosteric modulator	Chacha® 1.8 EC	Orange	400
Chlorantraniliprole	28 (Diamides)	Ryanodine receptor modulator	Coragen® 20 SC	FMC	50
Chlorpyrifos	1B (Organophosphate)	Acetylcholinesterase inhibitor	Chopat® 40 EC	Orange	1000
Deltamethrin	3A (Pyrethroids)	Sodium channel modulator	Decis Super® 100 EC	Bayer	80
Emamectin benzoate	6 (Avermectins)	Glutamate-gated chloride channel allosteric modulator	Proclaim® 019 EC	Syngenta	200
Fipronil	2B (Phenylpyrazoles)	GABA-gated chloride channel blockers	Termal® 5 SC	Star Agro Sciences	480
Lambda cyhalothrin	3A (Pyrethroids)	Sodium channel modulators	Lambda® 2.5 EC	FMC	250
Lufenuron	15 (Benzoylureas)	Chitin synthesis inhibitor (IGR)	Match® 050 EC	Syngenta	200
Profenofos	1B (Organophosphate)	Acetylcholinesterase inhibitors	Curacron® 500 EC	Syngenta	750

*According to IRAC MoA Classification Version 10.2, March 2022.

Screening bioassay against C. heimi

In first bioassay, nine different synthetic insecticides having different modes of action were tested against *C. heimi* as per their label-recommended doses. Although most of these insecticides do not have recommended dose rates against termites, we tested them in our preliminary screening as per their label-recommended dose rates against other target insect pests. In the control treatment, tap water was used. Filter paper disc bioassay method as described in Akbar *et al.* (2019) was used to assess the toxicity of these insecticides against *C. heimi*. Experimental design was completely randomized (CRD) with eight replications per treatment. Filter paper discs were dipped into each treatment solution for 5–10 sec and were air-dried at ambient temperature (26 ℃) for 10–15 min before placing them into Petri-plates (9 × 2.5 cm) lined with 1.0 mm layer of 1.5% agar. Ten active and healthy termite individuals (9 workers and 1 soldier) were released on each treated filter paper disc and Petri-plates were incubated at 25±2 ℃ and 65±5% RH in an environment chamber. Mortality data was recorded after 3, 6, 12, 24, 48 and 72 h of exposure. Moribund individuals showing no movement were considered as dead.

Four most effective insecticides resulted out from the screening experiment were further bioassayed using five concentrations (*i.e.,* 5, 10, 20, 40 and 80% of the label-recommended dose) of each insecticide. Experimental protocol was similar as described above for the first bioassay except number of replications for each treatment was six in this bioassay. Mortality data were recorded at regular time intervals as mentioned above.

Statistical analyses

Mortality data of termites in response to different insecticidal treatments were analyzed by factorial analysis of variance (ANOVA) keeping insecticides and exposure time as main factors. The percent mortality was corrected with Abbott's formula (Abbott, 1925). Means were further compared by Tukey HSD test at 95% significance level. The analyses were performed by using Minitab 17.0 software. Median lethal concentration (LC_{50}) and time (LT_{50}) values were calculated by probit analysis using PoloPlus® software.

RESULTS

In screening bioassay, all insecticides exhibited significant (F = 530.5, $p < 0.001$) mortality of *C. heimi* individuals at different exposure time (F = 242.4, $p < 0.001$). The highest mean mortality of *C. heimi* individuals was recorded by chlorpyrifos (89.0%) and fipronil (85.0%) recorded at 48 h of application and this mortality was increased to 100.0 and 95.0%, respectively at 72 h post-exposure. Deltamethrin, lambda-cyhalothrin, lufenuron and emamectin benzoate exhibited intermediate mortality (65.0–76.0%) of *C. heimi* at 72 h of application. The least effective chemicals were chlorantraniliprole and profenofos causing 26.0 and 37.0% mortality, respectively at 72 h post-exposure (Fig. 1).

The toxicity of four most effective insecticides with different concentrations was tested against *C. heimi* individuals in second toxicity bioassay. Moreover, this mortality trend increased along with the insecticidal concentrations and exposure time. At minimum concentration, chlorpyrifos and fipronil exhibited 34.9 and 31.2% mean mortality of termites, respectively which increased to 69.6 and 60.5% at their higher concentrations followed by 53.5% by lambda-cyhalothrin and 37.8% by lufenuron. Lower concentrations of lambda-cyhalothrin and lufenuron caused less than 20.0% mean mortality of termites (Table II).

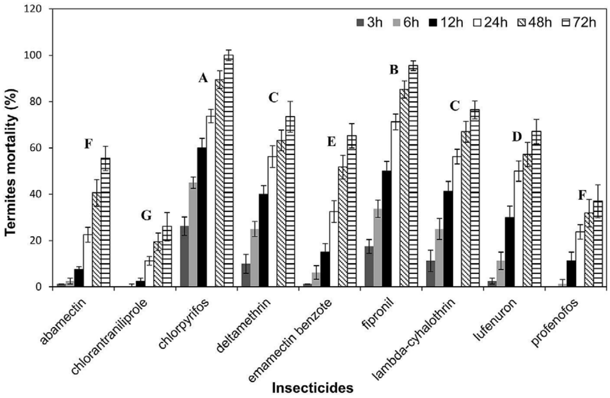

Fig. 1. Percent corrected mortality (mean ± SE; n = 8) of worker individuals of *Coptotermes heimi* termites exposed to different synthetic insecticides recorded at different post-exposure time intervals. Letters indicate overall statistical difference among the insecticidal treatments (factorial ANOVA; HSD at α = 0.05).

According to the dose response probit regression analysis of the mortality data, lowest LC_{50} value was recorded in the case of chlorpyrifos at 72 h (1.29%) and 48h (5.36%) than other insecticides. In case of fipronil, LC_{50} values were 2.04% at 72 h and 8.06% at 48 h post-exposure. LC_{50} values for lambda-cyhalothrin were 3.68% at 72 h and 10.23% at 48 h, while the minimum effectiveness was observed by lufenuron having maximum values of LC_{50} (Table III). Similar trend of effectiveness was observed in case of LT_{50} values. Minimum LT_{50} values were recorded for chlorpyrifos (6.58 h) and fipronil (10.11 h), while lambda-cyhalothrin and lufenuron showed LT_{50} values of 14.14 and 30.14 h, respectively (Table IV).

Table II. Percent corrected mortality (means ± SE) of *Coptotermes heimi* after application of synthetic insecticides at different concentrations.

Treatment Conc. (%)	Corrected mortality (%)			
	Chlorpyrifos	**Fipronil**	**Lambda**	**Lufenuron**
80	69.61±1.90a	60.58±1.46a	53.54±1.42a	37.89±1.13a
40	60.08±1.73b	51.05±1.53b	45.68±1.36b	35.36±1.28a
20	51.64±0.98c	43.74±1.35c	38.89±1.13c	29.50±1.04b
10	41.50±0.88d	36.30±0.93d	32.87±0.89d	25.03±1.13c
5	34.97±0.58e	31.27±0.82e	25.56±0.24e	20.36±0.82d

Treatment means sharing similar letters are not significantly different at $p \leq 0.05$.

Table III. Median lethal concentration (LC$_{50}$) values for different synthetic insecticides bioassayed against *Coptotermes heimi* worker individuals under laboratory conditions.

Insecticides name	Time (h)	LC$_{50}$ (%)	Lower and upper 95% fiducial limits	Slope±SE	χ2 (df = 3)	P value*
Chlorpyrifos	12	28.16	21.15 - 39.81	0.91 ± 0.14	0.20	<0.001
	24	11.05	7.40 - 14.87	0.91 ± 0.14	0.41	<0.001
	48	5.36	2.92 - 7.74	1.17 ± 0.16	3.01	<0.001
	72	1.29	0.35 - 2.44	1.27 ± 0.24	2.91	<0.001
Fipronil	12	89.34	50.43 - 316.86	0.61 ± 0.14	0.25	<0.001
	24	19.37	13.82 - 26.98	0.82 ± 0.14	1.03	<0.001
	48	8.06	2.81 - 13.33	1.07 ± 0.15	4.18	<0.001
	72	2.04	0.06 - 4.77	1.25 ± 0.20	4.90	<0.01
Lambda-cyhalothrin	12	106.72	60.03 - 364.30	0.67 ± 0.14	0.66	<0.001
	24	31.87	21.69 - 55.22	0.67 ± 0.13	1.52	<0.001
	48	10.23	6.50 - 14.07	0.86 ± 0.13	1.93	<0.001
	72	3.68	2.04 - 5.32	1.21 ± 0.16	1.18	<0.001
Lufenuron	12	444.71	152.21 - 9981.6	0.59 ± 0.15	0.17	<0.001
	24	444.71	152.21- 9981.6	0.56 ± 0.13	0.46	<0.001
	48	102.65	53.67 - 515.22	0.56 ± 0.13	0.46	<0.001
	72	Incalculable	Incalculable	0.50 ± 0.19	0.49	<0.001

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

DISCUSSION

Subterranean termites such as *C. heimi* cause considerable economic loss to agricultural crops and wooden infrastructures worldwide, and are considered as major threat to agro-forestry and urban sectors, particularly in the tropical and subtropical countries (Evans, 2021). Although many control tactics including chemical, physical and biological techniques can be employed to manage termite infestations, however chemical termiticides have been commonly used combating subterranean termites (Ahmed *et al*., 2006, 2020; Su, 2011; Kuswanto *et al*., 2015).

We conducted a preliminary survey in district Sargodha to assess the local farmers and civil community's perception about subterranean termites, their infestation, identification and control measures. Unfortunately, most of the community did not know how to identify and how to combat termite infestations. Conventional insecticides were being used by them as sole control option with no or unsatisfactory control of termites and other insect pests as documented previously (Manzoor *et al*., 2012; Majeed *et al*., 2022). Furthermore, *Coptotermes* and *Odontotermes* and *C. heimi* were found as the most important subterranean termites' genera and the most abundant termite species, respectively in the study area.

Table IV. Median lethal time (LT_{50}) values for different synthetic insecticides bioassayed against *Coptotermes heimi* worker individuals under laboratory conditions.

Treatments	Conc. (%)	LT_{50} (h)	Lower and upper 95% fiducial limits	Slope ± S.E	X^2 (df=4)	P value*
Chlorpyrifos	80	6.58	4.67 - 8.59	1.85 ± 0.15	5.92	<0.001
	40	10.08	7.16 - 13.44	1.57 ± 0.13	6.08	<0.001
	20	15.11	10.63-21.21	1.65 ± 0.13	8.73	<0.01
	10	24.31	19.02 - 31.92	1.69 ± 0.13	5.01	<0.001
	5	32.85	23.39 - 51.41	1.73 ± 0.14	9.57	<0.01
Fipronil	80	10.11	7.24 - 13.54	1.73 ± 0.14	8.71	<0.001
	40	15.43	11.26 - 20.98	1.69 ± 0.13	7.46	<0.001
	20	22.37	15.82 - 33.30	1.45 ± 0.13	7.75	<0.001
	10	31.15	22.65- 46.72	1.66 ± 0.14	7.98	<0.001
	5	41.01	28.99 - 67.94	1.57 ± 0.14	7.87	<0.001
Lambda-cyhalothrin	80	14.14	12.24 - 16.27	1.91 ± 0.14	3.50	<0.001
	40	19.73	13.78 - 28.94	1.75 ± 0.13	10.69	<0.01
	20	28.23	22.05 - 37.72	1.53 ± 0.13	7.22	<0.001
	10	39.14	28.62 - 60.20	1.48 ± 0.14	5.86	<0.001
	5	53.98	44.67 - 68.47	1.70 ± 0.16	3.67	<0.001
Lufenuron	80	30.14	22.91 - 42.18	1.44 ± 0.13	4.70	<0.001
	40	34.08	28.58 - 41.75	1.54 ± 0.14	3.69	<0.001
	20	44.50	34.39 - 62.62	1.60 ± 0.15	4.08	<0.001
	10	55.54	45.72- 71.06	1.67 ± 0.16	3.95	<0.001
	5	74.25	45.96-223.04	1.73 ± 0.14	3.27	<0.001

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

These findings are in line with previous studies (Manzoor *et al*., 2011, 2013; Rasib and Ashraf, 2014; Dugal and Latif, 2015; Sarmad *et al*., 2020).

We evaluated some currently available synthetic insecticides including some reduced-risk insecticidal formulations against *C. heimi* in the laboratory. Termites showed a differential response to all tested insecticides. This difference in toxicity of insecticides would definitely be due to their differential chemistry and modes of action (Li *et al*., 2012; Rashid *et al*., 2012; Iqbal and Saeed, 2013). Out of nine tested chemicals, chlorpyrifos and fipronil exhibited highest toxicity against *C. heimi* termites. Our findings are in accordance with Iqbal and Saeed (2013) and Zhang *et al*. (2022) who also reported chlorpyrifos and fipronil as the best insecticidal options to manage subterranean termites *Microtermes mycophagus* and *Coptotermes formosanus*, respectively. Some insecticides including chlorantraniliprole, avermectins and profenofos were least effective and caused lowest termite mortality. These differences in susceptibility of various insecticides could be the result of differential rates of penetration, insensitivity of target sites and metabolic resistance due to some enhanced level of detoxifying enzymes (Valles *et al*., 2000; Osbrink *et al*., 2001; Valles and Woodson, 2002; Zhou *et al*., 2021). However further biochemical studies are needed to better understand this phenomenon.

In the second bioassay, different concentrations of the four most effective insecticides were tested against *C. heimi*. The results showed that the mortality of termite individuals was greater with the application of a higher concentration (80%) of chlorpyrifos and fipronil. Lower LC_{50} and LT_{50} values also confirmed the higher toxicity of these chemicals. In previous studies, the toxicity of chlorpyrifos and fipronil has been reported against termites (Ahmed *et al*., 2005; Khan *et al*., 2021). Upadhyay *et al*. (2010) reported that fipronil is an effective chemical to manage the infestation of *O. obesus*. Similarly, chlorpyrifos is a very effective chemical to control termite infestation, and it has been confirmed by previous studies as well (Ahmed *et al*., 2017). It is a repellent termiticide and controls termites' movement in the soil and limits the access of termites to food leading to termites' mortality

(Ahmed *et al*., 2015). Similarly, Ahmed *et al*. (2017) also reported that fipronil is an effective compound to control subterranean termites as a pre-construction treatment; it does not allow the termites to penetrate through the treated soil.

CONCLUSION

Our findings showed that chlorpyrifos and fipronil at recommended doses were the most effective synthetic insecticides against *C. heimi* termites. Overall, this laboratory study suggests that subterranean termites' infestation such as of *C. heimi* can be eliminated by using these two synthetic insecticides in agricultural sector and in urban dwellings.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Muhammad Asam Riaz for identification of the termite species.

Funding

The study was financially supported by the internal grant of the Department of Entomology, University of Sargodha, Pakistan.

Ethical statement

Authors declare that this study did not require ethical committee's approval or any other ethical considerations.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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