Research Article

Comparative Toxicity of Selected New-chemistry Insecticides against Subterranean Termites *Odontotermes obesus* Ramb. (Isoptera: Termitidae)

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Abstract | Subterranean termites cause considerable damage to a wide number of agricultural crops, tree plantations and wooden infrastructures all over the world including Pakistan. This study was aimed to evaluate the comparative efficacy of 10 commercial formulations of new-chemistry insecticides against *Odontotermes obesus* (Isoptera: Termitidae), one of the most economic subterranean termite species in Indo-Pak region. Label-recommended dose rates of insecticides were tested against worker termites using modified filter paper disc method according to completely randomized design. All insecticides caused significant mortality of termites as compared to control treatment. Formulations of chlorantraniliprole, chlorfenapyr and pyriproxyfen exhibited maximum termite mortality, ranging from 31.18±5.67, 28.33±7.03 and 21.67±4.01%, respectively at 3 h post-exposure to 100% at 24 h, followed by triflumuron (81.67±3.07%) and indoxacarb (63.33±5.58%). Moreover, chlorantraniliprole, chlorfenapyr, pyriproxyfen, emamectin, indoxacarb and triflumuron showed LT_{50} values of 15.62 h (10.66 – 20.58), 18.48 h (14.72 – 22.93), 27.34 h (22.34 – 33.97), 28.22 h (23.37 – 34.53), 31.91 h (26.68 – 38.90) and 32.82 h (28.03 – 39.11), respectively. On the contrary, spirotetramat, pymetrozine and spinosad were least effective against *O. obesus* termites. Conclusively, based on the results of this study, chlorantraniliprole, chlorfenapyr, pyriproxyfen, triflumuron and indoxacarb are recommended to be incorporated in future integrated pest management programs against subterranean termites.

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Introduction

With 3,000 described species, termites are important fauna of most of the tropical and subtropical ecosystems and play a crucial role in nutrients cycling, plant litter decomposition, organic matter turnover and soil conditioning (Jouquet et al., 2011; Brauman et al., 2015). However, in spite of great ecological significance, many species of termites are noxious agricultural and urban pests. Particularly, subterranean termites are severe pests of a wide number of agricultural crops, tree plantations and wooden structures (Rouland-Lefèvre, 2010). In Pakistan, these termites cause considerable damage to many agricultural crops including sugarcane, cotton, gram, wheat, maize and sesame. Moreover, they pose a serious threat to wooden infrastructures in urban and rural areas (Ahmed et al., 2005 and 2011; Iqbal and Saeed, 2013). *Odontotermes*, *Coptotermes* and *Microtermes* are the most common genera and *Odontotermes obesus*, *O. guptai* and *Microtermes obesi* are the most economic species of subterranean termites in arid and semi-arid

regions of Pakistan (Ahmed et al., 2005 and 2011; Manzoor et al., 2011; Aihetasham et al., 2017).

Farmers in Pakistan mostly rely on conventional synthetic insecticides for the prevention and eradication of subterranean termites such as organophosphates, organochlorines, carbamates and pyrethroids (Ahmed et al., 2006; Manzoor et al., 2012). Due to extensive and irrational use of these synthetic and persistent agro-chemicals, many insects including termites have developed resistance against them (Kranthi et al., 2002; Zhu et al., 2016). Synthetic insecticides have been an integral part of crop protection around the globe. Without these agrochemicals, it is almost impossible to ensure agricultural production to feed world's ever-growing population (Carvalho, 2006). However, sole reliance on these synthetic chemicals has been creating many problems including environmental contamination, biodiversity eradication, secondary pest outbreaks and insect pest resistance (Desneux et al., 2007; Edwards, 2013).

Therefore, there is a need to explore novel environment-friendly options for controlling insect pests such as new-chemistry insecticides. Insecticides having novel chemistry and with the mode of action different than the conventional ones are emerging as promising alternatives to be incorporated in bio-based integrated pest management programs. These insecticidal formulations are often less persistent, quickly biodegradable, more target-specific and relatively safe to non-target fauna and humans (Grafton-Cardwell et al., 2005; Ishaaya and Degheele, 2013). These insecticides can be effective IPM tools to combat insecticide resistance problems.

In view of the damage potential of subterranean termites and the ecological consequences of synthetic persistent insecticides, this study was aimed to evaluate and screen out the most effective new-chemistry insecticidal formulations under laboratory conditions which can be recommended to indigenous farmers for the management of subterranean termites.

Materials and Methods

Insect culture

Intact colony of subterranean termites (*Odontotermes* spp.) was collected from the damaged stubbles of sugarcane (*Saccharum officinarum* L. var. BF-237) from surroundings of College of Agriculture, University of Sargodha (32°07'58"N; 72°41'32"E). It was ensured that the sugarcane field from which termite colony was collected was not treated with any pesticide for at least 4 months. This colony along with the termite individuals was maintained for few days in a plastic box at 27±2°C and 65% relative humidity. Only healthy and active termite individuals were utilized in bioassays.

Toxicity bioassays

The commercial formulations of selected new-chemistry insecticides were procured from the authorized dealers of pesticide market of District Sargodha (Punjab, Pakistan). Slightly modified standard filter paper disc method (Yuan and Hu, 2012) was used to assess the toxicity of these insecticides against subterranean termites. Bioassays were laid out according to CRD with 6 replications per treatment. In brief, filter paper discs were dipped for 5-10 sec in insecticidal solutions which were made according to their label-recommended dose rates as described in Table 1. Then these filter paper discs were allowed to dry at room temperature (25°C) for 15-20 min prior to their placement in glass petri plates (9 cm diameter). Distilled water was used to prepare treatment solutions and same was used in control petri-plates. Fifteen healthy and active worker termites were released on each of the treated filter paper discs and petri plates were incubated in an environment chamber at 27±2°C and 65% relative humidity and under a 16:8 h light–dark photoperiod. Mortality of termites was recorded at 3, 6, 12, 24 and 48 h post-exposure. Moribund insects unable to move their body appendages upon touching with camel hair brush tip were considered as dead.

Statistical analysis

Apart from graphical presentation of percent mortality of termites in response to new-chemistry insecticides, mortality data was subjected to statistical analysis using Statistix® 8.1 (Analytical Software, 2005). Median lethal time (LT_{50}) values were calculated by probit analysis using POLO-PC® (LeOra Software, 1987) regression software. Prior to probit analysis, termite mortality was corrected using Abbott's formula (Abbott, 1925) after correction for the control treatments. Moreover, means of the treatments were further compared by one-way and factorial analysis of variance using 5% probability level, followed by Tukey's highest significant difference (HSD) test using time and treatment as factors.

Table 1: *Selected new-chemistry insecticides evaluated under laboratory conditions against worker subterranean termites (Odontotermes obesus).*

Chemical Name (active ingredient)	Chemical family*	Mode of Action	Brand Name	Company	Label Dose (a.i. ha^{-1})
chlorantraniliprole	28 (diamides)	Ryanodine receptor modulators	Coragen® 18.5 SC	DuPont	375 ml
chlorfenapyr	13 (pyrroles)	Uncoupler of oxidative phosphorylation	Pirate® 360 SC	Swat Agro Chemicals	500 ml
emamectin	6 (avermectins)	Glutamate-gated chloride channel (GluCl) allosteric modulators	Proclaim® 1.9 EC	Syngenta	500 ml
indoxacarb	22A (oxadiazines)	Voltage-dependent sodium channel blockers	Steward® 150 EC	DuPont	375 ml
pymetrozine	9B (pyridine azomethine derivatives)	Chordotonal organ TRPV channel modulators	Planum® 50 WG	Syngenta	125 g
pyriproxyfen	7C (pyriproxyfens)	Juvenile hormone mimics (IGR)	Admiral® 10 EC	FMC	75 ml
spinosad	5 (spinosyns)	Nicotinic acetylcholine receptor (nAChR) allosteric modulators	Tracer® 240 SC	Dow Agro Sciences	150 ml
spirotetramat	23 (tetramic acid derivatives)	Acetylcholinesterase (AChE) inhibitor	Movento® 240 SC	Bayer CropScience	800 ml
triflumuron	15 (benzoylureas)	Chitin synthesis inhibitor (IGR)	Alsystin® 480 SC	Bayer CropScience	800 ml

**according to Insecticide Resistance Action Committee (*www.irac-online.org*) IRAC MoA Classification Version 8.3, July 2017.*

Results and Discussion

Ten different types of insecticides with differential mode of action were evaluated against worker individuals of subterranean termites (*O. obesus*) under laboratory conditions. Percent mortality of termites recorded at different post-treatment time intervals was subjected to factorial analysis of variance which revealed that both insecticidal treatment ($F_{(9, 245)}$ = 88.14, P = < 0.001) and time ($F_{(4, 245)}$ = 535.80, P = < 0.01) factors and their interaction ($F_{(36, 245)}$ = 9.75, P = < 0.001) had significant impact on the termite mortality (Table 2).

All insecticides caused significant mortality of termites as compared to control (Table 3). Mortality in control treatments varied from 0.00 to 11.67%. At 3 h of exposure, chlorantraniliprole, chlorfenapyr and pyriproxyfen gave maximum mortality (31.18±5.67, 28.33±7.03 and 21.67±4.01%, respectively) followed by spirotetramat, indoxacarb and emamectin, while spinosad exhibited the minimum mortality (Table 3). At 6 h exposure, again chlorantraniliprole (48.33±3.07%), chlorfenapyr (43.33±4.94%) and pyriproxyfen (40.00±5.16%) caused maximum mortality while spinosad (16.67±2.11%) and spirotetramat (18.33±3.67%) gave minimum mortality.

Table 2: *Analysis of variance comparison table for mean mortality of worker individuals of subterranean termite (Odontotermes obesus) exposed to label-recommended dose rates of selected new-chemistry insecticides under laboratory conditions.*

Source	DF	SS	MS	F-value	P-value
Replication	5	384	76.8		
Treatment	9	57681	6409.0	88.14	<0.001
Time	4	155851	38962.8	535.80	<0.01
Treatment * Time	36	25535	709.3	9.75	<0.001
Error	245	17816	72.7		
Total	299	272244			
Grand Mean	33.80				
CV	25.23				

P < 0.001 (highly significant) and P < 0.01 (significant); two-way factorial ANOVA at α = 0.05.

Similarly, after 12 h of exposure, former three insecticides gave the maximum mortality (66.52±6.33, 53.33±2.11 and 51.67±4.01%, respectively) followed by indoxacarb (45.00±5.01%) and triflumuron (43.33±4.22%) while spirotetramat and spinosad exhibited minimum mortality of termites. According to observation at 24 h, again chlorantraniliprole, chlorfenapyr and pyriproxyfen exhibited 100% mortality followed by triflumuron (81.67±3.07% and indoxacarb (63.33±5.58%). Similar trend of mortality

Table 3: *Percent mortality of worker individuals of subterranean termite (Odontotermes obesus) exposed to label-recommended dose rates of selected new-chemistry insecticides.*

Treatments	3h		6h		12h		24h		48h	
chlorantraniliprole cd	31.18 ±	5.67* a	48.33 ±	3.07 a	66.52 ±	6.33 a	100.00 ±	00 a	nd	
chlorfenapyr a	28.33 ±	7.03 a	43.33 ±	4.94 a	53.33 ±	2.11 a	100.00 ±	0.00 a	nd	
emamectin b	11.67 ±	3.65 abc	20.00 ±	2.58 bc	23.33 ±	2.11 c	38.33 ±	3.07 d	69.16 ±	3.65 b
indoxacarb b	11.67 ±	3.33 abc	35.00 ±	4.28 ab	45.00 ±	5.01 ab	63.33 ±	5.58 c	98.33 ±	1.67 a
pymetrozine c	8.33 ±	2.11 abc	30.00 ±	2.58 b	35.00 ±	2.24 b	46.67 ±	3.33 d	58.33 ±	8.72 b
pyriproxyfen b	21.67 ±	4.01 ab	40.00 ±	5.16 a	51.67 ±	4.01 a	100.00 ±	0.00 a	nd	
spinosad d	3.33 ±	2.11 bc	16.67 ±	2.11 c	21.67 ±	3.07 bc	35.00 ±	2.24 d	61.67 ±	4.77 b
spirotetramat d	11.67 ±	3.07 abc	18.33 ±	3.07 c	25.00 ±	3.42 bc	45.00 ±	4.28 d	66.67 ±	4.22 b
triflumuron b	10.00 ±	6.15 bc	31.67 ±	3.07 b	43.33 ±	4.22 ab	81.67 ±	3.07 b	91.67 ±	4.01 a
control e	0.00 ±	0.00 c	1.67 ±	1.67 e	3.33 ±	2.11 d	8.33 ±	1.67 e	11.67 ±	1.67 c

**values are means of six independent replications for each treatment ± standard errors. Means within a column bearing different letters are significantly different from each other (2-way factorial ANOVA for overall treatments comparison and one-way ANOVA for comparison of treatments at each time interval; Tukey's HSD at α = 0.05). nd = not determined.*

was recorded at 48 h post-exposure. According to factorial ANOVA and Tukey HSD test, the most effective insecticides against *O. obesus* subterranean termites were chlorantraniliprole, chlorfenapyr, pyriproxyfen, indoxacarb and triflumuron while emamectin, spirotetramat and spinosad were the least effective ones (Table 3).

The same pattern of effectiveness of insecticides against subterranean termites was also more or less reflected by their median lethal time (LT_{50}) values. According to probit analysis, the most effective insecticides were chlorantraniliprole, chlorfenapyr, pyriproxyfen, emamectin, indoxacarb and triflumuron with LT_{50} values of 15.62 h (10.66 – 20.58), 18.48 h (14.72 – 22.93), 27.34 h (22.34 – 33.97), 28.22 h (23.37 – 34.53), 31.91 h (26.68 – 38.90) and 32.82 h (28.03 – 39.11), respectively. On the other hand, maximum LT_{50} values were recorded for pymetrozine (46.28 h), spinosad (63.03 h) and spirotetramat (104.09 h) (Table 4).

Screening of available control tactics against insect pests have been a crucial aspect of any effective plant protection program. Insecticides with a mode of action and chemistry different from those of conventional ones have always been promising pest control tools to be incorporated in different insecticide resistance management programs against insect pests of economic importance including subterranean termites (Delgrade and Rouland-Lefevre, 2002; Li et al., 2012; Iqbal and Saeed, 2013; Paul et al., 2018). This study was aimed to evaluate the comparative efficacy of ten available new-chemistry insecticidal molecules with different modes of action against subterranean termites, devastating pests of a wide range of industrial and agricultural crops, orchard and forest plantations and wooden structures (Rouland-Lefèvre, 2010).

Table 4: *Median lethal time (LT_{50}) values of selected new-chemistry insecticides evaluated against worker individuals of subterranean termite (Odontotermes obesus) under laboratory conditions.*

Treatment	LT_{50} (hr)	Lower and Upper 95% Fiducial Limits (hr)	X^2 (df = 28)*	P-value
chlorantraniliprole	15.62	10.66 – 20.58	536.28	< 0.001
chlorfenapyr	18.48	14.72 – 22.93	425.13	< 0.001
emamectin	28.22	23.37 – 34.53	247.27	< 0.001
indoxacarb	31.91	26.68 – 38.90	277.71	< 0.001
pymetrozine	46.28	38.75 – 57.62	165.11	< 0.001
pyriproxyfen	27.34	22.34 – 33.97	347.01	< 0.001
spinosad	63.05	52.17 – 81.11	164.37	< 0.001
spirotetramat	104.09	71.50 – 194.88	149.37	< 0.001
triflumuron	32.82	28.03 – 39.11	171.08	< 0.001

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

Results revealed that all insecticides at their label-recommend dose rates were effective against *O. obesus* termites. The order of average mortality caused by tested insecticides from highest to lowest was chlorantraniliprole > chlorfenapyr > pyriproxyfen >

triflumuron > emamectin > indoxacarb > spirotetramat > spinosad > pymetrozine. This differential toxicity of these insecticides to *O. obesus* individuals might be due to their differential chemistry and mode of action (Iqbal and Saeed, 2013). These findings are in line with the results of Hu (2005), Li et al. (2012), Rashid et al. (2012) and Iqbal and Saeed (2013).

Nevertheless, it was found that chlorantraniliprole, chlorfenapyr and pyriproxyfen exhibited higher toxicity than triflumuron, indoxacarb and emamectin which is in line with the findings of Rashid et al. (2012) and Iqbal and Saeed (2013). It was probably due to more recent introduction of chlorantraniliprole, pyriproxyfen and chlorfenapyr formulations against termites in Pakistan than later chemicals. Low mortality response of *O. obesus* to formulations of spinosad and emamectin might be due to the increased incidence of resistance in indigenous subterranean termite populations (Manzoor et al., 2009). Similarly, ineffectiveness of pymetrozine and spirotetramat corroborates the fact that these insecticides are more effective and are usually recommended against sucking insect pests rather than soil-inhabiting chewing insect pests (Fuog et al., 1998; Nauen et al., 2008).

Nevertheless, this study evaluated the tested insecticides on the basis of their *in-vitro* toxicity measured in terms of mortality of the exposed termite workers. However, slow-acting insecticides are usually recommended for termites' control. Interestingly, most of the new-chemistry insecticides found effective in this study against *O. obesus* termites behave as well as slow-acting toxicants when applied in the form of baits or formulations to be applied against subterranean termites in urban setting. Chlorantraniliprole, chlorfenapyr, pyriproxyfen, triflumuron and indoxacarb have been demonstrated to be effective for longer period of time as slow-acting toxicants (Hu, 2005; Rust and Saran, 2006; Quarcoo et al., 2010).

Conclusions and Recommendations

Conclusively based on the results of this study, chlorantraniliprole, chlorfenapyr, pyriproxyfen, triflumuron and indoxacarb are recommended to be incorporated in future integrated pest management programs as effective chemical insecticidal tools along with concurrent monitoring of resistance in local populations of subterranean termites to these insecticidal formulations as demonstrated by Iqbal and Saeed (2013). However, apart from their *in-situ* evaluation, the determination of synergistic action of these new-chemistry termiticides as demonstrated by Li et al. (2012) constitutes the future perspectives of this research work.

Author's contribution

Muhammad Shahzad Akbar and Muhammad Zeeshan Majeed conceived the idea and planned the experiment. Muhammad Shahzad Akbar performed experiments and wrote first draft of the manuscript. Muhammad Zeeshan Majeed performed statistical analyses and technically revised the manuscript. Muhammad Afzal provided technical assistance and proofread the manuscript.

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