Pakistan J. Zool., vol. 45(3), pp. 709-713, 2013

Toxicity of Six New Chemical Insecticides Against the Termite, *Microtermes mycophagus* D. (Isoptera: Termitidae: Macrotermitinae)

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Abstract.- The termite, *Microtermes mycophagus* D., is an economically important species causing damage to wooden structures and agricultural crops in Pakistan. Toxicities of six new chemical insecticides were evaluated to determine the lethal concentration (LC_{50}) against *M. mycophagus* collected from four locations (tree plantation, untreated building, treated building, agriculture area) of Multan, Pakistan. The population collected from agricultural area was more tolerant to all insecticides compared to those of other three locations. The order of average toxicity of insecticides from highest to lowest was: chlorfenapyr > spinosad > thiamethoxam > fipronil > indoxacarb > imidacloprid. The study provides an opportunity to compare the toxicities of some new chemical insecticides and to use them effectively in the termite management programme.

Keywords: New chemical insecticides, lethal concentration, toxicity ranking, *Microtermes mycophagus.*

INTRODUCTION

Termitidae is the largest family of termites comprising eight subfamilies, about 250 genera and over 2000 species (http://164.41.140.9/catal/). The members of this family are abundantly found in tropical forests where they play an important role in decaying and recycling of plant materials. Unfortunately, they become serious economic pests when their appetite for wood extends to agriculture and urban structures in most subtropical and tropical countries (Lee *et al*., 1999, 2003; Su and Scheffrahn, 2000; Ahmed *et al*., 2004, 2006; Ahmed and Farhan, 2006).

Billions of dollars are spent annually on termite prevention, control, and repair measures all over the world (Su *et al*., 1987; Su and Scheffrahn, 1990; Ahmed *et al*., 2007). Although the tolerance or action threshold for most of the urban pests is considered low in developed countries, control measures for termites with the same population level may not be economically feasible, especially in countries where these harmful pests are most abundant (Robinson, 1996). In developing countries like Pakistan, severe termite infestation to structures is often tolerated because the cost of control may exceed the replacement of damaged lumber or renewal costs. But with the increase of earnings and living standards in big cities of Pakistan, the trend has changed and people are now very conscious of termite damage to their valuable structures.

In Pakistan, 53 termite species have been described but only 11 species have been found to cause economic damage. *Microtermes mycophagus* D. is a fungus growing termite and is more common in arid/desert localities of Pakistan (Akhtar and Sarwar, 1993). It has been reported from various districts of Pakistan like Multan, Bahawalpur, Bahawalnagar, Mianwali, Bhakkar, Lahore, Muzaffargarh, Khanpur, Chichawatni, Pirawala, Jhelum, Sibi, Karachi and Hyderabad (Sheikh *et al*., 2005). This species has been found to cause serious damage to buildings, agricultural crops and trees (Ahmed *et al*., 2004; Manzoor *et al*., 2011).

Protection of valuable timber structures and crops from termites in Pakistan is still dependent upon the application of highly persistent insecticides like organochlorine, organophosphate (chlorpyrifos) and synthetic pyrethroid (bifenthrin) (Ahmed *et al.*, 2006; Ahmed and Qasim, 2011). However, in many countries this trend has changed over recent years because of environmental and public health concerns associated with the use of these insecticides. For this reason, numerous studies with

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0030-9923/2013/0003-0709 $ 8.00/0

different objectives and methodologies, have been conducted to examine the efficacy of new insecticides against different termite species (Remens and Su, 2005; Wang *et al.*, 2007; Mao *et al.*, 2011). Little information is available on the toxicity of new chemicals against *M. mycophagus*, particularly in Pakistan. The present study was, therefore, conducted to evaluate the toxicity of some new chemical insecticides against *M. mycophagus*.

MATERIALS AND METHODS

Termites

Foraging workers of *M. mycophagus* were collected using collection traps described by Sornnuwat *et al*. (1996) with some modifications. The trap unit consisted of a PVC pipe containing toilet paper roll and simal (*Bombax cieba*) wood stacks. Multiple collection traps were randomly installed in each of the four localities. The first locality (L1) was three hectare mixed tree plantation ($\geq$ 6 years old) with approximately 1000 m of barren land on each side. The second locality (L2) was a building where no insecticide was ever applied in the surrounding area of approximately 500 m^2. The third locality (L3) was also a building where soil treatments with chlorpyrifos, bifenthrin, and imidacloprid had been done for termite prevention. The fourth locality (L4) was an agricultural area receiving organophosphate, carbamate, synthetic pyrethroid and new chemistry insecticides each year. The infested wood stacks and paper rolls were collected twice weekly from the traps and brought back to laboratory where termites were separated from the debris into plastic trays containing moist paper towels. Termites were then immediately shifted to 100 liter plastic containers having moistened simal (*B. cieba*) wood blocks and paper roll. Distilled water was sprayed on the inside walls of the container to keep the relative humidity (RH) above 80% (Acda, 2007). All termites were tested within seven days of collection based on the statement in Valles and Woodson (2002a) and only healthy and active termites were used for bioassays.

Insecticides

Fipronil (Regent® 5% SC, Bayer Crop Science), imidacloprid (Confidor 20 SL, Bayer Crop Science), chlorfenapyr (Squadron 360 SC, FMC United), thiamethoxam (Actara 25 WG, Syngenta), indoxacarb (Steward 150 SC, Du-Pont) and spinosad (Tracer 240 SC, Arysta Life Science) were purchased from a commercial source.

Lethal concentration estimation

A no-choice feeding bioassays were performed to determine the trends in mortality of *M. mycophagus*. Five to six concentrations (causing >0 % and <100 % mortalities) were prepared by serial dilution in distilled water for each of the six insecticides. Only the distilled water was considered to calculate the ppm. One ml of an insecticide concentration was spread on the Whatman No. 1 filter paper placed on the bottom of a Petri dish (9 cm diameter by 1.5 cm high). Filter paper moistened with same quantity of distilled water was served as control. Filter papers were dried in the fume hood for 24 hours. Then 20-30 workers of at least the third instar plus two soldiers were introduced into the dish. Each concentration was repeated 10 times with a total of 50-60 dishes per insecticide. There were five to six control dishes for each insecticide. The dishes were then placed in an incubator at 25±2°C and 70±5 % RH. A filter paper underneath the glass cover of the dish was wetted with distilled water daily during treatment. The mortality was recorded after three days of exposure. Workers were considered dead when they showed no movement upon probing with a fine brush.

Data analysis

The mortality data were corrected using Abbott's formula (1925), if the mortality in the control was more than 5 %. Data were analyzed by probit analysis using SPSS software (Version 10.0 for windows, SPSS Inc., Chicago, USA) to determine median lethal concentrations (LC_{50}). Susceptibility ratios were calculated by dividing the LC_{50} value of a field strain to the LC_{50} value of the most susceptible field strain.

RESULTS

Based on LC_{50} values, the L1 population exhibited the lowest LC_{50} values for all the insecticides tested, and hence was used as a reference strain to calculate the susceptibility ratios.

Table I.- Toxicity of different insecticides against field populations of *Microtermes mycophagus* after 3 days exposure on treated filter paper.

Insecticide	Location	LC_{50} (95% FL)	^{2}	d.f.	P	SR* LC_{50} (95% FL)	N**
Fipronil	L1	3.72(3.41-4.05)	6.56	4	0.16	1	1947
	L2	4.74(4.35-5.16)	5.64	4	0.23	1.27	1970
	L3	4.17(3.56-4.908)	6.94	4	0.14	1.12	1997
	L4	5.88 (4.76-7.46)	9.27	4	0.05	1.58	2028
Chlorfenapyr	L1	2.80(1.95-3.77)	7.34	3	0.01	1	1579
	L2	2.95(2.19-3.83)	5.36	3	0.15	1.05	1543
	L3	3.04(2.18-4.03)	6.11	3	0.12	1.09	1577
	L4	3.45 (3.04-3.88)	4.14	3	0.25	1.23	1635
Thiamethoxam	L1	3.11(2.79-3.48)	4.08	3	0.25	1	1577
	L2	3.56 (2.66-5.07)	6.94	3	0.07	1.14	1533
	L3	3.44(3.06-3.89)	3.9	3	0.27	1.11	1489
	L4	5.37(4.63-6.41)	3.51	3	0.32	1.73	1569
Imidacloprid	L1	12.59(10.61-15.53)	3.91	3	0.27	1	1519
	L2	14.37(12.02-17.92)	5.22	3	0.15	1.14	1490
	L3	17.71(14.46-22.94)	3.51	3	0.32	1.41	1483
	L4	24.06(19.02-32.81)	4.15	3	0.24	1.91	1617
Indoxacarb	L1	8.72(7.45-10.45)	2.95	4	0.56	1	1909
	L2	8.98(6.60-13.53)	7.41	4	0.12	1.03	1706
	L3	9.06(6.64-13.73)	8.09	4	0.08	1.04	1899
	L4	9.57(6.91-15.01)	7.86	4	0.09	1.1	1771
Spinosad	L1	3.24(2.48-4.12)	5.87	3	0.12	1	1503
	L2	3.34(2.44-4.42)	7.31	3	0.06	1.03	1385
	L3	3.67(2.65-5.01)	9.07	3	0.03	1.13	1420
	L4	3.72(3.33-4.14)	1.18	3	0.75	1.15	1416

*Susceptibility ratio = LC_{50} of field population/LC_{50} of susceptible strain
**Number of workers tested in a bioassay

This population, however, may not be truly susceptible, yet its LC_{50} values were quite low and can be used as baseline data for future control programmes.

The results of the concentration–mortality tests of different insecticides are presented in Table I. The toxicity of fipronil to kill 50 % workers of *M. mycophagus* collected from different localities was ranged from 3.72-5.88 ppm. Workers from the L2, L3 and L4 were comparatively 1.27, 1.12 and 1.58 times less susceptible, respectively. The LC_{50} values of chlorfenapyr for different termite colonies were ranged from 2.80-3.45 ppm with the susceptibility ratios of 1.05, 1.09 and 1.23 for the colonies of L2, L3 and L4, respectively. For thiamethoxam, the LC_{50} values were ranged from 3.11-5.37 ppm. The results in table I revealed that the workers of L2, L3 and L4 were less susceptible with the susceptibility ratios of 1.14, 1.11 and 1.73 times, respectively (Table I).

Laboratory bioassay of imidacloprid against *M. mycophagus* workers showed that the LC_{50} values were in the range of 12.59-24.06 ppm and the susceptibility ratios were 1.14, 1.41 and 1.91 times for the population of L2, L3 and L4, respectively. The LC_{50} values of indoxacarb were ranged from 8.72-9.57 ppm. The susceptibility ratios of L2, L3 and L4 were 1.03, 1.04 and 1.1 times, respectively. The LC_{50} values of spinosad were ranged from 3.24-3.72 ppm. The susceptibility ratio values for spinosad were 1.03, 1.13 and 1.15 for the population of L2, L3 and L4, respectively (Table I).

DISCUSSION

The control of subterranean termites through the use of conventional and highly persistent insecticides caused severe public health and environmental concerns (Pearce, 1997). In the present study we evaluated the toxicities of six insecticides having novel modes of action against *M. mycophagus* collected from the four localities in Multan, Pakistan. The LC_{50} values of the tested insecticides revealed small differences among the tested localities which could be due to the genotypic or phenotypic differences in the populations. The phenotypic differences in an insect population can result from age, time since last molt, or induction caused by feeding history (Matsumura, 1985). The susceptibility differences also arise from differential rates of insecticide penetration, target-site insensitivity and metabolic resistance (Osbrink *et al.*, 2001). In the present study some of the termite populations were less susceptible and may possess mechanisms that enhance detoxification of the insecticides by certain enzymes (Valles and Woodson, 2002b)

The order of average toxicity of insecticides from highest to lowest was: chlorfenapyr >spinosad >thiamethoxam>fipronil>indoxacarb >imidacloprid. The difference in the toxicity of the tested insecticides might result because of the difference in their modes of action. Our results showed that the workers of the L4 showed comparatively high LC_{50} values and susceptibility ratios than the workers of the other three localities. One probable reason for this phenomenon could be due to the fact of collection of the said population from agro eco-zone which received high concentration of insecticides for the management of agricultural insect pests each year. The pest might have become more tolerant to insecticides due to enhanced level of detoxifying enzymes (Valles *et al*., 1998, 2000; Obsrink *et al*., 2001), however it should be confirmed biochemically in the future studies.

In Pakistan, *M. mycophagus* is a serious pest of agricultural crops and wooden structures in residential areas. However, no effort has been taken to explore the effect of different insecticides to formulate chemical based management strategies against this harmful pest. In the current study, we have explored the susceptibility level of *M. mycophagus* to new chemical insecticides bearing novel mode of action in Punjab, Pakistan. The results presented here could be useful in the management of subterranean termites in Punjab, Pakistan.

ACKNOWLEDGEMENTS

This research was funded by Higher Education Commission (HEC) PhD Indigenous Scholarship Batch IV. Authors are also thankful to Hafiz Azhar Ali Khan Ph.D Scholar Agri-Entomology for his help in this research

REFERENCES

ABBOTT, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.,* **18:** 265-267.

ACDA, M.N., 2007. Toxicity of thiamethoxam against Philippine subterranean termites. *J. Insect Sci.*, **7:** 26, available online: insectscience.org/7.26

AHMED, S. AND QASIM, M., 2011. Foraging and chemical control of subterranean termites in a farm building at Faisalabad, Pakistan. *Pak. J. Life soc. Sci.,* **9**: 58-62.

AHMED, S. AND FARHAN, M., 2006. Laboratory evaluation of chlorpyrifos, bifenthrin, imidacloprid, thiamethoxam and flufenoxuron against *Microtermes obesi* (Isoptera: Termitidae). *Pak. Entomol.,* **28:** 45-50.

AHMED, S., MUSTAFA, T., RIAZ, M.A. AND HUSSAIN, A., 2006. Efficacy of insecticides against subterranean termites in sugarcane. *Int. J. Agri. Biol.,* **8**: 508-510

AHMED, S., AKBAR, W. AND RIAZ, M.A., 2004. Effect of crop rotation and intercropping on subterranean termites in wheat at Faisalabad. *Int. J. Agric. Biol.*, **26:** 25-30.

AHMED, S., KHAN R.R. AND. RIAZ M.A., 2007. Some studies on field performance of plant extracts against termites (*Odontotermes guptai* and *Microtermes obesi*) in sugarcane at Faisalabad. *Int. J. Agric. Biol.,* **9:** 398-400.

AKHTAR, M.S. AND SARWAR, G., 1993. *Termites of desert zones of Pakistan*. Final Technical Report, Project No. P-PU/Bio/172, pp. 1-283.

LEE, C. Y., NGEE, P.S. AND LEE, L.C., 2003. Foraging populations and territories of a mound building subterranean termite, *Microtermes pakistanicus* (Isoptera: Macrotermitinae). *Sociobiology,* **41:** 307-316.

LEE, C.Y., YAP, H.H., CHONG, N.L. AND JAAL, Z., 1999. *Urban pest control – a Malaysian perspective*. Universiti Sains Malaysia Press, Penang, Malaysia.

MANZOOR, F., CHAUDHARY, M., SHEIKH, N., KHAN, I.A. AND KHAN, T., 2011. Diversity and proportion of termite species in garden trees and wheat crop in District Bhakkar, Pakistan. *Pakistan J. Zool.,* **43**: 537-541.

MATSUMURA, F., 1985. *Toxicology of insecticides*. Plenum, New York.

OSBRINK, W.A., LAX A.R. AND BRENNER R.J., 2001. Insecticide susceptibility in *Coptotermes formosanus* and *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). *J. econ. Ent.*, **94:** 1217-1228.

PEARCE, M. J., 1997. *Termites: Biology and pest management*. CAB International, London

REMEN, L.N. AND SU, N.Y., 2005. Time trends in mortality for thiamethoxam and fipronil against formosan subterranean termites and eastern subterranean termites (Isoptera: Rhinotermitidae). *J. econ. Ent.,* **98**: 911-915

ROBINSON, W. H., 1996. Integrated pest management in the urban entomology. *Am. Entomol.,* **42:** 76-78.

SHEIKH, N., BANO, A.S. AND AKHTAR, M.S., 2005. Morphometric analysis of population samples of *Microtermes mycophagus* (Desneux) from Pakistan. *Punjab Univ. J. Zool.,* **20**: 169-175.

SORNNUWAT, Y.K., TSUNODA, K., YOSHIMURA, T., TAKAHASHI, M. AND VONGKALUANG, C., 1996. Foraging populations of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in an urban area. *J. econ. Ent.,* **89:** 1485-1490.

SU, N.Y, TAMASHIRO, M. AND HAVERTY, M., 1987. Characterization of slow-acting insecticides for the remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. econ. Ent.,* **80**: 1-4.

SU, N.Y. AND SCHEFFRAHN, R.H., 1990. Comparison of eleven soil termiticides against the Formosan Subterranean termite and eastern subterranean termites (Isoptera: Rhinotermitidae). *J. econ. Ent.,* **83:** 1918-1924.

SU, N.Y. AND SCHEFFRAHN, R.H., 2000. Termites as pests of buildings. In: *Termites: evolution, sociality, symbioses, ecology* (eds. T. Abe, D.E. Bignell and M. Higashi), Kluwer Academic Publishing, Dordrecht, pp. 437-453.

VALLES, S.M. AND WOODSON, W.D., 2002a. Group effects on insecticide toxicity in workers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. *Pest Manag. Sci.,* **58**: 769-774.

VALLES, S.M. AND WOODSON, W.D., 2002b. Insecticide susceptibility and detoxication enzyme activities among *Coptotermes formosanus* Shiraki workers sampled from different locations in New Orleans. *Comp. Biochem. Physiol.,* **131**:469–476.

VALLES, S.M., OI, F.M. AND WAGNER, T., 2000. Toxicity and *in vitro* metabolism of t-permethrin in eastern subterranean termite (Isoptera: Rhinotermitidae). *J. econ. Ent.,* **93**: 1259–1264

VALLES, S.M., OSBRINK, W.L.A., OI, F.M., BRENNER, R.J. AND POWELL, J.E., 1998. Cytochrome P450 monooxygenase activity in the Dark Southern Subterranean Termite (Isoptera: Rhinotermitidae). *J. econ. Ent.,* **91**: 1131–1135.

WANG, X., WEI, J., MO, J., MAO, W. AND YE, T., 2007. The Susceptibility of *Reticulitermes chinensis* (Isoptera: Rhinotermitidae) to sulfluramid, imidacloprid and ivermectin. *Sociobiology,* **50**: 1-12.

(Received 16 February 2013, revised 20 March 2013)