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Estimation of Degradation of Different Termiticides under Field Conditions using TLC Method

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Abstract

The use of soil termiticides has improved nowadays with the recruitment of persistent, slow acting and non-repellent termiticides. In this study, the field efficacy and persistence of three commonly used termiticides, i.e., Imidacloprid, Chlorpyrifos and Chlorfenapyr were analysed using thin layer chromatography (TLC) detection method. Investigations of termiticide degradation were conducted at Jallo Forest Park, Lahore. Solvent extraction and residue methods were used to analyse the termiticide residues in Imidacloprid, Chlorpyrifos and Chlorfenapyr, respectively. Chromatographic analyses were carried out with TLC and retention factor (R_f) values of each termiticide which were calculated and compared with standard R_f values. The results showed that Imidacloprid was a more persistent termiticide even after 1.8 years, while Chlorpyrifos was least stable and converted into primary metabolites (TCP) after the same period.

Keywords: Pesticides, Residues, Chlorfenapyr, Chlorpyrifos, Imidacloprid.

Introduction

Termites are troublesome pests and cause intense damage to agricultural crops and urban infrastructure (Lewis, 1997). For a long time, man has been trying to find a solution in order to get rid of termites. The use of soil termiticides is a common preventive control measure. These treatments typically involve creating termiticides barriers in and around buildings, which may be horizontal and vertical. Concentrated termiticides are diluted in suitable solvents – usually water – and applied to soil surface to create a horizontal barrier and incorporated in to soil trenches to create vertical barriers (Horwood, 2007). In recent years, termiticides such as phenyl pyrazole (Fipronil), pyrole (Chlorfenapyr), neonicotinoid (Thiamethoxam) and chloronicotinyl (Imidacloprid) have been popular insecticides to control subterranean termites. These termiticides are less hazardous than chlorinated hydrocarbons and organophosphates and have a limited life in soil. These compounds are slow acting and non-repellent (Shelton and Grace, 2003). The efficacy of any soil termiticide depends upon its bioavailability and degradation rate. For this purpose, the soil samples from treated areas were collected and analysed by different quantitative techniques, such as HPLC, GCMS and TLC etc., to detect termiticide availability.

Present study was carried out to check the field persistence and efficacy of selected slow acting insecticides, i.e., Imidacloprid (20% A.I. (active ingredient)), Chlorfenapyr (36% A.I.) and Chlorpyrifos S.C. tm (24% A.I.) and to analyse the soil samples for residues to determine the extent of degradation with the passage of time.

Materials and Methods

Investigation of termiticide degradation was conducted at Jallo Forest Park, Lahore, Pakistan. The test plots were selected on the basis of their different soil texture, vegetation and termite pressure. Prior to termiticide application, the plots were checked for termite infestation by placing poplar stakes at an interval of 4-feet in each plot, showing the highest termite activity.

Termiticides and soil treatments

At each test location, 0.5% concentration of all termiticides, i.e., Imidacloprid, Chlorfenapyr and Chlorpyrifos, was applied in accordance with the recommendation of the manufacturer, i.e., 34 ml/17 litres, 20.4 ml/17 litres and 10.2 ml/17 litres for Imidacloprid, Chlorpyrifos and Chlorfenapyr, respectively. For persistence test of

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termiticides, the procedure was the same as that adopted by Gold et al. (1998). Square plots at the selected site were cleared from debris and each concentration of termiticide was replicated three times. At the selected sites, 3 holes (24cm in diameter and 30.5cm in depth) were drilled. The diluted samples were mixed thoroughly with the soil. The treated soil was placed in to predrilled holes and soil was levelled to surrounding ground. For the monitoring of termite infestation and treatment efficacy, a 25cm long poplar stake was inserted into the hole and covered with a precast concrete block (5cm x 30.5cm x 30.5cm). Controls were also included and handled similar to the treated soils.

The study was carried out for about 18 months to check the persistence of each termiticide. During the study, Lahore had warm and humid climatic conditions with an average summer temperature of 30.6 °C and an average winter temperature of 24.14 °C. The relative humidity was 45.67%. The soil type at the site was Prentiss silt loam (38% sand, 58% silt, 4% clay) with an average pH of 7.32. Samples were analysed soon after the application and then again after 18 months.

Soil Sampling

Soil samples were collected from the plot of each termiticide immediately after treatment (0 month) and again after 18 months. Samples were collected to a depth of 10-20cm, using a steel tube from each of three depths (Top, Middle and Bottom) and sealed properly in labelled plastic bags.

Sample analysis

Prior to extraction, all soil cores were sieved by passing them through a 2mm mesh. To analyse the Imidacloprid residues, the solvent extraction method of (Baskaran et al. 1999), was adopted with some modifications. 25g of treated soil and 8ml Acetonitrile:Water (80:20 by volume) was taken in a conical flask which homogenised the sample on a horizontal shaker for 4 hrs. (37°C, 8000 rev/min). The supernatant was collected by centrifugation (6000rpm). The extract was cleaned by passing through anhydrous sodium sulphate packed in Pasteur pipettes stopped with glass wool. The solvent was evaporated by placing extract in a rotary evaporator at 45°C for 15 minutes. Similarly, analysed Chlorfenapyr and Chlorpyrifos residues method of Cao et al. (2005) was used with some modifications. 25g of soil and 100ml of Acetone:Water (70:30 by volume) was taken in a conical flask which homogenised the sample on a horizontal shaker (37 °C, 8000 rev/min) for 1hr. The extract was filtered by first passing it through Whatman 1No.1 filter paper and then evaporating the solvent by placing in a rotary evaporator. Extracts were cleaned by transferring once to a separator funnel containing 4% NaCl (50ml) and thrice with dichloromethane (25, 15 and 5ml), respectively, diluted with methanol to make the volume with 50 ml and vol. of 5 ml(?). Each organic phase was separated and transferred to a beaker containing 10ml *n*-hexane, 1g activated charcoal and 1g of anhydrous sodium sulphate. The slurry was allowed to settle. The clear slurry was slowly passed through the glass column packed with silica gel. The charcoal was also washed 3 times with *n*-hexane and passed through the column as well. The extracts were combined and again evaporated using rotary evaporator until dryness and dissolved in 5ml methanol. The extracts were ready and analysed using thin layer chromatography (TLC).

TLC Method

For Imidacloprid residues, TLC method of Oi (1999) was used. Similarly, for Chlorpyrifos, TLC method of Kale et al., 2002, was used while no TLC procedure for Chlorfenapyr has been reported in the literature and the method developed has been obtained by hit and trial method.

For the detection of termiticide residues, Pre-coated TLC plates (silica gel 60 F_{254}, 0.25 mm layer thickness, 20 cm × 20 cm, Merck Ltd., Germany) were used. Acetonitrile:Ethylacetate:Water (70:23:7 by volume) mixture was used as solvent system for Imidacloprid residues, Methanol for Chlorfenapyr while Toulene:Methanol:Hexane (90:5:5 by volume) was used for Chlorpyrifos residues. The samples were spotted 1cm apart with a volume of 5µl on TLC plates with fine capillary tubes along with the same volume of each pure termiticide as standard. The plates were dried and the chromatogram was developed in a pre-saturated tank containing the solvent system as mentioned above. After developing the plates, the solvent front (distance travelled by the solvent) was

immediately marked and the extra solvent was evaporated (dried) in fume hood. The plates were then exposed to UV at 254nm for 16-20 minutes. A brown spot with yellow background was clearly visible. The spots were marked. The distance travelled by solvent and eluted compounds was noted and R_f values of each compound was calculated and compared with the standard.

Results and Discussion

The results of present study regarding residues of selected termiticide are shown in Table 1 and in Chromatograms 1-6 of Fig. 1. The calculated R_f values of all the tested samples obtained from the TLC plates are summarised in Table 1. The R_f values of all the tested samples are in close agreement with those reported earlier. The conditions of TLC have to be controlled in order to get the required efficiency (Ambrus et al., 1981). For this purpose, standards run along with samples. In chromatogram No. 1, the standard Imidacloprid in lane No.1 run along with samples. The R_f value obtained for Imidaloprid was 0.84, which was near to the reported value by Oi (1999) and standard Chlorpyrifosin lane No. 1 of chromatograms 3 and 4 while Chlorfenapyr in lane No. 1 of chromatograms 5 and 6 was spotted along with the samples. The R_f values of the sample spots were compared with the standard R_f value obtained. The intensity of the spots appeared were correlated with the concentrations used.

Table 1. Comparison of R_f values of Imidacloprid, Chlorpyrifos and Chlorfenapyr (obtained and reported).

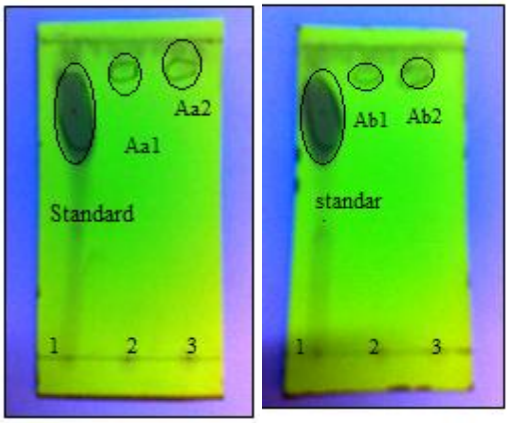
Chemicals	Standard R_f value	R_f value obtained After 0 month treatments			Mean	%CV	R_f value reported After 1.8 months			Reported R_f values
	Lane 1	Lane 2	Lane 3	Lane 4			Lane 2	Lane 3	Lane 4	
Imidacloprid	0.84	0.89	0.88	0.88	0.88	0.65	0.88	0.88	0.88	0.84 by Oi (1999)
Chlorpyrifos	0.76	0.73	0.70	0.70	0.70	0.79	0.28	0.29	0.28	0.76 (Chlorpyrifos) and 0.28 (TCP) by Kale et al.,2002
Chlorfenapyr	0.76	0.77	0.76	0.76	0.76	0.75	0.69	0.67	0.65	Not reported

In chromatogram 1, the spotted samples were taken from plot Nos. 1 and 2 of the experimental site. Spots of A_{a1} and A_{a2} in lane Nos. 2 and 3 showed 0.88 average R_f which was near to the standard Imidacloprid spotted in lane No. 1 with the R_f value of 0.84. In chromatogram 2, the spotted samples were taken from the experimental site after 1.8 years. Spot A_{b1} in lane No. 2 showed the average R_f value of 0.88, spot A_{b2} in lane No. 3 showed the R_f value of 0.85. The average R_f values reported after 18 months was 0.88. The R_f values of all the spots obtained were near to the standard Imidacloprid spotted in lane No. 1 with the R_f value of 0.84.

In chromatogram 3, the spotted samples were taken from the experimental sites soon after analysis. Spot B_{a1} in lane No. 2 showed the R_f value of 0.76, spot B_{a2} in lane no. 3 showed the R_f value of 0.77. The R_f values of all the spots obtained were near to the standard Chlorpyrifos spotted in lane No. 1 with the R_f value of 0.76 as reported by Kale et al., 2002. In chromatogram 4, the Chlorpyrifos samples were analysed, after being taken from the experimental sites after 1.8 years. The average R_f value of spots B_{b1}, B_{b2} and B_{b3} in lane Nos. 2, 3 and 4 was 0.28. The R_f values of all the spots obtained were different from the standard Chlorpyrifos spotted in lane No. 1 with the R_f value of 0.76 but it was close to the TCP major chlorpyrifos metabolite with R_f value of 0.31 as reported by Kale et al., 2002.

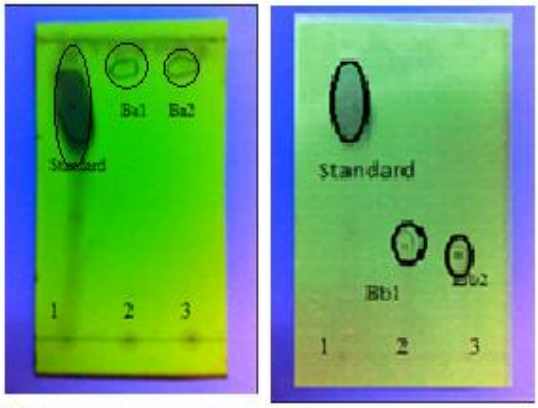
In chromatogram 5, the spotted samples were taken from the experimental sites soon after analysis. Spot C_{a1} in lane No. 2 showed the R_f value of Chlorfenapyr 0.77, spot C_{a2} in lane No. 3 showed the R_f value of 0.76 and spot C_{a3} in lane No.4 showed the R_f value of 0.76. The R_f values of all the spots obtained were near to the standard Chlorfenapyr spotted in lane No. 1 with the R_f value of 0.76. Chromatogram 6 showed the R_f values of Chlorfenapyr after 18 months analysis. The average R_f value of spots C_{b1}, C_{b2} and C_{b3} in lane Nos. 2, 3 and 4, was 0.67. From the results, it was revealed thatR_f values for Chlorfenapyr were little different from standard. Thus, it can be concluded that Chlorfenapyr degraded to some extent but not the same as Chlorpyrifos. Same trend was true for other plots and replicates for all termiticides.

From the R_f values, it is reported that Imidacloprid was very stable and Chlorfenapyr was stable to some extent, while Chlorpyrifos displayed least persistence. After 18 months, primary metabolites of Chlorpyrifos, i.e., 3, 5, 6-trichloro-2-pyridinol (TCP) with R_f value 0.28 were detected. However, soil treated with Imidacloprid gives same R_f values as that of standard even at the end of the study. Results indicated that Imidacloprid and Chlorfenapyr persist even after 1.8 years. Different environmental factors may be involved in the break down process. Temperature is the most important factor. During the study, temperature was extremely hot. The results of Chlorpyrifos correlated with the results of Baskaran et al. (2003). They found that Chlopyrifos degraded more rapidly with increasing depth. Chlorpyrifos losses were attributed to an associated increase in soil pH found deeper in the soil profile. Similarly, in the present study, alkaline soil was used, which played a vibrant role in Chlorpyrifos degradation. Published estimate of chemical half-lives also support the present results as it was reported that approximate half-lives of Chlorpyrifos and Chlofenapyr was 315 and 462 days, respectively, while the half-lives (life?) for Imidacloprid half-lives were 990-1230 days (Racke et al., 1994: Baskaran et al., 1999 and Murry et al., 2001). These findings have important implications for the regulation of termiticide applications.



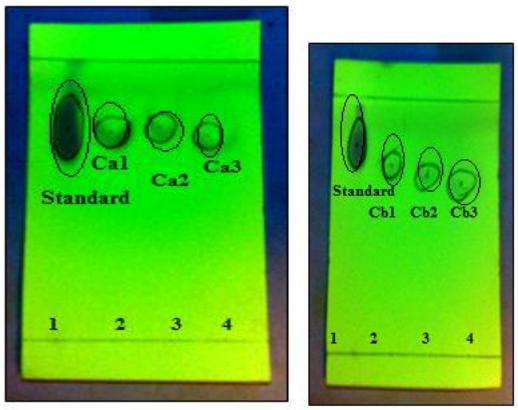
Chromatogram 1 Chromatogram 2

Chromatograms 1 and 2 show Imidacloprid R_f values at 0 and 1.8 months, respectively.



Chromatogram 3 **Chromatogram 4**

Chromatograms 1 and 2 show Chlorpyrifos R_f values at 0 and 1.8 months, respectively.



Chromatogram 5 **Chromatogram 6**

Chromatograms 1 and 2 show Chlorfenapyr R_f values at 0 and 1.8 months, respectively.

Fig. 1: Showed comparison of R_f values of Imidacloprid, Chlorpyrifos and Chlorfenapyr with standard at 0 and 18 months interval.

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