

Leaching of Termiticides Containing Bifenthrin, Fipronil and Imidacloprid in Different Types of Soils under Laboratory Conditions

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ABSTRACT

Termiticides need to persist in the soil to give continuous protection to the building structures or plantations. Leaching contributes to massive contamination that can further lead to underground water contamination. The leaching activity of three different termiticides (bifenthrin, fipronil and imidacloprid) in different types of soils (sandy loam and loamy sand) under laboratory conditions was evaluated. Leaching activity of the termiticides using a soil column method revealed that bifenthrin had a good adsorption characteristic as its concentration at the top of the column was higher (Sandy loam = 892.77mg/L; Loamy sand= 1060.93 mg/L) compared to fipronil and imidacloprid. There was no significant difference between soil types ($p= 0.131$) but there was a significant difference between termiticides used ($p= 0.00$). The concentration of bifenthrin was higher in the treated area (0-5 cm – top layer) due to the higher Koc value and lower water solubility compared to imidacloprid and fipronil. Thus, bifenthrin is recommended during a rainy season for soil treatment.

Keywords: Termiticides, leaching, imidacloprid, fipronil, bifenthrin.

INTRODUCTION

Subterranean termite infestation can cause great damage to a wide variety of human structures such as houses and wood-based furniture. Moreover, the infestation of this insect can also adversely affect agricultural sectors such as oil palm and rubber tree plantations. The infestation is practically controlled by creating a chemical barrier around the building structures (Horwood 2007; Kamble and Saran 2005; Spomer and Kamble 2010). Nevertheless, excessive application of termiticide to control subterranean termite can lead to environmental contamination. Termiticide contamination occurs in several ways: precipitation, leaching and dust (Arias-Estévez *et al.* 2008; Delcour *et al.* 2015; Gustafson 1989; Huseth and Groves 2014). Among these, leaching appears to contribute the most to massive environmental contamination that further leads to underground water

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contamination (Bajeer 2012). In general, termiticides need to be persistent in the soil to give continuous protection to human structures or plantations.

The environmental fate of termiticide is mainly controlled by its behaviour in the soil, where several physio-chemical and biological processes control the movement and dissipation in other environmental components such as air, water and biota (Tang *et al.* 2012). It is crucial to recognise the fate of termiticide in the field to prevent a maximum effect on non-target insects as well as to avoid any environmental contamination. Uncontrolled termiticide treatment may also lead to other problems. For example, leaching of termiticide is toxic to non-target organisms, causing indirect accumulation, which simultaneously affects the human population through food chains. Polluted soil, surface and ground water are harmful to the environment and human health (Bajeer 2012).

Many countries in Southeast Asia rely heavily on the use of soil termiticide against subterranean termites in the urban environment including Malaysia (Lee 2002). Imidacloprid (chloronicotinyl class), bifenthrin (pyrethroid class) and fipronil (phenyl pyrazole class) with a novel mode of action are among the most commonly used termiticides for controlling subterranean termites. Imidacloprid has a higher water solubility (510 mg/L at 20 °C) (Fernández-Bayo *et al.* 2007; Peterson 2007) compared to fipronil (1.9 to 2.4 mg/L at 20 °C) (Husen *et al.* 2009) and bifenthrin (0.1 mg/L at 20 °C) (Fecko 1999). Furthermore, bifenthrin has a greater K_{oc} value (1.31-3.02 x 10⁵) (Fecko 1999) followed by fipronil (803) (Connelly 2001) and imidacloprid (132-310) (Fossen 2006).

Soil texture has an important impact on termiticide performance, but the effects differ within termiticides. According to Wiltz (2010), the clay content in soils is significantly related to termite mortality across all termiticides, application rates, and exposure times. In assays conducted with bifenthrin, fipronil and chlorfenapyr, *C. formosanus* mortality was the highest when clay content was low (Wiltz 2010). According to Osbrink and Lax (2002), the mortality of *C. formosanus* workers was higher in the fipronil-treated sand than in treated mixture of soil and clay. Sorption is greatly influenced by the amount of clay and organic matter. Fipronil indicates a significant decrease in adsorption coefficient as the soil clay content decreases, thereby, increasing its bioavailability (Bobé *et al.* 1997; Cox *et al.* 1998; 2001).

Based on a previous study, the most economic important termite pest *Coptotermes* sp. was found in two types of soil- sandy loam and loamy sand (Ab Majid & Ahmad 2013a). These two types of soil are also commonly treated with imidacloprid, fipronil and bifenthrin base termiticides known as soil corrective treatment to prevent termite infestation of the building structure (Ab Majid & Ahmad 2013a; Ab Majid & Ahmad 2013b; Ab Majid & Hassan 2008; Ab Majid & Ahmad 2011). Therefore, the objective of this study was to evaluate the leaching activity of three different termiticides (bifenthrin, fipronil and imidacloprid) in two types of soils (sandy loam and loamy sand) under laboratory conditions by evaluating the termiticides residues left in different parts (top, middle, bottom) of the soil column and the concentration in the leachates.

MATERIALS AND METHODS

Soil Samples

Soil samples were collected from two sites i.e. Durian valley, Universiti Sains Malaysia (USM) (5°21.35'N; 100°18.16'E) for sandy loam and Teluk Bahang, Penang (5°26.47'N; 100°13.04'E) for loamy sand. The soils were taken approximately 10 cm from the top layer (A-horizon). The remaining debris such as stones, vegetation and macro faunas were removed. The soils were air-dried at room temperature (20-25 °C), sieved through a 2-mm sieve, air dried and stored at ambient temperature. The soils were subsequently analysed for particle size, pH and organic matter content. Soil pH was determined using a pH meter (HANNA HI 8424, Romania). The soils were mixed with distilled water at a ratio of 1:2 and left overnight to obtain the pH value.

Soil Texture

Soil texture was analysed following the method of Bouyoucus (1962). Soil equivalent to 50 g was mixed with 100 mL of 6% hydrogen peroxide (H₂O₂) in a 500 mL beaker. The mixture was left overnight at room temperature. Then, the beaker was placed on a hot plate at 90 °C for 10 min. Then, 50 mL 1M NaOH were added into the beaker, followed by the addition of distilled water to achieve a final volume of 400mL. The mixture was left to rest for 20 min.

The mixture was then stirred for 10 min, transferred into a 1 L measuring cylinder to which was added distilled water to achieve a final volume of 1 L. The mixture was allowed to rest to achieve a thermal equilibrate and the temperature was recorded. The measuring cylinder was inverted several times to ensure the contents were thoroughly mixed. Then, a hydrometer was immediately lowered into the suspension and readings were recorded after 40 sec. After 2 h, the hydrometer was placed into the measuring cylinder. The buoyancy of soil particles and temperature were then recorded using hydrometer and thermometer respectively. The percentage of sand, silt and clay was obtained from readings collected from the hydrometer and thermometer. Soil texture was determined by using USDA textural triangle based on the proportion of sand, silt and clay. The interception percentage of sand, silt and clay indicates the soil texture.

Organic Matter Content

Organic matter content was obtained using a loss on ignition technique (Ball 1964; Pallasser *et al.* 2013). Three replicates of 1st soil and an empty can were weighed and recorded, respectively. Each can was placed into a muffle furnace (Lab-Heat BLUEM) for 16 h at a temperature of 440 °C for the ignition process and cooled overnight. The cans with ash were weighed and recorded. The calculation for organic matter content is shown below:

$$\%TOM = \frac{(mass\ of\ soil) - (mass\ of\ ash)}{mass\ of\ soil} \times 100$$

Termiticides

Three soil treatments were carried out using termiticides containing different active ingredients: (1) 20% Imidacloprid, (Prothor); (2) 5% Fipronil, (Chalcid 5.0); and (3) 10% Bifenthrin (Maxxthor). Formulated products of Prothor 200 SC (Ensystem (Malaysia) Sdn. Bhd., Kuala Lumpur), Maxxthor 100 SC (Ensystem (Malaysia) Sdn. Bhd., Kuala Lumpur) and Chalcid 5.0 SC (Hextar Chemicals Sdn. Bhd., Selangor, Malaysia) were purchased from a local distributor. Table 1 shows the sorption parameters for the termiticides used in this study.

TABLE 1
Soil sorption parameters of termiticides tested

Termiticide	Koc(L/kg)	Log Kow	H ₂ O Solubility (mg/L)	Henry's law constant (atmm ³ /mol)	Reference
Bifenthrin	1.31 x 10 ⁵ - 3.02 x 10 ⁵	6.0	0.1 (25°C)	7.2 x 10 ⁻³	Fecko (1999)
Fipronil	3.8 x 10 ³ - 1.2 x 10 ⁴	4.01	2.4 (pH 5) 2.2 (pH 9)	3.7 x 10 ⁻⁵	Connolly (2001)
Imidacloprid	1.3 x 10 ² - 3.1 x 10 ²	0.57	514 (20°C, pH 7)	6.5 x 10 ⁻¹¹	Fossen (2006)

Preparation of Columns

Soil column apparatus was prepared as shown in Figure 1. Untreated soils were air-dried and sieved. Then, the soils were packed in the columns up to the height of approximately 20 cm. The soils were added by using a spoon to create an optimum packing. The total weight of the soils packed in the columns was determined and the weight of all duplicate columns was set at 500 g. Then, the soil columns were pre-wetted with artificial rain from bottom to top to remove air pockets within the soils. Five mL (1000 mg/L) of each termiticide was poured on the top of the soil columns. The top surface of the column was covered with a filter paper, to allow the soils to evenly distribute when artificial rain was poured into the columns. Then, artificial rainfall was poured into the soil columns at 100 mL/L with the aid of a peristaltic pump (Bajeer 2012). After 5 h, the soils were left to drain, the column was divided into three sections (top= 0-5 cm, middle= 10-15 cm and bottom= 25-30 cm) for further analysis (Figure 2).

Termiticide Extraction from Soils

The collected samples were placed in a black plastic and immediately stored in a freezer (below -14 °C) prior to analysis to prevent termiticide degradation. Then, the soil samples were removed from the freezer and air-dried for 24 h. Ten grams of the soil samples for each replicate was weighed into a 200 mL conical flask. About 40 mL of acetonitrile (ACN) (HPLC grade) were then added to each soil sample. The flasks were covered with aluminium foil and were agitated overnight at 200 rpm using a shaker at 20 °C.

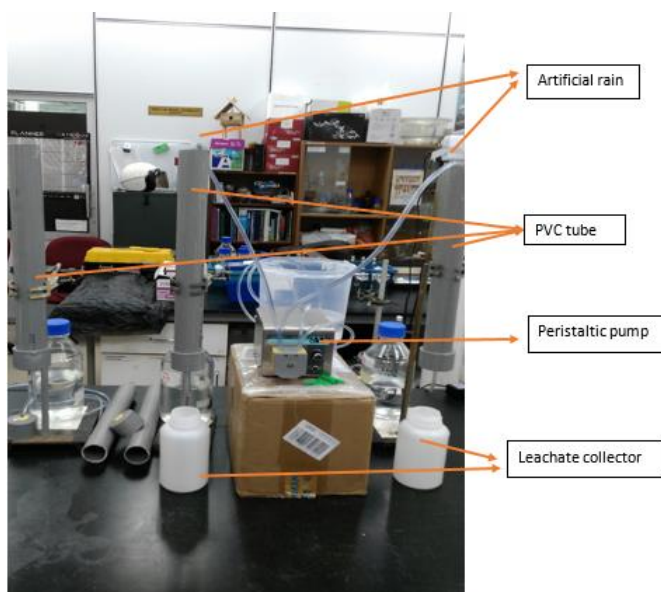


Figure 1: Setup of apparatus for the leaching soil column experiment

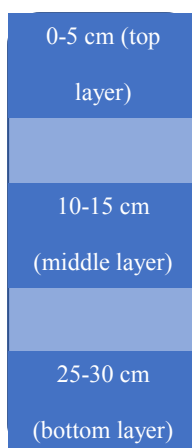


Figure 2: Schematic diagram of the soil column showing different layers.

The samples were left to stand for 1 h to allow soil particles to settle. A total of 1.5 mL of clear supernatant was filtered through 0.22 μm Choice Syringe Filters with nylon Membrane (Thermo Scientific, China) into a 2.0 mL microcentrifuge tube. Aliquots were centrifuged (Eppendorf Centrifuge 5424) at 12 000 rpm $\times$ g for 20 min. One mL of supernatants was transferred into 2 mL auto-injector vial and was sealed with a PTFE lined screw cap after passing through a 0.22 μm Choice Syringe Filters with nylon membrane (Thermo Scientific, China). Samples were analysed after the extraction procedure.

Termiticide Extraction from Leachate

A liquid-liquid extraction was done according to the modified version from EPA method 3350 (USEPA, 2007)(not in ref list). The collected leachate was stored in -14°C to prevent degradation. About 10 mL of the leachate was mixed with 10 mL of acetonitrile in a universal bottle. Then, the mixture was vortexed on Grant-bio (Type: PV-1) for 10 sec. The mixture in the universal bottle was allowed to settle down for 30 min following which 10 mL of supernatant was obtained. The supernatant was transferred to a new universal bottle to which was added another 10 mL of ACN. The new mixture was sonicated with an ultrasonic cleaner (Model: CE-7200A). One mL of the new supernatant was filtered through 0.22 µm Choice Syringe Filters with nylon Membrane (Thermo Scientific, China) and transferred into a 1.5mL vial for UPLC analysis.

Residual Analysis

Technical grade of bifenthrin (98.8%), fipronil (97.9%) (Sigma-Aldrich, Malaysia) and imidacloprid (99.5%) (Chem Service) were used as standards. Acetonitrile (Fisher Chemical, Malaysia) was used as a universal solvent to dissolve the termiticides. Extracted termiticides were separately analysed using an ACQUITY UPLC systems (Waters) coupled with PDA detector. A BEH C18 stainless steel column, (2.1 mm x 100 mm) of 1.7 µm particle size was used. The injection volume was set at 5.0 uL and the flow rate at 1.0mL/min.

A mobile phase, UV wavelength and retention times for all termiticides are summarised in Table 2 (Baskaran *et al.* 1999; Saran and Kamble 2008).

Data analysis

The termiticide residue was measured following a standard formula by CEPA, (1993)(not in ref list) and Ong et al. (2016):

$$R = \frac{(Spl\ H)(Std\ C)(Std\ V)(V)}{(Std\ H)(Spl\ V)(Spl\ W)},$$

TABLE 2
UPLC setting for termiticides used in this study

Termiticide	Mobile phase (Water: CAN)	Wavelength (nm)	Retention time (minutes)
Bifenthrin	60:40	204	1.082
Fipronil	20:80	280	1.756
Imidacloprid	30:70	270	1.190

where R is termiticide residue of part per million (mg/L), $Spl\ H$ is the sample peak height area, $Std\ C$ is the standard concentration in mg/L, $Std\ V$ is the standard volume injected in μl , V is the volume after the injection in mL, $Std\ H$ is the standard peak height area, $Spl\ V$ is the sample volume injected in μl , and $Spl\ W$ is the sample weight in g.

Atwo-way ANOVA (SPSS version 21) was conducted to compare significance of differences between the factors examined in this study. Data normality was evaluated using IBM SPSS Statistics version 21. Data transformation was applied as the results were abnormal. Analysis of variance (ANOVA) was performed to determine the significance of differences between types of soils, termiticides and different parts/layers (top, middle and bottom).

RESULTS

Table 3 summarises the characteristics of two soil samples used in this study. The tested soil was sandy loam, containing 80% of sand, 16.4% clay and 3.6% silt, while, the other soil was loamy sand, containing a slightly higher percentage of sand (84.6%) with lower clay (11%) and silt (4.4%) than sandy loam. Sandy loam was more acidic (pH 4.4) compared to loamy sand (pH 4.8). The organic matter content for sandy loam and loamy sand were 11.4% and 8.5%, respectively.

The concentration of each termiticide in the soil column is shown in Figure 3. Bifenthrin noted the highest residual termiticide in the top layer of the soil column (sandy loam=892.77mg/L; loamy sand = 1060.93 mg/L). Meanwhile, fipronil presented a slightly lower level than bifenthrin with a concentrations of 585.67 mg/L in sandy loam and 66.59 mg/L in loamy sand. Imidacloprid, on the other hand, showed the lowest level of termiticide residual (sandy loam= 16.37 mg/L; loamy sand= 0.97 mg/L) compared to bifenthrin and fipronil.

All factors significantly affected the termiticide concentrations, except

TABLE 3
Characteristics of soil samples used in this study

Characteristics	Loamy sand	Sandy loam
pH	4.4±0.05	4.8±0.04
Organic matter content (%)	11.4±0.14	8.5±0.45
Sand (%)	80.0	84.6
Clay (%)	16.4	11.0
Silt (%)	3.6	4.4

for soil types ($p= 0.131$) (Table 4). Termiticides used had a significant effect on termiticide concentration ($p= 0.001$). Bifenthrin significantly showed a higher concentration in the soil column and leachate compared to fipronil and imidacloprid. The parts of soil column also showed a significant difference ($p= 0.001$), where termiticide concentrations were significantly higher at the top than at the middle and bottom parts of the soil columns. The interaction of termiticide, soil and parts affected the termiticide concentration ($p=0.001$). The termiticide concentration reported higher levels at the upper part of soil in the loamy sand soil treated with bifenthrin compared to fipronil and imidacloprid. (Please note that in Table 4 sig values are not given in bold though you say they are given in bold in your notes at the bottom of the table)

Figure 4 shows the leaching patterns of bifenthrin in this study. Based on 2-way ANOVA, no significant difference ($F= 0.674$, $df= 1$, $P= 0.424$) was observed between sandy loam and loamy sand. Bifenthrin concentrations remained high in the top of the column for both soil types, while the concentrations degraded at the middle and bottom parts of the columns at an average of 97.07 mg/L. The concentrations of leachate, however, remained high in both soils (SL= 756.53; LS= 517.49 mg/L).

TABLE 4
Effects of termiticide, soil type and part of the soil columns on termiticide concentration

Variable	df	Mean Square	F-value	P-value
Termiticides	2	1813.222	265.94	0
Soils	1	16.055	2.355	0.131
Parts	3	672.424	98.622	0
Termiticide * soil	2	45.602	6.688	0.003
Termiticide * Parts	6	239.171	35.079	0
Soil * Parts	3	52.971	7.769	0
Termiticide * soil * Parts	6	39.056	5.728	0

Notes: df = degree of freedom; significant values are given in bold

Fipronil also showed the highest concentration at the top of the soil column in both soils (SL= 585.67 mg/L; LS= 66.59 mg/L) compared to the middle and low parts as well as in the leachate. This finding indicates that most of the fipronil remained in the termiticide-treated area (Figure 5).

A similar pattern was also shown by imidacloprid, except that the top of the soil column of sandy loam showed a higher concentration (16.37 mg/L) compared to loamy sand (0.97 mg/L). Both soils lost almost 98.36% to 99.90% at the top of the soil columns. Imidacloprid indicated a low concentration (1.55 mg/L) at the middle, bottom and leachate of the soil column in both soils (Figure 6).

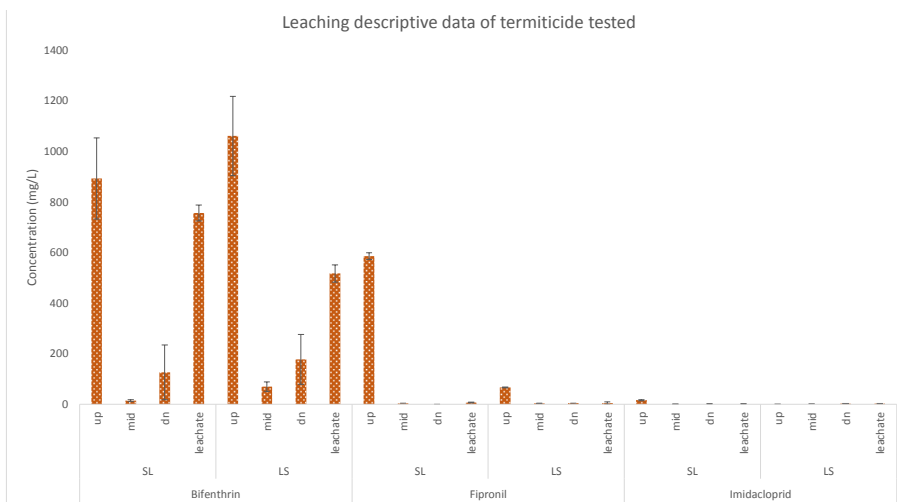


Figure 3: The concentrations of bifenthrin, fipronil and imidacloprid in soil columns (SL= Sandy loam; LS= Loamy sand)

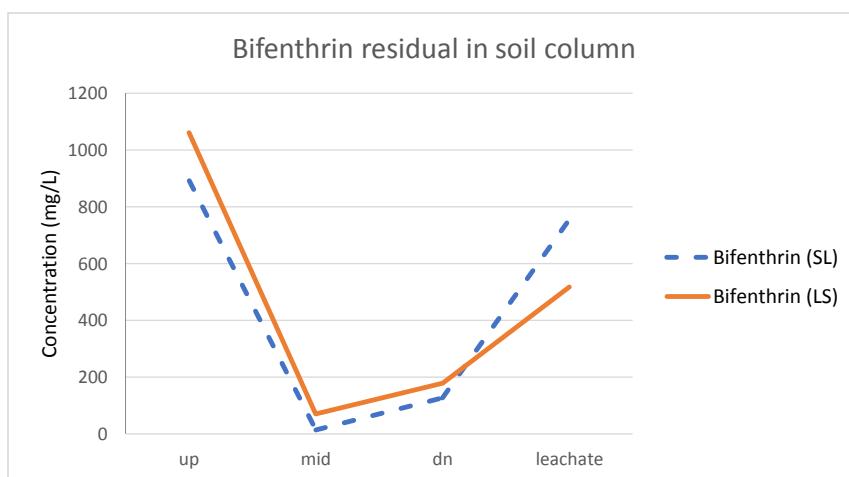


Figure 4: Leaching patterns of bifenthrin in sandy loam (SL) and loamy sand (LS) in upper layer (up), middle layer (mid), down layer (dn) and leachate

DISCUSSION

Bifenthrin showed a higher concentration in the top part of the soil column compared to fipronil and imidacloprid, indicating that bifenthrin is the most persistent among termiticides tested. A similar finding was reported by Horwood (2007) who found that bifenthrin was higher than chlorfenapyr, chlorpyrifos, fipronil and imidacloprid. According to Kamble and Saran (2005), as bifenthrin

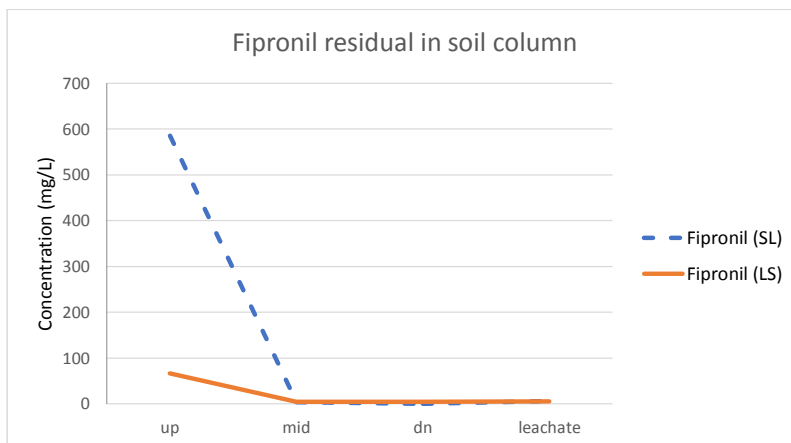


Figure 5: Leaching patterns of fipronil in sandy loam (SL) and loamy sand (LS) soil in upper layer (up), middle layer (mid), down layer (dn) and leachate

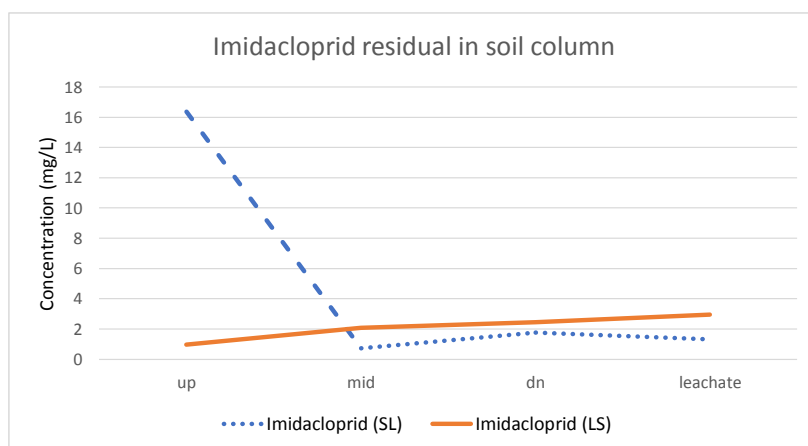


Figure 6: Leaching patterns of imidacloprid in sandy loam (SL) and loamy sand (LS) soil in upper layer (up), middle layer (mid), down layer (dn) and leachate

has a higher Koc value, it has a high capability to remain in the soil particles, resulting in low leaching potential. A study by Meyer *et al.* (2013) also found that bifenthrin bonded to soils and sediment due to the strong binding capability towards soil particles.

As artificial rain was applied on the top of the column, termiticide with low water solubility probably remained on the treated site. Bifenthrin has the lowest water solubility (0.1mg/L) (Kamble and Saran 2005) compared to fipronil (1.9 to 2.4 mg/waL) (Zhu *et al.* 2004) and imidacloprid (510 mg/L) (Ping *et al.* 2010) and is also hydrophobic (Lee *et al.* 2002; Liu *et al.* 2004). Due to its hydrophobicity, bifenthrin repelled the water molecules from the artificial rain used in this study,

causing it to remain at the top of the column. The efficacy of termiticides is also influenced by volatilisation capability (Chai *et al.* 2013; Delcour *et al.* 2015; Husen *et al.* 2009; Joshi *et al.* 2016; Richman *et al.* 2006). In field treatment, the actual concentration of termiticides fails to reach the target species if the termiticides rapidly volatilise after the application on the soil. In this study, the top column was uncovered, thus, bifenthrin might volatilise during the treatment period. The concentration of bifenthrin remained high in the top part of the treated soils in the column, which due to low ability, has the potential to volatilise in the environment (Fecko 1999; Gan *et al.* 2005)

Interestingly, the concentration of fipronil in the top part of the column containing sandy loam was higher than in the top part of the column containing loamy sand, while, the middle part, bottom part and leachate showed no difference in its concentrations. Generally, fipronil has a moderate Koc value, ranging from 396 to 825 L kg⁻¹ (Spomer and Kamble 2010; Ying and Kookana 2001). In this study, fipronil had good capability to hold onto soil particles after the artificial rain was applied into the soil column. The adsorption of fipronil is also influenced by the soil organic matter (Shuai *et al.* 2012). Previous studies have indicated that pesticide sorption increases when soil organic matter (SOM) increases (Bekb and Yenig 1999; Gonzalez and Ukrainczyk 1996; Mallawatantri and Mulla 1992). Sandy loam showed a higher percentage of organic matter than loamy sand with 11.4% and 8.5%, respectively. Thus, fipronil was able to retain its higher concentration in sandy loam compared to loamy sand.

Moreover, fipronil has a moderate water solubility, ranging from 1.9 to 2.4 mg/L compared to bifenthrin and imidacloprid (Kamble and Saran 2005; Zhu *et al.* 2004). Thus, fipronil might moderately leach out from the treated soil. A study by Shuai *et al.* (2012) indicated that fipronil was desorbed from the treated soil through a leaching process or runoff due to artificial rain simulation. Another study indicated that fipronil lost 96% of its concentration during the experiment (Horwood 2007).

Imidacloprid showed the highest dissipation process either by leaching or degradation. Imidacloprid has the lowest Koc value compared to other termiticides tested in this study. The Koc values of imidacloprid are in the range of 156 to 960, depending on multiple factors such as soil types, water application and concentration (Cox *et al.* 1998; Kamble and Saran 2005; Leiva *et al.* 2015; Oliver *et al.* 2005). Low Koc values indicate low sorption between imidacloprid and soil surface, which further suggests high leaching potential (Fernández-Bayo *et al.* 2007). The above statement clearly explains the small amount of imidacloprid retained in the treated soils. Imidacloprid applied on a well-drained sandy soil region was reported to be easily accessible in the groundwater (Huseth and Groves 2014). In addition, a survey conducted from 1999 to 2005 by the U.S. Geological Survey (USGS) reported that 13% of imidacloprid was detected in groundwater due to the leaching process, in an area that was dominated by sandy and excessively drained soil (Leiva *et al.* 2015).

Furthermore, imidacloprid has higher water solubility compared to bifenthrin and fipronil (Kamble and Saran 2005; Ping *et al.* 2010; Kurwadkar *et al.* 2014). Therefore, considering its high-water solubility and a weak sorption at the soil surface, the leaching of imidacloprid from the treated soils used in this study was easier. The massive loss of imidacloprid might also be affected by soil properties such as clay content, organic matter and characteristics of pesticides such as chemical characteristics, water solubility, charge distribution on pesticide molecules and molecular charge (Das *et al.* 2015; Murphy *et al.* 1992). Clay is important to avoid the occurrence of leaching, as it provides a large surface area. In this study, the clay content for sandy loam was higher (16.4%) than loamy sand (11%). Thus, the concentration of imidacloprid at the top of the column containing sandy loam was higher than the top column containing loamy sand.

Organic matter is an important element attributed to absorption and leaching process (Bajeer 2012). Liu *et al.* (2006) indicated that organic matter was the primary sorptive medium for imidacloprid. This statement was also supported by Kurwadkar *et al.* (2014) who mentioned that low sorption of imidacloprid could be affected by the presence of low organic matter content. Soil organic matter is closely related to cation exchange capacity (Bajeer 2012; Kurwadkar *et al.* 2014). High organic matter content leads to high exchange capacity. Therefore, the higher amount of cation exchange capacity and organic matter in soil could increase the adsorption of termiticide, and simultaneously reduce the leaching probability.

CONCLUSION

Bifenthrin had a higher concentration in the treated area (0-5 cm; top layer) than fipronil and imidacloprid due to its higher K_{oc} value and lower water solubility compared to imidacloprid and fipronil. There was no significant difference for soil tested (sandy loam and loamy sand). Thus, soil type used in this study did not affect the leaching process in the soil column.

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