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Residual Effects of Termiticides on Mortality of Formosan Subterranean Termites, Coptotermes Formosanus Shiraki, in Substrates Subjected to Flooding

Rajani Sapkota
Louisiana State University and Agricultural and Mechanical College

RESIDUAL EFFECTS OF TERMITICIDES ON MORTALITY OF FORMOSAN SUBTERRANEAN TERMITES, *COPTOTERMES FORMOSANUS* SHIRAKI, IN SUBSTRATES SUBJECTED TO FLOODING

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ABSTRACT

Formosan subterranean termites (*Coptotermes formosanus* Shiraki) are one of the most important economic insects in the Southern US since they cause severe and costly damage to properties. Soil treatment with termiticides is the most widely used and reliable method for termite prevention, but their efficacy after a home floods, an all too common event for a homeowner in recent years, has prompted many pest control companies to suggest a new treatment is necessary. This can be expensive and possibly unnecessary. However, several factors such as termiticide water solubility and binding properties, soil type and soil leachability and the inherent toxicity of different termiticides, even at low concentrations, must be considered. We set out to address this little studied issue in a controlled laboratory setting. Colonies of *C. formosanus* were collected from Brechtel Memorial Park in New Orleans using a termite milk crate trap method. The termiticides chosen for study are among the most commonly used today and included: fipronil, imidacloprid, chlorantraniliprole and bifenthrin. Evaluation of 1, 10 and 25 ppm of each termiticides in sand and soil (Wt/Wt basis; 3 replications for each combination using two colony groups) was done. Comparison of the mortality of *C. formosanus* in no- choice bioassays exposed to flooded (for 1 week) and unflooded substrates were done. The results indicate that for bifenthrin and fipronil mortality of *C. formosanus* was not affected by flooding regardless of soil type except at the lowest concentration tested. Chlorantraniliprole toxicity was lower after a flood at 1 ppm in sand but otherwise similar at higher concentrations. In soil, mortality was generally low but unexpectedly higher at 10 ppm and 25 ppm in flooded compared to unflooded soil. For all concentrations of imidacloprid treated sand, mortality of *C. formosanus* was reduced after a flood. However, at high concentrations in soil, flooded conditions increased its toxic effects on *C. formosanus*.

CHAPTER 1. INTRODUCTION

1.1 Termites

Termites (order- Blattodea) are a huge group with 281 genera and more than 2600 described species in 7 families and 14 subfamilies that were formerly recognized under the order Isoptera (Kambhampati and Eggleton 2000). Family Termitidae includes more than 75% of the total termite species with the remaining species in other six families (Bourguignon et. al 2014). Due to similarities in the presence of internal oothecae in the ancestors of termites and the wood feeding cockroaches, and coprophagous behavior along with gregariousness, termites are now considered eusocial cockroaches in the order Blattodea (Inward et al. 2007a) Similarly, molecular analysis has further confirmed the Blattodea- Isoptera clade (Inward et al. 2007b). Termites diverged from *Cryptocercus* roaches 170 million years ago and subsequently gave rise to the modern termite families including Termitidae (Bourguignon et al. 2014).

The termite family Rhinotermitidae Froggatt is believed to have originated in the Cretaceous period about a hundred million years ago (Krishna and Grimaldi 2003). The dispersal of termites is documented through multiple processes that include oceanic dispersal and human introduction (Bourguignon et al. 2016). Termites are superorganisms with a distinct division of labor among a caste system that includes workers, soldiers and reproductives (Eggleton 2010). Workers in a colony are responsible for foraging, locating food sources, and feeding of soldiers, reproductives and immatures. Soldiers are responsible for active colony defense, whereas reproductives are responsible for the maintenance of the colony population through reproduction. The caste system in termites is probably a result of post-embryonic development and under the control of hormones and pheromones (Noirot and Pasteels 1987, Traniello and Leuthold 2000, Eggleton 2010).

1.2 Formosan subterranean termites

1.2.1 Distribution

Formosan subterranean termites (*Coptotermes formosanu*s Shiraki) belong to the family Rhinotermitidae. These termites were officially recorded in 1913 from Honolulu, Hawaii for the first time. The *Coptotermes formosanus* is an invasive species and it is believed to have been introduced from South China or Formosa through the sandalwood trade (Yates and Tamashiro 1999). However, DNA sequencing studies document that *C. formosanus* has two distinct lineages of introduction to the United States, one being introduction through Hawaii and other being introduction through continental US (Jenkins et al. 2002, Austin et al. 2006). The *C. formosanus* is found in 11 different states in the US: Alabama, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas (Suiter et al. 2002).

The *C. formosanus* was first reported in 1966 in Louisiana from two different locations: New Orleans and Lake Charles (Spink 1967). From there, the dispersal of these termites has increased dramatically into several parishes in Louisiana with Ouachita parish (Monroe, LA) being the northern most parish for the distribution of *C. formosanus* (Messenger et al. 2002).

1.2.2 Economic importance

Out of the many described species of termites, only 183 species are reported to damage buildings with 83 species causing severe damage to property. Among the economically important termite species, 28 are in the genera *Coptotermes* and cause significant damage. This is the largest number causing damage from a single genus (Su and Scheffrahn 2000).

The *C. formosanus* is one of the most destructive and economically important pests. The annual cost incurred to customers for preventive and remedial measures for management of these

termites is over US $ 1 billion (Lax and Osbrink 2003). The *C. formosanus* is a ground dweller and its attack on food sources is highly aggressive (Spink 1967). Much damage can be observed in a relatively short period of infestation because of the large population size which may range from average of 2 to 10 million individuals in a single colony (Yates and Tamashiro 1999).

The primary food of termites is cellulose derivatives including cork, nuts, paper and even living plants (Yates and Tamashiro 1999). Damage from *C. formosanus* is not limited to untreated wood only but they are capable of damaging creosoted telephone poles at salt water dams (Henderson 2008). Elimination of the entire colony of *C. formosanus* is very difficult, and as a result have successfully established in a wide range of areas (Su 2003).

1.2.3 Control measures

1.2.3.1 Physical control

Physical control measures for the management of termite populations received increased attention due to the ban on the use of persistent organochlorine insecticides. For prevention of termites foraging into the wooden structures, Basaltic termite barrier (Ameron HC&D, Honolulu), a particle barrier to *C. formosanus,* could be useful to create an effective barrier against these termites (Yates et al. 2000). Similarly, at different research stations in Hawaii, a grade 316 steel, TERMI-MESH (TERMI-MESH Australia Pty. Ltd., Queensland, Australia), was also found to be effective in reducing the penetration by subterranean termites (Grace et al. 1996).

1.2.3.2 Biological control

Biological control methods to manage target termite populations include the use of pathogens (fungi, bacteria, nematodes), predators, parasitoids (Verma et al. 2009). Due to the underground habitat of *C. formosanus*, the potential for use of predators and parasitoids is

negligible (Grace 1997, Culliney and Grace 2000). However, because of the soil habitat, the use of pathogens that include entomopathogenic fungi like *Beauveria bassiana* and *Metarhizium anisopliae* have potential for the control of *C. formosanus*. The entomopathogen *M. anisopliae* was able to grow in the presence of anti-fungal volatiles that are present in the nest of *C. formosanus*, adding a prospect to biological control of these termites using the entomopathogen (Wright et al. 2000). Nevertheless, the potential of biological control of termites has always been controversial. Social behaviors present in termites like grooming and trophallaxis may help in the transfer of conidia of entomopathogens and increase mortality in the colony (Wright et al. 2002). However, the same social behaviors that are believed to help in spreading of fungus may also be an efficient defense against the infection of entomopathogens. The spores of entomopathogens on cuticle of termites are removed by the mutual grooming behavior of the nest mates which imparts defensive mechanism against the pathogens (Yanagawa et al. 2009, Chouvenc and Su 2010). Therefore, the successful control of termite colonies by entomopathogens is compromised by eusocial behaviors (Culliney and Grace 2000). Therefore, the use and prospect of entomopathogens for colony level management of populations remains controversial.

1.2.3.3 Chemical control

Chemical control measures for subterranean and drywood termites are different due to their different habitat. Localized treatment with a liquid insecticide is most common for the control of drywood termites (Lewis 2003), while insecticide barriers and baiting are used for soil dwelling subterranean termites. Presently, non-repellent and slow-acting baits are becoming popular for the management of subterranean termites around homes (Su and Scheffrahn 2000). Slow acting bait toxicants like noviflumuron, and hexaflumuron are very effective in controlling entire colonies of subterranean termites (Su and Scheffrahn 1998, Su 2005). Similarly,

diflubenzuron and lufenuron are effective bait actives against *C. formosanus* (Gautam and Henderson 2014). Materials that can be used in a termite bait matrices include cob (waste product from biofuel and food industry) along with cardboard and wood (Wang and Henderson 2012). Another study suggests that exposure to lufenuron can suppress the resistance of *C. formosanus* to bacterial strains of *Pseudomonas aeruginosa* (Wang et al. 2013a).

Soil treatment with liquid termiticides is popular in the US. Non-repellent termiticides are widely used compared to repellent termiticides used in the previous generation. Liquid termiticides provide an effective barrier against the termites and hence provide a good chance for population control (Horwood et al. 2010). However, efficacy is dependent on concentrations applied (Su et al. 1987).

Fipronil is a broad-spectrum toxicant from the phenylpyrazole group used for the control of many insects. It interrupts the normal functioning of GABA gated chloride channels (Gant et al. 1998, Gunasekara et al. 2007). Non-repellent termiticides like fipronil allow termites to penetrate the treated substrates causing delayed mortality (Hu 2005, Remmen and Su 2005). The slow action of non-repellency increases the chances of interaction with other naïve termites, thus resulting in horizontal transfer of the toxin from exposed to unexposed individuals (Ibrahim et al. 2003, Gautam et al. 2012). Fipronil concentrations greater than 10 ppm are effective in the lab (Shelton and Grace 2003) but the population size of a termite colony is an important factor to be considered for horizontal transfer and colony management (Wang et al. 2013b).

Imidacloprid is a systemic and contact insecticide which is used to control a wide range of insects. It is a stimulator of nicotinic acetylcholine receptors and has low mammalian toxicity (Elbert et al. 1991, Mullins 1993). Imidacloprid is also a slow acting termiticide which has the potential of horizontal transfer of toxin within a colony, but only at concentrations higher than 10

ppm (Shelton and Grace 2003). Imidacloprid was capable of suppressing termite colonies located away from the termiticide application site (Parman and Vargo 2010) which provides an evidence to the slow action of imidacloprid.

Chlorantraniliprole is a member of the anthranilic diamide class of insecticides that acts on ryanodine receptors. It is widely used on agricultural crops. It is highly selective to insects (Bentley et al. 2010). The delayed toxic reaction of termites to chlorantraniliprole-treated sandy clay soil provides enough time for social interaction. This action facilitates the greater transfer of the toxin within the poisoned and non-poisoned individuals (Neoh et al. 2012). The horizontal transfer of chlorantraniliprole in Eastern subterranean termites was efficient at concentrations of 25 and 50 ppm (Buczkowski et al. 2012). However, the toxicity and horizontal transfer of chlorantraniliprole is dependent upon the type of substrate treated (Gautam and Henderson 2011).

Bifenthrin is a pyrethroid whose target site is voltage gated sodium channels (Vijverberg and vanden Bercken 1990). Bifenthrin is a repellent termiticide that can create an effective barrier against the termites to keep them away from the treated property (Yeoh et al. 2006, Yeoh and Lee 2007, Smith et al. 2008). Bifenthrin is persistent in nature and can help in preventing the termite population from foraging in the treated area for long periods of time and hence provide longer control (Su et al. 1999, Baker and Bellamy 2006).

Even though fipronil and bifenthrin are persistent termiticides, degradation is greatly dependent on the moisture content of the substrate. Dissipation of both termiticides under water-saturated conditions is faster than at 20% field capacity moisture (Soudamini and Ahuja 2009, Soudamini and Ahuja 2010, Soudamini et al. 2010). However, Baskaran et al. (1999) observed that soil moisture had little effect on degradation of bifenthrin and imidacloprid through time in a

laboratory condition. In a study by Shuai et al. (2012) the half-life of fipronil was in the range of 28-34 days at 75% field capacity level, which is less than at normal conditions. They also observed, in a simulated rainfall condition for 24 hours, soil with lower carbon contents had 29% chemical loss which was the greatest obtained in their experiment. A greenhouse study by Keefer and Gold (2014) reported that imidacloprid was completely absent from soil and leachate after 3 months of application when applied at the labelled rate. The degradation of bifenthrin was not dependent on the type of substrate (Soudamini and Ahuja 2009). Similarly, the rate of degradation of imidacloprid was also found to be faster under submerged conditions (Mahapatra et al. 2017). When applied at labelled rate, chlorantraniliprole was found to be persistent in soils 700 days or more after a treatment (Spomer and Kamble 2011).

Termiticides eventually degrade after application in soil but when it is incorporated into soil its degradation is much delayed compared to on top of the soil or crop. Degradation may be dependent on water solubility of the termiticide, type of substrates used, half-life of chemical, application rate, environmental factors (light, heat, etc.), microbial activities, and pH. The area treated with water soluble termiticides possess a high risk of termite attack in water prone areas that are the usual habitat of termites. Flooding and water stagnation are frequent problems in water prone areas like Louisiana. Flooded substrates might result in leaching of the active ingredients from chemical treated areas and could lead to damage due to termite attack after a flood which poses a serious problem in the management of termites. However, there are very few published data available on residual effects of termiticides in flooded soils. The studies on flooding with the chemicals by a group of researchers did not study the effect of prolonged flood with on the residuals of chemicals. Similarly, the residuals of chemicals after application in the

field condition might not have uniform distribution of the chemicals due to several field limitations.

The research proposed here was undertaken to evaluate the performance of fipronil, imidacloprid, chlorantraniliprole and bifenthrin in flooded substrates. No-choice bioassays were conducted on the substrates that were obtained after flooding treatment in a laboratory setting. This research has relevance to areas prone to flooding that are also subject to frequent termite infestations like Louisiana. Areas that are subjected to flooding and water stagnation for a several days may have an increase in prevalence of termite attack after the water drains due to water solubility and leaching of the termiticides. Therefore, this research aims to investigate the effects of flooding on efficacy of termiticides with different water solubilities under laboratory conditions. The results of this research will help homeowners and pest control companies ascertain the need for re-application of termiticides in flooded areas. This research has significance to Louisiana and to other areas of the world which are invaded by Formosan termites and have frequent rainfall.

Concerning the water prone environment in Louisiana, we wanted to test the efficacy of termiticides in pre- and post- flood conditions by the following three approaches:

- By comparing termiticide concentrations in pre- and post- flood conditions.
- Chemical analysis of how much chemical was removed after flooding.
- By assessing the mortality of termites in the pre- and post- flood substrates through no-choice bioassays.
- By assessing the amount of food (filter paper) consumption in the pre- and post- floods conditions.

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CHAPTER 2. RESIDUAL EFFECTS OF TERMITICIDES ON MORTALITY OF FORMOSAN SUBTERRANEAN TERMITES, *COPTOTERMES FORMOSANUS* SHIRAKI, IN SUBSTRATES SUBJECTED TO FLOODING

2.1 Introduction

Tremendous amounts of property loss are associated with the damage by the Formosan subterranean termite (*Coptotermes formosanus* Shiraki). Because of the economic losses, control measures that include physical, biological and chemical approaches have been developed. Persistence in the environment due to widespread use of organochlorines led to interest in the use non-chemical control methods (physical and biological) to manage populations of *C. formosanus* (Logan et al. 1990). Sized particle barriers- granite or crushed basalt (Ameron HC&D, Honolulu) and Steel mesh (TERMI-MESH, a grade 316 steel, TERMI-MESH Australia Pty. Ltd., Queensland, Australia) are approved in Hawaii and Australia, can be effectively used in Hawaii to create physical barriers against *C. formosanus* (Grace et al. 1996b). Similarly, from field and laboratory studies, the use of graded sintered glass particles as a physical barrier could be a potential tool against *Coptotermes* species in Australia (Shiday and French 2011). Physical barriers, although they show potential for prevention, are not effectively used for management of the colony since greater care needs to be taken while installation of the barriers (Yates et al. 2000) and colony elimination is not observed.

Biological methods of termite control include the use of pathogens (fungal, bacterial and nematode), predators, and parasitoids (Grace 1997, Verma et al. 2009) Due to the underground habitat of subterranean termites, control with predators and parasitoids fail to control the colony, providing more limitations to the use of biological control approaches (Grace 2003). However, the use of entomopathogens (*Beauveria bassiana* and *Metarhizium anisopliae*) is widely studied in the laboratory (Lenz 2005) among the other measures. The use of entomopathogens is limited

by several biological characteristics possessed by eusocial insects like termites (Culliney and Grace 2000). Social activities like grooming, cellular encapsulation and gut antifungal activity in the termites reduce the transfer and germination of conidia within the termite population and provide resistance in the termites against entomopathogens (Chouvenc and Su 2010). Grooming of each other in a colony helps in the removal of fungal conidia of *M. anisopliae* from the cuticle. In addition, the conidia reaching guts could not germinate to infect the *C. formosanus* due to the gut's antifungal activity (Yanagawa and Shimizu 2006).

Due to presence of limitations of biological and physical control measures, chemicals are predominantly used for termite management. Soil treatment with liquid termiticides and baiting are commonly used control measures. The use of baits is increasing and appears to be a promising method of control (Su 2002). Chitin synthesis inhibitor like diflubenzuron, hexaflumuron, lufenuron, and triflumuron restrict the molting process and cause delayed mortality in termites (Xing et al. 2014, Vahabzadeh et al. 2007). Among the several other chitin synthesis inhibitors, hexaflumuron accounts for one of the successful baiting chemical used for the management of a termite colony (Evans and Iqbal 2014). Similarly, diflubenzuron and lufenuron are other effective baiting chemicals against *C. formosanus* (Gautam and Henderson 2014). For colony-level population suppression, the use of baits has great potential but immediate mortality cannot be obtained by using baits (Grace et al. 1996a). However soil treatment with termiticides have been used over the last half century to achieve immediate effect on the colony population and to create an effective barrier against termites (Smith and Rust 1990, Su and Scheffrahn 1998).

Soil treatment with liquid termiticides is the most widely used and effective method of management of subterranean termites (Racke et al. 1994, Peterson et al. 2006). However,

efficacy of soil termiticides is limited by various soil and termiticidal properties (Su and Scheffrahn 1998). Persistence and degradation is dependent on soil moisture, pH (Racke et al. 1996), type of termiticide, soil type, organic matter content (Forschler and Townsend 1996) and rate of initial termiticide application (Saran and Kamble 2008). In continuous exposure bioassays, bioavailability is reduced when the termiticides are applied at lower concentrations in high clay, organic matter and pH substrates (Henderson et al. 1998).

Some of the commonly used liquid chemicals for the management of *C. formosanus* include fipronil, bifenthrin, chlorantraniliprole, cyantraniliprole, imidacloprid, chlorfenapyr and indoxacarb (Mao et al. 2011). Fipronil is generally considered to be a non-repellent insecticide (Hu 2005, Yeoh et al. 2006, Yeoh and Lee 2007) although it was found to be repellent at its highest labelled rate of 0.125% in treated sand (Ibrahim et al. 2003). Bifenthrin is a repellent insecticide that prevents the foraging of termites in the treated area and thus maintains a barrier against attack by Formosan subterranean termites (Yeoh et al. 2006). Imidacloprid and chlorantraniliprole are non- repellent insecticides and cause delayed mortality with ample time for horizontal transfer (Yeoh and Lee 2007).

Persistent termiticides with low mammalian toxicity are desired for termite control. But as discussed earlier, persistence is limited by several factors, one of which is flooding. Flooding may reduce the efficacy of termiticides through leaching of the chemicals. Therefore, water prone areas which are the usual habitat of subterranean termites and that have been treated with water soluble termiticides possess a high risk of termite attack after a flood. The half-life of termiticides in submerged and normal conditions varies depending on the persistence of the active ingredient.

A study on fipronil under submerged conditions in soil claims the loss of toxicity of chemical to be much more rapid than under normal field conditions at 20% moisture level (Soudamini and Ahuja 2010). In a study by Shuai et al. (2012), they simulated the rainfall condition for 24 hours in soil treated with fipronil. They observed up to 29% chemicals loss from soil with lowest carbon content. Similarly, they also reported the half-life of fipronil between 28-34 days at 75% field capacity level. The rate of dissipation of imidacloprid from sterile and non-sterile soil after 60 days of incubation was found to be 66.2-79.8% under submerged conditions (Mahapatra et al. 2017). However, the concentration of imidacloprid and bifenthrin when applied at termiticidal application rate was not affected by varying moisture levels in treated soil and bedding materials used in Australia during an incubation period of 24 months (Baskaran et al. 1999). Soudamini and Ahuja (2009) stated that the degradation of bifenthrin is also independent of the type of substrate. There are very few published data available on residual effects of termiticides in flooded soils. Likewise, there is few studies on the efficacies of termiticides in the simulated rainfall or flooding conditions which may last for few days in the water prone environment like Louisiana. Therefore, we conducted an experiment to address this little-studied issue and compared the mortality of *C. formosanus* in pre-and post-flooded substrates using four commonly used termiticides.

2.2 Material and methods

2.2.1 Termiticides

Termidor (BASF Corp. Florham Park, NJ), Altriset (DuPont™ Corp. Wilmington, DE), Premise (Bayer Corp. Pittsburgh, PA), and Talstar Pro (FMC Corp. Philadelphia, PA) were used in the experiment. Most chemicals were available in the urban entomology laboratory at Louisiana State University (LSU) and Talstar was provided by FMC. The active ingredient of

Termidor is fipronil, of Altriset, chlorantraniliprole and of Premise, imidacloprid. These belong to the non- repellent class of insecticides. Talstar Pro has bifenthrin as the active ingredient and is a repellent termiticide.

2.2.2 Termites

Two colonies of *C. formosanus* were used in this experiment. One colony was initiated using a collection from Brechtel Memorial Park, New Orleans, LA in 2017 made with the milk crate trap technique (Gautam and Henderson 2011b). The second colony had been established in Parker Coliseum in 2013 and was also collected from Brechtel Memorial Park. Termite colonies were maintained in trashcans with wet wood until the experimental setup in the Parker Coliseum, Louisiana State University, room number 130, at 25ºC- 28ºC.

2.2.3 Substrates

Clay soil (Westwego soil series) and construction sand were used as substrates in the experiment. Construction sand was fine-grade Masonry sand (Louisiana Cement Products, LLC; Greenwell Springs, LA) and clay soil was collected by shovel from Brechtel Memorial Park, New Orleans, LA. Both substrates were autoclaved (12 cycles at 250˚K for 60 minutes) to sterilize. Substrates were placed in incubator at 60˚C for 24 hours for drying after being autoclaved.

2.2.4 Substrate treatment

Based on a preliminary experiment, the amount of substrate to be treated and the amount required for the flooding experiment were determined. Different amounts of termiticides based on calculations were used to make three different concentrations. These concentrations were determined from the literature. In clay soil, a 20% moisture level, and a 10% moisture level in sand, were prepared and maintain similar to Bhatta et al. (2016). At moisture levels of 9.5% in

sandy soil and 15.4-25.1% in clay soil, workers of *Macrotermes* were able to obtain water (Lys and Leuthold 1994). For all the control substrates used in the experiment, deionized water and substrates were mixed gently in a sealed Ziploc bags by hand and were handled similarly as the treated substrates. Six Ziploc bags (3 for sand and 3 for soil) were used for control. The percentage of moisture level maintained in the controls was same as the treated substrates. Treatment of substrates with termiticides and handling was done as described below.

2.2.4.1 Termidor

To attain 1, 10 and 25 ppm fipronil with a 20% moisture level in soil, 2.64µl, 26.4µl and 65.93µl Termidor (a.i fipronil 9.1%) was added to each of 40ml deionized water. The slurry was mixed and added to 200g soil in three Ziploc bags (S.C. Johnson & Son, Racine WI). Using the same product and concentrations with 10% moisture in sand, 3.02µl, 30.21µl and 75.54µl Termidor were added to 25ml of deionized water. The solution was then added to 250g sand in three different Ziploc bags. A total of six Ziploc bags were used for fipronil. The treated substrates (sand and soil) were mixed gently in sealed Ziploc bags by hand. There were 6 treatments of fipronil (3 in soil and 3 in sand).

2.2.4.2 Premise

Final concentrations of 1, 10 and 25 ppm of imidacloprid were made in both soil and sand. For soil, 0.00032mg, 0.0032mg and 0.008mg Premise (a.i imidacloprid 75%) were added to separate aliquots of 40 ml deionized water and the slurries were added to 200g soil in each of three different Ziploc bags. The Ziploc bags were sealed and the soil and insecticide were mixed. Similarly, for sand, 0.00037mg, 0.0037mg and 0.0091mg Premise were added to 25ml of deionized water to make final concentrations of 1, 10 and 25 ppm of imidacloprid. The solution was then added to each of 250g sand kept in three different Ziploc bags. The Ziploc bags were

sealed and sand and insecticides were gently mixed by hand. In total, six Ziploc bags were used for imidacloprid. There were 6 treatments of imidacloprid (3 in soil and 3 in sand).

2.2.4.3 Altriset

Three different quantities of Altriset (a.i chlorantraniliprole 18.4%), 1.31µl, 13.04µl, and 32.60µl, were added to 40ml deionized water to make final concentrations of 1, 10 and 25 ppm of chlorantraniliprole in each of 200g soil, respectively. The solution was then added to 200g of soil in three different Ziploc bags. The Ziploc bags were sealed and soil and insecticide were mixed thoroughly. Using the same product and concentrations, 1.49µl, 14.94µl and 37.36µl Altriset were added to 25ml of deionized water to make final concentrations of 1, 10 and 25 ppm of chlorantraniliprole in the 250g of sand. The solution with different concentrations were added to sand in 3 different Ziploc bags. The Ziploc bags were then sealed and sand and insecticide were gently mixed by hand. Total of six Ziploc bags were used for chlorantraniliprole. There were 6 treatments of chlorantraniliprole (3 in soil and 3 in sand).

2.2.4.4 Talstar

To attain 1, 10 and 25 ppm of bifenthrin with 20% moisture level in soil, 3.04µl, 30.38µl and 75.95µl Talstar (a.i bifenthrin 7.9%) were added to each of 40ml deionized water. The slurry was then added to separate aliquots 200g of soil in three Ziploc bags (S.C. Johnson & Son, Racine WI) respectively. Soil and insecticide were mixed gently. Using the same product and concentrations with 10% moisture in sand, 3.48µl, 34.81µl and 87.03µl of Talstar were added to separate aliquots of 25ml deionized water. The solutions were then added to each of 250g sand in three different Ziploc bags and sand and insecticide were mixed gently by hand. Total of six Ziploc bags were used for bifenthrin. There were 6 treatments of bifenthrin (3 in soil and 3 in sand).

After the substrates were gently mixed with water-termiticide slurries at different concentrations in sealed Ziploc bags, they were opened and kept under fume hood for drying for 2 days. During drying stirring of substrates was done by hand. The visibly dry substrates obtained were used without further processing for experiments. Thirty grams (5g in each petri dish) of dried substrates was used to set up the no-choice bioassay for unflooded substrates, while 175g sand and 125g soil were subjected to flooding for the no-choice bioassays using flooded substrates and chemical analysis, and 25g of each treated substrate was taken for chemical analysis, for a total of 24 Ziploc bags.

2.2.5 Flooding treatment

Termiticide-treated and untreated (control) substrates were subjected to simulated flooding treatments in cups in the following manner (figure 2.1). Flooding was simulated using 9.6cm tall plastic cups (Better Living Brands, LLC, CA). Twenty-one small holes were made and placed on the bottoms of cups that contained substrates using a sewing needle of 1.1mm diameter. Holes were equidistant from one another at 5.4mm and were placed in the center of bottoms of the cups (3.6cm diameter). The purpose of the holes was to allow water to drain from cups. The bottoms of the cups were filled with 2.7cm of pebbles (TERM- Particle Barrier, Polyguard, Ennis, Texas) for easy flow of water. The next 4.2cm was used for either sand (175g) or soil (125g) and the remaining 2.7cm was allocated for adding deionized water to the cup. The pebbles were washed for this experiment.

After each cup was filled with substrate and pebbles, it was placed inside of a second cup (with no holes) to retain the flood water. Then 130ml deionized water for cups containing both sand and soil were added to the cups containing the substrates. For draining of water, the filled cups were lifted and the stagnant water passed through the layer of substrate and pebbles and

drained through the holes present in the inner filled cup. After an hour the bottom cup was removed to allow water to drain off completely. Drained water was collected in a sealed waste container for disposal. A second time, the substrate cups were placed inside of new cups without holes and 90ml deionized water for cups with sand and 70ml for cups with soil was added. This water was retained for one week. The cups with the treated substrates were not disturbed until day 7, when the water was drained. After one week of exposure to water, the cups were drained as described previously and substrates (sand and soil) were collected. For the collection of substrates, the cups were cut by using scissors and the lump of wet substrate was removed and placed on open plastic containers available at the urban entomology laboratory at LSU.

To obtain control flooded substrates, flooding was carried out by using untreated substrates. Similar plastic cups were used to flood the untreated substrates and all the procedure used was as described earlier except untreated substrates were used in the 4.2cm of the cups. The control substrates were handled in a manner similar to the treated substrates. There were total of 30 cups (6 cups for each termiticide- 3 for sand and 3 for soil, each concentration had one Individual cup for flooding, and 6 cups were for control- 3 for sand and 3 for soil).

Figure 2.1. Flooding of substrates treated with four termiticides in cups

The following is the schematic of flooding in the laboratory setting that indicates times of exposure.

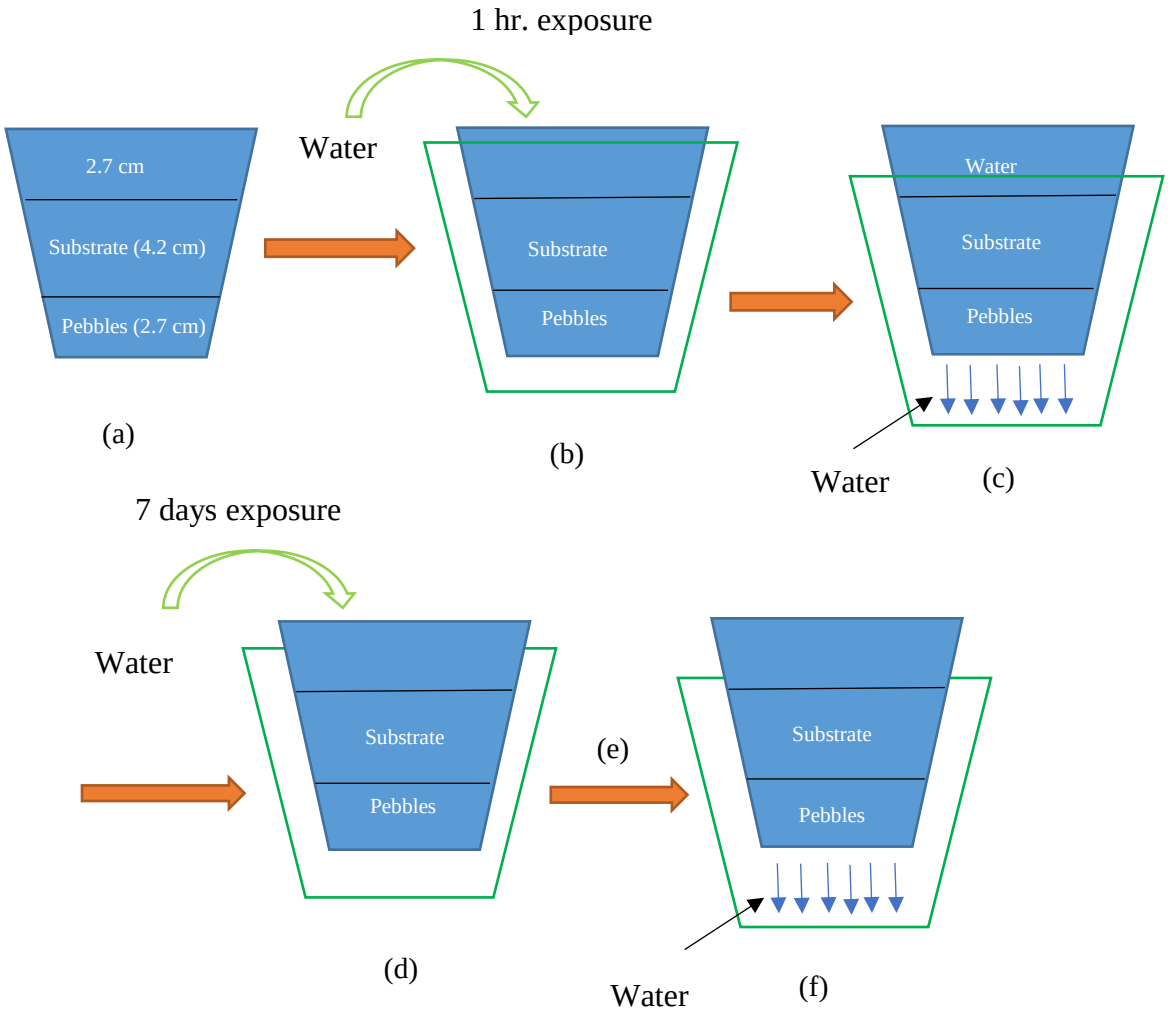


Figure 2.2. Schematic of flooding treatment showing steps used: (a) cup filled with substrate, (b) water added to cup, (c) draining of water by lifting the second cup, (d) water again added to the cup after draining for the first time, (e) water in cup allowed to sit for a week, and (f) water draining by lifting of the cup.

The collected substrates were kept under a fume hood for 4 days to dry. On the fifth day, the dried sand and dried soil was churned into fine powder using a clean mortar and pestle. For each treated sample 25g of each concentration was taken to LSU AgCenter Agricultural Chemistry Laboratory for chemical analysis. An additional 30g (5g in each Petri dishes) portion

of substrate was used to conduct a no- choice bioassay (flooded condition) and remaining portion of treated substrate was stored in the urban entomology laboratory before it was disposed through LSU Environmental Health and Safety. Similarly, the control substrates were obtained and 5g of each was used in each Petri dishes in no-choice bioassays.

2.2.6 Chemical analysis

For chemical analyses, 25g of the flooded and unflooded substrates were collected in 48 Ziploc bags (24 for unflooded substrates and 24 for flooded substrates) and taken to the LSU AgCenter Agricultural Chemistry Laboratory for residual analysis. Residual analysis of different chemicals was done through two chemical analysis methods by Amy B. Hernandez, Program Coordinator at LSU Agricultural Chemistry Department.

GC/MS analysis (fipronil and bifenthrin)

An Agilent 6890 gas chromatograph (GC) interfaced with an Agilent 5973 quadrupole mass spectrometer (MS) was used for the analysis of samples. An Agilent 7683 series autosampler was used to inject sample extracts and standards onto a 30m long Restek 5MS GC column with internal diameter of 0.25mm and film thickness of 0.25µm. Instrument control and quantitative data analyses were carried out in Agilent Chemstation software (Agilent, Santa Clara, CA). Injection volume of the extracts was 2.0µl with pulsed split less injection with 20psi pressure pulse for 0.74 minutes. The injector temperature was 250°C and transfer line temperature was 280°C. The carrier gas in the line was helium with the constant flow rate of 1.2ml/min. The mass spectrometer was operated in electron impact ionization (EI) mode with the MS ion source at 230°C and quadrupoles at 150°C. The electron multiplier was set 200V above the PFTBA auto-tuned setting. For screening and quantitative analysis, selected ion monitoring (SIM) mode was used. For initial identification of the pesticide, detection of the characteristics

ion peaks, their relative abundances (%) and the comparison of retention times (RT) with those observed in the analytical standard were used.

LC/MS/MS analysis (imidacloprid and chlorantraniliprole)

For sample analysis of imidacloprid and chlorantraniliprole, a Waters UPLC Aquity liquid chromatograph interfaced with a Waters TQD triple quadrupole mass spectrometer/mass spectrometer was used. Two different injection rates of imidacloprid and chlorantraniliprole were required.

For imidacloprid, the injection volume of extract was 10µl with water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B. Flow rate of 0.3ml/min was used in the beginning at 98%A/2%B changing to 2%A/98%B over 8 minutes. Thereafter, conditions were changed back to 98%A/2%B over 0.5 min and these conditions were maintained for 12 minutes. Similarly, for chlorantraniliprole, the injection volume of extract was 10µl with water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1%. Flow rate of 0.3ml/min was used in the beginning at 95%A/5%B changing to 65%A/35%B over 2 minutes. These conditions were held at 65%A/35%B for 1 minute and changed to 5%A/95%B over 1 min and fully changed to original condition and equilibration for 7 minutes.

The triple quadrupole operated in electrospray positive mode with capillary at 3.84kV and extractor at 3.66V for imidacloprid and 2.0V for chlorantraniliprole were used. Source temperature at 120°C, desolvation temperature of 400°C and nitrogen flow of 500L/hr was maintained for both the chemicals. The collision gas used was argon with the flow of 0.18ml/min. For comparison of ion peaks and their relative abundances as well as comparison of retention time (RT) with those observed in the analytical standard, multi reaction monitoring (MRM) was used.

2.2.7 No-Choice bioassays

No- choice bioassays were conducted for flooded, unflooded and control substrates. For soil, 5g of substrate in each Petri dishes was used for bioassays. Filter papers (Ahlstrom qualitative filter papers, grade 615, diameter 7.5cm) available in the lab were placed on the bottom of each Petri dishes followed by 5g soil to which 1ml of de-ionized water was added (20% W:W).

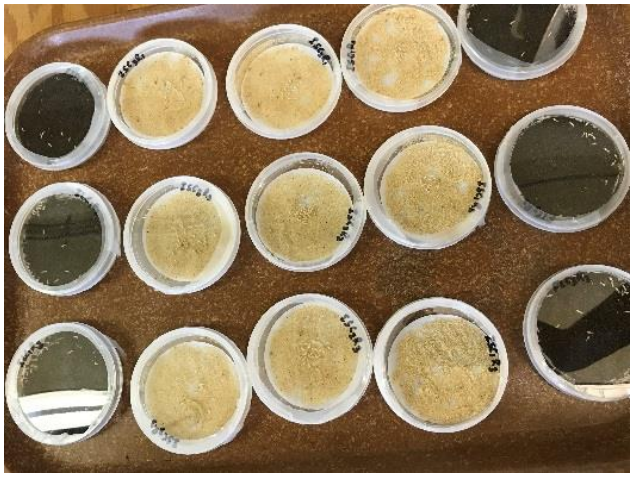


Figure 2.3. Setup for no-choice bioassays with imidacloprid (I) and fipronil (F) at 1 (C1), 10(C2) and 25 ppm (C3) with filter paper in sand and soil

The procedure used for sand was similar to that used for soil except 0.5ml (10% W:W) water was used to wet the sand. Similarly, for controls the procedure used for treated sand and soil was similar except the untreated substrates were used in the Petri dishes. There was a total of 312 experimental units (144- treated and unflooded, 144- treated and flooded, 32 control- 12- untreated and unflooded and 12- untreated and flooded).

On top of the substrates, 31 termites (20 workers and 11 soldiers) were added and the Petri dishes were sealed using Parafilm (Bemis flexible packaging, Neenah, WI) to prevent the moisture loss from and contaminants into the dishes. The mortality of termites was recorded

daily until day 6. Termites which did not show movement for 5 seconds were regarded as dead. Readings were taken by observing the termites without opening the Petri dishes.

Following is the schematic of the experiment.

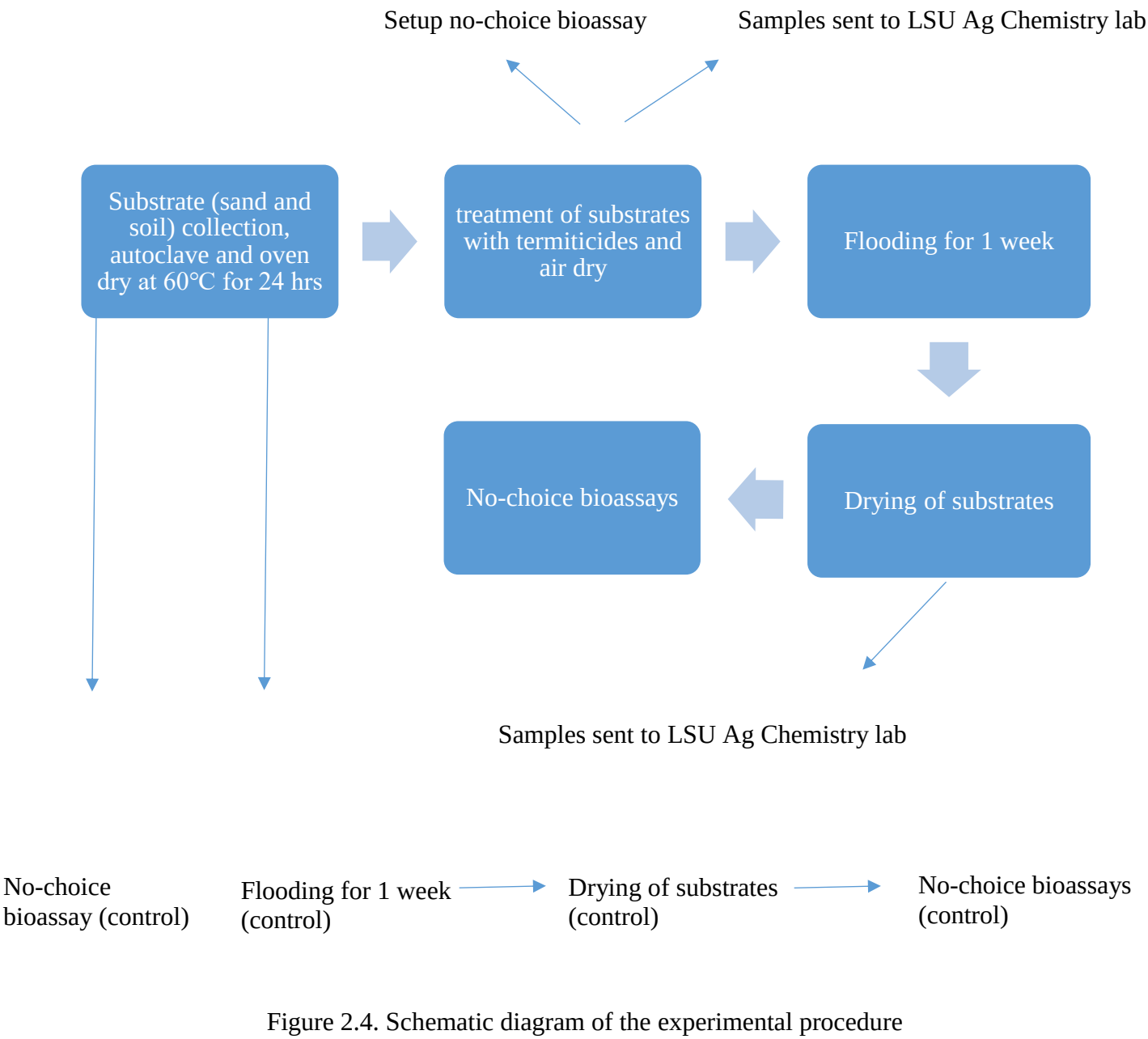


Figure 2.4. Schematic diagram of the experimental procedure

2.2.8 Filter paper consumption

Observations on food consumption were made from the filter papers used in no-choice bioassays as mentioned earlier. For this, filter papers were dried, weighed, labelled and kept in each Petri dishes in the no-choice bioassays. This was the initial reading. The filter papers were used to feed the termites in each Petri dish. Filter papers were removed from the no-choice bioassays after dismantling of the Petri dishes to assess the amount of food consumed during an experiment, and were dried. Substrate or dirt present was removed using a small soft brush and filter papers were weighed. This weight was the final reading; the difference in the initial and final weight was the amount consumed by termites during the experiments. The amount of filter paper consumed by termites in the controls was handled similar to treatments.

2.3 Statistical analysis

A 4 way ANOVA was used to test the effect of flooding on the mortality of termites in the no-choice bioassays, followed by Tukey means comparisons for the comparison of mortality for each treatment combination. The four independent variables in the analysis were insecticide, dose, flooding and days after treatment. In addition, a 3- way ANOVA was used to test the effect of flooding on filter paper consumption with independent variables insecticide, dose and flooding, was followed by Tukey analysis for the comparison of consumption for each treatment combination.

2.4 Results

2.4.1 Chemical analysis

Chemical analyses were conducted by the LSU AgCenter Agricultural Chemistry Laboratory. Since only a single replicate of each flood treatment-insecticide-insecticide concentration combination was submitted for chemical analysis, statistical analyses could not be

done. The trends in the results, however, indicate loss of chemicals due to flooding was greater in sand than in soil in all the chemicals except for bifenthrin (table 2.1), which had greater (approx. 32%) loss in 10 ppm of soil than in 10 ppm of sand (approx.10%).

Table 2.1. Concentrations of fipronil, imidacloprid, bifenthrin and chlorantraniliprole in soil and sand samples subjected or not to one week of simulated flooding.
Note: '-' indicates lower concentration of chemical in substrates subjected to flooding; a '+' indicates higher concentration of chemical in substrates subjected to flooding.

Chemical	**Substrates**	**Non-flooded sample concentration (in ppm)**	**Non-flooded sample concentration (in ppm)**	**Difference in concentration (%)**
fipronil 1ppm	sand	1.06	0.0052	-99.50943
fipronil 10ppm	sand	8.78	3.62	-58.76993
fipronil 25ppm	sand	27.69	10.1	-63.52474
fipronil 1ppm	soil	1.042	0.67	-35.70058
fipronil 10ppm	soil	8.237	6.55	-20.48076
fipronil 25ppm	soil	21.26	17.52	-17.59172
imidacloprid 1ppm	sand	1.3	0.0034	-99.73846
imidacloprid 10ppm	sand	12.58	0.031	-99.75358
imidacloprid 25ppm	sand	26.16	0.12	-99.54128
imidacloprid 1ppm	soil	0.997	0.459	-53.96189
imidacloprid 10ppm	soil	7.81	4.07	-47.88732
imidacloprid 25ppm	soil	29.72	12.45	-58.10902
bifenthrin 1ppm	sand	1.68	1.388	-17.38095
bifenthrin 10ppm	sand	7.84	7.05	-10.07653
bifenthrin 25ppm	sand	29.41	25.94	-11.79871
bifenthrin 1ppm	soil	1.03	1.236	+20
bifenthrin 10ppm	soil	7.56	5.11	-32.40741
bifenthrin 25ppm	soil	20.59	19.83	-3.691112
chlorantraniliprole 1ppm	sand	3.304	1.925	-41.73729
chlorantraniliprole 10ppm	sand	16.542	13.82	-16.45508
chlorantraniliprole 25ppm	sand	36.81	23.09	-37.27248
chlorantraniliprole 1ppm	soil	3.41	2.44	-28.44575
chlorantraniliprole 10ppm	soil	13.84	13.47	-2.67341
chlorantraniliprole 25ppm	soil	26.09	18.85	-27.7501

In both sand and soil, imidacloprid was the most leachable and bifenthrin the least leachable chemical among the four used in this experiment. Leaching of fipronil and chlorantraniliprole was intermediate. Similarly, the lowest concentration showed the greatest percent loss for all chemicals except bifenthrin and imidacloprid in soil. Bifenthrin had 20% increase in concentration in soil at 1ppm after a flood, a result which is probably attributable to the lack of replication in our samples or to uneven distribution of chemical prior to flood. Table 2.1 summarizes the percent differences in the concentrations of four chemicals in flooded and non-flooded substrates.

2.4.2 No-choice bioassays

The control treatments consisted of flooded and unflooded sand and soil not treated with insecticides. The mortality of *C. fomosanus* observed in the controls in the no choice bioassays was less than 5% in both flooded and unflooded substrates. Since the purpose of this research was to compare the efficacies of termiticides in flooded and unflooded conditions, the mortality of *C. fomosanus* from controls were not included in the statistical analyses but mortality in controls is presented in the graphs below for reference.

The effect of the four-way interaction of insecticide, flooding, days after treatment and dose on mortality of *C. fomosanus* was significant in treated sand ($F_{12, 360}$=8.90; P<0.0001) but was not significant in treated soil ($F_{12, 360}$=0.23; P=0.9967). There are two-way and three-way interactions that are significant among the main effects insecticides, doses, flooding and days after treatment which are presented in the table 2.2 (a and b). The main effect of flooding on mortality of *C. fomosanus* was significant on termiticide-treated sand ($F_{1, 360}$=281.38; P<0.0001) but was not significant on termiticide-treated soil ($F_{1, 360}$=0.04; P=0.8365). The main effect of insecticide had a significant effect on both treated sand ($F_{3, 360}$= 1963.13; P<0.0001)

and treated soil ($F_{3, 360}$= 142.95; P<0.0001). The dose of insecticide had significant effect on mortality of *C. fomosanus* in both treated sand ($F_{2, 360}$= 461.95; P<0.0001) and treated soil ($F_{2, 360}$= 182.03; P<0.0001). Similarly, the main effect of days after treatment also had significant effect on mortality of *C. fomosanus* in both treated sand ($F_{2, 360}$= 471.33 P<0.0001) and treated soil ($F_{2, 360}$= 146.68; P<0.0001). Mortality of *C. formosanus* was analyzed on 1, 3 and 6 days after treatment, but mortality of only day 6 is presented, since cumulative mortality was recorded for all treatment combinations until day 6.

Table 2.2. Four-way ANOVA for mortality of termites in termiticide- treated and unflooded or flooded: (a) sand and (b) soil.

(a)

Type 3 Tests of Fixed Effects(sand)				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Insecticides (I)	3	360	1963.13	<.0001
Doses (D)	2	360	461.95	<.0001
I*D	6	360	50.12	<.0001
Flooding(F)	1	360	281.38	<.0001
I*F	3	360	52.88	<.0001
F*D	2	360	31.81	<.0001
I*F*D	6	360	62.49	<.0001
Days aft treatment(T)	2	360	471.33	<.0001
I*T	6	360	59.05	<.0001
T*D	4	360	15.92	<.0001
I*T*D	12	360	22.65	<.0001
F*T	2	360	7.87	0.0005
I*F*T	6	360	5.41	<.0001
F*T*D	4	360	3.93	0.0039
I*F*T*D	12	360	8.90	<.0001

(b)

Type 3 Tests of Fixed Effects(soil)				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Insecticides (I)	3	360	142.95	<.0001
Doses (D)	2	360	182.03	<.0001
I*D	6	360	25.94	<.0001
Flooding (F)	1	360	0.04	0.8365
I*F	3	360	2.25	0.0827
F*D	2	360	1.67	0.1905
I*F*D	6	360	0.32	0.9243
Days after treatment(T)	2	360	146.68	<.0001
I*T	6	360	17.81	<.0001
T*D	4	360	17.15	<.0001
I*T*D	12	360	4.01	<.0001
F*T	2	360	0.37	0.6878
I*F*T	6	360	0.67	0.6718
F*T*D	4	360	1.02	0.3960
I*F*T*D	12	360	0.23	0.9967

In fipronil-treated sand, a highly significant difference in mortality of *C. formosanus* between flooded and unflooded conditions was observed at 1 ppm ($P<0.0001$). However, a significant difference in mortality between flooded and unflooded sand treated with fipronil was not observed at 10 and 25 ppm. Similarly, no significant differences in mortality of *C. formosanus* were observed at any concentrations of fipronil in treated soil (figure 2.5, A). Mortality of *C. formosanus* differed significantly between flooded and unflooded sand treated with 10 ppm ($P<0.0001$) and 25 ppm ($P<0.0001$) of imidacloprid but the effect was insignificant in 1 ppm. However, no significant differences in mortality were observed in soil treated with imidacloprid, although the percent mortality was slightly increased at higher concentrations (figure 2.5, B). Fungal hyphae were seen on dead termites in 7 Petri dishes with imidacloprid on flooded soil at 10 and 25 ppm. Flooding did not have significant effects on mortality in bifenthrin-treated substrates (both sand and soil). The mortality observed in sand with bifenthrin was 100% at all concentrations and was near 100% in soil at 10 and 25 ppm in both flooded and unflooded conditions (figure 2.5, C). Chlorantraniliprole-treated sand showed significant difference in mortality of *C. formosanus* at 1 ppm in unflooded and flooded substrates ($P=0.0002$). However, mortality was not significantly different between flooding treatments at 10 and 25 ppm in treated sand. Similarly, mortality was not significantly different in soil treated with chlorantraniliprole in unflooded and flooded conditions but a slight increase in the mortality after a flood at 10 and 25 ppm was observed (figure 2.5, D).

(A) Fipronil

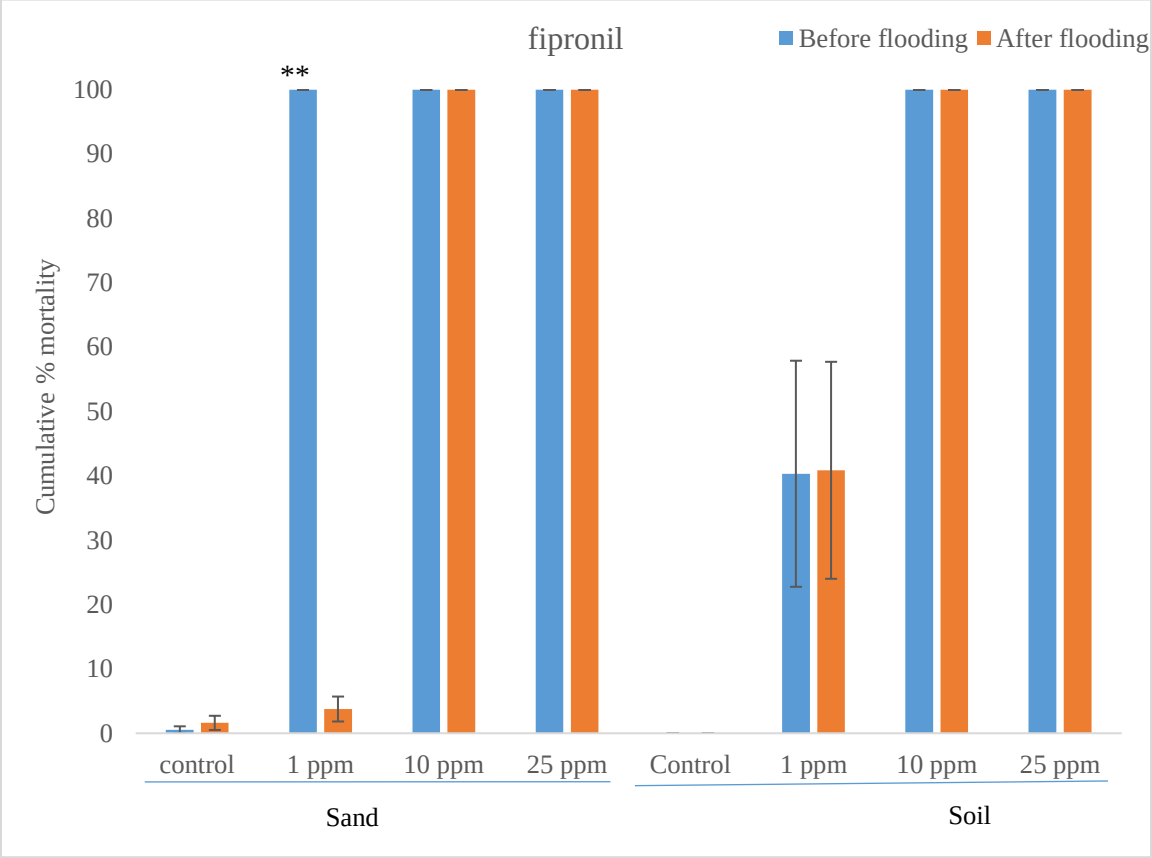
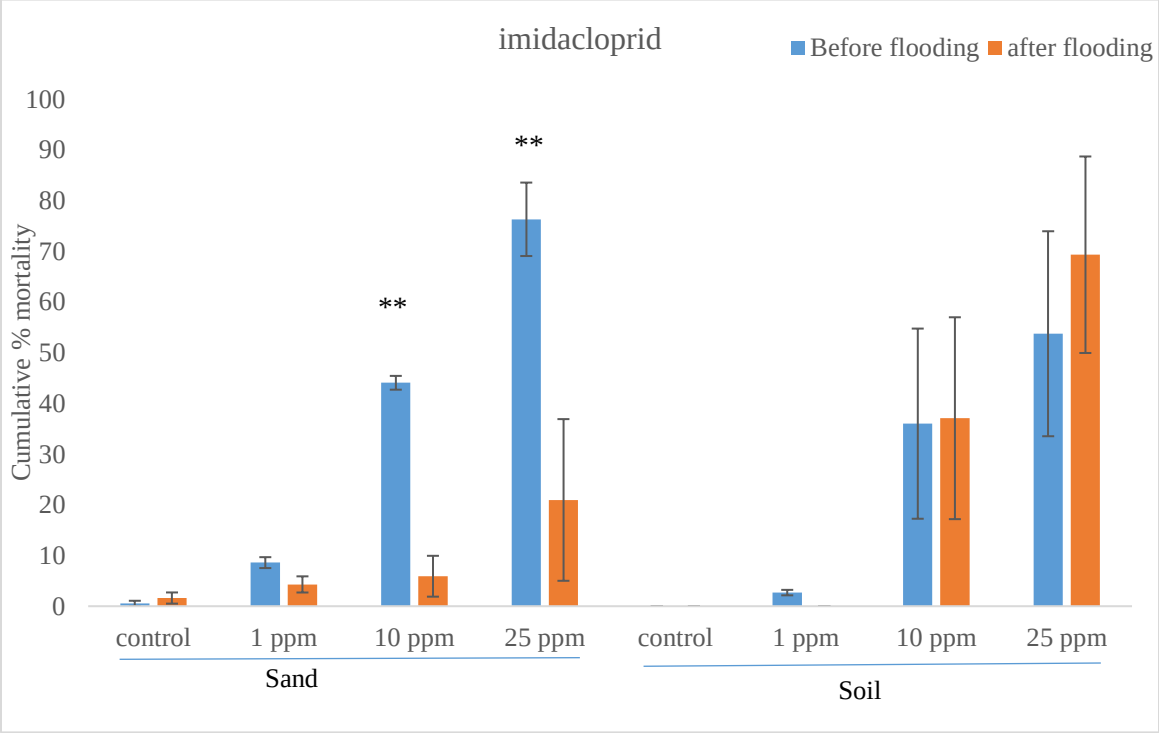


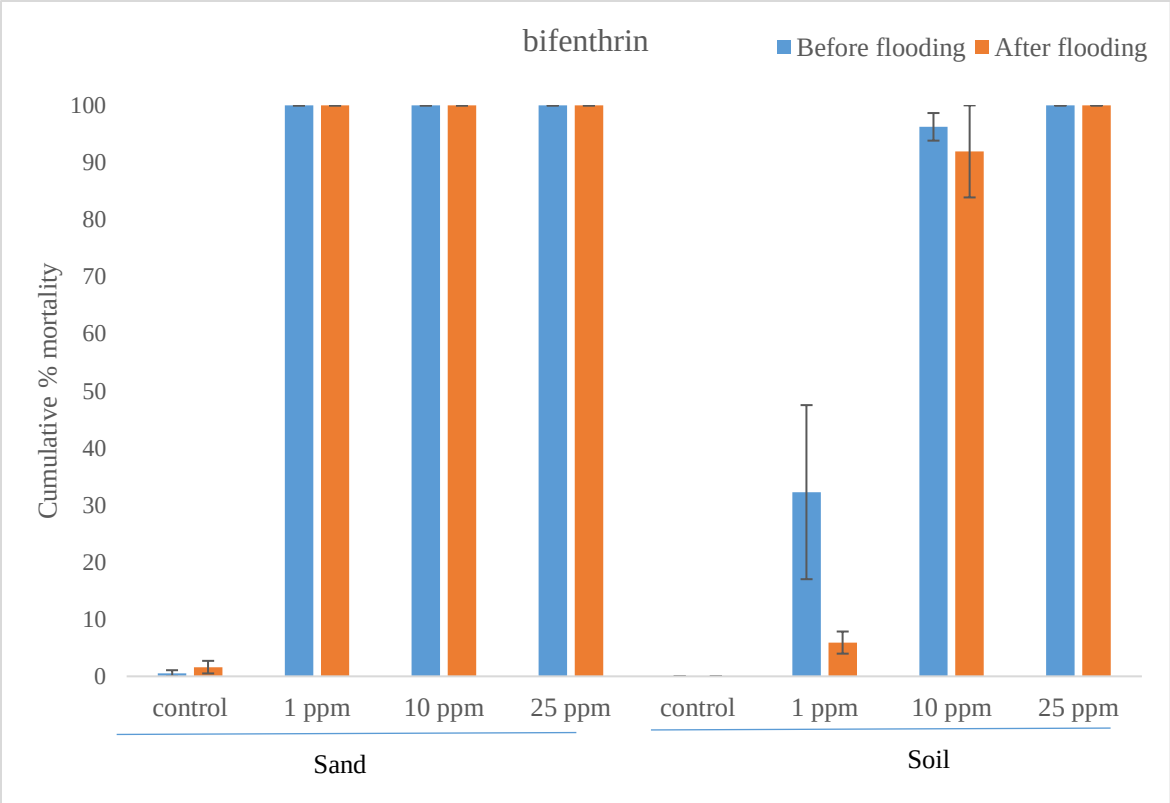
Figure 2.5. A. Cumulative mean percent mortality (±SEM) of *Coptotermes formosanus* in termiticide-treated or untreated, unflooded or flooded sand and soil on day 6. Means were compared using Tukey means comparisons procedure. **Note:** ** means the mortality of *C. formosanus* is significantly different between flooded and unflooded substrates at 0.001 level of significance.

Note: Figure 2.5 contd.

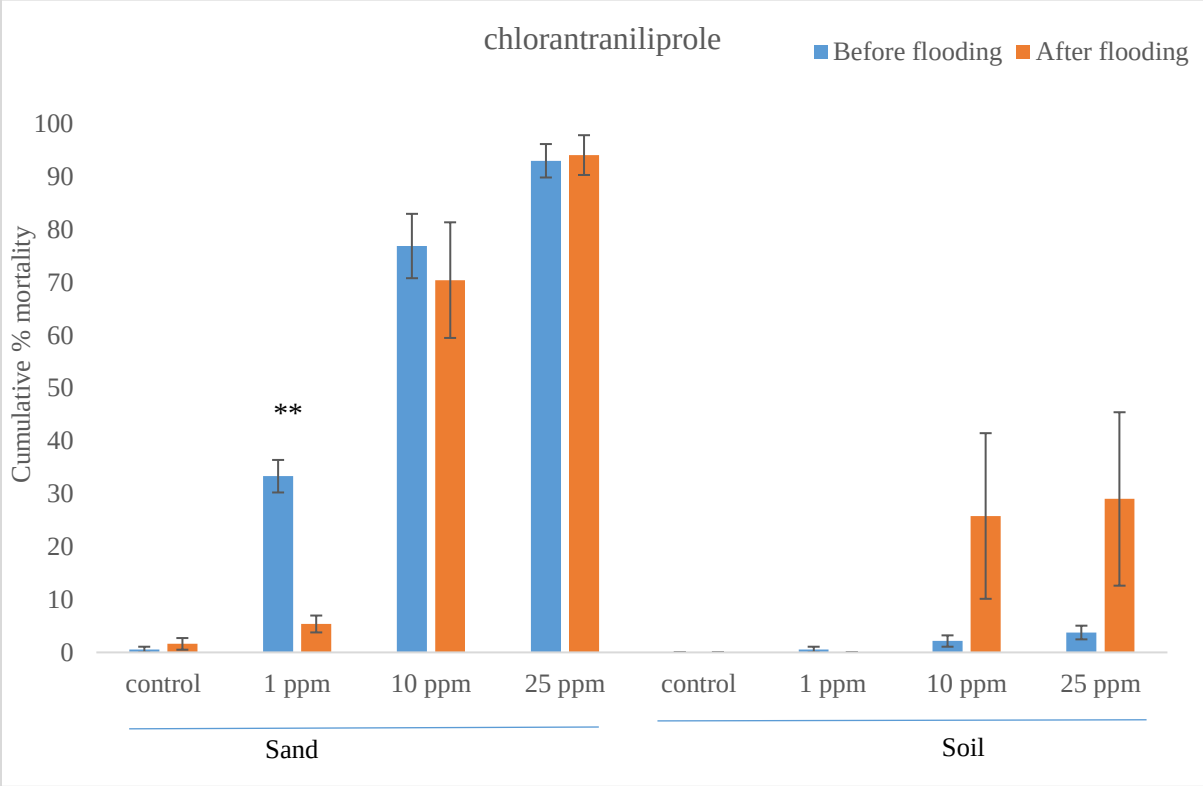
(B) Imidacloprid



(C) Bifenthrin



(D) Chlorantraniliprole



Based on the mortality observed from no-choice bioassays, in sand bifenthrin was found to be the most effective chemical after a flood, followed by fipronil and chlorantraniliprole while imidacloprid was least effective after a flood. In soil, bifenthrin and fipronil were similar in terms of effectiveness followed by imidacloprid. Chlorantraniliprole was least effective among the four tested in this experiment in soil. The effectiveness here was based on the mortality observed. In addition, the higher the dose of insecticide, the better was the effectiveness for all insecticides.

2.4.3 Filter paper consumption

Filter paper consumption by controls were obtained from the no-choice bioassays of controls in the experiment. The statistical analysis of filter paper consumption does not incorporate the analysis of filter paper consumption in control substrates since the main purpose

of this study was to observe the effect of flooding on the efficacies of insecticides on two substrates.

The effect of the interaction of insecticide, flooding and dose on filter paper consumption was not significant in treated sand ($F_{6,\ 120}$=1.45; P=0.2014) but the interaction was significant on consumption in treated soil ($F_{6,\ 120}$=2.64; P=0.0195). Similarly, there were other two-way interactions in the analysis which are presented in the table 2.3. Food consumption was significantly affected by the main effects of insecticide in treated sand ($F_{3,\ 120}$=14.94; P<0.0001) and treated soil ($F_{3,\ 120}$=3.01; P= 0.0328), dose in treated sand ($F_{2,\ 120}$=4.61; P=0.0118) and treated soil ($F_{2,\ 120}$=6.38; P=0.0023), and flooding in treated sand ($F_{1,\ 120}$=4.34; P=0.0394) and treated soil ($F_{1,\ 120}$=19.68; P<0.0001). A summary of the interactions is shown in Table 2.3 (a and b).

Table 2.3. Three-way ANOVA for analysis of food (filter paper) consumption in termiticide-treated and unflooded or flooded: (a) sand and (b) soil.

(a)

Type 3 Tests of Fixed Effects (sand)				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Insecticides (I)	3	120	14.94	<.0001
Doses (D)	2	120	4.61	0.0118
I*D	6	120	2.28	0.0402
Flooding (F)	1	120	4.34	0.0394
I*F	3	120	3.69	0.0138
F*D	2	120	3.49	0.0335
I*F*D	6	120	1.45	0.2014

(b)

Type 3 Tests of Fixed Effects (soil)				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Insecticides (I)	3	120	3.01	0.0328
Doses (D)	2	120	6.38	0.0023
I*D	6	120	2.02	0.0687
Flooding (F)	1	120	19.68	<.0001
I*F	3	120	2.48	0.0644
F*D	2	120	1.39	0.2528
I*F*D	6	120	2.64	0.0195

In the Tukey analysis for the consumption of filter paper in the flooded and unflooded experimental units, significant differences in consumption in individual treatment combinations was not observed in either substrate. In sand, consumption of food after a flood in fipronil at 1 ppm increased but consumption of food was decreased in flooded substrates at 10 and 25 ppm

(figure 2.6, A). In imidacloprid-treated sand, higher consumption was observed in the flooded treatment at all concentrations. Bifenthrin and chlorantraniliprole showed increased consumption in the flooded treatment at 1 ppm but consumption was decreased at 10 and 25 ppm for both chemicals. In soil, filter paper consumption was increased at 1 ppm of fipronil in the flooded treatment but decreased at 10 and 25 ppm. All concentrations of imidacloprid, bifenthrin and chlorantraniliprole in soil had reduced filter paper consumption in flooded treatments (figure 2.6, B).

(A) Sand

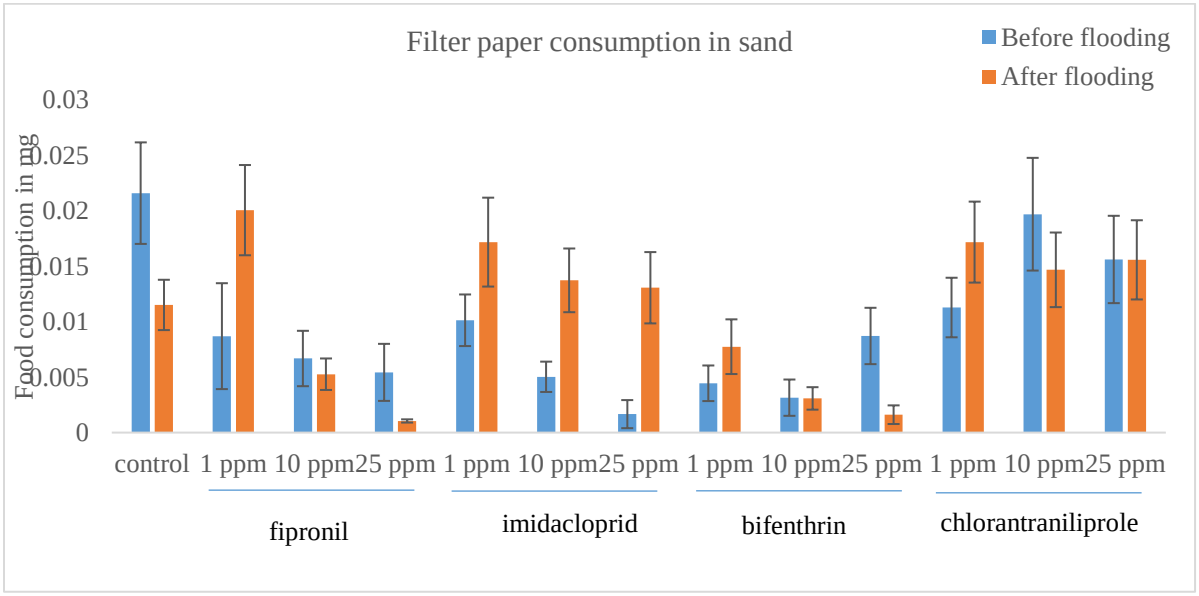
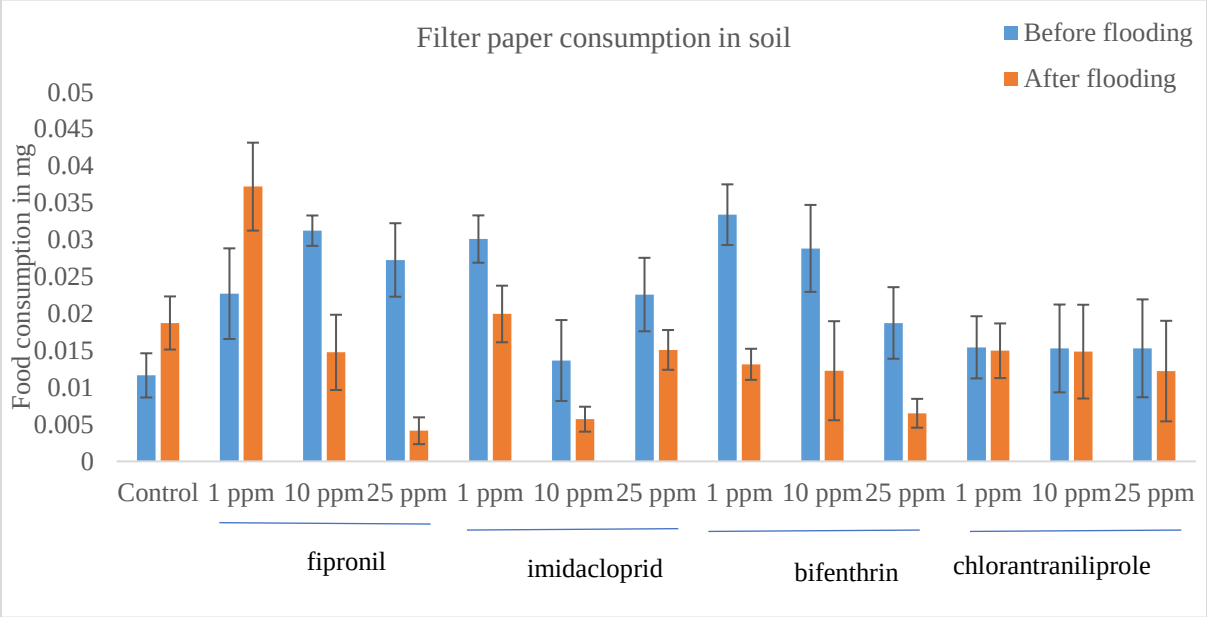


Figure 2.6. Cumulative mean consumption (±SEM) of filter paper in termiticide-treated or untreated and flooded or unflooded sand (A) and soil (B). No significant differences were detected by Tukey means comparisons procedure.

Note: Figure 2.6 contd.

(B) Soil



2.5 Discussion

The trend in loss of termiticides after a flood was higher in sand than in soil for most of the termiticide concentrations. The organic matter content in sand was lower than that in clay soil, which decreases the surface area for adsorption resulting in greater leaching of chemicals from the sand surface (Bajeer et al. 2012). Similarly, the adsorption capacity of soil is dependent on the clay content (Paszko 2006), which could be a reason for the differences in reductions in the concentrations of termiticides in sand and soil observed after the simulated flood. Even the most water-soluble chemical, imidacloprid, had higher loss in concentration from sand than from soil. Higher amount of chemical loss was observed from the lower concentrations treated with a few exceptions (bifenthrin 1 ppm in soil and imidacloprid 25 ppm in soil), which showed greater loss from higher concentration treated. Saran and Kamble (2008) reported that the rate of degradation and bioavailability of termiticides was dependent on initial concentration during the application. They studied the degradation and bioavailability of imidacloprid, fipronil, and bifenthrin after a 6 month of application. Seven days of flooding in our experiment showed

similar trend of loss of chemicals, as was observed in soil aged six months in laboratory conditions by Saran and Kamble (2008).

The loss of fipronil from sand was approximately73% (58, 63 &99%) varying on concentration applied while loss from soil was approximately 24% (17, 20 &35%). In soils treated with fipronil, while simulating a rainfall for 24 hours, Shuai et al. (2012) found 29% loss of fipronil from soil with lowest organic carbon through leaching. The lower loss in our study compared to Shuai et al. (2012) could be due to the type and organic carbon content of the soil used in experiments. However, we did not determine the organic carbon content of the soil we used in this experiment. The variation on the percent loss of concentrations in substrates was observed. Differences in the properties of sand and soil, have played a role in variation of the losses. Concentration loss of imidacloprid after a flood was higher than any other chemicals from both the substrates used in the experiment. The loss of imidacloprid in sand was 99% and 47-58% in soil after the flood. The leaching and retention of imidacloprid in soil was dependent on soil organic matter content (Liu et al. 2006, Samnani et al. 2013), which explains the variation in the loss of imidacloprid from sand and soil in our study. In a greenhouse experiment, imidacloprid was completely absent from soil or leachate in six months after the application when applied at labelled rate which indicates the leachability and water solubility of imidacloprid (Keefer and Gold 2014). A study by Baskaran et al. (1999) observed that soil moisture did not have any effect on degradation of imidacloprid in soil and bedding material which is common in Australia. This study supports the use of two different moisture levels in sand and soil to obtain similarity in the free water in the substrates without affecting the rate of degradation of imidacloprid. Bifenthrin had the lowest loss after a flood (except at 10 ppm in soil) among the chemicals tested in this experiment. Consistent with the findings of our study,

Baskaran et al. (1999) stated that soil moisture causes little degradation in the concentration of bifenthrin in treated soil and the bedding materials commonly used in Australia. Chlorantraniliprole was also found to be somewhat persistent in soil (with an approximate loss of 2-28%) and in sand (with an approximate loss of 16-41%).

In no-choice bioassays, fipronil was still effective after a flood at higher concentrations irrespective of the substrate but was ineffective at 1ppm. Termiticides when applied at higher concentrations during initial application has greater persistence (Saran and Kamble 2008), persistence of fipronil could have impacted on its bioavailability to the *C. formosanus* in flooded substrates in this experiment. Higher concentrations of fipronil were effective in causing mortality of *C. formosanus* in both sand and soil in flooded and unflooded conditions. A study by Bobé et al. 1997 observed the bioavailability and persistence of fipronil to be better in soil with higher organic matter, which supports the mortality results from no-choice bioassays in our study. Imidacloprid was ineffective after a flood in sand as the mortality of *C. formosanus* was significantly reduced at higher concentrations in the no-choice bioassays which is supported by the data observed on concentration loss in sand. In contrast, the mortality of *C. formosanus* was increased after a flood in the soil treated with higher concentrations of imidacloprid. The increase in mortality could be due to fungal blooms which may occur in clay soil treated with imidacloprid (Ramakrishnan et al. 1999). Some fungal hyphae were observed in the flooded clay soil with imidacloprid but the fungi were not identified in this experiment. Additionally, we hypothesize that the increased mortality could also be due to better distribution of the active ingredients by floodwater.

Additionally, bifenthrin was the most effective in flooded sand and effective in flooded soil among the four chemicals tested in this experiment. A study by Saran and Kamble (2008)

fipronil, bifenthrin and imidacloprid were applied at the labelled rates in loamy soil. Six months after the termiticide application, mortality was tested on the same soil and the fastest mortality of workers of *Reticulitermes flavipes* in continuous exposure bioassays was observed for bifenthrin. We also observed rapid mortality of *C. formosanus* from flooded and unflooded sand treated with higher concentrations of bifenthrin and fipronil (100% mortality was observed by day 4). Chlorantraniliprole caused an effective mortality after a flood at 10 and 25 ppm tested in sand but was not effective at the lowest concentration treated. Flooding did not have an apparent effect on the mortality of *C. formosanus* in soil with chlorantraniliprole but the mortality was not effective. However slight increase in percent mortality was observed in flooded soil at higher concentrations. From this increased mortality, we hypothesize that there could be a better distribution of active ingredients due to flood water making the active ingredient readily available to *C. formosanus*. The lower termite mortality in soil than in sand, even under unflooded conditions , could be due to the presence of organic matter, which may reduce the bioavailability of chlorantraniliprole to termites even at labelled doses (Gautam and Henderson 2011a, Spomer et al. 2009). Like chlorantraniliprole, chlorpyrifos, fenvalerate, cypermethrin and permethrin also demonstrated greater toxicity in sand compared to the soil in continuous exposure bioassays in a previous lab study (Forschler and Townsend 1996). Another study by Mao et al. (2011) observed mortality of termites to be more than 30% after a day of exposure in sand treated with chlorantraniliprole and cyantraniliprole even at 1.28 ppm, which supports the idea that chlorantraniliprole is effective in sand. The similarity in the mortality of *C. formosanus* in flooded and unflooded substrates suggests the persistent nature of chlorantraniliprole.

Food consumption in sand was found to be consistent with the results from no-choice bioassays and the chemical analysis. The chemical concentrations which allowed greater survival

of *C. formosanus* after a flood also showed greater consumption of food, for example in all concentrations of imidacloprid-treated sand. From this we conclude that food consumption in areas that have been flooded might be cause for concern, but the consumption is dependent on the type of chemicals used as repellent chemicals show rapid toxicity which lowers the rate of feeding while non-repellent chemicals with slow action provide room for consumption of greater amount of food. In soil, the consumption of filter paper was decreased after a flood in all the termiticides concentrations except at 1 ppm of fipronil, which had increased consumption. The flood water might have made a better distribution of termiticides in soil, making the toxin available to *C. formosanus* which might have caused reduction in the consumption. From the results on filter paper consumption, we assume that the activity of *C. formosanus* may decrease in the areas with clay soil after a flood. But in a prior choice test conducted in the laboratory by Wang and Henderson (2014), they observed that there is greater aggregation of *C. formosanus* in clay soil even when filter paper consumption was significantly decreased. The aggregation may increase the termite activity in the areas with clay soil even if food consumption is decreased.

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CHAPTER 3. SUMMARY AND CONCLUSION

The Formosan subterranean termite (*Coptotermes formosanus* Shiraki) is one of the most economically important pest of structures that causes a tremendous amount of property damage with a wide range of feeding habitats. Due to the severe economic loss by *C. formosanus*, several control measures are widely used. However, soil treatment with liquid termiticides have dominated over other control measures due to its quick action and most promising results against the *C. formosanus*. However, the efficacy of liquid termiticides applied as soil treatment is determined by several factors like temperature, moisture level, type of chemical, concentrations applied, flooding, etc. In this study, we designed an experiment to test the efficacies of termiticides impacted by flooding on *C. formosanus*. The primary purpose of this study was to determine if the efficacies of four termiticides were affected by flooding. Four commonly used termiticides- Termidor, Premise, Talstar and Altriset, varying in chemical properties were used at three concentrations, and were tested in both sand and soil substrates. The effects of flooding on the residual activities of the four termiticides were dependent on the type of termiticide, concentration applied, and type of substrate used. Based on both chemical analysis data and no-choice bioassays, among the four termiticides tested bifenthrin had the lowest loss of activity in flooded substrates followed by fipronil in both flooded and unflooded conditions irrespective of substrate, while imidacloprid was most affected by flood. Similarly, the higher doses of all the chemicals were more effective in causing mortality of *C. formosanus* after a flood.

In the first part, we tested the direct concentration loss of those four termiticides after a flood. For this, we had three different concentrations, 1, 10 and 25 ppm tested in two different substrates. From our chemical analysis report, we found that imidacloprid was the most

leachable termiticide and bifenthrin was the most persistent termiticide in both the substrates. The concentration loss of chlorantraniliprole and fipronil were in between these two extremes.

In the second part, we did the no-choice bioassay to compare the mortality of Formosan subterranean termites in pre-and post- flooded substrates. After a flood, both fipronil and chlorantraniliprole were in-effective at 1 ppm in sand but were effective at 10 and 25 ppm. However, in soil treated with fipronil, there was no effect of flooding since mortality observed was similar. In soil treated with chlorantraniliprole, there was a higher mortality after a flood than before. However, this increase in mortality was still less than an effective. Similarly, mortality was not affected by flooding in substrates treated with bifenthrin at any concentrations. After a flood, imidacloprid was in-effective at any concentrations treated in sand but in soil flood had no effect. The mortality of termites in soil were similar in both the conditions.

In the last part of our study, we compared the amount of filter paper consumption by termites. After a flood in sand, the consumption of filter paper was higher at concentrations, which had greater termite survival like in imidacloprid at all concentrations tested. There was slight decrease in consumption of filter paper in soil with imidacloprid and bifenthrin at all concentrations and fipronil at higher concentrations treated. However, this decrease was not significant.

From a practical standpoint it appears that the question of having to retreat after a flood or not depends on the chemical, soil type and concentration used. Our study supports the idea that termiticides with a higher water solubility may require a home be retreated after a flood to maintain an effective termite barrier. At all concentrations tested, chemical analysis results showed some loss in concentration of all termiticides. An interesting unexpected result that

warrants further investigation is the nature of some of the products that increased the mortality of termites after a flood.

VITA

Rajani Sapkota is originally from Nepal. She was born in a small town in Chitwan district. She completed her Bachelors of Science in Agriculture with Plant Breeding major from Institute of Agriculture and Animal Sciences, Tribhuvan University in 2014. After her undergraduate degree, she worked as a Field Officer in Forum for Rural Welfare and Agricultural Reform for Development (FORWARD Nepal). She started her master's degree in the fall of 2016 under the supervision of Dr. Gregg Henderson. Her research is focused on the study of residual effects of termiticides on mortality of Formosan subterranean termites in substrates subjected to flooding.