Stakes as Aggregation and Detection Tools for Control of Termites at Three Different Stations in Lahore, Pakistan

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Abstract: Some imported woods are well known for their durability and their characters have carried them far from their original homes. It is necessary to know the behaviour of wood species under different environmental conditions and their durability class before the timber or timber product is put into use. The extent of wood deterioration is dependent on various conditions such as soil, rainfall, altitude, temperature, and other environmental conditions under which the timber is put to use. Natural durability refers to the ability of wood species to resist attack by different agents, especially biological ones. This paper describes the natural resistance of important imported timber species of Pakistan against termites at different stations. Field experiments were carried out at three locations with the aim of evaluating the natural resistance of 18 species of imported woods. Wood stakes measuring $(L \times W)$ 19 inches x 3.0 inches were installed in the soil and observed for a period of 3 months alone and in different combinations with M.anisophilae, Fipronil and Imidacloprid. The termites active in the test yard and on the test stakes were collected and identified. Results revealed significant variation in rate of degradation of wood species with climatic zones producing significant variation as well. Termites collected belonged to three species with the single family Termitidae. While Odontotermes obesus Microtermes obesi and Coptotermes heimi was the dominant species in most of the locations.

Keywords: Wooden stakes, Fipronil, Imidacloprid, Metarhizium anisophilae, Termites

1. Introduction

The subterranean termite is truly an enigma. It is the most destructive, xylophagous pest of human structures and economically important plants [1], [2]. The most troublesome termites in agricultural are the fungus-growing termites (Termitidae). Termite fauna of Pakistan consists of 50 species. Of these nine termites species i.e. Odontotermes assmuthi, O. guptai, O. gurdaspurensis, O. horai, Microtermes mycophagus, M. obesi, M. unicolor, M. paradoxalis and Angulitermes debraensis were recorded from sugarcane field at Gojra, Punjab, Pakistan [3],[4]. They become economically important pests when they started to destroy the wood and wooden products of human homes, building materials, forests, agriculture crops and other commercial products [5]. The principal component of their diet is wood, which is also the dominant structural element of the building construction industry [6]. Their innate role as decomposers of wood and other vegetation in the natural ecosystem changes their status from beneficial insect to that of a "pest," depending on whether the subject of their attack is a fallen tree or the lumber in a home or other building. The continued growth and expansion of urbanization also creates conditions conducive to infestation by subterranean termites. Wood-framed structures, well-watered lawns, bark mulch adjacent to buildings, and firewood piles beckon to the termites to feast on the plethora [7]. The world's timber resources play an important role in the economic development of both wood-producing and importing regions [8].

Subterranean termites are widespread pests of wood structures and wood products. These wood destroying pests are a continuous threat to wood composite materials in residential and commercial structures. Wood composite panels used extensively in buildings. However, manufactured particleboard and other wood-based panel products are seldom evaluated for resistance to attack by subterranean termites. Because termites are cryptic, and severe damage often occurs but remains undetected until structural wood components are beyond repair, it is important to know the susceptibility or resistance of these materials to damage by termites [9]. As termites forage on cellulosic resources they can cause damage to living trees and many crop plants, but the fact that they can use dead wood makes them a major pest for timber used for construction purposes both outdoors and inside buildings. They are known to cause damage to buildings throughout the tropics, sub-tropics and temperate regions and have an increasing economic impact when present [10]. One factor that limits the utilization of wood for various end products is, without a doubt, its low natural durability. Knowledge of the natural resistance or susceptibility of wood is therefore of fundamental importance for recommendations concerning its most appropriate uses, particularly in the furniture and civil construction industries. Not only does this help to avoid unnecessary costs for the replacement of parts, but it also reduces the impact on residual forests by reducing deforestation [11].

Due to the organic nature of its constituent polymers, wood can have its durability compromised by a variety of biological, physical and chemical agents. By virtue of its structure and chemical composition, it suffers from attacks by various deteriorative organisms, principally fungi and termites [12]-[15]. Termites use wood as food and as a physical medium for shelter and nesting, and thus, in any circumstance, they cause damage to the wood. In contrast to fungi, termites destroy wood by burrowing in the wood structure to create galleries, consequently altering its mechanical resistance. The present study was aimed to observe the percentage damage of different stakes and the rate of infestation, by different termites. Such work may have

potential to attract maximum termites for their subsequent control through baiting technology. Wooden stakes are commonly used as the initial means of detecting subterranean termites at field sites. However, depending upon local climate and soil conditions, termite colony size, and the foraging behaviour of the particular.

2. Materials and Methods

2.1 Preparation of Wooden stakes as aggregation devices under field conditions

Wooden stakes of 15 different woods i.e. Pinus roxburghii (Chir), Morus alba (Mulberry), Mangifera indica (Mango), Populus euramericana (Poplar), Casia fistula (Amaltas), Azadirachta indica (Neem), Eucalyptus cameldulensis (Safaida), Butea monosperma (Dhaak), Syzygium cumini (Jaman), Heterophragma adenophyllum (Beri Patta), Erythrina subrosa (Gul-e-Nishtar), Albizia lebbeck (Shreen), Jacaranda mimosifolia (Gul-e-neelam), Bauhinia variegata (Kachnar) and Tectona grandis (Sagwan) were prepared. All stakes were measured to be (L x W) 19 inches x 3.0 inches respectively. At the FC College botanical garden at two different sites (station 1 and station 2) were driven vertically in a random distribution with one-half the length of each stake buried in the soil. Third site (station 3) for stakes installation was selected at Lahore canal bank in the vicinity of FC College dominated by P. euramericana plantation. Nine poplar trees were selected and five stakes per tree were driven at the base partially buried vertically (30cm deep) into soil. So a total of 45 different wooden stakes were driven. All these stakes were installed at an interval of 2 ft each. Any stake with signs of termite infestations/activity and galleries appearance after termite establishment was immediately treated independently with bait toxicant alone (Fipronil and Imidacloprid separately) and bait toxicant in combination with Metarhizium anisopliae at conidial concentration (1 \times 10⁷ conidia/ml) and the date of first infestation was recorded since the installation of stakes. Three replicates for each wooden stake were established and first served as control (Untreated), second treated with at low concentration using Imidacloprid and fipronil (0.03ppm each) separately at two different sites (as station 1 and station 2) and third treated with fungus bait toxicant in combination. The number of days from installation to first termite infestation/activity, bait treatment and feeding cessation for each station was recorded. The date of intoxication was recorded and then observations continued till the cessation of termite activity on the bait stations with further follow up observations to duration ascertain the exact of the termite elimination/suppression from the experimental sites.

3. Results

3.1 Termite activity

A total of 7546 collections of termites from 3 species and 3 genera were made during the study. The number of species increased at the 12 (week) from the date of installation of stakes. The extent of % termite infestation on different baits was 57.8% for *Odontotermes obesus*, 24.7 % for *Coptotermes heimi* and 16.5% for *Microtermes obesi* respectively. These findings clearly indicate that, for baiting

to work successfully, termites must find the bait stations so that the matrix with



Figure 1: Showed percentage damage (%) by *O.obesus*, *C.heimi* and *M. obesi* on different wooden stakes under field conditions.

toxicant can be added treated for termite consumption and transfer it back to the untreated nest mates. These bioassays were carried out at three different sites of Lahore (two at College botanical garden) and one along the canal bank in the vicinity of FC College Lahore, where *P.euramericana* plants are severely infested by *C.heimi* (Fig. 1).

3.2 Wooden stakes as aggregation devices under field conditions and termite activity

3.2.1 Station 1

Of the 45 wooden stakes 8 (17.7%) were attacked after 3 months. Mean termite activity varied significantly over time (P<0.001), with seasonal increases from June to August 2012 (Weeks 1-12). The overall mean percentage of damage was significantly different at different stakes (P<0.001), but effect of wooden stakes on termite activity interacted significantly with time (P<0.001). Average termite activity was significantly greater at *P.euramericana*, *H. adenophyllum*, *C. fistula*, *A. indica*, *E. camaldulensis* and *E. subrosa* wooden stakes respectively (Fig. 2, 3).

3.2.2 Station 2

Of the 45 wooden stakes 13 (28.8%) were attacked after 3 months. Mean termite activity varied significantly over time (P<0.001), with seasonal increases from June to September 2012 (Weeks 1-13). The overall mean percentage of damage was significantly different at different stakes (P<0.001), but effect of wooden stakes on termite activity interacted significantly with time (P<0.001). Average termite activity wooden was significantly greater at stakes of P.euramericana, В. variegata, Р. roxburghii, Н. adenophyllum, E. subrosa, M. alba, A. indica, J. mimosifolia, C. fistula, , M. indica , D. sissoo , A. excelsa and T.grandis respectively (Fig.2, 3).

3.2.3 Station 3

All the stakes partially driven vertically in to the soil (30cm deep) around the *P.euramericana* stem periphery at base of 9 plants (5 each) revealed average infestation on *Populus euramericana*> Azadirachta indica > Pinus roxburghii > Butea monosperma > Morus alba > Bauhinia variegata > Albizia lebbeck> Dalbergia sissoo > Heterophragma adenophyllum > Erythrina subrosa > Syzygium cumini >

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Mangifera indica > *Jacaranda mimosifolia* > *Eucalyptus camaldulensis* and *Tectona grandis* respectively. Of the 45 wooden stakes 15 (33.3%) were attacked by *C. heimi* after 3 months. Mean termite activity varied significantly over time (P<0.001), with seasonal increases from June to August 2012 (Weeks 1-12). The overall mean percentage of damage was significantly different at different stakes (P<0.001), but effect of wooden stakes on termite activity interacted significantly with time (P<0.001) (Fig.2, 3).



Figure 2: Showed termites activity on wooden stakes at different stations. Station 1: FC College Botanical garden; Station 2: FC College Botanical Garden; Station 3: Lahore canal bank

3.3 Treatment of stakes alone with standard sublethal concentration 0.3ppm of Fipronil

3.3.1 Station 1

Although termite infested almost every stakes to some extent, but average termite activity was significantly greater at P.euramericana, H. adenophyllum, C. fistula, A. indica, E. camaldulensis and E. subrosa wooden stakes respectively. The treatment of above infested stakes with 0.03ppm of fipronil significantly (P<0.05) effected the termite activity and gradual decline was observed after treatment. There was a significant (P<0.05) decreases in galleries construction and bimonthly observations indicated that there was no termite activity in the bait stations for an extended period of time. The complete elimination of O.obesus and M. obesi termites colony after treatment exhibited 175 days and follow up observations after elimination were carried out up to 62 days to ensure the complete elimination of the termite colonies. The performance of fipronil treated stakes revealed significant results as it had successfully eliminated termites (Table 1).

3.4 Treatment of stakes alone with standard sublethal concentration 0.3ppm of Imidacloprid

3.4.1 Station 2

Average termite activity was significantly greater at wooden stakes of *P.euramericana*, *B.variegata*, *P.roxburghii*, *H.adenophullum*, *E.subrosa*, *M.alba*, *A.indica*, *J.mimosifolia C.fistula*, *T.grandis*, *M.indica*, *D.sisso* and *A.excelsa* at station 2 respectively. The treatment of above infested stakes at station 2 with 0.03ppm of Imidacloprid significantly (P<0.05) effected the termite activity and gradual decline was observed after treatment. There was a significant (P<0.05) decreases in galleries construction. Bimonthly and seasonal observations indicated that there was no termite activity in the bait stations for an extended period of time. The complete elimination of *O.obesus* and *M.obesi* termites colony after treatment exhibited 180 days and follow up observations after elimination were carried out up to 58 days to ensure the complete elimination of the termite colonies. The performance of Imidacloprid treated stakes revealed significant results as it had successfully eliminated *O.obesus* and *M.obesi* colonies (Table1).

3.5 Treatment of stakes alone with standard sublethal concentration 0.3ppm of Imidacloprid

3.5.1 Station 3

C.heimi aggressively fed on a variety of wooden stakes indicating polyphagous behaviour of the termite. *Populus euramericana* > *Azadirachta indica* > *Pinus roxburghii* > *Butea monosperma* > *Morus alba* > *Bauhinia variegata* > *Albizia lebbeck*> *Dalbergia sisoo* > *Heterophragma adenophyllum* > *Erythrina subrosa* > *Syzium cumini* > *Mangifera indica* > *Jacaranda mimosifolia* > *Eucalyptus camaldulensis* and *Tectona grandis* were significantly effected by this termite. The treatment of infested stakes with sublethal concentration 0.03ppm of Imidacloprid



1st field feeding station





A. indica

E. camaldulensis





P.euramericana

C. fistula



H. adenophyllum



E. subrosa



J. mimosifolia



P. roxburghii



2nd Feeding station



C. fistulaP. roxburghiiM. albaA. excelaFigure 3: Wooden Stakes ranges of attack on different species by Odontotermes obesus, Coptotermes heimi and Microtermes
obesi at two different experimental sites under field conditions.

 Table 1: Showed days for activity of termites on wooden stakes treated with Imidacloprid and Fipronil at three different stations

Stations	Treatment	Stakes with termite Infestation	Termites Infestation	Installation to feeding cessation(Days)	Total days
*Station 1	Fipronil	P. euramericana, H. adenophyllum, C. fistula, A. indica, E. camaldulensis and E. subrosa	O. obesus and M. obesi	175	62
*Station 2	-	P. euramericana, B. variegata, P. roxburghii, H. adenophyllum, E. subrosa, M. alba, A. indica, J. mimosifolia C. fistula, A. excelsa, M. indica, D. sissoo and T. grandis	O. obesus and M. obesi	180 days	58
*Station3		P. euramericana, A. indica, P. roxburghii, B. monosperma M. alba, B. variegate, A. lebbeck, D. sissoo, H. adenophyllum, E. subrosa, S. cumini, M. indica, J. mimosifolia, E. camaldulensis and T. grandis	C. heimi	190days	55

*Station 1: FC College Botanical garden; Station 2: FC College Botanical Garden; Station 3: Lahore canal bank

significantly (P<0.05) impacted termite activity and galleries formation followed by gradual decline after treatment at week 16 and bimonthly observations up to week 32 indicated the suppression of the colony (Sep 2012-Jan.2013).The complete elimination of C.heimi termite colony after treatment exhibited 190 days and follow up observations after elimination were carried out up to 55 days to ensure the complete elimination of the termite colonies (Table 1).

3.6 Treatment of stakes with Fungus *M.anisopliae*-Imidacloprid combination

3.6.1 Station 1

When stakes of station 1 were treated to a combination of 0.03ppm Imidacloprid plus the entomopathogenic fungus *M.anisopliae* at 1×10^7 conidia/ml, a reduction in termite activity was observed, when compared to the control. The combination treatment of Imidacloprid and M.anisopliae played a significant role in decline the survivorship of M.obesi and O.obesus. In both O.obesus and M.obesi. Imidacloprid decreased Termites activity and survivorship to introduced M.anisopliae after 12 weeks (Sep-Nov 2012) of treatment to wooden stakes. Termites activity on different wooden stakes concurrently exposed to M.anisopliae and Imidacloprid was comparatively lower than the activity of termites exposed to either treatment alone. Furthermore, at last week of Sep 2012, the difference was more pronounced and hence decreasing activity in case of both termite species exposed to the highest concentration of *M.anisopliae* (1×10^7) conidia/ml-0.03ppm of Imidacloprid) in combination. After 24 week a follow up study (from Dec 2012-Mid May 2013) revealed complete absence of termite activity on all treated wooden stakes. Therefore, Lower termite activity was observed here and importantly a significant interaction between fungus and Imidacloprid with 100% termite elimination and termite activity (F=,p<). The number of days to first termite activity, on any one of the bait station installed in the field, for all bait systems, ranged from a low of 49 days to high of 84 days. There was no significant difference between groups when comparing (t-test) days to first termite activity data from three sites for each baiting system (p= 0.0, 0, 0) (Table 2).

3.7 Treatment of stakes with Fungus *M.anisopliae*-fipronil combination

3.7.1 Station 2

When stakes of station 2 were treated to a combination of 0.03ppm fipronil plus the entomopathogenic fungus *M.anisopliae* at 1×10^7 conidia/ml, a reduction in termite activity was observed, when compared to the control. The combination treatment of fipronil and *M.anisopliae* played a significant role in declining the survivorship of O.obesus and M. obesi. In both O.obesus and M. obesi, fipronil decreased termites activity and survivorship to introduced M.anisopliae after 11 weeks (Sep- 3rd week of Nov 2012) of treatment to wooden stakes. Termites activity on different wooden stakes concurrently exposed to M.anisopliae and fipronil was comparatively lower than the activity of termites exposed to either treatment alone. Furthermore, at 3rd week of Sep 2012, the difference was more pronounced and hence decreasing activity in case of both termite species exposed to the highest concentration of *M.anisopliae* $(1 \times 10^7 \text{ conidia/ml-0.03ppm of})$ fipronil) in combination. After 24 week a follow up study (from Dec 2012-Mid May 2013) revealed complete absence of termite activity on all treated wooden stakes. Therefore, Lower termite activity was observed here again and importantly a significant difference between fungus and fipronil with 100% termite elimination and termite activity (F=, p<). The number of days to first termite activity, on any one of the bait station installed in the field, for all bait systems, ranged from a low of 46 days to high of 80 days. There was no significant difference between groups when comparing (t-test) days to first termite activity data from three sites for each baiting system (p= 0.0, 0, 0). These findings clearly indicate that fipronil plus fungus interaction pronounced slightly faster results than the Imidacloprid plus fungus combination. So it is evident that Imidacloprid combination with fungus is comparatively much slower in action than that of fipronil fungus interaction (Table2).

3.8 Treatment of stakes with Fungus *M.anisopliae*-Imidacloprid combination

3.8.1 Station 3

When infested stakes (*Populus euramericana* > Azadirachta indica > Pinus roxburghii > Butea monosperma > Morus alba > Bauhinia variegata > Albizia lebbeck> Dalbergia sisoo > Heterophragma adenophyllum > Erythrina subrosa > Tectona grandis> Mangifera indica > Jacaranda mimosifolia

> Eucalyptus camaldulensis and Syzium cumini of station 3 were treated to a combination of 0.03ppm Imidacloprid plus the entomopathogenic fungus M.anisopliae at 1×10^7 conidia/ml, a reduction in termite activity was observed, when compared to the control. The combination treatment of Imidacloprid and M.anisopliae played a significant role in decline the survivorship of C.heimi. Imidacloprid decreased termites activity and survivorship to introduced M.anisopliae after 13 weeks (Sep-Nov 2012) of treatment to wooden stakes. Termites activity on different wooden stakes concurrently exposed to M.anisopliae and Imidacloprid was comparatively lower than the activity of termites exposed to either treatment alone. Furthermore, at Ist week of Oct 2012, the difference was much more evident and hence decreasing activity. After 24 week a follow up study (from Mar 2013-Mid May 2013) revealed complete absence of termite activity on all treated wooden stakes. These results indicated that a significant interaction between fungus and Imidacloprid exist on termite mortality with synergistic effects (F=p<).

The number of days to first termite activity, on any one of the bait station installed in the field, for all bait systems, ranged from a low of 52 days to high of 90 days. There was no significant difference between groups when comparing (t-test) days to first termite activity data from three sites for each baiting system (p=0.0, 0, 0) (Table 2).

Table 2: Showed days for activity of termites on wooden

 stakes treated with Imidacloprid and Fipronil in combination

 with *M.anisophilae* at three different stations.

with <i>M.anisophilae</i> at three different stations.							
Sites/	Treatments	Termite	Infestation	Termites			
Stations		Activity	period				
		days					
*Station 1	M. anisopliae-	49 to 84	Dec 2012-Mid	M. obesi			
	Imidacloprid	days	May 2013	and <i>O</i> .			
				obesus			
*Station 2	M. anisopliae-	46 to 80	Dec 2012-Mid	M. obesi			
	Fipronil	days	May 2013	and <i>O</i> .			
	_	-		obesus			
*Station 3	M. anisopliae-	52 to 90	Mar 2013-Mid	C. heimi			
	Imidacloprid	days	May 2013				

*Station 1: FC College Botanical garden; Station 2: FC College Botanical Garden; Station 3: Lahore canal bank

4. Discussion

The percentage of wood materials or wood stakes with signs of termite infestation was much greater than that with foraging termites. This might be related to the termite foraging behavior, change in suitability of wood materials for termite foraging, and other factors such as weather and predators. The wooden stakes were principally infested by the following subterranean termite species: Odontotermes obesus (Rambur), Microtermes obesi and Coptotermes heimi (Wasmann) (Isoptera: Rhinotermitidae) at different stations. The first species was collected 89 days after the experiment was started. The highest % termite infestation was observed by O.obesus, 57.8% while lowest infestation was observed by M. obesi i.e. 16.5% respectively while C.heimi showed highest infestation on P.euramericana which was 24.7% respectively. While during infestation period at station 1 and 2 of the 45 wooden stakes 8 (17.7%) and 13 (28.8%) were attacked after 3 months. However, at station 3, of the 45 wooden stakes 15 (33.3%) were attacked by C. heimi after 3 months. [16] investigated the resistance of different wooden stakes on the basis of visual damage assessment in the soil for a period of 4 year; they found Termites belonged to nine species under four genera and the single family Termitidae. While Odontotermes obesus was the dominant species in most of the locations. [17] noted that termite infestation was higher during June to September wood degradation by termites was most during the months of April to June, moderate from July to October and less from November to March. The more degradation from April to June might be attributed due to pre monsoon showers, which provides necessary amount of moisture required for the termite survival. Degradation was less from November- March which, might be possibly due to higher atmospheric temperature and lower amount of relative humidity both act as a constraint for the foraging behaviour [18]. It is possible the approximately one-month period between that installation of the stakes and inspection was not sufficient for the termites to locate the stakes. It is more likely, however, that the termites had sufficient wood and only attacked the stakes if a subterranean foraging tunnel directly contacted them. The condition of the wood stakes may also affect feeding activity and it is possible that the stakes had to age and decay to become as attractive as bark. Decomposing wood has greater concentrations of sucrose and more associated yeasts than sound wood [19]. In addition, fungi that are associated with wood decay are often consumed by termites [20], and these fungi are rich in urea [21].

Stakes when treated with sublethal dose of Imidacloprid and Fipronil termite activity was gradually declined. Station 1 and 2 was highly infested by O.obesus and M. obesi respectively. This suggests that agonistic response may have occurred between colonies of these two species, which probably resulted in a takeover of the O.obesus foraging site by M.obesi. Interspecificity encounters among termite colonies resulting in agonistic response were observed by [22], [23]. Field observation by [24] revealed two incidents in which foraging sites of *Reticulitermes flavipes* (Kollar) were taken over by Coptotermes formosanus Shiraki. Whereas, C.heimi was dominant station 3 because vegetation cover was surrounded by P.euramericana. Resistance is a critical determinant of life span of tree species. Many heartwood species are known for their resistance against degradation [25]. Tree species such as T. grandis [26]-[30], S. robosta [31], Shorea sp. [32], S. marcoptera, Dryobalanops sp., and Xylia dolabriformis [33]-[35], and Pterocarpus soyauxii [36] are well-known for their durability from ancient times. In our study, T.grandis was found to be moderately resistant against degradation under Pakistani conditions.

In our study the combined formulation or application of *M.anisopliae* with Imidacloprid and Fipronil played a significant role in decline the survivorship of *M. obesi O.obesus* and *C.heimi* at all stations and more effective as compared to alone treatment. Similarly, the findings of [37] also explained the integration of insecticides acetamiprid, Imidacloprid and Thiamethoxam with fungal conidia on the basis of compatibility in order to enhance the biocontrol potential of entomopathogenic fungi. The nature of fungal repellency by the species within the order Isoptera has been

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widely assessed in the laboratory [38]-[39]. This type of avoidance behaviour is very important for the management of insect pests like termites residing in difficult-to-reach locations, such as in underground nests. Similarly, [40] found that organic mulches supplemented with *M. anisopliae* significantly repelled foraging *C. formosanus* and reduced mulch consumption by up to 71%.

5. Conclusions

The present study clearly indicates that the addition of termiticides to *M. anisopliae* has pronounced positive effect in the control of termites in natural environment. The present investigation showed that biocontrol of termite pest with entomopathogenic fungus was impressive under experimental conditions and had high prospects in controlling termite. However, the efficacy of bio-agents is dependent on physio-chemical and biological properties of soil and climatic factors as well as the degree of susceptibility of host species [41], [42].

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