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TRG 11 Proceedings

Contents

Diversity and evolution of symbiotic protist communites in termites especially focused on genus *Reticulitermes*

by

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Abstract

The symbiotic protist communities of termites and wood-feeding *Cryptotercus* cockroaches are characterized by strong host specificity of its species composition. Cluster analysis based on the similarity of symbiont generic composition and a phylogeny comparison between the hosts and the symbionts revealed that cospeciation or cocladogenesis was the main determinant of the symbiont composition. Similarly, in *Reticulitermes* hosts in Japan, patterns of the symbiont communities reflected cospeciation and geohistorical events. Host hybridization induced symbiont community mixing and resulted in asymmetrical inheritance of protists from parent species. Hybridization or cannibalistic attack between different host lineages and following community mixing probably affected the evolution of the symbiotic protist communities.

Keywords: protist, symbiosis, community, cospeciation

Introduction

Lower termites and wood-feeding *Cryptocercus* cockroaches have symbiotic protist communities in their hindguts. The protist symbionts belong to two phyla Parabasalia and Preaxostyla and six orders Trichomonadea, Tritrichomonadea, Trichonymphea, Cristamonadea, and Spirotrichonymphea and Oxymonadea, respectively (Adl *et al*. 2012, Cepicka et al. 2010). Cellulases produced by the symbionts are essential for the host termites to digest their food, while the symbionts also depend on their hosts for food and an anaerobic habitat (Honigberg 1970, Inoue *et al*. 2000, Brune and Ohkuma 2011). The number of symbiont species in a host's hindgut range from one (several termite genera in the Rhinotermitidae) to 26 (*Cryptocercus punctulatus*) (Kitade 2004). The species composition of a symbiont community is usually specific to a host species (Kirby 1934, Honigberg 1970, Ohkuma and Brune 2010), probably reflecting the transmission mode of the symbionts. The anaerobic symbionts are transmitted between individuals in a colony through proctodeal trophallaxis, and a newly founded colony should follow the symbiont faunae of the king and queen (Inoue *et al*. 2000). Obligate mutualism between the termites and protists suggests long-time cospeciation (cocladogenesis) that should be reflected in the pattern of symbiont communities.

Diversity and similarity of protist communities and cospeciation between hosts and symbionts

Based on data from a checklist by Yamin (1979), taxonomic papers, and direct observation of 12 termite species, we evaluated the similarity of symbiont genetic composition between host genera to understand the overall patterns of symbiont community similarity (Kitade 2004). UPGMA cluster analysis based on Jaccard's Similarity revealed a clear tendency that symbiont composition of host genera belonging to the same family or monophyletic family groups were similar. This trend was particularly remarkable in the clades of Kalotermitidae and (Rhinotermitidae + Serritermitidae). These results suggest that host phylogeny is a main determinant for the pattern of symbiont composition. Intriguingly, the genus *Reticulitermes* (Rhinotermitidae) had a fauna closely similar to *Hodotermopsis* (Termiopsidae), suggesting the possibility of horizontal transfer of symbionts between ancestors of these lineages (Kitade 2004, Lo et al. 2011). The strong effect of host phylogeny in structuring protist community composition and traces of symbiont horizontal transfer were also suggested by the recent exhaustive investigation of protist SSUrDNA using pyrosequencing (Tai et al. 2015). Molecular phylogenetic analysis of Rhinotermitid hosts and the symbiont genus *Pseudotrichonympha* (Noda et al. 2007) inferred almost identical topology for hosts and symbionts, indicating vertical transmission of *Pseudotrichonympha* and cospeciation.

Diversity and similarity of protist communities of *Reticulitermes* spp. in Japan

Reticulitermes in the Japan Archipelago are the most intensively studied termites for diversity and similarity of symbiotic protist communities. Field investigations revealed species-specificity for the 15 species of protist from six species of *Reticulitermes* (Kitade & Matsumoto 1993). Intercolonial variation (beta-diversity) of the symbiont community is low. UPGMA cluster analysis based on the similarity of symbiont composition divided host species into four groups, which corresponded to the geographical distribution of hosts and the host phylogeny inferred from mitochondrial genes. The largest difference in the fauna corresponded to the Tokara Strait, the biogeographical boundary between the Paleoarctic and the Oriental regions. These results support the strong effects of the host phylogeny and a causal paleogeological event on the symbiont communities.

Community mixing caused by the host hybridization

Hybridization of the different host species followed by symbiont community mixing is a likely mechanism for the symbiont horizontal transfer. However, the process(es) that resulted in the re-organizing process of the symbiont communities after the community mixing is totally unknown. We artificially produced hybrid colonies using *Retculitermes speratus* and *R. kanmonensis* alates and observed the prostist species compositions of the members of the hybrid colonies. Hybridization resulted in symbiont community mixing between the king and queen and their mixed-type symbiont faunae were initially transferred to the offspring. However, after 700 days members of most colonies had symbiont communities closely similar to *R. speratus*. These results show that the final protist communities that resulted from the original mixing were not a random assemblage. The community structure of the parent host termites were stable, probably reflecting coadaptaion. The symbiont transmission mode in the community re-organization process was strongly asymmetric. Horizontal transfer with strongly asymmetrical symbiont succession must have occurred in the ancestors of *Reticulitermes* and *Hodotermopsis*, which resulted in *Hodotermopsis*-like symbiont communities in the *Reticulitermes*.

Conclusion

The similarity in patterns of the symbiotic protist faunae and phylogeny congruence between hosts and symbionts indicated that current symbiont species composition has been strongly structured by cospeciation. Hybridization or cannibalistic attack between host lineages followed by symbiont community mixing is regarded as an occasional but important determinant in symbiont community evolution. Protist community mixing and asymmetrical symbiont inheritance have possibly functioned as a mechanism by which host termites obtain novel ligno-cellulose degradation systems.

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Biogeography history, morphological variation of *Macrotermes* gilvus and patterns of interaction specificity with its fungal symbiont, *Termitomyces sp.* in Southeast Asia

by

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Abstract

The Southeast Asia Sunda archipelago harbours a rich biodiversity with a substantial proportion of endemic species. Various environmental forces were identified to drastically influence the evolutionary history of these species including tectonic movements, glacial cycles, climatic changes and volcanic activities. *Macrotermes gilvus* (Hagen), an indigenous subterranean and fungus-growing termite species of Southeast Asia is well suited to investigate the relative impact of these forces in shaping the natural distribution of this species across Southeast Asia. This paper summarizes the biogeography history, morphological variation, and fungal symbiont interaction specificity of *M. gilvus* which may serve as a model of generalisation for early terrestrial dispersal pattern across Southeast Asia and additionally provide an insight into the termite-symbiont mutualism in a complex insular environment of Southeast Asia.

Key words: termite, biogeography, Southeast Asia, dispersal

Introduction

Island archipelagos render dynamic ecosystems for the study of biological diversifications and have been the subject of many foundational works in the field of evolution and biogeography (Darwin 1859, MacArthur and Wilson 1967).The present distribution of plants and animal in Southeast Asia may owe much more to the last one million years or so in the Pleistocene epoch than the preceding 30 million years (Hall 1998). The significance of the periodic land connections to the evolution of the Sunda shelf fauna has been well demonstrated for some animal groups such as non-volant mammals, shrews, bats, water snakes and frogs (Heaney 1986, Ruedi 1996, Schmitt et al. 1995, Inger and Voris 2000), but remains to be investigated in a phylogeographical framework for the most diverse group of soil organism – the termites.

Termites are excellent indicators of biogeographic and evolutionary studies due to their wide functionality in native habitats and sensitivity to landscape disturbances reflective of historical patterns (Jones and Eggleton 2000, Gathorne-Hardy et al. 2002). *Macrotermes gilvus*, an indigenous fungus-growing termite of Southeast Asia offers great potential in understanding the phylogeographic history of regional fauna. This species is endemic and ubiquitous in distribution throughout Southeast Asia from Indochina to Malayan peninsula, Sumatra, Java, Borneo and the Philippines (Roonwal 1970). It is a poor disperser and transoceanic dispersal is improbable in this species (Neoh and Lee 2009). Therefore, the genetic signature from this species should reflect a historical vicariance rather than contemporary gene flows between different regions of Southeast Asia. This species cultivates fungal symbiont which gives additional perspective in understanding the termite-symbiont mutualism specificity in an insular environment such Southeast Asia.

Phylogeography becomes useful as a conceptual umbrella to weigh relative merits of oft-competing vicariance and dispersalist scenarios invoked to account for the origins of spatially disjunct taxa (Ronquist 1997). Vicariance is considered when related populations became detached as the continuous ranges of ancestral forms were sundered by environmental forces (Myers and Giller 1988). Alternatively, dispersal is important to phylogeography through rare long-distance movements which often implicates the colonization of new habitats such as oceanic islands (Briggs 1974, Freeland 2005). Knowledge on these historical processes is of utmost importance in understanding the natural history or evolutionary processes that shaped the spatial structure of local taxa.

This paper summarizes the natural history of *M. gilvus* by investigating its biogeography, morphological variation and pattern of interaction specificity with its fungal symbiont *Termitomyces sp*. across Southeast Asia by means of a phylogeographic approach.

Biogeography History of *M. gilvus*

The consistency of results between the mitochondrial DNA and microsatellites markers suggest that the phylogeographic pattern of *M. gilvus* was most likely relics from Pleistocene environmental changes. Even at microsatellite loci, contemporary processes seemed not to influence the historical processes that shaped the phylogeographic structure of this species. Most genetic differentiation was associated with Pleistocene geographical division, which remains distinct despite temporary connections. Gene flow between geographical groups was rare. An obvious exception to this is a strong genetic propinquity between Malayan peninsula and North Sumatra populations despite the presence of a formidable physiographic barrier – the straits of Malacca separating the two regions. This either suggests ongoing gene flow and/or a recent dispersal event in order to explain the narrow time factor suggested by the lack of distinct genetic differentiation. Unexpectedly, a significant genetic divergence was observed within populations on Borneo and Sumatra that can be best explained by multiple, independent dispersal events rather than vicariance. There was no direct connectivity inferred between Borneo and Sumatra. This finding is of paramount importance as it elucidates the position of Java as a central biogeographical link connecting the two major islands. Phylogeographic analysis also suggests a likelihood of M. gilvus introduction into the Philippines and/or northern Borneo from mainland Indochina.

The range expansion of *M. gilvus* was detected during the early Pleistocene, spreading out of Indochina towards Java and the Philippines reaching the modern distribution across Southeast Asia by the end of late Pleistocene.

Morphological Variations

Morphological variation in *M. gilvus* was highly concordant to the phylogeographic structure inferred by molecular data. Specifically, the genetic subdivision observed in Sumatra and Borneo was consistent with the significant morphological sub-structure displayed by discriminant function analysis. Moreover, the close genetic association between Malayan Peninsula, Singapore and North Sumatra was well supported by the morphological similarity between their soldier castes. Similarly, the inferred migratory route between Indochina and Java based on the mtDNA data was supported by the morphological similarity observed between the two regions. The Philippines termites stood out by being the smallest in size. By contrast, Riau and West Sumatra were among the largest, particularly in head size and tibial length. It is worth noting that the termites of Thailand were intermediate in size when compared to the rest of the geographical groups in the discriminant dimensions, suggesting a potential ancestral phenotype. Overall, present data displayed a clear correlation between genetic data and morphometric similarity on a large spatial scale, an indication that morphology matches genetics. Therefore, it can be hypothesized that the morphometric variation of M. gilvus across Southeast Asia was a result of geological evolution rather than a case of phenotypic plasticity. Nevertheless, on a small spatial scale in Java, previous records of various morphological forms or variants of M. gilvus can be relegated as a single geographical race most likely due to nutritional phenotypic plasticity.

Termite-Symbiont Mutualism

There are at least three genetically distinct types of fungal symbiont associated with this fungus-growing termite. The host termite was found to be promiscuous in symbiont acquisition. In turn, the symbionts were specific to the host. Such interaction may benefit the host by increasing chances of the foraging termites to pick up viable spores from the environment during colony establishment. Divergence dating demonstrated that the fungal symbiont co-diversified with host, *M. gilvus* during its initial radiation in Southeast Asia. Investigation of the host-symbiont interaction specificity revealed that the geographical variation of the fungal symbiont in relation to the host termite was not exclusive. On the one hand, the fungal distribution shows no support for the genetic subdivision of *M. gilvus* in Borneo, since only a single *Termitomyces* type was found (type A). On the other hand, the patchy distribution of type C in North Sumatra and type B in Riau/West Sumatra displayed excellent support for the termite subdivision in Sumatra. This means that genetic differentiation in the host termite may not necessarily reflect the genetic differentiation of the fungal symbiont.

Conclusion

Given the complexity of the natural history of Southeast Asia, there is no single force which single-handedly shapes the phylogeographic signature of *M. gilvus* in the region. In general, phenotypic variations were in accord with genotypic differentiation in *M. gilvus*. The divergence time suggests a co-diversification between the host, *M. gilvus* and its fungal symbiont, *Termitomyces* sp. however, the relationship was not completely exclusive. The natural history of *M. gilvus* has shed light on the early dispersal pattern of terrestrial fauna across Southeast Asia and additionally gives insight on the host-symbiont mutualism scenario in the frequently changing environment of Southeast Asia.

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No paper was received from the authors. Please contact the authors for further information.

Interaction between sexual and parthenogenetic incipient colonies of *Reticulitermes speratus*

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Abstract

Reticulitermes speratus can found a colony by two female dealates in the laboratory and reproduce by parthenogenesis. On the other hand, we could not find female-only colonies that reproduced exclusively by parthenogeneis during a survey of 149 field colonies in Japan. The reproductive success of the parthenogenetic field colonies, even if it is present, should be much smaller than ordinary sexual colonies. It is likely that the aggregated distribution of incipient colonies results in strong competition between sexual and parthenogenetic colonies, which the latter lose.

In this study, we produced 400 sexual (male-female) colonies and 242 parthenogenetic (female-female) colonies using *R. speratus* alates, and examined colony compositions after 300 days. We then transplanted 20 pairs of the sexual and parthenogenetic colonies adjacent to each other, and observed interactions for 90 days. Survival rate and size of the parthengenetic colonies (18.1% and 18.9 ± 6.9) on day 300 were significantly lower than the sexual colonies (42.8% and 42.2 ± 18.4). Nymphs and nymphoids differentiated only in the parthenogenetic colonies as the X-linked caste determination model predicted. During the colony interaction experiment, eight colony pairs fused. Both reproductives from the perthenogenetic colonies were killed in six of the fused colonies. Among 12 colony pairs without colony fusion, parthenogenetic colonies died in seven pairs and partial offspring incorporation occurred in nine colony pairs. During the interaction, the survival rate of reproductives from sexual colonies was 95.0%, while that from parthenogenetic colonies was 27.5%. Our results indicate that the parthenogenetic colonies are much less competitive during intercolonial interactions. On the other hand, frequent colony fusion and offspring incorporation should allow invasion by individuals with a "nymph-oriented genotype" into sexual colonies, which would have had a tendency to become secondary queens even in the presence of the original queen.

Keywords: parthenogenesis, intraspecific competition, incipient colony, *Reticulitermes*

Comparative efficacy of kempas and rubberwood dip-treated in proprietary insecticides cypermethrin and permethrin between short dipping durations under low or high humidity Malaysian H2 hazard class conditions against *Coptotermes curvignathus*

by

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Abstract

Publicly available water-borne organic agro-insecticides normally contain prescribed spray-on solution strengths for crop protection against specific insect crop pests and also termites. The objective of this study was to examine the efficacy of cypermethrin and permehrin products to protect dip-treated wood against *Coptotermes curvignathus* at the prescribed use rates meant for specific insect crop pests. This study compared dipping times (3 sec versus 60 sec) in organic water-borne insecticide products at concentrations not exceeding the prescribed spray solution strengths (prescribed for cypermethrin: 0.0055%g/g; permethrin: 0.0053%g/g) for termite control on kempas (*Koompassia malaccensis*) heartwood and rubberwood (*Hevea brasiliensis*) sapwood test blocks exposed to two variants [low (LH) versus high (HH) humidity] of H2 Hazard class (above-ground, protected from light, rain and flooding). Results showed that wood dip-treated for 60 sec. had higher insecticide retentions (%g/g) than that for 3 sec. although such differences were significant (P<0.05) only for treated rubberwood. Untreated rubberwood blocks were destroyed at both H2 hazard class exposures (HH: mean mass loss 93.9%, mean AWPA termite rating 1.2; LH: mean mass loss 91.7%, mean rating 0), while untreated kempas sustained rather severe attacks [HH: mean mass loss 39.0%, mean termite rating 5.5, LH: mean mass loss 48.2%, mean rating 5.7]. Furthermore, termiticides with prescribed or reduced concentrations regardless of dipping times and H2 hazard class situations, failed to protect rubberwood [mean mass losses >70%, with mean AWPA termite ratings ≤3.8]. Contrasted with kempas, based on AWPA ratings, the best termite control was not at the prescribed (maximum) solution strengths for these insecticides with no consistent trends between HH and LH H2 hazard classes, or dipping times [mean termite ratings of 8.7 (0.0055% cypermethrin at LH, and 3 sec; 0.00275% cypermethrin at HH and 3 sec) and rating 9.1 (0.001325% permethrin at LH and 60 sec)]. In conclusion, treated rubberwood was more susceptible to termites than treated kempas. Overall kempas treated with cypermethrin and permethrin at their best performing concentrations were quite well (but not completely) protected, implying that a higher minimum dip-treatment pesticide concentrations than that prescribed in the product labels for spray-on crop protection, would be required to prevent attack of both woods exposed to H2 hazard class situations.

Keywords: kempas, rubberwood, cypermethrin, permethrin, dip-treatment, H2 hazard class, *Coptotermes curvignathus*

Ectoparasite Fungi Species Found on Subterranean Termite *Resticulitermes* spp. in Japan

by

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Abstract

Reticulitermes spp. are one of the important termite pests in Japan. They cause damage to wooden houses and structures. Various deterrents and control strategies are currently used by applying chemical treatments or physical barriers against these pests. The extensive application of chemical pesticide can cause environmental problems and insecticide resistance. Therefore, over five decades studies on termite control using biological agents such as fungi have been reported, but none have investigated ectoparasitic species. There are 20 fungi species classified as parasites of termites, found all around the world. This study was conducted to develop the potential of ectoparasitic fungi as termite biocontrol agents by identifying those species occurring on *Reticulitermes* spp. in Japan. It would be the first national survey in Japan. Two to five colonies of *Reticulitermes* spp. were collected from 17 locations (from Okinawa Prefecture to Hokkaido Prefecture) in Japan. Termites nesting in tree branches were brought back to the laboratory and a subsample consisting of 500 workers and 20 soldiers were separated from the debris then frozen for two hours prior to observation. Termites were individually examined under a dissecting microscope to observe and identify ectoparasitic fungi. Three species of ectoparasitic fungi are commonly found, namely *Laboulbeniopsis termitarius*, *Antennopsis gallica*, and *Termitaria* sp. These fungi species are reported to cause negative impact to their hosts i.e. decline in the activity but rarely cause disease symptoms, although death can occur over time. Therefore, these fungi have potential to be developed as termite biocontrol agents.

Keywords: *L. termitarius*, *A. gallica*, *Termitaria* sp., ectoparasite, *Reticulitermes* spp.

Case Studies of Controlling Subterranean Termites Using Noviflumuron Baits

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Introduction. The first termite bait product became available in 1994 in the U.S. and soon became a common method for controlling subterranean termites in the U.S. (Su and Scheffrahn 1998, Grace and Su 2001, Thoms et al. 2009). This method of termite control uses much less insecticide than a liquid trench treatment. Here, I present two case studies showing the efficacy of noviflumuron baits in eliminating *Reticulitermes flavipes* (Kollar) colonies in single homes. I also discuss the challenges of the current baiting methods for termite control.

House 1 (West Lafayette, Indiana). This single home had two stories and a basement. Termite damage was noticed by home owner in 2001. In-ground stations (Sentricon Termite Elimination System™ , Dow AgroSciences, Indianapolis, IN. USA) were installed around the house perimeter at approximately 3 m intervals on 31 May 2001. Each monitoring station contained two pieces of wood as bait. The stations were monitored every 2-4 weeks. Termites were first found in one station three months after installation. Two additional stations were installed beside each station with termites during periodic inspections. Among the 38 stations installed, seven of them had active termites on 28 July 2002. Six of these active stations were replaced with 0.25% noviflumuron bait (Recruit III). Termite activity disappeared 56 days after treatment. A total of 3.6 tubes of baits were consumed before termites were eliminated.

House 2 (Frankfurt, Indiana). This single home was similar to House 1. Termite damage was noticed by home owner for many years prior to this study. The home owner sprayed pyrethroids to the damage sites in the past. In-ground Sentricon termite monitoring stations were installed around the house perimeter at approximately 3 m intervals on 22 May 2002. One above-ground station (AG station) was installed on the bathroom wall where live termites were found. The bait stations were monitored every 2-4 weeks. Termites were first found in one under-ground station two weeks after installation. Termites appeared in the AG station 36 days after installation. Red dyed paper rolls were installed in the AG station where found termites. Red colored termites were later found in three under-ground stations located on the west, northwest, and southeast of the house, indicating termites around the house belonged to one population. On 9 August 2002, the AG station was replaced with 0.5% noviflumuron bait (Recruit III). After 264 days, 3.75 AG stations were consumed and termite activity disappeared.

Discussion. Termite baits containing chitin synthesis inhibitors were effective in eliminating subterranean termite colonies (*Reticulitermes* and *Coptotermes* species) in previous studies (Messenger et al. 2005). A downside of the bait treatment is the need for periodic inspection of the monitoring stations. To reduce the need for periodic monitoring, more durable bait matrices were being adopted and toxic baits were installed on day zero instead of being installed when termites appear in the monitoring stations. However, this approach increases insecticide use, materials expense, and may potentially reduce efficacy as result of infrequent monitoring.

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Transcriptome analysis of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

by
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Abstract

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most economically important termite pests in the world. It has been studied using the transcriptome-based conventional Sanger sequencing method. Next generation sequencing methods, which could generate over one billion bases of high-quality DNA sequence have yet to been applied to *C. formosanus* transcriptome research.

To facilitate future research on this subterranean termite, a cDNA library of pooled castes - worker, soldier and reproductives was constructed and used for transcriptome sequencing. A total of 11.02 GB of 125-bp paired-end clean reads were generated using the Illumina HiSeq™ 2500 platform. The obtained reads were assembled into 189,421 unigenes with mean length and N50 length of 629 bp and 974 bp, respectively. In total 61,407 unigenes were annotated in the NCBI non-redundant protein (NR) database. Species distribution showed that 35% NR annotated unigenes were homologous to genes in *Trichomonas vaginalis*, which may be an important symbiont of *C. formosanus*. Moreover, 16,552 unigenes were assigned to 3,995 gene ontology terms, with 2,208 terms in biological processes, 511 in cellular components, and 1,276 in molecular functions. Using the Kyoto Encyclopedia of Genes and Genomes pathway database, 16,444 unigenes were mapped onto 221 pathways. A total of 9,054 simple sequence repeat (SSR) markers were identified. Mono-nucleotide repeats were the most common repeat motif, accounting for 42.42%, followed by di-nucleotide repeats at 29.20%.

Our data provides the most comprehensive sequence resource available for a subterranean termite and demonstrates that the Illumina sequencing allows *de novo* transcriptome assembly and gene expression analysis in a species lacking genome information. We anticipate that next generation sequencing technologies hold great potential for the study of the transcriptome of other non-model organisms.

Key words: *Coptotermes formosanus*, *de novo* transcriptome assembly, functional annotation, SSR markers

Nest-site preference and founding success of the western drywood termite, *Incisitermes minor* in six commercial timbers

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Abstract

An evaluation of nest-site selection preference and founding success of the western drywood termite, *Incisitermes minor* (Hagen) in natural conditions was conducted. Three Japanese timbers: Hinoki (*Chamaecyparis obtusa* Endl.), Karamatsu (*Larix leptolepis* Gord.), and Sugi (*Cryptomeria japonica* D. Don); and three USA timbers: Douglas fir (*Pseudotsuga menziessi* Mirbel), Western red cedar (*Thuja plicata* Donn ex D. Don) and Spruce (*Picea sitchensis* Bong. Carriere), with 80 pieces for each species, were used in this study. All timbers were made up of a combination of sapwood and heartwood with dimensions of 50 (R) x 50 (T) x 300 mm (L), and were laid at random positions in a highly infested attics in four houses in Wakayama Prefecture, Japan. Timbers were arranged in “a close gap (CG)” on the edge of two timbers and “an open gap (OG)” on the other edge. CG is a position in which the edge of two timbers are touching each other, while OG is a position in which the two timber edges are separated by a ~1 cm gap. Annual monitoring was conducted in November, two months after the *I. minor* swarming season, from 2012 - 2015. We recorded a total 344 *I. minor* nest founding events with 99.1% infestations from nuptial flights and 0.9% from colony invasion. The results suggest that the ranking of nest founding preference was Hinoki > Spruce > Western red cedar > Sugi > Douglas fir > Karamatsu. About 79.8% nest-founding activities were found in sapwood, while heartwood and borders encountered 16.0% and 4.2%, respectively. *Incisitermes minor* most preferred CG at 81.7%, followed by the bottom side, OG, upside, and cross section by 10.8%, 4.1%, 2.3% and 1.2%, respectively.

Key words: nest founding, nest-site selection, commercial timbers, *Incisitermes minor*

2D NMR study on structural alterations of wood cell walls during digestion by a lower termite, *Coptotermes formosanus* Shiraki

by

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Abstract

Termites are effective in degrading wood cell walls. Although degradation of cell wall polysaccharides in the termite digestive system has been extensively studied, lignin degradation by termites remains unclear. We applied modern multi-dimensional NMR techniques to further study cell wall degradation in termite digestive system. Sugi and Buna sapwood samples were fed to workers of *Coptotermes formosanus* Shiraki, and the fecal material subjected to detailed structural analyses using high-resolution 2D NMR as well as a series of wet-chemical analyses. Cell wood residues (CWRs) isolated from the termite fecal material and the original wood sample were comparatively characterized by 2D HSQC NMR using the direct cell wall dissolution/swelling method. The results suggested that lignin signals were much augmented, while polysaccharide signals were considerably depleted in the spectra of the fecal samples, compared to those of the original wood samples. The result was in line with the compositional data obtained by thioglycolic lignin assay and neutral sugar analysis, and collectively suggest that polysaccharides were preferentially decomposed over lignin during digestion by *C. formosanus*. In both the fecal and original wood cell wall spectra, typical lignin aromatic and side-chain signals were still clearly visible, suggesting that lignin decomposition/modification during termite digestion was not drastic. However, it appeared that syringyl lignin signals were slightly depleted over guaiacyl lignin signals in Buna faces compared to original Buna wood samples

Keywords: 2D NMR, wood cell walls, termite digestive system, *Coptotermes formosanus* Shiraki

A comparative study on the complete mitochondrial genome sequences of 5 species in the genus *Macrotermes*

by
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Abstract

The genus *Macrotermes* is group of fung-growing termites belonging to the family Macrotermitinae (Isoptera: Termitidae). The complete mitochondrial genome was sequenced and annotated for 2 species, *M. annandalci* and *M. yunnanensis* collected in Yunnan. These are the 4th and 5th whole termite mt genomes reported. Combined with GenBank data sets for *M. Barneyi* which is widely distributed in China, *M. subhyalinus*, and *M. natalensis* from Africa, the evolutionary tree of 5 *Macrotermes* termites were build using Maximum Likelihood (ML) and Bayesian Inference (BI), with *Odontotermes formosanus*, *Reticulitermes speratus* and *Coptotermes formosanus* as out groups. Comparing the different composition structure, coding and a non-coding regions of 5 *Macrotermes* species, the referred mitochondrial genome is important in understanding their radiation and speciation.

1. The obtained sequence length of the whole mitochondrial genome of *M. annandalci* and *M. yunnanensis* were 15375bps and 15965bps respectively.

2. The mitochondrial genome of two Yunnan native *Macrotermes* species contained an extra tandem repeat in the longest non-coding region. The difference between the two genomes promised to serve as a marker for distinguishing those two species.

3. The *genus Macrotermes* originated from *M. subhyalinus* and *M. natalensis* were at the basal position of the genus *Macrotermes*, The Africa species *M. annandalci* and *M. yunnanensis* were inferred to be closely related as a sister group, but *M. Barneyi* more evolutionary than the others.

4. Multiple gene NADH dehydrogenase complex among the 5 species and ATP8 harbored higher mutation rates and Ka/Ks index, suggesting an association with species and environmental adaptability.

5. The metagenomics sequence among 5 species showed high relativity with species distribution and habits.

Keywords: metagenomics; divergence times; *Macrotermes*; *Macrotermes annandalci* (Silvestri); *Macrotermes yunnanensis* Han

Results

Table 1 Primer sequence for PCR amplification of *M. Annandalci* mitogenomics

forward primer	forward primer sequences	reverse primer	reverse primer sequences	amplified region
LYS-C1-F	CACAAG(A)GAT(C)ATTGGAACACT	LYS-C1-R	TGTGTTCTGCTGGTGGT(G)AAG	COX1
LYS-N4-F	TCAACGGAAACCAG(A)TGT(C)AAA	LYS-N4-R	CGTGTTCAGGCTGGTATT(C)TA	ND4
LYS-CB-F	TA(C)AGACACATCTGCCGAGAC	LYS-CB-R	CGTGCT(C)CCGATTCATGTT	CYTB
DL-N2-F	ATGCCCAGA(T)AACTCAACC(T)AA	DL-N2-R	TTCCTGTTAATGAGATT(C)GATGA	ND2
LYS-N1-F	CGAAAGAGGTAAAAATCTC(T)C(T)	LYS-N1-R	GTTATGGTGGGTGTGGCT(C)T	ND1
LYS-CB-S	CTATTCGCATACGCAATCCT	LYS-N1-C	GT(G)GAGTCTGAG(A)CTTGTTTCT	CYTB-ND1
LYS-C1C2-F	TAACATTCTTCCCG(A)CAACA	LYS-C1C2-R	ATTGGTGATGCTCTGTCTTG	COX1-COX2
LYS-N5F	TCCGTTTACACTTCTAAAACAA	LYS-N5R	TTTCTGCTTTAGTTCATTCTTC	ND5
LYS-N4L-S	ACTACGAACCATAGAAACCA	LYS-CB-C	CCTCAAAATGATATTTGTCC	ND4-CYTB
D42-N112S-3S	AAGGTCCAACGCGGACTATC	D42-12SN2-2C	AATGTGGTGGATTTGGCTGG	ND1-ND2
LYS-12S-2S	TAAACGGCGGTATACAAACA	LYS-N2-2C	CACTTTTTAACAGGAGTGGG	12S-ND2
LYS-N1-2S	GAAGACCTCCTAACAAAGAA	LYS-12S-2C	CAGTGTTATTTGTTGTTTGG	ND1-12S
LYS-N5-2S	AAGACCC(T)AACTGTCTCAAAG	LYS-N4-2C	CCCTCTCTTAAT(C)CTTCTTGG	ND5-ND4
LYS-C1-S	TATTCCCAACCCAC(T)ACT(G)AGA	LYS-A6-C	GTGTTCGAATATGTGGTTTG	COX1-ATP6
D42-N1DL-2S	CACACACCAAAGGAGATAGCC	D42-DLN2-3C	GCCATGAATTGGAGGAGACAG	D-loop
LYS-N2-S	AACATTGACAGTCGTATCAG	LYS-C1-C	AAGG(A)GTTAGT(C)GATGGTGGT	ND2-COX1
LYS-C3-S	CTTCGCATCATTCTTCTGAG	LYS-N5-C	CAG(A)TATAAT(C)GGCTTAAGGGT	COX3-ND5
LYS-N4-S	AATAGGCAATCAACGACTTC	LYS-4L-C	TTTGTGGTGTTTGGGTG(T)TT	ND4-ND4L
DL-A6-S	CCA(C)TACATCTTCACC(T)AGAAC	DL-C3-C	GCTTGTAGTGCG(A)GTG(A)AAGT	ATP6-COX3
LYS-12S-F	CAGCCACTTTGTTACGACTT	LYS-12S-R	TTTGCGTGGTTTG(A)C(A)TCTTA(G,T)T	12S

Talbe 2 Primer sequence for PCR amplification of *M. Yunnanensis* mitogenomics

forward primer	forward primer sequences	reverse primer	reverse primer sequences	amplified region
LYS-N1-F	CGAAAGAGGTAAAAATCTC(T)C(T)	LYS-N1-R	GTTATGGTGGGTGTGGCT(C)T	ND1
LYS-C1-S	TATTCCCAACCCAC(T)ACT(G)AGA	LYS-A6-C	GTGTTCGAATATGTGGTTTG	COX1-ATP6
LYS-N2-S	AACATTGACAGTCGTATCAG	LYS-C1-C	AAGG(A)GTTAGT(C)GATGGTGGT	ND2-COX1
LYS-C1C2-F	TAACATTCTTCCCG(A)CAACA	LYS-C1C2-R	ATTGGTGATGCTCTGTCTTG	COX1-COX2
LYS-12S-F	CAGCCACTTTGTTACGACTT	LYS-12S-R	TTTGCGTGGTTTG(A)C(A)TCTTA(G,T)T	12S
LYS-CB-S	CTATTCGCATACGCAATCCT	LYS-N1-C	GT(G)GAGTCTGAG(A)CTTGTTTCT	CYTB-ND1
D50-C3-2S	ACTAGGATCAACATGACCGC	D50-N5-2C	TGGCTTAAGGGTATTTTTGG	COX3-ND5
D50-N5-2S	GAATAACACCACCAGCACAC	D50-N4-2C	CTCCCCCCTCTCTTAATCTT	ND5-ND4
D50-N4-2S	ACAAAAACACCACCAACCAA	D50-4L-2C	TTGGATGCAAGTCGTGTTAT	ND4-ND4L
LYS-N1-2S	GAAGACCTCCTAACAAAGAA	LYS-12S-2C	CAGTGTTATTTGTTGTTTGG	ND1-12S
LYS-12S-2S	TAAACGGCGGTATACAAACA	LYS-N2-2C	CACTTTTTAACAGGAGTGGG	12S-ND2
LYS-N4-F	TCAACGGAAACCAG(A)TGT(C)AAA	LYS-N4-R	CGTGTTCAGGCTGGTATT(C)TA	ND4
DL-N2-F	ATGCCCAGA(T)AACTCAACC(T)AA	DL-N2-R	TTCCTGTTAATGAGATT(C)GATGA	ND2
DL-A6-S	CCA(C)TACATCTTCACC(T)AGAAC	DL-C3-C	GCTTGTAGTGCG(A)GTG(A)AAGT	ATP6-COX3
LYS-CB-F	TA(C)AGACACATCTGCCGAGAC	LYS-CB-R	CGTGCT(C)CCGATTCATGTT	CYTB
LYS-N5F	TCCGTTTACACTTCTAAAACAA	LYS-N5R	TTTCTGCTTTAGTTCATTCTTC	ND5
LYS-N4L-S	ACTACGAACCATAGAAACCA	LYS-CB-C	CCTCAAAATGATATTTGTCC	ND4-CYTB
LYS-C1-FN	CACAAGGACATTGGAACACT	LYS-C1-RN	GTGTGGGTTGGGAATAGAAT	COX1

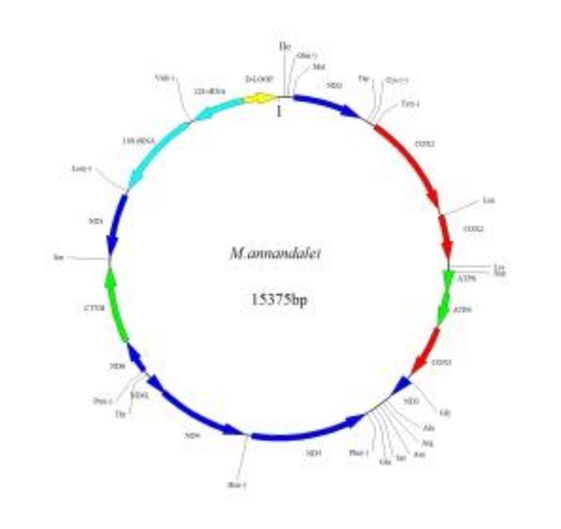

Fig.1 Gene map of the *M. annandalci*

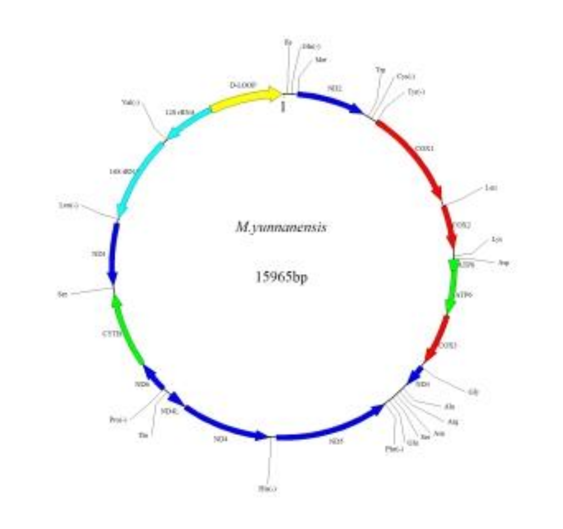

Fig.2 Gene map of the *M. yunnanensis*

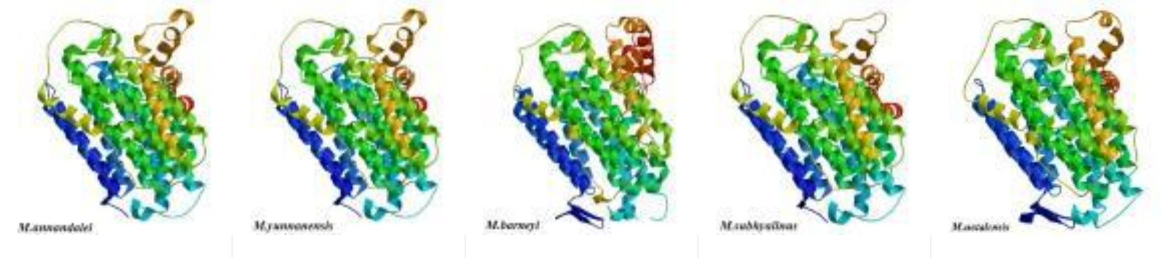

Fig. 3 Simulative tertiary structure of ND5

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Reporting the northern limit of the distribution of Subterranean Termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

By
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Abstract

Termites are insects which are sensitive to temperature change and their populations spread slowly. These characteristics suggest they can be an indicator insect for global warming. An intact colony of *Coptotermes* was detected June 27, 2013, in Suofang Villege (northern latitude 34°12′N, east longitude 118°53′E、altitude 6m), Guandun town, Shuyang City, Jiangsu Provine. Samples were collected and reared indoorsto obtain specimens for identification of the species. The field colony was also continuously monitored and alates collected from swarms that occurred in the same season for following 2~3 years. The final species was identified as *Coptotermes formosanus* by means of morphological characters and DNA barcoding. These observations have extended the known northern limit for the known distribution of this subterranean termite from the former northernmost collection at 33.5°N to 34°.12′N. As global warming intensifies, the northern limit distribution of *C. formosanus* should change as with other insects and more attention paid quarantine and control this pest. It was found that the critical climate factors that act on the distribution of *C. formosanus* were accumulated temperature, minimum temperature while precipitation had low impact on its distribution. Combined with analyzing the historic records, we concluded that the suitable regions for *C. formosanus* worldwide are located south of the line made up by Yibin, Chengdu, Chognqing, Sichuan Province, to Wuhan, Hubei Province, and to Shuyang, Jiangsu Province. The population of *C. formosanus* in Shuyang, Jiangsu Province should be included in the native geographical distribution of that species.

Key words: *Coptotermes formosanus*; North-distributing limitation; Global warming

Results

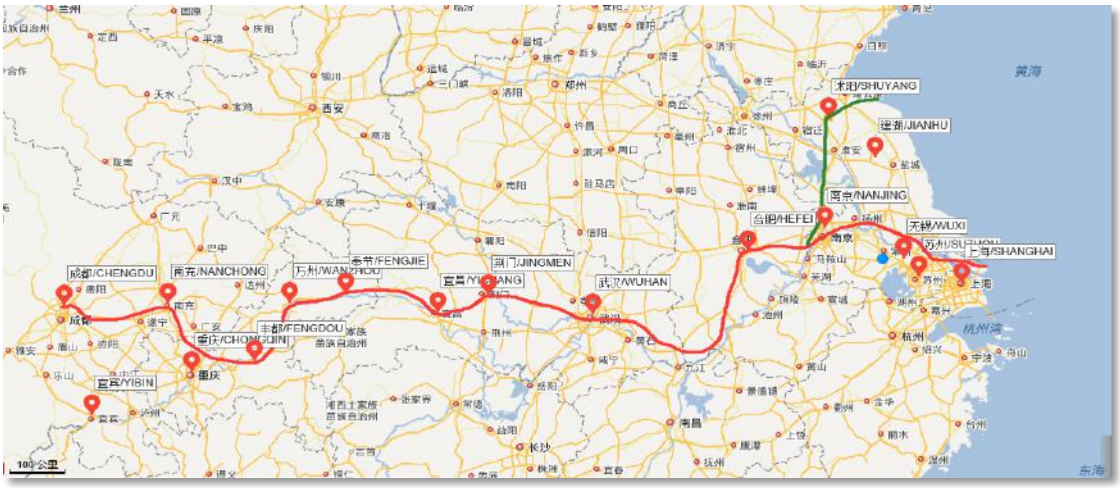

Fig. 1 The northern line of *C. formosanus* distribution in China

Fig.2 The photos of soldier
a. soldier; b. dorsal view of head; c. ventral view of head; d. mandibles (right, left); e. fontanelle; f. bristle of fontanelle; h. antenna; i. pronotum

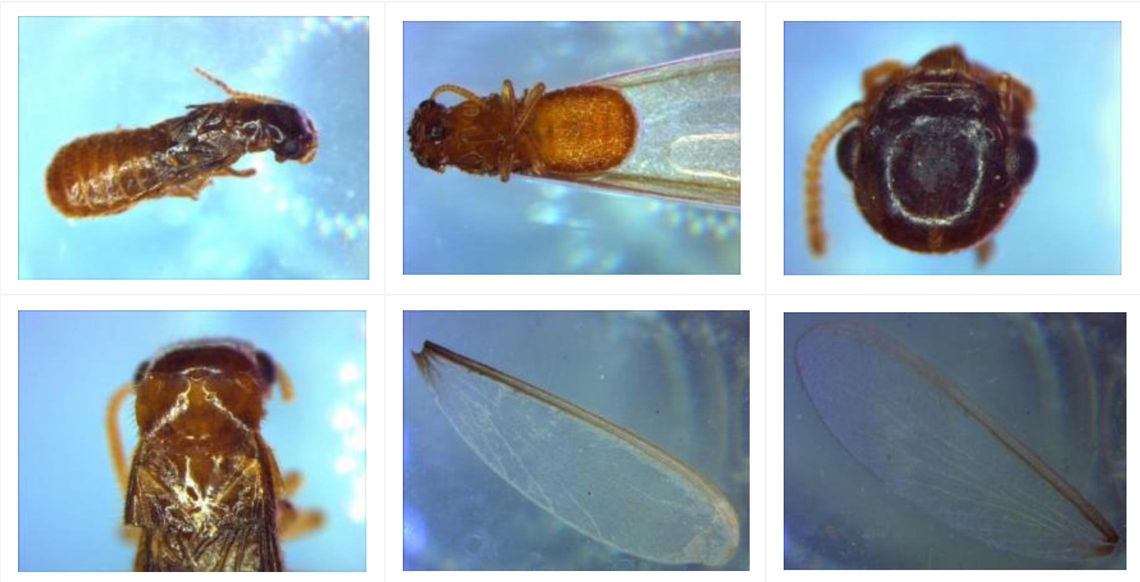

Fig. Photos of imago
a.imago; b. ventral view of imago; c. dorsal view of head; d. pronotum; e. forewing; f. underwing

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Chemical communication in the fungus-growing termites (Isoptera: Macrotermitinae)

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Abstract

Termites use trail pheromone to regulate foraging activities. In the dominant Chinese fungus-growing termite, *Odontotermes formosanus*, the trail pheromone is comprised of (3*Z*)-dodecen-1-ol (DOE), and (3*Z*,6*Z*)-dodecadien-1-ol (DDE), in ratios related to the context of foraging. When large numbers of labors are needed, the recruitment trail pheromone (DDE) is secreted in higher amounts (Wen et al., 2014). In *Odontotermes* spp, *Ancistrotermes* spp, DDE from the female sternal gland also is used as the sex pheromone for attracting males (Wen et al., 2012, 2015), but males can use additional minor components, such as DOE, to determine sex pairing (Wen et al., 2015). Minor isomerical components in this termites pheromone blend were identified (Wen et al., 2015). Beside the parsimony of structure, pheromones are quantitatively and qualitatively more diversified in the fungus-growing termites, *Odontotermes* spp. and *Ancistrotermes* spp.

Keywords: fungus-growing termites, pheromone, sex-pairing, foraging

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New discovery and review of termitophile fauna in Taiwan

by
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Abstract

The termite fauna in Taiwan has been studied since the early 20th century. However, termite-associated animals, termitophiles, in Taiwan has received little attention. This study summarizes the fragmented termitophile faunal data and reports a new discovery in Taiwan. We provide a preliminary check-list with 21 species or morphospecies of termitophiles from 7 insect families for Taiwan.

Key words: symbiont, social insect, Coleoptera, Diptera, Thysanura

Introduction

The termite fauna in Taiwan has been studied since early 20th century (Zhu 2005), and termite taxonomic studies and biodiversity surveys continue (Li et al. 2015). However, significantly less attention has been given to the termitophiles of Taiwan. Termitophiles refer to the symbionts that have obligatory relationship with termite societies (Kistner 1969). Depending on the relationship between symbionts and their host termite, the social insect symbionts can be divided into two categories, the integrated and non-integrated species (Kistner 1979). The biology of termitophiles was reviewed by Kistner (1982) and Wilson (1971) while the current study provides an overall review of termitophiles in Taiwan and reports a new finding. This study is the first step in our investigation of termitophile biology in Taiwan.

Materials and methods

We examined the termite specimens deposited in the National Chung Hsing University (NCHU) Termite Collection, Taichung, Taiwan to begin our investigation of the termitophile fauna in Taiwan. The NCHU Termite Collection was established by the second author in 2005. At the time of this investigation there were 3,341 termite colony specimens and associated animals deposited in NCHU Termite Collection, representing 4 termite families: Termitidae, Rhinotermitidae, Kalotermitidae, and Archotermopsidae. These specimens were collected through numerous termite biodiversity surveys in various regions of Taiwan. In addition, a comprehensive search for termitophiles in termite nest as well as field observations of termitophile behavior were conducted by the first author over the past two years. Termitophile collecting methods varied depending on the termite taxon (Kistner 1969).

Results and discussion

According to specimen examination and field survey, we discovered 13 morphospecies of termitophiles belonging to 10 genera. Coleoptera represented the greatest number and the rest were dipteran and thysanuran. A total of 21 termitophile species or morphospecies were found in Taiwan using the combination of new data and previous records (Table. 1). Six termite species, *Coptotermes formosanus* Shiraki, 1909, *Hodotermopsis sjöstedti* (Holmgren, 1911), *Nasutitermes kinoshitai* (Hozawa, 1915), *N. parvonasutus* (Nawa, 1911), *Odontotermes formosanus* (Shiraki, 1909), and *Reticulitermes flaviceps* (Oshima, 1908) are the hosts to termitophiles.

Table. 1. The list of termitophiles in Taiwan.

Termitophile species and taxonomic position	**Host termites**	**References**
Order Coleoptera		
Family Cerylonidae		
Cycloxenus sp.	*C. formosanus*	New record for Taiwan
Family Hydrophilidae		
Oreomicrus sp. 1	*N. kinoshitai*	New record for Taiwan
Oreomicrus sp. 2	*N. parvonasutus*	New record for Taiwan
Family Staphylinidae		
Termophidoholus formosanus Naomi & Hirono, 1996	*H. sjöstedti*	Naomi & Hirono (1996)
Corotocini sp.	*N. parvonasutus*	New record for Taiwan
Zyras sp.	*O. formosanus*	New record for Taiwan
Sinophilus sp.	*C. formosanus*	New record for Taiwan
Japanophilus sp.	*C. formosanus*	New record for Taiwan
Trichopsenius sp. 1	*R. flaviceps*	New record for Taiwan
Trichopsenius sp. 2	*R. flaviceps*	New record for Taiwan
Trichopsenius sp. 3	*R. flaviceps*	New record for Taiwan
Family Tenebrionidae		
Ziaelas formosanus Hozawa, 1914	*O. formosanus*	Hozawa (1914)
Order Diptera		
Family Keroplatidae		
Isoneuromyia sp.	*O. formosanus*	New record for Taiwan
Family Phoridae		
Achaetophora aristafurca Disney, 1996	*O. formosanus*	Disney (1996)
Bolsiusia spatulasetaeis Disney, 1996	*O. formosanus*	Disney (1996)
Clitelloxenia formosana (Shiraki, 1925)	*O. formosanus*	Shiraki (1925)
Clitelloxenia audreyae Disney, 1997	*O. formosanus*	Disney (1997)
Pseudotermitoxenia nitobei Shiraki, 1925	*O. formosanus*	Shiraki (1925)
Horologiphora sinensis Disney, 1997	*O. formosanus*	Disney (1997)
Selenophora shimadai Maruyama & Disney, 2011	*O. formosanus*	New record for Taiwan

Order Thysanura		
Family Nicoletiidae		
Nicoletiidae sp.	*O. formosanus*	New record for Taiwan

1. Coleoptera

1.1. Cerylonidae

The adults of Cerylonidae were commonly found in leaf litter, rotten debris or under the fungus infested bark, and a few species are associated with ants and termites. The termitophilous species belong to the genus *Euxestoxenus* or *Cycloxenus* strictly associated with fungus growing termites, mostly *Odontotermes.* (Kistner 1982, Leschen et al. 2011). Several species were found nesting in the fungus garden (Kistner 1982).

We found termitophilous Cerylonidae belonging to the genus *Cycloxenus.* These specimens were found in the fungus garden of *O. formosanus* and in the company of termite larvae. These beetles secret a white waxy substance from pores on their dorsal body surface, a phenomena also mentioned by Kistner (1982).

1.2. Hydrophilidae

The beetle family Hydrophilidae are mainly found in aquatic environments but nearly a third of the species are terrestrial (Short and Fikáček 2013) with a few species known to be associated with ants (Fikáček et al. 2015). Recently, the genus *Oreomicrus* had been confirmed to be a termitophilous group and several new species including Taiwanese species are on the way to being described (Fikáček et al., in prep.). Numerous samples were found inhabiting in the foraging tunnels of *N. kinoshitai* and *N. parvonasutus* in rotten wood.

1.3. Staphylinidae

The family Staphylinidae is one of the most diverse termitophile taxon. However, termitophilous Staphylinidae are poorly studied in Taiwan. The only related study was when Naomi and Hirono (1996) described, *Termophidoholus formosanus*, associated with *H. sjöstedti.*

A total of 7 morphospecies of termitophilous Staphylinidae were found in our investigation. One, Corotocini sp., was collected in the foraging tunnels of *N. parvonasutus* on bark. Two species, *Japanophilus* sp. and *Sinophilus* sp., belonging to the Termitohospitini were collected from the nest of *C. formosanus*. Three morphospecies of genus *Trichopsenius* were collected from the nests of *R. flaviceps*. One species in the genus *Zyras* was confirmed to be an obligate predator of *O. formosanus* in laboratory choice tests (Liang et al. unpublished data).

1.4. Tenebrionidae

Fairmaire (1892) established the genus *Ziaelas* with one species, *Z. insolitus.* Hozawa (1914) described a second species, *Z. formosanus*, based on three specimens collected in Heng-Chun

Peninsula, southern Taiwan from an *O. formosanus* fungus garden. Bremer (2014) indicated that these species might be synonyms according to their similarity morphology. However, the proper status should be determined using additional specimens of *Z. formosanus* from Taiwan.

We frequently observed *Z. formosanus* inside the fungus garden and walking on the chamber walls of *O. formosanus* nests in the field. This species is very integrated into the termite society without any aggressive behavior between termites and beetles. We successfully reared one beetle in an *O. formosanus* nest in the laboratory where the *Z. formosanus* probably feed on the fungus without damage to the media (Liang et al. unpublished data).

2. Diptera

2.1. Keroplatidae

The larvae of Keroplatidae were found in the foraging tunnels of *O. formosanus* in rotten wood. These larvae make webs from oral secretions and use the webbing to navigate the tunnel and catch termites. The larvae are probably specialized obligate predators of termites (Liang et al. unpublished data). Some larvae were successfully reared to adult, and identified to the genus *Isoneuromyia.* This is the first record of termitophilous Keroplatidae.

2.2. Phoridae

Shiraki (1925) described *Clitelloxenia formosana* and *Pseudotermitoxenia nitobei,* collected from the fungus garden of *O. formosanus* in Taiwan. The fauna of the termitophilous subfamily Termitoxeniinae in the oriental region was further investigated by Disney and Kistner (1997), and they described two more species, *Clitelloxenia audreyae* and *Horologiphora sinensis* from Taiwan. Disney (1996) described two species belonging to the subfamily Phorinae, *Achaetophora aristafurca* and *Bolsiusia spatulasetaeis.* The host termite of *C. audreyae*, *C. formosana*, *Horologiphora sinensis*, and *A. aristafurca* collected from Taiwan were identified as *Odontotermes hainanesis* (Disney 1996, Disney and Kistner 1997). However, according to our survey and identification with molecular tools, *O. formosanus* is the only fungus-growing termite found in Taiwan (Li at al. unpublished data). We believed *O. formosanus* is the only termite host of these phorid termitophiles in Taiwan.

Our investigation of *O. formosanus* fungus gardens resulted in discovering the fifth species of Termitoxeniinae, *Selenophora shimadai*, in Taiwan. *Selenophora shimadai* was recently described by Maruyama et al. (2011) based on specimens from Ishigaki-jima and Iriomote-jima, Japan.

3. Thysanura

3.1. Nicoletiidae

Nicoletiidae had been reportedly associated with numerous termites (Kistner 1982). In Taiwan, we recorded one morphospecies inhabiting the fungus garden of *O. formosanus.*

Conclusions

Based on a literature review and survey our new discovery brings the total species or morphospecies of termitophiles in Taiwan to 21 representing 7 families from 3 insect orders. Coleoptera, with 12 termitophile species, is the most diverse and species-rich order associated with termites. Along with understanding the nest structure and ecology of Taiwanese termites we expect to find additional, new termitophiles. Further investigation of the biology of these termitophiles and their interaction with their termite hosts will be our focus.

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Molecular phylogeography of *Reticulitermes* in Yarlung Zangbo Grand Canyon glacial refugia of Tibet

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Abstract

The genetic diversity of the genus *Reticulitermes* in Tibet was detected, reviewed and analyzed based on morphological methods and molecular evidence using DNA barcoding as well as a partial (658 bp) sequence of the mitochondrial COI gene from. 167 samples collected in Tibet. There were 29 representative samples selected to build a phylogenic tree based on COI sequence. The phylogenetic relationship of *Reticulitermes* was analyzed among Tibet and neighboring areas including Yunnan, Sichuan, Jiangxi, Jiangsu and Fujian. The *Reticulitermes* species were collected at altitude 2356m in Chayu which broke the original collecting record of 2020m. Molecular evidence supported the morphological results, there were 2 species in Tibet, *R. tibetanus* and *R. assamensis*, the former has 3 haplotyes and the latter 5 haplotypes. A total of 8 haplotypes were found but no strong phyloeographic structure was revealed. Intra- and inner-specific genetic distances among the 3 species were 0.090~0.097 and 0.002~0.005, respectively. The two Tibetan *Reticulitermes* species were distributed in both areas of Chayu City and Motuo City. *R. jiangchengensis* was a synonym for *R. assamensis.* Two samples from Chendu, *Reticulitermes* sp.-Chengdu CD1 and *Reticulitermes* sp.-Chengdu2 were considered a sister group with *R. assamensis*. The two *Reticulitermes* species in Tibet have a close relationship with other Chinese continental *Reticulitermes*, indicating that they are not relict species confined to Tibet.

Key words: *Reticulitermes*; *R. tibetanus*; *R. assamensis*; Tibet; mtDNA; Revison

Introduction

Climatic oscillations during the Quaternary could have important effects on the spatial distribution and genetic structure of organisms in the Yarlung Zangbo Grand Canyon (YZGC). However, how the termite species that occur there changed their distribution in response to the Quaternary climatic oscillation remains controversial. Motuo County and Chayu County located in the Linzhi District are the lowest elevation and offer the best termite environment in Tibet with tropic rain forest (Huang,1985).The termite species in Tibet were first surveyed by, Tsai & Huang (1975) who identified *Reticulitermes chayuensis* from their collections but since then there have been 20 species belonging to 3 families reported in Tibet (Huang, *et al.*, 1987; 2006) including 3 species of *Reticulitermes*. We conducted a study of the phylogeography of the *Reticulitermes*, endemic in the canyon using mtDNA molecular markers to resolve phylogenetic relationships at species levels.

Materials and Methods

Morphological identifcation

Based on the original description and review data (Tsai & Huang,1975; Huang & Han,1985; Huang, *et al*.,2000), specimens were identified to species.

Molecular identification

DNA templates were extracted with "TianGen"-kits and kept at -20℃. DNA barcoding COI universe primer pairs (Folmer *et al*,1994):

LCO1490 (5'-GGT CAACAAATCATAAAGATATTGG-3')

HCO2198(5'-TGATTTTTTGGTCACCCTGAAGTTT A-3')

PCR program: 94℃ 1 min, 46℃ 1 min, 72℃ 2 min, total 35 cycles and keep -4℃.

Data analysis

Sequences were edited with DNAStar software, homologous sequence searched via Blast on GenBank, an evolutionary tree was built using MEGA 5.0 software, the genetic distant, polymorphic site and haplotype were analyzed (Ding, 2013).

Table 1 Collecting information, and haplotypes for *Reticulitermes*

Province	sepcies	town	city	haplotype
Tibet	*R. tibetanus*	Bangxin	Motuo	X
		Shama	Motuo	Y
		Xigonghu	Motuo	Z
		Xigonghu	Motuo	Z
		Xigonghu	Motuo	Z
		Xigonghu	Motuo	Z
		Xiongjiu	Chayu	Z
		Xiongjiu	Chayu	Z
		Zongba	Chayu	Z
		Ziba	Chayu	Z
		Xiachayu	Chayu	Z
		Shams	Chayu	Z
	R. assamensis	Zala	Chayu	A
		Shama	Chayu	B
		Dengren	Chayu	C
		Dengren	Chayu	C
		Dengren	Chayu	C
		Miba	Chayu	C
		Shama	Chayu	C
		Guba	Chayu	D
		Migu	Chayu	D
		Zhuwagen	Chayu	D
		Aniqiao	Motuo	D
		Didong	Motuo	D
		Bangxin	Motuo	D
		Gelin	Motuo	D
		Beibeng	Motuo	D
		Xirang	Motuo	E
		Motuo	Motuo	E
Yunna	*R. jiangchengensis*	-	Nuzu	YN1
		-	Tengchong	YN2
Sichuan	*R.* sp.-Chengdu	-	Chengdu	CD1
		-	Chengdu	CD2
Jiangsu	*R. qingjiangensis*	-	Nanjing	JS
Fujian	*R. leptomandibularis*	-	Wuyishan	FJ
Jiangxi	*R. leptomandibularis*	-	Jiulanshan	JX

Results and Discussion

Morphological identifcation

Morphological characters of 29 samples suggested two species groups, *R. tibetanus* and *R. assamensis.*

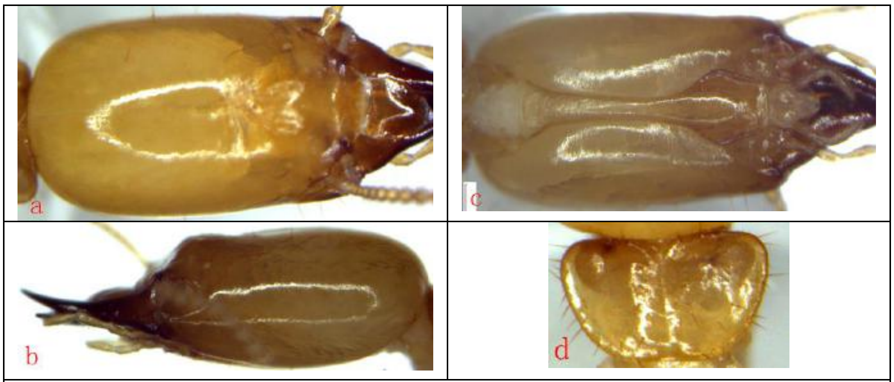

Fig.1 Morphological characteristics of the soldiers' head and the pronotum of *R. speratus*
Head of *R. tibetanus*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum

Fig.2 Morphological characteristics of the soldiers' head and the pronotum of *R. assamensis*; Head of *R. assamensis*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum

Molecular identification

A standard DNA barcode was obtained using a 658bp COI fragment and the fragment averaged G+C 42.0%, and A+T content 58.0%. *R. tibetanus* had 3 haplotyes, *R. assamensis* had 5 haplotyes.

Table 2 Haplotype variation at 5 nucleotide sites among *R. assamensis*

Haplotype	39	40	518	556	640
D	T	C	G	A	T
A	.	.	.	G	.
B	A	.	A	.	.
E	.	A	.	.	.
C	.	.	.	.	C

Table 3 Genetic distance pairwise-species and within-species of *Reticulitermes* in Tibet

No.	Specie	1	2	3	4	5	6	7	8
1	*R. tibetanus* X	—							

2	*R. tibetanus* Y	0.003	—						
3	*R. tibetanus* Z	0.002	0.002	—					
4	*R. assamensis* A	0.093	0.095	0.093	—				
5	*R. assamensis* B	0.095	0.097	0.095	0.005	—			
6	*R. assamensis* C	0.090	0.092	0.090	0.003	0.005	—		
7	*R. assamensis* D	0.091	0.093	0.092	0.002	0.003	0.002	—	
8	*R. assamensis* E	0.093	0.095	0.093	0.003	0.005	0.003	0.002	—

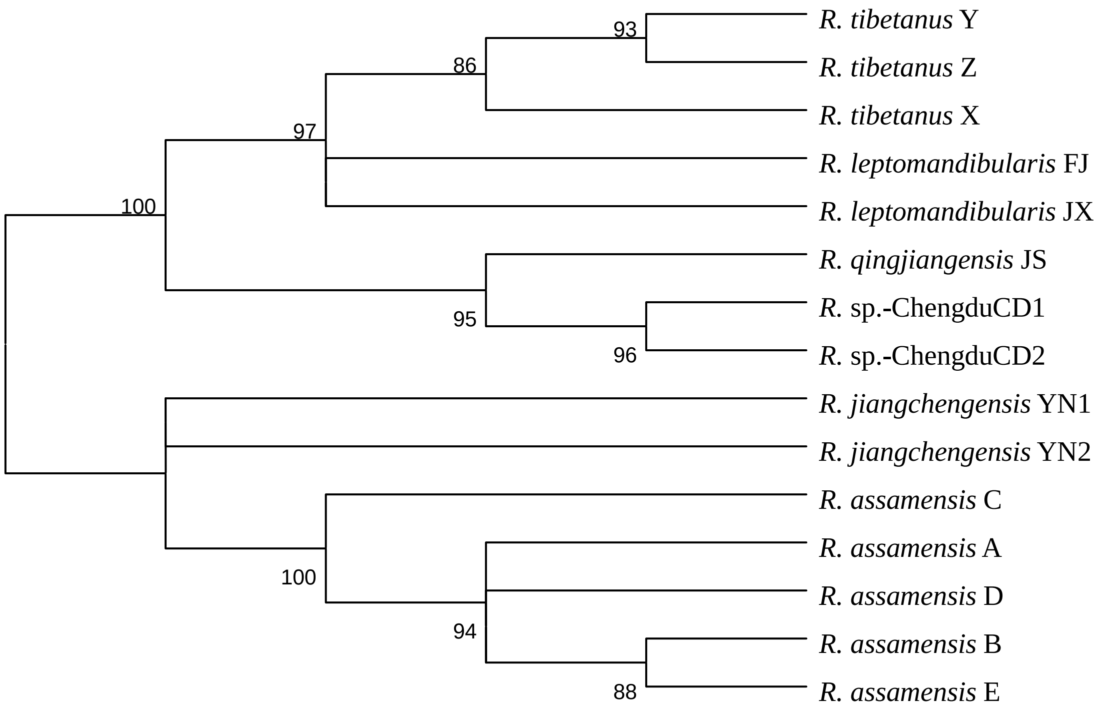

Fig.1 Maximum Likelihood tree based on the COI gene

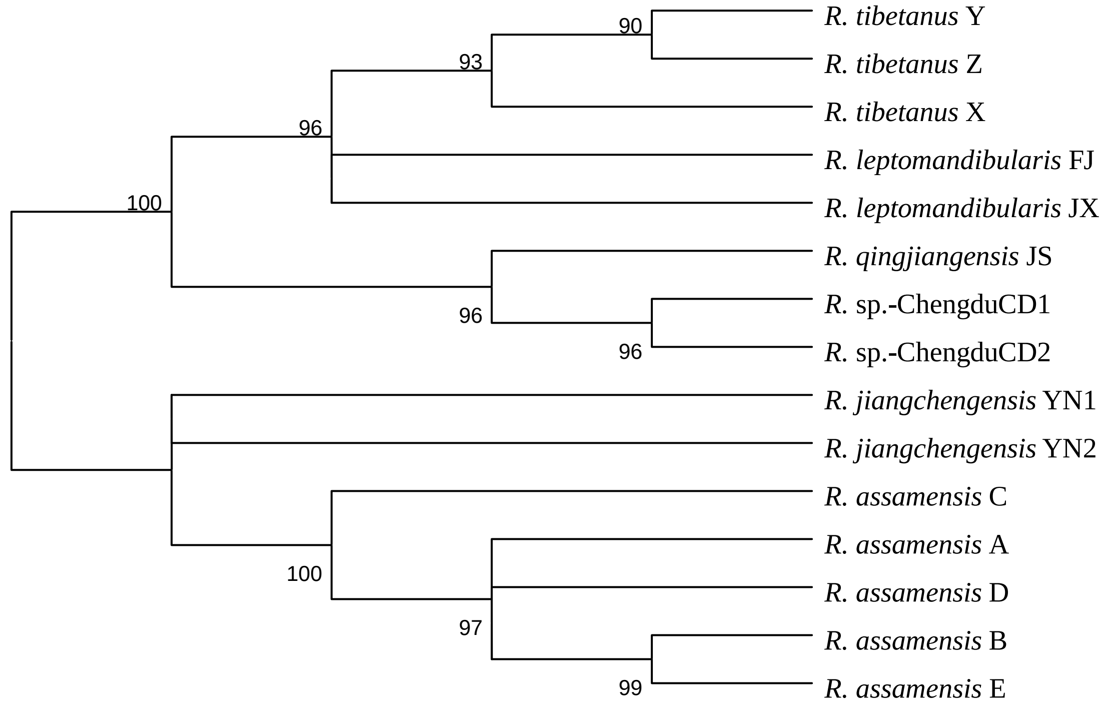

Fig.2 Neighbor-joining tree based on the COI gene

Conclusions

The phylogenetic relationship of *Reticulitermes* was analyzed among the termites collected in Tibet and neighboring areas, such as Yunnan, Sichuan, Jiangxi, Jiangsu and Fujian. Molecular evidence supported the morphological identification results, there were 2 species in Tibet. Intar- and inner-species genetic distances are 0.090~0.097 and 0.002~0.005, respectively, both *Reticulitermes* species were distributed in both Chayu City and Motuo City in Tibet. *R. jiangchengensis* is a synonym *R. assamensis*. Two samples from Chendu *Reticulitermes* sp. chengdu CD1 and *Reticulitermes* sp. Chengdu were closely related to *R. assamensis*, and were considered a sister group. The *Reticulitermes* species samples were collected at altitude 2356m in Chayu, which broke the original collecting record 2020m, that's the highest record in East Asia, but is lower than *Archotermopsis wronqhtoni* (Desneux) located in the Kashmir region and northeast of Kabul, Afghanistan that have been found at 2743m. In the samples from Chayu, we strongly suspect the species *R. chayuensis* should be synonymized because we could not detect either a morphological and molecular character that would separate this species., The two *Reticulitermes* species in Tibet are not relict species confined to Tibet.

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The impact of fire on the termite assemblage in a tropical, degraded peatland

By

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Abstract

Fire is a common feature in tropical peatlands, particularly, in these recent years, during drought in association with El Niño events and land conversion to oil palm plantations. The phenomenon poses a massive threat to invertebrates and disrupts the integrity and biological functioning of ecosystems. Termites are seen as major soil engineers and if their services could be harnessed efficiently these insects could be a value to the peatland ecosystem at post-fire. A first step is to understand the termite assemblage in disturbed tropical peatlands, and toward that end we collected termite samplings in Riau, Sumatra. A total of 21 species representing 10 genera were identified. The relative species richness in the fire-impacted peat decreased up to 40% compared to an undisturbed peat swamp forest. Only four to ten species could be found in fire-impacted peats and the termite assemblage mainly consisted of members from the Rhinotermitidae. The species evenness indices demonstrated that there were significant effects of fire on termite species evenness in peats when compared with natural peat swamp forests. Similarity index significantly separated the termite faunal composition in peat swamp forests and fire-impacted peats where a major division was delineated at 39%. The relative abundance of termites detected did not change significantly along temporal variation.

Key words: peat fire, disturbed peatland, oil palm plantation, fire resistance, biological functioning

Introduction

Over several millennia, fire has been of a common feature in the tropical peatlands, particularly during drought in association with El Niño events (Yulianti et al., 2012). The current event is also tightly linked to land conversion through slash and burn techniques to oil palm plantations (Langner and Siegert, 2009). Fire hotspot data captured by NASA MODIS illustrated that the highest hotspot density areas included the mass degraded peat lands in Kalimantan and Sumatra (Yulianti et al., 2012). The fires undisputedly pose a massive threat to invertebrates and indirectly disrupt the integrity and biological functioning of ecosystems.

Earthworms play role similar to termites and are also disturbance-sensitive. However, owing to the highly acidic peat ecosystem earthworms are absent from disturbed peat. Thus, termites are seen as major soil engineers post fire especially if their services could be harnessed efficiently. As a first step to understanding the termite assemblage in tropical disturbed peatlands, termite samplings were carried out in degraded peatlands in Riau, Sumatra.

Materials and methods

This study was conducted in the transition zone of the Giam Siak Kecil–Bukit Batu Biosphere Reserve (0°44'–1°11'N, 0°11'–102°10'E) that lies between 0 and 50 m above sea level. Five burned peatlands (Peat1-5) were surveyed in this study. For comparison, two transects were employed at two undisturbed peat forests (Forest1 and 2). The termite samplings were carried out using a standardized belt transect (Jones & Eggleton, 2000). The belt transect comprised a survey area of 100 × 2 m and was divided into 20, 5 × 2 m sections. A collector spent an hour on each section. Potential termite habitats such as dead tree branches, logs, soil under logs, termite galleries, and nests were surveyed. Soldier and worker termites were collected and stored in 80% ethanol until identified.

To observe the termite abundance over the dry and rainy seasons, four termite samplings were conducted in November 2012, February 2013 and June 2013, and March 2014 at Peat4 and Peat5. The sampling times were based on the in-situ environmental and climatic variations (Neoh et al., 2015). At each sampling, transects were set 10 to 20m away from previously sampling sites.

Estimated termite species richness, diversity index, and similarity index in each landscape were generated using EstimateS Version 8.2 (Colwell, 2005). We employed the Chao2 estimator to extrapolate species richness beyond the observed data in the study site. Diversity indices (i.e., Shannon diversity index and Simpson’s inverse diversity index) were calculated to infer species evenness at each surveyed site.

To determine whether the relative abundances of termites across seasons and locations were significant, we employed General Linear Mixed Models (GLMM) analysis with termite species as the random effect. When testing the significance level of the relative abundances of termites across seasons, time of sampling was a fixed factor and location was treated as the covariate. The procedure was employed vice-versa when testing the significance of relative abundances of termites between locations.

Results and Discussion

A total of 21 species representing 10 genera were collected. The relative species richness in fire-impacted peats decreased up to 40% compared to termite species found in peat swamp forest. Only four to ten species could be found in fire-impacted peats (Peat1-Peat5) and the termite assemblage mainly consisted of the subfamily Rhinotermitidae. Nevertheless, the differences in estimated species richness between forest and fire-impacted peats was not statistically significant as evidenced by overlapping of 95% confidence intervals.

The species evenness indices demonstrated that there were significant effects of fire on termite species evenness in peats when compared with natural peat swamp forests (Shannon's diversity Index: $t(5)=6.356$, $P<0.05$) except for Forest1 in which Simpson's inverse diversity index registering 5.78 ($t(5)=1.969$, $P>0.05$). The similarity index significantly separated the termite faunal composition in peat swamp forests and fire-impacted peats with a major division delineated at a similarity of 39% (Fig. 1).

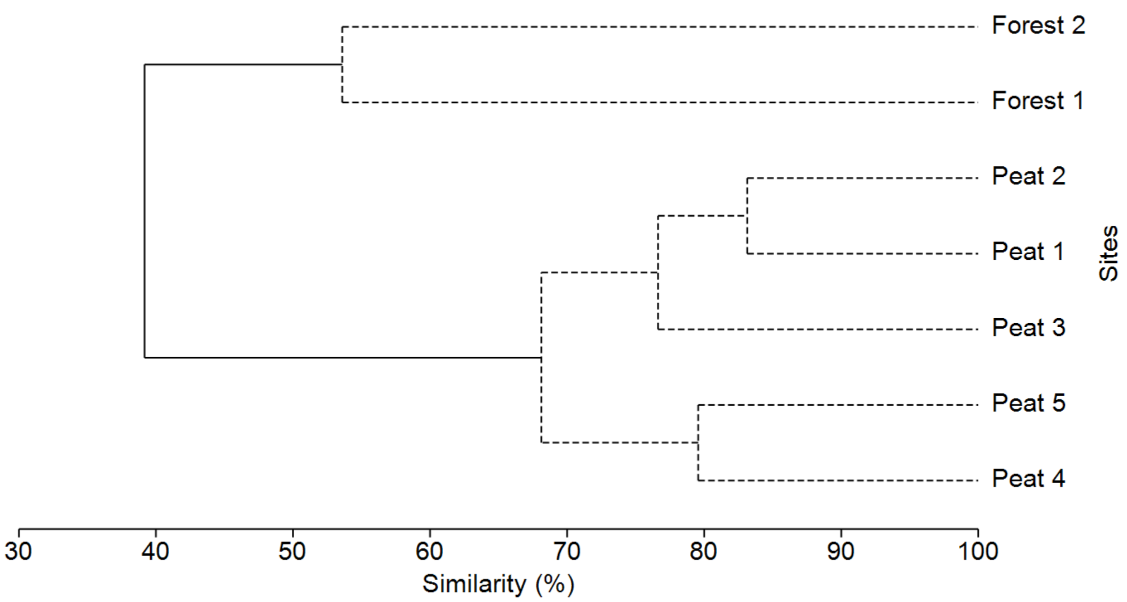

Fig. 1. Dendrogram of group-average clustering of the seven sites (two near forested peat sites and five fire-impacted peatlands based on means of Bray-Curtis distances. Solid lines represent significant differences among groups by SIMPROF tests ($P<0.05$)

The fire behavior in peatland is characterized by high temperatures and long-lasting duration. For example, the progression of temperatures has been found to be at ground level as high as 400°C and at 5-cm below ground 200°C, at 10-cm 90°C, 20-cm 60°C, 3-cm 50°C, and 4-cm 45°C during a peat fire event (Usup et al., 2004). Further, a fire could result in smoldering that usually spreads laterally and downward to a depth of 20-60 cm to form a bowl-shape depression. This is the depth where termites nest and forage (20-30 cm below ground) (Kon et al., 2012). The likelihood of the peat fire exterminating both the arboreal and subterranean nest building termites is high.

The GLMM analysis showed that the relative abundance of termites detected along transects did not change significantly throughout the sampling carried out at selected time intervals in Peat4 and Peat5 (GLMM; $F = 0.921$, $P > 0.05$) (Fig. 2). In contrast, significant difference in termite abundance in fire-impacted peatland cultivated with oil palm (Peat5) was detected when compared with abandoned fire-impacted peat (Peat4) (GLMM; $F = 4.341$, $P < 0.05$) (Fig. 2).

In a given tropical fire-impacted peatland, the water table may register 20-cm from the soil surface and soil moisture of more than 0.30 m^3 H_2O m^{-3} during the rainy season (Neoh et al., 2015). Peat inundation does not favor the survivorship of termites (Masijan, 2007). Nevertheless, the notion does not hold true in the present study as we found subtle changes in the termites represented in our temporal collections. We cannot explain precisely the reasons for this phenomenal but we should consider the nesting ecology of the surviving termites, i.e., tree bark.

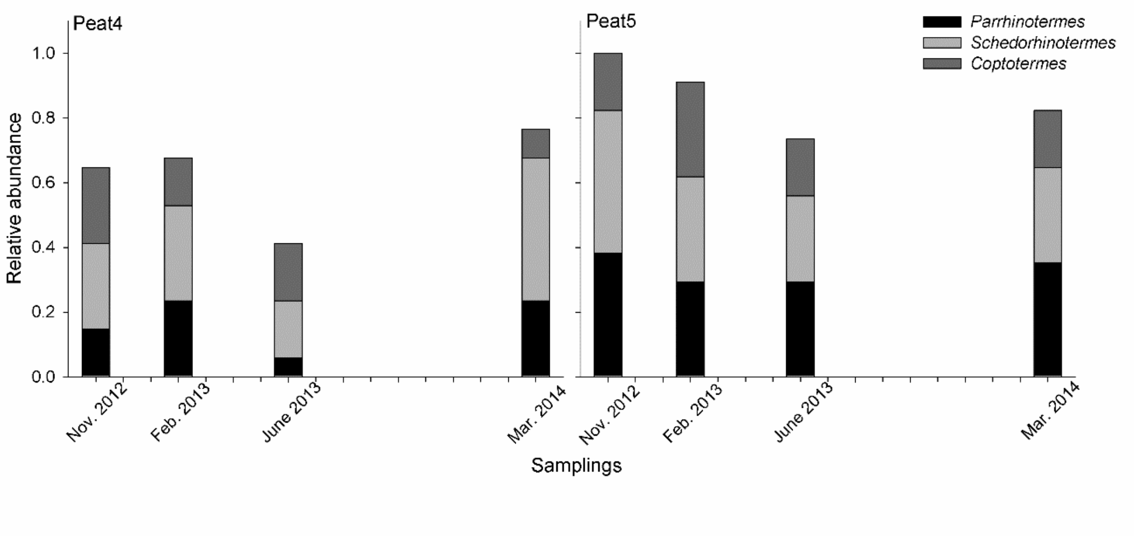

Fig. 2. Relative abundance of the termite assemblages based on the encounter rate on 20 sections in a standardized transect over time at long-burnt, abandoned peat (Peat4) and fire-impacted peat cultivated with oil palm (Peat5) in Sumatra. The termite species encountered were grouped by genera.

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Genetic Differentiation of *Reticulitermes speratus* in East Asia

By
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Abstract

In this study, samples of *Reticulitermes speratus* (Kolbe) were collected in China and a portion of the mitochondrial gene encoding for the cytochrome oxidase subunit II (COII) was sequenced. A phylogenetic tree was constructed using neighbor-joining (NJ) and Maximum likelihood (ML). To compare the phylogenetic relationship and migration route of *R. speratus* in East Asia, we used GenBank data of populations from Korea and Japan. Results indicated:

1 The 655bp length sequence consisted of 581 conserved sites, 74 variable sites, 62 information sites. G+C content averaged 37.0%, A+T content averaged 63.0%.

2 Results showed that *R. speratus* was mainly divided into two groups, with a correlation between phylogenetic distance and locality of collection. *R. speratus* of southern Japan and Korea flock together in one branch, *R. speratus* of northern Japan to other branch, *R. speratus* of China divided to two branches.

3 According to the comparative analysis of the sequences, we suggested that *R. speratus* is found in China and Japan, China and Korea, and there were at least two subspecies of *R. speratus* in China included nominate subspecies *R. speratus speratus and R. speratus kyushuensis*. 4 Our results indicated that *R. speratus* migrated into the Japanese main islands from East China, and that Korean populations diverged from Japanese populations.

Key words: *Reticulitermes speratus*; COII; migration route; phylogenetic relationship

Introduction

Reticulitermes speratus (Kolbe) was described by Kolbe 1885 where type locality was Japan, Snyder (1949) moved it into the genus *Reticulitermes* where it remains (Huang, *et al*., 2000).

This economically important termite pest widely distributed in northern China, Japan and Korea, and its speciation, genetic variation and migration route among those three countries is fascinating.

Materials and Methods

Materials information

Termite samples were collected from 6 cities in Liaoning and Shandong Province, 3~5 colonies per city. All specimens were kept in 75% ethyl alcohol for morphological comparison, and a backup sample kept in 75% ethyl alcohol at -20℃ for molecular comparison (Table 1).

Morphological comparison

Digital images for illustrations were taken using a NIKON SMZ1500 camera (NIKON Microsystems, Japan). Measurements data were compared and analyzed with original ones in taxonomy references to identify the samples.

Molecular comparison

DNA templates was extracted with "TianGen"-kits and kept at -20℃.

PCR amplification

DNA sequence COII universe primer pairs (Folmer *et al*,1994):TL-J-3037 (5-ATGGCAGATTAGTGCAATGG-3)，TK-N-3785 (5-GTTTTAAGAGACCAGTACTTG-3) (Xing *et al.,* 2001).PCR program: 94℃ 1 min, 46℃ 1 min, 72℃ 2 min, total 35 cycles and keep -4℃.

Table 1 List and sampling localities of the termites used in this study

Species	Sites	Reference	GeneBank No.
R. speratus	Korea	Kim *et al*.,2012	GU732288
R. speratus	Korea	Kim *et al*.,2012	HM560007
R. speratus	Korea	Kim *et al*.,2012	HM560010
R. speratus	Korea	Ohkuma *et al*.,2004	AB109530
R. speratus	North Japan	Yashiro *et al*.,2007	DQ493737
R. speratus	North Japan	Yashiro *et al*.,2007	DQ493740
R. speratus	North Japan	Austin *et al*.,2002	AF525344
R. speratus	North Japan	Xing *et al*.,2001	AB050706
R. speratus	South Japan	Yashiro *et al*.,2007	DQ493736
R. speratus	South Japan	Yashiro *et al*.,2007	DQ493739
R. speratus	South Japan	Yashiro *et al*.,2007	EF016101
R. speratus	Yantai, CN	—	KU061240
R. speratus	Longkou, , CN	—	KU061241
R. speratus	Gongzhuling	—	KU061242
R. speratus	Dalian, CN	—	KU061243
R. speratus	Qixia, CN	—	KU061244
R. speratus	Dalian, CN	—	KU061245
R. speratus	Qingdao, CN	—	KU061246
R. speratus	Changsha, CN	Long *et al*.,2009	FJ423457
R. speratus	Changsha, CN	Long *et al*.,2009	FJ423463
R. speratus	Changsha, CN	Long *et al*.,2009	FJ423464
R. speratus	Beijing, CN	Chen *et al*.,2012	JX142152
R. speratus	Beijing, CN	Chen *et al*.,2012	JX142153
R. speratus	Beijing, CN	Chen *et al*.,2012	JX142154
R. aculabialis	Hangzhou, CN	Chen *et al*.,2012	JX142172○
R. chinensis	Beijing, CN	Xing *et al*.,2001	AB050705○

○With potential for wrong identify.

Data analysis

The sequences were edited with DNAStar software (Burland,1999), homologous sequence searched via Blast on GenBank, an evolutionary tree was built by MEGA 5.0 software (Tamura et al., 2011), the genetic distant, polymorphic sites and haplotypes were analyzed.

Results and discussion

Morphological comparison

The samples in this study were *R. speratus* revealed similar morphologies with description in the key.

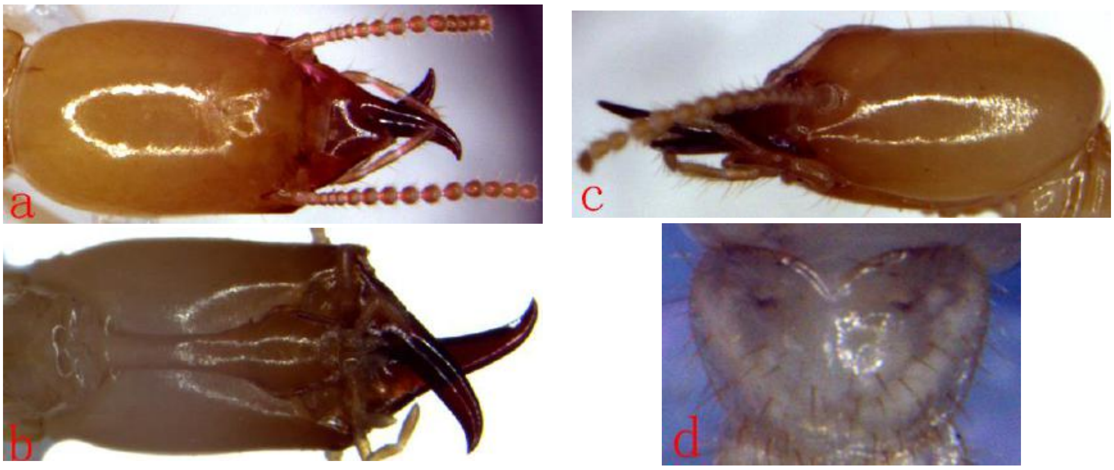

Fig.1 Morphological characteristics of the soldiers' head and the pronotumof *R. speratus*
Head of *R. speratus*: a. dorsal view; b. ventral view; c. lateral view. d. Pronotum.

Molecular analysis

The 655bp length sequences were selected a phylogenetic tree constructed and analyzed, which consisted of 581 conserved sites, 74 variable sites, 62 information sites with the G+C average content of 37.0%, while A+T averaged 63.0%.

The tree was constructed by neighbor-joining method (NJ) and Maximum likelihood (ML) method (Fig. 2~3).

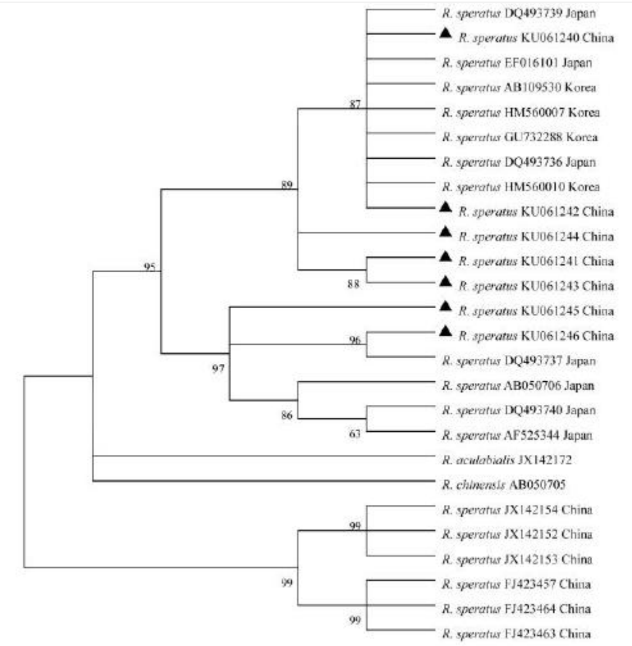

Fig.2 Maximum Likelihood tree based on the COII gene
▲: The specimens were collected in China, the same below.

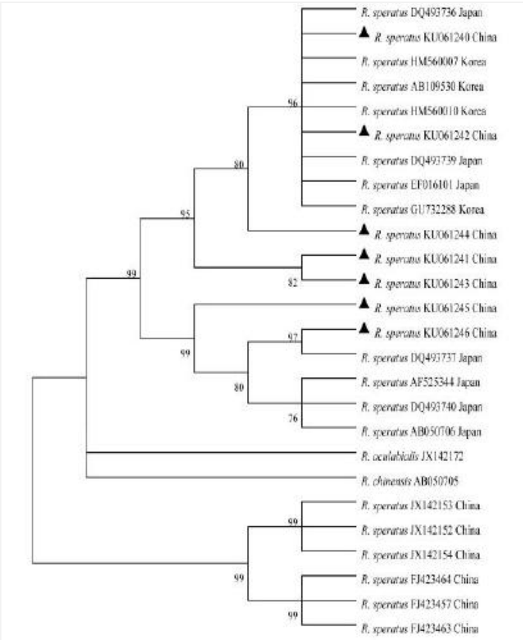

Fig. 3 Neighbor-joining tree based on the COII gene

3

Analysis results showed that *R. speratus* was mainly divided into two groups, with correlation the phylogenetic distance and the locality of collection. *R. speratus* of southern Japan and Korea flock together into one branch, *R. speratus* of northern Japan to other branch, *R. speratus* of China divided to six branches.

Discussion

Subspecies discuss

According to the comparative analysis of the sequences, we suggested that the same *R. speratus* subspecies are existed in China and Japan, China and Korea, respectively, there were at least two subspecies of *R. speratus* in China including *R. speratus speratus and R. speratus kyushuensis*.

Migration route of R. speratus in East Asia

Our results supported the hypothesis that ancestral *R. speratus* separated into northern and southern Japanese populations after its migration into the Japanese main islands from East China during the early Pleistocene via the East China Sea basin, which may have been exposed during that period. The Korean populations seem to have diverged recently from southern Japanese populations; this may explain the current distribution of *R. speratus* in the Japanese Arachipelago (Park *et al*,2006).

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Table 2 Genetic distances among the populations of *Reticulitermes speratus*

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	*R. speratus* DQ493736 Japan	—																								
2	*R. speratus* DQ493739 Japan	0.002	—																							
3	*R. speratus* EF016101 Japan	0.002	0.000	—																						
4	*R. speratus* HM560007 Korea	0.002	0.000	0.000	—																					
5	*R. speratus* HM560010 Korea	0.003	0.002	0.002	0.002	—																				
6	*R. speratus* AB109530 Korea	0.002	0.000	0.000	0.000	0.002	—																			
7	*R. speratus* GU732288 Korea	0.002	0.000	0.000	0.000	0.002	0.000	—																		
8	*R. speratus* KU061242 China	0.002	0.000	0.000	0.000	0.002	0.000	0.000	—																	
9	*R. speratus* KU061240 China	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	—																
10	*R. speratus* KU061244 China	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.002	—															
11	*R. speratus* KU061241 China	0.005	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.002	—														
12	*R. speratus* KU061243 China	0.005	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.002	0.000	—													
13	*R. speratus* KU061245 China	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	—												
14	*R. speratus* KU061246 China	0.027	0.025	0.025	0.025	0.027	0.025	0.025	0.025	0.025	0.023	0.025	0.025	0.014	—											
15	*R. speratus* DQ493737 Japan	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	0.012	0.002	—										
16	*R. speratus* DQ493740 Japan	0.025	0.023	0.023	0.023	0.025	0.023	0.023	0.023	0.023	0.022	0.023	0.023	0.012	0.011	0.009	—									
17	*R. speratus* AF525344 Japan	0.036	0.035	0.035	0.035	0.036	0.035	0.035	0.035	0.035	0.033	0.035	0.035	0.025	0.023	0.022	0.012	—								
18	*R. speratus* AB050706 Japan	0.028	0.027	0.027	0.027	0.028	0.027	0.027	0.027	0.027	0.025	0.027	0.027	0.012	0.011	0.009	0.003	0.015	—							
19	*R. speratus* JX142153 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	—						
20	*R. speratus* JX142154 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	0.000	—					
21	*R. speratus* JX142152 China	0.060	0.058	0.058	0.058	0.060	0.058	0.058	0.058	0.058	0.057	0.058	0.058	0.074	0.076	0.074	0.074	0.084	0.077	0.000	0.000	—				
22	*R. speratus* FJ423457 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	—			
23	*R. speratus* FJ423464 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	0.000	—		
24	*R. speratus* FJ423463 China	0.065	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.062	0.063	0.063	0.075	0.077	0.076	0.075	0.085	0.079	0.032	0.032	0.032	0.000	0.000	—	
25	*R. aculabialis* JX142172	0.046	0.045	0.045	0.045	0.046	0.045	0.045	0.045	0.045	0.043	0.041	0.041	0.055	0.056	0.055	0.051	0.063	0.055	0.063	0.063	0.063	0.068	0.068	0.068	—
26	*R. chinensis* AB050705	0.044	0.043	0.043	0.043	0.044	0.043	0.043	0.043	0.043	0.041	0.040	0.040	0.053	0.051	0.050	0.050	0.061	0.053	0.065	0.065	0.065	0.068	0.068	0.068	0.045

Result of controlling drywood termites (*Cryptotermes domesticus* Haviland) using a novel method

by
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Abstract

The efficacy of injecting and liquid termiticide and covering the treatment area with cotton cloth saturated with insecticide for 48 hours was conducted in 2 areas of Tan Da Resort (central Area and Lac Viet area) that were damaged by drywood termite. The results showed that the drywood termite *Cryptotermes domesticus* was completely eliminated 5 days after the injection and covering treatment and there have been no signs of termite activity for 3 months.

Introduction

Tan Da Spa Resort (Tan Da Resort) is typical of the ecological zones considered urban area of Hanoi, it is 60 km West of Hanoi city. Tan Da Resort was designed to provide the style of an open, peaceful space and harmonious landscape. The ecological resort area is separated from the residential area. The environmental conditions of the construction area fall in line with the natural environment (wide space, many big trees, etc.) and most of the construction includes materials containing cellulose (wood, bamboo, neohouzeauna, palm leaf, etc.), food of many types of termite. The research reported by Trinh Van Hanh et al. (2014) showed that the drywood termite *Cryptotermes domesticus* Haviland was one of the main harmful termite species collected at the Tan Da Resort.

The Management Unit of Tan Da Resort has attempted in the past to limit the destructive ability of drywood termites traditional pesticide surface and injection treatments. However, the efficacy of these treatments did not meet the contract requirements. Termite only "temporarily left" the place of pesticide treated for a short period of time. In front of such a situation, in 2015, the Institute of Ecology and Works Protection researched the application of new method to treat this harmful termite. The result showed that 3 months after treatment, no termite were found at the locations where they previously appeared.

Materials and Methods

Time of research: The research is carried out from January to April, 2015 at 27 wooden structural and decorative items on the property of the Tan Da Resort.

Materials*:* Permethrin 50 EC, Cislin 2.5 EC (deltamethrin, 25g/l), cotton cloth, nylon, adhesive tape, etc.

Methods*:*

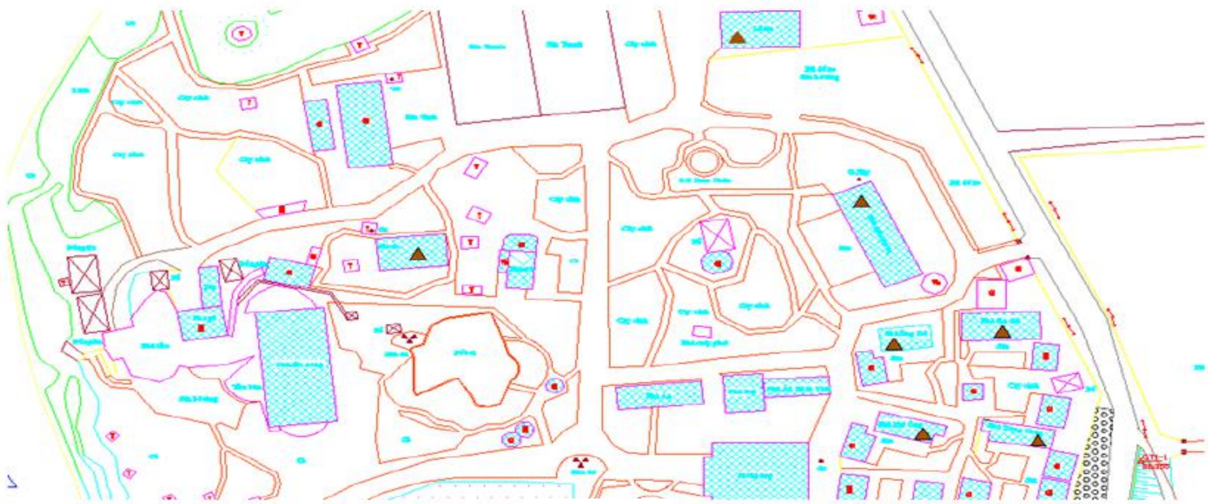
+ According to the method of Nguyen Quoc Huy et al. (2014).
+ Surveying and marking the position of dry wood termite galleries (based on position of the feces) and determining the quantity of termite fecal before treatment.
+ Injecting a solution of 0.5% Cislin 2.5 EC to saturate the termite galleries
+ Covering the entire known, infested area with cotton cloth (thickness of 3 mm) saturating it with a 1% Permethrin 50 EC solution.
+ Covering the cotton cloth with nylon (1mm thickness) for 48 hours.
+ Using adhesive tape to fix the nylon layer.
+ Removing treatment materials at treatment locations after 48 hours
+ Checking the quantity of termite feces at treated and not-treated positions on the 1st, 3rd, 5th, and 7th day after removing the covering material.
+ Comparing the structural and decorative items treated by only injecting Cislin (not covering) and items not treated.

Data was processed using Excel 2010.

Results and Discussion

Actual state of dry wood termite damage in Tan Da Resort

A total of 42 structural and decorative items were investigated at the Tan Da Resort and 27 were damaged by drywood termites. The damaged items were focused mainly in the Central area of the Resort, including the House on stilts, ancient houses and working room (figure 1). At the affected areas, we realized that drywood termite caused damage at 3 main locations within buildings namely the wooden dividing walls in rooms of the ancient houses, wooden pillars and collar beams of the houses on stilts, and the reception house and management house.



Center area of Tan Da resort

▲ Sites of *Cryptotermes domesticus*

Figure 1. Position of dry wood termite damaging in the Central area of Tan Da Resort

The location of drywood termite infestations was conducted by visual search based on signs of termite feces and marking those positions to arrange testing the treatments.

The test is carried out on 3 different types of wooden structures damaged by the termite *Cryptotermes domesticus* including: wooden pillars (V1), collar beams (V2) and wooden dividing walls (V3). The test was repeated 3 times for each type of structure for those areas treated with Cislin and not-treated controls.

The results in Figure 2 shows that at the time of treatment, the quantity of drywood termite feces discharged at the wooden dividing wall was more than that in 2 other groups in that structure. The quantity of termite feces was the least from the collar beam group at before and after treatment. One day after treatment, the quantity of termite feces at all treatment positions decreased (decreased on average 8.6g/structural element in comparison to before testing). The quantity of termite feces continued to decrease (on average 14 g/structural element) after 3 days of treatment. Five days after treatment, there were no feces of drywood termite at treatment points. Meanwhile, in comparison, the quantity of feces was slightly reduced but increased gradually after testing. For the measure on injecting Cislin through the knock-out holes, the quantity of discharged feces was reduced in comparison to the periods 1 and 3 days before testing on average, 6.3 g/structural element after treatment. The efficiency of injecting Cislin against the drywood termite was 17.5% after 5 days which indicated that the colony was reduced but not eliminated (Figure 3). Meanwhile the injecting covering, and, binding treatment achieved 100% efficiency after 5 days.

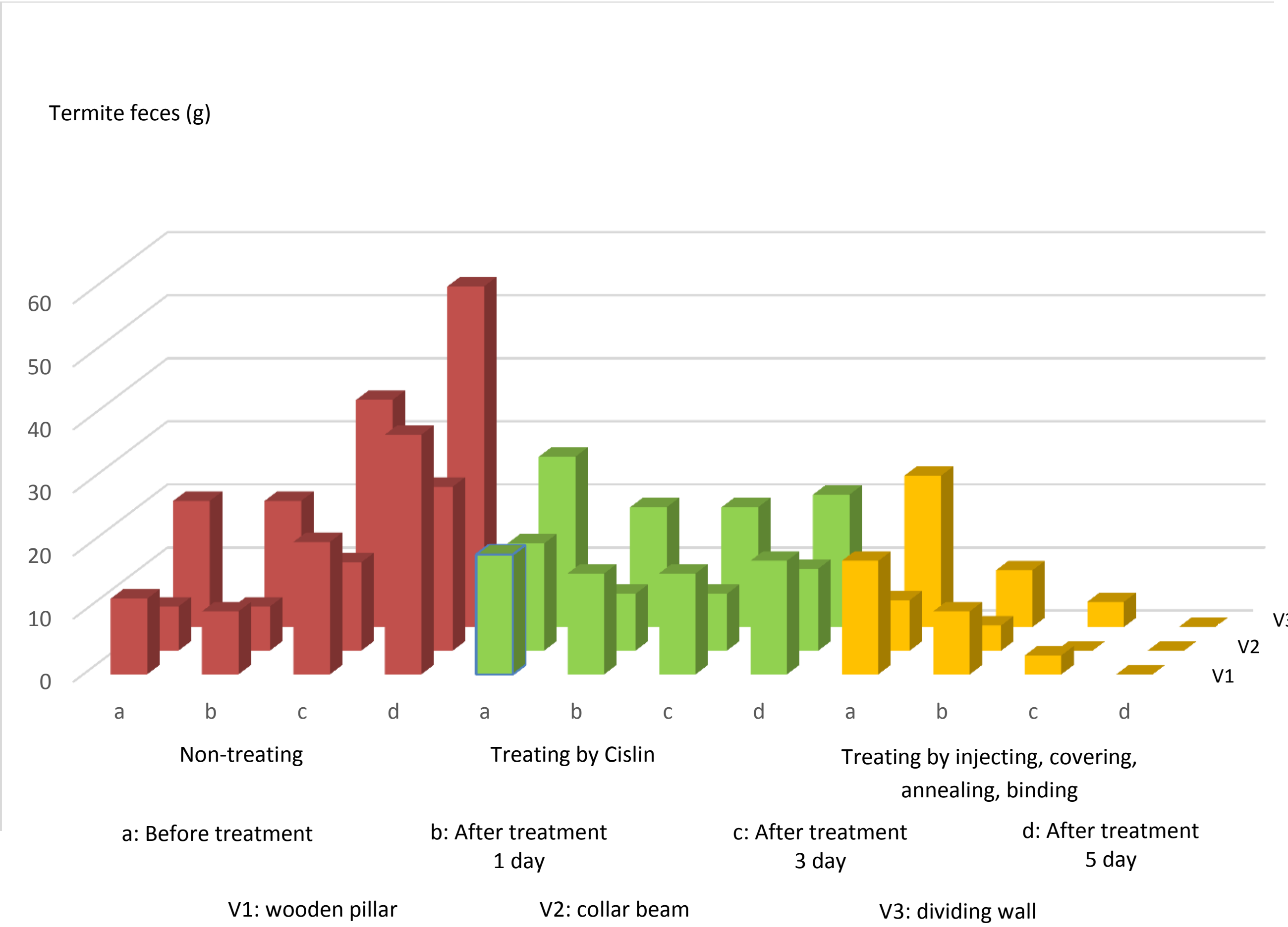


Figure 2. Quantity of termite feces at treatment positions according to the time

World-wide, there are many reports on methods for preventing and controlling drywood termite. For example, Vernard et al. (1996) evaluated chemical and non-chemical methods for controlling the drywood termite *Incisitermes minor* (Hagen) and judged that 3 days after treatment 100% control was achieved using steam and 2 fumigants (sulury1 fluoride and methyl bromide). According to Lewis (2003), the method of injecting pesticides as local treatments as proposed by Scheffrahn et al. (1997) is easy to apply, economical and popular.

Vernard R. Lewis (2005) evaluated the efficiency of Thiamethoxam 2SC (0.1%) in controlling dry wood termite *Incisitermes minor*. The results showed that the locations treated with Thiamethoxam decreased or eliminated the infestations of the drywood termite *Incisitermes minor*.

Figure 3. Wing termite and head of worker termite died at position of wooden pillar at house on stilts of Tan Da Resort

In Vietnam, method of treating drywood termite in structural elements and new architecture by applying the solutions in a local treatment method only reduces infestations. The method of injecting, covering, and binding as proposed for the first time according to the basic process of preventing infestations of the beetle *Stromatium longicorne* used by the Vietnam of Institute of Ecology and Works Protection (2014) actually eliminated infestations. Nguyen Quoc Huy et al. (2014) also proposed a similar method to prevent drywood termites at relic areas of Thanh Hoa Province. The research result from Tan Da Resort is significance by affirming the efficiency of injecting, covering, and binding for controlling drywood termite (*Cryptotermes domesticus*) in Vietnam.

Conclusion

Application of the method of injecting, covering, and binding two types of termiticide for treating drywood termite (*Cryptotermes domesticus*) at buildings of Tan Da Resort showed 100% efficiency at eliminating infestations after 5 days, which was more effective than only injecting a termiticide (achieving 17.5%).

Acknowledgements. We sincerely thank the expenditure support of the subject *"Collaboration research on technology to manage pests (termites, ants, cockroaches) in urban areas".*

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Tender Documentation for Termite Inspection and Control in the Built Environment

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Abstract

Tender documentation for termite management and control aims to collect competitors' expertise, duration, production and fee. Practices in the construction industry have provided examples to collect form of tender, job references, payment schedule and method statement to focus on the expertise, duration, production and fee tendered for by the pest control operators. No matter how well-equipped a tender document is, there is always room for value –adding. The features of the case study project warrant a meticulously thought-out document: it is in the vicinity of a school building and the trees are on a very steep slope. Termite treatment will be done once and for all in a termite nest and colony. There is no chance for giving it a trial. If work is not properly done, it will invite a repeated visit, termites might have spread to other areas which will take another cycle to find them and will waste time. Before and after tender, it is prudent to identify grey areas, ambiguous term, and the presence of precautionary advice that tenderers can provide. As such the document will be fully thought out to cover for all these arduous and complicated works that ensure.

Key words: Termite Management, Form of Tender, Master Program, Payment Schedule

Introduction

In the course of termite management and control, institutional organizations often have two options to engage a pest control operator: either by competition or negotiation. Competition may be restricted to a few selected firms or open to almost any firm who wishes to submit a tender. The pest control operators (PCOs) who are newcomers to the market will be able to attempt the work. Single PCOs are selected and given the tender documentation to be priced in the usual way. The priced documents are then passed to the project manager to check for the reasonableness of the rates and prices.

The paper will focus on the competition route to determine what should go into the tender documentation so as to engage a competitive pest control operator. Tender documentation refers to all the documents necessary for completing tendering procedure up to engaging a competitive pest control operator, including form of tender, letter of invitation, specification and drawing.

Termites, the white ants, have always been the most destructive pests. In Hong Kong, owing to suitable temperature and humidity, termite attack is one of the major problems of property management in the built environment.

Land resource is scarce in urban cities. Many built-up areas are converted from natural habitat or hills traditionally infested with termites. With no proper termite control and management the built environment will have to tolerate the co-existence of termites and humans. The obvious remained features attacked by termites are trees. Original trees left on hills near to built-up areas can be very dangerous. Once the trees are rotten in the tree trunk or near the root stump, they can fall down any time when there are heavy rain and strong wind in tropical cyclone season.

One recent incidence reported in a newly completed primary school building is that there was a 10-metre tree fell and landed on the verandah railing, broke and damaged a section of the metal handrail and corridor balustrade. Termites were seen running away from the fallen tree trunk. That triggered the need to look at the possibility of further damage done to the campus building by other trees and the need to reduce the conducive conditions for termite existing adjacent to the school building; and to avoid the possibility of colonization of the built-up area by the termites in the vicinity.

Since termites are very sensitive to the conditions of survival, once a tree has fallen, the nest survivors will escape to the next tree or to the next possible habitat for foraging and infestation.

The work to control and manage termite infestation must base on the fundamental concept that there is a one-time chance to eliminate the termite colonies. Any failed attempt will waste the chance of complete elimination. As such the pest control operator so chosen has to be the most competent one and one who can provide the 'silver bullet' to effectively eliminate all.

The aims of this research are to collect data about how to find the competitive pest control operator so that the work can be performed most effectively and efficaciously. Such result hinges on the appropriate content of the tender documentation.

Methodology

The method of the research was based on the standard procedure used in building construction and the comparison of the practice to a case study to add or edit out what is actually required.

Cook (1991) has described the purpose and types of tender documents most appropriately: tender documents are intended to provide each builder with common data in sufficient detail to suit the circumstances of each project. The intention with such documents is to obtain a number of competitive tenders that can be compared objectively in order to select a suitable bidder. Cook (1991) expects that upon the pest control operator's return of tender it will be able to cover PCO's expertise, duration, production and fee.

Expertise – a statement of experience and expertise within the organization followed by details of the management approach to be employed on this project;

Duration – master program for the work and staff deployment;

Production – details of the method of termite control and management;

Fee – the financial reimbursement required for managerial services and a separate sum to cover for providing both common site facilities and a supportive general workforce; and the related schedule of payment percentages.

Brook (2006) cherishes the time spent to preparing good quality documents, which aid the pest control operator's understanding of the works and will benefit the finished product.

Combining the literature review and a case study, it is possible to choose the better practice of the tender documentation for the purpose of termite management and control.

Findings

On return of PCOs' submitted tenders for the protection of the primary school building and external works in October last year, the following information was present: form of tender, expertise, payment schedule, method statement.

Form of Tender

The Form of Tender is the PCO's offer in response to the invitation of tender. It is a standard pro-forma duly filled in with information and will form part of the contract tender documents later. The pro-forma has got individual company's identification information including company names, their titles and positions, commercial licencing registration number and the notification of the law to prevent bribery. Comparing these with the Conditions of Contract of the Institution of Civil Engineers, there is still a big difference in the available information. Because the standard tender form (Ashworth, 1996) will usually contain the following:

(a) the contractor's price;
(b) commencement date and time for completion;
(c) salient particulars of the offer.

Aims of the service

The aim is clearly stated to identify any termite activity in the site boundary. The scope of work is sequential as to include (1) identify termite activity either past or present; (2) estimate damage level; (3) motion detection by ultrasound or microwave or equivalent to individual trees; (4) motion detection by ultrasound or microwave or equivalent to all hand-reach wooden fixtures (e.g. wall panels, cabinets, timber floor, doors, doors frames, etc.); (5) provision of scaffolding and temporary working platform for complete execution of work; (6) dismantling of the scaffolding and temporary working platform upon completion of works; and (7) provision of comprehensive report with photos and drawing plans showing all finding.

Service area

Service area has to be demarcated. It is divided into within site boundary and outside site boundary. Within boundary will include all areas such as planters, rooms, common areas, corridors washrooms, staircases, plant rooms, switch rooms, hall and backstage, furniture, equipment, musical instruments, fixtures, fitting out, door and door frames, flooring, etc.

Outside site boundary include the hill slopes as marked on the tender drawing.

Results and discussion

Tender documentation attempted to be complete, but the returned documentation showed two grey areas: the detail and the procedure of termite control. There are also good practices. The aims of service requires both the scaffolding and working platform but when only scaffolding is quoted the detail does not seem to have a way to control this absence of the platform. Attention must be given as this is a statutory industrial safety requirement. There is a submitted use of an approved termiticide to treat all areas of active infestation and the entry points with it. But such word as 'approved' can be quite dubious and unclear.

There are two features in this project that the tender documentation will need to address: one is the trees infested by termites are near to a school building and the next one is the trees are on very steep slopes. The efficacy of a termite treatment system is closely related to the number of stations installed and the frequency of visits and the tender documents must reflect all these.

The submitted method to carry out termite management and control shall need to be described in full detail. The returned tender showed a good practice to show all perceived details as follows: 'scaffolding company will build up a bamboo scaffold and platform on the whole slope. During the service, workmen have to climb up the scaffolding and drill hole (about ½ feet depth) at the bottom of each tree at the slope. After that, workmen will place bait stations and hide up inside the hole. After completion of installation of bait stations, we would carry out regular inspection (bi-weekly for the initial two months, then monthly to quarterly basis for the following months). The service is for one year starting from completion of installation of bait station'. The detail becomes an added value to the tender documentation.

The returned tender documentation can provide some precautions which is absent in the scope of service. The precaution advice is: 'termites are very sensitive, once their living activity is disturbed. They will find a new place to build up their nest. Therefore, during the elimination period (estimated about 3 months), all construction work / renovation work / any activities surrounding the baiting stations with active termites (~ 3 meters) are highly recommended to be stopped / avoided.' This is a second added-value to the document and allow efficacy to the final outcome.

Conclusion

From the literature review, tender documentation for termite management and control aims to collect competitors' expertise, duration, production and fee. The above case study shows that the retuned document has obtained the form of tender, expertise, payment schedule and method statement. No matter how well-equipped a tender document is there is always room for value – adding. The features of the project are: it is in the vicinity of a school building and the trees are on a very steep slope. Termite treatment will be done once and for all in a termite nest and colony. There is no chance for giving it a trial. If work is not properly done, it will invite a repeated visit, termites might have spread to other areas which will take another cycle to find them and will waste time. Before and after tender, it is prudent to identify any grey areas, particularly in the detailed procedure, like 'working platform'; in the use of term like 'approved' and in the number of precautionary advice that tenderers can provide. As such the tender will be fully thought out to cover for all these arduous and complicated works that ensure.

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Field Trials in Thailand on the Efficacy of some Soil Termiticides to Prevent Subterranean Termites

by

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Abstract

A study on the efficacy of some soil termiticides available in Thailand market was made in order to evaluate their effectiveness in preventing invasion of subterranean termites foraging underneath buildings. The Modified Ground Board Test (MGB), a method representing slab-on-ground construction, a practice widely used for houses was used in the study. Five test sites were chosen as experiment plots to compare different climate and land use practices common around the country. The first test site was in the northeast followed by the second test site in the west, the third in the east, while the fourth and the fifth were in the south. Synthetic pyrethroids was the major group of termiticide used in the study (alpha-cypermethrin, cypermethrin, permethrin, bifenthrin and fenvalerlate). One organophosphate and carbamate termiticide was used in the study chlorpyrifos and fenobucarb, respectively. Four newly introduced products were also testing including; fipronil, imidacloprid, chlorfenapyr and chlorantraniliprole. Results from yearly observations reveal that the pyrethroids bifenthrin 3 EC effectively prevented termite attack for more than 8 years, alpha-cypermethrin 4 SC, 8 SC and cypermethrin 10 MC lasted 9 years, permethrin 30 EC lasted 5 years and fenvalerate 10 EC lasted 4 years. The organophosphate chlorpyrifos 40 EC lasted 7 years and the carbamate fenobucarb 20 EW lasted 5 years. The newly introduced products fipronil 2.5 EC, 5 SC, 5 SL and 80 WDG lasted more than 7 years, imidacloprid 5 SL, 10 SL and 17.8 SL lasted more than 7 years, chlorfenapyr 2 SC lasted between 5-7 years and chlorantraniliprole 17.8 SC lasted more than 8 years.

Key words: subterranean termite, termiticide, efficacy, prevention

Introduction

Soil treatment in Thailand can be dated back only until early 1960's (Vongkaluang, 1990). Before that time termite damage to buildings was considered as less important because of many reasons, the most obvious were type of buildings and the durability of timber used as construction materials. In those days, homes and buildings were one stories with high post and timber used were highly durable species such as teak, ebony, rose wood ects. Changing of construction design later to slab on ground and replacement of durable timber by non-durable timber resulted in higher degree of termite infestation in buildings and prevention of termite damage then started to gain more attention from all concerned. Soil treatment was the practice recommended to be used and chemicals used for treatment during those time were mostly insecticides used for agricultural crops such as aldrin, dieldrin and chlordane (Vongkaluang, 2004). The organophosphate group entered into practice about a decade later followed by synthetic-pyrethroids in late 1980's. By that time the chemicals used for soil treatment for termite management were switched from agricultural insecticides to termiticide which were readily available in the market. After the expiration of patented of many synthetic pyrethroids and the most popular termiticide (fipronil), various generic termiticides were introduced into the country. It was therefore necessary to evaluate the efficacy of these soil termiticides to be used as guideline for both home owners and pest control operators whenever they came to the point to select soil termiticides in their construction deals.

Materials and methods

1. Materials

1.1 Termiticide

The various concentrations of commercial soil termiticides tested in the 5 permanent plots designated by Royal Forest Department belong to 7 chemical classes. The chemical class, common name of the active ingredient and product formulations used were:

Synthetic pyrethroid:	α-cypermethrin	(0.15 EC, 2 EC, 4 EC, 4 EW, 4 SC, 5 EC, 5 SC, 7 EC, 8 SC, 10 EC)
	cypermethrin	(4 EC, 4 SC, 5 EC, 7, 8 SC, 10 EW, 10 MC, 15 MC, 16 SC, 25 EC, 25 EW, 25 SC, 35 EC)
	permethrin	(25 EC, 30 EC)
	bifenthrin	(1 EW, 1 EC, 2 EW, 2.5 EC, 2.5 SC, 3 EC, 3 SC, 3 SP, 4 EC, 5 EC, 5 EW, 5 SC, 8 SC, 10 EC, 10 EW, 10 SC, 10 ME, 15 EC, 15 EW, 15 SC, 20 EC, 20 EW, 20 SC, 24 EC, 24 SC, 25 SC, 60 SC)
	fenvalerate	(10 EC, 10.5 SC)
Organophosphate:	chlorpyrifos	(40 EC)
Carbamate:	fenobucarb	(20 EC, 20 EW, 20 SC)
Phenyl pyrazole:	fipronil	(2.5 EC, 2.5 SC, 4 EC, 5 EC, 5 SC, 5 SL, 10 EC, 10 SC, 20 SC, 80 WDG, 80 WP)
Chloronicotinyl:	imidacloprid	(2.5 SL, 5 EC, 5 SC, 5 SL, 10 EC, 10 WP, 10 SC, 10 SL, 17.8 SL, 18.3 EC, 20 SC, 20 SL, 25 SL, 25 WP, 35 SC, 60 SC, 70 WDG)
Pyrroles:	chlorfenapyr	(2 SC, 24 SC)
Anthranitic diamide:	chlorantraniliprole	(17.8 SC)

1.2 Rubber wood block (*Hevea brasiliensis* Muell.Arg.) size was 5 x 5 x 2.5 cm^3 for treatments and control.

1.3 Construction tools.

2. Methods

Modified Ground Board Test (MGB)

This test representing field condition of building with slab on ground. Test methods are:-

2.1 Install 1 x1 x 0.2 m^3 cement block on designation plot.

2.2 Fill in the sand into the ditch and using construction tools to level the sand surface to the top of the plot height (20 cm).

2.3 Mix chemical to required concentration.

2.4 Evenly pour test chemical by the watering pot on the surface of the sand 5 liter to 1 square meter (1 plot).

2.5 Put the plastic sheet on the entire surface of the sand and the outer edge of the concrete blocks.

2.6 Put the PVC pipe in the middle of the ditch and pure concrete 8 cm thick on top of the plastic sheet (around the PVC pipe).

2.7 Cut out the plastic sheet inside the PVC pipe.

2.8 After the concrete hardened, put one block of rubber wood (5 x 5 x 2.5 cm^3) inside the hole of the PVC pipe and cover the pipe.

2.9 Comparatively evaluation the result on the rubber wood block in treated sand and untreated sand (Control) at 1 year and once every year thereafter.

3. Test Sites

The experiment was located at five locations

3.1 Khon Kaen Province (KK) in the northeast, at Extension & Development Utilization of Small Timber and Non-Wood Forest Products Station, Muang Distric.

3.2 Ratchaburi Province (RB) in the west, at Extension & Development Utilization of Small Timber and Non-Wood Forest Products Station, Muang Distric.

3.3 Chonburi Province (CB) in the east, at Nong Ta Yoo Forest Plantation, Sri Racha District.
3.4 Phuket Province (PK) in the south, at Bang Kanoon Plantation, Talang District.
3.5 Surat Thani Province (SR) in the south, at Surat Thani Silvicultural Research Station, Muang Distric.

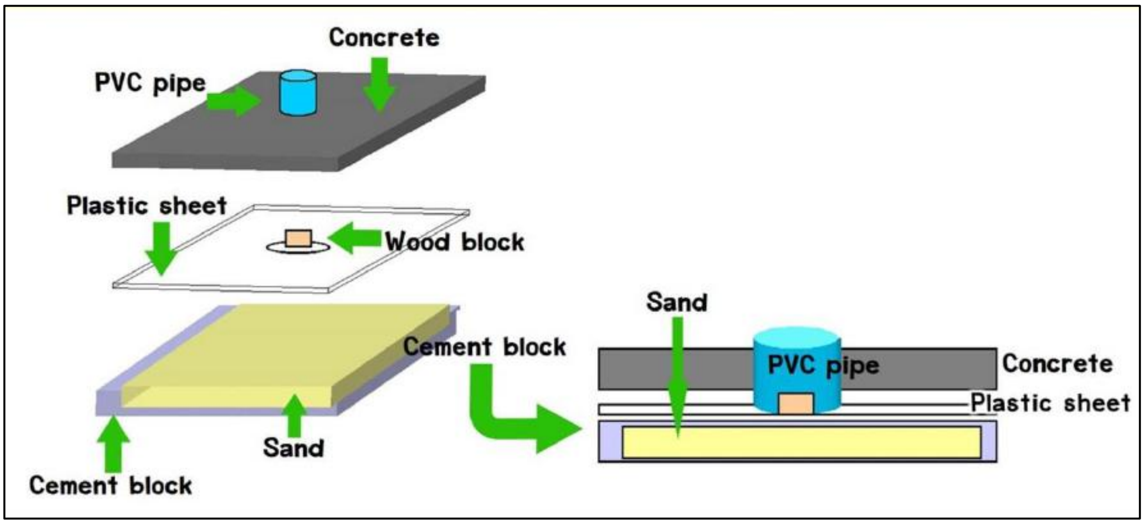

Figure 1 Modified Ground Board Test.

Figure 2 Plot of termiticides test by Modified Ground Board (MBG).

Result and discussion

Figure 3 - 6 show the results of the annual efficacy evaluation of the tested chemicals as soil termiticides using the Modified Ground Board Test (MGB). Number of years (Times on the Y axis) shown in the figures for each chemical indicate the longest period of time that chemical actively prevented the invasion of termites as demonstrated by the appearance of termites into at least one permanent plot.

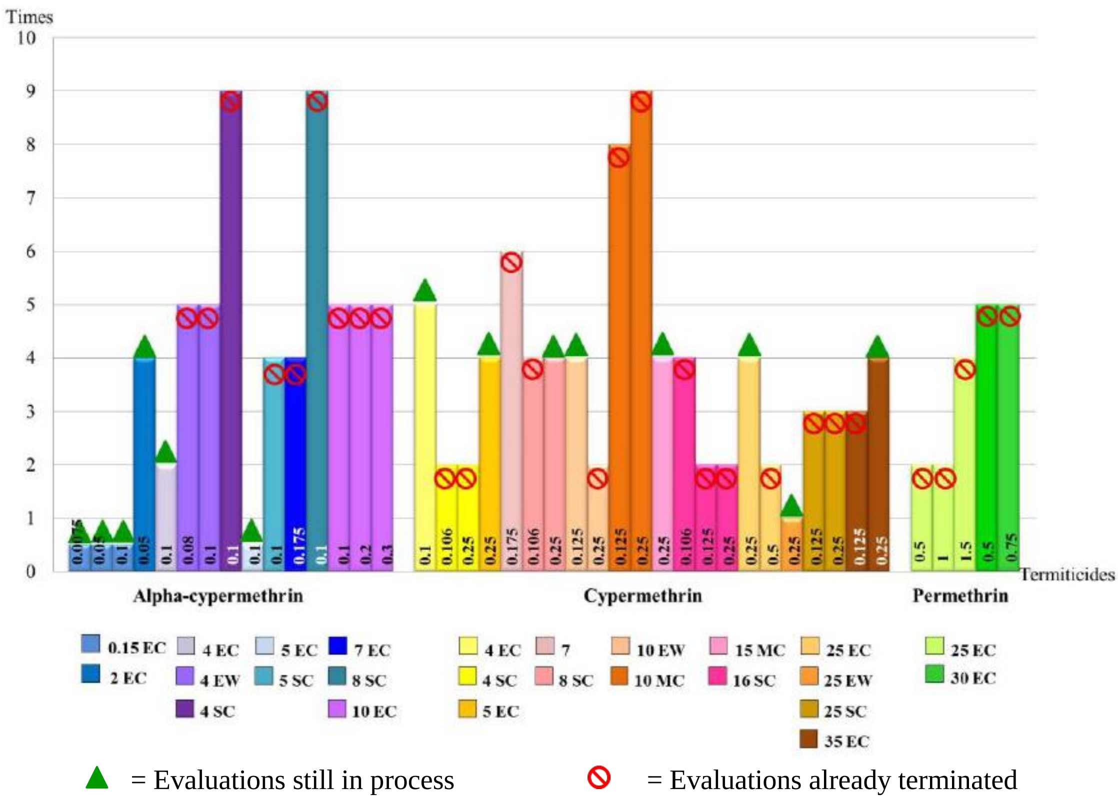

Figure 3 MGB efficacy results for the synthetic pyrethroid termiticides by formulation and concentration in time (years) when termites were first observed in at least one plot.

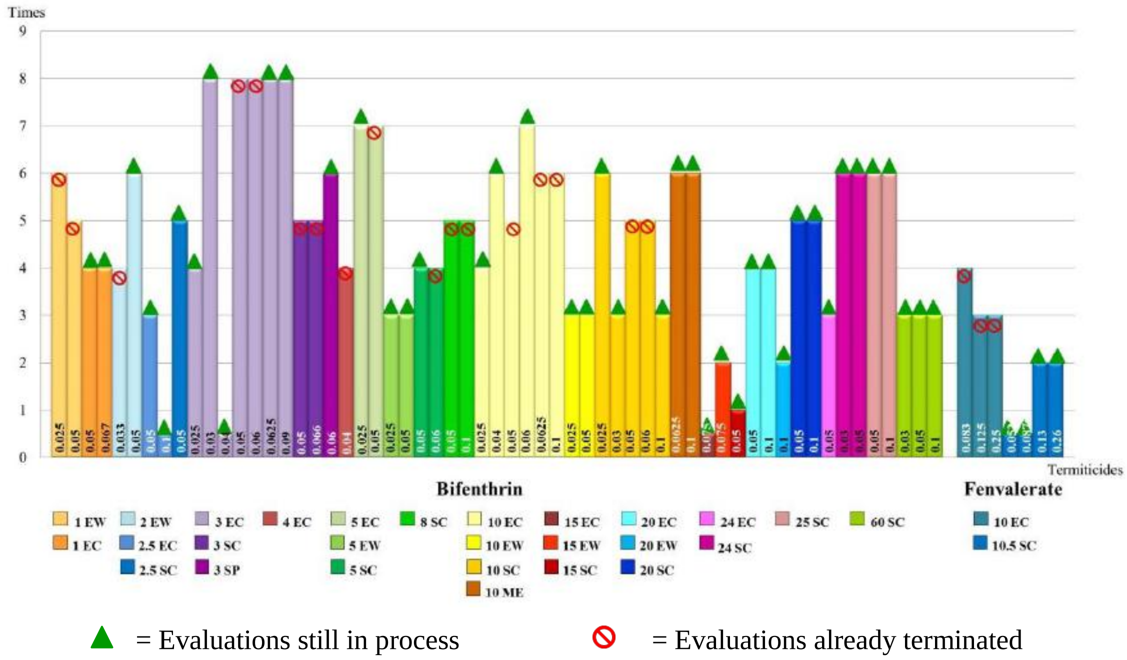

Figure 4 MGB efficacy results for the synthetic pyrethroid termiticides by formulation and concentration in time (years) when termites were first observed in at least one plot.

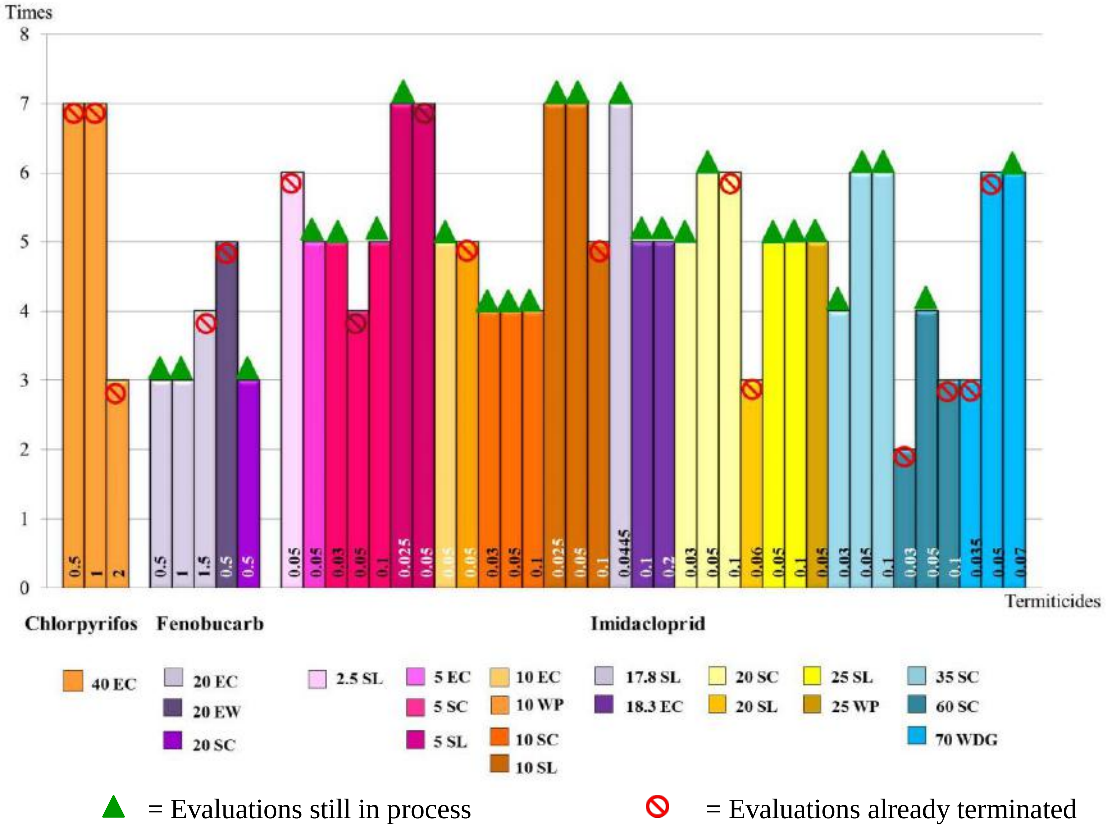

Figure 5 MGB efficacy results for the organophosphate, carbamate, chloronicitinyl termiticides by formulation and concentration in time (years) when termites were first observed in at least one plot.

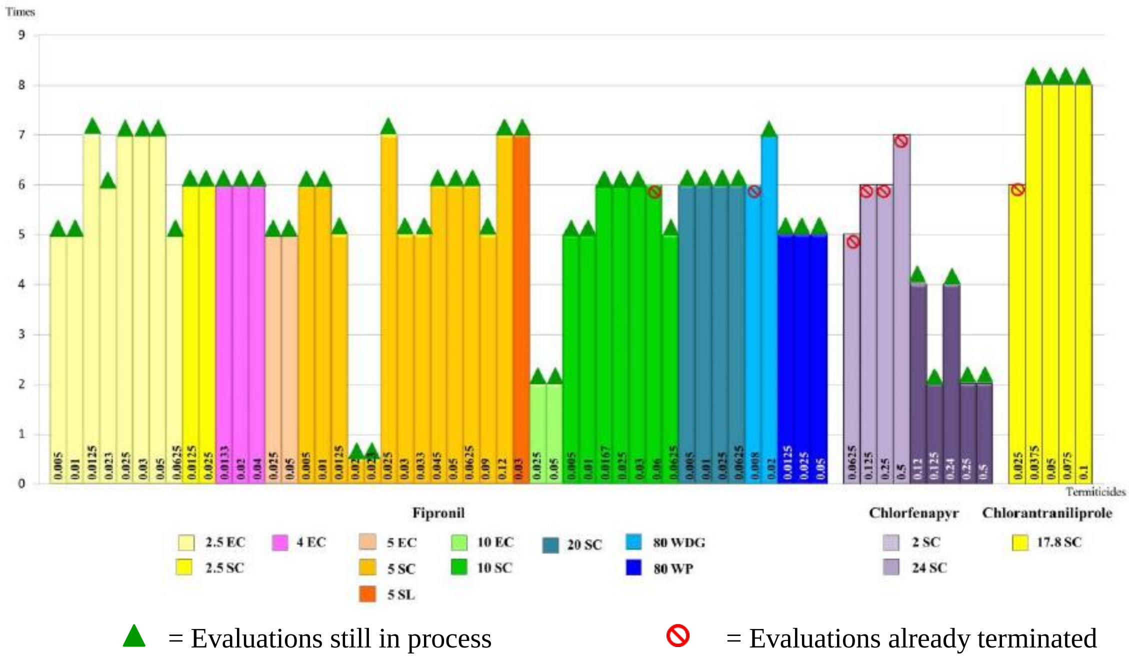

Figure 6 MGB efficacy results for the phenyl pyrazole, pyrroles, antranitic diamide termiticides by formulation and concentration in time (years) when termites were first observed in at least one plot.

From the results as shown in Figures 3, 4, 5 and 6, it is quite obvious that some of the soil termiticide formulations and concentrations used in the "Modified Ground Board Test" performed for over 5 years especially the termiticides in synthetic pyrethroid, organophosphate, phenyl pyrazole and nicotinoid groups. The result revealed that bifenthrin is the most popular synthetic pyrethroid used by Thailand's pest control business. Bifenthrin 3 EC showed longer a preventive role (more than 8 years), while some formulations and concentrations revealed 4-6 years protection. Other termiticides in this group such as alpha-cypermethrin 4 SC, 8 SC and cypermethrin 10 MC lasted for 9 years, while fenvalerate 10 EC and permethrin 30 EC lasted 4 and 5 years respectively.

The organophosphate chlorpyrifos formulation 40 EC at the 0.5% and 1% concentration prevented termite attack for 7 years while the 2% concentration failed at 3 years, the carbamate fenobucarb 20 EW at 0.5% lasted 5 years while the 20 EC at 1.5% failed in three years.

The newly introduced termiticides such as the pyrroles and anthranitic diamide came into the Thailand market but still not very well recognized. Chlorfenapyr 2 SC lasted between 5-7 years and showed a concentration-dependent response with the lowest rate (0.0625) failing at 5 years and the high rate (0.5) at 7 years. Chlorantraniliprole 17.8 SC is still providing protection after 8 years at concentrations higher than 0.25%.

The concentration of termiticide and formulation played a role in the performance of the products in the MGB field trials. Termiticide such as fipronil (phenyl pyrazole) 2.5 EC, 5 SC, 5 SL and 80 WDG prevented attack for more than 7 years at various concentrations. Imidacloprid 5 SL, 10 SL and 17.8 SL lasted more than 7 years, while some formulations and concentrations lasted between 2-6 years, so it should be taken into consideration that the formulations and concentrations of termiticide may probably effect the degradation of termiticide when applied in field condition.

The record of observations as shown in Figures 3, 4, 5 and 6 revealed that results from different test site varied. Factors such as soil type, annual rainfall, pH of soil and land utilization can account for the variation of results from place to place.

Taking into consideration the discussion given above, it is recommended that factors involved in the selection of a termiticide for soil treatment should include the chemical group of the product, as well as the formulation, concentration and location.

Conclusions

Field trials in Thailand on the efficacy of selected soil termiticides to prevent subterranean termite infestations using the Modified Ground Board Test (MGB), which are modified from the USDA forest service guidelines fit with construction practice in Thailand were conducted at five sites in Thailand. The MGB can provide recommendations for soil treatments for houses with slab on ground. The study compared termiticide groups available to the termiticide industry in Thailand. Results revealed that bifenthrin 3 EC effectively prevented attack of termite for more than 8 years, alpha-cypermethrin 4 SC, 8 SC and cypermethrin 10 MC lasted 9 years, permethrin 30 EC lasted 5 years and fenvalerate 10 EC lasted 4 years. chlorpyrifos 40 EC lasted 7 years, fenobucarb 20 EW lasted 5 years. fipronil 2.5 EC, 5 SC, 5 SL and 80 WDG lasted more than 7 years, imidacloprid 5 SL, 10 SL and 17.8 SL lasted more than 7 years. chlorfenapyr 2 SC lasted between 5-7 years and chlorantraniliprole 17.8 SC lasted more than 8 years.

Acknowledgment. The authors would like to express our sincere appreciation to the companies in Thailand for supplying chemicals and financial for the establishment of the permanent plots for the evaluation. We would also like to thank all the staff of the test sites for their hospitality and helping hands extended to us while we work in their properties.

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First evaluation of baits as a preventative tool to control *Coptotermes* termites in Vietnam

by
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Abstract

This research was conducted in construction sites to illustrate the effectiveness of bait in controlling subterranean termites as a preventative tool. Bait was applied around three houses that were under Coptotermes attack in urban areas. The houses represented different design styles, and they were monitored over 1- year period. As a result, termite colonies were declined gradually after 18- 24 weeks, after about 12 to 17 weeks foraging in the bait stations containing only wood attractant blocks. The bait stations would also be maintained to monitor concealed entry of termites into the structures. Overall, this finding is considered as the initial research that confirmed protective function of Vietnam termite baits used to protect structures under infection of subterranean termites.

Introduction

Subterranean termites, particularly the genus Coptotermes, are one of the most important pests for constructions in Vietnam. They are widely distributed in different regions but abundant in urban areas (Nguyen Duc Kham 2007, Trinh V. Hanh et al., 2010). According to recent studies, there are two Coptotermes species causing serious damage to structures in the North part of Vietnam, including *Coptotermes gestroi* and *Coptotermes formosanus* (Nguyen Duc Kham et al 1985, Trinh Van Hanh et al. 2014). The cost of termite control and repairs was reported at about $1.7 million USD for only private houses (WIP, unpublished data, 2011). Hence, preventing subterranean termites for houses under construction can significantly reduce economic losses as well as environmental problems and human health issues related to frequent use of pesticides.

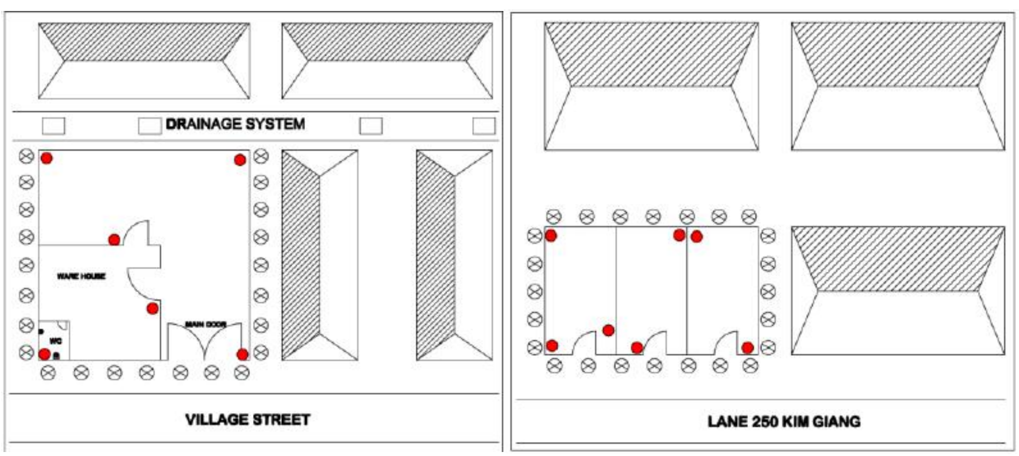
Fortunately, termite bait was evaluated as an effective tool in termite control. The use of the termite baits has been improved over 20 years (Buczkowski 2014), and incessantly developed in association with understanding termite foraging behavior and social activities (Wang & Henderson 2012). However, termite bait treatment in Vietnam was not well published to the world, particularly as a function of a preventative barrier for new building construction. There are several reports about bait application for structures with the active ingredient hexaflumuron including an assessment of BDM, made in Vietnam, used to treat termites infesting buildings at a resort (Hanh et al. 2014). While demand for innovative bait applications are increasing in Vietnam in relation to environment concerns, understanding and enhancing bait effectiveness has become a priority. To promote a wide use of domestic termite bait in Vietnam, it is necessary to design field trials to show the advantage of baits to protect structures from termite attack.

Materials and Methods

Materials
There were two termite bait components used in this study: i) bait stations containing, on average, 15 pine blocks 20cm x1.5cm x1.5cm in size treated with an attractant to lure termite foragers, ii) termite bait, named Mobahex, supplied by WIP containing the active ingredient hexaflumuron.

Investigation of termite damage status
Prior to conducting field trials and to gain data for quantifying control, investigation of termite damage status was undertaken in three local areas of Hanoi using questionnaires and face-to-face interviews. They included A1 block (Hoang Mai district), A13 block (Thanh Xuan district) and homes on the campus of University of Trade Union (Dong Da district) and Hanoi Open University (Hai Ba Trung district) for a total of 320 houses, averaging 80 apartments per area or block. Households used in the interviews were selected randomly and investigated based on a SPSS survey (2004) combined and improved with actual situations like: House type? Having termite or not? etc.

Field trials of termite bait treatment
Wood stations, containing no bait, were installed as a field trial control around the houses. These stations found strong *Coptotermes* activity after for 12 weeks. These houses included a private modern house with dynamic decoration style, an antique house used as a church by one family with a big wooden altar in the left-hand-side room and wood decorations inside, and a small building having 4 floors with the ground floor used as a garage and storage place. The wood blocks, 20cm × 1.5cm × 1.5cm in size, used were pine treated through a cycle of submersion and drying then compressed to increase attractive capacity to *Coptotermes* species (Mo 2014). Wood stations were placed at 2m internals and 0.3 – 0.5m from structural food depending on the house design and mapped for each construction site (Fig.1). The monitoring interval of this phase was 1 week and lasted for 12 weeks. The time period of termite observation was from March 2014 to June 2014 and considered as pre-treatment phase (Su 2007). Wood consumption rate was estimated based on number of wood blocks infected by termite foragers per station (WIP, unpublished data).



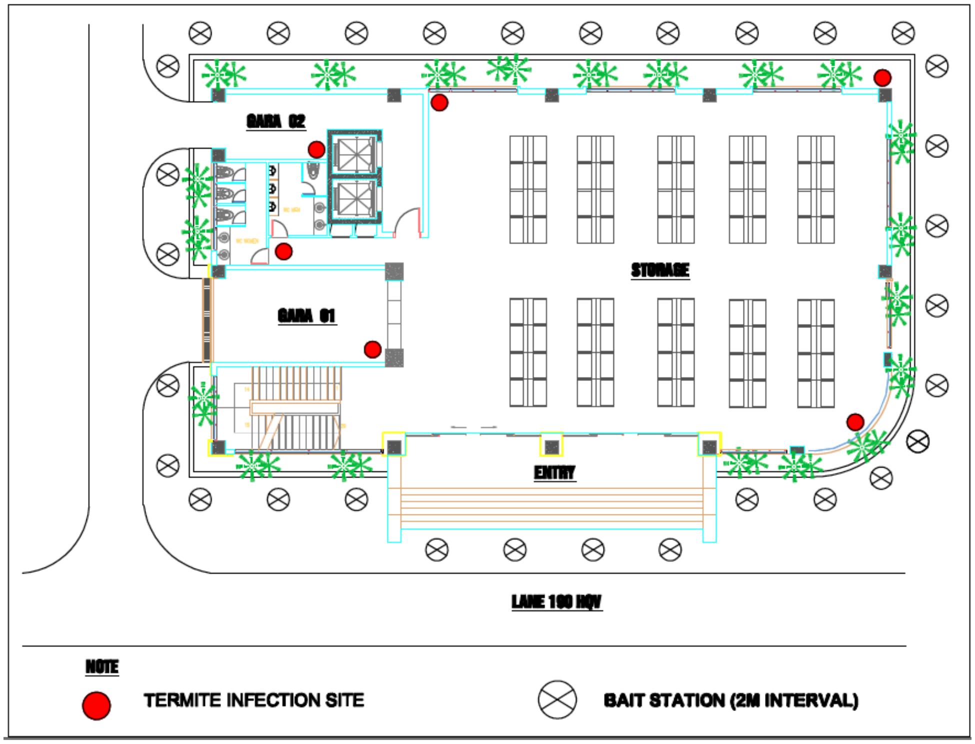


Fig 1. Treatment of in-ground monitoring stations around structures (scale 1:250);
Top left- private house installed 23 stations, top right- antique house installed 24 bait stations and small building installed 32 bait stations.

After that, baits containing active ingredient were introduced into the wood stations. The monitoring interval was longer than the pre-treatment phase, 2 weeks. After bait elimination, bait stations were maintained and monitored for 13 weeks (approximately every 3 months) until June 2015 to test re-infestation of termites. Effectiveness of the in-ground termite bait was evaluated based on the decline of termite activity around the houses, following the method of Grace and Su (2001) and Su (2007).

Statistical analysis

Data was processed using SPSS (ver 2.0) (http://www-01.ibm.com/software/analytics/spss/) to analyze current status of termite pests in urban areas of Vietnam, while bait effectiveness was assessed through statistical analysis using Excel 2011. The output data was collected and saved for general evaluation system at WIP, Vietnam.

Result and discussion

Investigation of termite infection in urban areas

Termites were found in over 25% of the 320 structures investigated. However, this rate was not equal in the different house types, with a high rate of termite appearance in private houses and old houses compared to new and antique houses of the Old Quarter in Hanoi. This rate in the private houses and old houses are 8.07 and 8.38, respectively (Fig.2). When analyzed under cross tabulation, there is a significant difference in the percentage of new houses and old houses ($P = 0.0001 < 0.05$), indicating a preference of termite pests to house types that use a high portion of cellulosic materials to construct or decorate. The reason could be that the old style of structural design generates a humid environment around footing areas, especially ground pavement. Also, a low ceiling and old construction materials, such as carton, lime mortar, might create attraction for termites. Surprisingly, this result was not the same for antique houses, with a very low infestation rate, 4.97%. To explain for this result, the city council in this area implemented annually several area-wide control programs to preserve the antique view for the Old Quarter tourism. Therefore, the percentage of termite damage decreased. However, to understand the preference trends of termite with different types of construction, there is a need to investigate more houses in wider areas in future. Additionally, the percentage of houses under attack by termites only indicates the incidence of termite infestations, not the damage level. According to Grace & Su (2001), whenever termites are found, they set these structures under threat or a zero tolerance action threshold. These results could be an initial part of integrated pest management programs, being a first step to designing appropriate solutions and gaining public awareness based on actual situations in Viet Nam.

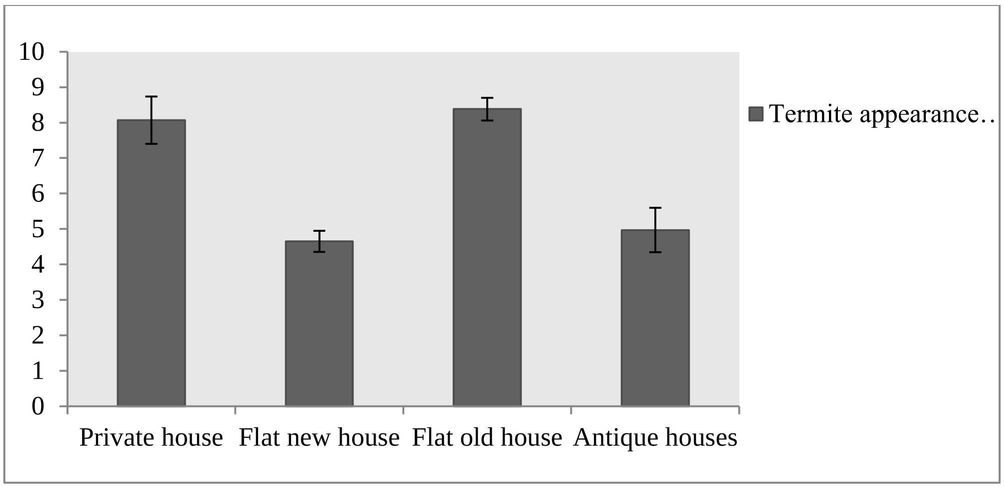


Fig.2. Percentage of house found to be attacked by subterranean termites

Effectiveness of bait treatment as protective tools

Wood consumption rate. There was a general pattern of increasing consumption of the wood blocks during 3 months in all three houses. Termites were first found in bait stations around the modern house, after 3 weeks, then the antique house and lastly the ground floor of the building. However, the wood consumption rate in the antique house was highest at week 12, with 20% of wood blocks consumed, and steadily increased over time. The same pattern was illustrated in the small building, except a slightly stability in weeks 7 and 8, whereas the consumption rate in the modern house increased very slowly and reaching over 6% of the wood blocks consumed by week 12th. This result might be due to internal conditions around the modern house where there was a convenience store on the ground floor with noise from a number of visitors everyday. Also, the store contained a number of big carton boxes, which could serve as an alternative food source for termites (Baker & Weeks 2006). Compared to previous studies on different termite bait systems, termites were found in monitoring stations of Sentricon bait after 1 month (4 weeks) at rate from 8 – 27% (Grace et al. 1996) and termites first foraged to Firstline and SentriconR bait stations at 8.7 weeks and 3.7 weeks, respectively (Glenn et al. 2008). Hence, the time period of 4.3 ± 1.5 on average to attract *Coptotermes* is short and the consumption rate of Mobahex bait is within the reported range. This finding might be attributable to seasonal conditions in Vietnam, when termites are at their most active. In addition, the density of termite bait around the house could be another reason for the successful attraction because wood volume can increase termite recruitment (Lenz & Evans, 2002)

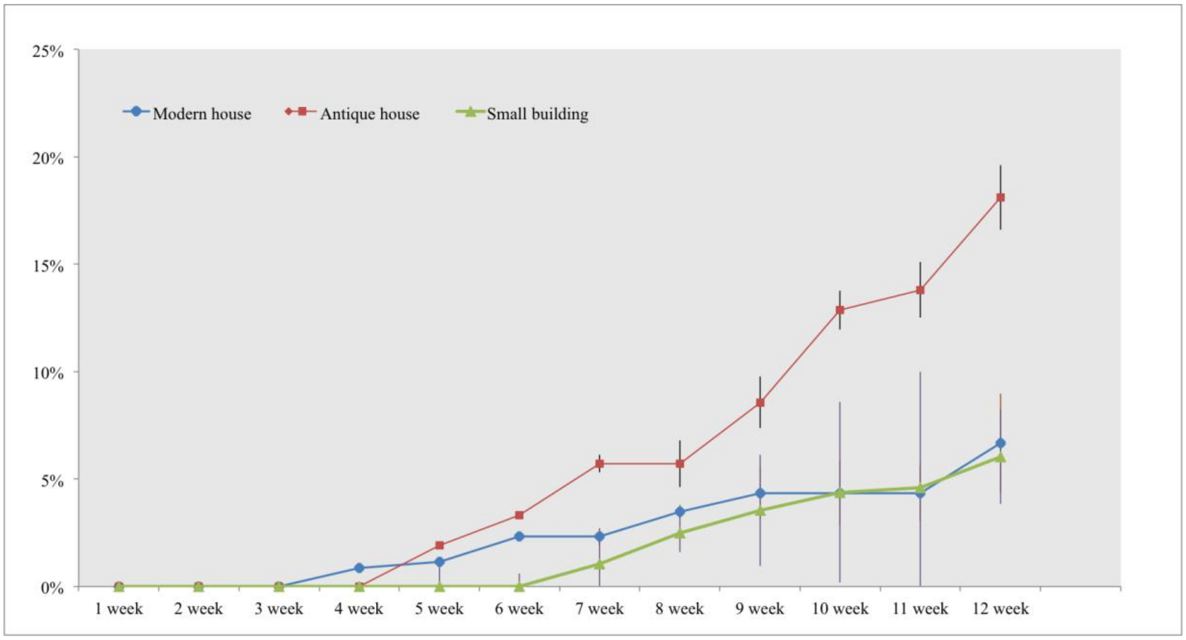


Fig. 3. Wood consumption rate per station of *Coptotermes* genus over 12 weeks in control sites.

Termite elimination by Mobahex. The effectiveness of Mobahex in controlling Coptotermes species was demonstrated after 21.3 ± 3.1 weeks (around 5 months) after installing bait stations (Table 2). There were no termites found in the area of the modern house after 18 weeks and in the antique house or the small building after 22 weeks. If considering only time after bait application, the time to elimination ranged from 6 – 10 weeks. Interestingly, despite termite foragers consuming significant wood in the stations around the antique house the elimination was generated later than at the other houses. However, to confirm a persistent control effect of Mobahex, it is necessary to maintain the bait stations over longer time to prevent concealed entry termite into the structures as illustrated by the case of termites appearing in the small building although it was not Coptotermes (shown as "+" letter in Table 1). This finding is in agreement with previous studies where termite population elimination by using hexaflumuron in large areas was reported in a relative short period, from 2 – 3 months (approximately 4 – 13 weeks). Furthermore, Osbrink, Conerlius & Lax (2011) asserted that the wide-area control using hexaflumuron bait can be maintained over two years and therefore act as an active barrier. In addition, the time period for Mobahex bait to be effective can also be compared to, for example, chlorfluazuron with over 6- 8 weeks (Sukartana, Sumarni & Broadbent 2009) or by week 12 (Peter & Fitzgerald 2003) on Coptotermes termites. Hence, this domestic bait can be recommended to control termites in wide areas.

The time variation reported for the amount of bait considered sufficient to control termites depends on different micro-environmental conditions at bait sites, building design and decoration styles. For instance, the antique house is in a quiet area and easier to install station systems including more favorable internal environment at bait stations. Whereas the surrounding at the small building includes a large garden with a high density of shrubs and trees that created a humid environment to make the bait moldy, which interfered with termite activities. In addition, control activities of the bait are related to population size, as well as more colonies that would require a longer time to elimination. To understand this hypothesis and preventative function of bait under a range of climate conditions, it needs further studies for longer time frames applied in a number of structures.

In comparison to the bait controls, it is clear to see from Table 1 and Fig. 2 that the termite bait killed all termite colonies after 4 months (around 16 weeks) as evidenced by a cessation of bait consumptions.

They also act as an active barrier to deal with termite problems around houses. This finding was first published about bait treatments applied against Formosan subterranean termites in field trials and can be a helpful reference for developing a national standard to protect new buildings under termite threats.

Table 1. Termite elimination of Mobahex bait over time periods

House types	**Termite activities per bait stations over time (week- wk)**								
	14 wks	**16 wks**	**18 wks**	**20 wks**	**22 wks**	**24 wks**	**36 wks**	**48 wks**	**60 wks**
Modern house	3	4	4	0	0	0	0	0	0
Antique house	4	6	7	7	3	0	0	0	0
Small building	4	4	5	4	2	0	0	0	+

Conclusion

The termite bait Mobahex can be used as an alternative barrier to prevent termite attacks into structures. To be effective, it requires a period from 16 to 24 weeks to eliminate all termite colonies around houses and the variation in time to elimination may depend on termite population size. The time of initial attraction to the termite bait stations was around 3 weeks. This is promising to apply bait to control termites in wide areas and developing this method is a key of integrated pest management along with education and public awareness in developing countries like Vietnam. Furthermore, these findings can be considered the first publication of the bait treatment method to prevent subterranean termites for urban structures. In the future further studies of termite bait systems should be tested over longer time periods and under different internal environmental conditions to investigate sustainable maintenance of the barrier.

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Eliminating subterranean termite infestation in ships and yachts across Philippine ports.

by
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Abstract

In the past few years incident of subterranean termite damages to seafaring vessels including boats, yachts and ships of different class have come to light across the Philippines. Inspection, collection and identification revealed that these infestations are primarily caused by two species *Coptotermes gestroi* and *Microcerotermes losbañosensis.* A number of successful treatments of yachts and ships were conducted using Requiem termite bait and the results prove the bait to be effective in eliminating termite colonies in a single feeding.

Key words : Subterranean termite, ships, boats, vessels, termite baiting

Introduction

Human aided dispersal by shipping vessels is one of the primary means by which termites are transported across oceans into new continents. Most notable within the last century are, establishment of 6 exotic termite species in Florida (Scheffrahn, and Su, 2005) followed by 5 species in Hawaii (Yeap et al. 2007), and one in Australia (Peters 1990).

Termite infestations in maritime vessels, including boats and yachts is an overlooked area in SE Asia. There are no records of such reports available to evaluate the seriousness of this subject. In the past 5 years the number of requests for termite treatment received from owners of ships and yachts, highlights the fact that infestations are much too common. As wood is extensively used in vessels to construct the interiors, it was expected that exposure to infestation and damage is naturally possible over time.

Generally termite damage to wood used in ships is notably from drywood termite species such as *Cryptotermes brevis* which is established in the Philippines. However subterranean termites are not commonly reported to be pests in such situations. A majority of ship-borne infestations are presumed to have originated from infested wood used in construction, renovation or repair of the vessels structure and interior. At times infestation from flying swarmers while berthed at port or at dry dock is considered a possibility.

Inspections over the past few years have also revealed the use of a number of susceptible timber varieties in boats and ships in the Philipines. This could be one more reason for manifestation of infestations. Following are some of the timbers commonly used, which have recorded various degrees of termite damages.

Scientific Name		Local Name
1	*Lagerstroemia speciosa (L.) Pers.*	Banaba
2	*Samanea saman (Jacq.) Merr.*	Raintree
3	*Anisoptera thurifera (Blanco) Blume ssp. Thurifera*	Palosapis
4	*Calophyllum inophyllum L.*	Bitaog
5	*Canarium luzonicum (Blume) A. Gray*	Piling-liitan
6	*Hopea foxworthyi Elm.*	Dalingdingan
7	*Petersianthus quadrialatus Merr.*	Toog

This article describes treatment of a subterranean termite infestation on a yacht berthed at the Manila Yacht Club in Manila Bay.

Material and Methods

Inspection
The infested yacht was inspected on receipt of an inqury from clients to identify the termite species. A treatment using the Labyrinth Baiting System with Requiem bait from Ensystex Inc was used. The Labyrinth Baiting system consists of a kit which includes 3 above-ground feeding stations and 1kg of Requiem termite bait.

Installation of baiting system
All infested sites were treated with the above-gound stations containing 140 gm of bait mixture. The bait mixture was prepared by mixing the bait with chlorine-free water in the ration of 1:3 to yield a consistency that does not drip water. As boats are expensive and contain delicate objects, care was taken not to install the stations in locations which would interfere with normal functioning of the ship.

Three (3) spots with active termites were chosen for installing the above-ground bait stations. Care was taken to make the bait less moist and a bit more dry because this happens to be the the preferred way to make subterranean termites feed on the bait quicker. The stations were then locked and left for monitoring. All feeding stations were also marked with a “Do not disturb “ sticker.

The first inspection was conducted 4 weeks after the installation. Stations were refilled again with the bait without disturbance. Final inspection was conducted 3 weeks after the first inspection. The stations were then removed and the treatment area cleaned for restoration.

Results and Discussion

Detection of Subterranean Termite infestation
Inspection, collection and identification revealed that yacht was infested by subterranean termite species *Coptotermes gestroi*. The workers of the termite were found at multiple feeding spots on structural timber as well as decorative timber used in the interior. But no indication of the colony location could be ascertained due to the nature of the site, in particular due to lack of access to all areas.

Installation of baiting system

Three (3) above ground stations were installed on this yacht. The first inspection was conducted 4 weeks after the installation. The bait consumption was complete with a few live soldiers noticeable. Sightings of only soldiers in a bait station is considered a sign of negative affect on a subterranean termite colony.

Final inspection was conducted after 3 weeks. No noticeable bait consumption was noticed. This was confirmed as colony elimination. The stations were removed and the treatment area cleaned for restoration. The total amount of bait mixture consumed was estimated to be 450 gms and the time taken for elimnation was 7 weeks.

However communication between and owners were kept open to know the status of the infestation. One year after this treatment the owner reported of no termite activity or termite damage on his property which confirmed a successful treatment of the termite colony.

Discussion

The nature of the infested site, a yacht berthed at sea, presented a limited scope for chemical treatment. Suggestion of fumigating the boat, followed by applying termite baits have been reported earlier (Mannes, 2012). The same report also quoted Dr. Scheffrahn and warned against fumigations, which are standard practice for drywood termites treatment as boats pose challenges and considerations that many pest controllers often overlook. For instance, rigging, cables and ropes make tenting the actual boat structure difficult. Most entrances on boats are sea-tight, but not airtight, which is another factor to consider in the fumigation process. Changes in tide could also affect how well a boat is sealed, and if a boat is not docked, pest controllerss must be aware of drifting or other changes in location and the effects such changes may have on resource availability and insurance regulations. Also awareness of the expensive contents and well maintained nature of these boats should be considerd, because any damage that occurs during fumigation could result in costly damages (Mannes, 2012).

In addition the intention of using chemical in a sea tight space was not thought safe. A strategy of a stand-alone bait method of to minimize insecticide was thought the safest alternative (Dhang, 2015). It was thus decided to carry out this treatment using termite baiting. Termite baiting has evolved in recent times as not only a sustainable method for managing termites but also as a quick action method in manmade structures of all kinds. Termite baiting involves the application of above ground baiting stations containing bait matrix on active termite feeding spots, mud tubes and mud galleries on the structure. This technique makes use of the inherent termite behaviors of interdependence, trophallaxis, mutual grooming and cannibalism to distribute the bait toxicant throughout the colony, resulting in drastic population loss, colony suppression and at times colony elimination.

The choice thus was to use an efficacious bait with a large, one-time bait loading to avoid refilling stations and to reduce follow up visits. A number of bait toxicants and baiting systems have been developed and evaluated over the last few years for the control of subterranean termite species in SE Asia, but the most efficacious I have tested is the Requiem termite bait (Dhang, 2011; Lee et al, 2014). The product uses the toxicant chlorfluazuron (1gm of AI per kg of bait matrix) and is manufactured by Ensystex Inc. (NC, USA) .

A number of successful treatments of yachts and ships were conducted over the past years and the result of using Requiem termite bait was proven to be suitable and effective. The bait completely eliminates the treated colony with a single feeding as in this case, the reason could be due smaller colony size in sites like vessels. A number of follow up inspections of dry dock and repair shops later had also reveled the presence of termites in stored wood stocks ready for use in vessels. Also boats staying for extended periods of time on land without proper protection had shown termite activity from subterranean colonies. It is however intriguing to note how these colonies continue to live inside boats while on water and how the colonies sustain without soil. Only a total dismantling of a boat can reveal information on this aspect.

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Field Evaluation of Hexaflumuron Bait for Colony Elimination of the Subterranean Termite *Coptotermes curvignathus* Holmgren in Oil Palm Plantation in West Kalimantan Indonesia

by
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Abstract

The number and size of oil palm plantations in Indonesia continues to grow. In West Kalimantan, most oil palm plantations are located in an area dominated by peat soils. This condition makes oil palm trees vulnerable to subterranean termite attack. *Coptotermes curvignathus* Holmgren has been identified as a major pest of oil palm. These termites attack the oil palm trunk, crown, fronds and fruit. Termite control in oil palm involves surface-spraying chemical pesticides, but this type of treatment proves uneconomical and hostile to the environment. In addition, surface sprays are not effective because the termite nest is below ground and the pesticide does not affect the termites inside the nest. Therefore, a new method such as baiting is needed to control termites in the plantation setting. The objective of this study was to evaluate the effectiveness of bait containing the active ingredient hexaflumuron to control *Coptotermes curvignathus* in an oil palm plantation. Three blocks of an oil palm plantation with heavy termite damage was chosen as the test site. Each block consisted of four oil palm trees and bait was placed on one tree within each block. Bait consumption and colony elimination was evaluated every seven days over four consecutive weeks. After colony elimination was determined, the site was evaluated every two weeks for an additional six months to evaluate re-attack of termites. Results showed that seven days after installation, the bait was consumed by termites and consumption remained high (average consumption of hexaflumuron bait from the third block of the test was 95.08%) from week one until week three. Consumption activity began to decline at the end of the fourth week. The decline in termite attack in the oil palm plantations was first observed at the third week where a high soldier to worker ratio was observed; termite population declined to less than 50 and termites shelter tubes were drying. Termites were not observed inside the oil palm fronds by the fourth week. After six months, there was no re-infestation from termites on the baited oil palm tree or the surrounding sample trees. It is concluded that Copton 0.5RB with 0.5% hexaflumuron is effective for colony suppression and elimination of *Coptotermes curvignathus* in oil palm plantations.

Key words: **baiting system, *Coptotermes curvignathus*, hexaflumuron, oil palm plantation, termites control**

Introduction

Subterranean termites are social insects that work together to sustain large populations. There are three castes in subterranean termites, i.e. worker, soldier and reproductive castes with the workers comprising the majority of a colony tasked with looking for food, sharing food with the colony, maintaining the nest, and taking care of the brood (Nandika et al 2015). Soldiers function to protect the colony and the castes responsible for reproduction. Subterranean termites are generally found in the soil and function as decomposers of organic matter in addition to increasing soil water infiltration and soil nutrients (Robert et al 2007).

Subterranean termites are one of the most economically important pests in buildings, plantations and forests. Lim and Silek (2001) stated that subterranean termites account for huge economic losses in oil palm plantations in Malaysia, especially those on peat soils. Diba (2015) found six species of termites which attack oil palm plantations in West Kalimantan, i.e. *Coptotermes curvignathus, Schedorhinotermes javanicus, Macrotermes gilvus, Odontotermes sp, Microtermes sp,* and *Nasutitermes sp*. On average, 75% of all infestations were caused by the termites *Coptotermes curvignathus*. Nandika (2014) reported *Coptotermes curvignathus* attacks oil palm plantations in Riau Province and caused an average economic loss of Rp 520.000/hectare. The cost for termite control is a big expense as shown by the annual cost of termite control in the United States ≈ $1.5 billion (Su 1991).

Traditional termite control in oil palm plantations consist of spraying termiticide on oil palm trees as a remedial approach. This method is very effective in controlling termites that contact the termiticide but much less effective to termites inside the trunk and below ground. Termites in the trunks of palm oil trees as well as the underground nest may attack other trees in the oil palm plantation. Termite baits are a new control technology utilizing chitin synthesis inhibitor (CSIs*)* active ingredients. These CSIs display a different mode of action providing an effective and environmentally friendly alternative. One of the active ingredients used in termite baits is hexaflumuron. Hexaflumuron was the first active ingredient registered with the US EPA as a reduced-risk pesticide and has been reported to successfully eliminate the Formosan and eastern subterranean termites in laboratory tests and field trials (Scheffrahn and Su 1991, Su and Scheffrahn 1993, Su 1994). Sajap et al (2000) reported hexaflumuron is effective against *Coptotermes curvignathus* in Malaysia. The objective of this research is to evaluate hexaflumuron in a baiting system for effectiveness against *Coptotermes curvignathus* in an oil palm plantation.

Materials and methods

Experimental site

The oil palm plantation was located in Purun District, Mempawah Regency, and West Kalimantan Province, Indonesia. The location was approximately 120 Km West of Pontianak City, Indonesia. The oil palm plantation was on peat soil with a total area of 2800 hectares. The peat ranged in depth from 0.75 m to 1.5 m with an average daily temperature of 26.5°C and 85% relative humidity. The research site was located in blocks with very high termite attack (termites attacked more than 20 oil palm trees in one block). The blocks consisted of Block H 40, H 41 and H 42. Four oil palm trees were used in each block.

Termite Baiting System

Termite baits used in this study were Copton 0.5 RB termite bait with 0.5% hexaflumuron obtained from Dow AgroSciences, LLC. One 30 g Copton 0.5 RB hexaflumuron bait was placed inside an oil palm frond infested with termites and left for one month (Figure 1).

Figure 1. Installation of Copton 0.5 RB termite bait with 0.5% hexaflumuron bait inside the oil palm frond

Evaluations of bait consumption and termite elimination were conducted every seven days. At one month the bait was removed from the tree, cleaned, measured, and weighed. Termite baits were weighed to determine the percentage of weight loss based on the formula of Sornnuwat et al (1996):

$$\text{Bait weight loss (\%)} = \frac{(W1-W2)}{W1} \times 100\%$$

W1 = initial weight of bait
W2 = weight of bait after one month installation

Termite colony vigour was analyzed according to Garcia et al (2007). The total number of termites, including the ratio of workers to soldiers, was recorded. Termites were classified as abundant, moderately abundant, few, and none (Table 1), at each monitoring interval.

Table 1. Classification of *Coptotermes curvignathus* Holmgren numbers in baits

Population	Classification
0	None
1 to 20	Few
21 to 50	Moderately abundant
Over 50	Abundant

Source: Garcia et al 2007

After bait removal, we monitored oil palm trees for re-infestation by termites. Rubber wood (2 cm x 2 cm x 30 cm) monitoring devices were installed around oil palm trees and evaluated for termite attack for six months (Figure 2). The top of the wood was colored with red paint to aid monitor locating.

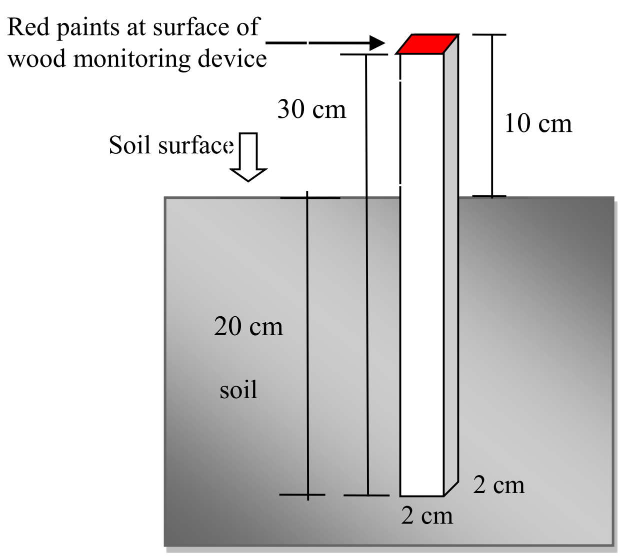

Figure 2. Installation of Wood Monitoring Device

Wood monitor stakes were installed vertically into the ground with 2/3 buried (20 cm). Each oil palm tree had four monitoring wood stakes; three within a 1.5 meters radius and one placed on the oil palm tree (Figure 3). Observations were made every two weeks for six months.

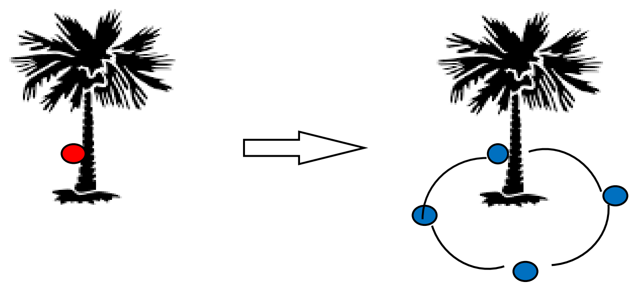

Figure 3. Schematic of monitoring device placement.

Termite bait - Hexaflumuron   Range ± 1.5 meter
Wood monitoring device   Oil palm tree

Coptotermes curvignathus Holmgren infestation was observed on the trunk, part of the fronds, the apical growth, and the fruit bunches of the palm trees. Hermawan et al (2014) states oil palm has high cellulose, with average holocellulose content of 82.534% ~ 88.328%, alpha cellulose content of 11.243% ~ 68.761%, and lignin content of 6.213% ~ 33.702%. The intensity of termite *C. curvignathus* attack on oil palm was categorized as heavy and very heavy. Heavy attack consisted of termite galleries on the trunk up to the apical growth but with green leaves and fronds. The category Very Heavy consisted of termite galleries on the trunk up to the apical growth with dry leaves and fronds. Cheng et al (2008) and Kirton et al (1999) reported that *C. curvignathus* is a pest of oil palm plantations in peat soil in Malaysia and Forest Plantation Area of *Acacia mangium* Willd.

The percentage of hexaflumuron baits infested with termites at the first observation (7 days after installation) was 83% (N=10) and 100% by the second observation (14 days after the installation). A large number of termite workers (greater than 50) were found in each bait. These observations indicate that Copton 0.5 RB termite bait with 0.5% hexaflumuron is not repellent to termites and acceptance rate was very fast. Diba (1999) revealed that hexaflumuron bait started to be consumed by termites 2 days after installation, and by the second week the entire bait was fully covered by soil and with greater than seventy percent consumed. The condition of hexaflumuron bait is presented in Figure 4.

Figure 4. Condition of Copton 0.5 RB termite bait with 0.5% hexaflumuron bait at 7 days after installation (a), 14 days after installation (b) and 21 days after installation (c)

One month after installation, average weight loss of Copton 0.5 RB termite bait with 0.5% hexaflumuron was 96.25% in Block H 40, 96.50% in Block H 41 and 92.50% in Block H 42. Average consumption across the three blocks was 95.08%. These results demonstrate that Copton 0.5 RB termite bait was readily accepted by termites. Hexaflumuron has a slow mode of action allowing termites to feed on the bait and spread the active ingredient throughout the colony before symptomology begins (Su et al 1995, Pawson and Gold 1996). These results are in agreement with Castillo et al (2013) who reported that termites choose the most appropriate type of cellulose containing food. Average consumption of Copton 0.5 RB bait by *C. curvignathus* for each block is presented in Figure 5.

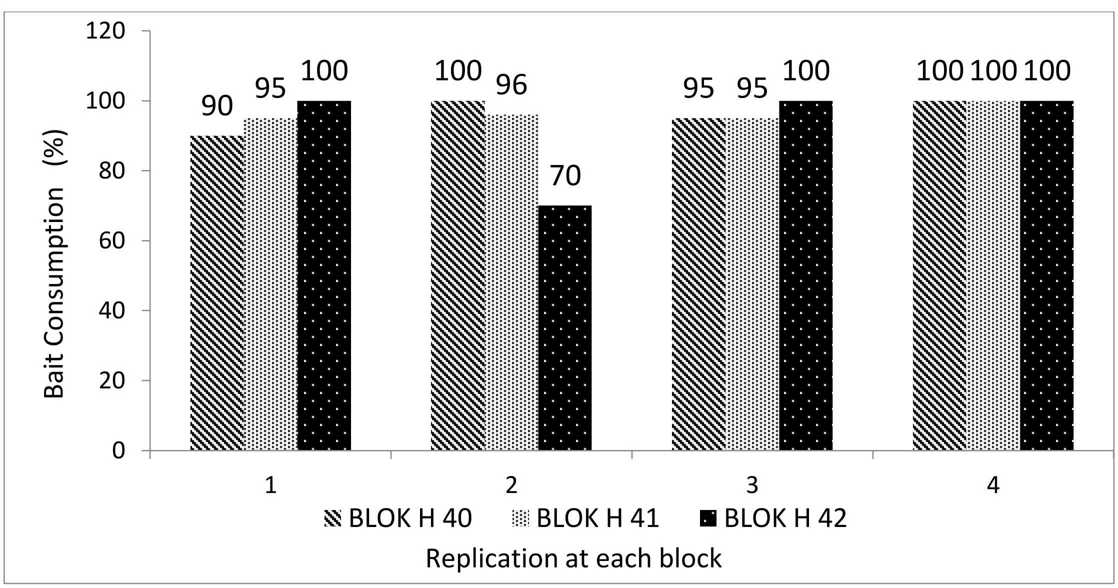

Figure 5. Copton 0.5 RB bait consumption by *Coptotermes curvignathus* in Oil Palm in West Kalimantan Province

The impact on termite colonies that consumed the bait increased from week one to week three as indicated by the higher soldier to worker (S/W) ratios**.** When greater than 50 termites were found in monitoring baits the population consisted mostly of workers. Populations found in baits of less than 50 had a higher proportion of soldiers. Consumption of bait began to decline by the end of the fourth week. It is suspected that by the end of the fourth week the hexaflumuron suppressed the termite colonies, as indicated by a reduced termite population, and a decline in termite attack on the oil palm trees. Evidence of dry soil mud tubes, and no termites found inside the oil palm fronds provided visual confirmation of reduced termite attack. The high soldiers to worker ratio are reported in Table 2.

Table 2. Estimation of termite population (P) and ratio of soldiers to workers (S/W) found at each oil palm tree during monitoring period

Oil Palm sample	Inspection period (days)									
	7		14		21		28		40 - 180	
	P	S/W	P	S/W	P	S/W	P	S/W	P	S/W
1	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
2	>50	S < W	<50	S > W	-	-	-	-	-	-
3	>50	S < W	<50	S > W	-	-	-	-	-	-
4	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
5	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
6	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
7	>50	S < W	>50	S < W	<50	S > W	<50	S > W	-	-
8	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
9	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
10	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
11	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
12	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-

Colony eliminations were noted four weeks after installation of Copton 0.5 RB termite bait and all activity was successfully eliminated at 40 days (Fig. 6). No oil palm tree re-infestation was observed at180 days.

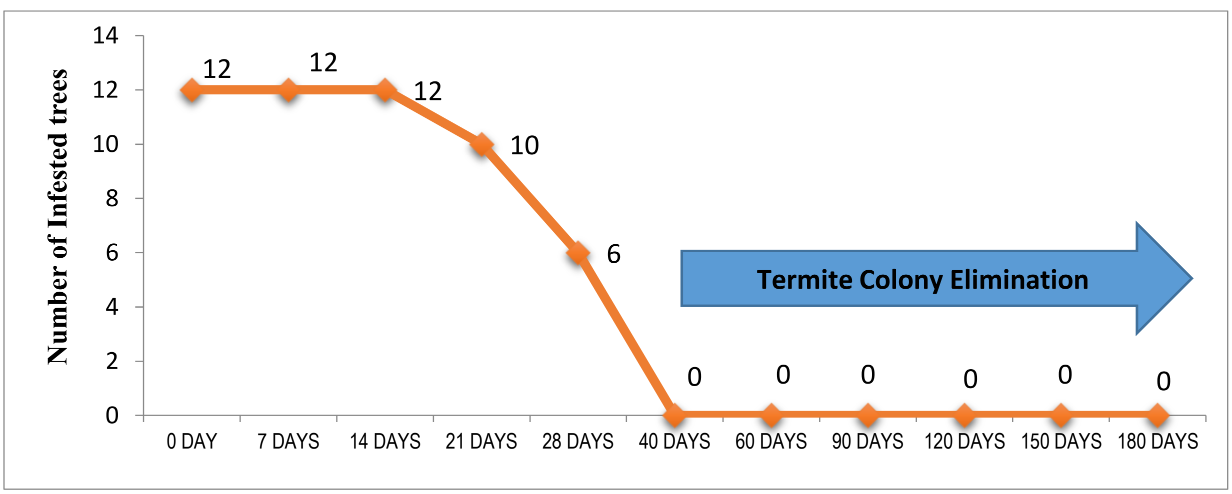

Figure 6. Colony suppression and elimination of *Coptotermes curvignathus* after consumption of Copton 0.5 RB termite bait in Oil Palm Plantation in West Kalimantan Province.

Termite re-infestation of oil palm trees was 0%, as shown by the absence of termite attack on the wood monitoring devices for six months. These results are in agreement with Sajap et al (2009) where a Preferred Textured Cellulose (PTC) matrix with 0.5% hexaflumuron was reported to eliminate *Coptotermes gestroi* and *Schedorhinotermes* sp colonies in oil palm plantations in Malaysia. Colony elimination occurred 42-77 days after bait installation with bait consumption between 22.93 to 167 grams. Nandika (2014) stated that the absence of re-infestation of oil palm trees by termites indicated that termites were eliminated.

C. curvignathus have been identified as the major pest in oil palm plantations. Immature oil palm trees are the most vulnerable to termite attack (Woei Kon et al 2012). Khoo et al. (1991) reported *C. curvignathus* also attack *Acacia mangium* and *Hevea brasiliensis* plantations. Current control methods for *C. curvignathus* in oil palm utilize liquid pesticide applications. Liquid pesticide application requires spraying and drenching the trunk, fronds, and leaves of oil palm. Treatment of the soil around infested trees is another control option to prevent termite attack. These treatments have proven to be non-economical because the termite nest is not directly treated leading to infestation of other oil palm trees. Therefore, new control strategies for termite control in plantations are needed. Termite baiting is an environmentally friendly method with reduced risk against non-target organisms. Copton 0.5 RB termite bait with hexaflumuron eliminated termite colonies in oil palm plantations as demonstrated by this research. Copton 0.5 RB utilizes the feeding behavior of termites to spread the active ingredient hexaflumuron throughout an entire colony. The natural behavior of food sharing, or trophalaxis, involves a relatively small number of termites in a colony feeding on a bait, and subsequent transfer of that toxic material to nest mates.Copton 0.5 RB termite bait has proven to eliminate *C. curvignathus* colonies in oil palm plantations.

Conclusions

The use of Copton 0.5 RB hexaflumuron termite bait is an effective and economical method to control *Coptotermes curvignathus* Holmgren in oil palm plantations. Copton 0.5 RB bait was tested and showed a high level of preference by *C. curvignathus.* Average bait consumption was 95.08% by one week and after three weeks we recorded high soldier to worker ratios. Termite activity was greatly reduced by four weeks indicating quick suppression of termite colonies. Copton 0.5 RB termite bait eliminated colonies of *Coptotermes curvignathus* Holmgren in oil palm plantations within 40 days.

Acknowledgment

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TERMIFILM® : A unique, effective product for preventing termite infestation of building construction

by

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Abstract

TERMIFILM® is a physico-chemical barrier that acts with lethal effect against many species of subterranean and tree-nesting termites. TERMIFILM® is a low-density waterproof polyethylene film 150 µm thick that contains the active ingredient permethrin. Grafting the encapsulated insecticide into the polyethylene plastic insures a barrier to termites and exempts the product of any toxicological and ecotoxicological classifications. TERMIFILM® is a patented invention that insures, years of termite protection for all types of construction (individual houses, collective housing, offices, industrial, commercial, agricultural, school buildings) for regions like Europe (Metropolitan France, Spain, Portugal), the South Pacific Ocean (Antilles: Guadeloupe, Martinique, French Guyana), the Indian Ocean (Reunion Island, Mauritius, Mayotte), South-East Asia (Thailand), the Caribbeans and Australia. Several field tests have proven the termite resistance of the TERMIFILM® barrier when exposed to different termites (*Reticulitermes, Coptotermes*, Mastotermes, *Microtermes, Microcerotermes, Allodontermes, Odontotermes, Nasutitermes, Cryptotermes, Heterotermes)* in different countries.

Key words: Termite resistance, Plastic material, Preventive treatment of buildings, Physical-chemical barrier, Subterranean termite

Introduction

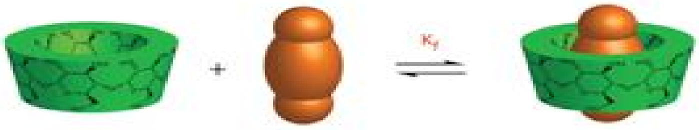
Plastic materials for termite prevention are a real alternative to spraying liquid termiticides and generally do not represent any risk of pollution to the ground or water tables. All plastic materials are not termite resistant during the service life of a building due to chemical structure, composition, hardness and surface finish. The number of termite species all over the globe makes it difficult to propose a unique plastic material solution against termite damage that can work in every region. The incorporation of an insecticidal active ingredient in the plastic material is almost mandatory. The laboratory of the BERKEM Group has formulated one original physical-chemical barrier called TERMIFILM® with the aim to provide an efficient and, above all, non-hazardous product for humans and the environment. A brief summary of the key results from six field tests in six regions in the world is provided in this document. The aim of those field tests was to evaluate the termite resistance of the permethrin-treated TERMIFILM® against several species of termites.

Materials and Methods

Materials

TERMIFILM® plastic sheeting film is treated with 1.0% permethrin trapped by LDPE (Low Density Polyethylene) molecules thus it is not sensitive to leaching, but still bioavailable (Figure 1). Permethrin has a high affinity for lipids and therefore migrates from the plastic onto the insect cuticle and through their body wall following simple contact. TERMIFILM® is considered a repellent. The mortality rate is dependent on the contact time between the insect and the film.

Figure 1: Dispersion of permethrin encapsulated in the LDPE matrix



TERMIFILM® has proved to be efficient for waterproofing according to EN13967 (2012).

TERMIFILM® is not classified dangerous for humans and the environment because of its physico-chemical properties. This non-classification for hazards allows the use of TERMIFILM® as confirmed by the OECD standards (for environmental effects: OECD 202, OECD 203, and OECD 201, for human effects: OECD 402, OECD 404, and OECD 406).

Methods

Six field tests were conducted in six different regions to evaluate the field performance of TERMIFILM® as a barrier to penetration by termites. TERMIFILM® plastic sheets that contained no insecticide were used for the control units.

1- Australia

Two trials were undertaken by Termite Research, CSIRO Entomology for the BERKEM Group.
The first trial was installed in August 1999 against *Mastotermes darwiniensis* and *Coptotermes acinaciformis* (mound building form) in wet-dry tropical northern Australia near Coolalinga, approx. 30km southeast of Darwin, Northern Territory. The second trial against *Coptotermes acinaciformis* (tree-nesting form) was installed in October 1999 in substropical eastern Australia within the Greenbank military training area approx. 23km south of Brisbane, Queensland. At both sites, subterranean termite species other than the targeted species are also abundant.

For each experimental unit, two sheets of TERMIFILM® measuring 700x600mm and 700x450mm were joined with clear 3M Tape (50mm wide) with a 200mm overlap. The largest sheets had a 50mm diameter hole which was fitted over a 55mm PVC pipe sealed at one end with stainless steel wire mesh. (Figures 2 and 3) TERMIFILM was fitted and secured onto the service pipe with a stainless steel pipe clamp. A piece of *Eucalyptus regnans* veneer bait wood was placed between the TERMIFILM® sheet and the concrete slab. Therefore only way the termites could reach the bait wood placed in the service penetration pipe was to penetrate the TERMIFILM. The top end of the pipe was closed with a PVC cap. Termites are able to sense the block of wood through the wire mesh providing a further stimulus for the termites to explore the barrier and attempt to reach the wood.

The experimental units at each colony site (six for each species) were installed at least a meter away from each other. Ten control units and ten treated experimental units were each distributed.
In total, forty experimental units (twenty for each target species) were installed at the Darwin site and twenty experimental units at the Brisbane site.

Figure 2: Diagrammatic representation of the TERMIFILM® experimental unit, TERMIFILM® installed as per the installation procedure for TERMIFILMTERMIFILM® with the overlap taped (with 3M Tape) on the inside, facing the concrete slab (Brisbane site)

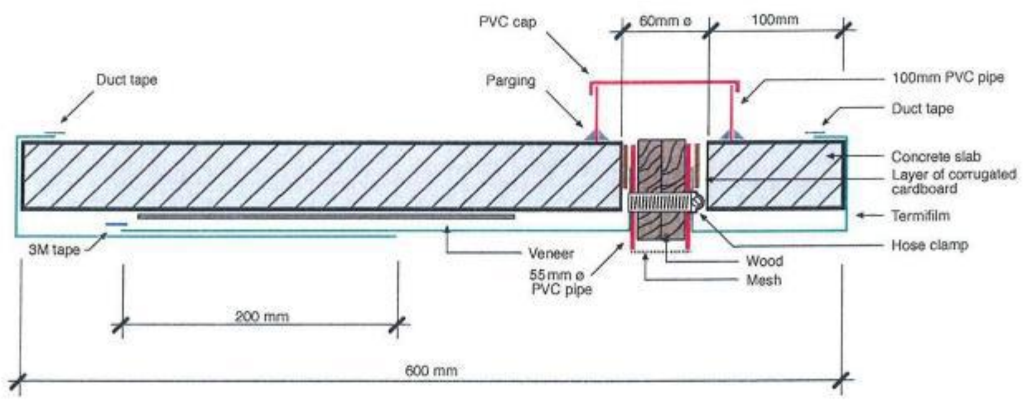
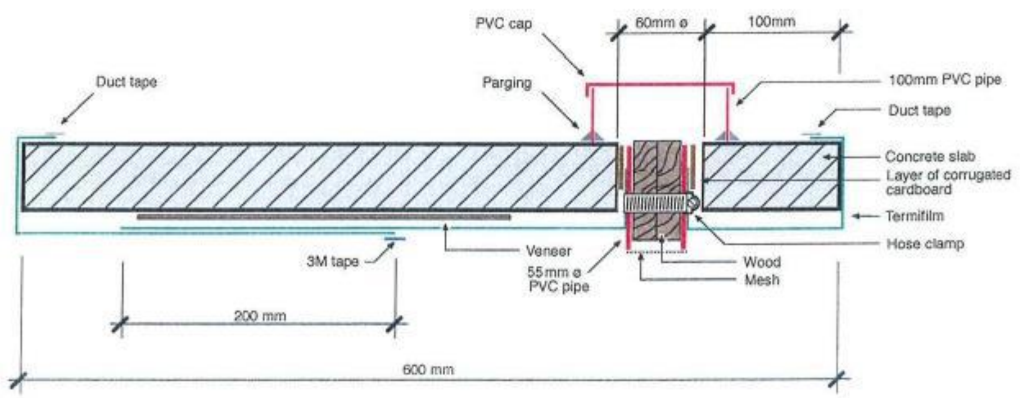


Figure 3: Diagrammatic representation of the TERMIFILM® experimental unit, the overlap taped (with 3M Tape) on the outside, facing the soil (Darwin site)



2- United States

This study was installed in the United States nationwide test sites (Arizona, Florida, Mississippi, South Carolina) in 1998 and was undertaken by the Wood Products Insect Research Forestry Sciences Laboratory of the Forest Service of the United States Department of Agriculture in cooperation with the BERKEM Group. The concrete-slab and concrete-block test methods were used to install the barrier. In the concrete-slab test, the barrier was placed between the soil and the concrete slab. In the concrete block method, the barrier was applied to the block of wood.

3- South Africa

This trial was undertaken by the South African Bureau of Standards using the test method SABS Method 859 except that:

- Four holes each measuring 350x300x150mm deep were dug. The holes were lined with TERMIFILM® and the ends protruding over the side of the hole were covered with soil.
  Six Saligna wood bait specimens coded A-F were placed out on the TERMIFILMTERMIFILM® as indicated below:

A	B	C
D	E	F

  The hole was then covered with a fibre cement sheet and the sides covered with soil.
- Another four holes except with no TERMIFILM® were used. These served as untreated controls.

The treatment was carried out on the 8th of July 1997. The termite species identified at the research station were *Microtermes sp.*, *Microcerotermes sp.*, *Allodontermes sp.* and *Odontotermes transvaalensis*.

4- Thailand

The trial was carried out by the Section of Development on the Prevention and Control of Forest Pest Insects of the Forestry Research and Development Office of the Royal Forest Department located in Bangkok, Thailand. The trial was located at the Extension & Development Utilization of Small Timber and Non-Wood Forest Products Station, Royal Forest Department, Ratchaburi Province, Thailand. The RFD standard methods on efficacy testing of chemicals used as soil treatment for prevention of subterranean termites were used with four replicates. The duration of the trial was three years (February 2011-March 2014). Tested organisms were subterranean termites.

5- French Guyana

The field tests in French Guyana were conducted in two areas:

- an urban area (PARIACABO + Campus) infested by *Nasutitermes spp, Cryptotermes sp* and *Coptotermes testaceus*,
- a forested area (PARACOU) mainly infested with *Nasutitermes spp* and *Heterotermes tenuis*.

The posts were put in the soil to a depth of 10-cm in July 1998 observations made once a month for one year. The inspection visits were made with the cooperation of the experts of CIRAD, France.

Installation of the traps with TERMIFILM® in the urban area

Traps with TERMIFILM® were installed at three sites. At each site, there were: two control samples of Poplar, two control samples of Kobe, one post of Kobe with TERMIFILM®. The two wood species Kobe (*Sterculia pruriens*) and Poplar (*Populous spp*) are sensitive to termite attack.

Installation of the traps with TERMIFILM in the forested area

Traps with TERMIFILM® were installed in three sites. At each site, there were: two control samples of Poplar, two control samples of Kobe, one post of Kobe with TERMIFILM®. All posts were randomly placed.

The site of the SIMKO in the village SARAMACA at Kourou also was treated with TERMIFILM® in 1996. It is located in an area known for attacks by *Coptototermes subterranean termites*.

6- Metropolitan France

The test was set up on April 1994 and was the first field trial undertaken to validate the protective effectiveness of TERMIFILM®.

The test site is located on the Ile d'Oléron, in the forest of Saint-Trojan-Les-Bains (ONF site).

The test TERMIFILM® was installed directly on the ground. The test area was naturally infested by *Reticulitermes flavipes* and displayed high termite activity. Nine test devices are monitored (8 with TERMIFILM® and one control without any termite protection). In each test device, small planks of maritime pine and blocks of scots pine were used to provide a potential source of food to termites.

Two test devices are installed only with TERMIFILM® :

- Device 1 : 1- 4 holes in TERMIFILM® on a median line,
- Device 2: 2- 16 holes in TERMIFILM® in a rectangular grid.

Results and discussion

1- Australia

At the annual inspection, the experimental units were carefully lifted and examined for signs of damage to the TERMIFILM®. The bait wood was inspected and termite damage noted.

The results of inspections carried out for the period 1999 to 2004 follows:

- Termite activity and pressure by the target species on the experimental units was high at the tropical Darwin, Northern Territory and subtropical Brisbane, Queensland, sites with a number of non-target subterranean termite pest species also present around the TERMIFILM experimental units.
- Control units with untreated TERMIFILM® were penetrated by *Mastotermes darwiniensis* after two or three years, by the mound-building form of *Coptotermes acinaciformis* (Darwin) within the first year and the tree-nesting form (Brisbane) within one to two years.
- Bait wood was not replaced after the inspection following three years of exposure to termites (2002). The TERMIFILM® units remained in situ exposing the TERMIFILMTERMIFILM continuously to the local climate and soil conditions at both the tropical and subtropical sites. After inspection in 2004 (five years), bait wood was re-installed under the experimental units.
- All TERMIFILM® experimental units resisted termite attack and remained intact in the fifth year after they were exposed to termite pressure.
- The repellent effect of the insecticidal active ingredient contained in TERMIFILM® was clearly evident in the trials at all sites and against all target species of termite. Termites consumed much of the bait wood underneath the TERMIFILM® sheets. Although the tape was not termite resistant, it also was not attacked by termites, no doubt due to the repellency of the permethrin treatment in TERMIFILM®.

2- United States

Eight annual inspections were made since 1998 with the eighth inspection made between February and September 2006. A summary of results is presented below:

Table 1: Percentage of TERMIFILM® barriers penetrated by subterranean termites for (A) concrete slab, (B) concrete block, and (C) controls the eighth year in field tests (2006) of TERMIFILM®

Sample	Arizona (2006)		Florida (2006)		Mississippi (2006)		South Carolina (2006)	
A. Concrete slab								
TERMIFILM® control unit	0		0		10		10	
TERMIFILM® 1% permethrin	0		10*		0		0	
CS[1]	10		0		30		0	
B. Concrete block								
Treatment	Center control block	Barrier penetrated	Center control block	Barrier penetrated	Center control block	Barrier penetrated	Center control block	Barrier penetrated
TERMIFILM® control unit	70	0	10	0	50	0	20	0
TERMIFILM® 1% permethrin	30	0	80	0	60	0	30	0
Controls								
CS[2]	50		70		60		20	

[1]6-mil-thick plastic polyethylene sheet vapor barrier only, not cut

[2]6-mil-thick plastic polyethylene sheet vapor barrier only, cut

*1% TERMIFILM barrier was cracked and brittle in all 10 concrete slabs in 2006

Table 2: Cumulative percentage (1999-2006) of TERMIFILM® barriers penetrated by subterranean termites for (A) concrete slab, (B) concrete block, and (C) controls attacked a four study sites

Sample	Arizona (2006)		Florida (2006)		Mississippi (2006)		South Carolina (2006)	
A. Concrete slab								
TERMIFILM® control unit	10		0		10		0	
TERMIFILM® 1% permethrin	10*		10		0		0	
CS[1]	0		0		0		30	
B. Concrete block								
Treatment	Center control block	Barrier penetrated	Center control block	Barrier penetrated	Center control block	Barrier penetrated	Center control block	Barrier penetrated
TERMIFILM® control unit	80	10	100	0	100	20	70	0
TERMIFILM® 1% permethrin	80	0	100	0	100	0	80	0
Controls								
CS[3]	50		100		100		100	
CB :no barrier, bare soil	80		100		100		90	

[1]6-mil-thick plastic polyethylene sheet vapor barrier only, not cut

[2]6-mil-thick plastic polyethylene sheet vapor barrier only, cut

*1% TERMIFILM barrier was cracked and brittle in all 10 concrete slabs in 2006

These trials demonstrate that TERMIFILM® is a potentially effective candidate for termite control in the United States.

3- South Africa

A forty-two month survey was carried out on the 12th of January 2001 to determine if TERMIFILM® was still effective.The results obtained are given in Table 4:

Table 3: 42 Month observation in South Africa field trial - TERMIFILM®

Sample	Repli-cates	Position of baits specimens and termite activity						Number of bait specimens attacked	Remarks
		A	B	C	D	E	F		
TERMIFILM®	1	Na	Na	Na	Na	Na	Na	0	Replicate 1 : Fungi on bait samples
	2	Na	Na	Na	Na	Na	Na	0	
	3	Na	Na	Na	Na	Na	Na	0	Replicate 2 : Dead beetles
	4	Na	Na	Na	Na	Na	Na	0	
Untreated control	1	Va	Va	Va	Va	Va	Va	6	Replicate 2: Fungi on bait samples
	2	Na	Na	Na	Na	Na	Na	0	
	3	Va	Wa	Va	Wa	Wa	Wa	6	
	4	Va	Va	Va	Va	Va	Va	6	
Legends: Va=very active; Sa=slightly active;Wa=were active; Na=not active; Td=totally destroyed									

The biological efficacy of TERMIFILM® was proven against termite attack from *Microtermes sp.*, *Microcerotermes sp.*, *Allodontermes sp.* and *Odontotermes transvaalensis*.

4- Thailand

The TERMIFILM® plastic barrier prevented subterranean termite attack in Thailand in the field trial for three years. The results (Table 4) at three years revealed no damage in treatment areas while the control was one hundred percent destroyed. Therefore the efficacy of TERMIFILM® was proven.

Table 4: Result Thailand Field trial of percentage of damages - TERMIFILM®

Sample	Percentage of destruction				Average percentage (%)
	Replication				
	1	2	3	4	
TERMIFILM® 1% Permethrin	0.00	0.00	0.00	0.00	0.00
Control	100.00	100.00	100.00	100.00	100.00

5- *French Guyana*

Inspections done in the period 1997-2005 revealed no trace of attack by insect or termites, and no fungal attack (frequency of inspection: one time per year).

Table 5: Results of efficacy of TERMIFILM® in the six sites in French Guyana

Sites	Kobe Control sample 1	Kobe Control sample 2	Poplar Control sample 1	Poplar Control Sample 2	Kobe with TERMIFILM®
1	No termites	No termites	Presence of termites	Presence of termites	No termites
2	Presence of termites	Presence of termites	Presence of termites	Presence of termites	No termites
3	Presence of termites	Presence of termites	Presence of termites	Presence of termites	No termites
4	Presence of termites	Presence of termites	Presence of termites	Presence of termites	Presence of termites
5	Presence of termites	Presence of termites	Presence of termites	Presence of termites	No termites
6	Presence of termites	Presence of termites	Presence of termites	Presence of termites	Presence of termites

The tests made on site 1 were inconclusive on the efficacy of TERMIFILM® because none of the Kobe control samples were attacked by termites. Whereas the five others studied sites, a termite presence was noticed on some of the samples. Depending on the site, between twenty-five and eighty percent of the control samples were attacked. TERMIFILM® seemed to be efficient because at the six-month period, at five sites there was no attack on the TERMIFILM® protected samples.
The presence of termites on the Kobe sample with TERMIFILM® was certainly due to leaf-drop which covered the ground which allowed termites to reach the wood posts put in a soil with TERMIFILM®. No termite attack was noticed at the site SIMKO in the village SARAMACA at Kourou.

6- *Metropolitan France*

The date of the last visit was May 2015 twenty-one years after the installation of the test. During this inspection, the inside of the unprotected control device appeared colonized by termites. In the two devices, no evidence of termite activity was found. No mud tunnels on the foundation walls. A lot of termite activity was observed during the inspection. , TERMIFILM® protected the wood specimens from termite attacks in the devices after twenty-one years of exposure.

Conclusions

TERMIFILM®, a permethrin-treated physico-chemical barrier, has an original and unique composition that can insure more than twenty-one years of termite protection for buildings in addition to waterproofing. The efficacy of the repellent product TERMIFILM® has been proven by laboratory tests and field tests in all regions invaded by termites: Europe, South Pacific Ocean, Indian Ocean, South-East Asia, Caribbean and Australia. The tox-ecotox profile of TERMIFILM® is classified as a non-dangerous product for humans and the environment.

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Interspecific competition between *Coptotermes gestroi* and *Odontotermes formosanus* in a forest ecosystem

by

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Abstract

Coptotermes gestroi (Wasmann) is an invasive termite species, and *Odontotermes formosanus* (Shiraki) a species endemic to Taiwan. Both are found in the Xiaping Tropical Botanical Garden, Nantou, Taiwan. Among 581 termite-monitoring wood stakes in Xiaping Tropical Botanical Garden, 280 were not consumed by termites, while 384 were exclusively occupied by *O. formosanus*, 5 by *C. gestroi*, and 12 stakes were fed upon by both species. The later 12 stakes were assumed to represent their interspecific territory. In the interspecific territory, succession between *O. formosanus* and *C. gestroi* on wood stakes was observed, and these two species rarely co-occured on a wood stake at the same time. Both species have different seasonal activity patterns and responded to climatic factors in the intraspecific territories which indicates that seasonality maybe the main factor affecting .succession, Only *C. gestroi* displayed seasonal activity in the interspecific territory, and the seasonal activity pattern of *O. formosanus* was different in interspecific and intraspecific territories. Thus, termite succession was not fully explained by seasonal activity. A control strategy of *C. gestroi* was conducted with Recruit HD termite baits starting in October 2014, and *O. formosanus* rapidly foraged on the wood stakes in the intraspecific territory of *C. gestroi*. It supports that once *C. gestroi* populations are weakened, *O. formosanus* may quickly invade its territory. The interspecific competition between endemic *O. formosanus* and invasive *C. gestroi* was documented in this study.

Keywords: invasive species, endemic species, interspecific competition, interspecific territoriality, multi-genera termite fauna, species succession

Introduction

Coptotermes gestroi (Wasmann) is a widespread termite pest in the world. *C. gestroi* is invasive to Taiwan (Li et al. 2009), and has become one of the major structural pests in southwestern Taiwan (Yang and Li 2012, Termite Identification Service 2015). In addition, *C. gestroi* was recently reported in Taiwanese forests and infesting living trees (Li et al. 2011, Li et al. 2015), including some native and economically valuable tree species, such as *Fraxinus formosana* Hayata and *Calocedrus formosana* (Florin) making *C. gestroi* a potential forest pest in Taiwan.
In February 2013 four long-term monitoring plots were set up in a forest of Nantou, Taiwan to conduct a risk assessment of *C. gestroi* damage in the forest. The fungus-growing termite *Odontotermes formosanus* (Shiraki), a common, endemic species in Taiwan (Chiu et al. 2010,

Yang and Li 2012), also occurred in the monitoring plots. *C. gestroi* and *O. formosanus* both fed on wood stakes in the monitoring plots, and their succession on wood stakes was recorded. Since October 2014, Recruit HD termite baits were placed near *C. gestroi* colonies within the monitoring plots to control their populations. The assumption was that elimination of a *C. gestroi* colony would open the niche previously occupied by *C. gestroi* for *O. formosanus*. The invasion of other termite genera during the baiting process has been reported in previous studies (Sornnuwat et al., 1996; Messenger et al., 2005; Lee et al., 2007). The succession of *C. gestroi* and *O. formosanus* on wood stakes provided a chance to study the interactions between an invasive termite and an endemic termite in a natural environment. Two possible mechanisms of termite succession, seasonal activity and interspecific competition, were examined in this study.

Material and Methods

A total of 581 wood stakes (143–148 stakes per plot) were installed in four long-term monitoring plots in the Xiaping Tropical Botanical Garden, the Experimental Forest, National Taiwan University, Nantou, Taiwan, in February 2013. The wood stakes were inspected monthly until October 2015, and the termite species infesting each wood stakes was identified.

Wood stakes occupied by only one termite species in the observed period were defined as intraspecific territory, and the stakes previously occupied by both termite species were defined as interspecific territory.

Seasonal activity

The monthly number of wood stakes occupied by termites in the intraspecific territory were used for analysis of seasonal activity from February 2013 to September 2014. Stepwise regression was used to find potential climatic factors affecting seasonal activity of the termites. Six climatic factors, humidity, rainfall, mean temperature, number of rainy days, evaporation, and range of temperature were included in the full model.

To test whether termites displayed general seasonal activity in the interspecific territory, the seasonal activities in intraspecific and interspecific territory were compared using Pearson correlation analysis.

Post effects of *C. gestroi* elimination

Three colonies of *C. gestroi* were marked in the monitoring plots, and treated with Recruit HD termite baits beginning in October 2014. To test the post effects of *C. gestroi* elimination on foraging of *O. formosanus*, the foraging pattern of *O. formosanus* in four area types, including non-termite-occupied areas, the intraspecific territory of *C. gestroi*, the intraspecific territory of *O. formosanus* and the interspecific area, were compared with a Kolmogorov-Smirnov test. The monthly cumulative number of wood stakes infested by *O. formosanus* from October 2014 to October 2015 was used for analyzing the foraging pattern of *O. formosanus*.

Results and Discussion

The intraspecific territories of *O. formosanus* and *C. gestroi* contained 387 and 5 wood stakes, respectively, showing that *O. formosanus* was the dominant species in this area. The interspecific area contained 12 stakes.

Eleven species succession events occurred in the interspecific territory during February 2013 －September 2014, 6 events involved *C. gestroi*, replacing *O. formosanus* and 5 events were when *O. formosanus* replaced *C. gestroi* . In the interspecific territory, wood stakes were mostly occupied by one species (28 observations) and rarely occupied by two species at the same time (2 observations), which indicates that these two species are not likely to share a wood stake. Agonistic behavior between *C. gestroi* and *O. formosanus* was observed in the laboratory, supporting the contention that these two species are competitors.

In the intraspecific territory, seasonal activity of *O. formosanus* responded negatively to mean temperature, and activity of *C. gestroi* provided a positive response to mean temperature, range of temperature, but negatively to number of rainy days (Table 1). The two species provided different responses to the climatic factors we examined, and their distribution in a habitat may therefore separate naturally. However, we found that the seasonal activity of *O. formosanus* in the interspecific territory was different to that in interspecific territory (Fig. 1A), and the seasonal activity of *C. gestroi* in intraspecific and interspecific territories were similar (Fig. 1B). Thus, termite succession in the interspecific territory was not fully explained by the seasonal activity data. We propose that, in the interspecific territory, agonistic interactions between *O. formosanus* and *C. gestroi* affect the seasonal activity of *O. formosanus*.

Table 1. Testing the effects of climatic factors on termite seasonal activity with stepwise regression. Six climatic factors, humidity, rainfall, mean temperature, number of rainy day, evaporation, and range of temperature were included in the full model.

Dependent variable	Independent variable	Slope	t value	P
No. of stake occupied by *O. formosanus*	Rainfall	-0.02	-1.64	0.12
	Mean temperature	-1.91	-2.88	<0.05
No. of stake occupied by *C. gestroi*	Mean temperature	0.28	5.26	<0.001
	No. of rainy day	-0.06	-2.41	<0.05
	Range of temperature	0.24	4.89	<0.001

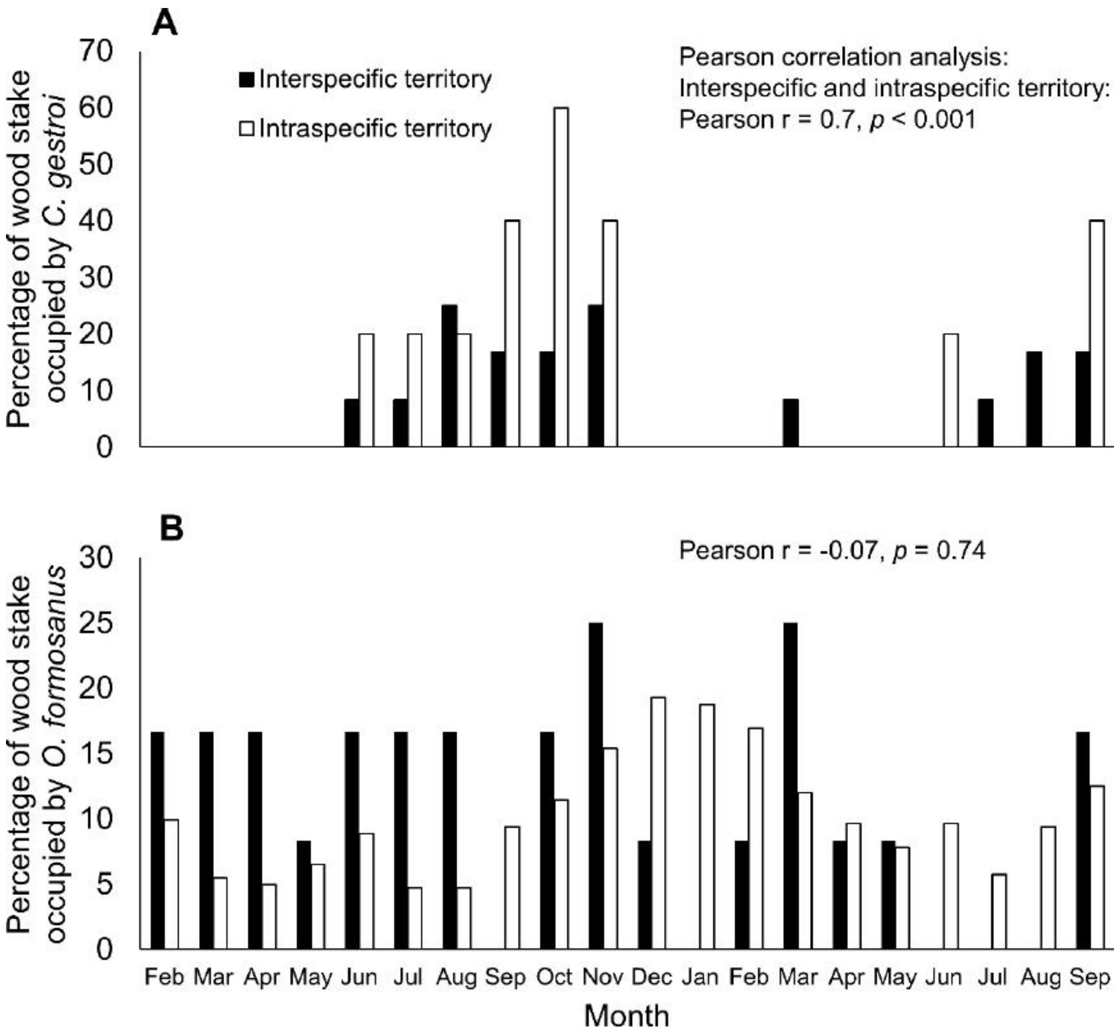

Fig. 1. Comparing seasonal activity at wood stakes in interspecific and intraspecific territories of *O. formosanus* (A) and *C. gestroi* (B). Seasonal activity of *O. formosanus* in the intraspecific territory was different in the interspecific territory, and seasonal activity of *C. gestroi* in the interspecific and intraspecific territories were similar.

After the *C. gestroi* colonies were baited, *O. formosanus* rapidly took over stakes in the intraspecific territory previously occupied by *C. gestroi* (Fig. 2). The foraging territory replacement rate of *O. formosanus* in the intraspecific territory of *C. gestroi* was significantly higher than in the non-termite-occupied area, the intraspecific territory of *O. formosanus*, and the interspecific territory (Fig. 2; Kolmogorov-Smirnov test: non-termite-occupied area: D = 0.89, $p < 0.001$; intraspecific territory of *O. formosanus*: D = 0.89, $p < 0.001$; interspecific territory: D = 0.67, $p < 0.05$). We propose that, foraging of *O. formosanus* in the intraspecific territory was inhibited by *C. gestroi*'s activity, and once *C. gestroi* was baited and being weaken, *O. formosanus* quickly invaded the abandoned *C. gestroi*'s territory through the empty subterranean gallery system .

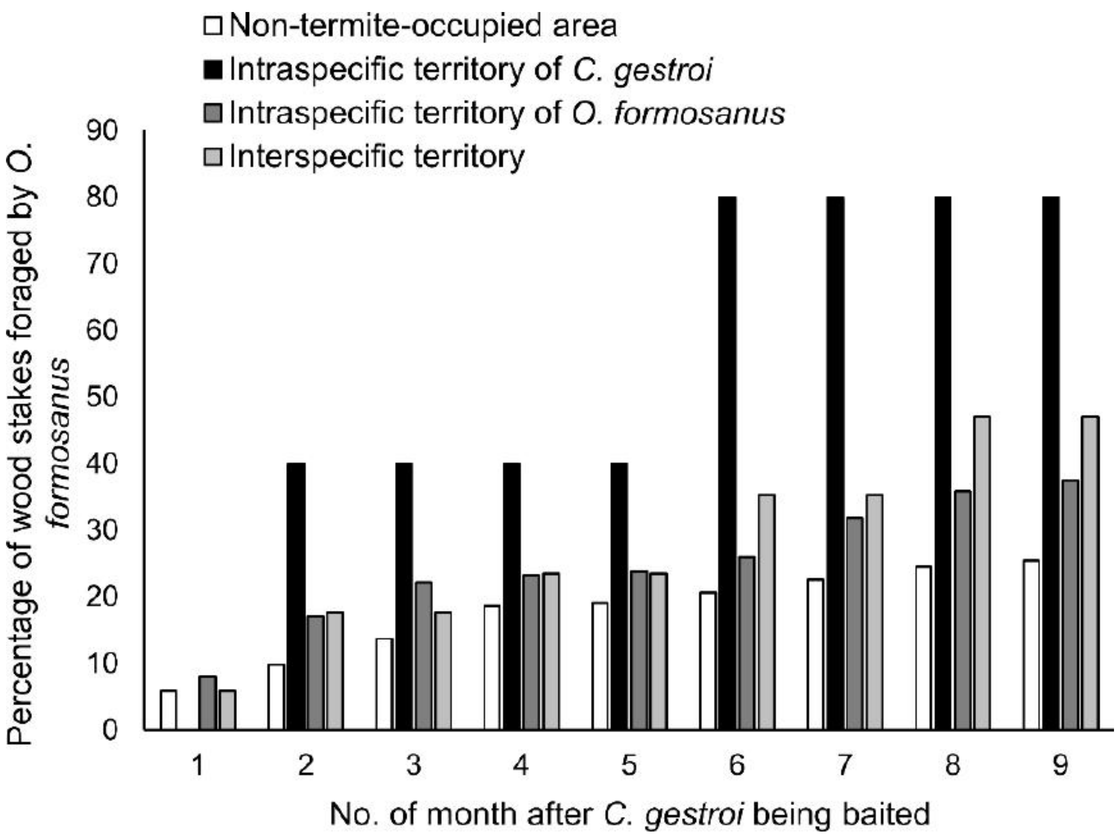

Fig. 2. Monthly cumulative number of wood stakes occupied by *O. formosanus* in non-termite-occupied area, the intraspecific territory of *C. gestroi*, the intraspecific territory of *O. formosanus* and the interspecific area after *C. gestroi* being baited. The foraging speed of *O. formosanus* in intraspecific territory is higher than that in the other three area types.

Conclusions

The current study documented the foraging interaction between invasive and endemic termites in a natural environment. In the forest study site, the territory of *O. formosanus* was larger than that of *C. gestroi*. In the overlapping intraspecific territory, the seasonal activity of *O. formosanus* was significantly affected by *C. gestroi* but not vice versa. The foraging behavior of the endemic higher termite, *O. formosanus*, was inhibited by the invasive subterranean *C. gestroi*. Once *C. gestroi* was baited, *O. formosanus* quickly occupied the territory left by *C. gestroi*. Further study is needed to more clearly understand the ecological effects of invasive termites on native fauna and flora.

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Temperature Fluctuation in *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae) Nests

by
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Abstract

One of the external factor which influence the survival of termites is temperature. Termites try to maintain the temperature of the nest within a certain range in order to survive. The objective of this research analyzed the temperature inside nests of the subterranean termite *Coptotermes curvignathus*. This research was conducted at the Laboratory of Termite-Department Forest Products-Bogor Agricultural University and simultaneously analyzed temperature data inside the nest of *C. curvignathus* and in the laboratory using a thermocouple. Data analysis was based on a model sinusoidal equation. The results showed that average temperature in the nest was 31.4 ^{O}C. which was 1.3 ^{O}C warmer than the temperature in the laboratory. The data showed that temperature alterations in termite nest were more stable than temperature alterations in the laboratory. It was determine based on value of the amplitude. Termites can maintain stable temperatures inside the nest.

Key Words: amplitude, degrees celsius, laboratory, sinusoidal equation, thermocouple

Introduction

Indonesia as tropical country located at 95^{o} – 141^{o} East Longitude and 6^{o} -11^{o} South Latitude (Sukojo 2003) characteristized by high temperatures and humidity throughout the year, with the lowest temperature being 18^{o} C (Suharsono 2008). Bogor is a city located in West Java with an average minimum altitude of 190 m and maximum 330 m above sea level. This city has warm climatic conditions with annual average temperatures between 25.1-26.4^{o} C and humidity of about 92 percent (BPS 2014) making it a nice place for termite to live.

The subterranean termite *Coptotermes curvignathus* have a high intensity of attacks in Indonesia (Nandika and Tambunan 1990), This termite can make secondary nests high in buildings. Ritalupa (2006) said that this termite is able to attack apartments and hotels in Jakarta-Indonesia up to floor 33. In addition, this termite can attack living trees and these attacks can lead to the death of the tree (Badaruddin 2007).

Weather elements such as temperature, humidity, and solar radiation affect the behavior of termites. According to Harris (1971), termites are able to maintain physical conditions in their nest so the temperatures inside and outside of the nest will be different. Differences of temperature can be used to determine the temperature range needed in order for the nest to survive. By knowing the characteristics of the termite nest, the damage caused by termites in the woods or houses can be reduced and avoided. Therefore, it is necessary to investigate the optimum temperature for *C. curvignathus* nests located in Bogor to hopefully develop an action plant to prevent termite attack to houses or buildings and the environment.

Materials and Methods

This research was conducted at the Termite Laboratory, Faculty of Forestry, Bogor Agricultural University, in a facility used for rearing *C. curvignahus*, during August, 2014. The temperature was measured in one of the termite nest rearing stations with a size of 150 x 100 x 100 cm as well as inside of the laboratory room where nest was located. The tools used were small-sized thermocouples that can be placed into termite nests (Figure 1) and a multimeter as the temperature reader. Microsoft Excel 2007 was used as a data processing software. Clock or timers were also used for the calculation of observation times.

Figure 1. Thermocouple instrument for measuring the temperature used during the study

Temperature measurements were carried out for 3, 24-hours periods and the temperature recorded every hour. The first temperature observations were conducted at 18.00 pm. The thermocouple was laid in the rearing chambers filled with termites which can be seen in Figure 2.

Figure 2. Thermocouples were laid in *Coptotermes curvignathus* rearing chambers in the Termite Laboratory

Data analysis was based on a model sinusoidal equation developed by Bahtiar *et all.* (2014 a, 2014 b) to fit the daily temperature cycle inside and outside the termite nest . The following model was chosen:

$$y = a + b\sin\left(\frac{\pi}{12}(t - t_0 - k_1)\right) + cz\sin\left(\frac{\pi}{12}(t - t_0 - k_2)\right)$$

Which:

- y = Temperature (T) (°C)
- a, b, c = Regression coefficient
- z = Dummy variable (binary variable which the value is 0 at night and 1 at daytime)
- t = Time of measurement (hour) (GMT+7)
- t_0 = Sunrise (hour) (GMT+7)
- k_1 = Additional phase for earth's surface energy effects (hour)(GMT+7)
- k_2 = Additional phase for sunrays radiation effects (hour) (GMT+7)
- L = Length of the day (hour)

Results and Discussion

The result of this research showed that the temperature inside of a nest on the first day ranged from 29.6-33.8^{O}C, on second day 29.9-33.1 ^{O}C, and third day 29.4-33.2 ^{O}C. While the temperature outside of the nest (temperature in laboratory room) provided the following respectively ranges 27.5-33.2 ^{O}C, 28.2-32.1 ^{O}C, and 28.2-32.1 ^{O}C (Table 1).

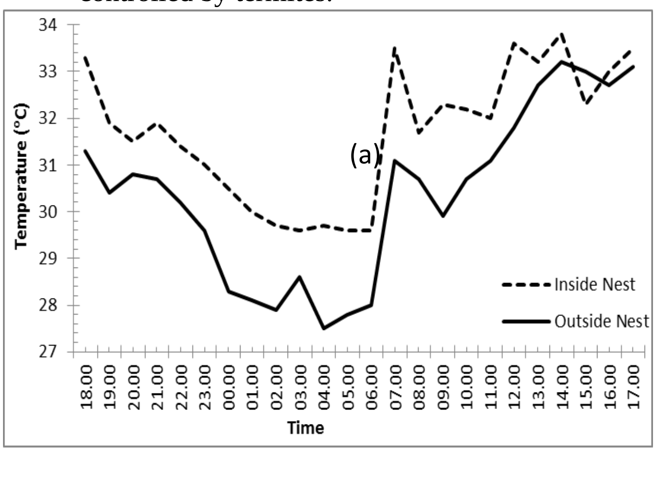
Table 1 The temperature inside and outside of nest *C. curvignathus*

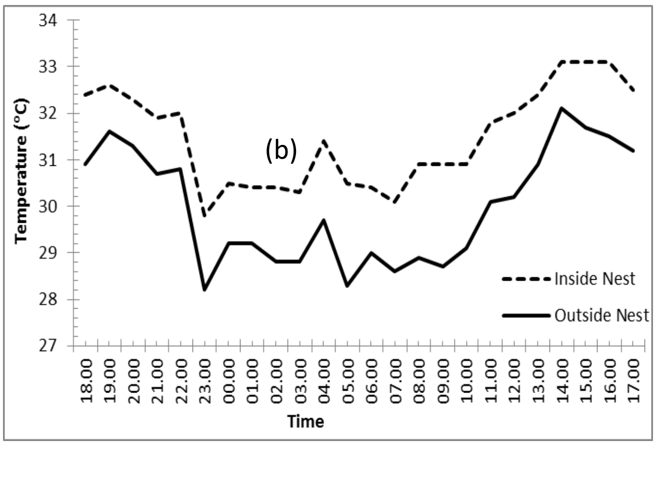
Observation time (day)	Temperature ranges (^{O}C)	
	inside nest	outside nest
1	29.6 – 33.8	27.5 – 33.2
2	29.9 – 33.1	28.2 – 32.1
3	29.4 – 33.2	28.2 – 32.1

Based on the temperature values in Table 1, changes in the nest were lower than outside. In this case, temperatures in nest ranged from 29.4-33.8 ^{O}C (changes 4.4 ^{O}C). While the temperature outside of the nest ranged from 27.5-32.2 ^{O}C (changes 5.7 ^{O}C). It means, termites can maintain more stable nest temperature. Generally, temperature inside of nest was warmer than outside (laboratory temperature). Lee and Wood (1971) said that diurnal temperature on the nest of termites varied daily but the temperature in the nest was higher than the soil or environment temperatures. Noirot (1970) also stated that the temperature was higher outside the nest (environment) than the area where termites resided.

Fluctuations in the temperature inside termite nests tend to follow fluctuations of the outside temperature. Termite nest temperature observations on the first day showed that temperatures reached their lowest at 06.00 and reach their maximum at 14.00. On the second day temperatures reached their lowest at 07:00 and reached the maximum at 14.00. Meanwhile, on the third day temperatures reached their lowest at 05.00 and reached the maximum temperature at 14.00. Temperature fluctuations inside and outside the termite nests are shown in Figure 3.

Temperature differences in termite nests by day and hour can be caused by activity, the number of individuals in the colony, the heat generated by the food collected by the termites (Nandika *et al.* 2015), termite metabolism (Noirot 1970), and the friction that occurs when the termites feed on the wood. In addition, Nandika *et al.* (2015), stated that one way to maintain the termite nest temperature is thermoregulation so the temperatures in some parts of the nest may be different but still can be controlled by termites.





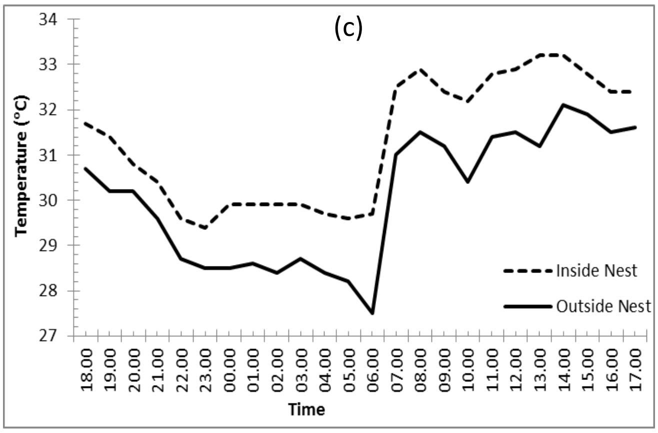


Figure 3. Fluctuations in temperature inside and outside of termites nest at firs day (a), second day (b), and third day (c).

Based on the data processing sinusoidal equation models we obtained two equations that describe the average temperatures and temperature fluctuations inside and outside the nest.
The equations are:

Inside Nest:

$$y = 31.3697 + 1.49492 \sin\left(\frac{\pi}{12}(t-6-3)\right) - 0.50917 \sin\left(\frac{\pi}{12}(t-6-9.3)\right)$$

Outside Nest:

$$y = 30.1487 + 1.82938 \sin\left(\frac{\pi}{12}(t-6-2.9)\right) - 0.02397 \sin\left(\frac{\pi}{12}(t-6-1.2)\right)$$

Based on these two models it can be seen that the average temperature of the termite nest is approximately 1.3 ^{O}C higher than the temperature outside the nest, which can be seen from the value of the regression coefficient a in the equation, where the average temperature of the termite nest is 31.4 ^{O}C, while the temperature outside the nest is 30.1 ^{O}C. It is stated that the temperature inside the termite nest is warmer than outside the termite nest. Woodrow and Grace (1999) showed that the temperature inside the galleries of dry wood termites *Cryptotermes brevis* is 24.33-37.04 ^{O}C. Optimum temperature for Macrotermes termite nests is 29-32 ^{O}C (Krishna and Weesner 1969).
While the fluctuations in temperature can be seen in the summed amplitude value of b and c. The amplitude value inside the nest is smaller than outside the nest. The mean amplitude value inside the nest was 0.98575 (from 1.98575 + (-0.50917)) and the mean amplitude values outside nest was 1.80533 (from 1.82938 + (-0.02397)). Temperature fluctuations measured and estimated iinside and outside of the termite nests during 3 x 24 hour observation periods are shown in Figure 4. Termites are able to maintain more stable nest temperatures when outside temperatures fluctuated. It showed

the insulating power of subterranean termites nest to ambient temperature. There are fluctuations of air temperature and relative humidity thatmake a cyclic loop every day. These fluctuations are caused by solar radiation and earth-surface energy. Bahtiar et al (2014a, 2014b) developed a sinusoidal equation to take into effect both sunrays radiation and earth surface energy on the daily cycles of air temperature and relative humidity.

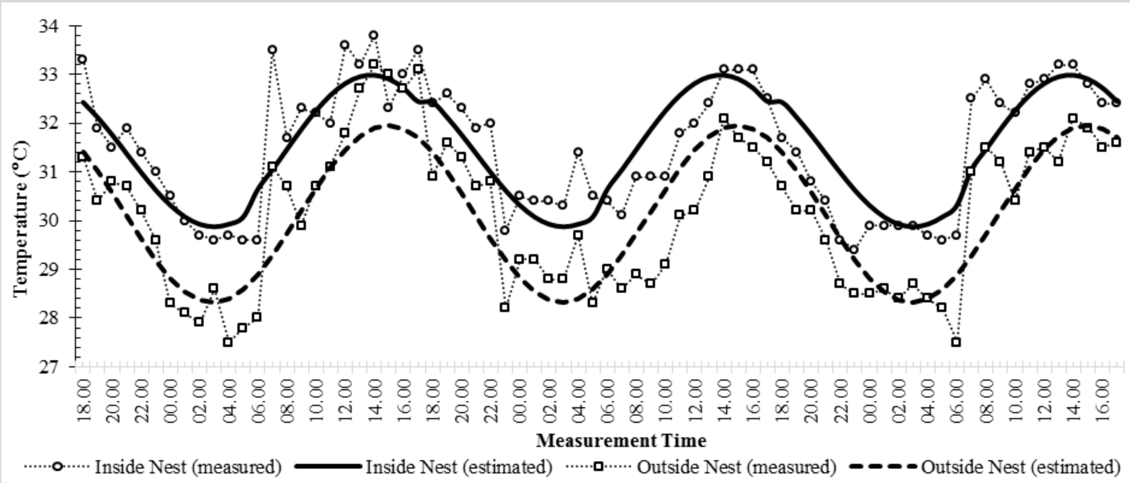


Figure 4. Temperature fluctuations measured and estimated inside and outside termite nests during 3, 24 hours observation periods.

Conclusions

Average temperature in the nest of *Coptotermes curvignathus* was 31.4 ^{O}C (ranged between 29.4 - 33.8 ^{O}C) This nest temperature was 1.3 ^{O}C warmer than the temperature on the outside surface of the nest or in the laboratory. Nest termite temperatures fluctuate following outside temperature, but termites can maintain a more stable nest temperature.

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A survey of the prevalence of endoparasitism by *Vertica fasciventris* Malloch (Diptera: Calliphoridae) in colonies of *Macrotermes carbonarius* (Hagen) (Blattodea: Termitidae) in Penang Island, Malaysia

by

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Abstract

A survey of the infestation rate of *Macrotermes carbonarius* (Hagen) (Termitidae: Macrotermitinae) colonies by the endoparasitoid *Vertica fasciventris* Malloch (Diptera: Calliphorida) was conducted in Penang Island, Malaysia from April to December 2015. Of the 313 *M. carbonarius* colonies surveyed, 105 (33.5%) were parasitized by *V. fastrivencis.* A high frequency was recorded in Balik Pulau (62.0%) and Youth Park-Botanical Garden (61.8%). Parasitized mounds were not found in Air Itam (28 surveyed mounds) and Jelutong (22 surveyed mounds). In all of the surveyed sites, the parasitized colonies were recorded as healthy. The height and diameter of parasitized mounds (n=105) was significant higher (t= -3.3; df= 311, P< 0.01) and larger (t= -3.2; df = 311; P < 0.01) than those of the not-parasitized ones.

Key words: endoparasitoid, *Macrotermes carbonarius*, mound size, infestation rate

Introduction

The fungus-growing termite *Macrotermes carbonarius* (Hagen) is widely distributed in Malaysia, Thailand, and Cambodia (Roonwal 1970). It is an open-air foraging species commonly found in forested and undisturbed areas (Lee et al. 2014). Parasitization by dipteran flies was first recorded by Kemner (1925), in which, a large larva parasitoid was found in the head of the soldiers *Macroteremes malaccensis* (Haviland). Recently, *Macrotermes barneyi* (Hagen) (Sze 2008) and *M. carbonarius* (Neoh & Lee 2011) were found to be parasitized by *Verticia fasciventris* Malloch in Hong Kong and Malaysia, respectively. *V. fasciventris* is an Oriental bengaline blow fly, which common in some Southeast Asian countries (Kurahashi et al. 1997). Neoh and Lee (2011) described the development of the larva parasitoid *V. fasciventris* in the head of *M. carbonarius.* The larva consume the entire content of the head of termite soldiers and eventually, fully fill the head capsule. The effect of the parasitoid *V. fasciventris* on the morphology of the host, *M. carbonarius*, was also described. Parasitized soldiers possess a square-like head with a pair of short mandibles.

Little is known about the infestation rate of *M. carbonarius* colonies by these larval parasitoids. Therefore, the goal of this study was to determine the prevalence and describe distribution of *M. carbonarius* parasitized by *V. fasciventris.*

Materials and Methods

Termite collection

The study was conducted on Penang Island, located on the northeastern coast of Peninsular Malaysia. A total of 313 *M. carbonarius* mounds were surveyed from April 2015 to December 2015. The coordinates of each termite mound was recorded by GPS (Garmin eTrex HCx Vista, USA). The details of the surveyed sites are presented in the Table 1.

M. carbonarius mounds were opened to determine whether the mounds were infested with *V. fasciventris*. The colony was considered infested with *V. fasiventris* if parasitized major or minor soldiers were sighted. Parasitized soldiers were characterized by a square-like head capsule and short mandibles and are commonly found in isolated, concealed chambers within infested termite mounds (Neoh & Lee 2011). Diameter and height of mounds were measured before excavation.

Status of colony health

Colony healthy was evaluated based on the ability of termites to repair damaged sections of the mound within 1-3 days. The mounds were classified as healthy if termites were able to repair the mound, and classified as unhealthy if the damaged part was not repaired.

Statistical analysis

A Student T-test was used to compare mound size (diameter and height) between parasitized and unparasitized mounds. Analyses were performed using SPSS version 20.1 for Windows (SPSS Inc. Chicago, IL, USA).

Results and Discussion

<u>Parasitism frequency, colony healthy of *M. carbonarius* mounds</u>

The number of surveyed *M. carbonarius* mounds and parasitism frequencies at each surveyed site are presented in Table 1. Of the 313 *M. carbonarius* mounds examined, 105 (33.5%) were infested with *V. fastrivencis*. The highest frequency of parasitism was recorded in Balik Pulau (62.0%) and Youth Park-Botanical Garden (61.8%), followed by USM Minden Campus (39.6%), Teluk Bahang (37.5%), Gelugor

(27.0%) and Bayan Lepas (19.5%). Parasitized mounds were not found in Air Itam (28 surveyed mounds) and Jelutong (22 surveyed mounds). Parasitized colonies were classified as healthy at all surveyed sites.

The mean height of parasitized (n = 105) and unparasitized (n = 208) mounds was 40.9 cm (range = 81 cm) and 34.8 cm (range = 85cm), respectively. The mean diameter of parasitized and not-parasitized was 107.1 cm (range=209 cm) and 92.0 cm (range=208 cm), respectively. The mound size (height and diameter) of parasitized colonies (n = 105) was higher (t = -3.3; df = 311, P < 0.01) and broader (t = -3.2; df = 311; P< 0.01) in comparison to not-parasitized colonies.

Table 1. Frequencies of parasitism at each surveillance site in Penang Island

Location	Longtitude	Latitude	No. mound surveyed	N. mound infested	Parasitism frequency %
Bayan Lepas	100 17.042	5 18 935	41	8	19.5
Gelugor	100 18 727	5 22 354	37	10	27.0
Balik Pulau	100 18.013	5 21.080	29	18	62.0
USM	100 18.380	5 21.634	106	42	39.6
Youth park-bontany garden	100 18.399	5 26.198	34	21	61.8
Air Itam	100 16.804	5 23.366	28	0	0
Jelutong	100 18.712	5 23.004	22	0	0
Teluk Bahang	100 13.255	5 27.720	16	6	37.5
Total			**313**	**105**	**33.5**

Discussion

The current study showed high parasitism frequencies in Balik Pulau and Youth Park-Botanical Garden, which are shaded areas with dense vegetation. Additionally, parasitized colonies were not found in areas with limited vegetation and in urban settings (Air Itam and Jelutong). Similar to our study, two blow flies *Chrysomyia albiceps* (Widemann) and *Chrysomyia marginalis* (Widemann) are usually in forested areas compared to open shrub lands (Braack & Retief 1960). Habitats with dense vegetation such as forests and undisturbed areas probably provide richer food resources than urban areas. Therefore, we suggest that the adult parasitoids prefer shaded, dense vegetation areas.

In colonies of Macrotermitinae, it is documented that mound size and termite numbers have a positive correlation (Josens & Soki 2010). Large and healthy populations are probably more preferred due to its rich resources for parasitoids exploiting. This premise was supported by the results of Smith and

Schwarz (2009), in which, the allodapine social parasites, *Inquilina schwarzi* Michener prefered to attack larger colonies of its host, *Exoneura robusta* Cokerell. Additionally, Cervo and Turillazzi (1996) also found that cuckoo paper wasps, *Plistes sulcider* Zimmermann, actively selected the larger and more developed colonies. In this study, we found that the size of parasitized colonies was significant larger than that of not-parasitized ones. In addition, the parasitized colonies were classified as healthy in all surveyed sites. It suggests that the parasitioid fly *V. fasciventris* might prefer to parasitize larger mounds and healthy colonies.

Conclusion

This study examined the distribution and parasitism frequency of *M. carbonarius* colonies parasitized by *V. fasciventris*. The adult parasitoid flies showed a preference for shaded areas with dense vegetation. The size of parasitized mounds was significant larger compared to the not-parasitized mounds. It suggests that the parasitoid *V. fasciventris* prefers to parasitize larger *M. carbonarius* colonies. In addition, the parasitoid flies tend to select healthy colonies to deposit their eggs.

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H2 hazard class field test of planted Malaysian hardwoods engkabang, kelempayan and rubberwood dip-treated with different concentrations of cypermethrin and permethrin against *Coptotermes curvignathus*

by

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Abstract

Some prescribed use rates of agro-insecticide products are meant to control specific insect pests, including termites, in crop or wood protection. The objective of this study was to determine the efficacy of agro-insecticidal dip-treated fast-grown Malaysian hardwoods against the subterranean termite *Coptotermes curvignathus* in an aboveground H2 hazard class field test. Proprietary agro-insecticides containing cypermethrin or permethrin were used to dip-treat wood of engkabang (*Shorea macrophylla*), kelempayan (*Neolamarckia cadamba*) and rubberwood (*Hevea brasiliensis*) for 60 seconds at their prescribed rates (cypermethrin: 0.0055%g/g; permethrin: 0.0053%g/g) and reduced concentrations. Field tests were conducted under low humidity in an H2 hazard class (shielded from light and rainwater/inundation wetting) situation for 6 months. Results showed that untreated rubberwood was completely destroyed (mean mass loss 100%; mean AWPA visual termite rating 0) while relatively severe attacks occurred in engkabang (mean mass loss 57.0%, mean rating 3.7) and kelempayan (mean mass loss 31.1%, mean rating 7.5). Based on termite rating scale wood protection using cypermethrin did not occur at the prescribed spray-on concentrations, yielding mean termite ratings of 7.0 for rubberwood at 0.00275%, a rating 7.3 for kelempayan at 0.00275% and 0.0055%, and a rating 9.2 for engkabang at 0.001375% and 0.0055%. Mean termite ratings within each wood species were significantly different ($P<0.05$) among cypermethrin concentrations. Permethrin concentrations up to 0.0053%, failed to protect rubberwood (mean mass loss ≥ 85%, mean visual rating ≤ 1.3) and showed reduced protection of both kelempayan and engkabang where the best control occurred at less than prescribed concentrations (mean visual rating of 6.8 for kelempayan at 0.00265%; a rating 8.3 for engkabang at 0.00265%). In conclusion, pyrethroid treated rubberwood was not resistant while both treated engkabang and kelempayan were moderately resistant to *C. curvignathus* even at less than prescribed concentrations of the insecticides. Further tests at higher concentrations for these insecticide products would be required to detect effective concentrations for complete H2 hazard class wood protection.

Keywords: engkabang, kelempayan, rubberwood, cypermethrin, permethrin, dip-treatment, H2 hazard class

Introduction

Southeast Asian countries like Malaysia have a wealth of low termite-resistant wood species that provide a number of wood products used under H2 biological Hazard Class conditions specified in the Malaysian wood protection Standards (Wong 2004, 2005) while untreated and treated Malaysian woods typically used outdoors aboveground and inground are predisposed to high decay and termite hazards (Kirton & Wong 2001). Particularly as termites are economically important structural pests in tropical countries, their destructive significance to wood products, by public perception, seem more serious than that of tropical fungal decay (Kirton & Wong 2001), with costs incurred in 2003 to control termites in Malaysia amounting to approximately USD10-12 million (Lee 2004). Consequently, there is a need for low-moderate durability woods to be protected with wood protecting chemicals or by other means. For low hazard class applications in wood usage, it may be sufficient to surface-treat the wood [e.g. spraying, brushing, dipping, steeping-immersion or soaking (Richardson 1993)] with water-borne synthetic pyrethroids (eg. cypermethrin and permethrin) recognized as contact nerve poisons. The advantages of using dipping are: simple technique, low treatment cost, and achieving deep penetration and loading depending to duration of immersion (Richardson 1993, Ma *et al* 2013), which may encourage production of biocidal surface-treated wood products for H2 hazard class exposures.

In Australia (south of the Tropic of Capricorn) and New Zealand, the wood products industry features termite-resistant bifenthrin surface-treated (envelope-treated) softwood framing timbers (Standards Australia 2005) and that further work on such treated wood has verified their high termite-resistance (Sukartana *et al* 2009). Limited laboratory termite tests on rubberwood surface-treatments using cypermethrin, permethrin, bifenthrin and chlorpyrifos at higher than recommended concentrations for dip or brush-treatments have also conferred complete wood protection (Sornnuwat *et al* 1994). Since many water-borne organic agro-insecticidal products are available from agro-pesticide retail outlets, with instructions for spray-on dosages for protection against specific insect pests and/or for termite control of plantation trees and vegetables, these products could be considered for wood protection against termites if minimum effective dosages are determined. The objective of this study was to determine termite resistance of two commercial agro-insecticides by dip-treating engkabang, kelempayan and rubberwood as measured by an aboveground field test simulating H2 hazard class conditions.

Materials and methods

Engkabang (*Shorea macrophylla*), kelempayan (*Neolamarckia cadamba*) and rubberwood (*Hevea brasiliensis*) wood were cut into 20 x 20 x 20 mm blocks. Two commercial agro-insecticides were obtained from a local agro-pesticides retail outlet. Trade names, formulations and mode of applications of the agro-insecticides are shown in **Table 1**. Concentrations of insecticides are shown in **Table 2**.

Engkabang, kelempayan and rubberwood blocks were oven-dried for 48 hours at 105˚C. Then wood blocks were dipped, one block at a time, into agro-insecticide solutions at various concentrations up to the prescribed product concentrations for exactly 60 seconds. After conditioning for 14 days at room temperature, wood blocks were arranged randomly on a perforated aluminium tray mixed with termite baits of corrugated board and softwood blocks, and then exposed to an aboveground H2 hazard class termite test assembly providing shade and cover in a, non-wetting internal environment except humidity (Wong 2005), positioned above a stack of keruing sawn timbers infested by *Coptotermes curvignathus* for 6 months at the Timber Research and Technical Training Centre, Sarawak Forestry Corporation. Thereafter the blocks were retrieved, cleaned of soil debris, and oven-dried for 48 hours at 105˚C, before percentage mass loss and termite visual rating were evaluated. The visual rating was based on AWPA E7-07 scale (**Table 3**). Data were analysed using One-way ANOVA from the MINITAB14 statistical software.

Table 1: Properties of the agro-insecticides

Trade Name	Composition	Mode of Application	Dosages (ml) in 10 litres
GARRISON™ 5.5 EC	Cypermethrin (5.5% w/w) Inert ingredient: (94.5% w/w)	Spray	*Aphis sp.* (10ml) *Leucinodes orbonalis* (10ml) (= 0.0055%g/g cypermethrin)
KENBUSH™ 26.3 EC	Permethrin (26.3% w/w) Inert ingredient: (73.7% w/w)	Spray	*Leucinodes orbonalis* (2ml) *Plutella xylostella* (2ml) *Aphis sp.*(2ml) *Earias phabia* (2ml) (=0.0053% permethrin)

Table 2: Concentrations of the agro-insecticides used

Agro-insecticides	Concentration of active (%g/g)			
Cypermethrin	0.00	0.001375	0.00275	0.0055
Permethrin	0.00	0.001325	0.00265	0.0053

Table 3: AWPA E7-07 termite rating scheme (AWPA 2008)

Rating	Description
10	Sound
9.5	Trace, surface nibbles permitted
9	Slight attack, ≤3% of cross sectional area affected
8	Moderate attack, 3-10% cross sectional area affected
7	Moderately severe attack and penetration, 10-30% of cross sectional area affected
6	Severe attack, 30-50% of cross sectional area affected
4	Very severe attack, 50-75% of cross sectional area affected
0	Failure (destroyed)

Results and Discussion

Results (**Table 4 & 5**) showed that untreated rubberwood was completely destroyed (mean mass loss 100%, mean AWPA termite rating 0) while untreated engkabang and kelempayan sustained rather severe attacks with mean mass losses of 57.0% (mean termite rating 3.7) and 31.1% (mean termite rating 7.5) respectively. There was a significant difference ($P<0.05$) in percentage mass loss and visual rating of both engkabang or kelempayan between concentrations of cypermethrin or permethrin, while for rubberwood significant differences occurred between concentrations of cypermethrin but not permethrin (**Table 4** *cf* **Table 5**). Comparisons between cypermethrin and permethrin would not be valid due to the different formulations. Based on termite ratings of cypermethrin-treated wood (**Table 4**), the best termite control did not always occur at the prescribed dosages, with no consistent trends among the concentrations (ie. best mean termite rating for rubberwood was 7.0 at 0.00275%, engkabang rated 9.2 at 0.001375% & 0.0055%, and kelempayan rated 7.3 at 0.00275% & 0.0055%). Cypermethrin-treated engkabang sustained the best, though not completely, termite control among the 3 wood species (**Table 4**).

Table 4: Mean percentage mass loss (%) and mean visual rating of engkabang, kelempayan and rubberwood dip-treated with cypermethrin

Species	Concentrations (%g/g)	Wood Mass Loss (%) Mean	Min	Max	Mean Visual Rating	Min	Max
Engkabang	Controls	57.0 (20.2) *b*	27.5	79.1	3.7 (3.2) *a*	0	8
	0.001375	20.2 (8.7) *a*	8.3	29.8	9.2 (0.7) *b*	8	10
	0.00275	25.4 (11.7) *a*	6.7	42.2	8.8 (0.7) *b*	8	9.5
	0.0055	20.5 (5.0) *a*	13.8	29.2	9.2 (0.6) *b*	8	9.5
Kelempayan	Controls	31.1 (8.3) *a*	21.8	45.1	7.5 (1.2) *b*	6	9
	0.001375	74.8 (9.6) *c*	64.5	84.7	5.0 (1.1) *a*	4	6
	0.00275	57.8 (5.9) *b*	49.5	66.5	7.3 (0.8) *b*	7	9
	0.0055	58.9 (4.8) *b*	53.8	66.9	7.3 (0.5) *b*	7	8
Rubberwood	Controls	100 (0) *c*	100	100	0 (0) *a*	0	0
	0.001375	76.2 (20.3) *b*	43.4	100	2.2 (3.4) *a*	0	7
	0.00275	42.2 (12.7) *a*	20.8	59.1	7.0 (1.1) *b*	6	9
	0.0055	85.8 (12.6) *bc*	63.6	100	2.3 (2.7) *a*	0	6

(…) = Standard deviation, n= 6.
Within-column values per wood species sharing same italicized letters denote no significant difference by LSD.
LSD values (% mass loss) for engkabang = 15.3, kelempayan = 8.9, rubberwood = 16.2
LSD values (termite rating) for engkabang = 2.0, kelempayan = 1.1, rubberwood = 2.7

Table 5: Mean percentage mass loss (%) and mean visual rating of engkabang, kelempayan, and rubberwood dip-treated with permethrin

Species	Concentrations (%g/g)	Mean Percentage Mass Loss (%)	Min	Max	Mean Visual Rating	Min	Max
Engkabang	Controls	57.0 (20.2) *b*	27.5	79.1	3.7 (3.2) *a*	0	8
	0.001325	16.0 (11.1) *a*	4.7	30.4	7.9 (0.9) *b*	7	9.5
	0.00265	20.7 (16.2) *a*	3.6	48.1	8.3 (0.9) *b*	7	9.5
	0.0053	32.8 (11.9) *a*	19.8	52.3	7.5 (1.0) *b*	6	9
Kelempayan	Controls	31.1 (8.3) *a*	21.8	45.1	7.5 (1.2) *c*	6	9
	0.001325	71.5 (11.5) *c*	56.4	86.1	5.7 (1.4) *ab*	4	7
	0.00265	57.5 (8.7) *b*	45.4	65.4	6.8 (0.8) *bc*	6	8
	0.0053	73.3 (7.4) *c*	62.8	81.8	4.7 (1.0) *a*	4	6
Rubberwood	Controls	100.0 (0.0) ns	100	100	0 (0) ns	0	0
	0.001325	100.0 (0.0) ns	100	100	0 (0) ns	0	0
	0.00265	87.2 (19.8) ns	60.9	100	1.3 (2.1) ns	0	4
	0.0053	86.3 (21.3) ns	58.6	100	1.3 (2.1) ns	0	4

(…) = Standard deviation, n= 6. ns= not significant ($P<0.05$)
Within-column values per wood species sharing same italicized letters denote no significant difference by LSD.
LSD values (% mass loss) for engkabang = 18.4, kelempayan = 10.9
LSD values (termite rating); engkabang = 2.2, kelempayan = 1.3

From **Table 5**, permethrin at the concentrations tested failed to protect rubberwood (mean termite rating ≤ 1.3, mean mass loss >85%). Based on the termite ratings, the best termite control for permethrin-treated engkabang and kelempayan was recorded at less than the prescribed concentration (engkabang and kelempayan at 0.00265%), with best mean termite ratings for engkabang and kelempayan of 8.3 and 6.8, respectively. Mean mass loss and visual ratings in **Table 5** revealed that permethrin treated engkabang was the least attacked among the 3 wood types tested.

High termite susceptibility of untreated rubberwood (**Table 4 & 5**) is typical of this wood species which was previously observed in other aboveground, inground or laboratory termite tests (Wong 2000, Grace *et al* 1998, Wong *et al* 1998). Rubberwood is indeed a suitable candidate substrate for termite baits or as a wood substrate for preservative testings as well as studies with bait toxicants. That these pyrethroids at those spray-on concentrations failed to protect engkabang, kelempayan and rubberwood is of concern, and should be re-tested at higher dosages. Read and Berry (1984) revealed that a 0.1% concentration of cypermethrin emulsion using surface application was sufficient against *Reticulitermes* termites and this contrasts with the extremely low dosages adopted in the present study, suggesting that prescribed product concentration of cypermethrin (0.0055%) was clearly inadequate for termite control. Zaidon *et al* (2008) found that exposure of rubberwood particleboard, Empty Fruit Bunch (EFB) particleboard and Rubberwood-EFB particleboard sprayed with 0.2% permethrin to *Coptotermes curvignathus* yielded low mean mass loss (range: 7.2 – 12.1%) unlike their untreated counterparts (range: 17.8 – 31.1%). Excellent protection was reported from a laboratory evaluation of 5-min dip-treated rubberwood blocks exposed to *Coptotermes gestroi* at 0.015, 0.25 and 0.5% cypermethrin and at 0.5, 1 and 2% permethrin (Sornnuwat *et al* 1994). In the present study, 60 sec. dipping time was adopted which reflects typical short duration dip-treatments of wood, yet it is useful to note that improved termite (and fungal) resistance of dip-treated wood do occur when dipping (or steeping) durations (and perceivable insecticide retentions in wood) increase appreciably [>1 min, a few or several hours (Sornnuwat *et al* 1994, Kamdem *et al* 1996, Ma *et al* 2013)]. Surface treatments rely on capillary action allowing a preservative solution to penetrate into the wood, with higher uptakes/absorptions and penetrations due to increased dipping times (Humar & Lesar 2009, Ma *et al* 2013). Since preservative performance against wood-degrading organisms depends mainly on wood species, target preservative retention (derived from concentrations and treatment methods used) and penetration of the preservative into the wood (Zabel & Morrell 1992), dip-treatments in water-borne insecticides can also enhance termite durability performance of wood under non-leaching (H2 hazard class) conditions when adequate dipping times and insecticidal concentrations are applied.

Conclusion

Both treated and untreated rubberwood were nonresistant while engkabang and kelempayan were respectively, non- and moderately termite resistant. Results shows that cypermethrin and permethrin also provide good protection to engkabang and moderate protection to kelempayan. This study revealed that agro-insecticides used at their prescribed spray-on concentrations meant for crop protection might not be adequate for wood preservation against termites. Further, similar wood dip-treatments at higher insecticidal concentrations would therefore be desirable to seek adequate concentrations (and practical dipping times) to prevent termite attacks.

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Efficacy of Crude Extracts from Indigenous Plant Species against Philippine Termites

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Abstract

The toxicity of extracts from four (4) selected Philippines plant species which include tubang-bakod *(Jatropha curcas*) Bayati *(Anamirta cocculus*), cashew nut shell *(Anacardium occidentale*) and river red gum (*Eucalyptus camaldulensis*) was evaluated against subterranean termites, Los Baños termites (*Microcerotermes losbañosensis),* Philippine milk termites *(Coptotermes gestroi)* and *drywood termites (Cryptotermes dudleyi)*by topical application method. Likewise yield of extracts from the plants was also determined.Results showed the volume of extracts obtained from the four (4) plant species varied. Likewise, toxicity of the extracts varied according to test insect, concentration and source of plant extract (plant species). Crude extracts of Jatropha methyl ester (JME), CNSL and *E. camaldulensis* were highly toxic to Los Baños termites while JME and cashew nut shell liquid (CNSL) were highly toxic to drywood termites andJME was highly toxic to Philippine milk termites (PMT). *Anamirta cocculus* was not toxic to the test insects.Identification of compounds that contributed to toxicity has to be further investigated. Likewise, formulated products from the extracts showed high potential as a promising insecticide.

Keywords: Natural products, plant crude extract Philippine termites, *Jatropha curcas,* Cashew nut shell liquid, *Eucalyptus tereticornis, Microcerotermes, Coptotermes, Cryptotermes*

Introduction

The use of preservatives for control of insect and fungal attack in wood and non-wood forest products has raised health and environmental concerns. Although the risk to workers and the environment can be reduced to acceptable levels through proper handling and use there is still a need to address those concerns to abate possible apprehension of manufacturers and exporters on the use of wood preservatives used in treating raw materials and finished products.

At present, new environment-friendly wood protection systems based on “green” technologies are considered necessary which create a major challenge for manufacturers of wood preservatives worldwide. Thus, the effortto develop eco-friendly wood preservatives from natural sources over the past decade.

This project involves selected Philippine plant species,River Red Gum (*E. camaldulensis*), Bayati *(A. cocculus*), Cashew nut shell *(A. occidentale*) and Tubang-bakod *(J. curcas*) which contain secondary metabolites in the leaves, bark, wood, flowers, fruits and/or seeds reported to be antagonistic to some insects. Through the use of appropriate extraction methods these substances can be made available and applied to wood or bamboo to prevent or control insect and fungal attack. The results presented were obtained from an on-going project funded by the Department of Science and Technology – Grants in Aide (DOST-GIA) of the Philippine Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), Los Baños, Laguna, Philippines.

Objectives

1. To determine the yield of the crude extracts from selected indigenous plants materials.

2. To evaluate the efficacy of the crude extracts against Philippine termites.

Materials and Methods

a. Collection and Preparation of Raw Materials

The raw materials from Bayati and cashew nut shells were collected from Quezon Province and Bataan, respectively. These raw materials were sorted, cleaned, and sliced/chopped into required particle size prior to extraction. *Jatropha* crude oil and methyl ester from seeds were solicited from the project on “Pilot production and testing of biodiesel from *J. curcas*of the Industrial Technology Development Institute (ITDI-DOST), Philippines. Leaves of *E. camaldulensis* were collected from San Fernando, Pampanga.

b. Extraction of compounds from five plant species

Jatropha

The solicited crude volatile oil was obtained from seeds of Jatropha, which were extracted using a mechanical expeller, filtered, and then stored in covered bottles for characterization (2010 ITDI).

Jatropha methyl ester was also obtained from ITDI. The *Jatropha*crude oil was subjected to the trans-esterification process that involved degumming with phosphoric acid;saponification of free fatty acid with lye, methanol and a catalyst was added to produce the methyl ester (ITDI 2010).

Bayatistems

The crude extract of finely chopped bayati was processed by the cold extraction method. A 100gsample was soaked in water for three days in 700 ml of water inside a refrigerated cabinet (8°C). After soaking, the solution was filtered using a Buchner funnel and the filtrate evaporated in a boiling water bath. The final dried samples were stored in labeled bottles.

Cashew Nut Shells

Thecashew nut shellswere cleaned and extracted using an expeller developed by FPRDI to produce crude CNSL.

***E. camaldulensis*Leaves**

Leaves were chopped into small pieces using a Moulinex cutting machine. The materials underwent steam distillation using a clavenger apparatus to produce essential oils. The steam-distilled essential oils were collected and used in the bioassay against the test insects.

c. Test on the Toxicity of Extracts Against Termites and Powder-Post Beetles

Preparation of crude extracts

The crude extracts were subjected to a toxicity test against wood destroying organisms. The toxicity of various plant extracts were evaluated against test insects using a manually operated topical applicator. The extracts were prepared at various concentrations (10%, 20%, 30%, and 40% v/v). These concentrations gave an initial indication of the efficacy of the extracts and its fractions.

Preparation of Test Insects

Workers of wood-destroying insects included the subterranean termites (Los Baños termites, *Microcerotermes losbañosensis* and Philippine milk termites, *Coptotermes gestroi*) and drywood termites (*Cryptotermesdudleyi*) were collected, kept in trays lined with filter paper and conditioned prior to testing. All test insects were conditioned for 24 hrs prior to toxicity test.

Procedure for Testing

Twenty healthy and active insects were used per treatment and replicated three times. One to two µL of extract were applied on the thoracic region of the test insects, after which they were placed in a petri dish lined with filter paper. The insects were observed after 24 hrs and the mortality recorded. Insects that did not move when touched gently with a soft brush were considered dead.

$$\text{Corrected Mortality} = \frac{\text{\% test mortality - \% solvent mortality}}{\text{100 - \% solvent mortality}} \times 100$$

Assessment of Toxicity of the Extracts

The degree of toxicity of the treatmentsto test insects was arbitrarily classified as follows:

% Insect Mortality	Classification of Toxicity
0	Not Toxic (NT)
1 - 25	Slightly toxic (ST)
26 - 50	Moderately Toxic (MT)
51 – 75	Toxic (T)
76 - 100	Highly Toxic (HT)

Results and Discussion

a. Yield of Extracts

Jatropha seeds had an average yield of 25.0% extracted oil (Almanzor et al 2010) (Table 1). Conversion of oil into methyl ester through the double stage trans-esterification process yielded 83.0%-89.0% methyl ester. About 1500ml of water extract from bayati stems yielded 70-80% and CNSL had an average crude extract yield of 38% to 40%. Chopped leaves of *E. camaldulensis* had an average yield of essential oils of 2% to 4%.

Table 1.Percentage Yield of Plant Extracts.

Plant Material	Common Name	Percent Yield
		Water orCrude Extract
J. curcas	Tubang-bakod	25.0
A. cocculus	Bayati	70.0 – 80.0
A. occidentale	Cashew	38.0 – 40.0
E.camaldulensis	River Red Gum	2.0 - 2.5

b. Toxicity of extracts against Philippinetermites

Toxicity of J. curcascrude extract

Results of the initial screening showed that *J. curcas* crude extract was slightly toxic to the Los Baños termites *M. losbañosensis* (Table 2). *Jatropha* crude extract regardless of concentrations provided 3.3% to 18.3% kill of *M. losbañosensis*. The standard, deltamethrin, was highly toxic and 100% mortality of test insects was noted after 24 hrs. The standard chemical was highly toxic while crude extract was only slightly toxic to termites. The untreated group had 0% mortality.

The 10% to 30% crude extracts were not toxic to milk termites, *C. gestroi* as shown by 0% mortality of test insects. The highest concentration, 40%, was slightly toxic and provided 6.7% mortality. The standard chemical eliminated 100% of *C. gestroi*. The 10% and 20% crude *J. curcas* extract was not toxic to drywood termites (*C. dudleyi)* while the higher concentrations of 30% and 40% were slightly toxic with 3.3% and 5.0% mortality, respectively. The toxicity of higher concentrations of *J. curcas*was significantly lower than the 100% kill provided by standard chemical.

Table 2. Toxicity of *Jatropha* crude extract to Philippine termites by topical application.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	M. losbañosensis	*C. gestroi*	*C. dudleyi*
1. 10% JcCE	3.3 ST	0 NT	0 NT
2. 20% JcCE	3.3 ST	0 NT	0 NT
3. 30% JcCE	5.0 ST	0 NT	3.3 ST
4.40% JcCE	18.3 ST	6.7 ST	5.0 NT
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0
Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic);26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)			

Based on the preliminary results, *Jatropha* crude extract was non-toxic to slightly toxic to *M. losbañosensis, C. gestroi* and *C. dudleyi.* The zero mortality in the untreated group suggests that the population used was ideal for testing.

Toxicity test of J. curcasmethyl ester

The 10% to 40%*Jatropha*methyl ester extract was as toxic as the standard chemical that provided 100% kill of *M.* losbañosensis (Table 3). All test concentrations except at 10% *Jatropha a*methyl ester were highly toxic to *C. gestroi* and *C. dudleyi* and the level of toxicity was comparable to the standard chemical. The 20% to 40% *Jatropha* methyl ester caused 65.0% to 85.0% to *C. gestroi* and 91.7% to 96.7% mortality to *C. dudleyi*. The lowest concentration of 10% methyl ester was slightly toxic to both test insects (8.3% to 16.7% kill). No termite mortality was recorded in the untreated group.

Table 3. Toxicity of *Jatropha* methyl ester to Philippine termites by topical application method.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	M. losbañosensis	*C. gestroi*	*C. dudleyi*
1. 10% Jc-ME	78.3HT	16.7 ST	8.3 ST
2. 20% Jc-ME	100HT	65.0 HT	91.7 HT
3. 30% Jc-ME	100HT	83.3 HT	96.7 HT
4. 40% Jc-ME	100HT	85.0 HT	95.0 HT
5. MeOH	25 ST	20.0 ST	1.7 ST
6. Deltam - Std	100 HT	100 HT	100 HT
7. Control	0	0	0
Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic);			

26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)

Toxicity test of A. cocculus crude extract

The crude extract of *A. cocculus*was not toxic to slightly toxic to three species of Philippine termites (Table 4). The crude extract regardless of concentration was slightly toxic to *M. losbañosensis*andmortality of test insects ranged from 3.3% to 13.3%. For *C gestroi* and *C. dudleyi,* the crude extract was not toxic to slightly toxic asshown by 0% kill in the former and 3.3% to the latter species of test insects. The standard chemical was highly toxic and caused 100% kill regardless test insect. There was no mortality of test insects in the untreated group.

Table 4. Toxicity of *A. cocculus*crude extract to Philippine termites by topical applicationmethod.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	M. losbañosensis	*C. gestroi*	*C. dudleyi*
1. 10% AcCE	3.3 ST	0 NT	0 NT
2. 20% AcCE	10.0ST	0 NT	0 NT
3. 30% AcCE	13.3ST	0 NT	0 NT
4.40% AcCE	13.3 ST	0 NT	3.3 ST
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0
Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic);26–50% (Moderately toxic); 1-25%(Slightly toxic) and 0% (Not toxic)			

Toxicity test of CNSL crude extract against subterranean termites and drywood termites

Table 5summarizes the results of initial toxicity of CNSL crude extract to Philippine termites. CNSL crude extract regardless of test concentration was as highly toxic as standard chemical with 100% mortality. Likewise, the 20% and 30% CNSL crude extract washighly toxic to *C. dudleyi*providing 95.2% to 100% kill. On the other hand, the crude extract was slightly toxic to *C. gestroi* with mortality ranging from 3.3% to 11.7%. The 40% CNSL crude extract encountered a problem in application because it was too viscous and difficult to apply. No mortality of test insects was noted in untreated group.

Table 5. Toxicity of CNSL crude extract to Philippine termites by topical application method.			
Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	M. losbañosensis	*C. gestroi*	*C. dudleyi*
1. 10% CNSLCE	79.5 HT	3.3 ST	32.9 MT
2. 20% CNSLCE	100 HT	6.7 ST	100 MT
3. 30% CNSLCE	100 HT	11.7 ST	95.2 HT
4. Deltam - Std	100 HT	100 HT	100 HT
5. Control	0	-	0
Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% 1 (Toxic);26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)			

Toxicity test of E. camaldulensis crude extract

Onlythe 40% concentration of the crude extract of *E. camaldulensis*was highly toxic to *M. losbañosensis*. It eliminated 98.3% of the test insects and as highly toxic as the 100% kill caused by the standard chemical (Table 6). Lower concentrations of10% to 30% were still toxic to termites but caused lower mortality at66.7% to 73.3% compared to standard chemical with 100% kill. Generally, the crude extract of *E. camaldulensis*was slightly toxic to*C. gestroi and C. dudleyi* with mortalities that ranged from 11.7% to 36% and 3.3 to 28.3%, respectively. All untreated test insects remained active at the end of the test.

Table 6. Toxicity of *E. camaldulensis*crude extract to Philippine termites by topical application method.			
Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	M. losbañosensis	*C. gestroi*	*C. dudleyi*
1. 10% Ec-CE	66.7 T	11.7 ST	3.3ST
2. 20% Ec-CE	73.3 T	16.7 ST	5.0ST
3. 30% Ec-CE	73.3 T	36.0 MT	15.0ST
4.40% Ec-CE	98.3 HT	60.0 T	28.3 MT
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0
Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic); 26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)			

Based on the results of the screening the toxicity of extracts and fractionated forms from five selected plants, the degree of toxicity varied according to plant species, and concentration. Subterranean termites *M. losbañosensis* were more susceptible than Philippine milk termites, *C. gestroi* and drywood termite *C. dudleyi.* Out of the 6 extracts from 5 species of plants3 of the indigenous materials were found highly toxic to *M. losbañosensis,* 2 extracts to drywood termitescompared to only 1treatment offered high toxicity to *C. gestroi*(Table 7).

Table 7. Toxicity test of the various crude extracts of selected indigenous plant materials against Philippine termites.	
	Test Insects

Treatment	*M. losbañosensis*	*C. gestroi*	*C. dudleyi*
1. *Jatropha* crude	ST	NT	ST
2. *Jatropha* Methyl Ester	HT(10-40%)	HT(20-40%)	HT(20-40%)
3. *A. cocculus* crude	ST	NT	NT
4. CNSL crude	HT (10-30%)	ST	HT(30%)
5. *E. camaldulensis* crude	HT(40%)	ST-T	ST-MT

Legend:

% Mortality	Level of Toxicity
0	Not Toxic (NT)3
1 - 25	Slightly Toxic (ST)
26 - 50	Moderately Toxic (MT)
51 - 75	Toxic (T)
76 -100	Highly Toxic (HT)

Conclusions

The volume of crude extracts obtained from the four (4) plant species varied. The toxicity of plant extracts from selected plant species varied according to test insect, concentration and source of plant extract (plant species). Out of the five (5) treatments, *Jatropha* methyl ester was highly toxic to Philippine termites regardless of test species. CNSL were highly toxic to *M. losbañosensis* and *C. dudleyi* but slightly toxic to *C. gestroi. E. camaldulensis* was highly toxic to *M. losbananosensis* but slightly toxic to *C. gestroi* and *C. dudleyi.* The crude extractsof *Jatropha* and *A. cocculus* were slightly to not toxic to Philippine termites.

The high toxicity of plant extracts indicates an insecticidal potential in preventing and controlling the attack of Philippine termites. However, follow-up screening is being conducted to verify the results of this toxicity of plant materials to Philippine termites.

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Subterranean Termite Resistance of Polystyrene Wood from Three Tropical Wood Species

by
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Abstract

Timber from a plantation forest has inferior resistant to subterranean termite attack compared to timber from a natural forest, because stands are cut at a young age and the timber consist of a lot of sapwood and juvenile wood in the heartwood. Polystyrene impregnation is a way to increase resistance to termite attack of the wood. Sengon (*Falcataria moluccana*), mangium (*Acacia mangium*), and pine (*Pinus merkusii*) were impregnated with polystyrene at polymer loadings of 26.0%, 8.6%, and 7.7%, respectively. The wood specimens were tested in the ground for three months. The weight loss of untreated sengon, mangium, and pine were 50.3%, 23.3%, and 66.4% %, respectively, and polystyrene wood 7.6%, 14.4%, and 5.1%, respectively. The polystyrene wood was more resistant than untreated wood to subterranean termite attack in the field test.

Key words: subterranean termite, polystyrene wood, weight loss, resistance class.

Introduction

Indonesian log production in 2013 reached 23.23 million m^3, and 84% was from plantation forest that included wood such as sengon (*Falcataria moluccana*), mangium (*Acacia mangium*), pine (*Pinus merkusii*), and gmelina (*Gmelina arborea*) (Ministry of Forestry, 2014). The logs were cut from young stands, 5 to 10 years old, resulting the timber had a lot of sapwood and juvenile wood in the heartwood (Fajriani, 2013).

Indonesia as a tropical country has a good environment for termites especially subterranean termites that could attack wooden building. Nandika (2015) mentioned that all parts of the country have been attacked by subterranean termites, including all districts in Jakarta. Furthermore, it has been mentioned that the economic loss was about US$ 1 million in 2015.

Juvenile wood has inferior characteristics in terms of physical-mechanical properties and therefore reistance to termite attack. Hadi et al. (2013; 2015a) mentioned that jabon wood (*Anthocephalus cadamba*) imprenated with methyl methacrylate had better physical-mechanical properties and resistance against subterranean termite (*Coptotermes curvignathus*) attack in laboratory tests according to the Indonesian standard. Furthermore Hadi et al. (2015b) treated mindi wood (*Melia azedarach*) from Indonesia with a density of 0.43 g/cm^3 and sugi wood (*Cryptomeria japonica*) from Japan with a density of 0.34 g/cm^3 of polystyrene resulting in those woods being more resistant against subterranean and drywood termites (*Cryptotermes cynocephalus*) in laboratory test according to the Indonesian standard.

The purpose of this study was to determine the resistance of polystyrene impregnated wood from three fast-growing tree species against subterranean termite attack.

Materials and Methods

Wood specimen preparation

The wood species sengon, mangium, and pine from Bogor, West Java, Indonesia, were used to determine resistance to subterranean termite attack. Test specimen size was 1 cm by 2 cm in cross section, and 20 cm in longitudinal direction according to Hadi et al. (2005). Impregnation of polystyrene was conducted to air-dried wood samples placed under vacuum at 600 mm Hg for 30 min, followed by immersion in monomer styrene at 10 kg/cm^2 for 30 min. The wood samples were then wrapped in aluminum foil and placed in an oven at 100 °C for 24 h. The aluminum foil was removed and samples weighed for polymer loading calculation.

In-Ground Test

The test specimens were placed vertically in the ground with half the length in the ground at the arboretum of Bogor Agricultural University Campus for three months. At the end of the test, each specimen was measured for weight loss according to the equation:

$$WL = (W1 - W2) / W1 \times 100\%$$

where W1 = weight of oven-dried wood prior to the test (g); and W2 = weight of oven-dried wood after the test (g).

Results and Discussion

The mean value and standard deviation of wood density, polymer loading, resistance class and percent wood weight loss to subterranean termite attack of untreated and polystyrened wood is shown at Table 1.

Table 1. Wood density, polymer loading, resistance class to subterranean termite attack, and wood weight loss of untreated and polystyrened woods.

Wood Species	Density (g/cm^3)	Polymer Loading (%)	Resistance Class*	Wood Weight Loss (%)	
				Untreated	Polystyrene
Sengon	0.34 (0.01)	26.0 (5.4)	V	50.3 (36.8)	7.6 (2.5)
Mangium	0.51 (0.01)	8.6 (4.8)	IV	23.3 (8.1)	14.4 (7.0)
Pine	0.69 (0.01)	7.7 (2.3)	V	66.4 (18.6)	5.1 (4.3)

Remarks: Values in the parentheses are standard deviation. * According to Arinana et al. (2012).

From Table 1 it can be seen that polymer loading of sengon was the highest followed by mangium and pine, and that order related to wood density from lower to higher values. Hadi et al. (2015) mentioned that a lower density wood was more easily penetrated by styrene monomer, borax, and acetic anhydride, because it has more voids compared to higher density wood.

Sengon and pine wood species had very poor resistance (class V) and mangium had poor resistance (class IV) to subterranean termite attack according to the SNI 2006 (Arinana et al. 2012) when test specimens were taken from trees 5 to 10 years old. The wood specimens were mostly sap wood as indicated by the light color of the wood (Fajriani et al. 2012).

Wood specimen weight loss of mangium (class IV) was lower than sengon and pine (both class V).. Even though wood density of mangium 0.51 g/cm^3 was lower than pine with 0.69 g/cm^3 but mangium had better resistant because wood resistance to biodeterioration was not related to wood density but the amount of toxic extractive content.

The average weight loss of untreated wood was 46.7% and polystyrene wood was 9.0%, indicated that polystyrene wood had much better resistant than untreated wood to subterranean termite attack and this result was in-line with Hadi et al. (2015).

Conclusions

The lower density wood had higher polymer loading of polystyrene compared to higher density wood. Sengon and pine woods had very poor resistance and mangium poor resistance to subterranean termite attack. Polystyrene wood had much better resistance than untreated wood to subterranean termite attack.

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Durability test of local, usable wood against termites

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Abstract

A durability test of 12 local, usable wood specimens and 1 commercial wood were conducted using termite no-choice bioassay (ASTM D3345-74) under laboratory and a random-placement -field test. One-way ANOVA was used to examine the difference in percent weight loss among wood species. A difference in subterranean termite feeding rates were found in 5 wood species including Durian, Bamboo, Mango tree, Rubber wood and Eucalyptus ($P<0.05$). Similarly, there was a difference in the percent weight loss among wood species ($F_{12, 64} = 49.3$, $P< 0.001$). Weight loss was significantly higher in 5 wood species, Durian (43.2 ± 8.3SE %), Bamboo (46.1 ± 9.8SE %), Mango tree (70.9 ± 7.1SE %), Rubber wood (39.5 ± 4.7SE %) and Eucalyptus (53.3 ± 9.1SE %), than the other species ($P<0.05$). in laboratory, 10 replicates were prepared for each timber species. A difference in feeding rates of termites was found in 1 wood species, Mango tree ($P<0.05$). Difference in the no-choice test weight loss was also found among wood species ($F_{12, 64} = 49.3$, $P< 0.001$), with weight loss significantly higher in 2 wood species, Mango tree (11.8 ± 1.9SE %) and Rubber wood (10.2 ± 1.4SE %) ($P<0.05$).
Keyword: Durability, local, usable woods, termites

Introduction

The termites are known to cause losses in agriculture and forest as well as to wooden structures resulting in expensive property damage in a short time (Bultman and Southwell, 1976),. Today, chemicals are used to control termites around residents by termite control businesses. Environmental pollution concern caused by the use of toxic chemicals, is resulting in the search for natural compounds as alternative termiticides (Carter, 1976, Ganapaty *et al*. 2004, Roszaini *et al.* 2009). The special properties of natural wood such as secondary metabolite, quinines, flavonoids and terpenoids (Scheffrahn, 1991, Balsiger *et al*. 2000), are thought to provide protection against wood damaging fungi, insects and other organisms (Hon and Shiraishi, 1991, Schultz and Nicholas, 2002). Other components from extractives, to wood density have been found to affect termites feeding (Bultman *et al.* 1979).

Humans have for many years used chemicals for termite control and today the service charge of termite control companies is dependent on chemicals used and type of wood to be protected. One objective of this study was to supply background information on the natural durability of different types of usable woods against termite damage. This study focused on wood consumption rates of termites offered different types of wood. The wood that we found to be seriously damaged by termites would be one good choice to use in termite control businesses.

In 1989, the government of Thailand declared a blockade on wood products from forests (Chairat, 2015) that meant the people and companies couldn’t use Thai wood to build or decorate dwellings nor could Thai wood be exported to other countries. Therefore, the best choices are used woods from plantation forests, agricultural fields and garden trees that ordinary people can apply to used. Secondary objective, the high natural resistance of woods that were used in the buildings and wooden makings to decorated the facilities.

Materials and methods

1. Wood types

Wood from a total of 13 different tree species were used including: Rubber wood (*Hevea brasiliensis*); Eucalyptus (*Eucalyptus camaldulensis*); Gurjin (*Dipterocapus* sp.); Neem Tree (*Azaduracta indica*); Mango tree (*Mangifera indica*); Bamboo (*Bambusa* sp.); Durian (*Durio zibethinus*); Casuarina (*Casuarina junghuhniana*); Siamese Sal (*Shorea obtuse*); Indian Walnut (*Albizia lebbeck*); Teak (*Tectona grandis*); Iron wood (*Xylia xylocarpa*) and Commercial lumber (*Pinus* sp.) was used as a control.

2. Field Test

Five circular test blocks measuring 70-cm in diameter were random take placed at the Technology Development and Transfer Division station in Ratchaburi Province. Thirteen wood specimens were cut into blocks measuring 25-mm in thickness, 100 mm in length, and 25 mm in width. All wood specimens were dried under 100±5 ^{o}C in 24 hours and dry weight recorded. Five replicates were prepared for each timber species with the test wood specimens placed above cement pads in each circular test block. At the end of six months the specimens were removed, cleaned, dried for 24 hours and reweighed.

3. No-choice laboratory test

Twelve wood specimens were random taken from sawmills and cut into by 6.4 mm thick, 25.4 mm length, and 25.4 mm width blocks, and including the Commercial wood (*Pinus* sp.) controls. All wood specimens were dried at 100±5 ^{o}C for 24 hours and dry weight recorded. Ten replicates were prepared for each timber species and subjected to termite bioassays according to the no-choice test procedure of ASTM D3345-74 (ASTM, 1988).

Termites were collected from the field using wood board traps and then maintained in a termite cement tank for approximately 4-6 weeks. Cups measuring 10-cm diameter by 8-cm high were filled with 100 g of sterilized sand and 30 ml distilled water. The cups were left overnight to equilibrate to laboratory conditions before test initiation. Each type of timber was placed on the surface of the sand in a cup and then 1 g (approximate 400 termites; 320 workers and 80 soldiers) termites were added to constitute a replication. At the end of four weeks the timbers were removed, cleaned, dried around 24 hours and reweighed.

4. Data analysis

One-way ANOVA is used to examine differences in the percentage weight loss among wood species under the field tests, as well as the no-choice laboratory bioassay. A general linear model (univariate) was performed to determine significant differences in weight before and after exposure to termites, between and among wood species. Data collection was log transformed to test homoscedastic assumptions of normal distribution. When ANOVA revealed a statistically significance difference between wood species (P<0.05), a Turkey HSD test was performed for each wood species as a post-hoc comparison. All statistical analyses were performed using SPSS 20.0 software package for windows (SPSS Inc., Chicago, USA).

Results

1. Field tests

Significantly different wood weigh loss rates before and after termite attack under field tests was affected by tree species ($F_{12, 104}$ = 18.1, P< 0.001; Figure 1). A large difference in termite feeding rates were found in the following five wood species Durian, Bamboo, Mango tree, Rubber wood and Eucalyptus (P<0.05). Similarly, there was a difference in the percentage weight loss among wood species ($F_{12, 64}$ = 49.3, P< 0.001; Figure 2). Percent weight loss was significantly higher in the same five wood species, Durian (43.2 ± 8.3SE %), Bamboo (46.1 ± 9.8SE %), Mango tree (70.9 ± 7.1SE %), Rubber wood (39.5 ± 4.7SE %) and Eucalyptus (53.3 ± 9.1SE %), than the other species (P<0.05).

2. No-choice laboratory test

A significant difference in termite feeding rates under no-choice laboratory tests were affected by plant species ($F_{12, 234}$ = 73.2, P< 0.001; Figure 3). A large difference in termite feeding rates was found in one wood species, Mango tree (P<0.05). A difference in weight loss was also found among wood species ($F_{12, 64}$ = 49.3, P< 0.001; Figure 4), with weight loss significantly higher in two wood species, mango tree (11.8 ± 1.9SE %) and Rubber wood (10.2 ± 1.4SE %) (P<0.05).

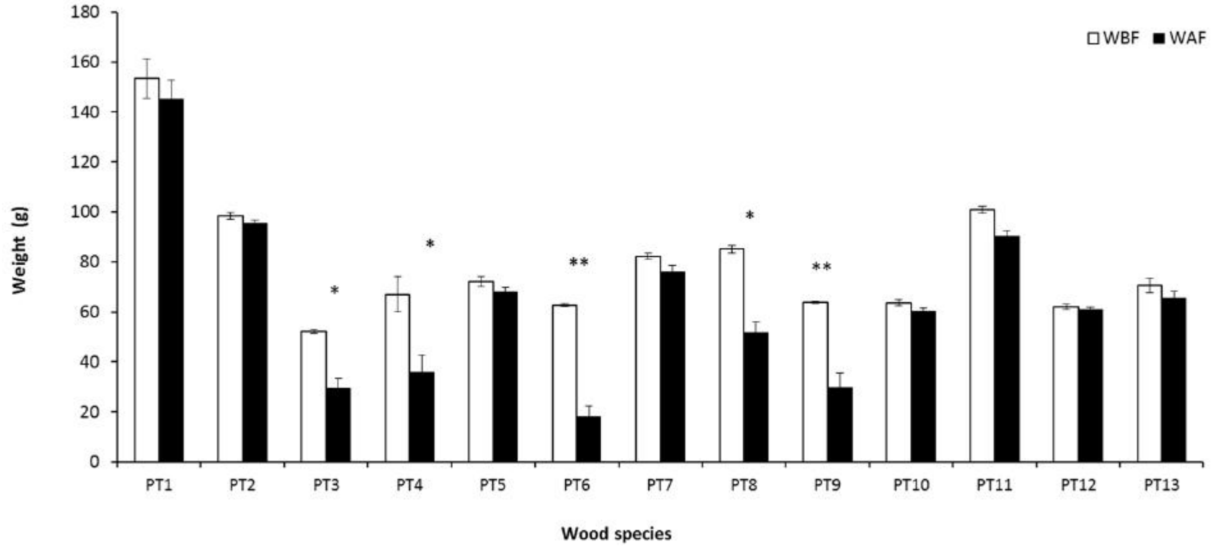

Figure 1 Comparison the wood weight before termite attack (WBF) and after termite attack (WAF) for each wood species from the field tests. Statistical differences between means are indicated by an asterisk (* = P<0.05 and ** = P<0.001). The wood species abbreviation are: PT1 = Iron wood, PT2 = Siamese Sal, PT3 = Durian, PT4 = Bamboo, PT5 = Indian Walnut, PT6 = Mango tree, PT7 = Keruing, PT8 = Rubber wood, PT9 = Eucalyptus, PT10 = Neem tree, PT11 = Casuarina , PT12 = Teak and PT13= Pine wood (commercial wood).

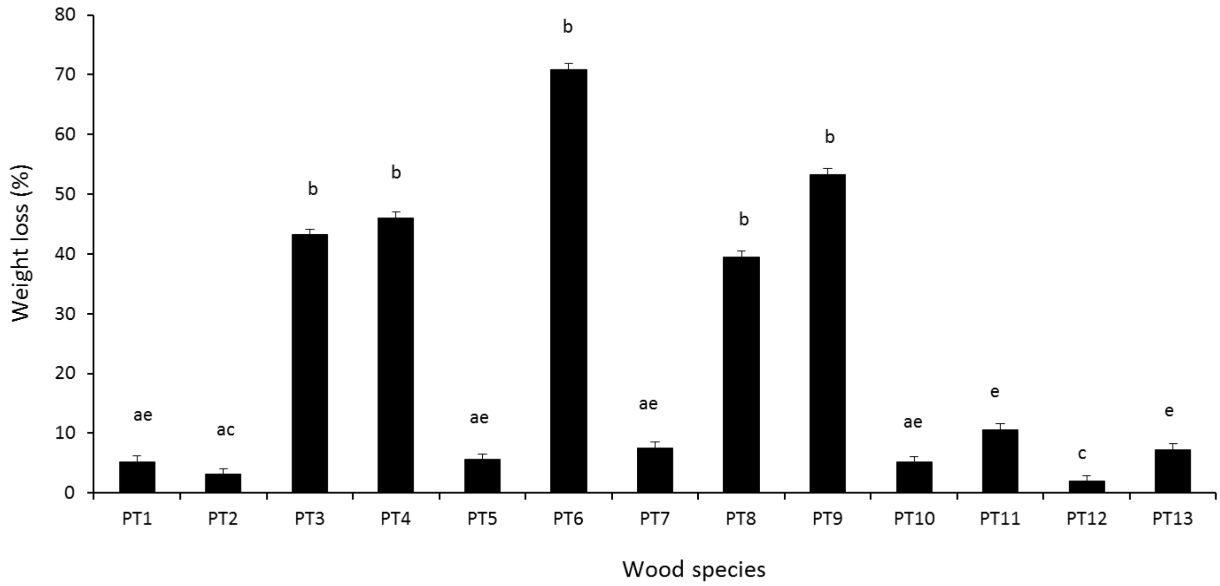

Figure 3 Change in percentage weigh loss in difference wood species from the field tests. Different lower case letters indicate a significant difference among wood species (P<0.05). The wood species abbreviations see in Figure 1.

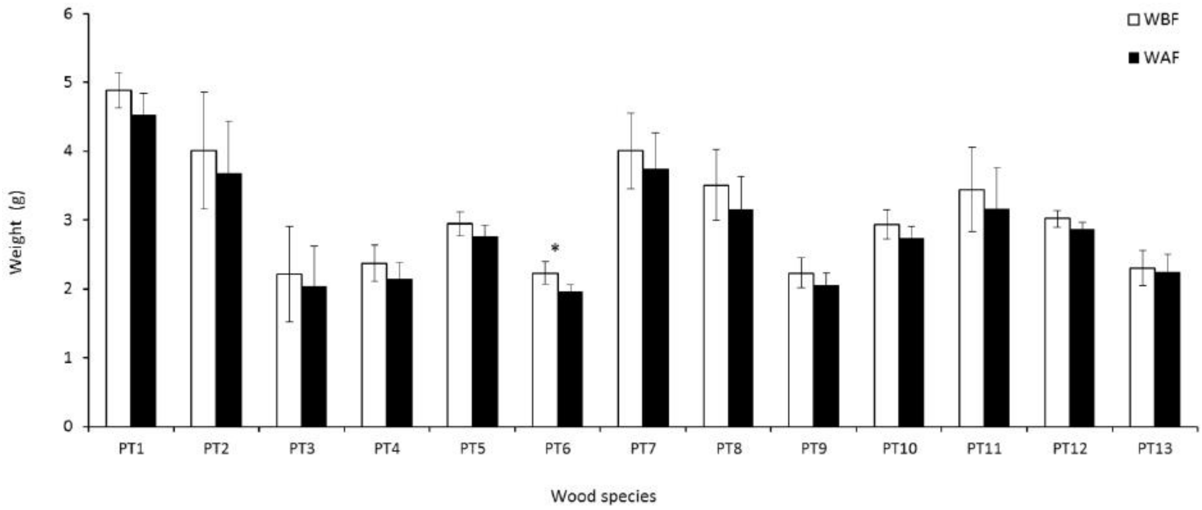

Figure 2 Comparison wood weight before termite attack (WBF) and after termite attack (WAF) of each wood species under no-choice laboratory tests. Statistical difference between means are indicated by an asterisk (* = $P<0.05$). The wood species abbreviations see in Figure 1.

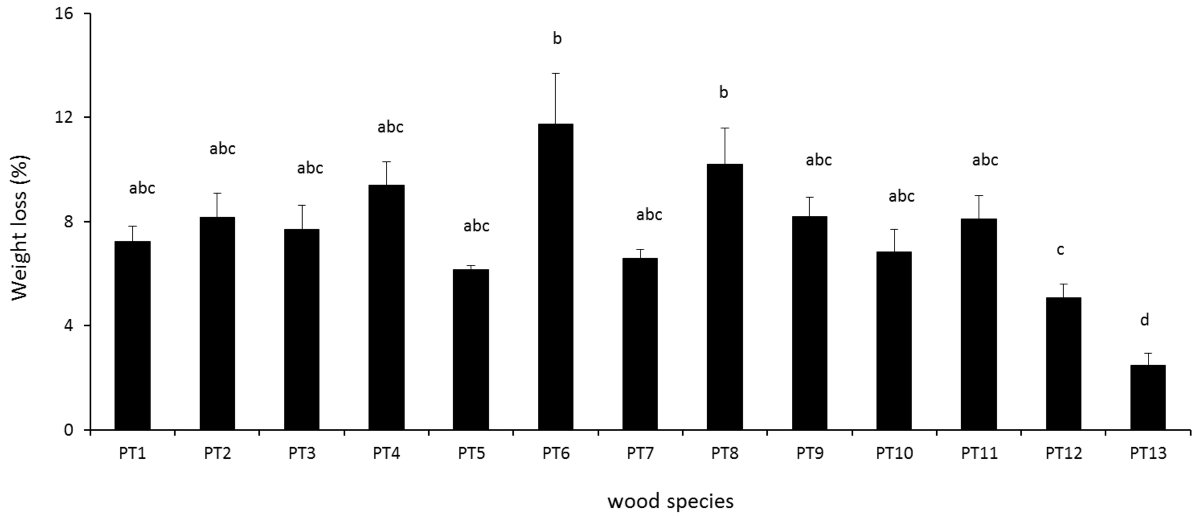

Figure 4 Change in percentage of weigh loss in difference wood species from no-choice laboratory tests. Different lower case letters indicate a significant difference among wood species (P<0.05). The wood species abbreviations see in Figure 1.

Discussion

The results presented herein show that the feeding rates of termites under field test conditions was greatest in five wood species, Durian, Bamboo, Mango tree, Rubber wood and Eucalyptus. Significant weight loss was also found in those wood species with respect to termite damage. Interestingly, termite feeding rates under no-choice laboratory bioassay was greatest in Mango tree, while significant weight loss was found in both Mango tree and Rubber wood.

All of those results indicated that Mango tree and Rubber wood might be an acceptable food for termites as indicated by a higher feeding rate and percentage wood weight loss in both field and no-choice laboratory tests. Thus, we would encourage that those wood species be used in wood-particle toxic baits for controlling termites.

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THE ROLE OF GUT MICROORGANISMS FROM THE SUBTERRANEAN TERMITE *Macrotermes gilvus* Hagen DURING COMPOSTING PROCESS

by

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Abstract

The role of termites in the degradation of woody components is not accomplished by the termites alone but in association with microorganisms inside the termites' gut. Microbes in the digestive tract have the potential to be utilized as a starter for composting. In this study, an observation on the composting process employing the microorganisms from the guts of termites was performed. The study was conducted by using a complete random block design. *Macrotermes gilvus* Hagen (worker caste) were collected and guts were taken by centrifugation. A starter culture was prepared by inoculating the supernatant into NB medium. Inoculant was incubated at the temperature of 30 °C for 4 days. The compost formulation involved solutions of 0%, 10%, 20%, 30%, 40%, and 50% from the microbial starter culture and 1-5 weeks of composting. Composting was done using a modification of the Takakura technique using perforated composting bins. Each treatment was conducted in triplicate. The total number of microorganisms cells/g of compost and compost profiles were determined. The results indicated the total number of microorganisms reached 92.67x 10^7 cells/g in a compost system that was coupled with the 30% starter culture at 5 weeks of composting. The maturity of compost was determined by the characteristics of black color, crumb texture, and soil-odor. The chemical profiles of compost were a C/N ratio of 11.77, pH 7.0 and moisture content 60.80%.

Keywords: microorganisms, termites' guts, *Macrotermes gilvus* Hagen, organic material degradation, compost

Introduction

Insects that live in woody environments have adjusted their physiological and biochemical pathways in a fascinating way that provides an efficient degradation of plant polymers, breakdown of lignocellulose, detoxification of plant secondary metabolites and enzyme inhibitors (Holt and Lepage, 2000). There are several orders of insects that have the ability to digest woody components, such as cellulose, lignocellulose and hemicellulose, i.e. Thysanura, Plecoptera, Orthoptera, Isoptera, Coleoptera, Trichoptera, Hymenoptera, Phasmida, Blattodea and Diptera (Scharf and Boucias 2010).

Termites (Blattodea: *Macrotermes gilvus* Hagen) are essential detrivores, feeding on a wide range of dead plant material at various stages of decomposition and are considered the best-known and most successful wood-degraders on earth. In termites a two-enzyme system plays an important role on the decomposition of plant polymers. Those systems are the endogenous insect enzymes and enzymes secreted by a variety of gut microorganisms, including protozoa and bacteria (Inward et al. 2007, Ni and Tokuda 2013). In fact, there are three stages involved in termite digestion of lignocellulosic materials. The first stage occurs in the insect guts as hydrolysis, followed by oxidation and/or fermentation, and finally acetogenesis and/or methanogenesis involving the participation of the Archaea (Watanabe and Tokuda 2010). The populations and diversity of microorganisms are influenced by characteristics of the termite gut, and immune system, including the gut pH, intestinal structures, and diet (Noirot and Darlington 2000).

In fact, the association of mutual symbiosis, i.e. termites together with the microorganisms in both their guts and the environment allow utilization of a wide array of food resources. Termites activities such as nest construction involves processes like carbon mineralization and nutrient recycling, especially in tropical areas (Noirot and Darlington 2010). On the other way, the nest of termites, i.e. mounds building oby *Macrotermes gilvus* Hagen provides suitable climatic conditions for the colony and its symbionts. It protects them from predators and may promote a strong influence on soil profile development. Through the activity of mound construction, soil translocation and soil repacking results in increased soil porosity and soil water content (Bignell & Eggleton, 2000). The capacity of termite gut microorganisms to produce enzymes for organic material degradation, support the ability of termites to translocate soil and highlights their potential for application in organic waste decomposition or composting.

In Indonesia, waste, especially organic waste is a growing problem magnified by the growth of Indonesians human population. Open dumping and burning of waste are traditional forms of waste management. Other constraints faced by the government are the lack of landfills, limited transportation capacity, and the duration of the composting process. The management of waste in this country has been improving with the help of innovative methods of composting. Commonly, a traditional composting process requires 3-4 months, whereas drum rotation, Takakura, and closure methods take only 1 month. There are biological agents, such as worms, bacteria, fungi and insects employed to produce organic fertilizer by the composting method (Simanungkalit et al. 2009). Some termites are able to modify the chemical properties of soil by secreting saliva and fecal material that enrich surface soils with nutrients useful to plants (Lisa & Conacher 2000). Therefore, it is important to explore the microorganisms in termite guts and their application in the composting process. In this study, the role of termite guts microorganisms used as a starter for composting, composting duration and compost profiles is described.

Materials and Methods

Collection of termites' guts

The termites used were *Macrotermes gilvus* Hagen, workers collected from Universitas Negeri Semarang. After collection in a clean bottle, termites were treated using 70% ethanol and washed using sterilized distilled water. Termite guts were taken aseptically using micro-tweezers and macro-tweezers were used to hold the termites. The guts were placed in sterilized microtubes.

Propagation of guts' microorganisms

A culture of microorganisms from the gut of *M. gilvus* was performed according to Tay et al (2010). The digestive tracts collected in microtubes were mixed with 1-ml of sterilized distilled water, and centrifuged at 1,000 rpm for 1 min to separate the supernatant from the debris. Subsequently, 0.3 ml aliquots of supernatant were suspended in NB medium and observed in NA medium. Finally, the microbial suspension was maintained at 30 °C for 4 days.

Preparation of microbial starter culture for stock solution and working solution

A starter culture preparation of the gut microorganisms was obtained by taking 1 ml of the aforementioned microbial suspension added to 9 ml NB medium. The mixture was incubated at 30 °C for 48 h and a working solution of the starter culture prepared by dilution. Various concentrations of 0%, 10%, 20%, 30%, 40% and 50% were obtained by mixing the stock solution with NB medium. The 0% dilution consisted of 10 ml NB medium that acted as a control.

Large scale composting

Large scale composting was conducted in a composting room using perforated bins in a modification of the Takakura method. The bin was lined with cardboard to prevent insect escape. All experiments were done in triplicate. Each of the working solutions of the microbial culture (0%, 10%, 20%, 30%, 40% and 50%) were dissolved in molasses and sterilized distilled water at the ratio of 1:1:50, respectively. Organic litter was chopped into the particle size ± 2-cm in diameter. The base-line part of each bin was lined with husk paddings to absorb the compost leachate. Over this padding 200 g compost mixture and 300 g chopped organic litter was spread. Compost and litterbins were then mixed with 2.6-l of the microbial solutions at the ratio of 1:1:50 molasses, microbial stock solution and water, respectively. The duration of composting was 1-5 weeks. The parameters temperature, pH, and moisture were measured every 2 days. Other subjective parameters were also measured including the smell, color, texture of the compost. Lastly, we measured the C/N ratio and the number of bacterial cells per 1 gram of compost. During the composting process, stirring was carried out once every two days. Furthermore, the top of the compost was covered with husk pillows, a black cloth, and a bucket lid (Nurulita and Budiyono, 2012; Ying and Ibrahim 2013).

Results and Discussion

Microbial numbers and compost maturity

Microbial growth as measured by cell numbers was variable depending on the concentration of the starter culture working solution and composting duration (Figure 1). The most abundant number of microorganisms was found in compost containing starter culture solution compared to the controls. The addition of higher concentrations of starter culture also increased the total number of microbial population in the compost.

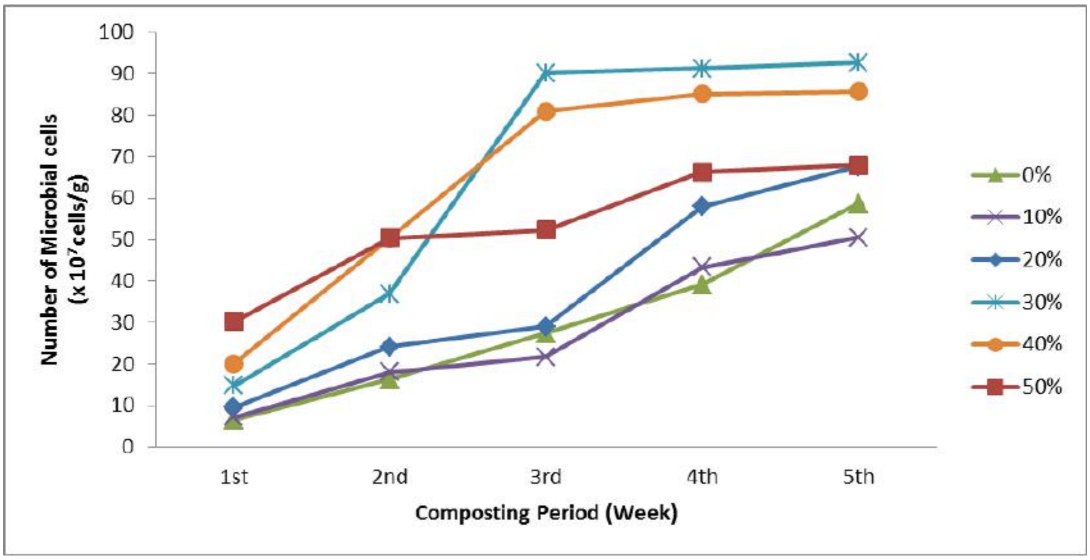

Figure 1. Number of microbial cells (x 10^7 cells/g) during composting were affected by the concentration of starter culture and composting period

An increased number of microbial cells was found at all starter culture concentrations during the 5 weeks of the composting process. A slow growth rate was found at the first and second week of the composting period. This phenomenon illustrated that the microorganisms were in an adaptation phase or lag phase where their metabolic pathways had adjusting to the environmental nutritive elements. After the second week of composting, the total number of microorganisms at 3rd, 4th, and 5th week showed significant growth. This stage represented the exponential growth or log phase where the mass and volume of the cells increased in accordance to nutrition and environment conditions. The speed of cell growth was up to 2-5 times higher than the initial cells number. At this phase, cells reproduced and the enzymes for lignocellulosic degradation process were secreted. In fact, the environmental conditions including temperature during this composting period averaged 32-34 °C. The exponential growth phase might also have occurred during days 6-15 depending on the availability of nutrients and environmental conditions (Firman et al. 2013)

According to the results, the optimum concentration of microbial starter culture concentration was 30%. This concentration gave the highest number of microbial populations in the compost, and it promoted faster organic material degradation to reach compost maturity. The concentration of microbial starter culture added to the compost and organic litter affected the compost maturity level. Organic litter coupled with microbial starter at 30%, 40% and 50% reached maturity at 3 weeks old; whereas the organic litter, at starter concentrations of 10% and 20%, reached maturity at 4 weeks. We conclude that a three-week composting duration is optimal for the composting process with the addition of 30% microbial starter from the gut of termites *M. gilvus* . The composting duration at 5 weeks showed the best composting results compared to the composting durations of less than 5 weeks. It can be concluded that based on our observations, the starter culture concentration and the duration of composting were in line with the maturity of compost. The higher starter concentration added to the process and the longer the duration resulted in faster compost maturity. In accordance with SNI (2004), the maturity of compost reached a maximum level when its color is black, the texture is crumb, and the smell is similar to soil. The scale of maximum maturity was assessed at the scale 4 in this study to quantitatively compare the quality of compost among treatments. Based on the assessment of compost quality and maturity, a four scale was found at compost treatment with 30% microbial starter culture solution.

Compost profiles

The quality of mature compost was reinforced with the results of compost profile assays that included the level of organic-C, N-total, P-total, C/N ratio, pH, and the moisture. The results of the compost profiles verified that the product qualified as mature compost (Table 1).

Table 1. Compost profiles obtained from compost using 30% microbial starter culture solution

Compost profiles	Value
Organic-C	48.87 ± 1.23 %
N-total	4.15 ± 0.11 %
P-total	4.21 ± 0.08 %
C/N ratio	11.77 ± 0.67
pH	7.0 ± 1.17
Moisture content	60.8 ± 1.81 %

The compost humidity reached 60.80%, higher than the standard of compost released by the Ministry of Agriculture, which states that the moisture content of compost should be between 15-25%. High moisture was observed due to the damp condition of the compost before treatment. Drying the compost is best accomplished in direct sunlight and it was difficult to conduct the drying process since this study was conducted during the monsoon period. Drying with oven or direct sunlight exposure could trigger the release of carbon in the organic materials. Some microorganisms that play an important role in the degradation of organic material are sensitive to heat and direct sunlight. However, a moisture content during the composting process of 60.80% is suitable for microorganisms according to Saithep et al. (2009)..

Compost maturity is determined by carbon content and our results showed that the carbon content was quite high, almost half of the compost by weight. In the process of composting, the degradation of organic compounds produces carbon dioxide released to the atmosphere. Thus, it would decrease the total carbon content gradually until it reached a static value, which is the indication of compost maturity (Hendri et al. 2009).

The nitrogen content is also an indicator of compost maturity. Based on the results obtained, the total nitrogen of our compost was in accordance with the standard level of NPK ratio, around 4%. A C/N ratio of compost that has a value less than 20 indicates the occurrence of mineralization on the organic compounds. The results showed that the C/N ratio was 11.77 ± 0.67. Microbes use the nitrogen available in compost for cell reproduction. An increased number of microorganisms would increase the decomposition activity of organic materials by decreasing the organic material reserves, one might improve the availability of N and other nutrients in the final compost. Besides that, evaporation of N could lead to lower N content and increased C/N ratio. Therefore, the composting system must be completed in a closed place to prevent the immobilization of nitrogen (Illiyin et al. 2012).

Testing of C/N ratio in this study was done from compost that was in the excellent maturity category. Compost at 5-weeks and the addition of 30% starter concentration showed the most excellent compost maturity. The longer the composting time, the degradation process of organic materials is optimized. Based on the observation (data not shown) a composting process of more than 5 weeks resulted in a decreased microbial population. A good quality compost is not only indicated by its physical form, but also the growth of microorganisms. At the moment when the microbial population decreases the degradation process has been stopped.

Microorganisms associated with composting were mainly bacteria, yeast, fungi, as well as protozoa. Microorganisms at the surface of the composting bin were confronted with fluctuating microclimatic conditions, and they may be exposed to stress in particular with regard to desiccation and ultraviolet radiation. Therefore, we performed frequentl stirring in order to overcome this problem (Nurulita and Budiyono 2012). Anaerobic and facultative anaerobic bacteria consume a portion of the acetate, and the other organic acids, that have been released by the microorganisms. However, anaerobic bacteria use oxygen diffusing into the bin for oxidation processes maintaining the anoxic status of the internal composting regions.

Conclusion

Microorganisms obtained from guts of *Macrotermes gilvus* Hagen play an important role in the composting process together with the microorganisms indigenous to compost and litter. The optimum composting process was obtained in 5 weeks of with the addition of 30% microbial starter culture. The optimum maturity level of compost was observed through the physical form, chemical and biological characteristics including organic-C, N-total, P-total, C/N ratio, pH, and moisture content.

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Field Evaluation of Hexaflumuron Bait for Colony Elimination of the Subterranean Termite *Coptotermes curvignathus* Holmgren in Oil Palm Plantation in West Kalimantan Indonesia

by
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Abstract

The number and size of oil palm plantations in Indonesia continues to grow. In West Kalimantan, most oil palm plantations are located in an area dominated by peat soils. This condition makes oil palm trees vulnerable to subterranean termite attack. *Coptotermes curvignathus* Holmgren has been identified as a major pest of oil palm. These termites attack the oil palm trunk, crown, fronds and fruit. Termite control in oil palm involves surface-spraying chemical pesticides, but this type of treatment proves uneconomical and hostile to the environment. In addition, surface sprays are not effective because the termite nest is below ground and the pesticide does not affect the termites inside the nest. Therefore, a new method such as baiting is needed to control termites in the plantation setting. The objective of this study was to evaluate the effectiveness of bait containing the active ingredient hexaflumuron to control *Coptotermes curvignathus* in an oil palm plantation. Three blocks of an oil palm plantation with heavy termite damage was chosen as the test site. Each block consisted of four oil palm trees and bait was placed on one tree within each block. Bait consumption and colony elimination was evaluated every seven days over four consecutive weeks. After colony elimination was determined, the site was evaluated every two weeks for an additional six months to evaluate re-attack of termites. Results showed that seven days after installation, the bait was consumed by termites and consumption remained high (average consumption of hexaflumuron bait from the third block of the test was 95.08%) from week one until week three. Consumption activity began to decline at the end of the fourth week. The decline in termite attack in the oil palm plantations was first observed at the third week where a high soldier to worker ratio was observed; termite population declined to less than 50 and termites shelter tubes were drying. Termites were not observed inside the oil palm fronds by the fourth week. After six months, there was no re-infestation from termites on the baited oil palm tree or the surrounding sample trees. It is concluded that Copton 0.5RB with 0.5% hexaflumuron is effective for colony suppression and elimination of *Coptotermes curvignathus* in oil palm plantations.

Key words: **baiting system, *Coptotermes curvignathus*, hexaflumuron, oil palm plantation, termites control**

Introduction

Subterranean termites are social insects that work together to sustain large populations. There are three castes in subterranean termites, i.e. worker, soldier and reproductive castes with the workers comprising the majority of a colony tasked with looking for food, sharing food with the colony, maintaining the nest, and taking care of the brood (Nandika et al 2015). Soldiers function to protect the colony and the castes responsible for reproduction. Subterranean termites are generally found in the soil and function as decomposers of organic matter in addition to increasing soil water infiltration and soil nutrients (Robert et al 2007).

Subterranean termites are one of the most economically important pests in buildings, plantations and forests. Lim and Silek (2001) stated that subterranean termites account for huge economic losses in oil palm plantations in Malaysia, especially those on peat soils. Diba (2015) found six species of termites which attack oil palm plantations in West Kalimantan, i.e. *Coptotermes curvignathus, Schedorhinotermes javanicus, Macrotermes gilvus, Odontotermes sp, Microtermes sp,* and *Nasutitermes sp*. On average, 75% of all infestations were caused by the termites *Coptotermes curvignathus*. Nandika (2014) reported *Coptotermes curvignathus* attacks oil palm plantations in Riau Province and caused an average economic loss of Rp 520.000/hectare. The cost for termite control is a big expense as shown by the annual cost of termite control in the United States ≈ $1.5 billion (Su 1991).

Traditional termite control in oil palm plantations consist of spraying termiticide on oil palm trees as a remedial approach. This method is very effective in controlling termites that contact the termiticide but much less effective to termites inside the trunk and below ground. Termites in the trunks of palm oil trees as well as the underground nest may attack other trees in the oil palm plantation. Termite baits are a new control technology utilizing chitin synthesis inhibitor (CSIs*)* active ingredients. These CSIs display a different mode of action providing an effective and environmentally friendly alternative. One of the active ingredients used in termite baits is hexaflumuron. Hexaflumuron was the first active ingredient registered with the US EPA as a reduced-risk pesticide and has been reported to successfully eliminate the Formosan and eastern subterranean termites in laboratory tests and field trials (Scheffrahn and Su 1991, Su and Scheffrahn 1993, Su 1994). Sajap et al (2000) reported hexaflumuron is effective against *Coptotermes curvignathus* in Malaysia. The objective of this research is to evaluate hexaflumuron in a baiting system for effectiveness against *Coptotermes curvignathus* in an oil palm plantation.

Materials and methods

Experimental site

The oil palm plantation was located in Purun District, Mempawah Regency, and West Kalimantan Province, Indonesia. The location was approximately 120 Km West of Pontianak City, Indonesia. The oil palm plantation was on peat soil with a total area of 2800 hectares. The peat ranged in depth from 0.75 m to 1.5 m with an average daily temperature of 26.5^{o}C and 85% relative humidity. The research site was located in blocks with very high termite attack (termites attacked more than 20 oil palm trees in one block). The blocks consisted of Block H 40, H 41 and H 42. Four oil palm trees were used in each block.

Termite Baiting System

Termite baits used in this study were Copton 0.5 RB termite bait with 0.5% hexaflumuron obtained from Dow AgroSciences, LLC. One 30 g Copton 0.5 RB hexaflumuron bait was placed inside an oil palm frond infested with termites and left for one month (Figure 1).

Figure 1. Installation of Copton 0.5 RB termite bait with 0.5% hexaflumuron bait inside the oil palm frond

Evaluations of bait consumption and termite elimination were conducted every seven days. At one month the bait was removed from the tree, cleaned, measured, and weighed. Termite baits were weighed to determine the percentage of weight loss based on the formula of Sornnuwat et al (1996):

$$\text{Bait weight loss (\%)} = \frac{(W1-W2)}{W1} \times 100\%$$

W1 = initial weight of bait
W2 = weight of bait after one month installation

Termite colony vigour was analyzed according to Garcia et al (2007). The total number of termites, including the ratio of workers to soldiers, was recorded. Termites were classified as abundant, moderately abundant, few, and none (Table 1), at each monitoring interval.

Table 1. Classification of *Coptotermes curvignathus* Holmgren numbers in baits

Population	Classification
0	None
1 to 20	Few
21 to 50	Moderately abundant
Over 50	Abundant

Source: Garcia et al 2007

After bait removal, we monitored oil palm trees for re-infestation by termites. Rubber wood (2 cm x 2 cm x 30 cm) monitoring devices were installed around oil palm trees and evaluated for termite attack for six months (Figure 2). The top of the wood was colored with red paint to aid monitor locating.

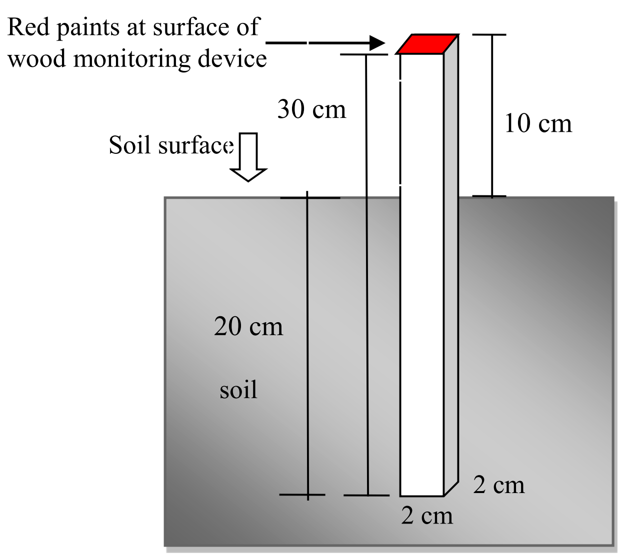

Figure 2. Installation of Wood Monitoring Device

Wood monitor stakes were installed vertically into the ground with 2/3 buried (20 cm). Each oil palm tree had four monitoring wood stakes; three within a 1.5 meters radius and one placed on the oil palm tree (Figure 3). Observations were made every two weeks for six months.

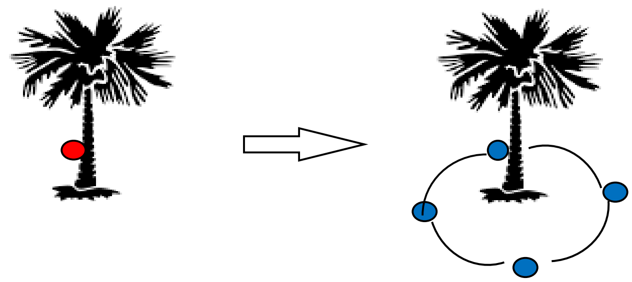

Figure 3. Schematic of monitoring device placement.

Termite bait - Hexaflumuron — Range ± 1.5 meter
Wood monitoring device — Oil palm tree

Coptotermes curvignathus Holmgren infestation was observed on the trunk, part of the fronds, the apical growth, and the fruit bunches of the palm trees. Hermawan et al (2014) states oil palm has high cellulose, with average holocellulose content of 82.534% ~ 88.328%, alpha cellulose content of 11.243% ~ 68.761%, and lignin content of 6.213% ~ 33.702%. The intensity of termite *C. curvignathus* attack on oil palm was categorized as heavy and very heavy. Heavy attack consisted of termite galleries on the trunk up to the apical growth but with green leaves and fronds. The category Very Heavy consisted of termite galleries on the trunk up to the apical growth with dry leaves and fronds. Cheng et al (2008) and Kirton et al (1999) reported that *C. curvignathus* is a pest of oil palm plantations in peat soil in Malaysia and Forest Plantation Area of *Acacia mangium* Willd.

The percentage of hexaflumuron baits infested with termites at the first observation (7 days after installation) was 83% (N=10) and 100% by the second observation (14 days after the installation). A large number of termite workers (greater than 50) were found in each bait. These observations indicate that Copton 0.5 RB termite bait with 0.5% hexaflumuron is not repellent to termites and acceptance rate was very fast. Diba (1999) revealed that hexaflumuron bait started to be consumed by termites 2 days after installation, and by the second week the entire bait was fully covered by soil and with greater than seventy percent consumed. The condition of hexaflumuron bait is presented in Figure 4.

Figure 4. Condition of Copton 0.5 RB termite bait with 0.5% hexaflumuron bait at 7 days after installation (a), 14 days after installation (b) and 21 days after installation (c)

One month after installation, average weight loss of Copton 0.5 RB termite bait with 0.5% hexaflumuron was 96.25% in Block H 40, 96.50% in Block H 41 and 92.50% in Block H 42. Average consumption across the three blocks was 95.08%. These results demonstrate that Copton 0.5 RB termite bait was readily accepted by termites. Hexaflumuron has a slow mode of action allowing termites to feed on the bait and spread the active ingredient throughout the colony before symptomology begins (Su et al 1995, Pawson and Gold 1996). These results are in agreement with Castillo et al (2013) who reported that termites choose the most appropriate type of cellulose containing food. Average consumption of Copton 0.5 RB bait by *C. curvignathus* for each block is presented in Figure 5.

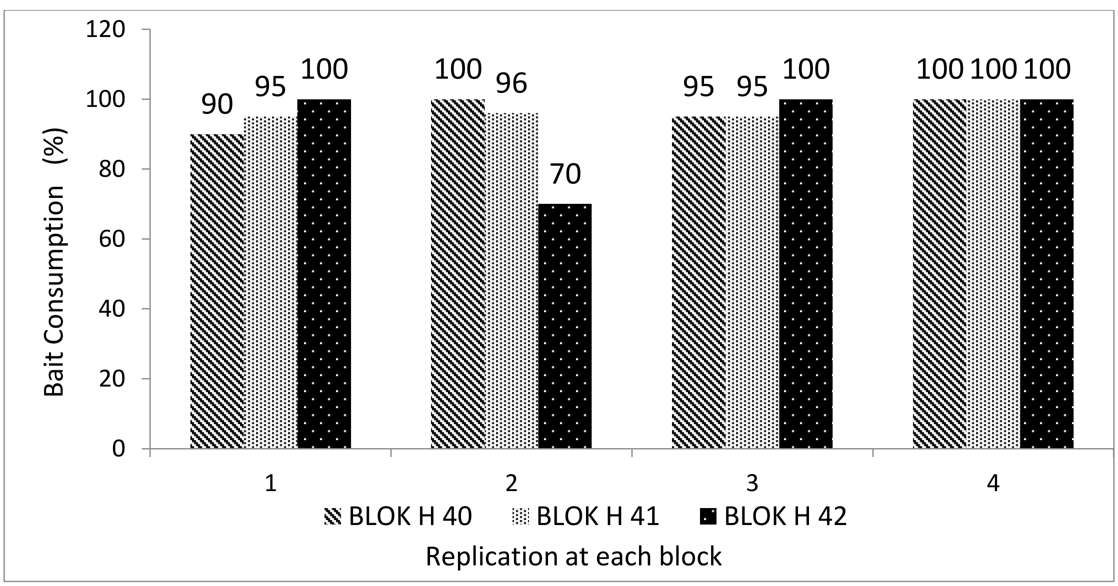

Figure 5. Copton 0.5 RB bait consumption by *Coptotermes curvignathus* in Oil Palm in West Kalimantan Province

The impact on termite colonies that consumed the bait increased from week one to week three as indicated by the higher soldier to worker (S/W) ratios**.** When greater than 50 termites were found in monitoring baits the population consisted mostly of workers. Populations found in baits of less than 50 had a higher proportion of soldiers. Consumption of bait began to decline by the end of the fourth week. It is suspected that by the end of the fourth week the hexaflumuron suppressed the termite colonies, as indicated by a reduced termite population, and a decline in termite attack on the oil palm trees. Evidence of dry soil mud tubes, and no termites found inside the oil palm fronds provided visual confirmation of reduced termite attack. The high soldiers to worker ratio are reported in Table 2.

Table 2. Estimation of termite population (P) and ratio of soldiers to workers (S/W) found at each oil palm tree during monitoring period

Oil Palm sample	Inspection period (days)									
	7		14		21		28		40 - 180	
	P	S/W	P	S/W	P	S/W	P	S/W	P	S/W
1	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
2	>50	S < W	<50	S > W	-	-	-	-	-	-
3	>50	S < W	<50	S > W	-	-	-	-	-	-
4	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
5	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
6	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
7	>50	S < W	>50	S < W	<50	S > W	<50	S > W	-	-
8	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
9	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
10	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-
11	>50	S < W	>50	S < W	<50	S > W	-	-	-	-
12	>50	S < W	>50	S < W	>50	S < W	<50	S > W	-	-

Colony eliminations were noted four weeks after installation of Copton 0.5 RB termite bait and all activity was successfully eliminated at 40 days (Fig. 6). No oil palm tree re-infestation was observed at180 days.

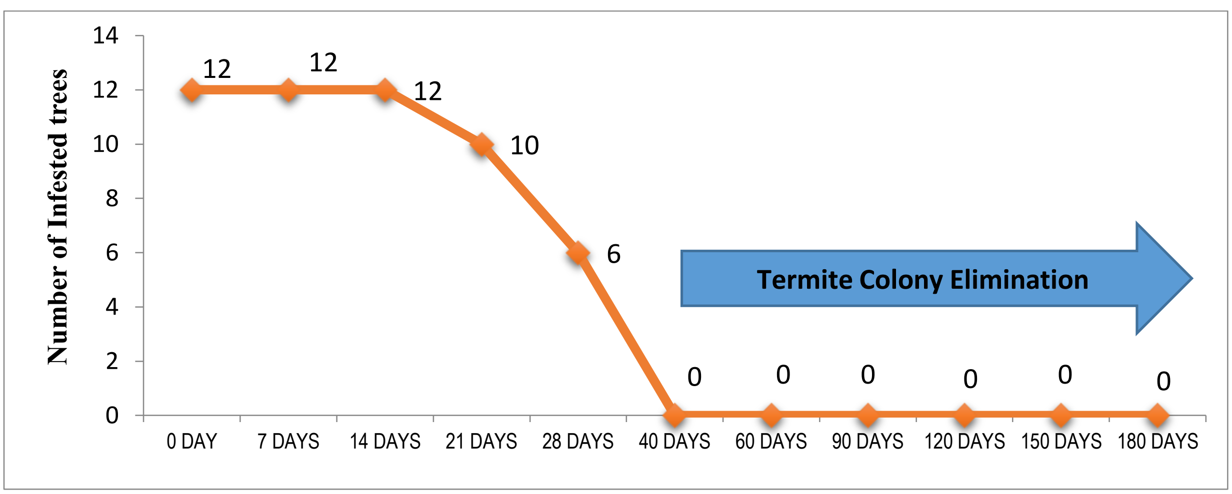

Figure 6. Colony suppression and elimination of *Coptotermes curvignathus* after consumption of Copton 0.5 RB termite bait in Oil Palm Plantation in West Kalimantan Province.

Termite re-infestation of oil palm trees was 0%, as shown by the absence of termite attack on the wood monitoring devices for six months. These results are in agreement with Sajap et al (2009) where a Preferred Textured Cellulose (PTC) matrix with 0.5% hexaflumuron was reported to eliminate *Coptotermes gestroi* and *Schedorhinotermes* sp colonies in oil palm plantations in Malaysia. Colony elimination occurred 42-77 days after bait installation with bait consumption between 22.93 to 167 grams. Nandika (2014) stated that the absence of re-infestation of oil palm trees by termites indicated that termites were eliminated.

C. curvignathus have been identified as the major pest in oil palm plantations. Immature oil palm trees are the most vulnerable to termite attack (Woei Kon et al 2012). Khoo et al. (1991) reported *C. curvignathus* also attack *Acacia mangium* and *Hevea brasiliensis* plantations. Current control methods for *C. curvignathus* in oil palm utilize liquid pesticide applications. Liquid pesticide application requires spraying and drenching the trunk, fronds, and leaves of oil palm. Treatment of the soil around infested trees is another control option to prevent termite attack. These treatments have proven to be non-economical because the termite nest is not directly treated leading to infestation of other oil palm trees. Therefore, new control strategies for termite control in plantations are needed. Termite baiting is an environmentally friendly method with reduced risk against non-target organisms. Copton 0.5 RB termite bait with hexaflumuron eliminated termite colonies in oil palm plantations as demonstrated by this research. Copton 0.5 RB utilizes the feeding behavior of termites to spread the active ingredient hexaflumuron throughout an entire colony. The natural behavior of food sharing, or trophalaxis, involves a relatively small number of termites in a colony feeding on a bait, and subsequent transfer of that toxic material to nest mates.Copton 0.5 RB termite bait has proven to eliminate *C. curvignathus* colonies in oil palm plantations.

Conclusions

The use of Copton 0.5 RB hexaflumuron termite bait is an effective and economical method to control *Coptotermes curvignathus* Holmgren in oil palm plantations. Copton 0.5 RB bait was tested and showed a high level of preference by *C. curvignathus.* Average bait consumption was 95.08% by one week and after three weeks we recorded high soldier to worker ratios. Termite activity was greatly reduced by four weeks indicating quick suppression of termite colonies. Copton 0.5 RB termite bait eliminated colonies of *Coptotermes curvignathus* Holmgren in oil palm plantations within 40 days.

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Comparison of Termite Species Composition and Diversity of 8 Forest Types in Tianmushan National Nature Reserve

By

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Abstract

Termites are ecosystem engineers in tropical and sub-tropical environments, and play an important role in soil processes, energy flow and matter cycling. To examine the differential between the 8 forest types in Tianmushan National Nature Reserve, species diversity and assemblage structure of termite were investigated using with active searching transects in different forest types in Tianmushan National Nature Reserve, including Cunninghamia lanceolata forest(SML), Pinus massoniana forest(SSL), mixed coniferous and broadleaf forest(HJL), evergreen broad-leaved forest(CLK), Moso bamboo forest (MZL), mixed evergreen and deciduous broad-leaved forest (CLH), deciduous broad-leaved forest(LKL) and deciduous broad-leaved shrub(LAL) in Tianmushan National Nature Reserve. The results shows that: 1) A total of 264 encounters from 2 families were found in the 24 transects, the dominant group was the wood-feeders of *Reticulitermes* in the family Rhinotermitidae；2）CLK hosted the highest numbers of species and encounters, while LAL, which attitude was between 1111—1156m, hosted lowest number of termites encounters, and only *Reticulitermes* were found in the transects. 3) *Ahmaditermes* were found only in MZL and CLK, which altitude was between 300-500m.Termite assemblages reflected the differences among the 8 forest types, and termites may be act as an indicator for different forest types.

Key words: Tianmushan National Nature Reserve, termite assemble, termites diversity

Introduction

Termites are abundant arthropods in tropical and subtropical ecosystems (Bignell and Eggleton, 2000). The consumption of dead plant matter makes termites important mediators of decomposition and nutrient cycling (Huang *et al.* 2000). By fragmenting litter, they create fresh surfaces for microbial colonization and increase the proportion of the litter mass that is accessible to microbial attack, accelerating the mineralization process (Chapin *et al.* 2002).The construction of mounds and their foraging activity in the soil profile have a strong impact on the structure and chemical composition of soils (Holt and Lepage, 2000; Schuurman, 2005). These functions are largely dependent on the species composition of the termite assemblage, and different feeding habits termites have as different ecological functions (Donowan *et al*. 2002).

Tianmushan National Nature Reserve is a mountain in Lin'an County 83.2 kilometers west of Hangzhou, Zhejiang, in eastern China. China's Tianmushan National Nature Reserve lies on the northwest portion of the mountain. It is a UNESCO Biosphere Reserve as part of UNESCO's Man and the Biosphere Program. The mountain has a lush sub-tropical climate with an annual rainfall of 1,767 millimeters (69.6 in) and an annual temperature of 17.3°C (63°F). And Tianmushan National Nature Reserve, whose elevation is more than 1,500 meters.

Along with the changes of altitude, heat and water conditions, Tianmushan National Nature Reserve has formed four obvious perpendicular band spectrum from the foothills to the top of the mountain, including 8 forest types (including Cunninghamia lanceolata forest(SML), Pinus massoniana forest(SSL), mixed coniferous and broadleaf forest(HJL), evergreen broad-leaved forest(CLK), Moso bamboo forest (MZL), mixed evergreen and deciduous broad-leaved forest (CLH), deciduous broad-leaved forest(LKL) and deciduous broad-leaved shrub(LAL)). The aim of this study was to compare termite species composition and diversity of 8 different forest types in Tianmushan National Nature Reserve.

Materials and Methods

Investigations were conducted in eight forest types of Tianmushan National Nature Reserve, including Cunninghamia lanceolata forest(SML), Pinus massoniana forest(SSL), mixed coniferous and broadleaf forest(HJL), evergreen broad-leaved forest(CLK), Moso bamboo forest (MZL), mixed evergreen and deciduous broad-leaved forest (CLH), deciduous broad-leaved forest(LKL) and deciduous broad-leaved shrub(LAL) in Tianmushan National Nature Reserve.

Termite assemblages were sampled using a protocol similar to the active searching transects (Jones and Eggleton, 2000). Each transect consisted of a 100 m x 2 m strip and was divided into ten sections. And each section was searched for termites for thirty person minutes. All termite species were recorded as well as the number of occurrences, and an occurrence was recorded when a population of termites of one species in one section of transect was samples.

All sampled termites were identified to species using soldier castes according to the Fauna Sinica (Huang *et al*., 2000), and confirmed at the Termite Collection of Guangdong entomological institute, China. Voucher specimens are lodged at Guangdong entomological institute, China. Differences in termite assemblage composition were compared between forest types. And, assemblage structures were analyzed using CLUSTER in Primer v. 6 (Clarke & Gorley 2006).

Result and Discussion

The basic information of 8 forest types in Tianmushan National Nature Reserve was seen in table1. 8 forest types, total 24 transects were mainly comprised of the pine tree, Camphor tree, maple, China fir, bamboo and Sycamore. The altitude of the 8 forest types was between 300-1200m.

Samples of termites were collected from soil and all possible collected habitats. From morphological identification, a total of 264 encounters, which belonged to 2 families, 6 genera, and 10 species were recorded (table 2). The dominant family in 8 forest types of Tianmushan National Nature Reserve was Rhinotermidae, and 5 species were identified by morphology (Fig. 1). *Reticulitermes* was a common group, and the genera *Reticulitermes* contained the most diverse species, with the proportion of 81.06%. And the second was *Odonotermes*, representing 10.98% of the total relative abundance (Fig. 1).

Table 1 The basic information of 8 forest types in Tianmushan National Nature Reserve

Forest types	Longitude and latitude of Mid-transect	Altitude (m)	Main trees	DBH of the big tree /Normal tree (cm)
SSL	30°19'35.83" 119°27'22.37"	337-514	pine tree	38/18
SML	30°18'40.96"; 119°26'38.37"	350-404	China fir	18/5
MZL	30°19'19.09"; 119°26'36.41"	426-479	Bamboo & China fir	57/12
HJL	30°18'41.79" 119°26'37.63"	327-341	pine tree, maple, Camphor tree	38/15
CLK	30°18'47.07" 119°26'44.53"	345-389	Cyclobalanopsis glauca, maple	150/18
CLH	30°20'2.70"; 119°26'10.88"	617-967	China fir, Daphniphyllum	98/12
LKL	30°20'45.42"; 119°26'4.76"	803-1010	China fir, Teak, IlexchinensisSims	110/55
LAL	30°20'23.91" 119°26'47.96"	1111-1156	Castanea seguinii Dode, Photinia	18/8

DBH: diameter at breast height

Table 2 Termite species results of 8 forest types in Tianmushan National Nature Reserve

Family	Genera	Species
Rhinotermitidae	*Reticulitermes*	*R. leptomandibularis* Xia *et* Fan,1965
		R. flaviceps Oshima, 1911
		R. fukinensis Light,1924
		R. labralis Hsia *et* Fan, 1965
		R. curvatus Xia *et* Fan, 1985
Termitidae	*Odontotermes*	*O. formosanus* (Shiraki),1909
	Sinocapritermes	*S. mushae* Oshima *et* Maki, 1919
	Pericapritermes	*P. nitobi* Shiraki,1909
	Ahmaditermes	*A. foveafrons* Gao,1988
		A. tianmuensis Gao,1988

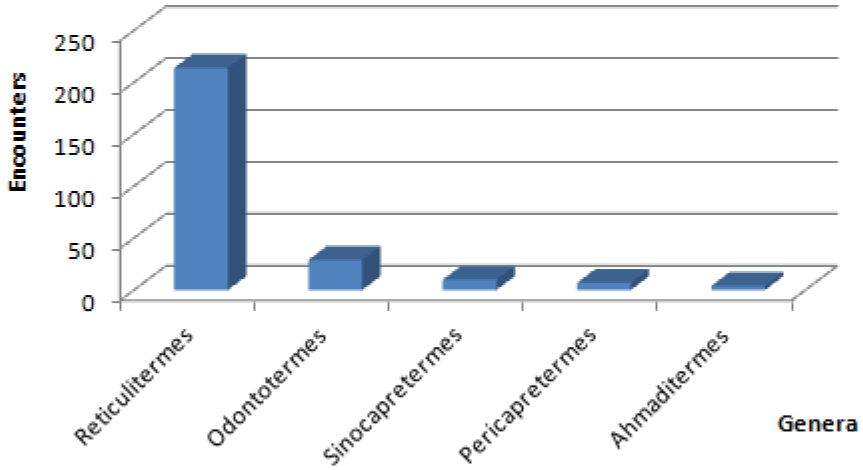

Fig.1 Sampled encounters from genera in 8 forest types.

The termite assemblages in the different forest types including 1) *Reticulitermes* was commonly seen in 8 forest types in Tianmushan National Nature Reserve, the encounters of *Reticulitermes* in LAL were lowest, only 9 encounters were seen in the three transects, while SML hosted the highest encounters of *Reticulitermes*, with 43 encounters in the three transects. 2) *Ahmaditermes* were only seen in MZL and CLK, which altitude was between 300-500m. 3) Both the number of species and relative abundance in CLK were the most, while LAL was composed of a single genus (*Reticulitermes*). SML, SSL and HJL have the Similar community composition (Fig.2).

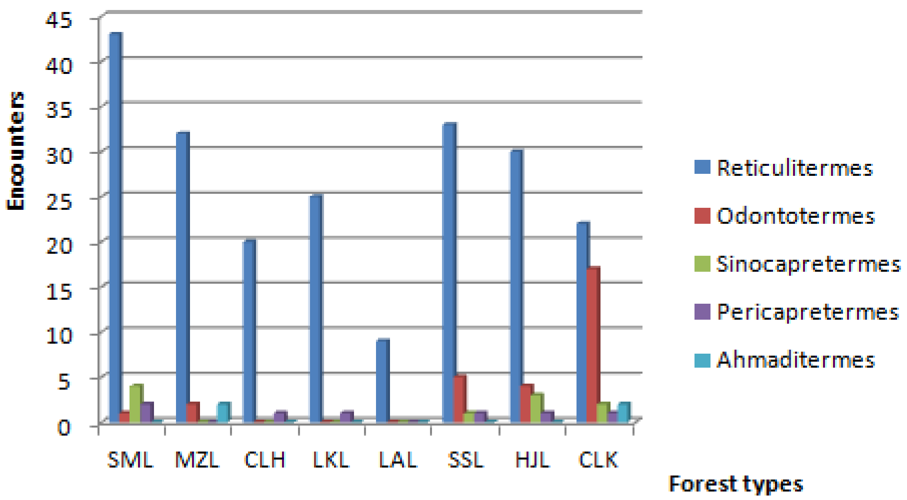

Fig.2 Termite species abundant of 8 forest types in Tianmushan National Nature Reserve

Similarity of termite assemblage structure was carried out in the species composition of 8 forest types. The results of the CLUSTER analyses showed that 1) The CLK hosted the highest numbers of species and encounters, and form a distinctly group. 2) LAL, CLH and LKL, hosted lowest numbers of termite encounters, form another group. 3) The rest 4 forest types, SSL, SML, MZL and HJL form the rest group (Fig.3).

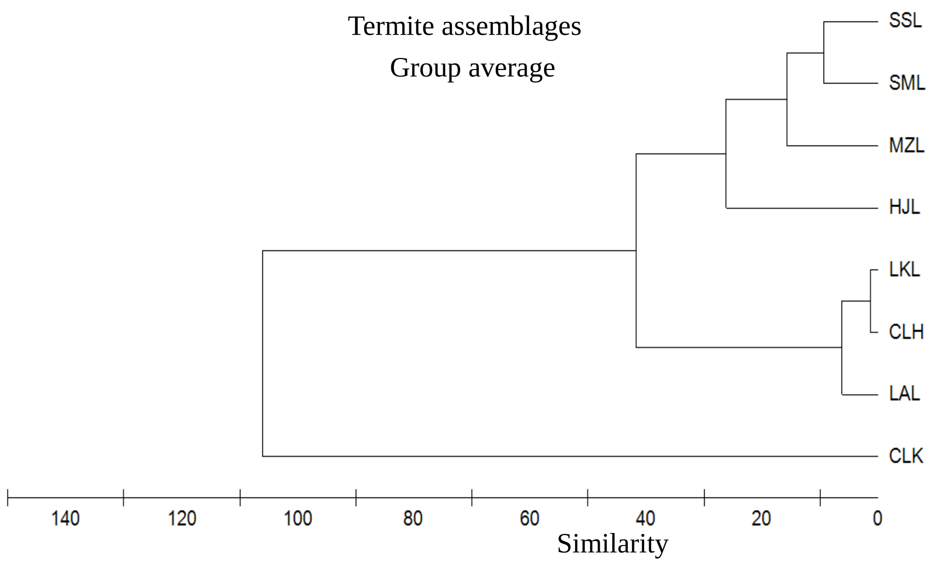

Fig.3 Dendrogram of termite diversity in 8 forests in Tianmushan National Nature Reserve

Conclusions

Termite fauna in Tianmushan National Nature Reserve was studied by several researchers (Gao *et al*. 1986,Yu *et al*. 1992, Xu *et al*. 2002), and 15 termite species were recorded previously. Three termite species, *Sinocapritermes tianmuensis*, *Mironasutitermes tianmuensis*, *Ahmaditermes tianmuensis*, were named of Tianmushan. Tianmushan National Nature Reserve was the type locality of four termite species, including *R. leptomandibularis*, *R.curvatus*, *R. flaviceps* and *O. formosanus*. In this study, ten species were recorded in Tianmushan National Nature Reserve. And so far, 15 of the 23 Hangzhouese termite species were found in the Reserve, Which indicates that Tianmushan National Nature Reserve has the highest termite diversity in Hangzhou.

Biodiversity is one of the important indexes of forest health, and the insect diversity is an important part of biodiversity (Wang *et al*. 2008). Termites play an important role in soil processes, energy flow and matter cycling as ecosystem engineers, and are major decomposers in tropical and subtropical regions (Sugimoto *et al*. 2000, Schuurman 2005). Different kinds of termite inhabit, feeding habits is different, so as lead to the community composition of different forest termites is different. Termite assemblages are considered as complex systems, which can respond to the differences of weather conditions and vegetation types（Bignell and Eggleton 2000, Dabues *et al*. 2003; Inoue *et al*. 2006）. In this study, 8 forest types in Tianmushan National Nature Reserve had also showed different termite assemblages. Especially, great changes of termite assemblages have taken place from CLK to LAL. CLK has the highest termite diversity while LAL has the lowest termite diversity in 8 forest types. Termite assemblages reflected the differences among the 8 forest types, and termites may be act as an indicator for different forest types.

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Soil-termite Control Practices Using Bait Toxicants in South China

By

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Abstract

Two chitin synthesis inhibitors, hexaflumuron and triflumuron, were evaluated in field trial as bait toxicants against the termites of *Odontotermes formosanus*(Shiraki), *Macrotermes barney*i Light and *Ancistrotermes dimorphus* Tsai et Chen. The eucalyptus-barks treated with the acetone solution containing 0.1 - 0.15% hexaflumuron or 0.1 - 0.15% triflumuron can significantly eliminate the active colonies of *Odontotermes formosanus*, *Macrotermes barney*i. The barks treated with the acetone solution containing 0.1% hexaflumuron can completely controlled the termites of *Odontotermes formosanus*, *Macrotermes barney*i in reservoir dams, *Odontotermes formosanus*, *Macrotermes barney*i and *Ancistrotermes dimorphus* in sugarcane fields.

Key words: *Odontotermes formosanus*, *Macrotermes barney*i, *Eucalyptus exserta*, hexaflumuron and triflumuron

Introduction

The family Termitidae is the largest and most diverse among Isoptera and some of the species in this family are used to be called soil-termite in China. The termites of *Odontotermes formosanus* （Shiraki）and *Macrotermes barney*i Light are responsible for greater economic losses, in aggregate, than all the other species in this family. This is not only due to the fact that they attack crops (such as sugarcane) and forest but also that they construct nests and tunnels inside the earth dams of rivers and reservoirs, sometimes cause water infiltration and even the collapse of dams (Zhong &Liu, 2001,). In southern China, more than 50% of the dams were suffered from termite infestation, especially in Fujian, Jiangxi, Guangdong, Guangxi, Yunnan, Hainan and other provinces, termite infestation rate reached more than 90% (Zhong & Chen, 1997).In sugarcane production area of Guangxi province， the dominant species were *Odontotermes formosanus* ,*Macrotermes barneyi* and *Ancistrotermes dimorphus*, by which more than 1 million hectares of sugarcane were affected(Fu et al., Unpublished).

Before the year 2000, the primary means of controlling the termites of *Odontotermes formosanus* and *Macrotermes barneyi* is the use of mirex baits, of which with 10-20 grams (0.1-0.2 g a.i. mirex) is able to eliminating the colony of soil-termite. Following the banning of Mirex which can cause effects on the nervous system and the liver, and also caused cancer in laboratory animals (Fujimori et al., 1982; Ebel, 1987), there is an urgent need to find the alternatives to mirex for controlling the termites of *Odontotermes formosanus*, *Macrotermes barneyi* and other soil-termite .

One strategy that is frequently used for subterranean termite management is baiting systems which the chitin synthesis inhibitors is employed as the active ingredient (Su and Scheffrahn, 1998). Since 2002, The effects of various concentrations of baits containing one of two chitin synthesis inhibitors(CSIs)--hexaflumuron or triflumuron were investigated against the colony of *Odontotermes formosanus*. In addition, the trials of baits for controlling the termites of reservoir dams and sugarcane fields were made.

Materials and Methods

Trial 1. CSIs bark-baits against termite colony

The dried and weighed barks of *Eucalyptus exserta* in 5 x 20 CM were soaked in CSIs-Acetone solution which containing 0.0, 0.1%, 0.15%, 0.25% of hexaflumuron or triflumuron for 20 minutes and weighed. When acetone in barks is completely volatized, 10 barks were placed in the soil near the swarming holes of the active colony of *Odontotermes formosanus* or *Macrotermes barneyi* . Check the barks once a week until termites disappear. The barks were collected 3-month later and then were re-dried and re-weighed, and the amount of bait remainder were recorded. Results were analyzed using Analysis of Variance (ANOVA) at $P < 0.05$.

Table 1. Attractiveness of barks plus acetone solution of CSIs concentrations to termites of *Odontotermes formosanus* in a single colony (Trial 1), 2002.

Compound		Colony	Treated barks consumed by termites(g) — Treated bark number										Mean*
			1	2	3	4	5	6	7	8	9	10	
blank		1	12.5	14.2	13.6	12.8	13.3	15.2	11	12.9	10.5	15.3	13.44 a
		2	13.5	12	13.5	15.1	14.1	12.3	15.1	13.9	11.8	13.5	
		3	14.3	11.9	14.6	13.7	13.9	14.8	12.8	14.6	12.7	13.9	
Hexaflumuron	0.10%	1	11.3	10.6	137	12	8.4	12.1	10.3	9.8	11.4	11.3	10.70 b
		2	10.3	7.9	12.1	10.6	13.1	10.3	9.8	10.6	9.1	10.3	
		3	12.3	9.4	7.9	12.3	12	11.5	10.6	11.9	10.7	12.3	
	0.15%	1	6.2	7.3	6.5	7.8	5.9	7.3	4.2	7.6	5.2	6.2	6.00 c
		2	5.4	5.2	6.7	5.3	6.1	4.9	6.5	5.4	6.3	5.4	
		3	6.1	4.7	5.9	5.8	4.6	7.1	5.2	6.8	6.1	6.1	
	0.25%	1	3.5	2.3	0	4.2	0	0	0	3.5	0	3.5	1.07 e
		2	0	0	3.1	2.2	0	0	1.9	2.5	0	0	
		3	1.2	2.5	0	0	1.6	2.4	0	0	1.3	1.2	
triflumuron	0.10%	1	10.5	7.2	9.5	9.5	10.2	11.1	13.3	11.3	10.1	9.3	10.01 b
		2	9.2	13.1	7.2	11.3	8.9	10.2	9.6	10.7	9.5	10	
		3	7.2	11	10.8	12.2	9.1	9.2	12.1	8.5	11.3	9.5	
	0.15%	1	5	6.3	5.4	4.1	5.8	6.5	0	5.3	6.5	3.6	5.40 d
		2	6.2	5.9	4.9	5.8	7.2	4.3	3.9	0	4.3	4.4	
		3	6.9	6.2	5.3	4.6	5.3	6.1	5.2	6.2	4.8	5.1	
	0.25%	1	2.5	0	0	0	0	2.6	2.1	2.3	0	0	1.01 e
		2	0	4.1	2.1	0	0	0	0	1.3	4.1	0	
		3	2.1	0	2.3	3.1	0	0	0	0	0	1.7	

* Means in column followed by the same letter are not significantly different using Analysis of Variance (ANOVA) at $P < 0.05$ ($P = 0.0$).

Trial 2. Hexaflumuron-bark-baits controlling reservoir termites in Guangdong and Fujian

The field trials were conducted using the Eucalyptus barks treated with Acetone solution which containing 0.1% of hexaflumuron. Since 2003, a total of seven reservoirs in Guangdong and Fujian province were selected as demonstration bases for termite colony eliminating trial (Table 3.). The treated barks were buried at intervals of 3 x 3 m in earth dam. For the convenience of future inspection, the bark of 3/4 were buried in the reservoir dam and the rest was left on the surface of the soil. Check the barks and the activities of termites once every month, If the bark is found to be eaten by termites, the new bark is added.

Trial 3. Hexaflumuron bark-baits controlling sugarcane termites in Guangxi

Since 2013, More than 200 hectares of sugar cane were selected as demonstration bases for termite colony eliminating trials in sugarcane production area of Guangxi province (Table 3.). The treated barks were buried at intervals of 2 x 2 m in sugarcane field in spring. In the sugarcane harvest season, check the sugarcane cut which whether to be damaged by termites or not and calculate the damaged rate by termites and the sugarcane yield of the trial fields.

Results and Discussion

Trial 1. CSIs bark-baits against termite colony

The blank numbered compound was much preferred by termite in field colonies of *Odontotermes formosanus* (Table 1.). Numerically, the lowest concentration (0.1%) was more attractive to termites than the higher concentrations. The differences in weight of bark-bait consumption were not significantly different between 0.1% hexaflumuron and 0.1% triflumuron, but were significantly different between 0.15% hexaflumuron and 0.15% triflumuron.

CSIs bark-baits can significantly eliminate the active colonies of *Odontotermes formosanus* beginning 3 months after application, and from that date the Hexaflumuron performed statistically similar to the triflumuron with the 0.1% and 0.15% ((Table 2.).

Trial 2. Hexaflumuron-bark-baits controlling reservoir termites in Guangdong and Fujian

Trials of seven reservoirs shows that barks treated with 0.1% Hexaflumuron can completely controlled the termites in reservoir dams, whether it is *Odontotermes formosanus* or *Macrotermes barneyi*. In Xikeng reservoir, we also found 30 sites of the fungus of *Xylaria polymorpha* which often growing from the dead fungus-growing termite colonies such as *Odontotermes formosanus*, *Macrotermes barneyi* or *Ancistrotermes dimorphus*. The control effect of the trials will be maintained for more than 3 years(Table 3).

Trial 3. Hexaflumuron bark-baits controlling sugarcane termites in Guangxi

Trials of 6 sugarcane plots show that barks treated with 0.1% hexaflumuron can completely controlled the termites in sugarcane fields in Guangxi, whether it is *Odontotermes formosanus* or *Macrotermes barneyi*, or *Ancistrotermes dimorphus*. The control effect will be maintained for more than 1 years and the termite damage rate of sugarcane cut are below 5%, and. Increase yield per ha is 6.75 to 15.75 ton (Table 3).

Table 2. Status of the colonies of *Odontotermes formosanus* following Consuming CSIs bark-baits (A=active colony,N=no termites found) ,2002-2003

Compound		Colony	Consumed by Termites(g)		Status of the trial colonies			
			Barks	A.I.	1-month	3-month	6-month	1-year
blank		1	131.3	0	A	A	A	A
		2	134.8	0	A	A	A	A
		3	137.2	0	A	A	A	A
Hexaflumuron	0.10%	1	110.9	0.1109	A	N	N	N
		2	104.1	0.1041	A	N	N	N
		3	110.9	0.1109	A	N	N	N
	0.15%	1	64.2	0.0963	A	N	N	N
		2	57.2	0.0858	A	N	N	N
		3	58.4	0.0876	A	N	N	N
	0.25%	1	17	0.0425	A	A	N	N
		2	9.7	0.0243	A	A	A	A
		3	10.2	0.0255	A	A	A	A
triflumuron	0.10%	1	102	0.1020	A	N	N	N
		2	90.5	0.0905	A	N	N	N
		3	91.4	0.0914	A	N	N	N
	0.15%	1	48.5	0.0728	A	N	N	N
		2	46.9	0.0704	A	N	N	N
		3	55.7	0.0836	A	N	N	N
	0.25%	1	9.5	0.0238	A	A	A	A
		2	11.6	0.0290	A	A	A	A
		3	9.2	0.0238	A	A	A	A

Table 3. Demonstration trials of controlling reservoir termites in Guangdong and Fujian,2003-2013

Reservoir*（volume，m^3）	Status of active termite	Trial date	Number of bark-bait			Status of active termite following 1-3 year**
			1st	2nd	3rd	
1（1300）	everywhere visible	2003.10.23	1400	84	0	N
2（1100）	everywhere visible	2006.11.9	3000	184	0	N
3（350）	everywhere visible	2006.11.11	3000	124	0	N
4（1905）	everywhere visible	2010.10.19	3000	636	122	N
5（3850）	everywhere visible	2010.10.21	5400	894	450	N
6（169）	everywhere visible	2010.10.24	2200	186	64	N
7（127）	everywhere visible	2010.10.25	1800	206	74	N

* 1-Magang Reservoir，Y angjiang，Guangdong；2-Xikeng Reservoir，Enping，Guangdong；3-Naji Reservoir，Enping，Guangdong；4-Chisha Reservoir，Haifeng，Guangdong；5-Yahu Reservoir，Zhaoan，Fujian；6-Dingliao Reservoir，Zhaoan，Fujian；7- Guguang Reservoir，Zhaoan，Fujian。

** N-No active termites and tunnel found.

Conclusions

Our studies show that the chitin synthesis inhibitors including hexaflumuron and triflumuron etc. can be completely used as an alternative to Mirex for controlling the termites of the family Termitidae. For the colony eliminating, it will take much longer time then Mirex, but it is completely safe and harmless to humans. Compared to the residential property owners who are not satisfied with too slow to control the house termites , the period of controlling the termite in reservoir dam or sugarcane field can also be accepted.

Using Eucalyptus barks as baits, the application efficacy in autumn is better than in spring, due to that termite foraging activities will be affected by frequent rain in spring.

Longer duration of efficacy of controlling termite can be obtained by using high density of bait buried, because the high density bait buried can not only eliminate the old termite colonies, but also eliminate the young colonies.

Table 3. Demonstration trials of controlling sugarcane termites in Guangdong and Fujian,2011-2015

Trial plot	Bark	Number of sugarcane cut damaged by termites Every 300 sugarcanes				Yield per *ha* (ton)	Increase yield per *ha*(ton)
		1	2	3	Mean(%)		
1	treated	5	9	7	2.77a	82.95	7.8
	untreated	28	35	29	10.15b	75.15	
2	treated	10	11	6	2.97a	91.5	14.85
	untreated	32	34	29	10.27b	76.65	
3	treated	11	8	12	3.76a	76.5	9
	untreated	60	69	54	21.32b	67.5	
4	treated	11	13	11	4.06a	73.5	6.75
	untreated	49	56	50	18.56b	66.75	
5	treated	10	15	13	4.43a	78	7.5
	untreated	54	50	57	17.89b	70.5	
6	treated	12	11	7	3.43a	76.5	15.75
	untreated	77	79	72	25.83b	60.75	

* Means in column (same plot) followed by the same letter are not significantly different using Analysis of Variance (ANOVA) at $P < 0.05$ ($P = 0.0$).

The barks of *Eucalyptus exserta* as baits for termite control are not easy to get mouldy and termites more like to eat than other natural materials, but in recent years, the number of *Eucalyptus exserta* is gradually decreasing. Looking for alternatives to *Eucalyptus exserta* is one of our research contents. Recently we have found a cheap alternative which have being used in Guangxi sugarcane fields to *Eucalyptus exserta*.

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Comparative Analysis of Microbial Community Structure and Composition in Sugarcane Harmful Termites by Illumina 16s RNA Sequencing

by

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Abstract

Guang xi province is the dominated sugarcane planting and sugar-producing areas of China. And the termite infestation is a serious factor in decline of sugarcane production and quality. Diet was an important community structuring factor for termite symbiotic community. In this study, we characterized the variation of symbiotic bacterial community by comparatively analyzing sugarcane harmful termites with the termites of same species living on pine, using amplicon libraries of 16s rRNA genes from12 nests of higher termites and lower termites. The analyses result suggested that: a) the diet high in sugar effectively re-shaped the bacteria community structure of *Coptotermes formosanus* and *Odontotermes formosanus*; b) family *Porphyromonadaceae* (genus *Candidatus Azobacteroides* and *Dysgonomonas*), genus *Treponema* and family *Enterobacteriaceae* are most likely to be the biomarker of glucose metabolism regulation in gut of termite.

Key words: bacteria community, *Coptotermes formosanus,* Illumina 16s RNA sequencing,

Odontotermes formosanus

Introduction

Termites are eusocial insect that have specialized on a diet of lignocellulose (Brune 2014). For lower termites, the symbiotic flagellates are considered the mainly decomposer of ligocellulose. And numerous prokaryotic microbes either attach to the surface of or live within the protozoan cell, which were play important role in acetate related energy circulation and nutrition factors synthesizing for lower termites (Brune and Ohkuma, 2011; Brune 2015). But for higher termites, gut microbiota is entirely prokaryotic who play critical role in digestion. Recent studies suggest that both host phylogeny and diet can be important determinants of bacterial community structure in termite guts (Dietrich et al. 2014; Otani et al.2014; Rahman et al. 2015).

Guang xi province is the dominated sugarcane planting and sugar-producing areas of China. And the termite infestation had been a serious factor in decline of sugarcane production and quality. Our previous survey of sugarcane harmful termites in Guang xi found that *Odontotermes formosanus* is the most distributed and damaged termite specie in sugarcane field. It is said that the high oligosaccharide diet would lead to metabolic feedback inhibition (Josef, 2008) and reduction in lignocellulases activity of *Coptotermes formosanus* (Zeng et al, 2011). How the bacteria community of termites living on sugarcane got over the metabolic feedback and adapted to living on high sugar plant? In this study, we comparatively analyzed representatives of sugarcane harmful termites with the termites living on pine to identify the candidate biomarkers of bacterial community in the hindgut, using amplicon libraries of 16s rRNA genes from 12 nests of *Odontotermes formosanus* and *Coptotermes formosanus*.

Materials and methods

Termites: collection, identification and DNA extraction Termite workers of *O. formosanus* and *C. formosanus* from 12 colonies from were sampled in the sugarcane filed(Beihai, china) and pine tree(Guangzhou, China). DNA of abdomen (25 per sample) were extracted and purified by using TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0 (Tianjin, China).

Pyrotag sequencing and data analysis The V4 region of the 16S rRNA gene was amplified from each sample. Purified PCR products were mixed and sequenced commercially (Illumina HiSeq). Only reads with a minimum length of 200 bp were selected and separated by sample using the sample-specific barcodes included in the sequences. After removal of barcodes and primers, the reads from each sample were clustered at a threshold of 97% sequence similarity to form operational taxonomic units (OTUs) using UPARSE. For the analysis of taxonomic composition of each sample, the OTUs were classified using the RDP classifier and GreenGene reference database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). Rarefaction curves and the Shannon, Chao-1, Simpson, ACE, goods coverage, Rarefaction Curve and Rank Abundance curve were calculated to evaluate the completeness and sequencing depth of the sampling effort. PCA, PCoA, Anosim, MRPP of the gut communities were calculated to described the structure difference between groups. OTU cluster analyses were performed on all quality-filtered and classified reads using QIIME (version 1.8.0). T-test, MetaStat and LEfSe were using to characterize the bacteria pool that were most represent and mostly affected differences in community structure between termites.

Results

Bacterial diversity and richness of sugarcane and pine tree termites

A total of 890,820 taxon tag were obtained for all samples and clustered into operational taxonomic units [OTUs] (Table 1) at 3 % genetic distance. Bacterial diversity and richness for the selected sequences from each sample (Table 1) was evaluated by rarefaction as shown in Fig1. Rarefaction curves and rank abundance curve were close to reach saturation. And the good-coverage of each sample was more than 99% which also indicated the sequencing depth is enough for this study. Results of alpha diversity (Shannon, simpson, chao1 and ACE) suggested greater microbiome diversity in *O. formosanus*. Both *O. formosanus* and *C. formosanus* sampled from sugarcane showed slight greater alpha diversity.

Table 1 Number of sequences, observed OTUs, the estimated richness and alpha diversity

Group	Sample name	TaxonTag	OTUs	Shannon	Simpson	chao1	ACE	Goods coverage
1	OfGX1	68855	1183	7.666	0.985	1393.076	1363.223	0.994
	OfGX3	108752	1062	7.647	0.988	1148.006	1178.002	0.996
	OfGX2	54315	1193	7.664	0.984	1344.622	1347.359	0.994
3	CfGX1	47731	859	4.469	0.72	1152.708	1157.045	0.993
	CfGX2	74745	977	5.188	0.77	1144.654	1167.077	0.994
	CfGX3	88448	769	4.553	0.738	1003.745	979.832	0.994
2	OfGZ1	105344	1118	8.051	0.992	1201.877	1218.116	0.996
	OfGZ2	84275	1038	7.185	0.979	1267.814	1249.874	0.994
	OfGZ3	91380	1128	7.988	0.991	1259.75	1252.144	0.995
4	CfGZ1	52464	740	3.397	0.596	868.727	879.557	0.995
	CfGZ2	34608	818	3.436	0.604	1417.28	1214.002	0.992

	CfGZ3	79903	618	3.101	0.587	744.23	786.872	0.995

OfGX, *Odontotermes formosanus* sampled from sugarcane field. OfGZ, *Odontotermes formosanus* sampled from pine tree; CfGX, *Odontotermes formosanus* sampled from sugarcane field; CfGZ, *Odontotermes formosanus* sampled from pine tree.

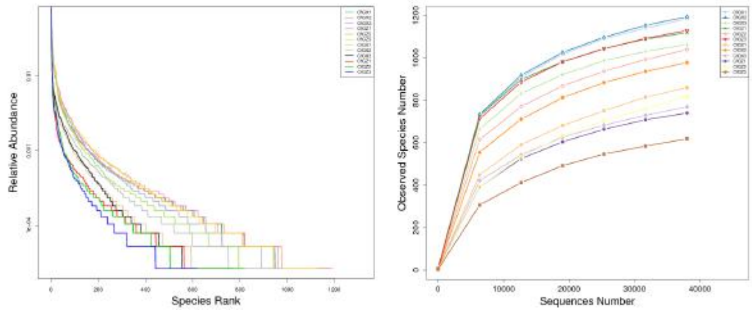

Fig.1 Rank abundance curve (A) and rarefaction curve and (B). OfGX, *Odontotermes formosanus* sampled from sugarcane field. OfGZ, *Odontotermes formosanus* sampled from pine tree; CfGX, *Odontotermes formosanus* sampled from sugarcane field; CfGZ, *Odontotermes formosanus* sampled from pine tree.

Bacteria community divergence between sugarcane and pine tree termites

Comparison between any pair of bacterial communities using Weighted Unifrac PCA and Weighted Unifrac PCoA exhibited a distinct clustering by diet environment and species (Fig. 2). For O. *formosanus* and *C. formosanus*, the statistic analysis of grouping distance (Anosim and MRPP**)** showed significant different between sugarcane and pine tree termites groups(Table 2). Heatmap of hierarchical clustering in phylum and genus levels also suggested the community structures were changed by the diet both in *O. formosanus* and *C. formosanus* (Fig. 3).

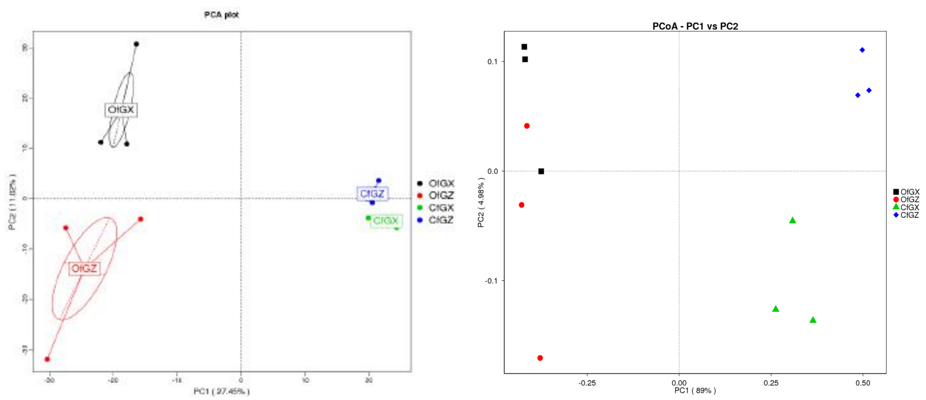

Fig. 2 Weighted Unifrac PCA (A) and Weighted Unifrac PCoA (B) visualizing bacterial community similarities across termite species. OfGX, *Odontotermes formosanus* sampled from sugarcane field. OfGZ, *Odontotermes formosanus* sampled from pine tree; CfGX, *Odontotermes formosanus* sampled from sugarcane field; CfGZ, *Odontotermes formosanus* sampled from pine tree.

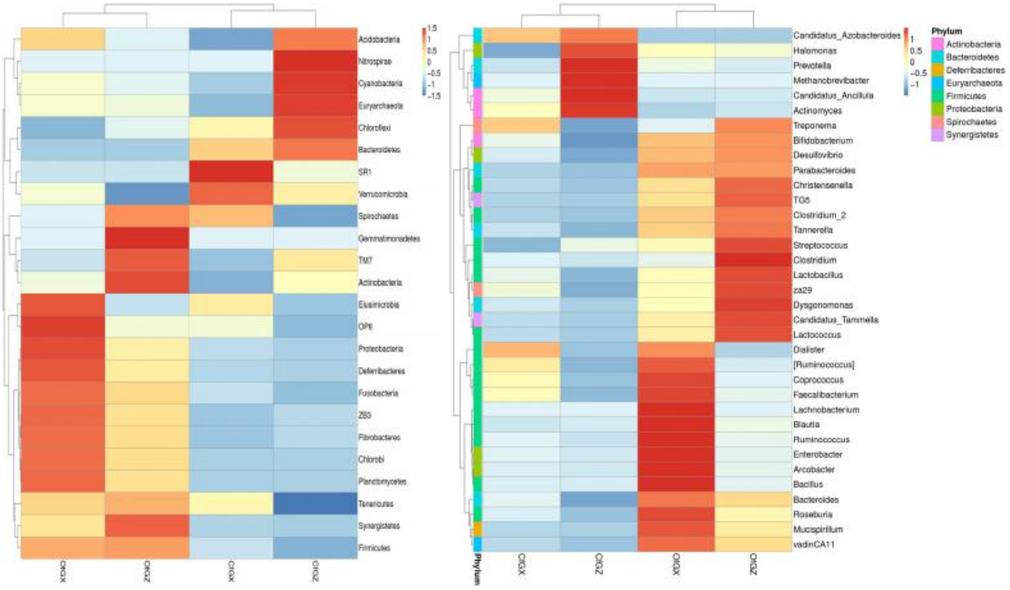

Fig. 3 Heatmap shows hierarchical clustering in phylum (A) and genus (B) levels (Relative abundances ranked in top 35). The scale bar represents color saturation gradient based on the relative abundances. OfGX, *Odontotermes formosanus* sampled from sugarcane field. OfGZ, *Odontotermes formosanus* sampled from pine tree; CfGX, *Odontotermes formosanus* sampled from sugarcane field; CfGZ, *Odontotermes formosanus* sampled from pine tree.

Table 2 statistic analysis of grouping distance

Statistic method	Group	R-value		
Anosim	CfGZ-CfGX	1.00		
	OfGZ-OfGX	0.41		
	Group	A	observed-delta	expected-delta
MRPP	OfGX-OfGZ	0.07	0.38	0.41
	CfGX-CfGZ	0.33	0.15	0.23

R-value among(1,-1)，R-value >0 indicates significant different between group. R-value<0 indicates difference of inter-group is greater than difference between group. The smaller observe delta is, the inter-group different smaller will be; the larger expected delta is, the different between group greater will be. A>0 indicates difference of inter-group is smaller than difference between group.

Candidate bacterial pool of glucose metabolism regulation in termite

The statistic method included T-test ($p<0.05$), Metastat ($p<0.05$) and LEfSe (LDA score>4) had been used to screening the biomarker which significantly influenced the difference between bacteria community of groups. In addition to this, relative abundances of bacterial groups (at phylum, class, order, family and genus levels) in the samples were used to evaluate the biomarker either. We set a threshold to eliminate the bacteria groups 1) relative abundance < 1%, 2) relative abundance difference between sugarcane and pine tree termites groups too small. Family *Porphyromonadaceae* (genus *Candidatus Azobacteroides* and *Dysgonomonas*), genus *Treponema* and family *Enterobacteriaceae* are most likely to be the biomarker of glucose metabolism regulation in gut of termite (fig.4).

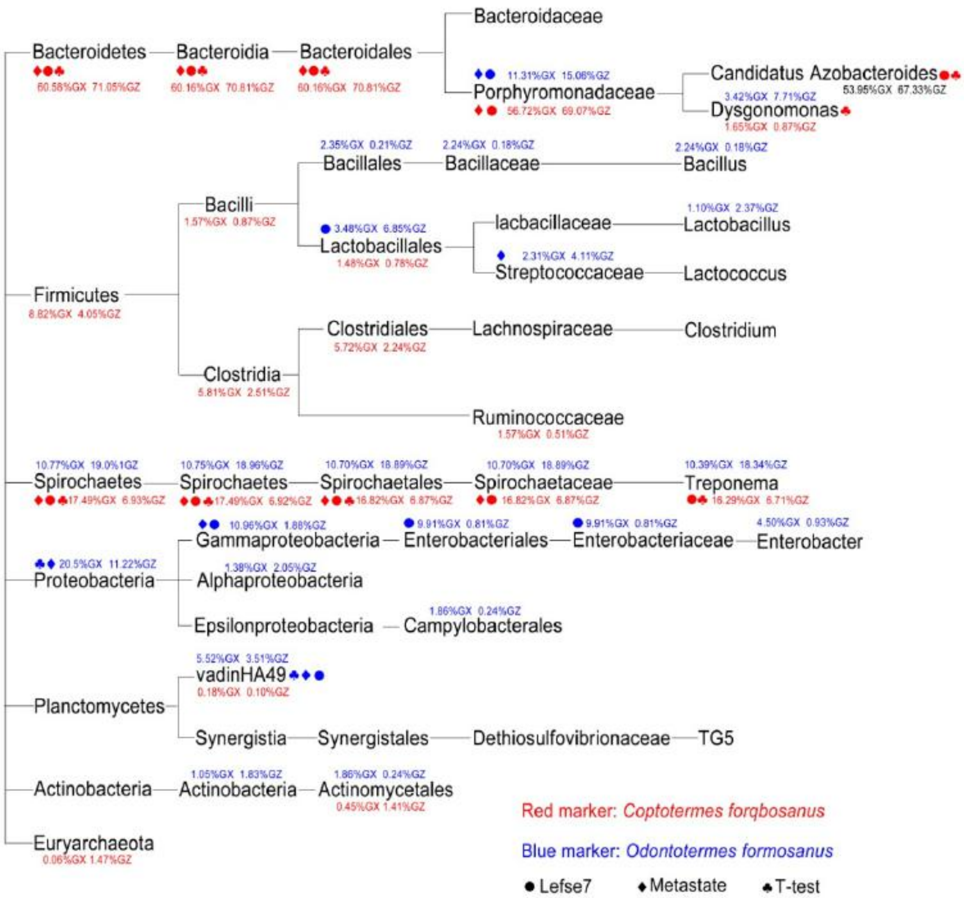

Fig. 3 Schematic diagram of candidate bacterial pool for glucose metabolism regulation in termite. The scale bar represents color saturation gradient based on the relative abundances. GX, sampled from sugarcane field. GZ, sampled from pine tree.

Conclusion

Both host phylogeny and diet can be important determinants of bacterial community structure in termite guts. In this study, we characterize the variation of symbiotic bacterial community by comparatively analyzing sugarcane harmful termites with the termites of same species living on pine, using amplicon libraries of 16s rRNA genes from 12 nests of higher termites and lower termites. The analyses result suggested that: a) the diet high in sugar effectively re-shaped the bacteria community structure of *Coptotermes formosanus* and *Odontotermes formosanus*. Both *O. formosanus* and *C. formosanus* sampled from sugarcane showed slight greater in community diversity; b) family *Porphyromonadaceae* (genus *Candidatus Azobacteroides* and *Dysgonomonas*), genus *Treponema* and family *Enterobacteriaceae* are most likely to be the biomarker of glucose metabolism regulation in gut of termite.

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Experimental verification and molecular basis of active immunization against fungal pathogens in termites

By

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Abstract

Termites are constantly exposed to many pathogens when they nest and forage in the field, so they employ various immune strategies to defend against pathogenic infections. Here, we demonstrate that the subterranean termite *Reticulitermes chinensis* employs active immunization to defend against the entomopathogen *Metarhizium anisopliae.* Our results showed that allogrooming frequency increased significantly between fungus-treated termites and their nestmates. Through active social contact, previously healthy nestmates only received small numbers of conidia from fungus-treated individuals. These nestmates experienced low-level fungal infections, resulting in low mortality and apparently improved antifungal defences. Moreover, infected nestmates promoted the activity of two antioxidant enzymes (SOD and CAT) and upregulated the expression of three immune genes (*phenoloxidase*, *transferrin*, and *termicin*). We found 20 differentially expressed proteins associated with active immunization in *R. chinensis* through iTRAQ proteomics, including 12 stress response proteins, six immune signalling proteins, and two immune effector molecules. Subsequently, two significantly upregulated (60S ribosomal protein L23 and isocitrate dehydrogenase) and three significantly downregulated (glutathione *S*-transferase d1, cuticle protein 19, and ubiquitin conjugating enzyme) candidate immune proteins were validated by MRM assays. These findings suggest that active immunization in termites may be regulated by different immune proteins.

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The combination technology of magnetic solid-phase extraction and high performance liquid chromatography and the application in the detection of termiticides residues in aqueous solutions

by

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ABSTRACT: Fe_3O_4 nanoparticles were prepared by solvothermal method with $FeCl_3$ as the iron resource. Carbon coated Fe_3O_4 nanocomposites (Fe_3O_4/C) were prepared by hydrothermal reaction with the glucose as the carbon resource. The nanoparticles were characterized by transmission electron microscopy (TEM), X-ray diffraction spectra (XRD), Fourier transforminfrared spectra (FT-IR), and thermogravimetry mass-differential scanning calorimetry spectrometry (TG-DSC). The nanomaterial was then applied in the magnetic solid-phase extraction of termiticides in water such as chlorpyrifos, deltamethrin and bifenthrin. Various MSPE parameters were optimized including amount of Fe_3O_4/C nanoparticles, pH of sample solution, adsorption time, elution solvent and reusability. Good recoveries (82.9–106.2%) were achieved with the relative standard deviations range from 2.1–9.6% with the optimal conditions. It is confirmed that the combination of Fe_3O_4/C nanocomposites and the high performance liquid chromatography can be used in the determination of termiticides in water.

Keywords: Magnetic solid-phase extraction; Carbon coated Fe_3O_4 nanoparticles; Pyrethroid; Organophosphorus pesticide; High performance liquid chromatography

磁固相萃取与高效液相色谱联用测定水体中防治白蚁农药残留

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[摘要] 以 $FeCl_3$ 为铁源，用溶剂热法合成了磁性 Fe_3O_4 纳米粒子，再以葡萄糖为碳源，水热法制备了 Fe_3O_4/C 纳米复合粒子。通过透射电镜（TEM）、X-射线衍射（XRD）、红外光谱（FT-IR）和差热-热重（TG-DSC）等分析手段对材料进行了表征。将材料应用于水体中防治白蚁药物毒死蜱、溴氰菊酯、联苯菊酯的磁固相萃取，考察了材料用量、pH、萃取时间、洗脱溶剂、重复性等条件对回收率的影响，在优化的实验条件下，将该方法应用于实际水样的测定，样品的平均加标回收率在 82.9-106.2%，相对标准偏差为 2.1-9.6%。实验表明，Fe_3O_4/C 纳米复合粒子与高效液相色谱联用的方法可以测定水体中防治白蚁药物残留。

[关键词] 磁固相萃取；碳包四氧化三铁纳米粒子；拟除虫菊酯；有机磷农药；高效液相色谱

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1 引言

白蚁是世界五大害虫之一，它会蛀食木材，腐蚀钢筋，对城市房屋、水库堤坝、农林作物等的危害尤为严重。我国目前防治白蚁普遍采用化学土壤屏障法，使用的药物主要为有机磷、拟除虫菊酯等[1-2]。有机磷类农药多为磷酸酯或硫代磷酸酯，种类多、药效高，在防治植物病、虫、害等方面应用十分广泛。对人畜危害轻者视力模糊、头痛、晕眩，严重患者可因昏迷和呼吸衰竭而死亡。拟除虫菊酯类药物是模拟天然除虫菊酯的化学结构衍生的合成酯类，其强烈的触杀及胃毒作用可有效地防治多种虫害，应用日益普遍。但其会通过影响神经轴突的传导而导致肌肉痉挛，长时间皮肤吸收，口服可引起中毒，严重的会导致呼吸衰竭[3]。因此，选择灵敏度高，准确度好的分析方法，对环境水体中的有机磷及拟除虫菊酯类药物残留量进行检测具有十分重要的意义。

目前用于检测有机磷及拟除虫菊酯类药物的方法主要有气相色谱法[4-5]（GC）、高效液相色谱法[6]（HPLC）、液相色谱-质谱联用法[7]（HPLC -MS）等。随着现代科学技术的发展，复杂基质中（如废水、土壤等）分析样品的种类和数量在增加，各类低浓度的分析样品对灵敏、快速测定的需求也在增加[8]。但在对这些痕量组分进行分析时测定时，往往会因仪器灵敏度不够或基质干扰造成分析困难[9]。因此样品前处理方法的选用非常重要，会影响最终结果的准确性和可靠性[10-12]。固相萃取法（Solid-phase Extraction，SPE）是目前应用最广泛的固态和水溶性样品中分析物的分离、净化和浓缩技术之一。样品与 SPE 柱的吸附剂相互作用被保留，其后使用少量有机溶剂洗脱即可，其回收率与富集倍数较传统的液液萃取有所提高，同时因分离迅速、操作简便等特点，在分析化学中得到了广泛的应用[13-14]。以磁性或可磁化的材料作为吸附剂基质的一种分散固相萃取技术称为磁固相萃取技术（Magnetic Solid-phase Extraction，MSPE）。近年来的研究主要集中于新型磁性吸附材料的合成与应用[15-16]，如磁性基体上包覆碳材料包括活性碳[17]、C60[18]、多壁碳纳米管（MWCNTs）[19-20]、石墨烯[21-22]，有机高分子材料[23-25]；金属有机框架材料[26]等。Razmi 等[27]以 Fe_3O_4 和葡萄糖为原料，采用水热合成法制备了石墨化碳包覆 Fe_3O_4/C 磁性纳米粒子，用来萃取水样中的有机磷农药；Wang 等[28]通过化学共沉淀法合成了磁性石墨烯复合材料 G-Fe_3O_4 并将其应用于水体中氨基甲酸酯类药物的萃取；胡斌课题组[22]制备了苯乙烯和甲基丙烯酸共聚物改性的磁性微球 Fe_3O_4/P(St-co-MAA), 并将其作为磁固相萃取吸附剂萃取果汁中的有机磷农药。相对于其他萃取技术而言，MSPE 有如下优点：原材料制备简单，同时具有良好的超顺磁性；吸附和萃取时间短，分离和收集过程不易产生二次污染；表面易于修饰，相比普适性的萃取吸附剂，其选择性强；仅使用少量有机溶剂即可完成低浓度的微量萃取，是一种环境友好的前处理方法[29-30]。

目前 Fe_3O_4/C 磁性纳米粒子在拟除虫菊酯类药物方面的研究尚未报导，本课题主要研究 Fe_3O_4/C 纳米粒子对水体中防治白蚁药物毒死蜱、溴氰菊酯、联苯菊酯的磁固相萃取过程，并与 HPLC 联用对其进行分析。通过电镜（TEM）、X-射线衍射（XRD）、红外光谱（FT-IR）和差热-热重（TG-DSC）等分析手段对材料进行了理化性质的表征，考察了材料用量、pH、萃取时间、洗脱溶剂、重复性等条件对回收率的影响，建立了 MSPE-HPLC 联用技术分析环境水样中防治白蚁药物残留的方法。

2 实验部分

2.1 实验材料和试剂

三氯化铁（$FeCl_3•6H_2O$，AR）、醋酸铵（CH_3COONH_4，AR）、醋酸（CH_3COOH，AR）、乙二醇（$C_2H_6O_2$，AR）、葡萄糖（$C_6H_{12}O_6•H_2O$，AR）购于国药集团化学试剂有限公司，毒死蜱（Chlorpyrifos，CP，99.0%）、溴氰菊酯（deltamethrin，DM，97.4%）、联苯菊酯（Bifenthrin，BF，97.3%）购于上海市农药研究所；色谱级乙腈购于美国天地公司；实验用水为娃哈哈纯净水（杭州娃哈哈有限公司）。所有水样于 2015 年 11 月采集于徐州市，雨水、自来水采自徐州市区，湖水取自徐州市云龙湖。所有水样随机取样并用 0.45 μm 微孔滤膜过滤除去悬浮物，在 24 小时内完成分析。

2.2 仪器

纳米材料形貌观察和微观结构表征采用 JEM-200CX 透射电子显微镜（TEM，日本 JEOL 公司，加速电压 200 kV）；X 射线粉末衍射图在岛津 XRD-6000 粉末衍射仪上测得（XRD，日本岛津）；傅里叶变换红外光谱图在 Tensor 27 型红外光谱仪（FT-IR，德国布鲁克）上压片法测得；用 STA449 F3 热重分析仪对材料进行热重-差热分析（德国耐驰），氮气气氛，升温速率 10 °C·min^{-1}，最高温度为 1000 °C。防治白蚁药物的定量分析在安捷伦 1100 型高效液相色谱仪（HPLC，美国安捷伦公司）上进行。

2.3 Fe_3O_4/C 纳米颗粒的制备

材料按文献[22,31]的方法稍作改进制得。将 1.5 g$FeCl_3·6H_2O$ 和 3.3 g 无水乙酸钠加入到 40 g 乙二醇中，超声和磁力搅拌交替进行 30 min 后，放入聚四氟乙烯内胆中，198 ◦C 水热反应 6 h，产物自然冷却后，用纯净水和无水乙醇清洗多次，干燥得 Fe_3O_4 磁性纳米粒子。将制得的 Fe_3O_4 分散于 0.5 mol·L^{-1} 葡萄糖溶液中，交替超声和搅拌 30 min，转移至聚四氟乙烯内胆中，180 ˚C 水热反应 5 h，产物自然冷却后，用纯净水和无水乙醇清洗多次，干燥得 Fe_3O_4/C 磁性纳米材料。

2.4 磁固相萃取实验

称取 30 mg Fe_3O_4/C 磁性纳米粒子到小烧杯中，加入 100 mL 待测水样，超声使纳米粒子均匀分散。10 min 后，用钕铁硼磁铁至于烧杯底部，磁性材料和溶液很快分离，弃去上清液，使磁性材料在空气中干燥后，加入 2 mL 乙腈到烧杯中，超声 2 min 后，再次磁分离，取上清液过滤，测定滤液中分析物的浓度。在 MSPE 条件优化实验中，以 100 mL0.04 mg·L^{-1} 分析物水溶液代替 100 mL 水样。

2.5 HPLC 条件

分析物的定量分析采用 HPLC 法，反相 C18 色谱柱（XB-C18 型，250 mm × 4.6 μm，5 μm，美国热电），流动相为乙腈:水 ＝80:20（V/V），流速为 1.0 mL·min^{-1}，进样量为 20 μL，柱温 30 ºC，采用二极管阵列（DAD）检测器，检测波长为 230 nm。

3 结果与讨论

3.1 磁性纳米粒子的表征

3.1.1 TEM

图 3.1 是 Fe_3O_4 和 Fe_3O_4/C 的 TEM 图，由图可见，两种纳米材料均呈近似球形，粒径分布均匀。Fe_3O_4 平均粒径约粒径为 100-120 nm，Fe_3O_4/C 的平均粒径约为 150-200 nm。Fe_3O_4 外层浅色的包裹层为碳层，说明 Fe_3O_4/C 是核壳结构。

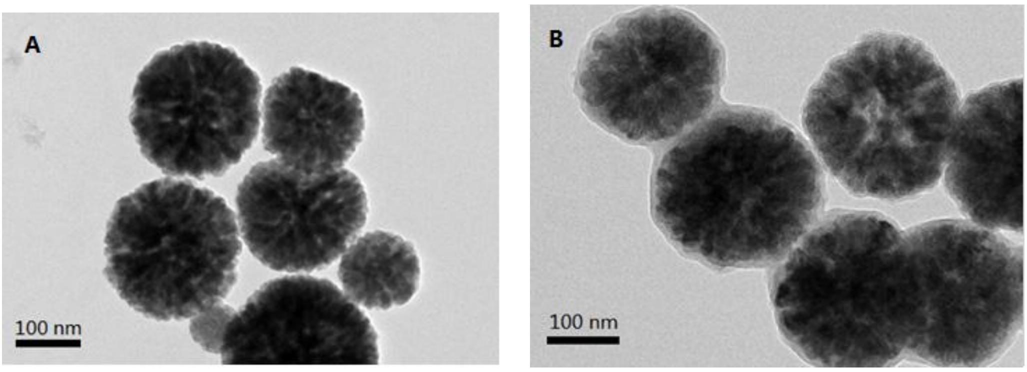

图3.1. Fe_3O_4 (A)和Fe_3O_4/C (B)纳米粒子的透射电镜图.

3.1.2 XRD

为了确定材料的晶型，对材料进行了XRD分析。由图3.2A和3.2B可知，Fe_3O_4和Fe_3O_4/C的2θ衍射角在30.25°, 35.58°, 43.21°, 54.39°, 57.09°, 62.92°和75.19°处的特征峰分别归属于Fe_3O_4的（220）、（311）、（400）、（422）、（511）、（440）和（553）特征衍射峰，粒子晶型与标准Fe_3O_4晶体（JCPDS No. 19-0629）和文献[32]完全一致。Fe_3O_4和Fe_3O_4/C的衍射峰没有明显的区别，说明碳层的包裹没有改变Fe_3O_4的晶型。

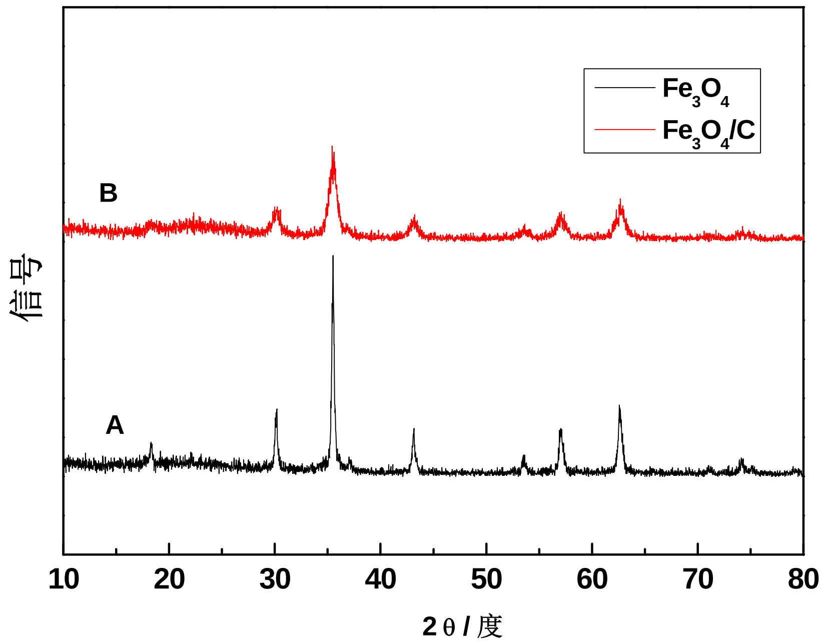

图 **3.2.** Fe_3O_4 (A) and Fe_3O_4/C (B)纳米粒子的 XRD 图.

3.1.3 FT-IR

为研究磁性纳米材料表面官能团，对材料进行了 FT-IR 光谱研究，图 3.3A 和 3.3B 分别为 Fe_3O_4 和 Fe_3O_4/C 纳米粒子的 FT-IR 光谱图，两条谱线中均显示 588 cm^{-1} 处有 Fe-O 伸缩振动峰 [32]，说明 Fe_3O_4 的形成。同时，在 3400cm^{-1} 处材料表面均有 O-H 伸缩振动，为其在水溶液或有机溶剂中均匀分散提供了依据。Fe_3O_4 纳米粒子在包覆碳层后，表面 C=O（1702 cm^{-1}）、C=C（1617 cm^{-1}）和 C-O（1400 cm^{-1}）伸缩振动明显，说明在水热过程中有含碳、含氧官能团聚集于粒子表面，使 Fe_3O_4/C 粒子又带有疏水性，易吸附疏水性物质，为其对有机磷和菊酯类药物的固相萃取提供了依据。

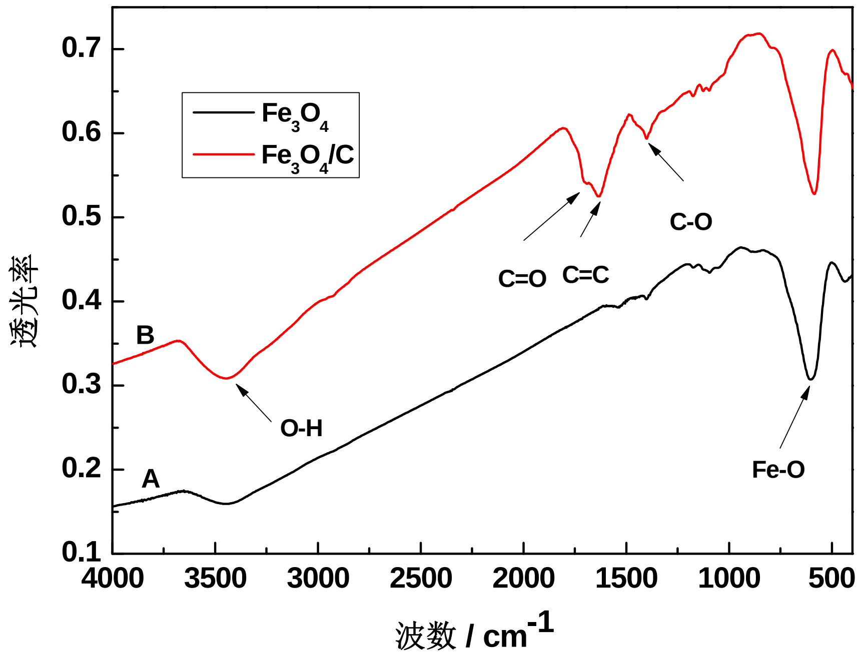

图3.3. Fe_3O_4 (A)和Fe_3O_4/C (B)纳米粒子的红外光谱图.

3.1.4 TGA-DSC

热分析方法可研究了 Fe_3O_4/C 纳米粒子表面的碳的含量及吸放热信息。图 3.4A 中曲线 A 是 Fe_3O_4 纳米粒子的 TG 曲线，显示在 550-600 ºC 温度范围略有失重，这可能与该温度下 Fe_3O_4 转变成 α-Fe_2O_3 [33]有关，图 3.4B 显示了 Fe_3O_4/C 纳米粒子的 TG 曲线，在 170 ºC 起的失重是由于材料表面失去了吸附水，550-700 ºC 有一明显失重是由于碳的失去，伴随碳被氧化失重的过程中，有一吸热峰产生（图 3.4C）。

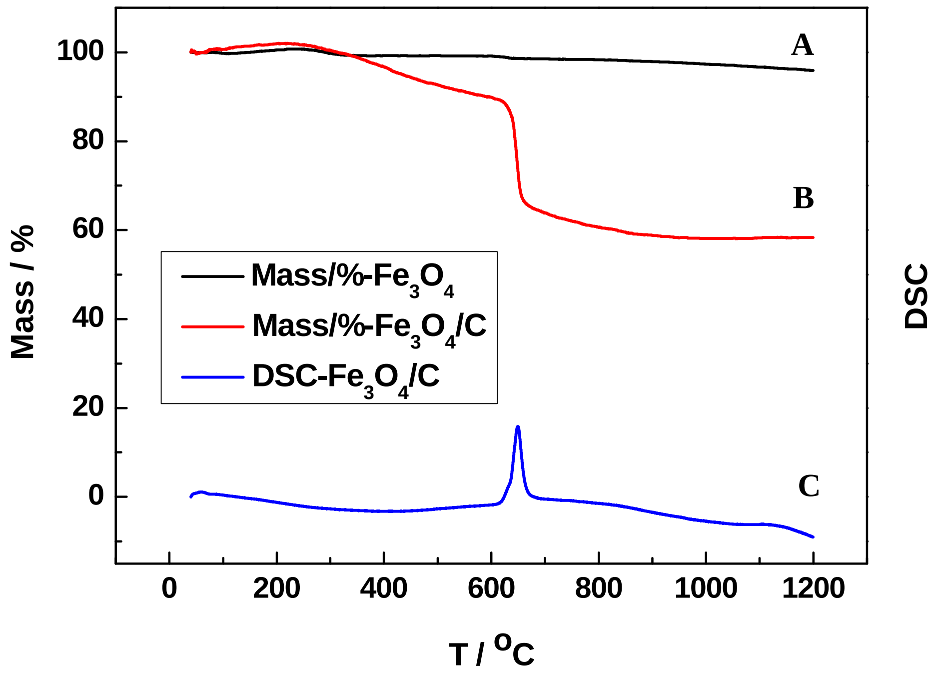

图 3.4. Fe_3O_4 和 Fe_3O_4/C 纳米粒子的热分析图.

3.2 磁固相萃取条件优化

本实验研究了 Fe_3O_4/C 纳米粒子对 CP、DM 和 BF 的磁固相萃取效率。每种分析物的初始浓度为 0.04 mg·L^{-1}，样品浓度用 HPLC 测定。为了得到最优化的萃取效率，我们对材料用量、重复使用性、样品溶液 pH、洗脱溶剂、吸附时间等一系列条件进行了优化。

3.2.1 材料的用量

取5-50 mg的Fe_3O_4/C吸附剂用于MSPE实验，随着Fe_3O_4/C用量的增加，分析物的回收率增加，当用20 mg Fe_3O_4/C时，回收率达到最大值（图3.5）。在后续实验中均使用了30 mg Fe_3O_4/C以保证对分析物的充分与稳定吸附。

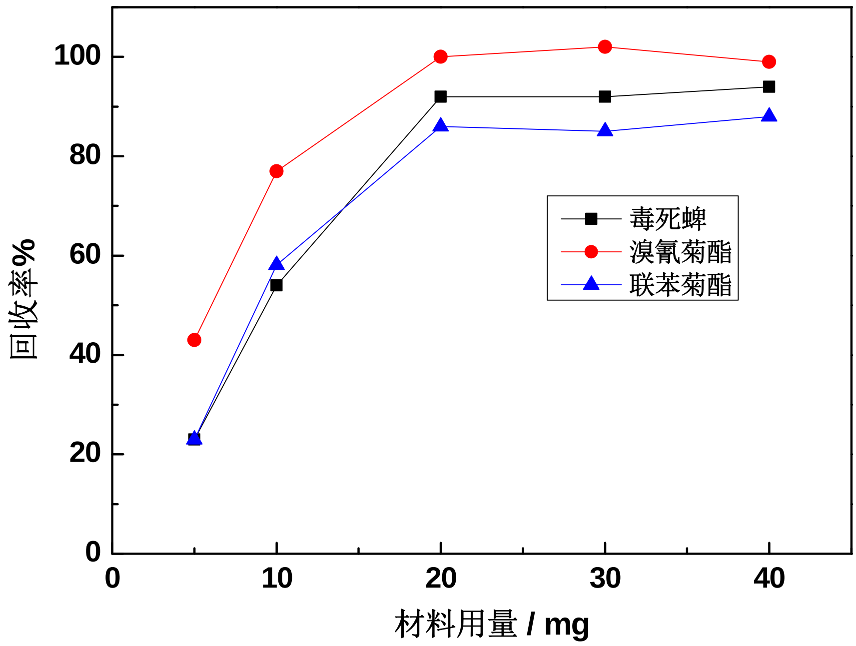

图 3.5. Fe_3O_4/C 材料用量对毒死蜱，溴氰菊酯和联苯菊酯的萃取效果.

3.2.2 pH的影响

为了考察MSPE过程中pH对防治白蚁药物的影响，实验中用1.0 mL 1.0 mol·L^{-1}乙酸铵和乙酸或氨水作为缓冲溶液调节水样的pH为5.0到9.0之间，图3.6显示，在中性和弱酸性条件下，回收率均在80%以上，而在pH为8-9时，回收率降低，这与药物在碱性条件下不稳定有关。且为了保证Fe_3O_4/C的使用寿命，以下实验均选择在pH为7.0时测得。

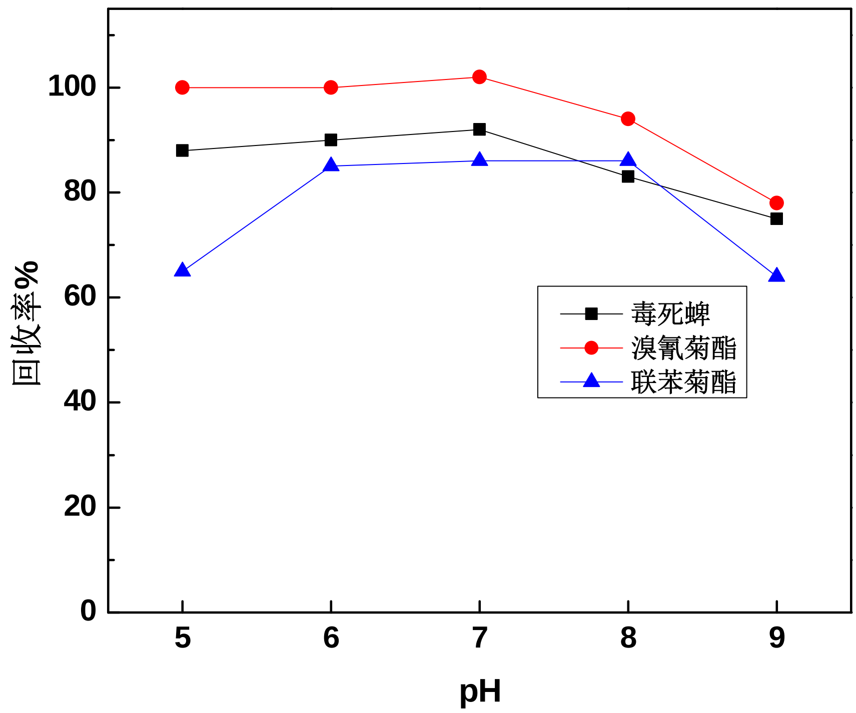

图 3.6. 溶液 pH 对毒死蜱，溴氰菊酯和联苯菊酯的萃取效果.

3.2.3 洗脱溶剂选择

实验中选用甲醇、乙醇、异丙醇和乙腈分别作为洗脱溶剂检验了洗脱回收率。图3.7表明，异丙醇的洗脱效果最差，而乙腈的洗脱效果最佳，可能由于乙腈与分析物之间的疏水作用更强，更容易将分析物从材料表面洗脱下来，本实验用2 mL的乙腈进行洗脱实验时，三种药物的回收率均超过80%，因此选乙腈作为洗脱溶剂。

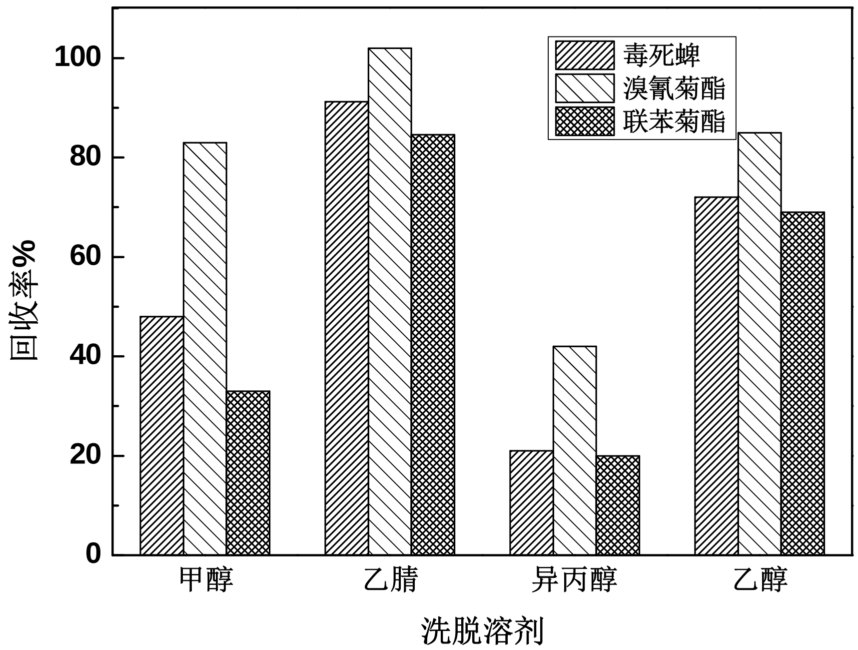

图 **3.7.** 洗脱溶剂对毒死蜱，溴氰菊酯和联苯菊酯的萃取效果.

3.2.4 吸附时间

由于材料的超顺磁性，只需在烧杯底部放上一个钕铁硼磁铁即可达到磁分离的效果。为了研究材料对药物吸附时间的影响，分别选2、5、8、10和15分钟，结果表明吸附时间为8分钟及以上时，对水样中的药物吸附效果更好，回收率为80%以上（图3.8），为了保证对分析物的充分吸附，后续实验中吸附时间选为10分钟。

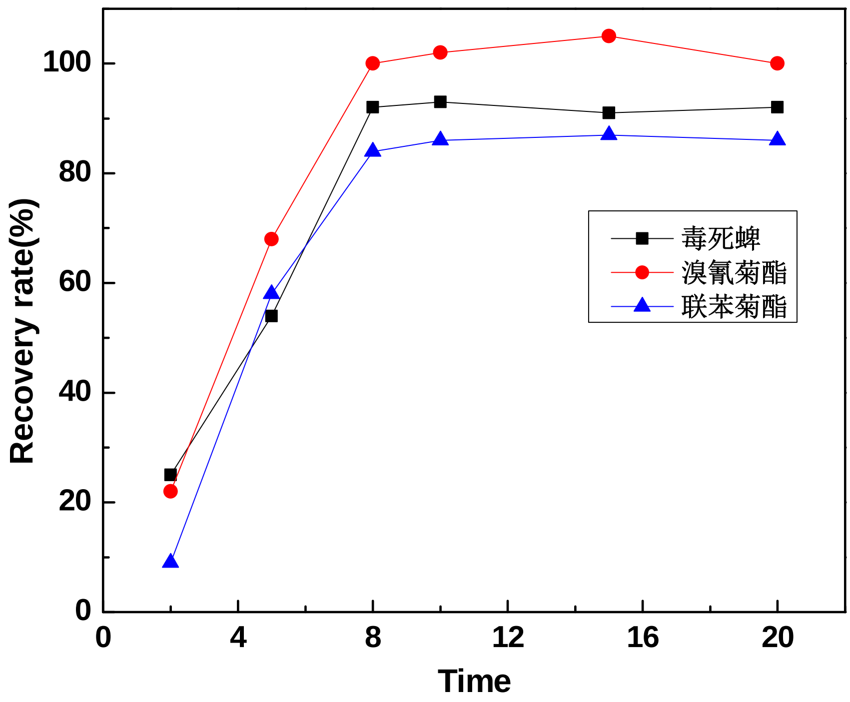

图 **3.8.** 吸附时间对毒死蜱，溴氰菊酯和联苯菊酯的萃取效果.

3.3.5 重复使用性

为了检验磁性材料对药物固相萃取后的重复使用性，实验中将用过的Fe_3O_4/C纳米材料用水和乙醇反复交替清洗后，干燥并重复使用。实验表明同一份Fe_3O_4/C纳米材料重复使用6次以上，分析物的回收率没有明显变化（图3.9）。这种良好的重复性也显示了材料的稳定性和耐用性。

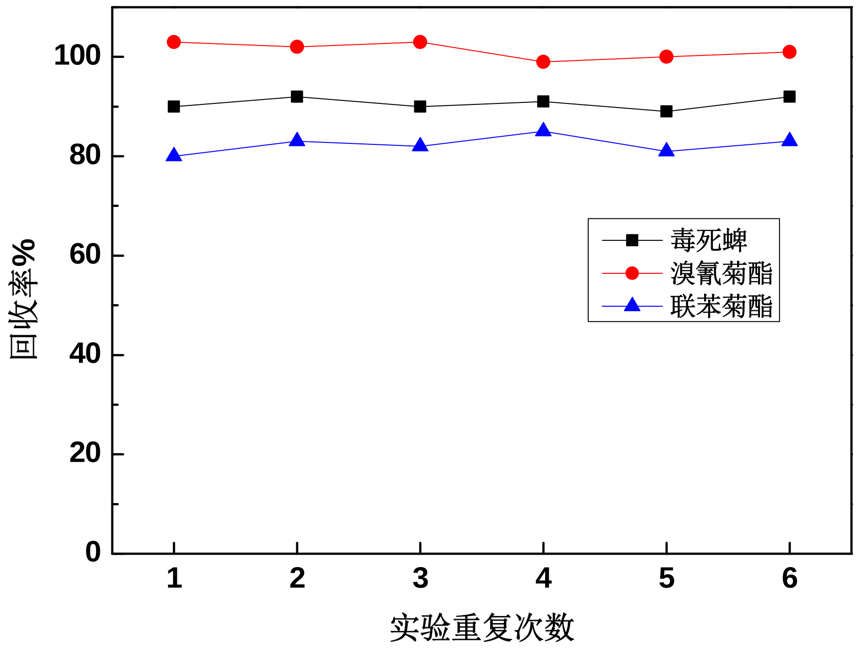

图 3.9. 重复试验对毒死蜱，溴氰菊酯和联苯菊酯的萃取效果.

3.4 环境水样的分析

在以上优化的MSPE和色谱条件下，对三种药物作了工作曲线。线性范围为0.01-0.1 mg·L^{-1}，相关系数（r^2）为0.9991-0.9995，检测限（LOD，3倍信噪比）在0.2-0.3 μg·L^{-1}之间（见表3.1）。

将该方法用于检测不同的环境水样（自来水，雨水和湖水）中的药物，分析结果列于表3.2，为验证该方法的可靠性，进行了 0.01 mg·L^{-1} 和 0.04 mg·L^{-1} 水平的加标试验（图 3.10），三种分析物的加标回收率为 82.9%-106.2%，相对标准偏差为 1.4%-9.6%，尽管环境水样中未测得三种防治白蚁药物，该方法显示出良好的准确性和可靠性。

表3.1. 毒死蜱，溴氰菊酯和联苯菊酯的标准曲线.

分析物	标准曲线	相关系数R^2	检测限 / g L^{-1}
毒死蜱	y=2446.8x-3.1617	0.9991	0.2
溴氰菊酯	y=2765.7x+12.817	0.9993	0.3
联苯菊酯	y=2939.5x+0.905	0.9995	0.3

表3.2. 实际水样中毒死蜱，溴氰菊酯和联苯菊酯的检测.

实际	加标 /mg L^{-1}	检测浓度/mg L^{-1}			回收率%（相对标准偏差%）		
		毒死蜱	溴氰	联苯	毒死蜱	溴氰	联苯
自来水	0	nd*	nd	nd			
	0.01	0.0093	0.0101	0.0083	93.4(8.2)	101.2(9.6)	83.3(2.1)
	0.04	0.0376	0.0390	0.0332	94.1(2.0)	97.6(2.9)	82.9(3.0)
湖水	0	nd	nd	nd			
	0.01	0.0093	0.0099	0.0091	93.1(7.9)	99.5(3.6)	90.8(4.9)
	0.04	0.0368	0.0400	0.0374	92.1(6.6)	99.9(8.2)	93.5(8.8)
雨水	0	nd	nd	nd			
	0.01	0.0093	0.0106	0.0085	93.2(8.8)	106.2(8.5)	85.4(7.8)
	0.04	0.0374	0.0392	0.0350	93.5(4.0)	98.0(6.3)	87.4(1.4)

* nd: not detected

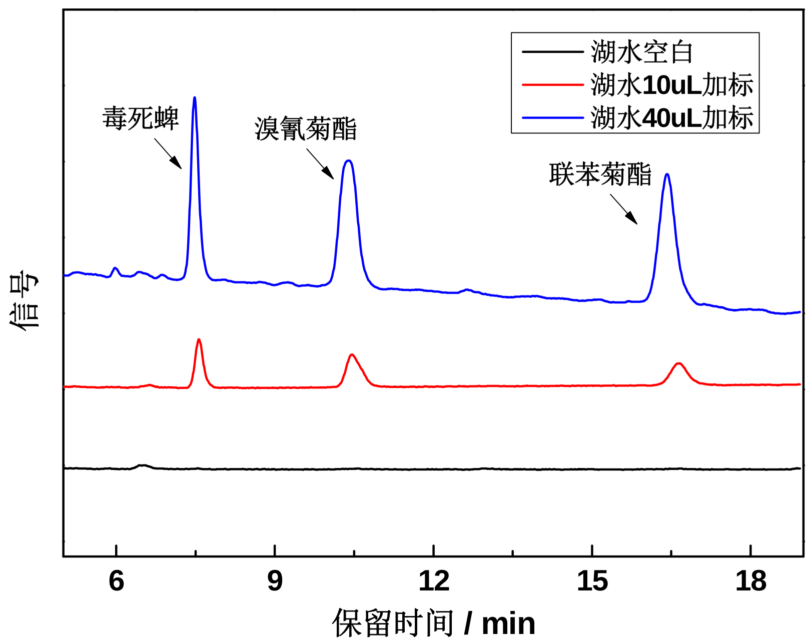

图 3.10. 实际水样中毒死蜱，溴氰菊酯和联苯菊酯的加标测定色谱图.

3.4 结论

Fe_3O_4/C纳米粒子具有良好的吸附-解吸性能，可用于对有机磷和菊酯类药物中毒死蜱，溴氰菊酯和联苯菊酯的磁固相萃取，在本文中，Fe_3O_4/C纳米粒子被成功应用于环境水样中毒死蜱、溴氰菊酯和联苯菊酯的MSPE-HPLC-UV分析检测，实验讨论了材料用量、pH、吸附时间、萃取溶剂、重复性等条件对萃取回收率的影响，优化了MSPE条件。实验结果显示，该方法回收率高、重复性好。

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南通功成精细化工有限公司始建于1999年，坐落于美丽的南黄海之滨、江苏省南通市洋口化工园内，占地面积34000平方米，注册资本5000万元，是工信部定点的农药生产企业。主要从事农药原药及制剂，特别是卫生杀虫剂的研发、生产、销售、技术服务和进出口贸易，是国内最优秀的公共卫生杀虫制剂生产企业之一，也是较早通过ISO 9001、ISO 14001、OHSAS 18001认证的企业，产品覆盖面及销量稳居国内同行业前列，并出口东南亚及非洲部分国家和地区。

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of

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第 11 次环太平洋地区白蚁研究组织学术研讨会议程

2016, April 18-19
Kunming, China

The 11th Pacific-Rim Termite Research Group Conference (TRG 11 Kunming)
18 – 19 April 2016

Programme

第11次环太平洋地区白蚁研究组织学术研讨会议程

2016年4月18 - 19日

17 April 2016 (Sunday)

1600 – 1830 Executive Committee (EC) meeting.
1830 – 2030 Dinner (EC members only).

18 April 2016 (Monday)

0730 – 0830 Registration: outside the International Hall 3/F
0830 – 0900 Speeches by Local Organizing Chairman, the President of PRTRG, and the group photography.

0900 – 1030 **Kunio Tsunoda Memorial Lectures (30 minutes each)**
Session Moderator: Prof Sulaeman Yusuf (Indonesia Institute of Science)

0900 – 0930 **Osumu Kitade** (Ibaraki University, Japan) – Diversity and evolution of symbiotic protest communities in termites especially focused on genus *Reticulitermes.* 散白共生原生生物群落多样性及进化

0930 – 1000 **Junhong Zhong** (Guangdong Entomological Institute, China) – Soil termite control practices using bait toxicants in South China.利用诱杀饵剂防治华南地区土白蚁的实践

1000 – 1030 **Veera Singham K Genasan** (Universiti Sains Malaysia, Malaysia) -- Biogeography history, morphological variation of *Macrotermes gilvus* and patterns of interaction specificity with its fungal symbiont, *Termitomyces* sp. in Southeast Asia. 南亚地区大白蚁生物地理史及形态变异，以及与鸡枞菌共生体相互作用的特异性模式

1030 – 1045 Tea break

1045 – 1200 **Session 1: Biodiversity and systematics (12 min + 3 min Q&A)**
Session Moderator: Dr Houfeng Li (National Chung Hsing University, Taiwan)

1045 – 1100 **Wei-Ren Liang and Hou-Feng Li** (National Chung Hsing University, Taiwan) - New discovery and review of termitophile fauna in Taiwan. 台湾鸡枞菌新记录及综述;

1100 – 1115 **Deng Feng, Liu Yansheng, Pang Zhengping, Ping Zhengming and Wang Jianguo** (Jiangxi Agricultural University, China) - Molecular phylogeography of *Reticulitermes* in Yarlung Zangbo Grand Canyon glacial refugia of Tibet.西藏雅鲁藏布江大峡谷冰川避难所散白蚁分子系统地理学研究。

1115 – 1130 **Kok-Boon Neoh, Lee-Jin Bong, Ahmad Muhammad, Masayuki Itoh, and Osamu Kozan, Yoko Takematsu and Tsuyoshi Yoshimura (National Chung Hsing University, Taiwan)** - The impact of fire on termite assemblage in tropical degraded peatland. 火对热带退化泥炭地的白蚁群落的影响。

1130 – 1145 **Deng Feng, Liu Yansheng, Pang Zhengping, Ping Zhengming and Wang Jianguo** (Jiangxi Agricultural University, China) - Genetic differentiation of *Reticulitermes speartus* in East Asia.东亚地区栖北散白蚁遗传变异

1145 – 1200 **Liu Bingrong, He Ying,Li Qiujian ,Zhong Junhong, Yuan Xiaodong, Hou Shoupeng, Cao Tingting, Gao Siwei and Yu Jie** (Guangdong Entomological Institute, China) - Comparison of Termite Species Composition and Diversity of 8 Forest types in Tianmushan National Nature Reserve. 天目山国家级自然保护区的8种森林类型白蚁物种组成和多样性比较

1200 – 1330 Lunch break

1330 – 1400 **Poster Presentation Session I** (poster presenters – please stand near your poster)

1400 – 1545 **Session 2: Termite management (12 min + 3 min Q&A)**
Session Moderator: Dr Foong-Kuan Foo (Alliance Pest Management, Singapore)

1400 – 1415 **Trinh Van Hanh, Do Tien Manh, Tran Thi Thu Huyen and Nguyen Thuy Hien** (Institute of Ecology and Works Protection, Vietnam) - Result of controlling dry wood termite (*Cryptotermes domesticus* Haviland) by new method. 截头堆砂白蚁防治新方法

1415 – 1430 **Eric Cheng** (City University of Hong Kong) - Tender Documentation for Termite Inspection and Control in the Built Environment. 在建筑环境中的白蚁检查和控制招标文件

1430 – 1445 **Napalai Samerjai, Khwanchai Charoenkrung and Charunee Vongkaluang** (Royal Forest Department, Thailand) - Field Trials in Thailand on the Efficacy of some Soil Termiticides to Prevent Subterranean Termites. 泰国土壤白蚁灭杀药剂预防地下白蚁的野外试验

1445 – 1500 **Nguyen H. Yen, Nguyen T. Hien, Trinh V. Hanh, Nguyen T. Vuong & Nguyen T. My** (Institute of Ecology and Works Protection, Vietnam) - First evaluation of termite baits as a protective tool to control *Coptotermes* termites in Vietnam. 越南首次使用白蚁饵剂作为保护工具防治乳白蚁的评价

1500 – 1515 **Partho Dhang** (Makati City, Philippines) - Eliminating subterranean termite infestation in ships and yachts across Philippine ports. 危害菲律宾港口船只及游艇白蚁的灭治

1515 – 1530 **Farah Diba, Iliyin Toni and M. Yuli Irianto** (Tanjung Pura University, Indonesia) - Field evaluation of hexaflumuron bait for population suppression of subterranean Termites *Coptotermes curvignathus* Holmgren in Oil Palm Plantation in West Kalimantan Indonesia. 氟铃脲诱饵抑制印度尼西亚西加里曼丹的油棕榈种植园乳白蚁密度的现场评价

1530 – 1545 **Daouïa Messaoudi, Neébil Bourguiba and Olivier Fahy** (Berkem Group, France) - TERMIFILM A unique effective product for preventive treatment of constructions against termites. TERMIFILM——一种用于预防建筑物白蚁有独特效果的产品

1545 – 1600 **Long Liu, Ganghua Li, Pengdong Sun, Chaoliang Lei and Qiuying Huang** (Huazhong Agricultural University, Wuhan, China) - Experimental verification and molecular basis of active immunization against fungal pathogens in termites. 病原真菌对白蚁主动免疫的实验验证和分子学机制

1600 – 1630 Tea break and **Poster Presentation Session II**

1630 – 1800 **Annual General Election of the PRTRG** (only members will be allowed to attend).
President's remarks
Report by Secretary-General
Report by Honorary Treasurer and approval of accounts.
Election and approval of the PRTRG New Office Bearers for 2016 – 2018.
Any other outstanding matters.

1900 – 2100 **The Gala Dinner of TRG 11** (Halal food will be available for Muslim delegates)
Short speech by the new elected President of PRTRG
Presentation of Certificates to the PRTRG Travel fund recipients.
Presentation of souvenirs to the sponsors of TRG 11
Presentation of memento to the Local Organizing Chairman.

19 April 2016 (Tuesday)

0900 – 0945 **Session 3 – Ecology and behavior (12 min + 3 min Q&A)**
Moderator: Dr Kok-Boon Neoh (National Chung Hsing University, Taiwan)

0900 – 0915 **Chun-I Chiu and Hou-Feng Li** (National Chung Hsing University, Taiwan) - Interspecific competition between *Coptotermes gestroi* and *Odontotermes formosanus* in a forest ecosystem. 哥斯特家白蚁和黑翅土白蚁在森林生态系统中的种间竞争

0915 – 0930 **Arinana, Ilmina Philippines, Effendi Tri Bahtiar, Yonny Koesmaryono, Dodi Nandika, Aunu Rauf, Idham Sakti Harahap and I Made Sumertajaya** (Bogor Agricultural University, Indonesia) - Fluctuation of subterranean termites nest *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae) temperature. 乳白蚁 *Coptotermes curvignathus*（等翅目：鼻白蚁科）巢的温度波动。

0930 – 0945 **Thi-Mai-Duyen To, Ching-Chen Lee and Chow-Yang Lee** (Universiti Sains Malaysia) - A survey of the prevalence of endoparasitism by *Vertica fasciventris* Malloch (Diptera: Calliphoridae) in colonies of *Macrotermes carbonarius* (Hagen) (Blattodea: Termitidae) in Penang Island, Malaysia. 马来西亚槟城大白蚁群体中丽蝇类（双翅目丽蝇科）内寄生的患病率调查

0945 – 1045 **Session 4 – Wood durability and preservation (12 min + 3 min Q&A)**
Moderator: Mr Jim Creffield (Onwood Entomology, Australia)

0945 – 1000 **Carlson A.D. Tawi and Andrew H.H. Wong** (Universiti Malaysia Sarawak, Malaysia) - H2 hazard class field test of planted Malaysian hardwoods engkabang, kelempayan and rubberwood dip-treated with different concentrations of cypermethrin and permethrin against *Coptotermes curvignathus*. 用不同浓度的氯氰菊酯和氯菊酯泡浸硬木 engkabang, kelempayan 及橡胶木防治乳白蚁的危险等级 2 的野外试验

1000 – 1015 **C.M. Garcia, M. Dionglay and M.R. San Pablo** (Forest Product Research and Development Institute, Philippines) - Efficacy of crude extracts from indigenous plant species against Philippine termites. 土著植物提取物对菲律宾白蚁的药效

1015 – 1030 **Yusuf Sudo Hadi and A. Arinana** (Bogor Agricultural University, Indonesia) - Subterranean termite resistance of polystyrened wood from three tropical wood species. 三种聚苯乙烯处理的热带树种木材对白蚁的抗性

1030 – 1045 **Suksawat Ponpinij** (Kasetsart University, Thailand) - Durability test of local usable woods against termites. 本地可用的木材对白蚁的耐久性试验

1045 – 1100 Tea break.

1100 – 1200 **Session 5 – Termites – Microbial Interactions (12 min + 3 min Q&A)**
Moderator:

1100 – 1115 **Niken Subekti and Fidia Fibriana** (Universitas Negeri Semarang, Indonesia) - The Role of Microorganisms from Guts of Subterranean Termites *Macrotermes gilvus* Hagen during Composting Process. 大白蚁肠道微生物在降解过程中的作用

1115 – 1130 **WenHui Zeng, QiuJian Li, BingRong Liu, ShaoFang Hu and JunHong Zhong** (Guangdong Entomological Institute, China) - Comparative Analysis of Microbial Community Structure and Composition in Sugarcane Harmful Termites by Illumina 16s RNA Sequencing. 利用 Illumina 16S RNA 测序方法比较分析甘蔗有害白蚁微生物群落结构和钩成

1130 – 1245 Lunch break

1300 – 1700 **Excursion to the Ethnic Minorities Village**. *Please assemble at the hotel lobby no later than 1250.*

1830 – 2030 Dinner (Halal food will be prepared for Muslim delegates).

Poster Presentation

Poster boards are in the conference hall (Level 3 – International Hall, Empark Grand Hotel, Kunming). All poster presenters must put up your poster no later than 10.45 am on 18 April 2016. All posters must be taken down by 11.00 am on 19 April 2016.

Poster No. 1
Osamu Kitade and Kenta Yabuki (Ibaraki University, Japan) - Interaction between sexual and parthenogenetic incipient colonies of *Reticulitermes speratus*.

Poster No. 2
Carlson A.D. Tawi and Andrew H.H. Wong (Universiti Malaysia Sarawak, Malaysia) - Comparative efficacy of kempas and rubberwood dip-treated in proprietary insecticides cypermethrin and permethrin between short dipping durations under low or high humidity Malaysian H2 hazard class conditions against *Coptotermes curvignathus*.

Poster No. 3
Ikhsan Guswenrivo, Hideki Sato and Tsuyoshi Yoshimura (Kyoto University, Japan) - Ectoparasite Fungi Species Found on Subterranean Termite *Reticulitermes* spp. in Japan.

Poster No. 4
Changlu Wang (Rutgers University, USA) - Case Studies of Controlling Subterranean Termites Using Noviflumuron Baits.

Poster No. 5
Wenjing Wu, Zhiqiang Li, Shijun Zhang, and Yunling Ke (Guangdong Institute of Applied Biological

Resources, China) - Transcriptome analysis of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae).

Poster No. 6
S. Khoirul Himmi, Masao Oya, and Tsuyoshi Yoshimura (Kyoto University, Japan) - Nest founding preference of the western drywood termite, *Incisitermes minor* against six commercial timbers.

Poster No. 7
Didi Tarmadi, Yuki Tobimatsu, Masaomi Yamamura, Takuji Miyamoto, Yasuyuki Miyagawa, Toshiaki Umezawa, Tsuyoshi Yoshimura (Kyoto University, Japan) - 2D NMR study on structural alterations of wood cell walls during digestion by a lower termite, *Coptotermes formosanus* Shiraki.

Poster No. 8
Yan-Sheng Liu, FengDeng, Zheng-Ping Pang , Jian-Guo Wang (Jiangxi Agricultural University, China) - A comparative study on the complete mitochondrial genome sequences of 5 species in the genus *Macrotermes*.

Poster No. 9
Pang Zheng-Ping,Hu Mao-Cheng, Jin Zhan-Bao, Deng Feng, Wang Jian-Guo (Wujin Institute of Termite Control, China) - Reporting the northern limit of the distribution of Subterranean Termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) in worldwide.

Poster No. 10
Wen Ping and Ken Tan (Chinese Academy of Science, Kunming, China) - Chemical communication in the fungus-growing termites (Isoptera: Macrotermitinae)