# Effect of chlorfluazuron bait against *Macrotermes gilvus*: Evidence for the presence of the toxicant compound in workers and larvae

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## Abstract

The alpha-cellulose powder containing 0.1% chlorfluazuron, a chitin synthesis inhibitor, was tested for efficacy against the mound-building termite, *Macrotermes gilvus* (Hagen). Mounds were dissected to determine the status of colony health after baiting. In the meantime, termites were also subjected to gas chromatography-mass spectrometry (GC-MS) to detect the presence of chlorfluazuron. After ≈4 months post-baiting, termite colonies removed an average of 610.0 ± 34.5 g of bait matrix. All of the treated medium-sized mounds were moribund in which dead termites were scattered throughout the mound, larvae were absent, and queens appeared unhealthy - flaccid with a dark yellow colours. Moderate to heavily consumed fungus combs were also found. The highest amount of chlorfluazuron was detected in workers from the royal chamber, followed by workers from the peripheral and nursery zones, and lastly in larvae from the nursery. Nevertheless, a limited treatment effect was shown in large mounds with comparatively lower levels of chlorfluazuron detected in workers and larvae.

**Key words**: colony size, mound-building termite, *Macrotermes gilvus*, baiting, GC-MS analysis

## Introduction

Subterranean termites are structural pests which cause serious damage to structures mainly in the tropics, sub-tropics and temperate regions. An estimated US$40 billion is spent annually to control and repair losses caused by termite pests around the world (Rust and Su 2012). Since the mid-1990's baiting systems have been widely used for termite management. Hexaflumuron, a benzoylphenylurea (BPU) compound, is one of the chitin synthesis inhibitor (CSI) active ingredients used in bait products. CSI's act by disrupting the synthesis of chitin in insect cuticle during molting (Yu 2008). Various studies have reported the elimination or suppression of lower termite species (Rhinotermitids) using BPU CSI's (Su 1994, Peters & Fitzgerald 2003, Evans 2010). However, published field studies successfully controlling higher termites (Termitids) using baits are limited (Neoh et al. 2011). Biological variation between higher termites and lower termites is possibly one of the main causes for the mixed result.

The fungus-growing termites of the subfamily Macrotermitinae are widely distributed throughout Africa, South Asia and South East Asia (Eggleton 2000). One of the common mound-building termites, *Macrotermes gilvus* (Hagen) (Isoptera: Termitidae: Macrotermitinae) is usually present on the perimeter of buildings and structures. However, *M. gilvus* can be a secondary pest, infesting houses after *Coptotermes* spp. (Rhinotermitidae) have been eliminated by termite baits (Lee 2002, Lee et al. 2007). Unlike the Rhinotermitids, *M. gilvus* generally shows poor response to termite baits (Ngee et al. 2004, Lee et al. 2007). We propose that efficacy of baits against *M. gilvus* depends on colony population size.

In this study, chlorfluazuron (CFZ) bait was tested for efficacy in eliminating *M. gilvus* colonies. Treated mounds were excavated to identify the level and distribution of bait toxicant in the colony. We also chemically analyzed the amount of active ingredient acquired by different termite castes using gas chromatography-mass spectrometry (GC-MS).

## Materials and Methods

The study was carried out in Bayan Lepas, Penang in northern Peninsular Malaysia (5°32′N, 100°29′E). 4 healthy medium (height, 21-40 cm; diameter, 51-80 cm) and 4 healthy large (height, 41-80 cm; diameter, 81-130 cm) *M. gilvus* mounds were selected, for this study. A total of 3 of each mound size were subjected to treatment (mound A–F) while the remaining mounds were considered not-treated control colonies (mound G & H). Termite traps were installed at each mound prior to testing. A wooden block consisting of Jelutong (*Dyera costulata*) stakes (15 by 4 by 2.2 cm) were placed inside a bottomless polyethylene container as the termite trap. Termite traps were installed as close as possible to the mounds. Once the trap was occupied by termites, the wooden block was replaced with a bait station. Bait stations consisted of a plastic container with predrilled holes (0.8 cm i.d) to allow termite access to the bait matrix.

The bait used in this study was 0.1% (wt:wt) CFZ-based alpha-cellulose powder (Ensystex Sdn. Bhd., Kuala Lumpur, Malaysia). Approximately 80 g of bait matrix mixed with 400 mL of water was introduced into the bait stations. Bait stations were inspected biweekly and baits replaced when the previously introduced bait was completely removed. The amount of bait matrix removed was visually estimated. The experiment was terminated after 4 mo baiting at which time all treated and control mounds were excavated and conditions inside the mounds recorded. Colony status was determined based on several criteria slightly modified from Evans 2010. Colony status was assessed as: (1) healthy, a normal and active colony; (2) weakening, a colony with reducing termite numbers; (3) moribund, a colony with impaired reproductive capacity and absent of immature castes; or (4) eliminated, a colony with all termite individuals dead.

During excavation, workers and larvae, if present, were collected from different areas of the mound (i.e., peripheral zone, nursery zone, and royal chamber). Three replicates of samples from each termite caste and mound part were chemically analyzed using the GC-MS assay developed in a previous study (Lee et al. 2013).

Concentrations of CFZ in each termite caste and mound part were analyzed using one-way analysis of variance (ANOVA), and means were separated using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$.

## Results and Discussion

After four months of baiting, *M. gilvus* attacked all of the bait stations in both the treated and control colonies. In treated mounds, some of the termites found inside the bait station were marbled-white colour. Termite workers started to show symptoms of benzoylphenylurea poisoning within one month after bait application. Workers appeared marbled-white in colour due to accumulation of whitish uric acid in the fat body (Noirot 1969). On average approximately 610.0 ± 34.5 g of the CFZ-based bait matrix and 750.0 ± 10.0 g of blank bait were removed by treated and control colonies, respectively.

In this study, 3 of the 6 treated colonies were moribund, none of them were completely eliminated in the 17 wk of the baiting period. Observations from the dissection of mounds showed that all of the treated medium colonies were in the moribund stage (Table 1). During inspection of the medium colonies, we observed small numbers of unhealthy workers and soldiers, while none of the immature castes such as larvae and nymphs were found inside the nest. CSIs are known to function as larvicides which cause abortive molting in larvae (Peppuy et al. 1998, Rojas and Ramos 2004). Dead and decaying termite cadavers were found in a number of spots inside the mounds. Heavily consumed, fragile or degenerated fungus combs were also observed. This is possibly due to poor maintenance of fungus combs by termite workers. Because worker and larval populations decreased, less energy might be invested in constructing fungus combs (Darlington 1991). The queens were dark yellow in colour, flaccid, and less physogastric compared with that in the untreated control mounds, which were turgid and creamy-white in colour.

Two of the large treated mounds were assessed as weakening after the bait application. We found smaller numbers of workers, soldiers, larvae, and eggs compared with the not-treated control colony, which had more live termites and eggs. There were a few dead termites inside the mound. In some instances, the fungus combs inside the large treated mounds were moderately consumed. While one of the treated large mounds and all the control mounds were assessed as healthy as indicated by large numbers of individual termites from different castes in addition to eggs without the presence of dead termites.

In general, significantly higher amounts of CFZ were detected in workers from the royal chamber, followed by workers from the peripheral and nursery zones, and lastly by larvae from the nursery zone (Table 1). By application of GC-MS, we showed that the amount of CFZ detected from workers ranged from 12.44 ± 3.18 to 86.22 ± 16.56 ng per termite. Kubota et al. 2008 reported that approximately 400 ng bistrifluron/termite was detected from moribund *Coptotermes formosanus* workers when exposed to bistrifluron-treated food (5000 ppm). The lower residue level of CFZ detected in our study compared to that of Kubota et al. 2008 could be due to direct the food-sharing behaviour in lower termites.

Table 1. Transmission as indicated by mean amount (ng per termite) of chlorfluazuron (CFZ) within individual termites in medium and large mounds, and colony status after baiting.

| Mound size | Colony | Mound height & diameter (cm) | CFZ concentration (ng/termite) in different termite castes from different nest areas | | | | Colony status |
|---|---|---|---|---|---|---|---|
| | | | P.Z wk[a] | N.Z wk[a] | R.C wk[a] | N.Z lv[a] | |
| Medium | A | 39 & 72 | ND | 74.15 ± 2.15a | 98.3 ± 15.61b | ND[b] | Moribund |
| | B | 31 & 78 | 42.57 ± 2.35a | 45.33 ± 1.74a | 76.2 ± 5.5b | ND | Moribund |
| | C | 34 & 69 | 53.33 ± 2.67a | 58.89 ± 0.59a | 90.67 ± 1.43b | ND | Moribund |
| Large | D | 56 & 90 | 16.72 ± 0.50b | 15.90 ± 3.38b | 35.63 ± 0.86c | 1.02 ± 0.14a | Weakening |
| | E | 75 & 129 | 10.64 ± 0.97b | 10.19 ± 0.89b | 16.50 ± 1.24c | 2.63 ± 0.31a | Weakening |
| | F | 51 & 130 | 18.79 ± 1.14b | 16.78 ± 1.03b | 25.05 ± 3.39c | 1.25 ± 0.14a | Healthy |

Values represent mean ± S.D., and mean followed by different letters in the same row are significantly different by the Tukey's HSD test ($P < 0.05$)

[a] P.Z wk: workers from peripheral zone; N.Z wk: workers from nursery zone; R.C wk: workers from royal chamber; N.Z lv: larvae from nursery zone

[b] ND, not determined due to absence of the caste

## Conclusions

In conclusion, population size or mound size played an important role in determining the efficacy of chlorfluazuron bait against *M. gilvus* colonies. All of the treated medium mounds were moribund, whereas treated large mounds were either in the weakening stage or remained healthy. Bait toxicant was successfully distributed among termite colony members.

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