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Residual efficacy of a Dual-Active Insecticide Formulation containing Bifenthrin and Imidacloprid in Mineral and Peat Soils against the Subterranean Termite *Coptotermes gestroi* (Blattodea: Rhinotermitidae)

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ABSTRACT

The persistence and bioavailability of insecticides are influenced by environmental factors, soil properties and ageing factor, which can affect the residual efficacy of soil-applied termiticides. This study assessed the residual efficacy of Bithor ® Dual Action against Coptotermes gestroi, in mineral and peat soils under different temperatures (30 and 40 °C), soil pH conditions (acidic, neutral, and alkaline), and ageing periods (1 day, 1 week, 1 month, 3 months, and 6 months). Treated soils were tested to a no-choice laboratory bioassay with termite mortality monitored over 7-days. Probit analysis was used to estimate lethal times (LT_{50}) while a four-way General Linear Model (GLM) was used to analyse the effects of ageing time, temperature, soil type, and pH. Termite mortality declined progressively with increasing ageing period, as demonstrated by increased LT_{50} values. Mineral soil consistently killed termites faster than peat soil, however higher temperatures (40 °C) lowered residual efficacy than at 30 °C. The GLM analysis showed that ageing time, temperature, and soil type significantly affected termite mortality ($p < 0.001$), whereas soil pH had no significant effect ($p > 0.05$). Significant interaction effects among environmental factors revealed that residual efficacy was influenced was dependent on their combined effects. Overall, ageing time and soil type are the major factors influencing residual performance of Bithor® Dual Action in soil. Reduced efficacy in peat soil may be attributed to decreased bioavailability associated with its high organic matter content. These findings provide useful information for optimizing soil termiticide application strategies in tropical environments.

***Key words**: Bifenthrin, Imidacloprid, residual efficacy, soil ageing, Coptotermes gestroi*

INTRODUCTION

The residual effect of insecticides in soils is an essential aspect influencing environmental persistence, agricultural safety, and ecological hazard. Soil-applied insecticides depend not only on the active ingredient and formulation, but also on soil properties, which significantly alter their persistence, mobility, and bioavailability. Comparing mineral and peat soils reveals substantial differences in how insecticides are adsorbed, degraded, and retained. Mineral soils, often characterized by lower organic matter content, frequently demonstrate faster degradation and shorter half-lives for many insecticides. In contrast, peat soils, which are known to contain a high percentage of organic carbon, exhibit greater sorption capacities but slower degradation rates (Dorado & Almendros, 2021; Fouad *et al*., 2023; Ngaini *et al*., 2024).

Differences in soil–insecticide interactions are especially relevant to subterranean termite control. Species such as *Coptotermes gestroi* pose a substantial threat in urban areas, particularly in tropical regions where warmth and humidity favour their growth and foraging. Their extensive underground galleries and ability to remain undetected at the surface complicate long-term control. Soil termiticides therefore remain an important defence, especially where baiting systems are not used or impractical (Himmi *et al*., 2013; Ab Majid & Ahmad, 2015; Wan Umar & Ab Majid, 2020; Chouvenc, 2024).

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To improve treatment performance, dual-active formulations are increasingly being evaluated and tested to enhanced efficacy, extend residual activity, and address issues relating to resistance and environmental impact (Peterson, 2011; Belsky *et al*., 2021; Deore *et al*., 2026). Dual-active insecticides contain two active ingredients with different modes of action, typically combining a fast-acting toxicant with a slower-acting compound. This combination allows for both immediate knockdown and delayed transfer effects within termite colonies, which believed to improve overall control efficiency (Taillebois & Thany, 2022; Oi, 2022).

Although dual-active insecticides are increasingly used, their long-term performance in mineral and peat soils remains poorly understood, particularly under tropical conditions. Understanding their behaviour in these soils is essential for optimising treatment strategies and ensuring consistent termite control in field-relevant settings. This study therefore aims to assess the residual efficacy of a specific dual-active insecticide against *C. gestroi* in mineral and peat soils. By monitoring termite mortality over time under controlled conditions, it seeks to clarify how soil characteristics influence insecticide persistence and efficacy and inform improved termite management practices.

MATERIALS AND METHODS

Insecticides

Insecticide employed in this study is Bithor® Dual Action (Ensystex, Malaysia Sdn. Bhd) which consisted of two active ingredients, namely bifenthrin and imidacloprid. Stock solutions were prepared by diluting the insecticide formulation with distilled water according to the concentrations recommended on the manufacturer's label.

Soil sampling

Soil samples were obtained from two sites, which included Durian Valley, Universiti Sains Malaysia (USM) for sandy clay soil (5.359616, 100.30534) and local distributor (1.725053, 103.625658) (Glow Green Trading, Johor) for peat soil. The soils collected around 10 cm from the top layer (A-horizon), while the remaining residue, which includes stones, vegetation, and macrofauna, were removed. The soils were air-dried at room temperature (20-25 °C), sieved through a 2-mm sieve being stored at room temperature before were sent to the Soil Analytic Sdn. Bhd. for the particle size, pH, and organic matter concentration and the soil texture analysis.

Soil pH adjustment

The soil pH was modified using hydrated lime powder (a liming agent) to increase its pH level. This study involves three manipulated pH categories: acidic, neutral, and alkaline. As the original soil used in the experiment is naturally acidic, adjustments were made only to achieve neutral and alkaline conditions. To increase the soil pH from acidic to neutral, a mixture of 5 grams of hydrated lime in 50 ml of water was applied per 500 grams of soil were applied to the mineral soil. While for peat soil, a different ratio is applied due to its higher organic matter and buffering capacity, requiring 8.75 g of hydrated lime to 100ml water per 500g soil.

Soil treatment preparation

Stock solutions for each insecticide were prepared using 1000 mL of deionized distilled water, following the concentration guidelines provided by the manufacturers. From these stock solutions, 100 mL of each insecticide was applied to 1000 g of soil and distributed into two square aluminium containers (162 mm × 162 mm × 64 mm). The trays were then placed in an oven set between 30°C and 40°C with each temperature setting included three replicates. As for control, 1000 g of soil was treated with 100 mL of deionized water. Soil samples were

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collected for analysis on day 1, day 7, and at the 1st, 3rd, and 6th months after treatment and kept in the freezer below 0 °C (Rashid & Ab Majid, 2018).

Test insect

Test insects employed in this study is an Asian subterranean termite, *Coptotermes gestroi*, where they were collected from monitoring stations installed at Universiti Sains Malaysia (USM), Penang. These stations consisted of cylindrical plastic containers (16 × 18 cm) embedded in the ground and were filled with pine stakes (*Pinus caribaea*) measuring 2.5 × 2.5 × 15 cm, that served as a food source for termite rearing (Rashid & Ab Majid, 2018). After 1–2 weeks, the pine stakes from the stations were brought and transferred to the Household and Structural Urban Entomology Laboratory (H&SUEL), School of Biological Sciences, USM, where they were acclimated to laboratory temperature conditions prior to use in the no-choice bioassay.

To obtain clean termites, individuals were separated from dirt and debris using the Tamashiro bridging method (Tamashiro *et al*., 1973; Rashid & Ab Majid, 2018). They were then counted (52 workers termite and 2 soldiers) and placed into plastic petri dishes (90 mm × 15 mm) containing moistened filter paper to serve as a food source before being use for the no-choice bio-assay.

No-choice bioassay

At each sampling interval (1 day, 7 days, 1 month, 3 months, and 6 months), 18 g of treated soil conditioned at 30 °C or 40 °C under acidic, alkaline, or neutral pH conditions was transferred to a Petri dish (**Figure 1**). Each dish received two soldier termites and fifty-two worker termites and was sprayed daily with 1 mL of distilled water to maintain moisture.

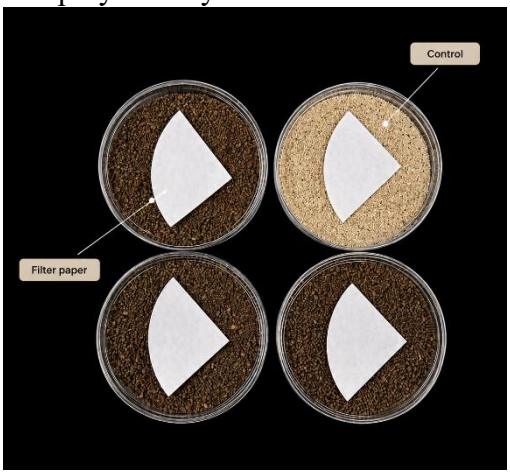


Figure 1. Experimental setup for the no-choice bioassay showing three replicates of treated mineral soil with filter paper as a food source

Termite mortality data were recorded at 1, 3, 6, and 12 hours after the exposure to the soil, and subsequently at 24-hour intervals over a 7-day period for each treatment and sampling time. The petri dishes were placed in a place set to 26°C and 65% relative humidity, and kept in complete darkness. Dead termites were removed daily from each petri dish throughout the experiment to reduce potential contamination and cannibalism.

Statistical analysis

Four-way factorial general linear model (GLM) was used to analyse the mortality data with ageing time, temperature, soil type and soil pH as fixed factors. The Probit analysis was used to estimate the LT_{50}. All statistical analyses were conducted with IBM SPSS Statistics version 29.

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RESULTS AND DISCUSSION

Soil characterization

Soil properties are important for understanding insecticide degradation, including that of bifenthrin and imidacloprid. This study used mineral soil from Durian Valley at Universiti Sains Malaysia (5.359616, 100.30534) and peat soil from Johor (1.725053, 103.625658). Their physicochemical properties are summarised in **Table 1**. Both soils were acidic, with mineral soil more strongly acidic (pH 4.6) than peat soil (pH 5.6), consistent with acidic soils commonly found in tropical regions such as Malaysia (Melling, 2015; Ratai *et al*., 2024). Carbon content was 2.32% in mineral soil and 35.96% in peat soil, reflecting their respective organic matter contents of 4.0% and 62.0%. Mineral soil also had more clay (36.0%) than peat soil (7.0%). These differences are expected to influence insecticide adsorption and bioavailability. The lower organic matter content of mineral soil generally favours greater insecticide bioavailability despite lower persistence. In contrast, the higher organic matter content of peat soil promotes sorption of hydrophobic compounds, reducing their availability to target organisms. Thus, differences in termite mortality observed in this study are more likely to reflect soil-related variation in insecticide availability than differences in application rates alone.

Table 1. Physicochemical properties of mineral and peat soils used in the study

Soil	Mineral	Peat
pH	4.6	5.6
% Organic Matter (OM)	4.0	62.0
% Carbon	2.32	35.96
% Clay	36.0	7.0
% Silt	16.0	-
% Sand	47.0	-

Bioavailability of insecticide

Termite mortality (%) over time

Mortality of *Coptotermes gestroi* generally increased with exposure time across all treatments. Freshly treated soils caused rapid mortality, with most treatments exceeding 90% mortality within the first few days. Aged soils caused slower mortality, indicating a gradual decline in residual insecticidal activity. Mineral soil consistently produced higher termite mortality than peat soil across all ageing periods. Residual efficacy was generally lower at an incubation temperature of 40 °C than at 30 °C. Differences among soil pH treatments were small, suggesting that pH influenced insecticide performance less than ageing time, soil type, and temperature.

Probit analysis and GLM (4-Way Factorial) results

LT_{50} values for Bithor in mineral and peat soils were estimated from termite mortality data collected after 1 day, 1 week, 1 month, 3 months, and 6 months of soil ageing. For each treatment and sampling interval, mortality was recorded at 1, 3, 6, and 12 hours after soil exposure, then every 24 hours over a 7-day period. Probit analysis generally showed increasing LT_{50} values with soil ageing, indicating declining efficacy of Bithor's active ingredients. Mineral soil generally yielded lower LT_{50} values than peat soil at all ageing intervals, with the same pattern observed at both temperatures (30 °C and 40 °C) and across acidic, alkaline, and neutral pH conditions. Factorial ANOVA showed that ageing time significantly affected termite mortality ($F = 567.83$, $p < 0.001$). Across all treatments, LT_{50} values were generally lower after 1 day of ageing than after 6 months.

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Table 2: LT_{50} values for *Coptotermes gestroi* exposed to Bithor's active ingredients at different time ageing, temperature, soil type and soil pH for laboratory study.

Ageing	Mineral soil		Peat soil	
	30°C	40°C	30°C	40°C
	Acidic / Neutral / Alkaline	Acidic / Neutral / Alkaline	Acidic / Neutral / Alkaline	Acidic / Neutral / Alkaline
Day 1	9.50 / 12.56 / 9.61	10.38 / 12.08 / 12.33	19.76 / 18.34 / 19.96	18.44 / 20.07 / 19.56
1 week	11.02 / 13.28 / 10.23	10.73 / 14.22 / 13.53	18.30 / 21.03 / 26.65	16.40 / 20.52 / 16.28
1 month	13.23 / 15.30 / 13.99	14.77 / 15.28 / 13.57	23.47 / 19.34 / 19.97	26.00 / 21.07 / 21.19
3 months	13.91 / 15.34 / 15.06	15.78 / 18.89 / 13.68	28.93 / 29.42 / 23.36	29.15 / 27.96 / 31.45
6 months	16.47 / 18.01 / 15.19	15.01 / 19.28 / 13.49	29.14 / 31.75 / 31.41	29.26 / 28.62 / 31.80

Notes: Values are LT_{50} (h); values within each cell are presented as acidic / neutral / alkaline soil pH.

Table 3: Effects of ageing time, temperature, soil type and soil pH on *Coptotermes gestroi* mortality for laboratory study.

Source	df	Mean Square	F	Sig.
Ageing Time	4	2345.104	567.83	**<0.001**
Temperature	1	155.496	37.65	**<0.001**
Soil Type	1	10503.228	2543.20	**<0.001**
pH	2	6.492	1.57	0.212
Ageing Time * Temperature	4	16.563	4.01	0.004
Ageing Time * Soil Type	4	2345.104	567.83	**<0.001**
Ageing Time * pH	8	62.759	15.19	**<0.001**
Temperature * Soil Type	1	155.496	37.65	**<0.001**
Temperature * pH	2	22.431	5.43	0.006
Soil Type * pH	2	6.492	1.57	0.212
Ageing Time * Temperature * Soil Type	4	16.563	4.01	0.004
Ageing Time * Temperature * pH	8	119.024	28.82	**<0.001**
Ageing Time * Soil Type * pH	8	62.759	15.19	**<0.001**
Temperature * Soil Type * pH	2	22.431	5.43	0.006
Ageing Time * Temperature * Soil Type * pH	8	119.024	28.82	**<0.001**

*Significant values are given in bold.

The four-way factorial General Linear Model (GLM) analysis (Table 3) showed that ageing time significantly affected termite mortality ($F = 567.83$, $p < 0.001$). LT_{50} values were generally higher after 6 months of ageing than after 1 day, indicating reduced residual efficacy over time. Soil type also had a significant effect ($F = 2543.20$, $p < 0.001$), with consistently higher mortality in mineral soil than in peat soil. In contrast, soil pH had no significant effect on mortality ($F = 1.57$, $p = 0.212$). The significant four-way interaction among ageing time, temperature, soil type, and soil pH ($F = 28.82$, $p < 0.001$) indicated that the effect of ageing time on mortality depended on the combination of the other three factors.

DISCUSSION

Ageing time effect

The present study shows termite mortality is greatly influenced by ageing time. LT_{50} values increased progressively from Day 1 to Month 6 under all soil types, temperatures and pH conditions, indicating a gradual decline in residual efficacy. This suggests that the residual efficiency of insecticides diminished over time, and termite mortality was often higher in freshly treated soil than in aged soil. Based on this, it is believed that the active substances gradually degraded or vanished during the ageing process. The drop in toxicity over time could be linked to a variety of environmental activities, including hydrolysis, microbial degradation,

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volatilisation, adsorption to soil particles, and photodegradation, all of which reduce the insecticide's bioavailability to termites (Xu *et al*., 2018; Raffa & Chiampo, 2021; Al-Hawadi *et al*., 2024). Previous research has also shown that as ageing time increases, the persistence and toxicity of soil-applied termiticides decreases. For instance, Mulrooney *et al*. (2006) reported that the efficacy of several termiticides was reduced against subterranean termites as a result of significant degradation over extended ageing periods. Similarly, a study by Manzoor and Pervez (2017) reported that concentrations and recovery of bifenthrin and fipronil decreased with increasing soil age, with differences in termiticide efficacy observed between soil types.

Lower mortality in aged soil may indicate a decline in bioavailable insecticide concentrations. As these concentrations decrease, longer exposure is needed to cause termite mortality, resulting in higher LT_{50} values. This pattern supports the view that insecticide persistence in soil strongly influences residual activity.

Soil type effect

Soil type was a major factor influencing termite mortality, with significantly higher mortality in mineral soil than in peat soil. The higher organic matter content of peat soil may bind pesticide molecules, reducing their bioavailability to termites. This reduced exposure may explain the lower mortality and higher LT_{50} values for *Coptotermes gestroi* in peat soil. Similarly, Ngaini *et al*. (2024) reported longer half-lives of λ-cyhalothrin in peat soil than in mineral soil, attributing this difference to higher organic carbon content and stronger adsorption. Pesticides have also been reported to persist longer in the surface horizons of organic peat soil (Fortin, 2002). Together, these findings indicate that soil composition significantly influences insecticide persistence and biological efficacy against *Coptotermes gestroi*.

Temperature effect

Temperature was also observed to have a significant effect on termite mortality in the current study. This could potentially be linked to the effect of temperature on the persistence and degradation of insecticides in soil. Higher temperatures can accelerate chemical and microbiological degradation, resulting in less residual action and a shorter insecticide half-life (Pérez-Lucas *et al*., 2019). This is additionally supported by the findings of Krieger *et al*. (2000), who discovered that increasing temperature accelerated florasulam breakdown in soil. Similarly, Chai *et al*. (2010) reported significantly faster acephate degradation at 35 °C than at 15 °C. They suggested that higher temperatures enhanced microbial and hydrolytic degradation, accelerating pesticide dissipation in soil.

pH effect

The GLM analysis showed no significant effect of soil pH on termite mortality ($F = 3.123$, $p = 0.051$), suggesting that the pH conditions tested did not substantially alter insecticide performance or termite tolerance to the insecticides. Similarly, Murray *et al*. (2001) reported that soil pH may have little influence on insecticide persistence in chlorpyrifos-treated soils, suggesting relative stability across pH conditions. The effects of pH on pesticide adsorption and bioavailability may also depend on interactions with clay content, organic matter, and cation exchange capacity (Ramakrishnan *et al*., 2000). Sharma *et al*. (2015) reported little variation in imidacloprid adsorption across pH levels, indicating a limited pH effect under some soil conditions. Overall, temperature, ageing time, and soil type may influence termite mortality more strongly than soil pH alone.

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CONCLUSION

Residual efficacy was mainly influenced by soil type and ageing time, with soil pH playing a minor role. Insecticide persistence alone did not determine biological performance: despite greater residue retention in peat soil, reduced bioavailability limited termite exposure. These findings underscore the importance of assessing both biological efficacy and chemical persistence when selecting soil termiticides for tropical environments.

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