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Evaluation of the repellent and antifeedant effects of selected insecticides against the subterranean termite *Microcerotermes diversus* (Blattoidea: Termitidae)

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Abstract

The subterranean termite, *Microcerotermes diversus* Silvestri (Blattoidea: Termitidae), is one of the most destructive pests in tropical regions, particularly in southwestern Iran, causing significant damage to palm trees and wooden structures. Traditional control methods, such as direct insecticide spraying, often fail because termites are cryptic. Toxic baiting systems that exploit termite trophallaxis offer a more sustainable solution, provided the insecticides used lack repellent and antifeedant effects. This study investigated the repellency and antifeedant effects of four insecticides, including thiacloprid (a neonicotinoid), imidacloprid (a neonicotinoid), emamectin benzoate (a macrocyclic lactone), and matrine (a plant-derived alkaloid), against worker termites of *M. diversus* under laboratory conditions. Termites were exposed to treated and untreated bait disks in soil-covered containers, and their food consumption and aggregation were recorded after 72 hours. The results showed that emamectin benzoate significantly reduced termite presence on treated baits by 20.89%, 26.08%, and 45.45% at concentrations of 0.001, 0.002, and 0.01 (L/L), respectively. Matrine also exhibited strong repellency, reducing termite settlement by 58.82% and 66.66% at concentrations of 0.002 and 0.01 (L/L), respectively. Both compounds also displayed antifeedant effects, as indicated by no significant weight loss in treated bait disks compared to controls.

In contrast, thiacloprid and imidacloprid demonstrated attractant properties, with termite density on treated baits being 6.8% to 213% higher than on control baits across all tested concentrations. Significant bait consumption was observed on disks treated with thiacloprid or imidacloprid, indicating no antifeedant effect. The findings suggest that emamectin benzoate and matrine are unsuitable baiting systems because of their repellent properties. Conversely, thiacloprid and imidacloprid, which do not repel termites and allow continued feeding, represent promising options for sustainable termite management in agricultural and urban environments.

Keywords: Subterranean termites, behavioral response, toxic bait, neonicotinoids, biopesticides

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بررسی اثرات دورکنندگی و ضدتغذیه‌ای چند حشره‌کش علیه موریانه زیرزمینی *Microcerotermes diversus* (Blattoidea: Termitidae)

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چکیده

موریانه زیرزمینی *Microcerotermes diversus* Silvestri (Blattoidea: Termitidae) یکی از مخرب‌ترین آفات در مناطق گرمسیری، به‌ویژه در جنوب غرب ایران محسوب می‌شود که خسارات قابل توجهی را به نخل‌ها و سازه‌های چوبی وارد می‌کند. روش‌های سنتی کنترل مانند سمپاشی مستقیم، اغلب به دلیل رفتار پنهان‌زیستی موریانه‌ها با شکست مواجه می‌شوند. روش استفاده از طعمه‌های مسموم که از رفتار تروفالاکسی موریانه‌ها بهره می‌برد، راهکار پایدارتری را ارایه می‌دهد، مشروط بر این که حشره‌کش‌های مورد استفاده فاقد اثرات دورکنندگی و ضدتغذیه‌ای باشند. در این پژوهش، اثرات دورکنندگی و ضدتغذیه‌ای چهار حشره‌کش از جمله تیاکلوپرید (نئونیکوتینویید)، ایمیداکلوپرید (نئونیکوتینویید)، امامکتین بنزوات (ماکرولاکتون) و ماترین (آلکالوئید گیاهی) علیه کارگران این موریانه در شرایط آزمایشگاهی بررسی شد. موریانه‌ها در معرض دیسک‌های طعمه‌ای تیمارشده و شاهد در ظروف پوشیده از خاک قرار گرفتند و تغذیه و تجمع آن‌ها پس از ۷۲ ساعت ثبت شد. نتایج نشان داد که طعمه‌های تیمار شده با غلظت‌های ۰/۰۰۱، ۰/۰۰۲ و ۰/۰۱ (لیتر بر لیتر) امامکتین بِنزوات، به ترتیب منجر به کاهش ۲۰/۸۹، ۲۶/۰۸ و ٤٥/٤٥ درصدی حضور موریانه‌ها شد. ماترین نیز خاصیت دورکنندگی قوی از خود نشان داد و در غلظت‌های ۰/۰۰۲ و ۰/۰۱ (لیتر بر لیتر)، استقرار موریانه‌ها را به‌ترتیب ۵۸/۸۲ و ٦٦/٦٦ درصد کاهش داد. هر دو ترکیب دارای اثر ضدتغذیه‌ای نیز بودند، که این امر از طریق عدم وجود کاهش معنی‌دار در وزن دیسک‌های طعمه‌ای تیمار شده نسبت به شاهد مشخص شد.در مقابل، تیاکلوپرید و ایمیداکلوپرید خاصیت جلب‌کنندگی روی موریانه نشان دادند، به‌طوری‌که تراکم موریانه‌ها روی طعمه‌های تیمار شده ٦/٨ تا ۲۱۳ درصد بیشتر از طعمه‌های شاهد بود. این اختلاف در تمام غلظت‌های مورد آزمایش مشاهده شد. مصرف قابل‌توجه طعمه‌های تیمار شده با تیاکلوپرید و ایمیداکلوپرید توسط موریانه‌ها، نشان‌دهنده عدم وجود اثر ضدتغذیه‌ای این ترکیبات بوده، بنابراین گزینه‌های مناسبی برای فرمولاسیون طعمه‌های سمی محسوب می‌شوند. با استناد به نتایج، امامکتین بنزوات و ماترین به دلیل دارا بودن اثر دورکنندگی برای استفاده در سیستم‌های طعمه‌گذاری مناسب نیستند، اما می‌توانند به‌عنوان موانع محافظتی مورد استفاده قرار گیرند. از سوی دیگر تیاکلوپرید و ایمیداکلوپرید به دلیل دارا نبودن اثر دورکنندگی و عدم تاثیر منفی روی تغذیه این موریانه، گزینه‌های امیدوارکننده‌ای برای مدیریت پایدار این آفت در زیست‌محیط‌های کشاورزی و شهری محسوب می‌شوند.

کلید واژه‌ها: موریانه‌های زیرزمینی، واکنش رفتاری، طعمه سمی، نئونیکوتینوییدها، سموم زیستی

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Introduction

Subterranean termites pose a serious threat to trees, shrubs, stored products, wooden materials, and tools in agricultural, forestry, urban, and domestic environments (Verma et al., 2009). Among these species, *Microcerotermes diversus* Silvestri (Blattoidea: Termitidae) is recognized as one of the most destructive termite species in tropical regions of Iran, particularly in Khuzestan Province in the southwestern part of the country (Habibpour et al., 2006; Habibpour et al., 2010; Cheraghi et al., 2013). Workers of this species feed on various parts of palm trees and create narrow galleries within the wood of trees or household wooden items, filling their internal spaces with fine granular frass (Habibpour et al., 2010).

Traditional control methods for this pest include toxic baits containing insecticides or direct insecticide spraying. However, spraying is often ineffective due to termites' cryptic behavior and the difficulty of accessing reproductive individuals (Oi, 2022). Consequently, toxic baits containing appropriate compounds are considered a more effective and sustainable approach for termite management in various environments (Su, 2019). Trophallactic behavior (mutual feeding) in termites ensures that reproductive individuals, including queens and males, receive sufficient amounts of active insecticide ingredients, ultimately leading to colony disruption and elimination (Verma et al., 2009). For this purpose, the insecticides used must lack repellent and antifeedant properties (Lee and Lee, 2025).

The repellent effects of various insecticides such as chlorfenapyr (Rust and Saran, 2006; Henderson et al., 2016), fenobucarb (Kubota et al., 2007), and acetamiprid (Rust and Saran, 2008), as well as chlorpyrifos, fipronil, carbofuran, propoxur, bifenthrin, cypermethrin, imidacloprid, fipronil, abamectin, and avermectin (Chen et al., 2015), and chlorantraniliprole (Henderson et al., 2016), have been studied on different subterranean termite species. Additionally, the antifeedant effects of insecticides such as chlorantraniliprole, chlorfenapyr, emamectin, indoxacarb, pyriproxyfen, spinosad, spiromesifen, and triflumuron (Akbar et al., 2019) have been investigated. In this study, the repellent and antifeedant effects of the insecticides emamectin benzoate, matrine, thiacloprid, and imidacloprid on worker individuals of *M. diversus* were evaluated.

Materials and Methods

A termite colony from the field laboratory of the Department of Plant Protection at the Agricultural Sciences and Natural Resources University of Khuzestan (coordinates: 31°35'56.565"N 48°53'23.528"E) in Bavi County (Khuzestan Province) was used for the experiments. Studies based on aggressive interactions among termites (Clément, 1986) confirmed that this area hosted a single large colony. Adult worker termites were collected from the colony using a soft brush and transferred to the laboratory in sealed containers containing date palm wood fragments and soil. Since termites separated from the mother colony are typically highly irritable. To ensure the termites were calm, we designed and implemented the following protocol: the termites were placed in an incubator set to 27°C and 60% relative humidity (under continuous darkness) for 24 hours before the experiment.

Date palm wood particles prepared from the rachis (petiole base) region were sieved to less than 3 mm in size and used as bait in the experiments. The wood particles were bonded together using a soil-water paste (clay). For this purpose, wood and clay were mixed at an 80:20 weight ratio. The procedure involved mixing 80 g of wood particles with 20 g of clay paste. Then, as attractants, glucose and cellulose were added

at a 5% weight ratio, and sodium bicarbonate at a 3% weight ratio was added to this mixture. The wood particles were shaped into circular disks with a diameter of 4 cm and a thickness of 0.4 cm (approximate weight: 2.5-4 g). These disks were then dried in an oven at 45°C for 24 hours. The specifications of the four insecticides used in the toxic bait preparation are shown in Table 1.

To evaluate the repellency potential of treated and control bait disks, we followed the method below: 3 mL of the determined concentration of each insecticide was added to 100 g of bait material to produce toxic bait disks. In the control bait disks, no insecticide was used during bait preparation. The bait disk containing the specified concentration (v/v) of each tested insecticide, along with another bait disk without insecticide (as a control disk), was placed 10 cm apart in a transparent rectangular plastic container measuring 15×20 cm and 5 cm high. Since termite feeding activity is more effective in soil environments and aggressive behaviors are reduced under such conditions, a 20% clay suspension was prepared and uniformly spread on the bottom of the containers to create an even soil layer (Figure 1). Thirty worker termites were released between the two bait disks in each test at a distance from each disk. After 72 hours, the number of live termites present on each bait disk was recorded. Environmental conditions during these experiments were maintained at 30°C temperature, 70% R.H., and continuous darkness. Four independent replicates were performed for each experiment.

Table 1. Insecticides used in toxic baits against the termite *Microcerotermes diversus*

Insecticide	Commercial name	Formulation	Company	Concentration (L/L)
emamectin benzoate	Pluto®	5% SC	Modern Insecticides Limited, India	0.001, 0.002, 0.01
imidacloprid	-	35% SC	Toseeh Sanaat Sam Azarbaijan, Iran	0.001, 0.002, 0.01
thiacloprid	Giscaia®	24% OD	Yancheng Shuangning Agro-Chemical, China	0.001, 0.002, 0.01
matrine	Rui Agro®	0.6% SC	Inner Mongolia Kingbo Biotech, China	0.001, 0.002, 0.01



Figure 1. Containers with soil-lined bottoms containing bait disks (insecticide-treated and control).

The percentages of repellency were computed using the following formula:

$$PR = [(Nc - Nt) \div (Nc + Nt)] \times 100$$

where: Nc and Nt are the number of termites on treated and control disks, respectively (Pascual-Villalobos and Robledo,1998).

In the feeding tests, the previously mentioned bait disks were weighed using a precision balance before being placed in the container. After the designated feeding time period (72 hours), live termites were counted, and the bait disks were re-weighed to measure consumption.

Data Analysis

All no-choice and free-choice tests were conducted in a completely randomized design with four replicates. A Student's t-test was performed in SPSS version 16 to compare means. Data normality was assessed using the Shapiro-Wilk test. For non-normally distributed data, a base-10 logarithmic transformation was applied.

Results

The results comparing the density of worker termites settled on toxic and control bait disks are presented in Table 2.

For the emamectin benzoate treatments, all tested concentrations significantly reduced termite settlement on toxic bait disks (20.89%, 26.08% and 45.45% lower settlement at concentrations 0.001, 0,002, and 0.01, respectively), indicating repellent properties against *M. diversus* workers at all concentrations. For matrine, the lowest concentration (0.1% L/L) showed no repellent effect, but higher concentrations (0.2% and 1% L/L) significantly reduced termite settlement on toxic baits (58.82% and 66.66% reductions at 0.002% and 0.01%, respectively), demonstrating concentration-dependent repellency.

Table 2. Number (mean ± SE) of settled individuals of the subterranean termite *Microcerotermes diversus* (per 30 individuals) in treated and control baits

Treatment	Concentration (L/L)	Treated bait	Control	PR (%)	$t_{(df=6)}$	P-value
emamectin benzoate	0.001	13.25±0.75 b*	16.75±0.75 a	11.66	-3.30	0.016
	0.002	12.75±1.10 b	17.25±1.10 a	15	-2.87	0.028
	0.01	10.50±0.50 b	19.25±0.75 a	29.41	-9.70	<0.001
matrine	0.001	13.25±1.10 a	16.75±1.10 a	11.66	-2.23	0.067
	0.002	8.75±2.39 b	21.25±2.39 a	41.66	-3.69	0.010
	0.01	7.5±1.44 b	22.5±1.44 a	50	-7.34	<0.001
thiacloprid	0.001	22.75±0.85 a	7.25±0.85 b	-51.66	12.83	<0.001
	0.002	19.25±0.85 a	10.75±0.85 b	-28.33	7.03	<0.001
	0.01	15.50±0.28 a	14.5±0.28 b	-3.33	2.44	0.050
imidacloprid	0.001	22.75±2.01 a	7.25±2.01 b	-50.83	5.43	0.002
	0.002	20.5±1.75 a	10.00±1.47 b	-34.42	4.58	0.004
	0.01	20.25±0.62 a	9.75±0.62 b	-35	11.80	<0.001

*Same letters in each column indicate non-significant difference at 0.05

Incontrast, thiacloprid and imidacloprid exhi bited no repellent effects. At all concentrations, worker termite density on toxic bait disks was significantly higher than on control disks (6.8%-213% more density), suggesting these neonicotinoids may have attractive properties for *M. diversus* workers.

The results of weight reduction in bait disks during the experimental period for each insecticide treatment and control are presented in Table 3.

Table 3. Weight loss (mean ± SE) of the subterranean termite *Microcerotermes diversus* before and after the experimental period in treated and control baits.

Treatment	Concentration (L/L)	Treated bait	Control	$t_{(df=6)}$	P-value
emamectin benzoate	0.001	0.59±0.008 a*	0.53±0.041 a	1.52	0.179
	0.002	0.49±0.01 a	0.55±0.06 a	-0.98	0.363
	0.01	0.38±0.01 a	0..43±0.01 a	-1.23	0.262
matrine	0.001	0.52±0.01 a	0.50±0.01 a	0.67	0.524
	0.002	0.50±0.09 a	0.43±0.05 a	0.68	0.520
	0.01	0.33±0.02 a	0.39±0.01 a	-1.69	0.141
tiacloprid	0.001	1.19±0.08 a	0.34±0.02 b	9.477	<0.001
	0.002	0.97±0.01 a	0.40±0.00 b	46.54	<0.001
	0.01	0.79±0.01 a	0.37±0.03 b	12.95	<0.001
imidacloprid	0.001	1.14±0.01 a	0.32±0.08 b	9.23	<0.001
	0.002	1.00±0.01 a	0.31±0.01 b	29.37	<0.001
	0.01	0.88±0.01 a	0.30±0.04 b	11.67	<0.001

*Same letters in each row indicate non-significant difference at 0.05

For emamectin benzoate and matrine treatments, no significant differences in weight loss were observed between toxic and control bait disks at any of the tested concentrations. These results indicate that no significant feeding occurred on the toxic bait disks containing these insecticides.

In contrast, thiacloprid and imidacloprid treatments showed significant weight reductions in toxic bait disks between pre- and post-experimental measurements (6.8%-213% and 6.8%-105% reductions, respectively), demonstrating substantial worker termite feeding activity on these treated baits.

Discussion

The data from this study demonstrated that both emamectin benzoate and matrine exhibit strong repellent and antifeedant effects. Consequently, these insecticides are unsuitable for use in toxic bait formulations aimed at sustainable control of *M. diversus* termites.

Emamectin benzoate induces repellency and antifeedant effects in *M. diversus* primarily through its neurotoxic mechanism of action, leading to early behavioral avoidance and feeding inhibition (Jansson et al., 1997). Its residual activity and strong physiological impact, even at low concentrations, prevent termites from interacting with treated bait, making it unsuitable for use in toxic baiting systems but potentially useful as a repellent barrier treatment. Limited research exists regarding its effects on termites. Although it has been identified as highly toxic to the subterranean termite *Psammotermes hypostoma* Desneux (El-Zoghby et al., 2022), its repellent and antifeedant properties have not been previously studied in termites. Interestingly, residual effects showed low repellency against the weevil *Hypera brunneipennis* (Ali, 2020), suggesting species-specific behavioral responses to environmental compounds that may explain these differential findings.

Matrine, a novel botanical insecticide with contact and stomach action, contains alkaloids derived from the roots of the shrubby sophora, Sophora flavescens, which can affect insect physiology and behavior by triggering nicotinic acetylcholine receptors (Akdeniz and Özmen, 2011; Sharifi et al., 2020). Studies on *Coptotermes formosanus* Shiraki revealed that plant extract residues

maintained toxic and repellent effects for up to 12 months (Mao and Henderson, 2007).

Both compounds show potential for structural protection by deterring forager entry, though their repellency precludes their use in baiting systems. The species-specific nature of behavioral responses to these compounds underscores the importance of tailored termite management strategies. These findings contribute to understanding insecticide-termite interactions while highlighting the need for non-repellent alternatives in bait-based control systems.

The insecticides thiacloprid and imidacloprid, due to their lack of repellent and antifeedant properties, are suitable candidates for incorporation into toxic baits for controlling *M. diversus* termites. One of the key requirements in the development of toxic baits for termite control is that the bait should be attractive and stimulate feeding behavior in worker termites. Therefore, the insecticide mixed with such baits should not interfere with or suppress this feeding-stimulating property of the bait (Su, 2019). This study marks the first investigation into thiacloprid effects on termites, while the impacts of other neonicotinoids, such as imidacloprid, have been previously examined.

For instance, subterranean termites *Reticulitermes virginicus* Banks exposed to sublethal doses of imidacloprid (10 or 100 ppm for 4 hours) and allowed a one-week recovery period showed no avoidance behavior upon re-exposure to the insecticide (Thorne and Breisch, 2001). Under natural conditions, termites that passed through imidacloprid-treated soil and later returned to the same area exhibited no avoidance, behaving similarly to untreated termites. In contrast, other neonicotinoids, such as acetamiprid and bifenthrin, demonstrated repellent properties, preventing termite entry (Smith et al., 2008).

In conclusion, the favorable characteristics of imidacloprid—including non-repellency, lack of antifeedant effects, and delayed mortality—make it an ideal candidate for sustainable control of *M. diversus* in olive orchards through baiting systems. Similarly, thiacloprid shows promise due to its non-repellent nature and ability to facilitate termite feeding on treated baits.

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