ANSF APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
16(4), 1402 - 1408 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Efficacy of insecticides spinosad and imidacloprid as baiting stations to control termite *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae)

Zaid Naji Hassan
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
Nassreen N. Mzhr
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
Maan Abdul Azeez Shafeeq*
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

*Corresponding author. E-mail: maanalsalihi@uomustansiriyah.edu.iq

Article Info
https://doi.org/10.31018/jans.v16i4.5788
Received: May 21, 2024
Revised: October 22, 2024
Accepted: October 27, 2024

How to Cite
Hassan, Z. N. *et al.* (2024). Efficacy of insecticides spinosad and imidacloprid as baiting stations to control termite *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae). *Journal of Applied and Natural Science*, 16(4), 1402 - 1408. https://doi.org/10.31018/jans.v16i4.5788

Abstract
The control of termite infestations, particularly *Microcerotermes diversus*, poses substantial challenges in agricultural settings, such as olive orchards, necessitating effective pest management strategies. This study investigates the efficacy of two insecticides, imidacloprid, and spinosad, at varying concentrations for controlling termite infestations in an olive orchard in Baghdad. The experiment utilized bait stations consisting of plastic containers buried within the soil of infested olive trees. Each bait station contained palm frond bundles treated with either imidacloprid (200, 400, 600 ppm) or spinosad (200, 400, 600 ppm) and the control group with no treatment. Palms were previously dried and placed in the bait stations, then inspected bi-monthly for termite infestation and weight loss over six months. After one month, the treatment with 200 ppm imidacloprid produced a 75% infection rate, with 3120 worker termites and a 55% reduction in palm frond weight. By six months, the infestation rates for imidacloprid and spinosad 600 ppm decreased to 0%, corresponding to 40% and 38% weight losses. The control group had 100% infestation and a peak of 5820 workers. Spinosad at 600 ppm proved most effective, achieving a total reduction in infestation by month four, demonstrating a significant difference in efficacy between the insecticides. The findings highlight the effectiveness of using bait stations with imidacloprid and spinosad to manage termite populations, providing a crucial method for safeguarding crops against severe pest threats. This study contributes valuable data on optimal pesticide application for sustainable agricultural practices.

Keywords: Imidacloprid, *Microcerotermes diversus,* spinosad, Tunnel, Worker

INTRODUCTION

For the subterranean termite *Microcerotermes diversus* (Silvestri) organization, preparations were primarily suitable for loams. The two major tactics used were (a) handling loams to discourage invasions and (b) implementing insecticides inside the invaded loams to regulate mighty reconditioned invasions. Although every design has advantages and disadvantages, the type of method chosen depends on the properties of the soil and the presence of pesticides (Henderson *et al*., 2016). It was determined that testing insecticides against termites was impractical and ought to wait until after death. Two insecticides that are part of an original group that acts as expellers when applied at recommended concentrations are imidacloprid, which belongs to a class of chemicals called the neonicotinoids, and spinosad, which is based on chemical compounds found in the bacterial species *Saccharopolyspora spinosa* (Cheraghi *et al*., 2013; Ahmed *et al*., 2014; Ahmed *et al*., 2015; Akbar *et al*., 2018).

These pesticides cause harmful attitude changes and abnormalities in termites, and their toxic effects spread from infected termites to uninfected people in the settlement, resulting in significant declines (Abd-Ella, 2020; Al-Jebury *et al*., 2023). Several methods are being used to study how insecticides affect termite poisoning. Termite technicalities for capturing insecticides include handling flatness, an accidental touch, reciprocated preparation and antennation, trophallaxis (rectal or stomach), nutrition and compound exchange, coprophagia, necrophoresis, carcass, anthropophagy, and contact with subaltern polluted superficies (Iqbal and

Saeed, 2013; Manzoor and Pervez, 2014a; Saran and Rust, 2014; Henderson *et al*., 2016; Misbah-Ul-Haq *et al*., 2016; Kumar, 2017; Ekhtelat *et al*., 2018; Iqbal and Evans, 2018; Iqbal *et al*., 2019; Aljedani, 2023). These educators describe Termites' efficiency in both situations' attraction and monitoring functions (Saran & Rust, 2014; Nisar *et al*., 2020; Mishra *et al*., 2021; Nisar *et al*., 2023). As evictor insecticides primarily rely on looking for termites in treated areas to achieve extremely deadly touch, non-expeller insecticides are currently preferred over them due to trophallaxis and late death rates (Shafiei Alavije *et al*., 2014; Udousung *et al*., 2022). Finding the optimal ppm concentration of the active ingredients in those two insecticides will help to minimize evaporation and optimize efficacy. This function uses either way feeding or transitory procedures to obtain and disseminate the pesticide within a specified area. The present study aimed to identify a unique spinosad concentration that will help the soil management of termites, *M. diversus*. The study intended to provide a distinctive and environmentally friendly remedy for termite annoyance.

MATERIALS AND METHODS

Bait station

Each bait station comprised a plastic container 16 cm tall and 6.5 cm in diameter. Five tiny holes, each with a diameter of 0.5 cm, were punched through the base of the container, and four side slots of 8 cm long and 4 cm wide (Cheraghi *et al*., 2013).

Bioassay of imidacloprid and spinosad for termite control in wood

An orchard in Al-Jadriyah, Baghdad, was the site of the experiment. A severe infestation of *Microcerotermes diversus* Silve. termites were found in six olive plants *Olea europaea*. Each tree stem had seven holes around it, each with a diameter of 6.5 cm and a depth of 15 cm. A plastic container was placed in each hole to represent the bait station. A palm frond, considered an attractive bait, was cut into pieces 15 cm long. 42 bundles were prepared, each consisting of tying three palm fronds with a metal wire. The bundles were buried in the soil after drying the wood in an electrical furnace at 120°C for 48 hours. Each of the three bundles was placed in one plastic container and buried in the soil, leaving only the cover visible. The inspection was performed every three days until all the palm fronds in the stations were infested with termites.

Seven large plastic containers with a height of 50 cm and a diameter of 25 cm were prepared. Six containers containing 2 liters of concentrations (200, 400, 600) ppm for the insecticide imidacloprid (75 WP) (The soil-applied termiticide, imidacloprid (Premise®, Hachikusan®) was registered for termite control in Japan in 1993). The control group did not receive any insecticide treatment The insecticide spinosad (240 SC) spinosad is the first spinosyn insecticide introduced to the market by Dow Agrosciences in 1997, spinosad effectively controls the insects of the groups Isoptera (termites) (20 ml/liter) for each of the two insecticides, each concentration in a container, there are two liters of distilled water in container 7. 42 bundles of palm fronds were prepared using the same previous methods mentioned above. Weighing each bundle individually, we recorded its weight. Six bundles of palm fronds were placed in each concentration of the two insecticides, and after 24 hours it was found that the bundles had absorbed an amount of 750 ml for each concentration, as well as distilled water. Following confirmation that all palm frond bait traps were thoroughly infested with termites, the packages were carefully retrieved and those treated with the two specified insecticides were positioned in the bait stations. Bundles of treated palm fronds were distributed randomly to the bait stations. The termites were then manually extracted from the infested palm frond packages by gently tapping and disassembling the fronds, and the collected termites were subsequently transferred and reintroduced into the treated packages placed within the bait traps. At the beginning of the 6 -month study, all tunnels made by termite workers were removed from the infested trees. A code for each treatment was written on the cover of the bait station likewise on a card placed inside the bait station.

The bait stations were inspected monthly to determine the change in the severity of the termite infestation, and the trees were used to count the number of termite individuals, including workers and soldiers, in the tunnels for a distance of 20 cm. After 6 months of the study, the bundles of palm fronds were removed from the bait stations and transported to the laboratory. The termite individuals were removed from the palm fronds using sieves with different openings placed one atop the other. At the bottom was a box with dimensions of 33 x 33 x 53 cm lined with sterile filter paper and moistened with distilled water for separation of termite individuals and their attraction to moisture. Termite individuals were counted separately for each treatment package. After completely removing the termite individuals from the palm fronds, the palm fronds were gently washed to remove dust and dirt and then dried in an oven at 120 °C for 12 hours. They were weighed and the weight loss percentages were calculated using the following equation. The duration of the study was from 4/2/2019 to 10/2/2019.

Damage wood percentage = Damage wood / Total wood X 100 Eq.1

Statistical analysis

The field experimentations were intended to approve the Randomized Complete Block Design (RCBD) and utilize the minimum important variance measure on the (0.05) grade to compare the outcomes. The impact of independent variables on research limitations was assessed using the Statistical Analysis System (SAS) Arithmetical Examination Arrangement in 2012. The Least Significant Difference (LSD) examination was utilized to compare income in this research. The SAS Arithmetical Examination (2012) methodology was employed to elucidate the influence of variance factors (Factorial experiment) on research constraints. The least significant difference (LSD) test for Analysis of Variance (ANOVA) was utilized to compare the revenues in this study.

RESULTS AND DISCUSSION

The study outcomes demonstrated that varying concentrations of imidacloprid and spinosad termiticides were notably efficacious in diminishing *M. diversus* infestation. Tables 1 and 2 present a significant increase in the percentage of infestation when the bait stations were examined one month after the trial started, especially when it came to the pesticide imidacloprid at a dosage of 200 ppm, where it peaked at 75%. This elevation was supported by the significant presence of 3120 worker insects and the correlated 55% drop in palm frond weight. Despite the low infestation rate, which reached 55% for the pesticide spinosad at a 600-ppm concentration, a 10% association was found between the percentage of infestation and the percentage of weight loss of the palm fronds. At the same time, 500 worker insects were present. When imidacloprid concentrations are 200 and 400 ppm, it is noticed that the infection percentage gradually declines. However, infestation was still present six months into the trial, with rates of 28% and 10%, respectively, ongoing observation. However, by the sixth month of the trial, the concentrations of the termiticides spinosad at 200 ppm and imidacloprid at 600 ppm. However, the concentrations of the termiticides spinosad at 200 ppm and imidacloprid at 600 ppm showed a similar decrease in palm frond infestation, reaching nil. In support of this, the weight loss percentages were 40% and 38%, respectively, and the corresponding populations of *M. diversus* workers were 2100 and 1850. The percentage of infestation of palm fronds treated with spinosad at a concentration of 400 ppm was initially at 60% after one month of the study commencing. Subsequently, the infestation gradually decreased and eventually reached zero after five months of study. The resulting percentage loss in the weight of palm fronds after six months was 25%, while the population of *M. diversus* workers tallied up to 1120. The results indicated that a spinosad concentration of 600 (ppm) was the most effective treatment tested in this study. After 4 months, the percentage of *M. diversus* infestation was observed to have reached zero percent under this spinosad concentration. Table 1 highlights notable variations in infection rates across different concentrations of insecticides and periods during the study. Moreover, significant differences were observed in the interaction between insecticide concentrations and study periods (P≤0.05). The control group consistently exhibited 100% infestation rates, an 80% reduction in palm frond weight, and a peak of 5820 workers over the study duration. Table 2 shows the variations in weight loss percentages of palm fronds attributed to different concentrations of two termite pesticides. These differences correspond to variations in the quantities of worker and soldier termites observed in each bait station across varying pesticide concentrations. Furthermore, significant differences in the total termite population within bait stations were observed for each concentration of imidacloprid and spinosad (P≤0.05).

Table 3 illustrates the tunnel lengths constructed by *M. diversus* workers. An average length of 20 cm was initially established for worker activity before treatment initiation. Interestingly, tunnel lengths remained constant at 20 cm for two months post-treatment commencement with the termiticides imidacloprid and spinosad across all concentrations, except for the 600-ppm concentration of spinosad. For this concentration, tunnel length reached zero after one month of treatment and persisted until the end of the trial. Analysis revealsed significant variations between treatments across different insecticide concentrations and study months. Statistical significance (P≤0.05) was observed when examining the interaction effects between treatments and periods. Table 3 illustrates the persistent presence of tunnels despite minimal lengths, suggesting impaired activity and functionality without worker termites. This observation indicated reduced tunnel construction capacity due to the impact of insecticidal treatments on termite populations over time. Following the construction of small tunnels, termites retreated to the interior of olive trees or their colony, where they inevitably perished. Consequently, tunnels might be visible even in the absence of worker termites. Unlike what they usually do, termites did not die inside the tunnels; instead, they returned to their nest or natural habitat before passing away. Termites typically hide in trees or the colony to discard deceased members, relying on other *M. diversus* workers within the settlement for removal.

About the *M. diversus* worker population within the tunnels, as delineated in Table 4, a reduction was noted after two months of treatment. Specifically, the worker count dropped to zero after one, two, and three months of exposure to spinosad insecticide concentrations of 600, 400, and 200 ppm, correspondingly, and this pattern continued throughout the study duration. The impact of imidacloprid on worker populations appeared to

Table 1. Percentage of infestation of bait stations with termites *Microcerotermes diversus* treated with different concentrations of the termiticide imidacloprid and the termiticide spinosad

Insecticide Concentrations	Termite infestation %					
	2/5/2019	**2/6/2019**	**2/7/2019**	**2/8/2019**	**2/9/2019**	**2/10/2019**
Imidacloprid 200	75[a]	72[a]	65[a]	50[a]	30[a]	28[a]
Imidacloprid 400	70[b]	61[a]	53[a]	40[a]	26[b]	10[a]
Imidacloprid 600	65[a]	50[a]	42[a]	28	10	0[a]
Spinosad 200	64[a]	48[a]	33[a]	22[b]	10[a]	0[a]
Spinosad 400	60[a]	48[a]	30[b]	15[b]	0[a]	0[a]
Spinosad 600	55[a]	20[a]	5[a]	0[b]	0[b]	0[b]
Control: Without any insecticide treqtment	100	100	100	100	100	100
LSDvalue (P≤0.05)	Concentration= 7.59 *; Time = 7.,02 *;Conc. X; Time = 12.93 *.					

be less significant than that of spinosad at the 600-ppm concentration while showing similar effects at the 200-ppm concentration. Consequently, the worker population was eradicated after 3, 4, and 5 months of imidacloprid exposure at concentrations of 600, 400, and 200 ppm, respectively, a trend that persisted until the conclusion of the research period. The tunnel lengths exhibit a diminishing trend toward zero for imidacloprid concentrations of 600, 400, and 200 ppm after 5, 5, and 6 months, respectively. Both the control group and treated groups with imidacloprid and spinosad experienced diminishing worker populations, resulting in a gradual reduction in tunnel lengths until reaching zero after 6 months of the study. In the impacted region, this served as strong proof of eradicating colonies, where termites had been infesting and damaging olive trees, showcasing their susceptibility to termiticide interventions. It is conjectured that a neonicotinoid insecticide could impact the foraging behavior of workers in the field, Carvacrol has been shown to produce potent, acute toxicity to insects, nematodes, and mites, potentially leading to a scenario where an insufficient number of workers can return to the colony for the horizontal transfer of this termiticide to other workers.

Orageno vulgare extract or its derivatives might be applied to exert control and manage some termite infestations and lessen or limit the quantity of additional poisonous pesticides currently in use (Salem *et al*., 2020). The role of *Metarhizium anisopliae* in influencing termite behavior remains ambiguous, specifically regarding its effects on mortality versus repellent properties in *Coptotermes formosanus*. This distinction is critical; if *M. anisopliae* functions solely as a repellent, its efficacy in eliminating termite populations, especially in structures like temples and historical monuments, would be significantly reduced (Wright and Cornelius, 2012). A laboratory bioassay was developed to test the toxicity, repellency, and tunneling behavior of imidacloprid against *Microtermes obesi* (Holmgren), >90% of termites died after 96 h exposure to 100 µg mL^{-1} of imidacloprid after 168 h all the termites died at all the tested concentrations.

Efficacy of imidacloprid and spinosad against *Microcerotermes diversus* on the stems of Olive trees (Manzoor *et al*., 2014b; Abbas *et al*., 2021). Nonetheless, it can prove effective as a soil barrier treatment. Imidacloprid has been associated with the cessation of termite feeding on baits, trophallaxis, and mutual grooming. These results suggest that chlorfenapyr, imidacloprid, and thiamethoxam may be used as soil termiticides, whereas fipronil can be used as soil termiticide and in termite baiting programs. (Iqbal *et al*., 2019; Oi, 2022). In various research studies, workers displayed immobility or reduced locomotion upon exposure to even minimal doses of imidacloprid. In additional studies, termites displayed immobility or decreased movement upon exposure to small amounts of imidacloprid. The reduced bait-feeding and negative impact of imidacloprid on termite movement makes it less suitable for termite baiting (Chand *et al*., 2018). However, upon comparing the influence of spinosad concentrations with that of imidacloprid across all months of the year, it was found that spinosad exhibited a significantly greater effect regarding both tunnel height and the number of workers. The efficacy of these active ingredients can be attributed to their unique and versatile mode of action, which sometimes positions them as the sole alternative to other termiticides.

Spinosad demonstrates efficacy against insects through contact, ingestion, or a mix of both methods (Trophallaxis and grooming) (Quarcoo *et al*., 2012; Ranjith *et al*., 2021; Udousung *et al*., 2022). Unlike other termiticides, spinosad does not affect areas where the performance of other termiticides is successful. Consequently, it operates through a unique mode of action. Its primary target sites include the nicotinic acetylcholine receptors (nAChRs) and, to a lesser extent, GABA neurotransmitter receptors (Saljoqi *et al*., 2014; Ravanshadi *et al*., 2023). Overall, the effectiveness of spinosad was notably pronounced in the extermination

Table 2. Percentage of weight loss in wood treated with different concentrations of imidacloprid and spinosad and the number of termites

Insecticide Concentrations (ppm)	Weight loss percentage	Number of workers (Worker/station)	Number of soldiers (Soldier/station)	Total number of termites for each station
Imidacloprid 200	55	3120	20	3140
Imidacloprid 400	48	2740	16	2756
Imidacloprid 600	40	2100	12	2112
Spinosad 200	38	1850	8	1858
Spinosad 400	25	1120	4	1124
Spinosad 600	10	500	2	502
Control: Without any insecticide treqtment	80	5820	35	5855
LSD value (P≤0.05).	6.791 *	81.663 *	4.517 *	108.558 *

Microcerotermes diversus after 6 months of treatment

Table 3. Tunnel lengths in olive trees surrounded by bait stations treated with varying concentrations of imidacloprid and spinosad over different treatment durations

Insecticide Concentrations	Lengths of tunnels formed / 20 cm						
	Before treatment	After1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 6 months
Imidacloprid 200	20	20	20	14	8	3	0
Imidacloprid 400	20	20	20	12	7	0	0
Imidacloprid 600	20	20	20	9	3	0	0
Spinosad 200	20	20	20	5	0	0	0
Spinosad 400	20	20	20	0	0	0	0
Spinosad 600	20	0	0	0	0	0	0
Control: Without any insecticide treqtment	20	20	14	9	5	1	0
LSD value (P≤0.05).	Treatment= 3.88 *, Time/Month = 3.88 *, Treatment. x Time = 6.21 *.						

Table 4. Quantification of termite worker populations in tunnels of olive trees surrounded by bait stations treated with various concentrations of imidacloprid and spinosad after varied treatment durations

Insecticide concentrations (ppm)	Number of termites in the tunnels						
	Before treatment	After1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 6 months
Imidacloprid 200	15	11	8	5	1	0	0
Imidacloprid 400	12	8	6	2	0	0	0
Imidacloprid 600	7	5	3	0	0	0	0
Spinosad 200	7	4	1	0	0	0	0
Spinosad 400	5	3	0	0	0	0	0
Spinosad 600	2	0	0	0	0	0	0
Control: Without any insecticide treqtment	20	16	12	8	5	1	0
LSD value (P≤0.05).	Treatment= 2.76 *; Time/Month = 2.76 *; Treatment. x Time = 4.36 *						

of *M. diversus* workers, which are known to cause significant damage to palm fronds and olive trees. As termites are major pests in tropical developing countries with substantial economic impact proportions, the cost-effective baiting strategy will most likely help in pest control at a more affordable cost, with minimal ecological impact.

Conclusion

This study demonstrated that varying concentrations of imidacloprid and spinosad are highly effective in reducing infestations of *Microcerotermes diversus* in olive trees. Notably, imidacloprid at 200 ppm initially led to a peak infestation rate of 75%, followed by a gradual decline at higher concentrations, while spinosad at 600 ppm achieved complete eradication by the fourth month, with zero % infestation reported. The corresponding reductions in palm frond weight and worker populations reinforced the efficacy of these insecticides, with significant differences observed across concentrations and study periods (P≤0.05). Despite lower initial effects, spinosad showed sustained efficacy, achieving a 25% weight loss in palm fronds after six months with a corresponding worker reduction. The persistent presence of tunnels, even in the absence of active termites, indicated impaired construction activity and difficulty in colony survival, highlighting the impact of insecticide treatment on termite behavior. Overall, this research confirms that both imidacloprid and spinosad are viable solutions for managing *M. diversus* infestations, with spinosad at higher concentrations providing the most effective long-term control. Future studies should explore the effects of these therapies on the environment and the possibility of resistance development to optimize their use in termite management strategies.

ACKNOWLEDGEMENTS

The authors thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad-Iraq for its support in this study.

Conflict of interests

The authors declare that they have no conflict of interest.

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