

Indoor Toxicity Test of Two Botanical Insecticides on Ghost Ants

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Research Article

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Abstract

Tapinoma melanocephalum (ghost ant) is a widespread invasive pest that causes significant indoor and outdoor issues, including the potential transmission of pathogens, aiding the spread of plant pests, and threats to native species. To identify effective and environmentally friendly control methods, this study evaluated the indoor contact toxicity of two botanical insecticides, rotenone and matrine, against the workers of ghost ants. The experiment employed the film residue method in a simulated environment at 30°C and 50% humidity, testing five concentration gradients from 6.25 µg/mL to 100 µg/mL. Mortality was recorded after 24 hours, and data were analyzed using Probit regression. Results indicated that both botanical insecticides exhibited effective contact toxicity against ghost ants. At the highest concentration of 100 µg/mL, rotenone achieved a corrected mortality of $92.73\% \pm 6.30$, while matrine reached $83.64\% \pm 16.36$. Furthermore, the LC_{50} of rotenone was 29.121 µg/mL, which was lower than the LC_{50} of 40.273 µg/mL for matrine. In conclusion, rotenone demonstrated significantly higher contact toxicity than matrine, making it a more suitable natural insecticide for controlling ghost ants.

Introduction

Tapinoma melanocephalum (Fabricius, 1793) is an ant species of the genus *Tapinoma*, subfamily Dolichoderinae, family Formicidae, order Hymenoptera. It is called ghost ant because of its light-colored abdomen and legs, dark brown head and thorax, and fast-moving speed. The geographic origin of ghost ants remains unclear, with possible origins in Africa or Asia, but they have indeed spread widely all over the world as an invasive tramp species due to human trade (Wetterer, 2009). Currently, they have been recorded in 97 countries and regions, spanning tropical, subtropical, and temperate zones in both the Old and New Worlds (Antmaps, 2026). *Tapinoma melanocephalum* is one of the most common invasive ant species. They are ubiquitous indoor and outdoor pests in temperate and subtropical regions and exist as indoor pests in temperate zones, ranking as highly invasive ant species second only to *Paratrechina longicornis* (yellow crazy ant) and *Monomorium pharaonis* (pharaoh ant) in terms of their invasive range (Wetterer, 2009; Klimeš & Okrouhlík, 2015). Ghost ants have remarkable adaptability, being capable of building nests in a wide range of confined spaces both indoors and outdoors, such as in flowerpots, under objects on the ground, under loose bark, and at the bases of palm fronds, cracks, spaces between books, wall voids, etc. (Nickerson et al., 2003). An ant colony typically consists of hundreds to thousands of worker ants and multiple queens (Harada, 1991). They have low territorial awareness towards conspecifics, and there is some evidence to suggest that they can form super colonies like *Linepithema humile* (Argentine ant), *Paratrechina longicornis* (yellow crazy ant), and *Solenopsis invicta* (red imported fire ant) (Debout et al., 2007). In terms of diet, ghost ants have a strong preference for carbohydrates and proteins, often feeding on sugar, honey, sweet foods, honeydew, insect corpses, etc. (Luo & Wang, 2011).

As a common household and outdoor invasive pest, ghost ants have various significant impacts on human life. Their indoor activities and feeding behaviors affect daily life and make them potential vectors for disease transmission. The research of Moreira et al. (2005) shows that ghost ants are the most common ant species in three medical institutions in Brazil, with 63.1% of specimens identified as

Tapinoma melanocephalum. 14 pathogenic bacteria species, including antibiotic-resistant strains, were detected on their bodies. In agriculture and forestry, the interspecific interaction between ghost ants and scale insects poses threats to plants. The studies by Zhou et al. (2014), Xu et al. (2019), and Shi et al. (2020) indicate that the behavior of ghost ants caring for the invasive scale insect species *Phenacoccus solenopsis* to obtain honeydew effectively aids the expansion and reproduction of the scale insect population and reduces the population of its natural enemies parasitic wasps and lady beetles. They pose similar hazards to plants grown in greenhouses and are considered impossible to control due to the restrictions on the use of toxic substances in these greenhouses (Nickerson et al., 2003). Liang et al. (2021) found that the attack and predation of *Tapinoma melanocephalum* on pollinators in Hawaii pose threats to 6 native plants and their native pollinators. Ghost ants are also harming other native ant species. Zhao et al. (2026) found that *Tapinoma melanocephalum* and several other invasive ant species significantly reduced the taxonomic, functional, and phylogenetic diversity of native ants and dominated the ant specimens collected from bait traps. Rahim and Ohkawara (2019) conducted a study on ants living on citrus and durian trees in Tarakan Island, North Kalimantan, and found that *Tapinoma melanocephalum* reduces the population of the native ant species *Oecophylla smaragdina*. Piva and De Carvalho Campos' (2011) research on ant populations in the urban area of São Paulo, Brazil, revealed that *Tapinoma melanocephalum* has replaced other native ant species as an invasive species. In the assessment of the socioeconomic and environmental impacts of invasive ants conducted by Gruber et al. (2022), the Environmental Impact Classification for Alien Taxa (EICAT) rating for *Tapinoma melanocephalum* was assessed as MR (Major), and the Socioeconomic Impact Classification for Alien Taxa (SEICAT) was assessed as MN (Minor).

Botanical insecticides are natural secondary metabolites produced by plants during their defense against harmful organisms. They are considered environmentally friendly, easily degradable, and low-residual insecticides due to their characteristics as natural products and have a long history of application in human history. At present, more than one thousand species of higher plants have been identified to contain substances exhibiting insecticidal activity (Li et al., 2022). In this research, I chose two natural botanical insecticides: rotenone, which belongs to the flavonoid class, and matrine, which belongs to the alkaloid class, to conduct indoor contact toxicity tests on workers of *Tapinoma melanocephalum*. In order to find effective and environmentally friendly botanical insecticides for chemical and biological control of ghost ants.

Material and Methods

Experimental Insects

The ants for the experiment were collected in Fangchenggang City, Guangxi Province, China, on August 21st, 2024. The specific coordinate of the collection site is 21°45'5.09" N, 108°19'8.71" E. Based on the description of Wu and Wang (1995), the ants collected are identified as *Tapinoma melanocephalum* (ghost ant). Before the experiment, the ants were kept in an artificial ant nest made of plaster for 8 d, and

were fed with sugar water, mealworms, and honey. Talcum powder was applied to the inner wall of the container to prevent the ants from escaping.

Test Insecticides

The purchased 95% pure rotenone technical material and 98% pure matrine technical material utilized in the experiment were manufactured by Meryer (Shanghai) Biochemical Technology Co., Ltd. Before the experiment, two technical materials were dissolved in acetone to prepare stock solutions with a concentration of 1000 µg/mL. The stock solutions were then diluted with acetone to five gradients: 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL.

Bioassay Methodology

The indoor contact toxicity tests of two insecticides against ghost ants were conducted from August 29th to August 30th, 2024, and from September 4th to September 5th, 2024, at the Pesticide and Weed Research Office of the Institute of Plant Protection, Gansu Academy of Agricultural Sciences. The experiment employed the film residue method for contact toxicity assay, referencing the method described by Liang et al. (2022), but modifying the container from $\varnothing 2.5 \times 7.5$ cm glass tube to $\varnothing 5.0 \times 7.0$ cm 100 mL glass beaker. Use a pipette to take 1 mL of acetone solution of the agent and add it to a beaker. Shake the beaker to ensure even distribution of the solution until it fully evaporates, forming a uniform layer of agent film. Take 20 test insects with a small brush and place them into a beaker. Apply talcum powder around the opening of the beaker and cover it with non-woven fabric to prevent the ants from escaping. Place a 2.5 mL centrifuge tube filled with DI water and blocked with a cotton ball to ensure water supply. Place the container inside the RXZ-380 artificial climate chamber produced by Ningbo Jiangnan Instrument Factory and set the temperature to 30°C and humidity to 50% to simulate the environment of tropical zone. Record the number of deaths after 24 h. Use a small brush to touch the insect body. Ants without spontaneous reaction are considered dead. Each concentration of the two insecticides was tested in triplicate, and a control group treated only with pure acetone was also established, with all other conditions being the same to calculate the mean mortality and corrected mortality.

Data Analysis

The data analysis followed the method of Jia (2006), utilizing the Probit regression analysis model in IBM® SPSS® Statistics 27.0 to analyze the experimental data. The toxicity regression equations, LC_{50} , 95% confidence intervals, χ^2 , standard error, and p -values for the two insecticides were calculated. The calculation of the corrected mortality rate was based on Abbott's formula mentioned by Piepho et al. (2024) and conducted using Microsoft Excel.

Results

The results of indoor contact toxicity tests on *Tapinoma melanocephalum* using the film residue method with two botanical insecticides rotenone and matrine, showed that both insecticides exhibited effective contact toxicity against the ghost ants. At the temperature of 30°C and the humidity of 50%, the corrected mortality of ghost ants exposed to the highest concentration of 100 µg/mL of two insecticides, rotenone and matrine, were 92.73% ± 6.30 and 83.64% ± 16.36, respectively (see Table 1). The LC₅₀ of rotenone is 29.121 µg/mL and the 95% confidence interval is 23.614–35.330 µg/mL. The LC₅₀ of matrine is 40.273 µg/mL and the 95% confidence interval is 32.735–49.767 µg/mL (see Table 2). From the data above, it can be seen that the contact toxicity effect of rotenone on ghost ants is significantly higher than that of matrine. In the chi-square goodness-of-fit test, with 3 degrees of freedom, the χ^2 value for rotenone is 5.155, with a *p*-value of 0.161, and the χ^2 value for matrine is 2.392, with a *p*-value of 0.495 (see Table 2). The *p*-values for both insecticides in the chi-square goodness-of-fit test were greater than 0.05. Within the context of this test, it signifies that the data exhibit good fit, with no statistically significant difference between the actual observed values and the values predicted by the model. The toxicity regression equations for both insecticides conform to the actual conditions.

Table 1
 24 h Corrected Mean Mortality of *Tapinoma melanocephalum* at Different Concentrations of Rotenone and Matrine

Insecticide	Corrected Mortality (%)				
	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Rotenone	15.76 ± 13.65	18.18 ± 5.45	32.73 ± 25.78	70.91 ± 37.13	92.73 ± 6.30
Matrine	7.27 ± 5.45	9.09 ± 8.33	36.36 ± 46.39	52.73 ± 27.45	83.64 ± 16.36
Note. The data in the table are presented as <i>x</i> ± <i>SD</i> .					

Table 2
 Indoor Contact Toxicity of Rotenone and Matrine against *Tapinoma melanocephalum* (24 h)

Insecticide	Regression Equation	LC ₅₀ (µg/mL)	95% Confidence Interval (µg/mL)	χ^2	Standard Error	<i>p</i> -value
Rotenone	y = – 3.361 + 2.295x	29.121	23.614–35.330	5.155	0.300	0.161
Matrine	y = – 3.570 + 2.224x	40.273	32.735–49.767	2.392	0.315	0.495

Conclusions and Discussion

This research aims to evaluate and contrast the indoor contact toxicity of two botanical insecticides, rotenone and matrine, against *Tapinoma melanocephalum*, with the objective of finding a natural insecticide that exhibits higher toxicity on ghost ants while being environmentally friendly. The experimental results showed that among the two insecticides tested at the highest concentration

designed, rotenone exhibited better performance with the corrected mortality rate of $92.73\% \pm 6.30\%$ against ghost ants. Its LC₅₀ of 29.121 µg/mL was also lower than that of matrine, which had an LC₅₀ of 40.273 µg/mL. In summary, rotenone is a more suitable insecticide for controlling ghost ants in this research.

There have been numerous studies in the academic community on the control of ghost ants. Klotz et al. (1996), Ulloa-Chacón and Jaramillo (2003), Wang and Luo (2011), and Cerioni et al. (2011) conducted researches on the control of ghost ants using baits such as boric acid, fipronil, hydramethylnon, diflubenzuron, imidacloprid, and borax, and determined that boric acid, borax, and fipronil are effective and suitable insecticides for controlling ghost ants. Although Wang and Luo (2011) believed in their study that imidacloprid baits were ineffective in controlling ghost ants, Braness (2002), Brooks et al. (2008), Bao (2011), and Klimeš and Okrouhlík (2015) reached the opposite conclusion in their studies, stating that imidacloprid is an effective and highly efficient stomach-toxic insecticide for controlling ghost ants. Scheurer et al. (1999) and Gusmão et al. (2008) both achieved good results in controlling ghost ants using fluorooctane sulfonamide (ALSTAR®) bait and methoprene bait, respectively. In terms of contact insecticides, Qiu et al. (2013) studied the contact toxicity of fipronil to ghost ants and concluded that ghost ants exhibit high sensitivity to fipronil, significantly higher than that of the other two ant species. In terms of biological control against ghost ants, Carvalho et al. (2018) studied the repellent effect of pygidial secretions of dung beetles on ghost ants and concluded that they could effectively repel ghost ants. Polanczyk et al. (2012) found that *Bacillus thuringiensis* could effectively control ghost ants. Brettell et al. (2017) extracted an RNA virus from ghost ants in Hawaii that could be used for their control. However, there is little research on the use of botanical insecticides or plant extracts for the control of ghost ants.

The two botanical insecticides, rotenone and matrine, used in this research have been proven effective against the invasive species, which also belongs to the Formicidae, the red imported fire ant (*Solenopsis invicta*), by Liang et al. (2022) in previous research. This study only evaluated their contact toxicity against ghost ants in a specific indoor environment. Further research is needed on the stomach toxicity and practical control effectiveness of ghost ants. In practical control scenarios, due to the influence of various factors such as terrain, temperature, and humidity, it may be considered to increase the dosage or combine with other proven insecticides to enhance control effectiveness.

Declarations

Conflict of interest

The authors have no financial or proprietary interests in any material discussed in this article.

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Author Contribution

The sole author designed the study, analyzed the data, and wrote the manuscript. The author has read and approved the final manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. 24 h corrected mortality is provided in Table 1, along with original reference describing the corrected mortality used in this study. Data of toxicity test is provided in Table 2, along with original reference describing the data of toxicity test used in this study.

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