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ASSOCIATION OF P-CYMENE WITH ZINC OXIDE NANOPARTICLES AFFECTING THE BEHAVIOR OF *Hypothenemus hampei* (COLEOPTERA: SCOLYTINAE)

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ABSTRACT

The coffee berry borer, *Hypothenemus hampei*, is one of the main pests of coffee crops due to its ability to colonize the interior of fruits and directly reduce yield and bean quality. This study evaluated the effect of the monoterpene p-cymene, in its pure form and associated with zinc oxide nanoparticles (ZnO-NPs), on the locomotor activity of adult females from ten Brazilian populations of *H. hampei*. The nanoparticles exhibited a semi-spherical morphology and a particle size distribution ranging from 5.1 to 53.2 nm, with a mean size of 24.16 nm, indicating a predominance of nanoscale particles with high relative surface area. In the behavioral assays, the association between ZnO-NPs and p-cymene reduced insect walking distance across all evaluated populations when compared to their respective controls. This pattern suggests that nanoengineering potentiated the interference of p-cymene on coffee berry borer locomotion. The observed response is consistent with studies demonstrating that chemical compounds can induce sublethal effects on insect locomotor behavior, including *H. hampei*, and that locomotor alterations may represent sensitive indicators of physiological stress or sublethal intoxication. Therefore, the association between p-cymene and ZnO-NPs shows potential as a complementary strategy for the behavioral management of *H. hampei*.

KEYWORDS: *Coffee berry borer*, Monoterpenes, Locomotor activity.

ASSOCIAÇÃO DE P-CIMENO COM NANOPARTÍCULA DE DIÓXIDO DE ZINCO AFETANDO O COMPORTAMENTO DE *Hypothenemus hampei* (COLEOPTERA: SCOLYTINAE)

RESUMO

A broca-do-café, *Hypothenemus hampei*, é uma das principais pragas da cafeicultura por colonizar o interior dos frutos e reduzir diretamente a produtividade e a qualidade dos grãos. Este trabalho avaliou o efeito do monoterpeno p-cimeno, em formulação pura e associado a nanopartículas de óxido de zinco (ZnO-NPs), sobre a atividade locomotora de fêmeas adultas de dez populações brasileiras de H. hampei. As nanopartículas apresentaram morfologia semi-esférica e distribuição de tamanho entre 5,1 e 53,2 nm, com média de 24,16 nm, indicando predominância de partículas em escala nanométrica com elevada área superficial relativa. Nos ensaios comportamentais, a associação entre ZnO-NPs e p-cimeno reduziu de a distância de caminhamento dos insetos em todas as populações avaliadas, quando comparada aos respectivos controles. Esse padrão sugere que a nanoestruturação potencializou a interferência do p-cimeno sobre a locomoção da broca-do-café. A resposta observada é compatível com estudos que demonstram que compostos químicos podem produzir efeitos subletais sobre o comportamento locomotor de insetos, incluindo *H. hampei*, e que alterações de locomoção podem representar indicadores sensíveis de estresse fisiológico ou intoxicação subletal. Dito isso, a associação entre p-cimeno e ZnO-NPs apresenta potencial como estratégia complementar para o manejo comportamental de *H. hampei*.

PALAVRAS CHAVE: Broca-do-café, Monoterpeno, atividade locomotora.

INTRODUCTION

Modern agriculture seeks to maximize the efficiency of control methods, the adaptation of emerging technologies, and the use of molecules with lower toxicity to humans and the environment. In this context, management strategies can achieve agronomic efficiency while simultaneously reducing impacts on natural enemies (JESCHKE, 2016). Among the country’s most traditional crops, coffee stands out as an example requiring imperative modernization throughout the production chain. Despite its longstanding importance, the control of key pests such as the coffee berry borer and the coffee leaf miner remains below satisfactory levels.

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytinae), is the most economically important pest in global coffee production, causing direct losses in both yield and the sensory quality of coffee beans (VEGA *et al*., 2015; JOHNSON *et al*., 2020). Damage begins when the colonizing female perforates the fruit for oviposition; subsequently, the larvae destroy the endosperm, favoring colonization by opportunistic microorganisms and accelerating fruit degradation (ALBA-ALEJANDRE *et al*., 2018). In Brazilian territory, the geographic distribution of the pest and its ability to survive in fruits remaining after harvest make its management a persistent challenge for the coffee sector (ARISTIZÁBAL *et al*., 2023).

The difficulty in controlling CBB lies primarily in its cryptic lifestyle. By remaining protected inside the fruits for most of its biological cycle, the pest’s exposure to conventional insecticides is severely limited (ADAMS *et al*., 2016; DAVI JÚNIOR *et al*., 2021). This natural barrier requires the use of molecules with high penetration capacity or persistence, factors that intensify the risk of resistant populations and adverse environmental impacts (BRUN *et al*., 1989; GUEDES, 2017). In this context, the development of nanoengineered systems has emerged as a promising approach to overcome such biological barriers and optimize the delivery of active compounds (AIGBEDION-ATALOR *et al*., 2024; MORENO-RAMIREZ *et al*., 2024).

Nanoformulated insecticidal systems with multifunctional modes of action offer an alternative that extends beyond the mere enhancement of intrinsic toxicity, by modifying the interactions between active compounds and the insect microenvironment (CHANG *et al*., 2012; WEI *et al*., 2025). In addition to their intrinsic effects, the association between nanoparticles and insecticides may alter physicochemical properties related to stability, bioavailability, and release kinetics (SABER *et al*., 2025; AHAMAD *et al*., 2026). In diamide insecticides, changes in release kinetics and environmental persistence have already been reported for nanoformulated systems (HOU *et al*., 2025).

Metal oxide nanoparticles and inorganic matrices may act through multiple mechanisms in plant protection, including the generation of reactive oxygen species (ROS), membrane disruption, and mitochondrial impairment (CHANG *et al*., 2012). This broad spectrum of physiological effects is strategic, as it may reduce the likelihood of resistance evolution under field conditions (THABET *et al*., 2025). Recent evidence indicates that pure ZnO, CuO, and CeO_2 nanoparticles, even when applied alone, are capable of inducing sublethal effects, repellency, and delays in the larval development of CBB (BUFALO *et al*., 2025; SOUSA *et al*., 2025). Therefore, associating the protective stabilization provided by these inorganic matrices with the bioactivity and volatility of botanical compounds emerges as a promising approach. Accordingly, this study evaluated the impact of the monoterpene p-cymene, in its pure and nanoengineered forms associated with zinc oxide nanoparticles (ZnO), on the locomotion and movement behavior of ten Brazilian populations of adult female CBB, aiming to determine the potential of this technology to modulate locomotor activity and sensory responses of the pest.

MATERIALS AND METHODS

Insect Population Collection

To obtain the coffee berry borer (CBB) populations, infested coffee fruits were collected in the states of Minas Gerais, São Paulo, Espírito Santo, Bahia, and Paraná. These states are among the largest coffee-producing regions in Brazil. In total, 10 CBB populations were obtained (Table 1).

TABLE 1. Collection locations of *Hypothenemus hampei* populations from different Brazilian states.

Population	Locate	Latitude (S)	Longitude (W)
1	Patrocínio/MG	18°56'12''	46°56'52”
2	Três Pontas/MG	18°43'96''	47°05'15'
3	Campo do Meio/MG	21°18'57''	45°22'26''
4	Ibiá/MG	21°08'82''	45°44'56''
5	Vermelho Novo/MG	19°25'44''	46°27'10''
6	Unaí/MG	16°19'31''	47°54'21''

7	Jaú/SP	19°26'10''	40°12'49''
8	Luís Eduardo Magalhães/BA	12°03'54"	45°54'12"
9	Londrina/PR	24°15'34"	53°46'48"
10	Linhares/ES	19°23'09"	40°03'59"

States of Brazil: MG: Minas Gerais, SP: São Paulo, BA: Bahia, PR: Paraná and ES: Espírito Santo

Collections were carried out in the lower-middle third of coffee plants during the production stage. The collected cultivars were Catuaí Vermelho IAC 144, Bourbon Amarelo, and Conilon. At each location, 5 kg of coffee fruits (dry, green, and ripe) were collected and placed in paper bags (30 × 45 cm) to obtain a high coffee berry borer population (>1000 borers per location). The samples were collected and sent to the Federal University of Viçosa, Rio Paranaíba Campus, Rio Paranaíba, Minas Gerais, Brazil, for colony establishment.

The infested fruits from each region were subjected to treatment with a 5% sodium hypochlorite solution to eliminate fungal contamination. Immediately afterward, the borers were extracted from the coffee beans, and the collection site of each CBB population was identified. The fruits were placed in PVC tubes (10 cm diameter × 25 cm length) with connection caps containing a nylon mesh (3 mm opening), attached to the tube using elastic bands. After extraction, the borers were transferred to an artificial diet. The artificial diet was prepared under aseptic laboratory conditions, adapted from Portilla and Streett (2006).

Coffee beans of the Catuaí IAC 144 cultivar were processed and ground using a 200 W electric grinder (model B55). After grinding, the material was weighed and mixed with previously sterilized agar for 25 minutes. Before solidification, the sterilized ingredients and the remaining solid ingredients were homogenized in a blender for 3 minutes, after which the liquid ingredients were added last, and the diet was further homogenized for an additional 1 minute in the blender. The diet was then placed in a white plastic tray (30 × 45 × 10 cm), exposed to UV light for 10 minutes, and dried in an oven at 55 ºC for approximately 10 hours.

The diet was then cut into pieces measuring 2.0 × 2.0 × 1.0 cm and transferred to 24-cell Gerbox containers (15 × 10 × 3.0 cm), which were maintained in a BOD-type germination chamber at a temperature of 25 ºC. One borer was added to each diet unit, and every 25 days the insects were transferred to new containers to increase the population. Diets showing the presence of contaminating fungi were separated and discarded.

Compounds

The reagent used for nanoparticle formulations was pure zinc oxide (ZnO) obtained from Sigma-Aldrich®. The secondary compound used was an analytical standard of p-cymene (purity >95%) from Sigma-Aldrich®. Solutions containing ZnO nanoparticle formulations and p-cymene were prepared by separately adding both compounds into the same solution.

Synthesis and Characterization of Nanoparticles Associated with p-Cymene

The p-cymene was used at a concentration of 50 µg/mL in ethanol. Tween 80 solution was used as a surfactant under constant stirring. Subsequently, the mixture was heated to boiling for 15 minutes, followed by cooling and filtration. ZnO synthesis was performed following an adapted method from Motelica *et al.*

(2022). Five grams of zinc acetate dihydrate were added to 45 mL of 1-butanol. The solution was then refluxed for 22 h under continuous stirring and allowed to rest for 22 h at 25 °C. The precipitate was separated by decantation and subjected to repeated centrifugation. Between centrifugation steps, the powder was washed with absolute ethanol, and this process was repeated six times. Subsequently, the obtained white powder was dried in an oven at 85 °C. An aliquot of 0.2 g of the obtained ZnO powder was impregnated with p-cymene (50 µL mixed with 200 µL of ethanol). The mixture was sonicated for 15 min and evaporated at 45 °C in a sterilization oven. The ZnO nanoparticles impregnated with p-cymene were then used in the behavioral assay. The size of the ZnO + p-cymene nanoparticles was measured using a high-resolution transmission electron microscope.

Behavioral Assay

The behavioral bioassay with CBB was conducted using a completely randomized design with 2 treatments and 20 replicates. The treatments consisted of nanoparticles associated or not associated with p-cymene (NPZnO + p-cymene), and the control treatment (NPZnO only). All Brazilian CBB populations were used.

The behavioral response of the mixed CBB population was evaluated in Petri dishes (90 × 15 mm) containing filter paper (Whatman No. 1). The filter paper was divided into two halves, with one half serving as the control and the other receiving the compound treatments (Nano + insecticides). A volume of 200 µL of each treatment was applied to the corresponding half. Subsequently, one adult female CBB was released at the center of the Petri dish, and after 15 minutes the insect was removed and replaced with another individual. Twenty insects were used for each treatment.

Walking activity was evaluated using a digital camcorder (XL1 3CCD NTSC, Canon, Lake Success, NY, USA) equipped with a ×16 video lens (Zoom XL 5.5–88 mm, Canon). A video-tracking system (ViewPoint LifeSciences, Montreal, Quebec, Canada) was used to analyze the videos and measure the distance walked by insects and the time spent resting on each half of the arena.

To interpret the results, the method proposed by Plata-Rueda *et al*. (2019) was used, in which treatments were considered repellent when insects remained for less than 1 s on the treated side, and considered irritant when the time spent on the treated surface was less than 50%.

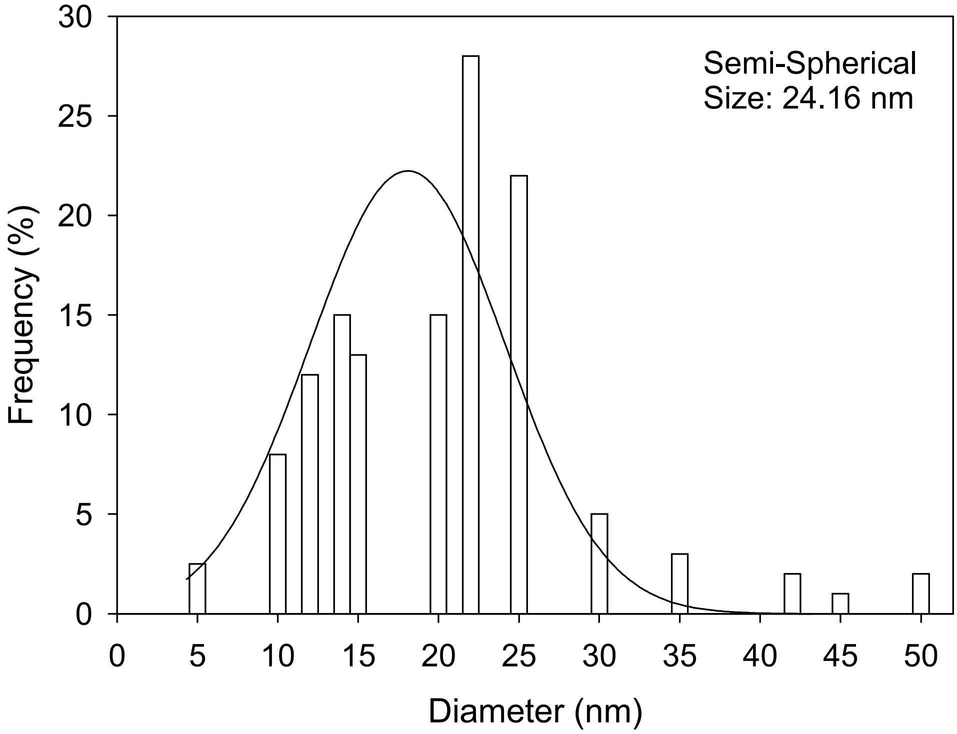
Statistical analyses

Behavioral response data were analyzed by one-way analysis of variance (ANOVA), and Scott Knott's test for comparison of means at the 5% significance level. The data were arcsine transformed to meet assumptions of normality and homoscedasticity and data were analyzed using SAS for Windows v. 9.0.31.

RESULTS AND DISCUSSIONS

The histogram (Figure 1) shows that the larger particles are distributed in clustered groups. The particle size ranged from 5.1 to 53.2 nm, with an average size of 24.16 nm.

FIGURE 1. Transmission electron showing the semi-spherical morphology of ZnO-NPs histogram of particle size distribution.



Source: Authors (2026).

Particles with diameters below 30 nm indicate that the synthesis resulted in a high relative surface area. This aspect is important because smaller particles tend to present a larger surface contact area, favoring the interaction between ZnO nanoparticles, p-cymene, and the insect body surface. In this way, they may contribute to greater dispersion, stability, and availability of the associated compound during behavioral exposure.

The semi-spherical morphology of ZnO-NPs also suggests a relatively adequate distribution for contact with the integument of CBB. The observed nanometric size allows us to infer that the formulation may have increased the physical interaction between p-cymene and the insect. This result is relevant for cryptic-habitat species such as CBB, whose residence inside the fruits limits direct exposure to conventional compounds and hinders traditional control strategies (Plata-Rueda *et al*., 2019).

Exposure to p-cymene associated with ZnO nanoparticles reduced the walking distance of adult females in all evaluated populations (Table 2).

TABLE 2. Walking distance (m) of Brazilian adult *Hypothenemus hampei* populations exposed to the monoterpene p-cymene and control treatments with and without association with nanoengineered systems.

Population	**p-cymene**	**Control**
Associated with nanoparticles[1]		
Patrocínio/MG	0.17 ± 0.003 Bb*b*	0.41 ± 0.002 Aa*a*
Três Pontas/MG	0.11 ± 0.005 Bb*b*	0.45 ± 0.005 Aa*a*

Campo do Meio/MG	0.09 ± 0.001 Bb*b*	0.48 ± 0.008 Aa*a*
Ibiá/MG	0.10 ± 0.005 Bb*b*	0.44 ± 0.002 Aa*a*
Vermelho Novo/MG	0.12 ± 0.005 Bb*b*	0.40 ± 0.000 Aa*a*
Linhares/ES	0.11 ± 0.005 Bb*b*	0.35 ± 0.002 Aa*a*
Jaú/SP	0.03 ± 0.005 Bb*b*	0.33 ± 0.005 Aa*a*
Luís Eduardo Magalhães/BA	0.25 ± 0.005 Bb*b*	0.32 ± 0.003 Aa*a*
Londrina/PR	0.14 ± 0.006 Bb*b*	0.31 ± 0.005 Aa*a*
Unaí/MG	0.12 ± 0.008 Bb*b*	0.29 ± 0.001 Aa*a*
Without association with nanoparticles		
Patrocínio/MG	0.55 ± 0.002 Aa*b*	0.40 ± 0.005 Aa*a*
Três Pontas/MG	0.40 ± 0.005 Aa*b*	0.42 ± 0.004 Aa*a*
Campo do Meio/MG	0.44 ± 0.000 Aa*b*	0.45 ± 0.008 Aa*a*
Ibiá/MG	0.55 ± 0.005 Aa*b*	0.46 ± 0.000 Aa*a*
Vermelho Novo/MG	0.14 ± 0.008 Aa*b*	0.42 ± 0.005 Aa*a*
Linhares/ES	0.17 ± 0.002 Aa*b*	0.41 ± 0.006 Aa*a*
Jaú/SP	0.55 ± 0.001 Aa*b*	0.49 ± 0.003 Aa*a*
Luís Eduardo Magalhães/BA	0.58 ± 0.003 Aa*b*	0.35 ± 0.001 Aa*a*
Londrina/PR	0.48 ± 0.001 Aa*b*	0.47 ± 0.001 Aa*a*
Unaí/MG	0.44 ± 0.004 Aa*b*	0.15 ± 0.005 Aa*a*

[1]Means followed by the same uppercase letter within columns (comparison among populations), lowercase letter within columns (comparison with and without nanoparticle association), and italic lowercase letter (comparison between compound and control) do not differ significantly at $p < 0.05$ (Scott–Knott test).

In the populations of Patrocínio, Três Pontas, Campo do Meio, Ibiá, Vermelho Novo, Linhares, Jaú, Luís Eduardo Magalhães, Londrina, and Unaí, locomotion values in the p-cymene treatment ranged from 0.03 to 0.25 m, whereas the corresponding controls ranged from 0.29 to 0.48 m. This pattern indicates a considerable reduction in locomotor activity when p-cymene was associated with ZnO-NPs.

The greatest reduction was observed in the Jaú/SP population, where insects exposed to p-cymene associated with nanoparticles moved only 0.03 m, whereas the control reached 0.33 m. Marked reductions were also observed in Campo do Meio/MG, Ibiá/MG, Três Pontas/MG, Linhares/ES, Vermelho Novo/MG, and Unaí/MG. Even in the Luís Eduardo Magalhães/BA population, which showed the highest value among insects treated with the nanoformulation (0.25 m), the distance was still lower than that of the control (0.32 m). Therefore, the effect of the ZnO-NPs + p-cymene system was consistent across different geographic origins, suggesting that the behavioral response was not restricted to a single population.

When p-cymene was applied without association with nanoparticles, the response pattern was less uniform. In some populations, such as Patrocínio/MG, Ibiá/MG, Jaú/SP, and Luís Eduardo Magalhães/BA, the distance traveled under the p-cymene-only treatment was equal to or greater than that of the control. In others, such as Vermelho Novo/MG and Linhares/ES, a reduction in walking activity was observed. This variation indicates that isolated p-cymene may present a population-dependent behavioral effect, whereas its association with ZnO-NPs conferred greater stability to the locomotor reduction pattern. These results are consistent with the study by Plata-Rueda et al. (2019), in which adult CBB exposed to the insecticide cyantraniliprole showed behavioral and physiological alterations, including changes in locomotor activity, reduced survival, and decreased respiratory rate.

The reduction in walking distance induced by the ZnO-NPs + p-cymene formulation interfered with the motor behavior of adult females. Since locomotion is directly related to fruit searching, colonization, and pest dispersal, reduced walking activity may represent an important effect for management, even when mortality is not assessed. Sfara et al. (2013) found that exposure to N,N-diethyl-3-methylbenzamide (DEET) reduced locomotor activity in Blattella germanica (Blattellidae; Blattodea) in a dose- and time-dependent manner. The effect persisted for at least 18 hours after the end of exposure, and it was observed after, rather than during, exposure to the repellent.

The variability in responses among Brazilian populations reinforces the importance of considering regional management history and the genetic sensitivity of each locality. The ability of nanoformulations to modulate the interaction between active compounds and the physiology of CBB represents a robust tool. The use of inorganic nanocarriers for p-cymene not only protects the active compound against abiotic degradation but also optimizes its environmental persistence, consolidating itself as a promising strategy for modernizing the behavioral management of the coffee berry borer in Brazil (AHAMAD *et al.,* 2026).

CONCLUSIONS

The results demonstrated that the association with ZnO-NPs potentiated the behavioral effect of p-cymene, causing a reduction in the locomotor activity of CBB. This alteration may represent a relevant sublethal effect, as it interferes with behaviors related to movement and fruit colonization. Therefore, p-cymene shows potential as a complementary technology for the management of CBB.

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