



Acute Soil Toxicity of a Commercial Lambda-cyhalothrin–Pirimicarb Formulation to the Harvester Ant *Messor barbarus*

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Abstract: Soil ants play key functional roles in agroecosystems, yet their sensitivity to insecticide applications remains insufficiently documented. This study evaluated the acute toxicity of a commercial formulation containing lambda-cyhalothrin (5%) and pirimicarb (10%) on the harvester ant *Messor barbarus* under controlled laboratory conditions in the Bouira region (Algeria). Soil physicochemical properties were characterized to contextualize exposure conditions.

Ants were exposed for 7 and 14 days to treated soil containing 0.10, 0.22, 0.90, and 1.80 mg/kg dry soil. Mortality increased significantly with concentration and exposure duration, with a significant concentration × time interaction ($p < 0.001$). After 7 days, the concentration–response model was significant, and the LC_{50} was estimated at 0.128 mg/kg dry soil (95% CI: 0.090–0.166). After 14 days, mortality approached saturation across most concentrations, preventing reliable estimation of an LC_{50} value. Complete mortality occurred at ≥ 0.90 mg/kg following prolonged exposure. In addition to lethal effects, marked behavioral alterations, including immobility and impaired social interactions, were observed.

These findings indicate a high sensitivity of *M. barbarus* to this commercial formulation under laboratory conditions and emphasize the importance of exposure duration in shaping toxic responses. Further investigations under field conditions are needed to assess potential ecological consequences for soil ant populations.

Key words: acute toxicity, agroecosystem, bioindicator, lambda-cyhalothrin, pirimicarb, *Messor barbarus*

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Introduction

Soil is a complex, living, and dynamic system that sustains essential ecosystem functions and harbors significant biodiversity. It acts as a reservoir, vector, and regulator of organic and inorganic substances, thereby supporting ecosystem functioning and agricultural productivity (Calvet et al. 2005). The sustainability of agroecosystems depends largely on soil integrity. However, the intensive use of plant protection products increasingly threatens soil biodiversity and compromises soil health and ecosystem quality (Chagnon et al. 2015, Gestel et al. 2021, Bertrand et al. 2025).

Each year, millions of tonnes of pesticides, including fungicides, insecticides and herbicides, are applied worldwide to reduce crop losses (Al-Assiuty et al. 2014). Global pesticide consumption increased by approximately 80% between 1990 and 2017 (Pelosi et al. 2021), reaching 3.70 million tonnes of active ingredients in 2022, representing a doubling since 1990 and a further 4% increase compared with 2021 (FAO 2023). Asia currently represents the largest producing and exporting region, while pesticide consumption in the Americas has risen by 10%. In India, production reached 299,000 tonnes, with more than 80,000 commercial products registered and a domestic consumption of 52,466 tonnes (Garud et al. 2024).

In Algeria, pesticide use has increased alongside agricultural intensification and expansion of cultivated land (Bettiche et al. 2019). More than 480 products are currently registered by the National Institute of Plant Protection (Ayad-Mokhtari 2012). Despite regulatory frameworks, pesticide exposure continues to pose risks to soil biodiversity and human health (Gunstone et al. 2021, Hodoşan et al. 2023, Moumene et al. 2024).

The environmental impact of pesticides extends beyond treated areas. Compounds may disperse through drift, evaporation, infiltration, and runoff, affecting groundwater, coastal waters, and terrestrial biodiversity (Pelosi et al. 2021). Soil contamination is particularly concerning because residues accumulate in the upper 15 cm, the most biologically active soil layer (Blasco & Pico 2009). These residues may persist for decades and bioaccumulate in soil organisms, plants, microorganisms, the atmosphere, and food chains, posing long-term ecological and health risks (McGinley et al. 2023).

Ecotoxicological assessments of soil contamination have traditionally focused on earthworms as bioindicators, evaluating endpoints such as survival, growth, reproduction, and avoidance behavior

(Kammenga et al. 2000, Schläppi et al. 2020, Haya-saka et al. 2022, Joimel et al. 2022). In contrast, other ecologically important soil taxa, including ants, remain comparatively underexplored despite their ecological importance.

Ants comprise nearly 25,000 described species worldwide and represent a substantial proportion of terrestrial animal biomass (Hölldobler & Wilson 1990, Del Toro et al. 2012, Ward 2014). As ecosystem engineers, they contribute to soil aeration, aggregation, nutrient redistribution, decomposition processes, and seed dispersal (Wills & Landis 2018). Their nesting and foraging activities ensure continuous contact with the soil matrix, potentially increasing exposure to soil-applied insecticides.

The present study evaluated the acute soil toxicity of a commercial formulation containing lambda-cyhalothrin and pirimicarb, two active ingredients commonly used in combination against agricultural pests. The harvester ant *Messor barbarus* (Formicidae) was selected as a bioindicator due to its ecological role in Mediterranean agroecosystems. Mortality and behavioral alterations, including immobility and disrupted social interactions, were assessed following exposure to treated soil in order to characterize concentration–response relationships and to evaluate potential ecological risks for soil ant populations.

Materials and Methods

1. Study Area

Ants were collected from a garden located in Lakh-daria municipality, approximately 40 km northwest of Bouira Province and 40 km southeast of Algiers (36°33'47"N, 3°35'41"E) (Fig. 1). The site was selected based on the presumed absence of recent pesticide applications, in order to minimize prior exposure and ensure the suitability of individuals for ecotoxicological assessment.

The region is characterized by a temperate Mediterranean climate, with hot, dry summers and cold, humid winters. Mean monthly temperatures range from 8.4 °C in January to 27.9 °C in July, with an annual average of 17.5 °C. Annual precipitation ranges between 645 and 665 mm, with a peak in November, contributing to local ecosystem dynamics and influencing agricultural activity.

Soil Sampling

A soil sample was collected from the same garden in Lakh-daria municipality where ants were sampled in order to characterize the physicochemical properties of the exposure substrate. Soil analysis was

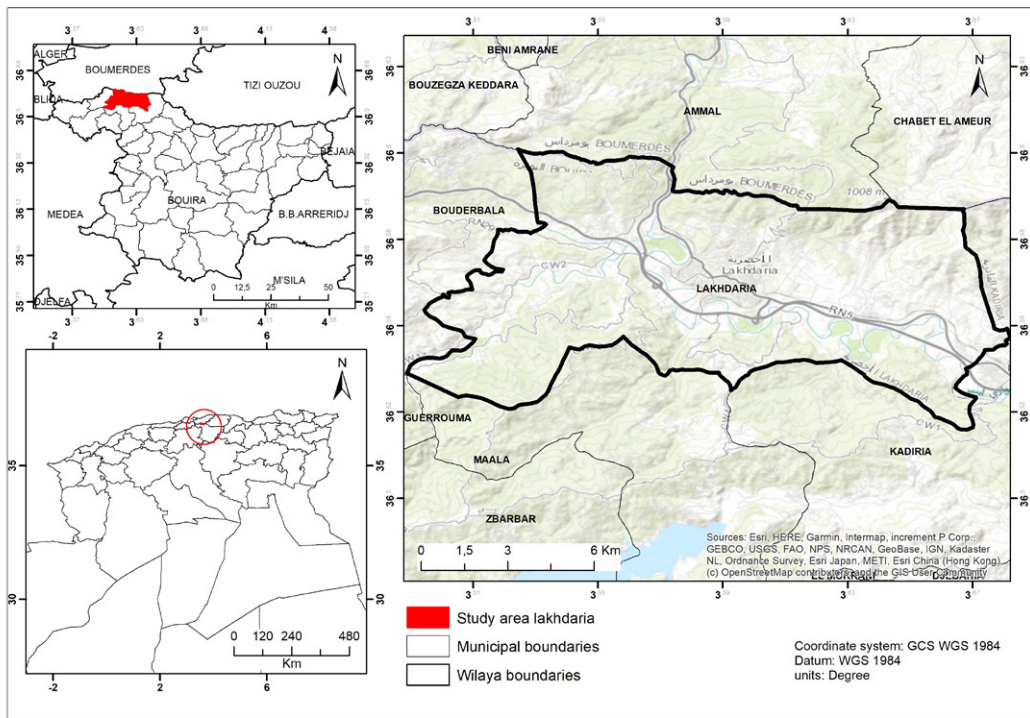


Fig. 1. Map showing the geographical location of the sampling site in Lakhdaria, Bouira Province, Algeria.

conducted to describe the edaphic conditions potentially influencing the biological responses observed during toxicity testing.

The collected soil was air-dried and sieved to obtain fine earth (≤ 2 mm). Soil temperature was measured at 30 cm depth using a digital probe thermometer. Total carbonate content was determined using Bernard's Calcimeter according to NF ISO 10693 (1995), based on the reaction of carbonates with hydrochloric acid (HCl). Soil pH was measured potentiometrically in a 1:2.5 soil–water suspension using a glass electrode (ISO 10390, 2021). Soil moisture content was determined gravimetrically as the difference in weight before and after drying at 105 °C (FAO 2023).

Organic matter (OM) content was determined by the loss-on-ignition method, based on weight loss after calcination at 375 °C for 16 h (Robertson 2011). Organic matter percentage was calculated as:

$$OM(\%) = \frac{(Dry\ soil\ weight - Weight\ after\ ignition)}{(Dry\ soil\ weight)} \times 100$$

Particle-size distribution was determined using the international Robinson pipette method according to AFNOR NF X31-107 (Buol et al. 2011). Soil texture was subsequently classified using the USDA soil texture triangle (USDA 2017; Chandra & Shrivastava 2024).

Total porosity was calculated from bulk density measurements performed on soil aggregates using the paraffin-coating method and assuming a particle density of 2.65 g/cm³.

Biological Material

The granivorous ant *Messor barbarus* (Formicidae) was selected as the biological model due to its abundance, wide distribution in Mediterranean ecosystems, and its functional role in soil processes, particularly seed dispersal and soil turnover. Through nesting and foraging activities, this species maintains continuous contact with the soil matrix, potentially increasing exposure to pesticide residues via contact and ingestion pathways. Despite their ecological importance, ants remain comparatively underrepresented in standardized pesticide toxicity assessments relative to other soil invertebrates.

Ant sampling was conducted manually in April at a garden site located in Lakhdaria (Bouira region, Algeria), following the approaches described by Lamotte & Bourlière (1969) and standard field techniques for ground-dwelling ants (Bestelmeyer et al. 2000). Sampling effort consisted of two hours per day over five consecutive days. Individuals were actively searched for and collected from the soil surface and various microhabitats (beneath stones, within dead branches, and under tree bark), including nests identified across the study site. A total of

250 adult workers were collected to ensure adequate representation of local populations and to reduce potential colony-level effects.

Following collection, ants were placed in plastic containers containing soil from the sampling site and transported to the laboratory for sorting and identification. Individuals selected for experimentation were maintained for three weeks to allow acclimatization to laboratory conditions. Acclimation was conducted at a controlled temperature (20 ± 2 °C) under lighting conditions similar to those used during the exposure phase. Ants were provided with seeds consistent with their natural granivorous diet. Prior to testing, individuals were sorted according to body size and weight to obtain experimental groups as homogeneous as possible.

Species identification was performed using a stereomicroscope based on diagnostic morphological characters and standard taxonomic keys (Cagniant 1968, 1969, 1973, 2005; Cagniant & Espadaler 1997a, b; Barech & Doumandji 2002; Barech et al. 2017). In the absence of a standardized protocol for this species, the experimental design was adapted from established soil ecotoxicity methods developed for *Lumbricus terrestris*, particularly with regard to soil exposure conditions, experimental containers, exposure duration, and mortality assessment.

Chemical Material

The insecticide tested in this study was LAZER, an emulsifiable concentrate containing two active ingredients: 5% lambda-cyhalothrin and 10% pirimicarb. Lambda-cyhalothrin is a pyrethroid insecticide acting primarily on the nervous system of insects, whereas pirimicarb is a carbamate compound that inhibits acetylcholinesterase activity. The formulation is used in agricultural practice for the control of various insect pests (Corbel et al. 2004; Bielza et al. 2007; Bonnet et al. 2009; Djènontin et al. 2009).

Experimental Setup

The acute soil toxicity test was conducted following a modified version of the SPE1/RM/43 soil toxicity method (Environment Canada 2004), originally developed for earthworms. Toxic effects were evaluated by recording the number of surviving individuals after 7 and 14 days of exposure. Mortality was calculated as the difference between the initial number of introduced individuals and the number of live individuals recorded at each observation time. In addition to mortality, behavioral alterations (e.g., immobility and disrupted social interactions) were qualitatively noted during each observation period.

The commercial formulation was diluted in distilled water to obtain nominal concentrations of 0.10, 0.22, 0.90, and 1.80 mg/kg dry soil. Distilled water served as the control treatment. Four independent replicates were performed for each concentration and for the control.

Toxicity tests were conducted in transparent polyethylene containers ($30 \times 20 \times 10$ cm) equipped with perforated lids to allow gas exchange. Each experimental unit contained 500 g of moist soil. Insecticide solutions were applied by spraying onto the soil surface, followed by thorough mixing to ensure homogeneous distribution before the introduction of ten adult *Messor barbarus* individuals per container. A total of 200 individuals were used in the experiment.

Soil moisture was maintained by periodic application of distilled water as required. Ants were exposed for 14 days, and mortality data recorded at days 7 and 14 were used for LC_{50} estimation.

Statistical Analysis

All statistical analyses were performed using RStudio (version 2025.09.1). Concentration–response relationships were modeled separately for each exposure period (7 and 14 days) using a two-parameter log-logistic model implemented in the *drc* package. Median lethal concentrations (LC_{50}) and their 95% confidence intervals were estimated using the delta method.

Normality of mortality proportions was assessed using the Shapiro-Wilk test. As the data deviated significantly from normality ($p < 0.001$), mortality proportions were transformed using the arcsine square-root transformation prior to parametric analysis.

A linear mixed-effects model was fitted to the transformed data using the *lme4* package, with concentration and exposure duration (week) included as fixed effects and experimental unit (box/replicate) as a random effect. The significance of fixed effects was evaluated using Type III analysis of variance with Satterthwaite's approximation for degrees of freedom. Post hoc pairwise comparisons were conducted using the *emmeans* package with Tukey or Dunnett adjustments where appropriate.

Results

Physicochemical Characteristics of the Soil

The physicochemical properties of the soil used in the experiment are presented in Table 1.

Ants were collected from soils with the following physicochemical characteristics (Table 1). The analyzed soil exhibited a temperature of 12 °C and a

moisture content of 22%. The soil was alkaline (pH 8.02) and contained 43.21% total carbonate (CaCO_3) and 3.28% organic matter. Total porosity was 44%.

Mortality and concentration–response analysis

Determination of LC_{50}

(Median Lethal Concentration)

Log-logistic regression models were fitted separately for each exposure period (Table 2, Figure 2). Each concentration was tested using four independent replicates with ten adult individuals per replicate ($n = 40$ per concentration).

Table 1. Physicochemical properties of the tested soil.

Analyzed parameters	Measured value
Temperature	12°C
pH	8.02
Moisture content	22%
Total carbonate content (%)	43.21%
Organic matter	3.28%
Total porosity	44%

During Week 1, the model was highly significant ($p < 0.0001$), indicating a concentration-dependent increase in mortality. The LC_{50} was estimated at 0.128 mg/kg (95% CI: 0.090–0.166 mg/kg).

In contrast, the model fitted to Week 2 data was not statistically significant ($p = 0.65$), and the estimated LC_{50} (0.046 mg/kg) was associated with a wide confidence interval overlapping zero. Mortality approached saturation across most concentrations after 14 days of exposure, limiting reliable parameter estimation. Consequently, the Week 2 LC_{50} was not interpreted further. The lack of model significance likely reflects near-complete mortality across treatments, which reduced the capacity of the log-logistic model to estimate stable parameters despite the significant interaction detected in the mixed-effects analysis

Effect of concentration and exposure duration on mortality

The linear mixed-effects model and estimated marginal means (EMMs) are presented in Figure 3. The model revealed significant effects of concentration and exposure duration (week), as well as a significant concentration \times week interaction ($F(4,24) = 58.87$, $p < 0.001$).

Table 2. Log-logistic dose–response model and LC_{50} estimates for each week

Week	Estimate	Std. Error	t-value	p-value	LC_{50} (mg/kg)	95% CI (Lower-Upper)
Week 1	1.6488	0.3128	5.27	< 0.0001	0.128	0.090 - 0.166
Week 2	4.8916	10.8976	0.45	0.65	0.046	- 0.112 - 0.206

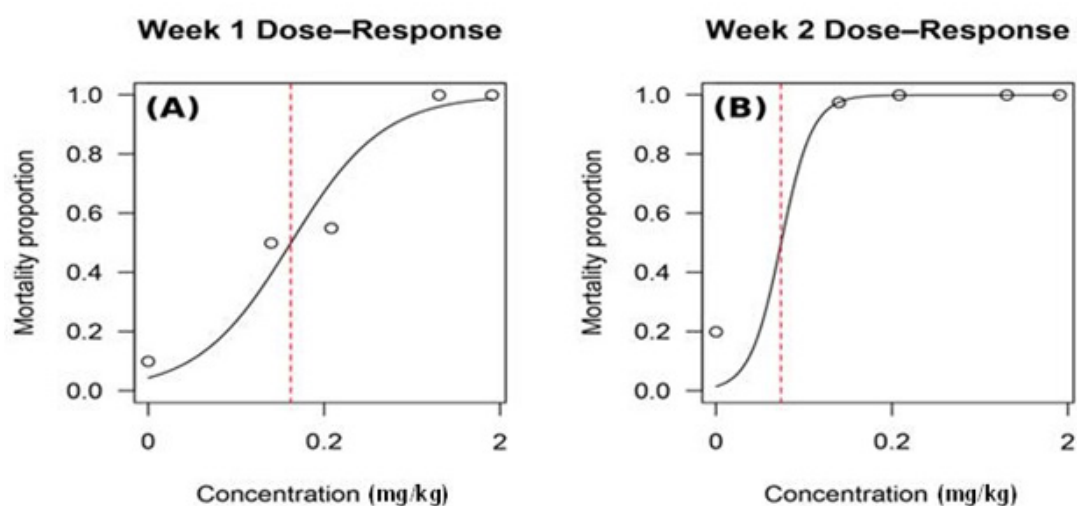


Fig. 2. Dose–response curves for mortality following exposure to the commercial formulation at Week 1 and Week 2. (A) Week 1: Log-logistic model fit (solid line) to observed mortality (open circles). The red dashed line indicates the $LC_{50} = 0.128$ mg/kg (95% CI: 0.090–0.166). (B) Week 2: Log-logistic model fitted to observed data. Due to near-saturation of mortality at most concentrations, the model was not statistically significant and the LC_{50} estimate was unreliable.

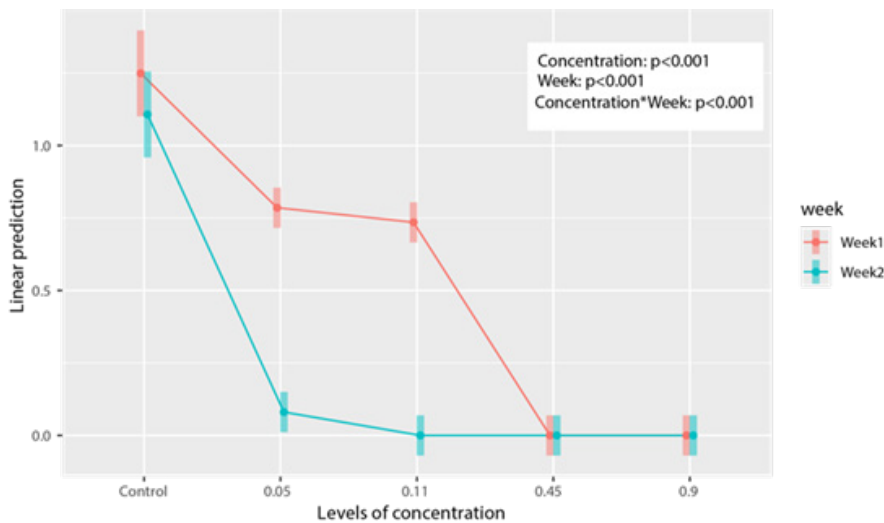


Fig. 3. Estimated marginal means for mortality across concentration levels and exposure periods derived from the linear mixed-effects model.

Predicted values are shown with 95% confidence intervals (Week 1 in red; Week 2 in teal). The inset displays Type III ANOVA results indicating significant effects of concentration, week, and their interaction ($p < 0.001$). Values are presented on the arcsine square-root-transformed scale.

During Week 1 (red line), predicted mortality increased progressively with concentration. The control group showed the lowest predicted response (EMM = 0.161, 95% CI: 0.071-0.251). Intermediate responses were observed at 0.10 and 0.22 mg/kg (EMM = 0.785 and 0.836, respectively), whereas the highest predicted responses occurred at 0.90 and 1.80 mg/kg (EMM = 1.571 for both concentrations). All treatment levels differed significantly from the control ($p < 0.001$).

During Week 2 (teal line), predicted mortality was higher overall, including in the control group (EMM = 0.393, 95% CI: 0.303-0.482; Week 1 vs Week 2: $p = 0.02$). Mortality reached values near the upper bound of the transformed response scale at concentrations ≥ 0.10 mg/kg and remained elevated at higher concentrations.

All EMM values are presented on the arcsine square-root-transformed scale used for statistical inference; consequently, estimates may exceed the biological 0-1 range of raw mortality proportions.

Behavioral and morphological alterations

Shortly after exposure to the commercial formulation, treated ants exhibited reduced mobility and diminished responsiveness compared to controls. At the highest concentrations, a localized dark discoloration around the mouthparts was observed (Figure 4), whereas this alteration was absent in control individuals. This discoloration was primarily noted in moribund or dead ants.



Fig. 4. Dark discoloration observed around the mouthparts of *Messor barbarus* workers following soil exposure to the commercial formulation at high concentrations.

No histological, biochemical, or microscopic analyses were performed; therefore, the origin and nature of this alteration cannot be determined within the scope of the present study. By the end of the 14-day exposure period, mortality reached 100% at the highest concentrations tested, and no recovery of activity was observed.

Discussion

The present findings confirm that *Messor barbarus* represents a functionally structuring taxon within Mediterranean terrestrial ecosystems. Through bioturbation, nest construction, seed harvesting and dispersal, and redistribution of organic matter, this species contributes to soil physical structure and nu-

trient fluxes, thereby influencing plant community dynamics (Farji-Brener & Werenkraut 2017; De Almeida et al. 2020a,b; Wiezik et al. 2013). During nest construction, substantial soil displacement alters soil structure and creates biopores, enhancing water infiltration and modifying soil aeration (Lobry De Bruyn & Conacher 1994). In Mediterranean agroecosystems, including those of Algeria, this ecological importance occurs in a context of agricultural intensification and sustained pesticide use (Bettiche et al. 2021; Soudani et al. 2020; Gaouar et al. 2021). According to FAOSTAT data, pesticide consumption in Algeria reached 6,067 tonnes in 2023, corresponding to approximately 0.71 kg/ha of cultivated land (FAO 2024), suggesting a potential for chronic exposure of non-target soil organisms.

Exposure to the commercial formulation combining lambda-cyhalothrin and pirimicarb induced marked acute toxicity in *M. barbarus*, with an LC_{50} of 0.128 mg/kg after 7 days and complete mortality at the highest tested concentrations. These results indicate a high sensitivity of this species to doses within ecologically relevant ranges and support its suitability as a model organism for assessing risks to soil arthropods.

The known modes of action of both active ingredients provide a mechanistic framework consistent with the observed effects. Pyrethroids such as lambda-cyhalothrin target voltage-gated sodium channels, leading to neuronal hyperexcitation, paralysis, and death (Burr & Ray 2004; Field 2017; Frank et al. 2018; Zhu et al. 2020; U.S. EPA 2020a,b; Poole & Schaffer 2024; Choi et al. 2024). Pirimicarb, a systemic carbamate, inhibits acetylcholinesterase, resulting in acetylcholine accumulation and neuromuscular dysfunction (FAO/WHO JMPR 2004; IRAC 2024; NCBI PubChem 2024). Concomitant exposure to both compounds may therefore enhance neurotoxic stress, which is consistent with the rapid immobilization and collapse observed in treated ants (Hodoşan et al. 2023; Mendarte-Alquisira et al. 2024).

Toxicity profiles obtained here are consistent with studies showing that pyrethroid–carbamate or pyrethroid–organophosphate combinations may amplify toxicity and accelerate the onset of paralysis and mortality (Corbel et al. 2006; Bielza et al. 2007; Cao et al. 2021). Beyond lethal outcomes, comparable sublethal effects have been reported in ants and other arthropods, including behavioral disorganization, oxidative stress, and reduced foraging efficiency (Schläppi et al. 2020; Liang et al. 2018; Wang et al. 2020; Silva et al. 2023). Inhibition of acetylcholinesterase activity has been documented

in other ant species exposed to bioactive compounds (Pantoja-Pulido et al. 2023), and secondary transfer of toxicants within colonies may further amplify impacts at the social level (Choe & Rust 2008). Collectively, these findings highlight that disruption of collective behaviors, locomotion, coordination, and foraging activity, constitutes a relevant ecotoxicological signal in social insects.

A localized dark discoloration around the mouthparts was observed at the highest concentrations. Cuticular and tegumentary alterations have previously been described in insects exposed to pyrethroids, including changes in permeability, thickness, or cuticular composition (Wood et al. 2010; Lilly et al. 2016; Yahouédo et al. 2017). In the absence of mechanistic analyses in the present study, this observation should be considered a visual marker associated with acute intoxication rather than evidence of a specific physiological process.

Soil physicochemical characteristics represent a key factor linking edaphic context to toxicological responses. Parameters such as pH, carbonate content, organic matter, and moisture influence pesticide adsorption, mobility, and persistence within the soil matrix, thereby determining the bioavailable fraction for edaphic organisms (Spark & Swift 2002; Farenhorst 2006; Arias-Estévez et al. 2008; FAO 2018). In the present study, the alkaline substrate (pH 8.02), high carbonate content (43.21% $CaCO_3$), organic matter content (3.28%), and moisture level (22%) may have influenced the bioavailability of the commercial formulation and consequently the effective exposure and sensitivity of *M. barbarus* (Pelosi et al. 2021). This relationship underscores that observed toxicity reflects an interaction between chemical properties and substrate characteristics rather than nominal dose alone.

In agroecosystems subjected to recurrent chemical pressures, potential implications extend beyond immediate mortality. Persistent reductions in abundance or activity may impair soil engineering functions performed by ants. Ants are also recognized as relevant bioindicators of environmental disturbance, as their activity reflects habitat quality and some species are capable of accumulating contaminants such as heavy metals (Skaldina et al. 2018). Long-term alterations in ant populations may therefore affect soil biogeochemical processes, trophic interactions, and overall agroecosystem functioning.

The sensitivity observed under controlled conditions suggests that minimizing impacts on soil fauna may benefit from optimized treatment regimes and wider adoption of integrated pest management strategies. In addition, the integration of be-

havioral endpoints and sublethal indicators into risk assessment frameworks would improve ecological realism, particularly for social organisms whose functioning relies on collective coordination.

Conclusion

Exposure of *Messor barbarus* to the combination of lambda-cyhalothrin and pirimicarb under controlled laboratory conditions induced pronounced adverse effects, highlighting the sensitivity of this species to neurotoxic stress. While the experimental design does not fully replicate field conditions, the observed responses suggest that such formulations may pose risks to non-target soil arthropods.

These findings underscore the importance of optimizing pest management strategies, including dose reduction, integrated pest management approaches, and the use of more selective alternatives. Incorporating ants as bioindicators in ecotoxicological assessments may provide a sensitive and ecologically relevant tool for monitoring soil biodiversity and ecosystem functioning, particularly in Mediterranean agroecosystems.

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Ethical of approval

The experiment was carried out by the guidelines for Experimental Animals approved by the Association Algérienne des Sciences en Expérimentation Animale (58 AASEA: N°45/DG-LPAG/ DVA/SDA/19).

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