The University of Tehran Press

Pollution

https://jpoll.ut.ac.ir/

Print ISSN: 2383-451X
Online ISSN: 2383-4501

Pesticide-Induced Selection Pressure on Soil Microbial Communities: An Assessment of Bacterial Tolerance and Diversity in Northeastern Algerian Agricultural Soils

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Article Info

Article type:
Research Article

Article history:
Received: 31 Jan 2026
Revised: 22 Jun 2026
Accepted: 10 Sep 2026

Keywords:
Algerian Soil,
Bacteria,
Pesticide,
Phylogenetic Analysis,
Toxicity

ABSTRACT

Pesticide-induced soil degradation is a major concern for intensive agriculture. This study assessed the impact of deltamethrin and thiophanate-methyl on the cultivable bacterial diversity of agricultural soils in northeastern Algeria. 48 bacterial strains were isolated, among which four representative species for this study —*Achromobacter marplatensis* EY-T10, *Bacillus* weihenstephanensis 261ZG8, *Arthrobacter sp*. dv7, and *Bacillus sp*. JDMASP42— were selected based on their metabolic potential. Growth assays, measured by Optical Density (OD_{600}), revealed distinct strain-specific responses: in the presence of deltamethrin, *Bacillus* strains reached a mean OD of 0.25, indicating a growth increase of 19% compared to the control. Conversely, thiophanate-methyl exerted a strong inhibitory effect, limiting the final OD to 0.059, for sensitive species, representing an important biomass reduction. Phylogenetic analysis confirmed that *Bacillus* and *Pseudomonas* were the dominant taxa among the pesticide-tolerant isolates. These quantitative results demonstrate that pesticide exposure acts as a strong ecological filter, significantly shifting the soil microbial community structure toward tolerant, spore-forming genera. This work provides critical data on the selection pressure exerted by agrochemicals on indigenous soil microflora.

Cite this article: Slimani, H., & Branes, Z. (2026). Pesticide-Induced Selection Pressure on Soil Microbial Communities: An Assessment of Bacterial Tolerance and Diversity in Northeastern Algerian Agricultural Soils. *Pollution*, 12(3), 865-873. https://doi.org/10.22059/poll.2026.408433.3276

 Publisher: The University of Tehran Press.
DOI: https://doi.org/10.22059/poll.2026.408433.3276

INTRODUCTION

Pesticides are routinely applied to enhance agricultural productivity, a practice that has intensified globally due to the growing food demand. However, this strategy has led to significant contamination of cultivated soils, as the continuous input of these xenobiotics introduces harmful compounds into the environment (Silva et al., 2019). Growing evidence indicates that repeated pesticide applications can alter the composition and functional activity of beneficial soil microbial communities, potentially affecting microbially mediated nutrient cycling and other key soil ecosystem processes (Imfeld & Vuilleumier, 2012; Jeyaseelan et al., 2024). Although these products are designed for specific targets, they often exert unintended adverse effects on non-target farmland biodiversity. Recent large-scale evidence indicates that pesticide residues substantially alter soil biodiversity and microbial functional diversity, thereby compromising key ecosystem functions and soil resilience (Köninger et al., 2026; Wan et al., 2025). Recent research has shown that pesticide exposure, even at environmentally

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relevant concentrations, can alter soil microbial activity and shift the diversity and composition of microbial communities. The extent of these changes depends on the type of pesticide, soil characteristics, and exposure history, but collectively they indicate that pesticides constitute a major ecological pressure on soil microbiomes (Jeyaseelan et al., 2024; Philippot et al., 2024). Furthermore, the persistence of pesticide residues in agricultural soils may impair microbial functions involved in nutrient transformation and other ecosystem processes, with potential consequences for soil quality and long-term agricultural sustainability (Silva et al., 2019; Chia et al., 2024). Because soil microorganisms regulate organic matter decomposition, nutrient cycling, and the natural suppression of soil-borne pathogens, disturbances in their diversity or activity can ultimately compromise soil fertility and crop productivity (Philippot et al., 2024; Jeyaseelan et al., 2024).Consequently, analyzing the shifts in microbial biodiversity serves as a crucial indicator of pesticide-induced environmental disturbance. This study investigates the impact of such chemical pressure on bacterial populations isolated from Algerian agricultural soils, focusing on how pesticide toxicity acts as an ecological filter.

MATERIEL AND METHODS

This study involved three agricultural stations that had been exposed to various pesticides over extended periods. A single sample was collected from the topsoil at each site. The sampling sites were. Immediately after collection, soil samples were transported to the laboratory for the isolation and identification of indigenous bacterial strains following standard microbiological procedures.

Soil samples were collected from the topsoil horizon (0–20 cm) in the Wilaya of El Tarf, eastern Algeria (36°47’16.34’’N, 7°52’00’’E). This depth was selected to ensure consistency with the zone of maximum microbial activity and pesticide accumulation. The sites were chosen based on their documented history of intensive and long-term pesticide usage. Key soil properties (pH, texture, and organic matter content) were analyzed and are summarized in Table 1.

For microbiological analysis, 10 grams of each soil sample were suspended in 90 ml of sterile 0.9% (w/v) NaCl solution. The mixtures were vigorously agitated at 150 rpm for 30 minutes at room temperature. Serial dilutions (10^{-1} to 10^{-6}) were then prepared using the same sterile saline solution. One-milliliter aliquots of the appropriate dilutions were dispensed into sterile Petri dishes, allowed to settle for 10 minutes, and then overlaid with Nutrient agar or Luria–Bertani (LB) agar. Plates were incubated at 30 °C ± 2 °C for 24 hours. Emerging colonies were purified by repeated streaking on the same medium (Slimani et al., 2022 a).

Identification of bacterial isolates was performed using classical methods, including morphological observation, Gram staining, oxidase and catalase tests, and biochemical profiling using API 20E, API 20NE, and API Staph systems (BioMérieux Inc., France). Molecular identification was carried out by sequencing the 16S rDNA gene using primers 1492R and 27F, as described by Batisson et al. (2009).

Multiple sequence alignments were generated using CLUSTAL_X (Thompson et al., 1997),

Table 1. General characteristics of the soil samples used in the study (Slimani et al., 2022 b).

Sampling Stations	Station 1	Station 2	Station 3
Soil texture classification	**Loamy Sand**	**Sandy Loam**	**Loamy Sand**
Water holding capacity (WHC) (%)	28.10	23.59	28.28
pH (in water)	7,77	7,39	7,81
Conductivity (µSimens/cm)	221	454	223
Organic Carbon (%)	1,42	1,26	1,42
Organic Matter (%)	2,44	2,17	2,44
Clay (<2µm) (%)	21.44	31.63	20.34
Loam (<2-50µm) (%)	71.42	53.97	69.52
Sand (50 – 2000µm) (%)	7.17	14.40	10.14

and phylogenetic analyses were performed with MEGA X Phylogenetic trees were constructed using the neighbor-joining method based on the maximum composite likelihood model. The robustness of the tree was evaluated through 1,000 bootstrap replicates (Tamura et al., 2011).

For strain selection and biodegradation assays involving deltamethrin and thiophanate-methyl, Rousseau et al.'s (2001) Mineral Salt Medium (MSM) with some modifications was used (Slimani et al., 2022 a). The medium contained:1.6 g K_2HPO_4, 1.6 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl, 0.02 g $CaCl_2$, 3.5 g $(NH_4)_2SO_4$, and 1 ml of $FeSO_4 \cdot 6H_2O$–EDTA stock solution (5 g and 7 g, respectively). The $FeSO_4 \cdot 6H_2O$–EDTA solution was sterilized separately and added aseptically after autoclaving. The pH was adjusted to 7.5. MSM containing 15 g/L agar was used for evaluating growth on solid medium.

Strain selection focused on microorganisms capable of degrading deltamethrin or thiophanate-methyl. For this purpose, MSM agar plates were supplemented with four pesticide concentrations (12.5, 25, 50, and 100 mg/L), using sterile pesticide solutions filtered through 0.45 µm membranes. Plates were incubated in the dark at 30 °C ± 2 °C for 24 hours to 15 days. Strains were classified as showing positive or negative growth. The most tolerant and fast-growing isolates on solid medium were then tested in liquid MSM.

For liquid culture tests, bacterial suspensions were prepared by resuspending colonies from fresh LB agar plates in sterile 0.9% NaCl (Ali et al., 2010). Growth experiments were conducted in 500-ml Erlenmeyer flasks containing 100 ml of sterile MSM. Deltamethrin and thiophanate-methyl (TM) were added to final concentrations of 50 mg/L and 25 mg/L, respectively, serving as the sole carbon and energy sources. Cultures were incubated at 30 °C ± 2 °C under shaking (150 rpm) in the dark to maintain aerobic conditions.

At 24-hour intervals, liquid samples were collected aseptically to monitor bacterial growth. Optical density (OD_{600}) was measured using a UV spectrophotometer (Secomam Uviline 9000). Non-inoculated flasks served as abiotic controls. All measurements were performed in triplicate (Calvayrac, 2011).

RESULTS AND DISCUSSION

Isolation and screening of pesticide-tolerant strains

In this study, we evaluated the growth performance of bacterial isolates under pesticide stress. It is important to clarify that these assays were designed to measure microbial tolerance and adaptive capacity to pesticide toxicity, rather than to establish exhaustive biodegradation kinetics. The observed growth of specific genera—particularly *Bacillus* and *Pseudomonas*—indicates that these bacteria possess physiological mechanisms to survive in highly contaminated environments (Slimani et al., 2022). The dominance of these resistant strains in our samples supports the hypothesis that pesticide application acts as an ecological filter, driving a shift in microbial community composition toward more tolerant species while potentially reducing overall biodiversity. While quantifying precise degradation rates via GC-MS would provide additional insights, our findings successfully highlight how pesticide-induced chemical pressure selects for specific microbial populations, providing a functional view of the ecological impact of these chemicals on native soil biodiversity.

Strains were enriched using a Mineral Salt Medium (MSM) with pesticides as the sole carbon and energy source. This selective strategy favors the development of populations adapted to chemical stress (Slimani et al., 2022). The screening process was conducted on MSM agar plates with concentrations ranging from 12.5 to 100 mg/L. Positive growth was the primary selection criterion for pesticide tolerance. Strains chosen for 16S rDNA molecular identification (*Achromobacter marplatensis*, *Bacillus weihenstephanensis*, *Arthrobacter sp.*, and *Bacillus sp.*) were selected based on their high resilience and consistent growth performance during these assays.

The growth curve obtained with thiophanate methyl clearly illustrates its inhibitory effect. As shown in Figure 1, a marked reduction in microbial biomass occurred after 24 hours of exposure, as determined by absorbance at 600 nm. Despite this inhibition, cell multiplication resumed, and during the exponential phase, turbidity reached 0.188 OD units after approximately 120 hours.

When exposed to 50 mg/L of thiophanate methyl, the *Arthrobacter* sp. dv7 strain showed an unusual growth pattern (Fig. 2).

.A slight increase in OD was detected two hours after inoculation, likely resulting from the consumption of residual carbon compounds transferred from the LB agar used for pre-culturing. This was followed by a decline in OD from 0.182 to 0.1 after 168 hours, a trend that was also

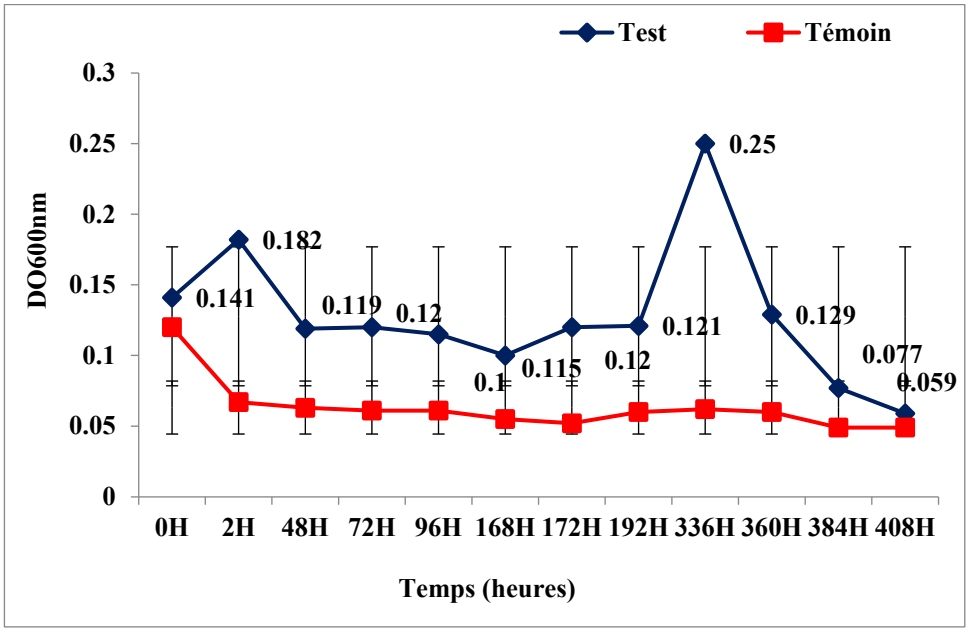

Fig. 1. Growth of *Bacillus* sp. strain JDMASP42 in the presence of 50 mg/l thiophanate methyl as the sole source of carbon and energy.

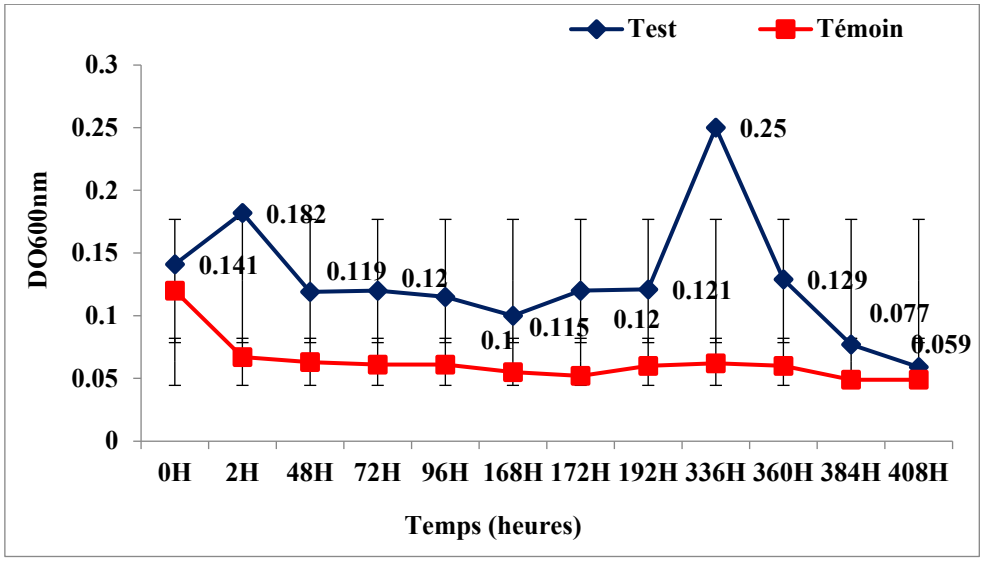

Fig. 2. Growth of *Arthrobacter* sp. dv7 in the presence of 50 mg/l of thiophanate methyl as the sole source of carbon and energy.

recorded for the *Bacillus* sp. JDMASP42 strain (fig. 1). A delayed increase up to 0.250 OD units after 336 hours suggests a slow adaptation to the toxic environment, limited assimilation of thiophanate methyl, and a persistent inhibitory effect.

Previous studies (Roberts et al., 1999) indicate that thiophanate methyl is degraded mainly through oxidation and hydrolysis, producing methyl benzimidazol-2-yl carbamate (MBC), a metabolite that retains fungicidal activity and persists in the environment. Only a few microorganisms, including *Enterobacter* sp. and *Bacillus* sp., can complete its degradation to CO_2 (Cycon et al., 2011). Thus, of all pesticides tested, thiophanate methyl generated the strongest toxic effect, likely linked not only to its active ingredient but also to the high proportion of adjuvants in its commercial formulation (30 g per 100 g of Pelt 70). Such additives are known to modify the environmental toxicity of pesticide preparations (Krogh et al., 2003; García-Ortega, Holliman & Jones; 2006). Laboratory assays often employ doses up to 100 times higher than field concentrations to reveal effects that are otherwise masked by soil heterogeneity or too subtle to detect at recommended agricultural levels (Crouzet et al., 2010; Cycoń, Piotrowska-Seget & Kozdrój, 2010; Singh et al., 2020).

In contrast, the growth patterns in Figure 3 indicate that deltamethrin did not exert noticeable toxicity on bacterial cells. The growth curves had an atypical bell shape without a stationary phase, suggesting rapid metabolic adaptation to the pesticide. The absence of a lag phase in *Bacillus weihenstephanensis* 261ZG8 implies fast acclimatization and efficient utilization or transformation of the pesticide. Maximum OD was achieved after 48 hours of incubation. All strains, however, eventually displayed a decline in OD, attributed to nutrient depletion and possible cell lysis.

Similarly, *Achromobacter marplatensis* EY-T10 displayed slow growth, with a significant decline in biomass (from 0.125 to 0.059 OD units) within the first 72 hours (fig 4). The strain then grew slowly, reaching its maximum OD after 384 hours. Compared to *B. weihenstephanensis*, this strain showed more difficulty adapting to deltamethrin and appeared less efficient in degrading the compound.

These observations are consistent with the findings of Zhang et al. (2009), who demonstrated

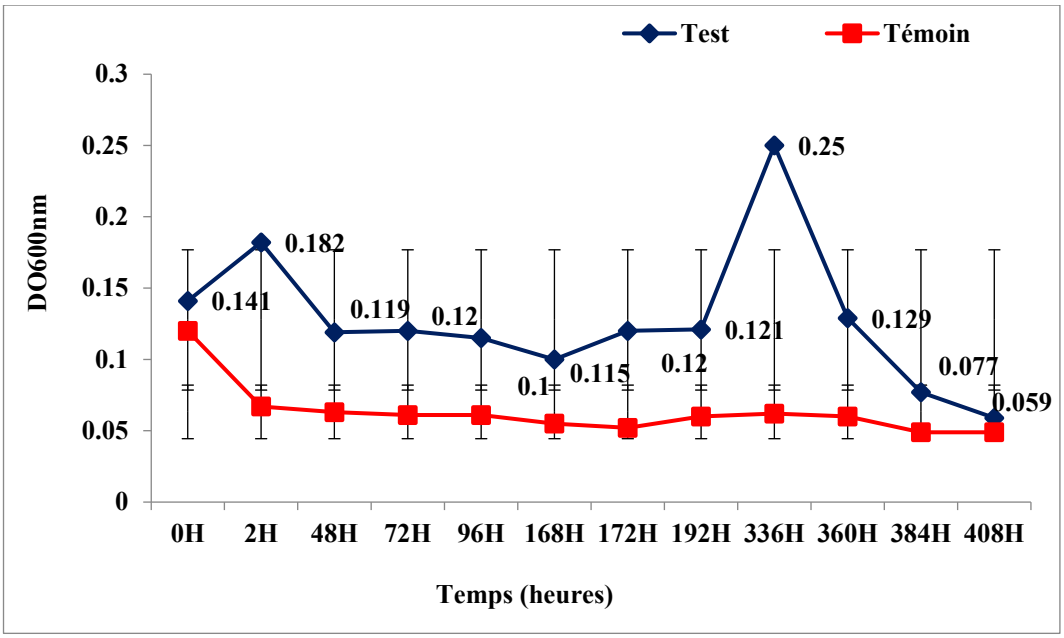

Fig. 3. Growth of *Bacillus weihenstephanensis* strain 261ZG8 in the presence of 25 mg/l deltamethrin as the sole source of carbon and energy.

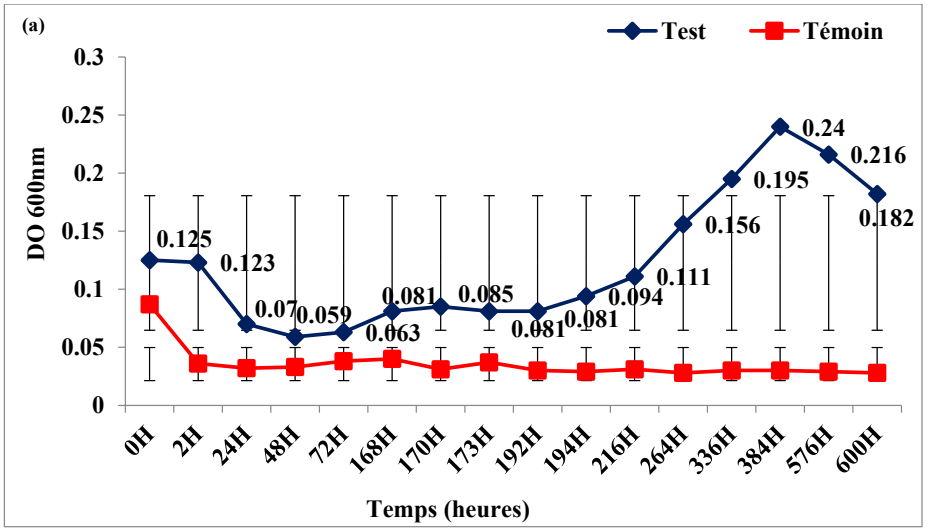

Fig. 4. Growth of *Achromobacter marplatensis* strain EY-T10 in the presence of 25 mg/l deltamethrin as the sole source of carbon and energy.

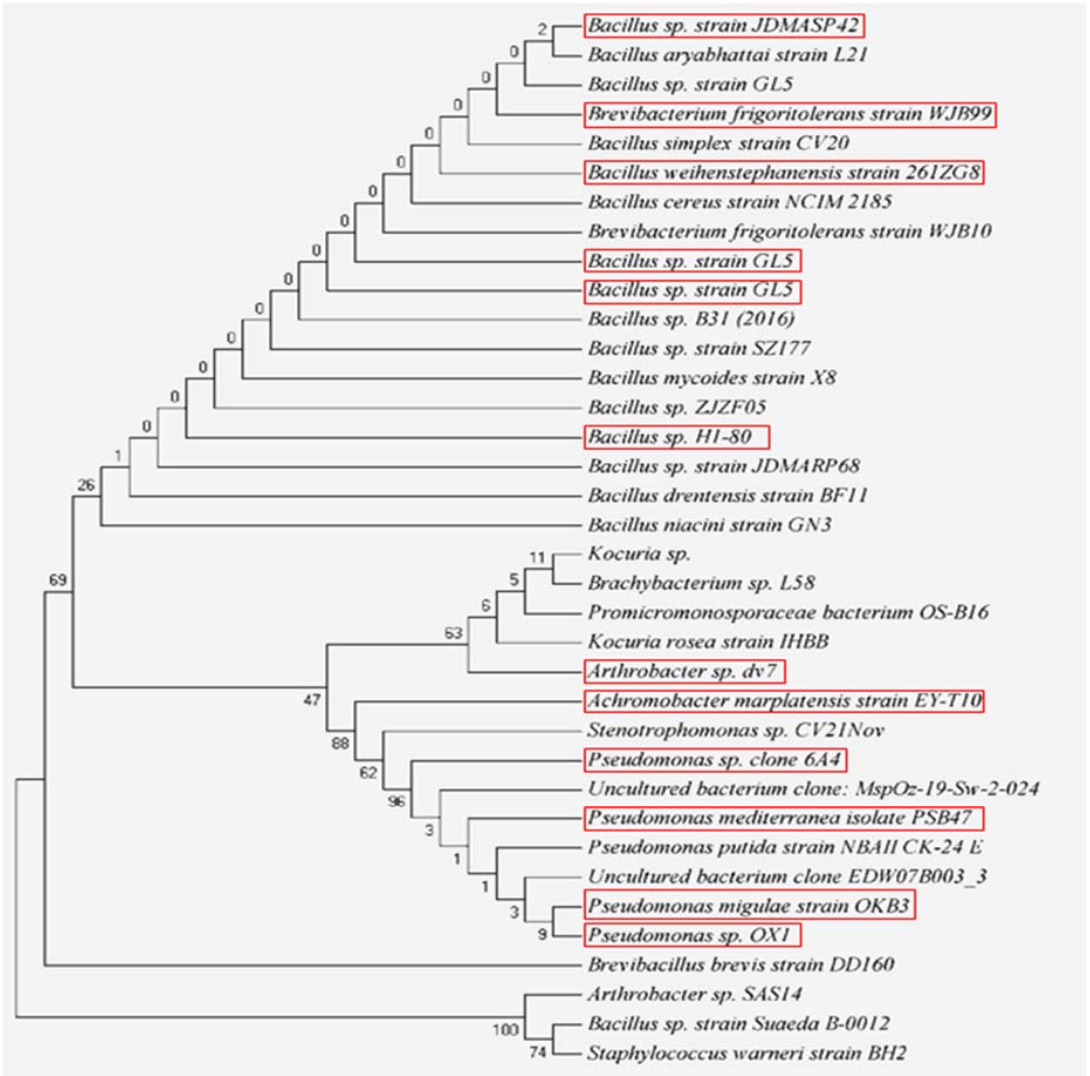

Fig. 5. Evolutionary relationships between interesting species and all the taxa identified.

that cypermethrin application significantly altered the composition of the pepper phyllosphere microbiome, resulting in a shift from Firmicutes-dominated communities toward bacterial groups dominated by Gram-negative taxa, particularly Bacteroidetes and γ-Proteobacteria.

Phylogenetic Analyses

The evolutionary relationships among isolates were inferred using the Neighbor-Joining method. The resulting tree had a total branch length of 0.28656512. Evolutionary distances were calculated using the Maximum Composite Likelihood model (Tamura et al., 2011) and expressed as the number of substitutions per site. Codon positions included the first, second, third, and non-coding regions (fig. 4). Sites with gaps or missing data were removed by full deletion, and the final dataset comprised 426 positions. All analyses were performed in MEGA X (Tamura et al., 2011).

Genetic profiling revealed a dominance of *Bacillus* species, representing 50% of all isolates. *Pseudomonas* ranked second (33%), followed by other genera such as *Arthrobacter* and *Staphylococcus*. Previous studies by our team showed that several strains belonging to these dominant genera could degrade other pesticides, including abamectin and chlorpyrifos (Slimani et al., 2022a; Slimani et al., 2022b).

Despite the essential role of pesticides in crop protection, their misuse places substantial pressure on soil ecosystems. A significant fraction of applied pesticides can miss the intended target and accumulate in soils, where residues persist and alter soil biodiversity and functioning (Köninger et al., 2026; Forger et al., 2023).

Even when applied at low doses, pesticides modify soil chemistry, biological activity, and microbial populations (Cycon et al., 2006; Singh et al., 2008; Cycon et al., 2010). Numerous studies have highlighted the decline in microbial diversity, richness, and evenness in soils contaminated with organophosphorus compounds and pyrethroids (Newman et al. 2016; Du et al., 2018; Wu et al., 2018; Malla et al., 2022).

CONCLUSION

Our culture-dependent tests using agricultural soil from Algeria that had been exposed to insecticides for long periods showed that indigenous microbial strains react differently to deltamethrin and thiophanate-methyl: some bacteria proved their tolerance with rapid adaptation and growth (thus demonstrating their degradation capacity), while other strains were strongly inhibited.

Among the isolated and identified strains, a remarkable predominance of the genera Bacillus and Pseudomonas suggests that these two genera can effectively participate in the elimination of pesticides in contaminated soils, also highlighting the effect of these toxic molecules on the presence and biodiversity of microbial communities.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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