Effect of herbicides on the growth of beneficial microorganisms in rhizospheric soil

Efecto de herbicidas sobre el crecimiento de microorganismos benéficos del suelo rizosférico

Efeito de herbicidas no crescimento de microrganismos benéficos em solo rizosférico

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Abstract

This research seeks to understand the impact of herbicides on beneficial soil microorganisms, essential for agricultural health, and contribute to more sustainable management practices. The study aimed to evaluate the effect of herbicides commonly used in conventional agriculture, such as glyphosate, paraquat, and MCPA, on the growth of beneficial soil microorganisms. The microorganisms were isolated from the rhizosphere soil of a CCN-51 cacao crop from a production farm in Ecuador, and identified through morphological characterization and molecular analysis. The detected beneficial microorganisms were seeded in culture media with field-used doses of the different herbicides mentioned above, and mycelial growth in fungi (*Trichoderma asperellum*) and colony extension in bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) were evaluated. The results showed that the herbicides had a significant inhibitory effect on the growth of these beneficial microorganisms. In the case of *T. asperellum*, paraquat and MCPA showed greater mycelial inhibition compared to glyphosate. Furthermore, all three herbicides significantly reduced the growth of *B. subtilis* and *P. fluorescens*, in contrast to the control groups, which maintained constant growth. These findings suggest that the herbicides studied may have a negative impact on the growth dynamics of beneficial microorganisms, which could affect the balance of the agricultural ecosystem. These results highlight the importance of considering the potential effects of herbicides on the soil microbiota in the management of sustainable agricultural systems.

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Resumen

La investigación busca comprender el impacto de los herbicidas en microorganismos benéficos del suelo, esenciales para la salud agrícola, y contribuir a prácticas de manejo sostenibles. El estudio tuvo como objetivo evaluar el efecto de herbicidas comúnmente utilizados en la agricultura convencional, como el glifosato, el paraquat y el MCPA, sobre el crecimiento de microorganismos benéficos del suelo. Los microorganismos fueron aislados del suelo rizosférico de un cultivo de cacao variedad CCN-51 de una finca productora en Ecuador, identificados por caracterización morfológica y análisis molecular. Los microorganismos benéficos detectados fueron sembrados en medios de cultivo con las dosis empleadas en campo de los herbicidas antes mencionados y se evaluó el crecimiento micelial del hongo (*Trichoderma asperellum*) y la extensión de las colonias en bacterias (*Bacillus subtilis* y *Pseudomonas fluorescens*). Los resultados mostraron que los herbicidas tuvieron un efecto inhibidor significativo en el crecimiento de estos microorganismos benéficos. Para el caso de *T. asperellum*, el paraquat y el MCPA mostraron una mayor inhibición del micelio en comparación con el glifosato. Por otro lado, los tres herbicidas redujeron significativamente el crecimiento de *B. subtilis* y *P. fluorescens*, en contraste con los grupos control que mantuvieron un desarrollo constante. Estos hallazgos sugieren que los herbicidas estudiados pueden tener un impacto negativo sobre la dinámica de crecimiento de microorganismos benéficos, lo que podría afectar el equilibrio del ecosistema agrícola. Estos resultados resaltan la importancia de considerar los efectos potenciales de los herbicidas sobre la microbiota del suelo en el manejo de sistemas agrícolas sostenibles.

Palabras clave: Glifosato, MCPA, paraquat, *Pseudomonas*, *Trichoderma*.

Resumo

A pesquisa busca entender o impacto dos herbicidas nos microrganismos benéficos do solo, essenciais para a saúde agrícola, e contribuir para práticas de manejo mais sustentáveis. O estudo teve como objetivo avaliar o efeito de herbicidas comumente usados na agricultura convencional, como glifosato, paraquate e MCPA, no crescimento de microrganismos benéficos do solo. Microrganismos foram isolados do solo da rizosfera de uma cultura de cacau CCN-51 de uma fazenda de produção no Equador, identificados por caracterização morfológica e análise molecular. Os microrganismos benéficos detectados foram semeados em meios de cultura com doses utilizadas em campo dos diferentes herbicidas citados acima e foram avaliados o crescimento micelial em fungos (*Trichoderma asperellum*) e a extensão das colônias em bactérias (*Bacillus subtilis* e *Pseudomonas fluorescens*). Os resultados mostraram que os herbicidas tiveram um efeito inibitório significativo no crescimento desses microrganismos benéficos. Para *T. asperellum*, paraquat e MCPA apresentaram maior inibição do micélio em comparação ao glifosato. Por outro lado, os três herbicidas reduziram significativamente o crescimento de *B. subtilis* e *P. fluorescens*, em contraste com os grupos controle que mantiveram o desenvolvimento constante. Essas descobertas sugerem que os herbicidas estudados podem ter um impacto negativo na dinâmica de crescimento de microrganismos benéficos, o que pode afetar o equilíbrio do ecossistema agrícola. Esses resultados destacam a importância de considerar os efeitos potenciais dos herbicidas na microbiota do solo no manejo de sistemas agrícolas sustentáveis.

Palavras-chave: Glifosato, MCPA, paraquat, *Pseudomonas*, *Trichoderma*.

Introduction

Herbicides are chemical compounds widely used in agriculture to control weeds that compete with crops for resources (Garcia *et al*., 2024). While their use has increased agricultural productivity, it has also raised concerns about their side effects on soil microbial biodiversity and the environment (González and Fuentes, 2022). In addition, it is important to consider that different herbicides have different modes of action, which may influence their impact on soil microbiota (Eceiza *et al*., 2024).

Herbicides affect not only plants, but also native, beneficial microorganisms that play essential roles in ecosystem health. Exposure to herbicides can alter their activity and diversity, which could have negative impacts on long-term agricultural productivity (Castañeda-García *et al*., 2024). Herbicide formulations, concentration and exposure time are factors that can significantly alter the development and functionality of beneficial microorganisms (Pérez-Hernández *et al*., 2024). Therefore, a detailed study of these factors is essential to understand the magnitude of the effect of herbicides on agricultural ecosystems. Alteration of soil microorganisms can interfere with their cellular functions resulting in a decrease in nutrient availability, which could compromise plant growth and disease resistance (Muñoz-Rojas *et al*., 2021).

Beneficial microorganisms, which include bacteria and fungi, are fundamental for the degradation of organic matter, soil fertility, as well as in the promotion of plant growth through their participation in nitrogen fixation, phosphorus solubilisation and phytohormone production (Reyes *et al*., 2024). As these microorganisms contribute to vital processes, they are important allies in sustainable agriculture (Sámano *et al*., 2024). The *in vitro* evaluation of the effect of herbicides on these microorganisms allows a more rigorous control over the experimental conditions, which facilitates the identification of specific responses of different microbial species.

This approach can provide valuable information on the tolerance and resistance of microorganisms to different types of herbicides, as well as their ability to recover after exposure. Bórtoli *et al*. (2012) studied the effect of glyphosate and concluded that its application at high concentrations alters the activity and structure of microbial communities in soil. Pazmiño *et al*. (2025) studied the effect of herbicides (glyphosate and paraquat) on the population of rhizospheric microorganisms in cocoa crops, observing that herbicides allow the development of pathogenic microorganisms and inhibit the *Trichoderma* fungus to a lesser extent. Alvear *et al*. (2006) evaluated the effect of different groups of herbicides, including MCPA on microbial biomass and some enzymatic activities, sustaining an imbalance in the biological activities in the soil biota, the microbial population is negatively affected by the use of these xenobiotics.

The study aimed to evaluate the effect of herbicides commonly used in conventional agriculture, such as glyphosate, paraquat and MCPA, on the growth of beneficial soil microorganisms.

Materials and methods

Obtaining biological material

The present study was performed in the Microbiology laboratory of the Facultad de Ciencias e Ingenierías of the Universidad Estatal de Milagro, located in Ecuador. The microorganisms were collected from rhizospheric soil with a sandy loam texture in a conventionally managed farm growing cocoa variety CCN-51, 8 years old, located in the parish of Lorenzo Garaicoa, Simón Bolívar canton, Guayas

province, Ecuador. The sampling technique was simple random sampling with five referential sampling throughout the field using a 2" Riverside type auger.

Isolation and morphological description of fungi

Five plastic traps were used to capture fungi, in which pre-cooked rice salt and fat free was introduced. They were strategically placed at 20 cm depth in soil close to the root system for 7 days, according to Chaparro-Montoya *et al*. (2020). Subsequently, the trap containing rice grains with colorimetric characteristics for the genus *Trichoderma* spp. was selected. These grains were sown on potato dextrose agar (PDA) medium (39 $g.L^{-1}$) and incubated at 28 °C for 7 days. Reseeding was performed until pure cultures were obtained by placing 5 mm^2 of fresh mycelium on new plates with PDA, and incubated at 28 °C for 7 days.

Macroscopic characterisation included the observation of shape, colour, texture and size of the strains on the PDA plates. While, for microscopic characterisation, fresh mycelium obtained from the surface of the strain with the help of a transparent Scotch™ tape was used. The mycelium was placed inside a drop of lactophenol blue and visualised under the microscope at 40X. Hyphae, conidiophores and conidia were observed.

Isolation and morphological description of bacteria

For bacterial capture, 1 kg of soil sample was collected close to the root system from five points (four corners and one central point) at a depth of 15 cm. 100 grams of rhizospheric soil were used in serial dilutions using sterilised peptone water (TM media, 20 g. L^{-1}), 100 µL of the dilutions with a concentration of 10^6 colony forming units (CFU) per millilitre were seeded on nutrient agar (NA) (TM media, 28 $g.L^{-1}$), the plates were incubated at 28 °C for 72 hours. Colonies were selected according to their macroscopic characteristics, inoculated onto new nutrient agar plates and sealed for a second incubation at 28 °C for 72 hours (Mora *et al*., 2020).

The pure strains on AN medium were used for macroscopic characterisation of colonies, observing shape, colour, texture and size. UV luminescence was used to detect *P. fluorescens*. Microscopic characterisation was carried out by Gram staining, differentiating between Gram-positive and Gram-negative bacteria.

Molecular identification of the isolated microorganisms.

DNA extraction was performed using conventional methods from 100 mg of sample (Contreras and Meneses, 2018). DNA quality and integrity was verified by spectrophotometry and visualisation on 1 % agarose gel. For PCR amplification of conserved DNA regions, the barcoding technique was used. In fungi, the BtuB gene, which codes for beta-tubulin, was amplified. In bacteria, the gyrB gene coding for DNA gyrase subunit B and the 16S ribosomal DNA gene were amplified (table 1).

PCR products were purified with the Merck™ GenElute™ PCR Clean-Up kit under the indications of the supplier Sigma-Aldrich® and sequenced in the 5' → 3' direction with the SANGER method (Valderrama Martín *et al*., 2020). The quality of the sequences was checked with the Geneious software version 11.1.2. Clean sequences were analysed by homology against sequences deposited in the GenBank database of the National Center for Biotechnology Information (NCBI).

Evaluation of the effect of herbicides on the growth of microorganisms

Microorganisms that were detected as beneficial, based on morphological description, molecular identification and literature review, were seeded on culture media with herbicides to evaluate the effect on their growth. The amount of each herbicide used in the Petri dishes (90 mm, area = 0.0063585 m^2) was based on the field dose recommended by the manufacturers (Ortiz-Martínez *et al*., 2013) (table 2). Treatments with 4-chloro-2-methylphenoxy acetic acid (MCPA) contained the commercial formulation Pamex® (480 $g.L^{-1}$), for glyphosate treatments Accion® (480 $g.L^{-1}$) was used and for paraquat treatment Hervax Inmonte® (240 $g.L^{-1}$) was used.

For fungi, a 5 mm^2 fragment of mycelium was inoculated into the centre of Petri dishes containing PDA with and without herbicide. The plates were incubated at 28 °C under darkness for 5 days. Every 24 hours, the growth diameter in millimetres was measured with a mechanical Vernier caliper, starting from the first day of sowing and continuing until the microorganism covered the entire surface of the box in any of the treatments (five days).

For the bacteria, a colony was inoculated into the Petri dishes with nutrient agar medium with and without herbicides. The plates were incubated at 28 °C in darkness for three days. Every 24 hours, the extent of the colony was recorded.

The control consisted of microorganisms grown on plates with culture medium without herbicides. Each treatment and control was repeated five times, which were distributed in a completely randomised design.

Statistical analysis

The growth of the microorganisms was subjected to a simple analysis of variance (ANOVA) to determine if there were significant differences between treatments. Where significant differences were found, the Tukey 5 % *post-hoc* test was applied to identify specific differences between groups.

Results and discussion

Morphological characterisation of the microorganisms

As a result of the purification of the strains of the isolated microorganisms, it was possible to obtain whole Petri dishes with specific attributes for bacteria of the genus *Bacillus* spp. and *Pseudomonas* spp. and the fungus *Trichoderma* spp. (table 3).

Table 1. Characteristics of oligonucleotides used in PCR assays for molecular identification of microorganisms.

Gene	PCR conditions	Oligonucleotides	Sequence (5' → 3')	Reference
gyrB	Conventional method	UP-1/UP-2r	UP-1: 5'-GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA-3' UP-2r: 5'-AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT-3'	Yamamoto and Harayama (1995)
16S	Conventional method	27F/1492R	27F: 5'-AGAGTTTGATCMTGGCTCAG-3' 1492R: 5'-GGTTACCTTGTTACGACTT-3'	Wilson *et al*. (1990)
BtuB	Conventional method	Bt2a/Bt2b	5'-GGTAACCAAATCGGTGCTGCTTTC-3' Bt2b 5'-ACCCTCAGTGTAGTGACCCTTGGC-3'	Glass and Donaldson (1995)

Table 2. Herbicides used during the evaluation of *in vitro* growth of native beneficial microorganisms.

Product	Active ingredient and concentration	Dose L.ha^{-1}	Dose µL. Plate^{-1}
Pamex®	MCPA - 4-chloro-2-methylphenoxy acetic acid 480 g.L^{-1}	0.5	0.3
Acción®	GLYPHOSATE - Isopropylamine salt of N-(phosphonomethyl) glycine 480 g.L^{-1}	2.5	1.6
Hervax Inmonte®	PARAQUAT 240 g.L^{-1}	2.25	1.4

Table 3. Morphological characteristics of the microorganisms isolated from the rhizosphere of a cocoa farm producing cocoa variety CCN-51 in Ecuador.

Characteristic	*Bacillus* spp.	*Pseudomonas* spp.	*Trichoderma* spp.
Colour	White to cream	Greenish cream	Dark green
Morphology	Convex colonies, rounded, smooth and shiny, with well-defined edges.	Convex colonies, rounded, smooth and shiny, with well-defined margins.	Circular colony with smooth margins, septate, thin, branched hyphae, branching conidiophores, and spherical conidia arranged in chains.
Size	Small colonies, 2 to 5 mm in diameter after 48 hours incubation at 28 °C.	Small colonies, 1 to 3 mm in diameter after 48 hours incubation at 28 °C.	Fast-growing colony, covering the surface of the medium in 5 days.
Texture	Smooth consistency	Mucous consistency	Cottony
Luminescence	Negative	Positive	Not applicable
Gram stain	Gram positive	Gram negative	Not applicable

The results of the morphological characterisation of *Bacillus* spp. and *Pseudomonas* spp. strains obtained in this study are consistent with previous descriptions in the literature. *Bacillus* spp. are characterised by large, smooth colonies, whereas *Pseudomonas* spp. have smaller, mucoid, often pigmented colonies. Microscopically, both are bacillary, but *Bacillus* spp. can form spores, which allows them to resist adverse conditions, while *Pseudomonas* spp. are motile thanks to their flagella. These characteristics not only facilitate their identification, but also reflect their adaptability and functionality in the environment, fundamental for biodegradation and plant growth, which is consistent with the descriptions of Jiménez-Pérez *et al*. (2023). On the other hand, *Pseudomonas* spp. colonies exhibited, characteristics that align with the properties of *Pseudomonas fluorescens* according to Esnard and Diaz (1997), this due to the fluorescence observed in *Pseudomonas* spp. colonies during the UV luminescence test which is attributed to the production of fluorescent pigments such as pyocyanin (Mayz and Manzi, 2017).

The morphological characterisation of the fungus *Trichoderma* spp. highlights the relevance of its macroscopic and microscopic characteristics, which are essential for its precise identification and for understanding its functionality. These characteristics, such as the texture of its colonies and the structure of its hyphae, allow it to be differentiated from other fungi and are indicative of its adaptability. The ability of *Trichoderma* spp. to efficiently colonise its environment is crucial for its role as a biocontroller of pathogens and in the degradation of organic matter, which makes it a valuable ally in sustainable agriculture, as documented in recent studies (Meléndez *et al*, 2024; González-León *et al*., 2023).

These species in the environment can have significant implications on their physico-chemical conditions, hence the importance of use within agricultural and biotechnological applications (Vera *et al*., 2024a; Vera *et al*., 2024b).

Molecular identification

High quality DNA was obtained for the amplification process, visualising bands of 1,090 bp, 1,198 bp and 326 bp, corresponding to the gyrB, 16S and BtuB markers, respectively (figure 1).

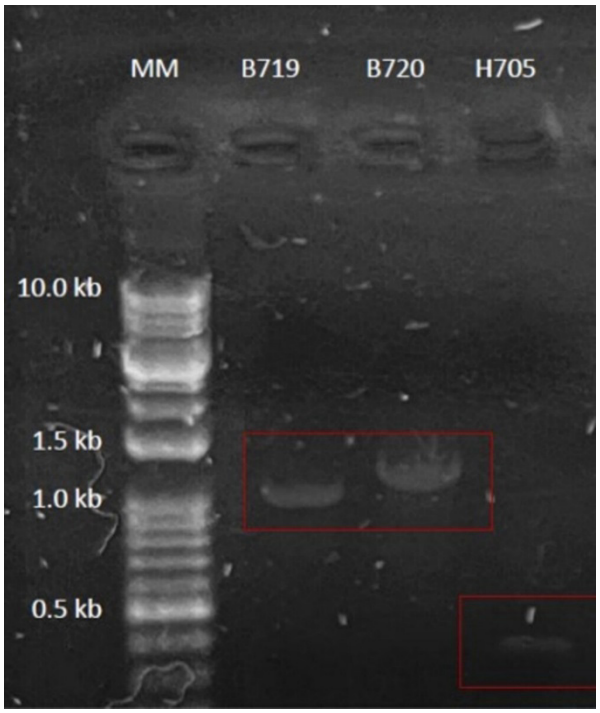

Figure 1. 1 % agarose gel with PCR products for the gyrB (B719), 16S (B720) and BtuB (H705) fragments of the microorganisms isolated from the rhizospheric soil of a CCN-51 cocoa farm. MM= Molecular weight marker.

From the reads resulting from SANGER sequencing, assembled sequences were obtained that allowed the identity of the isolates to be determined as shown in table 4.

Molecular identification of microbial strains is fundamental to understand their diversity and potential application in biotechnology, which highlights the reliability of molecular identification in the characterisation of microorganisms (Suárez-Contreras and Peñaranda-Figueredo, 2022).

Table 4. Summary of the molecular identification of the microorganisms isolated from the rhizosphere of a cocoa farm producing cocoa variety CCN-51.

Code	Quality	Organism	Gene	Identity	Accession	Coverage (%)	E-value
H705	100.00	*Trichoderma asperellum*	BtuB	100.00	PP596864.1	100	8e-162
B719	99.70	*Bacillus subtilis*	gyrB	100.00	CP009748.1	100	0.0
B720	85.80	*Pseudomonas fluorescens*	16S	99.92	MG977684.1	97	0.0

Effect of herbicides on the growth of microorganisms

Analysis of variance indicates that the mycelial growth of the fungus *T. asperellum* is significantly affected by the different herbicide treatments in relation to the control treatment according to $p<0.0001$.

Figure 2 shows the growth of the fungus *Trichoderma asperellum* under the effect of different herbicides over five days.

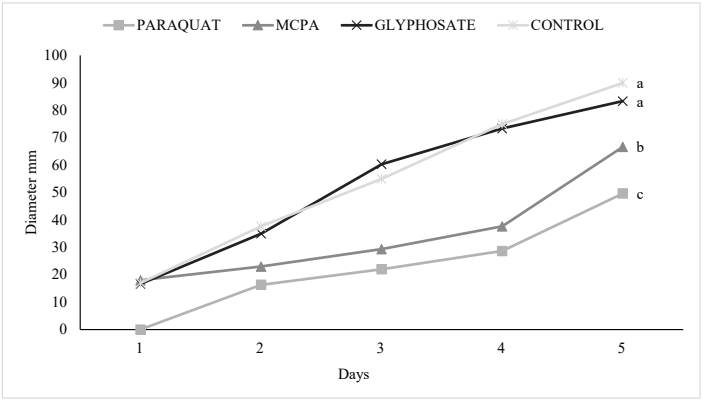

Figure 2. Growth dynamics of the fungus *Trichoderma asperellum* under the effect of different herbicides. Means with a common letter are not significantly different ($p > 0.05$).

The control treatment showed the highest constant growth throughout the five days, reaching 90 mm at the end of the period. This indicates that, without the presence of herbicides, the fungus grows faster. In the glyphosate treatment, growth is similar to that of the control, although slightly lower, suggesting that glyphosate has a moderate effect in inhibiting fungal growth or this strain has probably developed adaptation to this herbicide as reported by Amerio *et al*. (2020). The MCPA treatment shows slower growth compared to the control and glyphosate, possibly the dose did not alter the metabolism of these macroorganisms. While the Paraquat treatment shows the lowest growth among all treatments, indicating that paraquat has the most inhibitory effect on the growth of *T. asperellum*, it could be related to the possible inhibition of specific metabolic pathways or the lack of compatibility of this herbicide with the life cycle of the fungus (Martínez-Villarreal *et al*., 2016).

These herbicides interfere with metabolic processes of the microorganisms, generating severe oxidative stress and affecting cell synthesis (Pazmiño *et al*., 2025). Reyes *et al*. (2012) in their study on the compatibility of *Trichoderma asperellum* with herbicides most commonly used in rice cultivation indicated that herbicides affect the mycelial growth of *T. asperellum* strains and their sporulation from the recommended field dose. Likewise, López-Chávez *et al*. (2024) indicated that rhizospheric fungi are affected by glyphosate (50 mg acid equivalent.L^{-1}) inducing variations in the metabolic profile of soil nutrients.

Martinez *et al*. (2013) in their study confirmed that herbicides such as dicloran, 2,4-D amine salt and glyphosate inhibit the growth of *Trichoderma*, affecting its viability and cultural characteristics, while others, such as trifluralin, are compatible, despite the negative effects, *Trichoderma* can degrade certain herbicides, suggesting an interaction that could be beneficial for sustainable agriculture. These findings highlight the importance of a detailed analysis of herbicide-fungus interactions, as a deeper understanding of these mechanisms can guide the selection of herbicides that minimise the impact on beneficial microorganisms while controlling the weed population (Visentin *et al*., 2021).

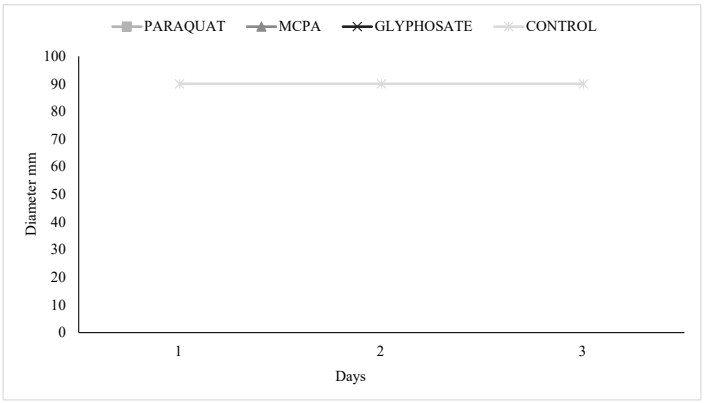

Figure 3. Growth dynamics of *Bacillus subtilis* and *Pseudomonas fluorescens* bacteria under the effect of the different herbicides evaluated.

The control group (control) maintained a constant growth on the surface of the 90 mm Petri dish after 24 hours and throughout the 72 hours (figure 3). Likewise, no bacterial growth is evident for the herbicides Paraquat, MCPA and Glyphosate. This graph suggests that herbicides could have a significant inhibitory effect on the growth of the bacteria studied, as supported by Florida *et al*. (2012) and López-Chávez *et al*. (2024) these herbicides affect the number of bacteria and fungi, as well as their edaphic properties. Likewise, Rivera *et al*. (2020) evaluated the effect of the herbicide glyphosate on beneficial bacteria in the soil and confirmed that the use of herbicides for weed control in crops can decrease yield by increasing the incidence of root diseases due to an alteration of the soil microbiota and its chemical composition.

These findings are consistent with studies by Alvear *et al*. (2006) who suggested that some herbicides can negatively alter the growth dynamics of beneficial microorganisms in the soil, which could affect the health of the agricultural ecosystem. The mode of action of the herbicide decreases the population of soil microorganisms, which can be total, contact, systemic and selective, as well as their degree of toxicity (Pazmiño *et al*., 2025). The application of herbicides must be carefully considered to avoid adverse effects on soil microbiota. Carranza-Patiño *et al*. (2023) suggested the search for more sustainable alternatives to partially replace synthetic pesticides in crops of economic interest.

Conclusions

Herbicides can have an adverse impact on the growth dynamics of beneficial microorganisms, but these depend on the type of herbicide applied, as was the case of *T. asperellum*, *B. subtilis* and *P. fluorescens*, which could influence their effectiveness as biocontrol agents, plant growth promoters, in the decomposition of organic matter, nutrient solubilisation and stress tolerance, affecting the agricultural ecosystem and which should be evaluated *in situ*.

Literature cited

Alvear, M., López, R., Rosas, A., and Espinoza, N. (2006). Efecto de la aplicación de herbicidas en condiciones de campo sobre algunas actividades biológicas. *Revista de la Ciencia del Suelo y Nutrición Vegetal*, *6*(1), 64-76. http://dx.doi.org/10.4067/S0718-27912006000100007

Amerio, N. S., Castrillo, M. L., Bich, G. A., Zapata, P. D., and Villalba, L. L. (2020). *Trichoderma* en la Argentina: Estado del arte. *Ecología Austral*, *30*(1), 113-124. https://ojs.ecologiaaustral.com.ar/index.php/Ecologia_Austral/article/view/945/432

Bórtoli, P. V., Verdenelli, R. A., Conforto, C., Vargas Gil, S., and Meriles, J. M. (2012). Efectos del herbicida glifosato sobre la estructura y el funcionamiento de comunidades microbianas de dos suelos de plantaciones de olivo. *Ecología Austral*, *22*(1), 33-42. https://ojs.ecologiaaustral.com.ar/index.php/Ecologia_Austral/article/view/1263/634

Carranza-Patiño, M., Contreras-Mora, M., Macias-Leon, M., Pincay-Pin, P., Rendón-Margallón, E., and Robinson, H. F. (2023). Uso de los pesticidas y su efecto en el cultivo de *Zea mays*: Una revisión de la literatura. *Código Científico Revista de Investigación*, *4*(E2), 1258-1286. https://revistacodigocientifico.itslosandes.net/index.php/1/article/view/219

Castañeda-García, B. A., Ramírez-Rangel, C. C., and Trujillo-Suárez, D. M. (2024). Efecto de los agroquímicos en la actividad enzimática del suelo agrícola: Una revisión bibliográfica. *Revista Sistemas de Producción Agroecológicos*, *15*(2), 1-16. https://revistas.unillanos.edu.co/index.php/sistemasagroecologicos/article/download/1082/1161

Contreras, L. Y. S., & Meneses, L. F. Y. (2018). Extracción de ADN de bacterias conservadas en el banco de cepas de la Universidad Francisco de Paula Santander sede Campos Elíseos. *Respuestas*, *23*(S1), 24-28. https://dialnet.unirioja.es/descarga/articulo/7001296.pdf

Chaparro-Montoya, E. E., Vera-Alcázar, M. M., Herrera-Córdova, F. B., and Barahona-Sánchez, J. C. (2020). Utilización de microorganismos eficientes para la elaboración de compost a partir de residuos orgánicos. *Sincretismo*, *1*(1). https://revistas.unam.edu.pe/index.php/sincretismo/article/download/15/13

Esnard Bolaños, S. C., & Díaz Rosa, O. E. (1997). Identificación y caracterización de bacilos Gramnegativos no fermentadores aislados en el medio hospitalario. *Revista Cubana de Higiene y Epidemiología*, *35*(1), 30-37. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-30031997000100006&lng=es&tlng=en

Eceiza, M. V., Jiménez-Martínez, C., Gil-Monreal, M., Zabalza, A., and Royuela, M. (2024). Expresión génica y actividad GST en el modo de acción de los herbicidas inhibidores de la síntesis de aminoácidos. *Revista de Ciências Agrárias*, *47*(1), 263-267. https://doi.org/10.19084/rca.34997

Florida, N., López, C., and Pocomucha, V. (2012). Efecto del herbicida paraquat y glifosato en propiedades del suelo que condicionan el desarrollo de bacterias y fungi. *Investigación y Amazonia*, *2*(1-2): 35-43. https://revistas.unas.edu.pe/index.php/revia/article/view/100/84

García, B., Parrales, L., Villón, H., Torres, S., Vera, J., Moyano, G., and Villamar, M. (2024). Comparison of weed control methods in rice in the rainy and dry seasons. *Revista de la Facultad de Agronomía de la Universidad del Zulia*, *41*(4), e244133. https://doi.org/10.47280/RevFacAgron(LUZ).v41.n4.02

Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, *61*(4), 1323-1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

González-León, Y., Ortega-Bernal, J., Anducho-Reyes, M. A., and Mercado-Flores, Y. (2023). *Bacillus subtilis* y *Trichoderma*: Características generales y su aplicación en la agricultura. *TIP Revista Especializada en Ciencias Químico-Biológicas*, *25*(1), 1-14. https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-888X2022000100318

González Ortega, E., & Fuentes Ponce, M. H. (2022). Dinámica del glifosato en el suelo y sus efectos en la microbiota. *Revista Internacional de Contaminación Ambiental*, *38*, 127-144. https://doi.org/10.20937/rica.54197

Jiménez-Pérez, O., Morales, G. G., Hernández-Castillo, F. D., Espinoza-Ahumada, C., del Angel, E. C., and Sanchez-Yañez, J. M. (2023). Actividad antagónica de *Pseudomonas donghuensis* y *Bacillus subtilis* para el manejo de fitopatógenos del "Damping off" del cultivo del chile 42. *Revista Bio Ciencias*, *10*. 1-15. https://doi.org/10.15741/revbio.10.e1382

López-Chávez, M. Y., Infante-Mata, D., Álvarez-Legorreta, T., Dunn, M. F., and Guillén-Navarro, K. (2024). Efecto del glifosato sobre la microbiota y la actividad enzimática en rizósfera de plantas riparias. *Acta Biológica Colombiana*, *29*(1), 119. https://doi.org/10.15446/abc.v28n1.90353

Martínez, B., Infante, D., and Reyes, Y. (2013). *Trichoderma* spp. y su función en el control de plagas en los cultivos. *Revista de Protección Vegetal*, *28*(1), 1-11. http://scielo.sld.cu/scielo.php?pid=s1010-27522013000100001&script=sci_arttext

Martínez-Villarreal, R., Garza-Romero, T. S., Moreno-Medina, V. R., Hernández-Delgado, S., and Mayek-Pérez, N. (2016). Bases bioquímicas de la tolerancia al estrés osmótico en hongos fitopatógenos: el caso de *Macrophomina phaseolina* (Tassi) Goid. *Revista Argentina de Microbiología*, *48*(4), 347-357. http://dx.doi.org/10.1016/j.ram.2016.05.004

Mayz, J., & Manzi, L. (2017). Bacterias hidrocarburoclásticas del género *Pseudomonas* en la rizósfera de *Samanea saman* (Jacq.) Merr. *Revista Colombiana de Biotecnología*, *19*(1), 29-37. https://doi.org/10.15446/rev.colomb.biote.v19n1.57408

Meléndez, V. E. P., Mora, F. J. C., Guerrero, G. T., and Villón, H. R. (2024). Avances en la evaluación de microorganismos como agentes biocontroladores de patógenos causantes de enfermedades en el cultivo de arroz. *Ciencia y Tecnología Agropecuaria*, *9*(1), 27-35. https://doi.org/10.24054/cyta.v9i1.2955

Muñoz-Rojas, M., Delgado-Baquerizo, M., and Lucas-Borja, M. E. (2021). La biodiversidad y el carbono orgánico del suelo son esenciales para revertir la desertificación. *Ecosistemas*, *30*(3), 2238-2238. https://doi.org/10.7818/ECOS.2238

Mora, K. I. G., Araujo-Abad, S., Collahuazo-Reinoso, Y., Salas, Y. L., Rigaud, X. J., Robles-Carrión, Á., and Urgiles-Gómez, N. (2020). Caracterización morfológica y fisiológica de microorganismos rizosféricos nativos de sistemas agroforestales de café. *Bosques Latitud Cero*, *10*(2), 124-136. https://revistas.unl.edu.ec/index.php/bosques/article/view/832

Ortiz-Martínez, J., Hernández-Ramírez, G., Cruz-Tobón, M., Figueroa-Rodríguez, K. A., Figueroa-Sandoval, B., and Hernández-Rosas, F. (2013). Inhibición *in vitro* de aislamientos nativos de *Trichoderma* en presencia de la cepa comercial T22. *Revista Colombiana de Biotecnología*, *15*(1), 126-136. http://www.scielo.org.co/scielo.php?pid=S0123-34752013000100013&script=sci_arttext

Pazmiño, E. P. V., Patiño, M. V. U., Ortega, D. V. U., Guamán, R. N. G., Abril, Á. F. V., and Cortázar, S. M. U. (2025). Efecto de los herbicidas sobre la población de microorganismos en el cultivo de *Theobroma cacao* L., en la parroquia Luz de América, Ecuador. *Siembra*, *12*(1), e7320-e7320. https://doi.org/10.29166/siembra.v12i1.7320

Pérez-Hernández, H., López-Valdez, F., Juárez-Maldonado, A., Méndez-López, A., Sarabia-Castillo, C. R., García-Mayagoitia, S., and Pérez-Moreno, A. Y. (2024). Implicaciones de los nanomateriales utilizados en la agricultura: una revisión de literatura de los beneficios y riesgos para la sustentabilidad. *Mundo Nano*, *17*(32), 1-50. https://doi.org/10.22201/ceiich.24485691e.2024.32.69720

Reyes, Y., Infante, D., García-Borrego, J., Del Pozo, E., Cruz, A., and Martínez, B. (2012). Compatibilidad de *Trichoderma asperellum* Samuels con herbicidas de mayor uso en el cultivo del arroz. *Revista de Protección Vegetal*, *27*(1), 45-53. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522012000100008&lng=es&tlng=es

Reyes, P., Oliveros, J., Racedo, C., and Giraldo, A. B. (2024). Uso de la biodiversidad para mejorar la agricultura: tendencias en la investigación y el mercado en biofertilizantes en Colombia. *Naturaleza y Sociedad. Desafíos Medioambientales*, (10), 185-211. https://doi.org/10.53010/nys10.06

Rivera, A. P. T., Murgas, R. E. C., Ríos, A. E. M., and Merini, L. J. (2020). Efecto del glifosato sobre la microbiota, calidad el suelo y cultivo de frijol biofortificado en el departamento del Cesar, Colombia. *Revista Argentina de Microbiología*, *52*(1), 61-71. https://doi.org/10.1016/j.ram.2019.01.006

Sámano, M. L., Nanjareddy, K., and Arthikala, M. (2024). Promoviendo la sostenibilidad alimentaria: a través de la fijación simbiótica de nitrógeno en plantas no leguminosas y mediante rizobacterias promotoras de crecimiento en los cereales. *Cuadernos de Educación y Desarrollo*, *16*(11), 01-28. https://doi.org/10.55905/cuadv16n11-053

Suárez-Contreras, L. Y., & Peñaranda-Figueredo, F. A. (2022). Identificación molecular de hongos filamentosos y su potencial biotecnológico. *Biotecnología en el Sector Agropecuario y Agroindustrial*, *20*(1), 194-206. https://doi.org/10.18684/rbsaa.v20.n1.2022.1914

Valderrama Martín, J. M. V., Ortigosa, F., and Cañas, R. A. (2020). Métodos de secuenciación de ácidos nucleicos: Primera generación. *Encuentros en la Biología*, *13*(173), 19-25. https://www.revistas.uma.es/index.php/enbio/article/view/17342

Vera, J., Barzallo, D., Villamar, M., Ortiz, J., Moyano, G., and Yugsan, J. (2024a). Productive and economic potential of the application of biofertilizer

in hybrid corn DAS 3383. *Revista de la Facultad de Agronomía de la Universidad del Zulia*, *41*(4), e244237. https://produccioncientificaluz.org/index.php/agronomia/article/view/42779

Vera Rodríguez, J. H., Barzallo, D., Villamar Aveiga, M. del R., and Barcia-Anchundia, J. X. (2024b). Efecto bioestimulante de los microorganismos sobre la germinación *in vitro* de semillas híbridas de pimiento. *Ciencia Y Tecnología Agropecuaria*, *25*(1), e3306. https://doi.org/10.21930/rcta.vol25_num1_art:3306

Visentin, A. P. V., Bertholdo, L. R., Hahn, R. C., Thomazoni, R. A., Touguinha, L. B. A., Branco, C. S. and de Barros, N. M. (2021). Comparative effectiveness of *Metarhizium rileyi*, novaluron, and glyphosate on immune system, development, and redox metabolism of *Anticarsia gemmatalis*. *Research, Society and Development*, *10*(6), e19810615611. http://dx.doi.org/10.33448/rsd-v10i6.15611

Wilson, K. H., Blitchington, R. B., and Greene, R. C. (1990). Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *Journal of Clinical Microbiology*, *28*(9), 1942-1946. https://doi.org/10.1128/jcm.28.9.1942-1946.1990

Yamamoto, S., & Harayama, S. (1995). PCR amplification and direct sequencing of gyrB genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Applied and Environmental Microbiology*, *61*(3), 1104-1109. https://doi.org/10.1128/aem.61.3.1104-1109.1995

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