



Scientific Article

## Effect of ornamental plants on the rhizospheric fungal microbiome and soil properties

Ramón Alejandro Murillo-Chaidez<sup>1</sup>, Mirella Romero-Bastidas<sup>1\*</sup>, Alejandra Nieto-Garibay<sup>2</sup>, Alejandro Palacios-Espinosa<sup>1</sup>, Laboratorio de Fitopatología, Departamento Académico de Agronomía, Universidad Autónoma de Baja California Sur, Carretera al Sur KM 5.5 Apartado Postal 19-B CP: 23080 La Paz, Baja California Sur, México<sup>1</sup>. Laboratorio de Fisiología vegetal, Centro de Investigaciones Biológicas del Noroeste. Km. 1 Carretera a San Juan de La Costa "El Comitan" CP: 23205, La Paz, Baja California Sur, México.

\*Corresponding Author:  
Mirella Romero-Bastidas  
miromero@uabcs.mx

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### RESUMEN

**Antecedent/Objective.** Ornamental plants are important for the aesthetics of green areas in Baja California Sur. However, there are few studies related to their influence on soil fungal diversity and edaphological characteristics. Therefore, the objective of this study was to evaluate the effect of six ornamental (*Euphorbia mili*, *Ruellia simplex*, *Catharantus roseus*, *Centaurea cyanus*, *Tagetes erecta* and *Calendula officinalis*) on the population, diversity and richness of fungi, as well as their relationship with soil physical and chemical characteristics.

**Materials and Methods.** Randomly obtained rhizospheric soil samples were subjected to serial dilutions for isolation, population counting, morphological identification, and the diversity and richness of fungi associated with each ornamental species. In addition, the soil's physical and chemical characteristics were determined at the beginning and end of the experiment. A Person correlation between the variables was also evaluated.

**Results.** The highest fungal population was in *C. cyanus* with 8,096.66 CFU g<sup>-1</sup> of soil. While, in *E. mili* and *T. erecta* it was reduced to 3,333.33 CFU g<sup>-1</sup> of soil. Seven genus were identified, with *Aspergillus* spp. being the most predominant. While, *E. mili* and *C. cyanus* stimulated the greatest diversity (12 and 13 isolates). The correlation analysis was negative.

**Conclusion.** This information confirms that the ornamental species *C. cyanus* has a significant influence on soil ecology by increasing the microbial population (from 2,833.33 to 8,096.66 CFU g<sup>-1</sup> of soil) and greatly stimulating its diversity (with seven isolates at the beginning and 13 at the end)

**Keywords:** Population, Fungi, Microbiote, Isolation, Rizosphere

## INTRODUCTION

Ornamental plants are important in the different ecological niches. They are characterized by their beauty, color and aroma. Different investigations have reported that, in addition to their esthetic value, they have an influence on the soil microbiology and characteristics (Francini *et al.*, 2022). Such is the case of *Euphorbia mili* and *Calendula officinalis*, used in the bioremediation of the soil, as they reduce heavy metals such as chromium, cadmium and lead (Afrousheh *et al.*, 2015), which in turn has an influence on the microorganisms found in the soil, since it leads to positive or negative effects on their survival. Additionally, this type of plant has been proven to favor the absorption of nitrogen and potassium by improving their assimilable forms and increase the content of organic matter (Sari and Küçük, 2019). Likewise, diverse studies have confirmed that the effect of ornamental plants on the physicochemical soil properties are associated to the diversity of the edaphic microbiota (Zhang *et al.*, 2023), where the fungal community stands out, and which plays a key role in the regulation of the diversity-productivity relation of plants (Mommer *et al.*, 2018), as it is one of the fundamental microorganisms in the decomposition of organic matter, favoring soil structure, fertility and health (Frac *et al.*, 2018). At the same time, the diversity of these organisms has been established to be affected by some plant species, which release secondary metabolites with an antimicrobial action such as saponins, which negatively alter the cell membranes of fungi, increase levels of Ca<sup>2+</sup> and oxygen reactive species (Turner *et al.*, 2013). In addition, it has been proven that coumarins, triterpenes, flavonoids, benzoxazinoids and phytohormones inhibit the growth of rhizospheric microorganisms (Voges *et al.*, 2019). Due to the importance of the plant-microbiome relation, there are currently numerous investigations in this regard. However, studies on the effect of ornamental plants on the fungal soil community, as well as their correlation with the edaphic characteristics are limited. Due to this, the aim of this study was to evaluate the biological response of ornamental plants on the diversity and richness of the fungal rhizospheric microbiome and the properties of the soil in Baja California Sur.

## MATERIALS AND METHODS

**Area of study.** The investigation was realized in the 2022-2023 cycle in the municipal area of La Paz, Baja California Sur, Mexico (24°7'0" N, 110°43'0" W and at an altitude of 0 to 27 masl meters above sea level). The region is characterized by an average annual rainfall below 200 mm and an average annual temperature ranging between 18 and 22 °C (INEGI, 2021). The climate is desert hot (BWh) semi-arid hot (BSh) according to Köppen's classification. The soil is considered loamy-sandy. The predominant vegetation is scrublands.

**Obtaining ornamental plants.** Six ornamental plant species were chosen for this investigation, considering characteristics such as commercial demand, low water need, intense light requirements, a short biological cycle and being a species introduced to the state. Two ornamental plants were acquired from cuttings: the crown of thorns (*Euphorbia mili*: Malpighiales-Euphorbiaceae) and the Mexican petunia (*Ruellia simplex*: Lamiales-Acanthaceae). While, the four remaining ornamental plants were

acquired from commercial seed lots (Vita®): Teresita (*Catharanthus roseus*: Gentianales-Apocynaceae), Brush (*Centaurea cyanus*: Asterales-Asteraceae), Cempasuchil (*Tagetes erecta*: Asterales-Asteraceae) and Pot marigold (*Calendula officinalis*: Asterales-Asteraceae).

**Experimental design.** The experimental work was carried out at the agricultural field level (with over 10 years without vegetable planting), by establishing seven plots, each with an area of 81 m<sup>2</sup>, consisting of three furrows, measuring 15 meters in length by 80 centimeters in width. The seeds of the second group of ornamental plants were directly planted in the furrows at a depth of twice its size (1-2 cm) with a planting distance of 30 cm. While, the 15 cm cuttings were transplanted at a depth of 10 cm, with a planting distance of 0.75 cm and 1.5 m. The treatments consisted in six ornamental species and a control (without a plant). Every treatment had three repetitions, each one of which consisting of one furrow. All treatments were arranged under a complete random block design.

**Sampling, isolation, quantification and identification of fungal isolates.** Nine rhizospheric soil samples were taken at random from each experimental unit at a depth of 30 cm before, during and after the experiment (n=27 samples/treatment) for an eight-month period (September-April). The fungal isolation was performed with seriated dilutions (10<sup>3</sup>) and planting in Potato-Dextrose-Agar (PDA) (MCD LAB, Oaxaca, Mexico) at a pH of 7. Ten days after planting (dap), the population was determined in UFC g<sup>-1</sup> of soil. Semipermanent preparations were generated from pure isolates on a slide and observed under an optic microscope (LABOMED-40x) for the fungal morphologic identification, which took place with a macroscopic (morphology and color of colonies) and microscopic characterizations (shape of mycelium, spores and fruit body) and they were compared with taxonomic keys (Barnett and Hunter 1972; Kirk *et al.*, 2008; Samson *et al.*, 2014).

**Diversity and species richness.** The fungal diversity in each one of the ornamental plants was determined with the frequency of occurrence of each species, calculated by dividing the number of colonies of a species, given between the total number of isolated communities in each sample, expressed as a percentage. While, the **richness** (S = total number of species) was determined using the formula proposed by Kumari *et al.* (2016) with diversity index by Shannon ( $H' = -\sum p_i \ln p_i$ , where  $p_i$  is the proportion of individuals of the  $i$  species) and Simpson ( $D = \frac{1}{\sum (n_i/N)^2}$ , where  $n_i$  is the total number of organisms of a particular species and  $N$  is the total number of organisms of all species).

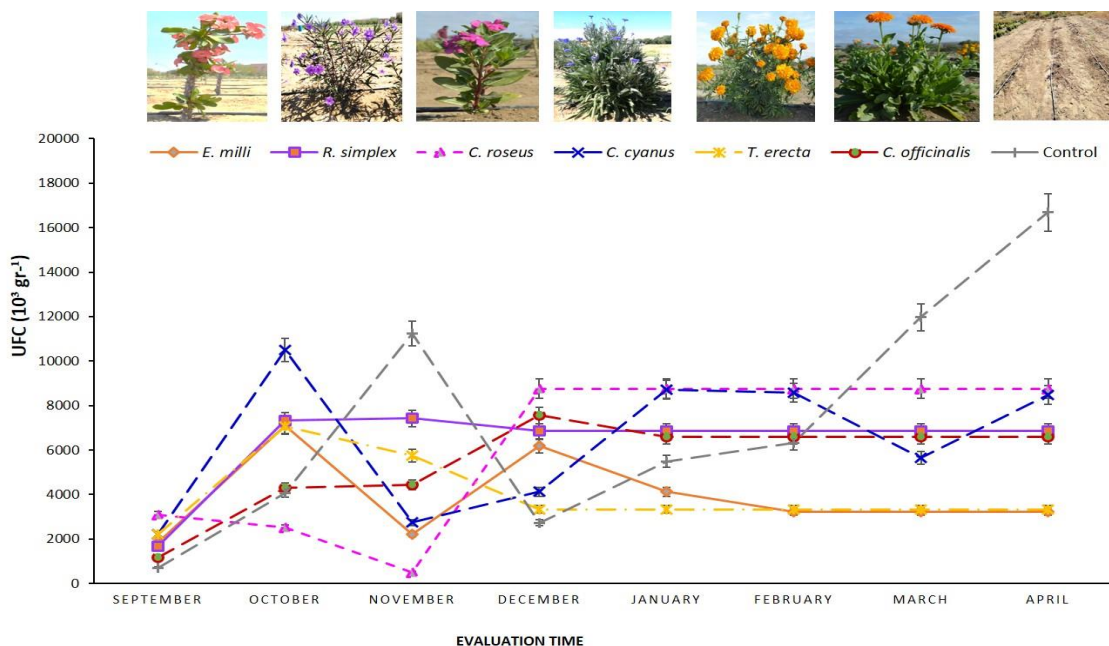
**Evaluation of edaphological characteristics.** The edaphological analysis of the soil was performed before the establishment of the plants and at the end of the experiment for a period of eight months. The effect of the ornamental plants on the soil properties was evaluated by determining the pH, organic matter (OM), Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and electric conductivity (EC). The soil pH was obtained with a potentiometer (VWR scientific products model sp20), whereas the organic matter was measured using the updated Walkley-Black method (Walkley, 1942). Likewise, Phosphorous was determined using the OLSEN method. Potassium was determined using the PECH method, and Calcium and Magnesium were determined using the EDTA

method. All the macro and microelements were measured in the spectrophotometer (Spectronic Instruments, model Spectronic 20 GENESYS) and electric conductivity was determined using the wheatstone bridge method with the help of a conductometer (Bridge model-31).

**Statistical analysis.** The data were organized in Microsoft Excel 2019 for processing. To identify significant differences in diversity, Simpson and Shannon indices, as well as chemical properties of the soil, a one-way analysis of variance was used, as well as a 0.05 confidence level with Tukey's test, using the statistical software Prism. Additionally, Pearson correlation coefficients between fungal diversity and soil properties in ornamental plants were analyzed.

## RESULTS AND DISCUSSION

**Population.** The fungal populations in ornamental species presented variations in time, where, in most cases, the population decreased in the month of October, which was related to changes in temperature and the permanence of moisture in the soil. *C. cyanus* increased the fungal population, going from 2,833.33 to 8,096.66 UFC g<sup>-1</sup> of soil (Figure 1). While, in *E. mili*, the population increase was lower (from 3,159 to 3,559 UFC g<sup>-1</sup> of soil). On the other hand, the species *C. roseus* presented a population of 3,126.66 UFC g<sup>-1</sup> of soil, and in the end, its population increased to 5,086.66 UFC g<sup>-1</sup>. In turn, *C. officinalis* began with 1,060 UFC g<sup>-1</sup> of soil and increased considerably to 6,633.33 UFC g<sup>-1</sup> of soil at the end of the experiment. Likewise, *R. simplex* maintained an initial population of 1,686.66 UFC g<sup>-1</sup> of soil and ended with 5,720 UFC g<sup>-1</sup>. The population of *T. erecta* went from 2,323.33 to 3,333.33 UFC g<sup>-1</sup> of soil at the beginning and the end, respectively. Finally, in



**Figure 1.** Effect of ornamental plants on the fungal population of the rhizosphere of plants.

the control treatment, a significant variation was presented in the fungal population, beginning with 720 UFC g<sup>-1</sup> of soil and ending with 12,483.33 UFC g<sup>-1</sup> of soil, exceeding the population in the ornamental plants (Figure 1). In general, most ornamental plants presented an increase in the fungal population at the end of the experiment, but none of them passed the control, where the microbiome increased significantly.

This response may only be related to the condition of moisture of the soil to activate the microbial population, and in which ornamental plants have an influence on the regulation of the fungal population, possibly due to the secondary metabolites that their roots secrete. This agrees with Ma *et al.* (2022), who report that the root exudates contain carbohydrates, amino acids, flavonoids and phenolic acids, which have different effects on the microorganisms of the roots. Among these, the amino acids and carbohydrates provide efficient sources of carbon and nitrogen that impact the number and the distribution of species of microbial populations in the soil. Likewise, Sasse *et al.* (2018) add that the root exudates gradually alter the microbiome by modifying the physicochemical properties of the soil by releasing large amounts of organic acids, which reduces the pH of the soil and affecting microbial growth and colonization. In addition, the root exudates not only solubilize phosphate, but also regulate the microbial absorption of phosphate in the rhizosphere. In addition, the concentration and types of exudates depend on the phenological stage of the plants, which may be related to the fungal population variation observed throughout time in each ornamental plant.

**Morphological characterization.** A total of 25 fungal isolates were obtained, with varied colony morphologies, presenting cottony, granulated textures and/or plane, circular or irregular in growth, the latter being fast, moderate or slow in some species. Different color tones were also displayed, which varied from olive green, light green to dark green, as well as white, yellow, purple, orange, black, salmon, brown and pink (Table 1). In the microscopic characterization, the fungi displayed differential shapes in their spores, mycelia and fruit bodies (Figures 2, 3 and 4), where, from these characteristics, 11 species of the *Aspergillus* genus, six of the *Trichoderma* genus, three of *Penicillium*, two of *Rhizopus*, one of *Mortierella* spp., *Fusarium oxysporum* and *Geotrichum* spp. were identified. Most of the fungi identified belong to the groups of saprophytes in which the genus *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp. and *Rhizopus* spp. stand out. This agrees with the report by Nicoletti and Trincone (2016), which mentions that *Aspergillus* and *Penicillium* represent the first and second most abundant fungus genus reported, respectively. The variety of colors of each fungus was evident, where these are associated to the environmental resistance and the type of feeding of the microorganism in its ecological niche. According to Pombeiro *et al.*, (2017), the fungal pigments related to the melanins are associated with the color gray; anthraquinones, hydroxyanthraquinones and quinones with maroon, bronze, and orange; azaphilones and oxopolyenes with yellow, purple and red; carotenoids with orange and yellow; and naphthoquinones with red and purple.

**Table 1.** Morphology of the rhizospherical fungi associated to ornamental plants.

Species	Colony		Mycelium		Spores
	Front	Back	Texture	Growth	
<i>Aspergillus</i> C-1	Purple	Purple	Plane	Slow	Globose
<i>Aspergillus</i> C-3	Green	Yellow	Plane	Moderate	Globose
<i>A. orizae</i>	Green	Green	Plane	Moderate	Globose
<i>A. terreus</i>	Salmon	Yellow	Plane	Moderate	Globose
<i>A. niger</i> C-1	Black	Black	Plane	Moderate	Globose
<i>A. niger</i> C-2	Black	Black	Granulated	Moderate	Globose
<i>A. candidatus</i>	White	Brown	Cottony	Moderate	Globose
<i>A. flavus</i>	Olive green	White	Plane	Moderate	Globose
<i>A. ochraceus</i>	Yellow	Crem	Granulated	Moderate	Globose
<i>A. flavipes</i>	White	Crem	Striated	Slow	Globose
<i>Trichoderma</i> spp.	Yellow	Yellow	Granulated	Fast	Globose
<i>T. atroviride</i>	Light green	White	Cottony	Fast	Subglobose
<i>T. harzianum</i>	Green	White	Cottony	Fast	Globose
<i>T. atroviride</i> C-1	Green	Green	Plane	Fast	Globose
<i>T. atroviride</i> C-2	Light green	White	Cottony	Fast	Globose
<i>T. asperellum</i>	Light green	White	Cottony	Fast	Globose
<i>Penicillium</i> C-1	Orange	Orange	Plane	Moderate	Oblong
<i>Penicillium</i> C-2	Yellow	Orange	Plane	Moderate	Globose
<i>P. digitatum</i>	Light green	Crem	Plane	Moderate	Globose
<i>Rhizopus</i> spp.	Brown	Brown	Cottony	Fast	Globose
<i>R. stolonifer</i>	Grey	Grey	Plane	Fast	Subglobose
<i>Mortierella</i> spp.	White	White	Plane	Slow	Globose
<i>Geotrichum</i> spp.	White	White	Plane	Fast	Bacillary
<i>F. oxysporum</i>	Pink	Pink	Plane	Moderate	Catenulated

**Fungal diversity and richness of the soil.** The fungal diversity indicated the presence of two phyla (Ascomycota and Mucoromycota), six families (Trichocomaceae, Nectriaceae, Mortierellaceae, Hypocreaceae, Endomycetaceae and Mucoraceae) and seven genus. The phylum Ascomycota was the most predominant (frequency of 71%), compared to the phylum Mucoromycota (29%). The results revealed that the fungal communities in the soil samples obtained from each ornamental plant were different at the time of evaluation (initial and final), and a change was observed, not only in the diversity of the microbiota of the ornamental plants in the initial and final evaluation, but also in the frequency of occurrence, modifying the population density by species of fungus.

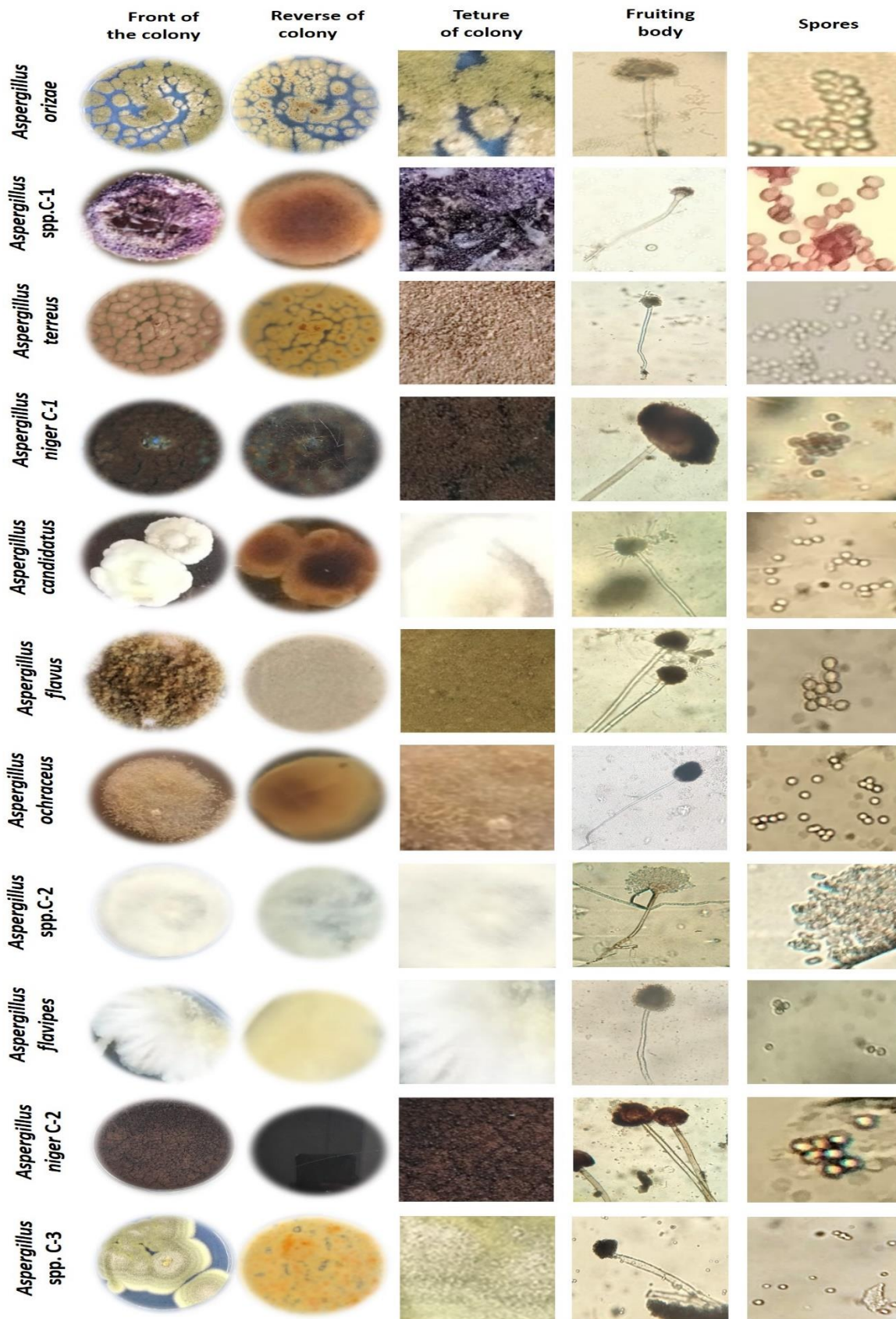


Figure 2. Microbiota corresponding to the *Aspergillus* genus found in the rhizosphere of ornamental plants.

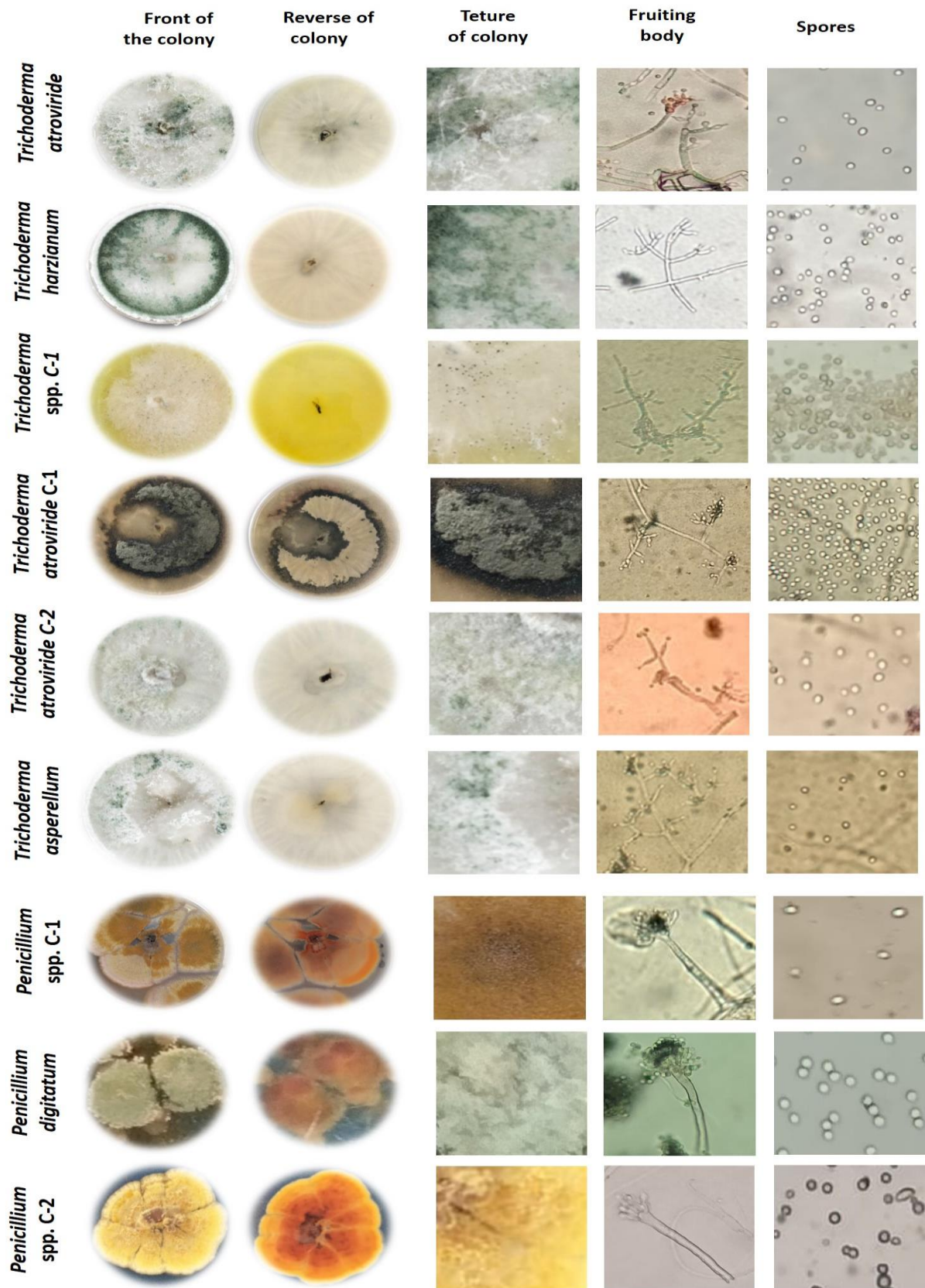
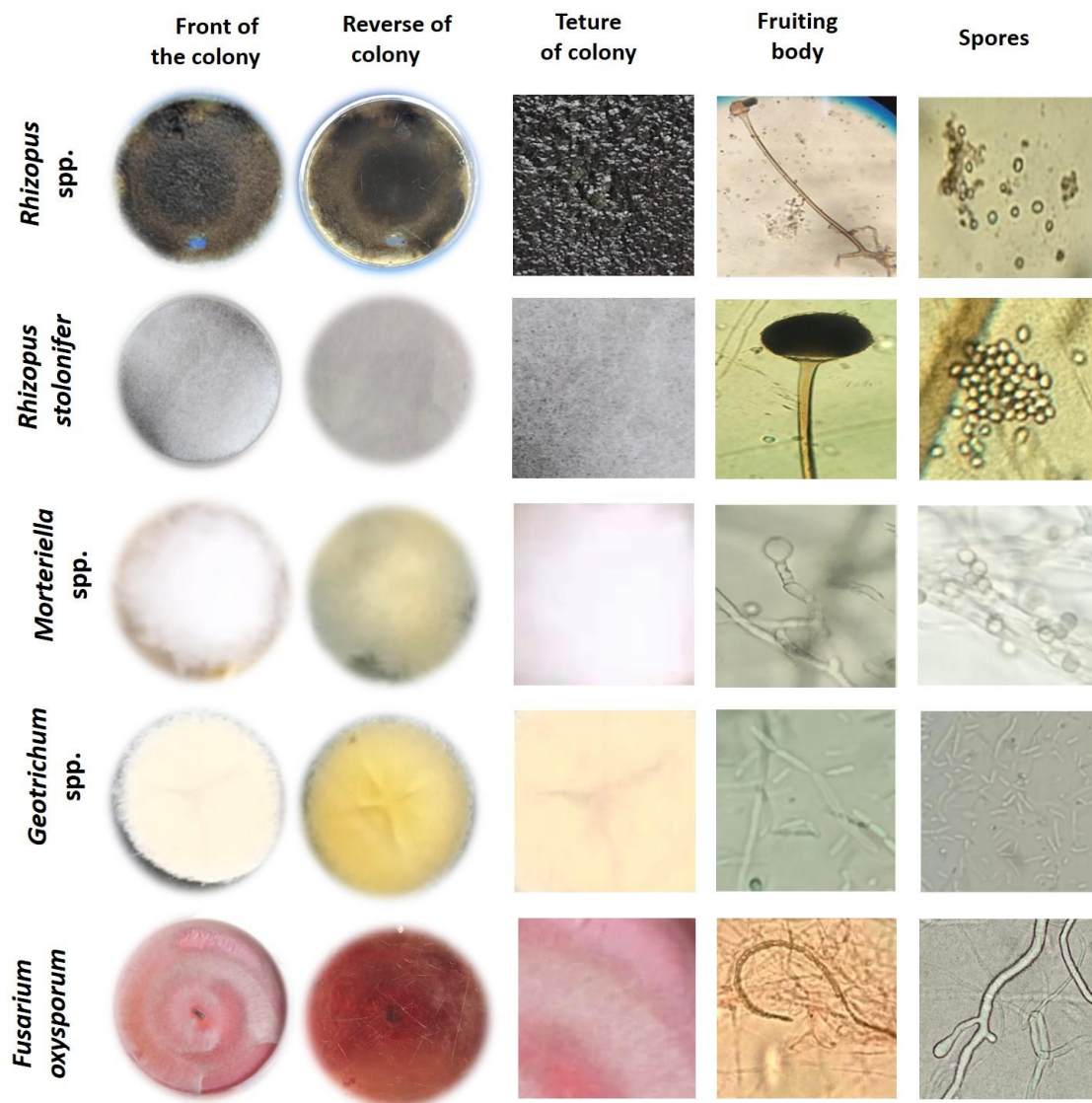


Figure 3. Microbiota corresponding to the *Trichoderma* and *Penicillium* genera of the rhizosphere in ornamental plants.



**Figure 4.** Microbiota corresponding to the genus with the least diversity in the rhizosphere of ornamental plants.

*C. cyanus* registered the greatest diversity of fungal species at the end of the experiment, presenting seven isolates at the beginning and 13 at the end of the study, where the genus *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma* highlighted, followed, in order of importance, by *E. mili*, presenting seven isolates at the beginning and 12 at the end. In turn, *C. officinalis* registered seven isolates, and at the end, it increased to nine isolates. In *R. simplex* and *C. roseum*, the number of isolates remained in seven and nine, respectively. While, in *Tagetes* (12-8 isolates) and the control treatment (9-6 isolates), diversity decreased (Figure 5). The diversity found in this type of species indicates that, despite the characteristics in the arid area, such as high temperatures, low amount of organic matter and low humidity, they had no impact on the genus *Aspergillus*, *Trichoderma* and *Penicillium*. This diversity is relevant for the plants established, as pointed out by Reinhart and Callaway (2006), when mentioning that the interactions between plants and soil microorganisms are dynamic by nature and based on coevolutionary pressures. In addition, Batten *et al.* (2006) point out that the rhizospherical communities differ among plant species, within the genotypes in the species and between

the different plant development stages, although it is unclear whether this is due to a greater heterogeneity of the habitat, the increase of the plant biomass commonly observed with very diverse communities or a greater diversity of carbon substrates and signaling compounds provided by the plants. Fitzpatrick *et al.* (2018) point out that the fungal microbiome is divided into three categories (mutualists, pathogens and saprophytes) based on the type of plant, which in turn depends on the antagonistic and synergic interactions within the microbiome of the soil, and it is confirmed that plants that grow in a soil with a common microbiome can alter the relative abundance of mutualists, pathogens and saprophytes in the soil.

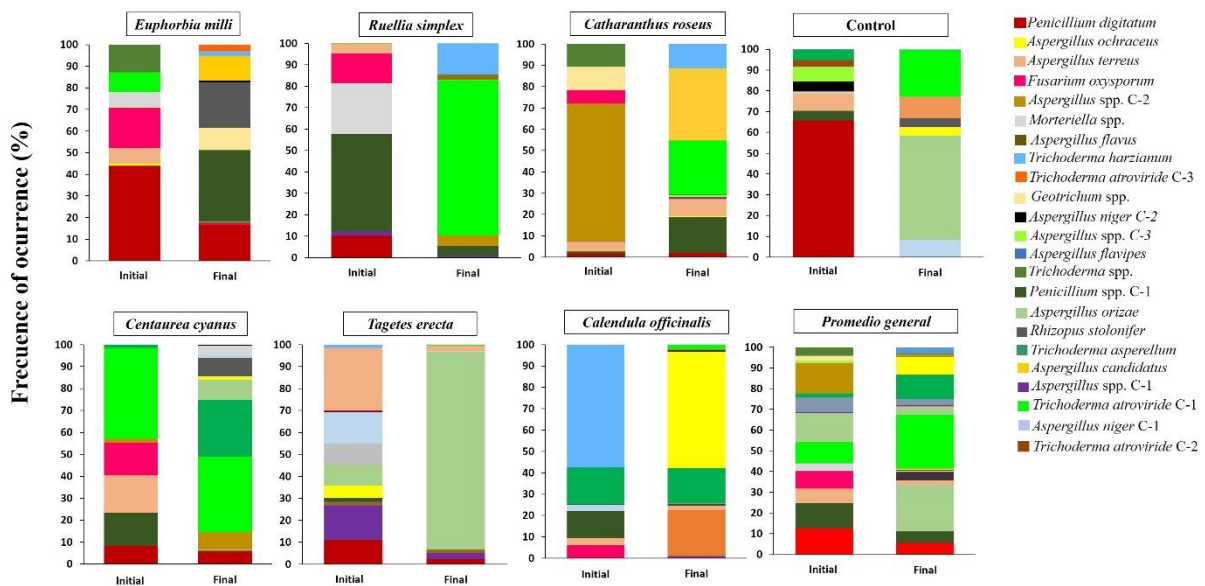


Figure 5. Fungal diversity and richness in the soil of ornamental plants.

**Shannon and Simpson Index.** The estimations of diversity in ornamental plants are shown in Table 2. The Shannon and Simpson indices were used to reveal the diversity and richness found in the rhizosphere of the ornamental plants. *E. mili* and *C. cyanus* presented 14 different isolates, whereas *T. erecta* displayed eight identified species. In addition, in *E. mili*, the species that presented the highest proportion were found to be *Penicillium spp. C-1* (33%), *Rhizopus stolonifer* (20.8%) and *Penicillium digitatum* (16.5%), whereas in *C. cyanus*, *Trichoderma atroviride C-1* (34%) and *Trichoderma asperellum* (25%) species were found. In *T. erecta*, the greatest proportion was found in the species *Aspergillus oryzae* (90%). The diversity calculated with the Shannon index presented similarity in the species *E. mili* and *C. cyanus*, whereas *T. erecta* displayed the lowest value in comparison with the rest of ornamentals. While, *C. officinalis* and *C. roseus* presented, in the end, a diversity of similar isolates (13 isolates). In turn, the dominance of species measured by the Simpson index showed that *C. cyanus* registered the highest value, whereas *T. erecta* displayed the minimum value. According to Margalef (1972), the Shannon index comprises values of 1-5, where values lower than 2 indicate a low diversity. Likewise, Silva *et al.* (2021) mention that the Shannon index increases as a higher number of its species and the proportion of individuals is more homogenous. In the Simpson index, Soler *et al.* (2012) indicate that it increases or decreases, depending

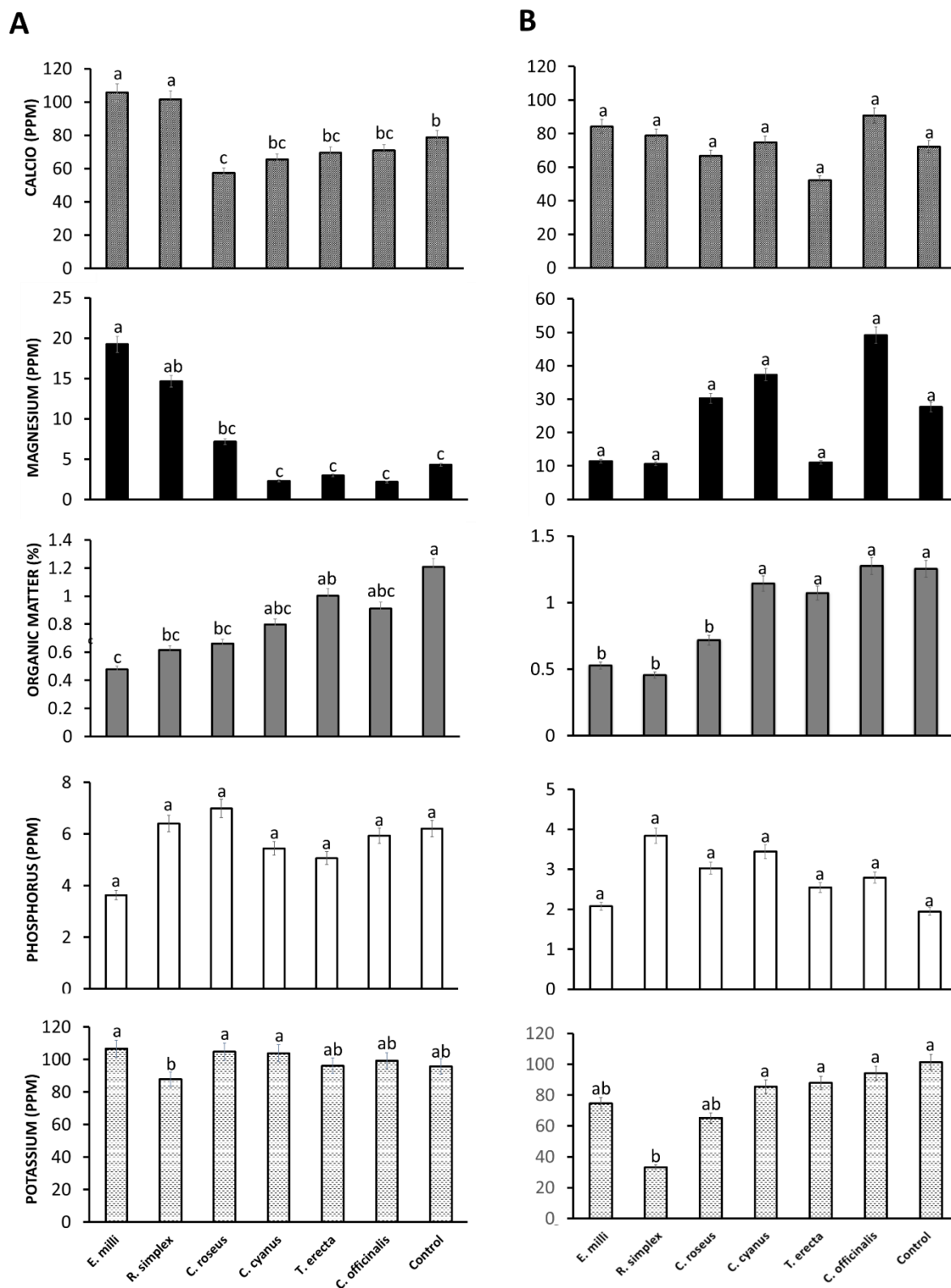
on whether the diversity increases or decreases, and it is really an index of dominance that overvalues the most abundant species of the total richness. Magurran (1998) adds that, as this value approaches zero, there is a greater possibility of the dominance of a species and of a population, and the closer to the unit, the lower the dominance, as was the case in *R. simplex* and *T. erecta*, where both presented a low diversity and a low species dominance.

**Table 2.** Fungal diversity and richness of the rhizosphere of ornamental plants.

Especie ornamental	Shannon index	Simpson index
<i>Euphorbia mili</i>	1.8774	3.9072
<i>Ruellia simplex</i>	0.7976	1.6496
<i>Catharanthus roseus</i>	1.6670	4.4036
<i>Centaurea cyanus</i>	1.8823	4.8416
<i>Tagetes erecta</i>	0.4890	1.2312
<i>Calendula officinalis</i>	1.2973	2.6898
Tratamiento control	1.4187	3.0936

**Chemical Properties of the Soil.** No significant differences were found regarding the content of phosphorous in the initial soil analysis or in the final analysis related to the six ornamental species and the control. In organic matter *C. officinalis* displayed the maximum values (1.07%), whereas in *R. simplex* it was lower (0.45 %). Likewise, *E. mili*, *R. simplex* and *C. roseus* displayed a significant difference with *C. cyanus*, *T. erecta*, *C. officinalis* and the control. In potassium, *R. simplex* displayed the lowest content in comparison with the rest of the ornamental plants (33.11 ppm) and the control (101.4 ppm). In addition, *R. simplex* displayed significant differences with the species *C. cyanus*, *T. erecta*, *C. officinalis* and the control treatment, whereas the species *E. mili* and *C. cyanus* displayed no significant differences with the rest of the treatments. In the contents of phosphorous, calcium, magnesium and electric conductivity, no significant differences were observed. In *R. simplex*, *E. mili* and *C. roseus*, in which organic matter was less, this can be associated to these being in constant production of biomass related to leaves, branches and flowers (Figure 6). This agrees with Adamczyk (2021), who points out that a greater proportion of roots can improve the amount and diversity of organic compounds such as nitrogen, improving the composition, the increase and stability of the organic matter in the soil, since this type of compounds secreted by the roots help to break down the molecules and/or ions associated with chemical elements found in the organic matter of the soil. Likewise, Coskun *et al.* (2017) point out that certain exudates can inhibit nitrification by selectively inhibiting nitrifying bacteria in the soil, by slowing down the reaction speed at which ammonium becomes nitrate in the soil.

For potassium content, *Calendula officinalis* presented the highest value, possible related to the secondary metabolites exudated by the roots of this plant, as pointed out by Ma *et al.* (2022), who claim that root exudates, rich in organic acids may exert a potassium-releasing effect on the minerals containing potassium. This response in the variation of the content of potassium may be due to some ornamental species having used this element in greater demand for drought resistance, the constant formation of roots, as

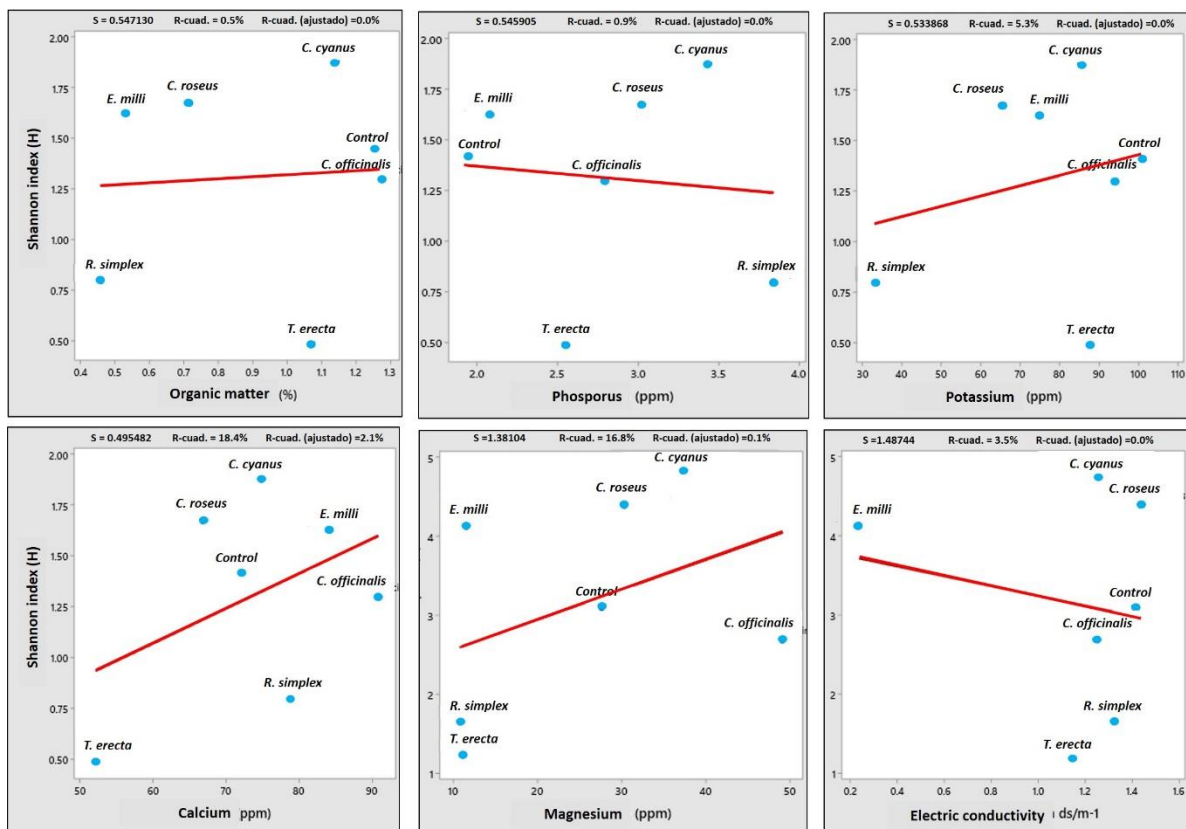


**Figure 6.** Effect of ornamental plants on soil nutrients. A) Initial and B) final analyses. The values are means  $\pm$  standard deviation. a b c d; means followed by the same letter for a given factor are not significantly different ( $p < 0,05$ ; Tukey's HSD test).

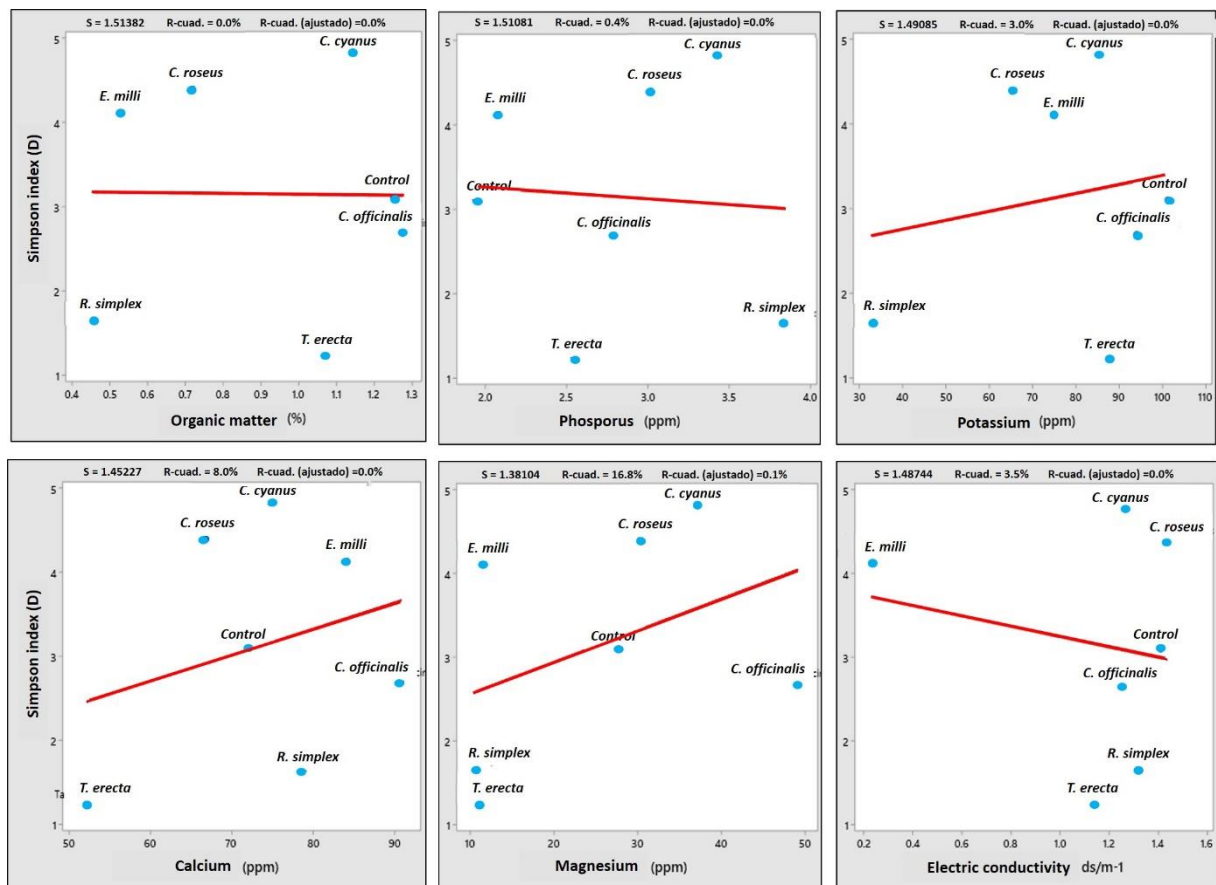
well as the continuous flowering presented in each one of the ornamental species. In this regard, Srivastava *et al.* (2019) indicate that potassium helps in the production of sugars, as well as in the formation and synthesis of proteins and reduces water loss. On the other hand, Anschutz *et al.* (2014) explain that potassium helps plants in drought resistance, as well as in stress caused by high temperatures. In the case of the remaining parameters, these showed no changes, possibly due to a need for more evaluation time, or that all ornamental species did not demand a higher amount of these elements, as in the case of organic matter and potassium.

### Analysis of correlation between the fungal diversity and the physical-chemical properties of the soil

The patterns found in the Pearson correlation diagrams point out that, both in the Shannon and Simpson indices, the edaphological characteristics of the soil (Figure 7 and 8) correlated negatively with the type of ornamental plant. Similar negative correlation results were reported by Pacasa-Quisbert *et al.* (2017) when evaluating the effect of the type of soil use, type of native vegetation and type of crop on the diversity and number of fungi related to the physical and chemical parameters of the soil (pH, texture, organic matter) and the concluded that microbial abundance is highly related to other factors such as soil type, weather conditions, other microorganisms and even the type of plant communities.



**Figure 7.** Correlation between Shannon's fungal diversity index and the characteristics of the soil associated to ornamental plants.



**Figure 8.** Correlation between Simpson's fungal diversity index and the characteristics of the soil associated to ornamental plants.

## CONCLUSIONS

The type of ornamental plant species is determined to have influenced on the diversity and richness of the fungal microbiota, as well as on some physicochemical soil properties. However, no positive relation was observed in the correlation of the three variables. *Ruellia simplex* was the ornamental plant with the least fungal diversity (7 isolate). While, the plant associated to *C. cyanus* presented the greatest increase in fungal diversity and richness (13 isolate). Likewise, ornamental plants *R. simplex* and *T. erecta* favored the presence of beneficial fungi in the soil with greater amounts of the genus *Trichoderma* spp. and *Aspergillus* spp. Therefore, it can be pointed out that some ornamental plants have a positive influence on the fungal diversity and richness.

### Conflict of interest

The authors declare that there is no conflict of interest in this investigation.

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### Contribution of the authors

Ramón Alejandro Murillo-Chaidez; research, preparation of the original draft. Mirella Romero-Bastidas; Validation, revision and editing. Alejandra Nieto-Garibay; supervision. Alejandro Palacios-Espinosa; Statistical data analysis.

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