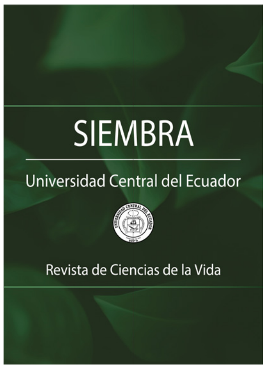
# *Effect of herbicides on the population of microorganisms in the cultivation of Theobroma cacao L., in the Luz de América parish, Ecuador*




## *Efecto de los herbicidas sobre la población de microorganismos en el cultivo de Theobroma cacao L., en la parroquia Luz de América, Ecuador*

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**Abstract**

Conventional agriculture is characterized by excessive use of machinery, agrochemicals, and environmentally unfriendly practices. We aimed to determine the effect of herbicides (glyphosate and paraquat) on the population of rhizosphere microorganisms in *Theobroma cacao* L. crops in the municipality of Luz de América, Ecuador. The research was carried out between June and November 2019 in a seven-year-old crop planted in a 4x4 m arrangement, with 6.36 % organic matter, silt loam clay loam soil, and pH of 5.93 with identical management throughout the plantation. The treatments were: T1 = systemic herbicide-glyphosate (1.5 l/ha); T2 = contact herbicide-paraquat (1.5 l/ha); and T3 = mechanical control-mower-control; each with six replicates. Three soil subsamples were taken and sent to the laboratories of the Instituto Nacional de Investigaciones Agropecuarias (INIAP). The variables measured were population growth, genus identification, and fungal population growth of sample. Statistical analyses were carried out using Tinn-R. Day 28 marked a significant difference between all observations (P-value=0.0269); the presence of microorganisms had a higher concentration when paraquat was used (1,894,001 CFU/ml). The presence of *Trichoderma* (higher in paraquat) and *Fusarium* (higher in glyphosate) was noted when evaluating the population development of the fungi. Considering the treatments and the days of observation, it was confirmed that the population of *Trichoderma* decreased in greater proportion when glyphosate was applied. We conclude that the two herbicides increase the development of *Fusarium*, although paraquat to a lesser extent, while paraquat favors the presence of *Trichoderma*.

**Keywords:** Population concentration, weed control, herbicides, rhizosphere microorganisms, *Theobroma cacao* L.

**Resumen**

La agricultura convencional se caracteriza por el uso excesivo de maquinarias, agroquímicos y prácticas poco cuidadosas con el ambiente. El objetivo de esta investigación fue determinar el efecto de los herbicidas (glifosato y paraquat) sobre la población de microorganismos rizósferos en el cultivo de *Theobroma cacao* L., en la parroquia Luz de América, Ecuador. La investigación se realizó en el periodo junio-noviembre de 2019; en un cultivo con siete años de edad, sembrado en arreglo 4x4 m, con 6,36% de materia orgánica, suelo franco limo arcilloso y pH de 5,93; con manejo idéntico en toda la plantación. Los tratamientos fueron; T1 = Herbicida sistémico-glifosato (1,5 l $ha^{-1}$); T2 = Herbicida contacto-paraquat (1,5 l $ha^{-1}$); y T3 = Control mecánico-chapeadora-testigo; cada uno con seis repeticiones. Se tomaron tres submuestras de suelo y se enviaron a los laboratorios del Instituto Nacional de Investigaciones Agropecuarias [INIAP]. Las variables medidas fueron; desarrollo poblacional; identificación del género; y desarrollo poblacional de los hongos de la muestra. El análisis estadístico se realizó con Tinn-R. El día 28 marcó diferencia significativa entre todas las observaciones (P-valor=0,0269); la presencia de microorganismos tuvo mayor concentración cuando se utilizó paraquat (1.894.001 UFC $ml^{-1}$). Se identificó la presencia de *Trichoderma* (mayor en paraquat) y *Fusarium* (mayor en glifosato); al evaluar el desarrollo poblacional de los hongos. Considerando los tratamientos y los días de observación, se confirmó que *Trichoderma* disminuye en mayor proporción su población cuando se aplica glifosato. Se concluyó que los dos herbicidas elevan el desarrollo de *Fusarium,* aunque paraquat en menor proporción, a la vez que este promueve la presencia de *Trichoderma*.

**Palabras clave:** Concentración poblacional, control de malezas, herbicidas, microorganismos rizósferos, *Theobroma cacao* L.

## 1. Introduction

In recent years, food security has experienced major challenges as a consequence of human overpopulation and minimal application of sustainable agricultural protocols. This both causes a short duration of natural resources, and limits production due to alterations of biotic and abiotic factors (Cruz Cárdenas et al., 2021). Knowingly the good use of resources, such as water and soil, allows producers to be self-sufficient (Guamán Guamán et al., 2020), since it is possible to cultivate without mitigating natural resources, through the application of environmentally friendly techniques (Waseem et al., 2020).

Conventional agriculture is characterized by the excessive use of machinery, agrochemicals and aggressive practices, bringing about modifications in the edaphic system as well as environmental instability (Alarcon et al., 2020). These cause an alteration in the soil-plant-microorganism interaction, which -under optimal conditions-, has the capacity to improve soil structure and allow the conservation of natural agroecosystems (Rosabal Ayan et al., 2021).

Within the same context, the current model of agricultural exploitation, is generally still associated with the use of agrochemicals, the most widely used being herbicides, fertilizers, insecticides and fungicides (MacLaren et al., 2020). In the case of herbicides, these are considered the best option when applying weed control on crops, a practice that is considered mandatory within a plantation, as weeds can occasion a reduction of up to 30% in agricultural production (González-Ortega & Fuentes-Ponce, 2022).

In this aspect, glyphosate is the main herbicide (non-selective systemic) applied in large extensions crops. Its frequency of use in South America is of 66%, regardless of the commercial brand, (Suárez Escobar et al., 2019). This agrochemical was declared persistent in soil, which has allowed it to be found in various aquatic environments thanks to runoff, affecting soil microbiota (Meena et al., 2020). Similarly, according to Caicedo Amazo (2021), this herbicide contaminates air, soil, water and food, directly affecting biodiversity. It is known that glyphosate is a broad-spectrum herbicide, harmful to health and likely carcinogenic when ingested, or when exposure to this toxic substance is prolongued (Aborisade & Atuanya, 2020).

Similarly, paraquat is one of the most widely used herbicides in North America and one of the most sold worldwide, despite its use is banned in European countries. It is used on more than 120 crops, mainly coffee, rice, pineapple, cocoa, palm, banana and pasture (Montero Rojas, 2018). This is a non-selective contact herbicide, it has high persistence and is inactivated upon contact with the soil, but tends to be stored in it. Knowingly this herbicide can remain in the soil for up to 7.2 years, and it is considered harmful to living beings (Stuart et al., 2023).

On the other hand, microorganisms present in the soil, also known as rhizospheric microorganisms (Granda Mora et al., 2021), have shown efficiency within treatments aimed at improving water quality, minimizing environmental odors, generating agrochemical-free food, managing waste produced by agricultural operations, recovering soil microfauna, among others (Morocho & Leiva-Mora, 2019). According to Saad et al. (2020). Soil microbial activity allows biochemical reactions related to physical, chemical and biological soil alterations to occur in greater, or lesser quantities. Additionally, they participate in the absorption cycles of carbon, nitrogen, phosphorus, iron, and sulfur, among others (Soria, 2016). This is why rhizosphorus microorganisms are considered a key factor in the conservation of resources, as they allow a reduction in the use of agrochemical, and an increase in crop productivity (Mandal et al., 2021). However, it is important to consider that soil microbial alterations are directly related to the inadequate use and dependence on agrochemicals applied in order to increase crop productivity (Bertola et al., 2021).

Globally, it is estimated that there is a community of approximately six million farmers engaged in the production of *Theobroma cacao* L. (cocoa). Between 2019 and 2020, its production worldwide was estimated at 4.7 million tons, mainly from Côte d'Ivoire, Ghana and Ecuador (García-Briones et al., 2021).

Cocoa is a highly important product in Ecuador, according to the Continuous Agricultural Surface and Production Survey (Instituto Nacional de Estadísticas y Censos [INEC], 2022) it occupies a surface of 626,962 hectares nationally. Cocoa is grown especially in the provinces of Los Ríos (21.85%), Manabí (20.74%), Guayas (18.41%), Esmeraldas (17.17%) and Santo Domingo de los Tsáchilas (4.95%). Santo Domingo de los Tsáchilas has a harvested area of 20,635.79 ha, either associated or in the form of monoculture, thus representing an important item within the province (García Vidal et al., 2017).

The main problems related to cocoa cultivation are high input costs, inadequate management plans, poor maintenance work, and loss of soil fertility (Tetteh & Amos, 2024). The latter is a consequence of soil erosion and damaged natural microfauna, which leads to low production and reduced product quality.

Considering the background information provided, the objective of this research was to determine the effect of herbicides (glyphosate and paraquat) on the population of rhizospheric microorganisms in the crop of *Theobroma cacao* L., in the Luz de América parish, Ecuador.

## 2. Materials and Methods

The study was carried out on the Santo Domingo-Quevedo road, km 24 (Hacienda Zoila Luz - Universidad de las Fuerzas Armadas ESPE, Sede Santo Domingo) in the rural parish Luz de América, belonging to the province Santo Domingo de los Tsáchilas, Ecuador, under the agroecological conditions described in the map in Figure 1. The main characteristics of the crop soil were: organic matter of 6.35%, clay loam loam soil type, and pH of 5.93 (slightly acidic). The research was carried out during the period June-November 2019, considered as dry season (low rainfall).

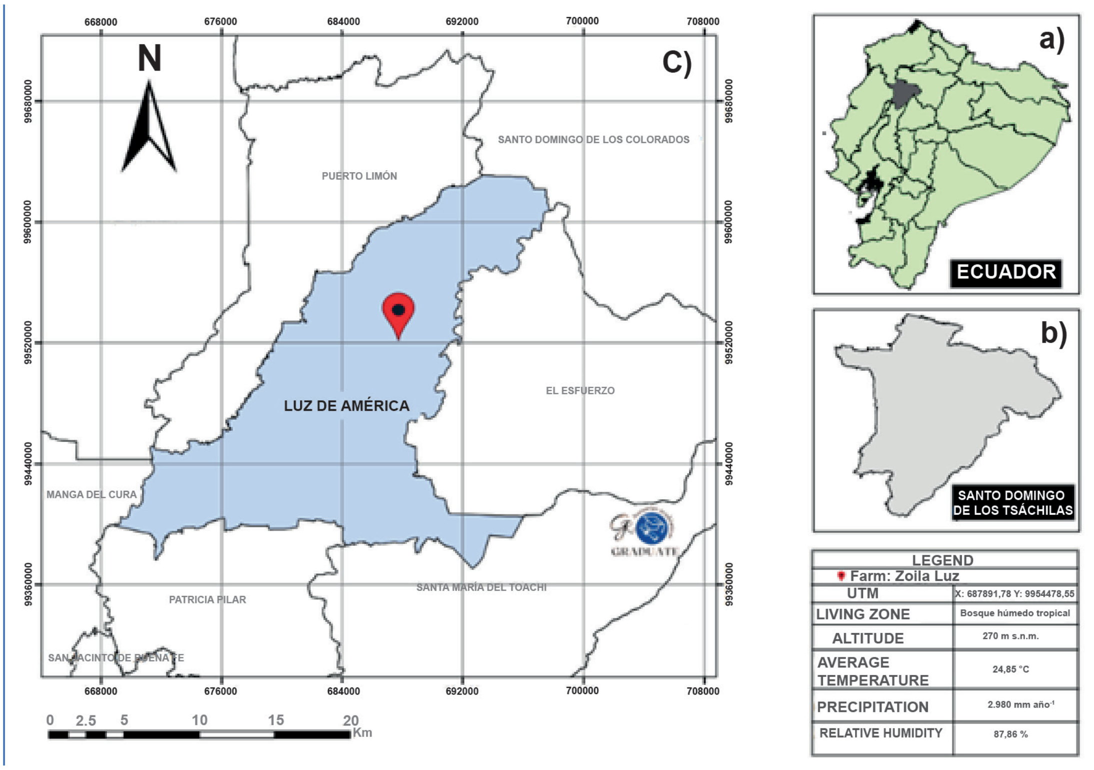


**Figure 1**. Location of the study area and agroecological characteristics of the site.

The CCN-51 cocoa variety crop was seven years of age, it was established on a flat topography (former cattle pasture), with a planting spacing of 4x4 meters. Crop management in terms of pruning, fertilization, irrigation, sanitary control, and weed control was identical throughout the plantation. It should be noted that the crop was pruned before setting up the trial (April 2019).

While, in order to provide both plants and microorganisms with an environment rich in nutrients, a soil analysis was previously performed (private institution), and a fertilization plan based on two applications was established, as detailed in Table 1. This was meant as a strategy to provide nutritional quality in the soil, before starting the trial.

**Table 1.** Soil fertilization plan for the cocoa crop plantation used in this research.

| Element | Soil content (without fertilization) | Crop requirements | Application | |
|---|---|---|---|---|
| | | (Mite Vivar, 2016) | 1° 40% at the beginning of the rainy season (December) | 2° 60% (June) |
| | | Kg ha$^{-1}$ | | |
| Nitrogen (N) | 21.0 | 150.0 | 60.0 | 90.0 |
| Phosphorus (P) | 15.7 | 50.0 | 20.0 | 30.0 |
| Potassium (K) | 112.0 | 125.0 | 50.0 | 75.0 |
| Magnesium (Mg) | 102.0 | 40.0 | 20.0 | 20.0 |
| Sulfur (S) | 9.4 | 60.0 | 20.0 | 40.0 |

### *2.1. Trial setup*

In order to provide different habitats in the soil of the cocoa crop, and to have references for comparison, three treatments or weed controls were established (T1 = systemic herbicide-glyphosate - 1.5 l ha$^{-1}$; T2 = contact herbicide-paraquat - 1.5 l ha$^{-1}$; and T3 = mechanical control-mower-testifier). The treatments were replicated six times, generating 18 experimental units with nine cocoa plants each (which were randomly drawn, identified and labeled). A total of 162 plants were evaluated in the trial. The area occupied by each experimental unit was 144 m2, and the total research area was 2,592 m$^2$. We worked with a Randomized Complete Block Design [RCBD].

### *2.2. Sample collection*

In each of the experimental units or plots, we performed the following manipulation on the nine selected plants:

- The leaf litter on the surface of the soil was removed, at a distance of 1 m from the stem of the cocoa plant, where three soil subsamples - extracted at a depth of 10 cm- were taken (random location);
- All the subsamples of each repetition were gathered (obtaining four samples per treatment), homogenized and a proportion of 200 g of each sample was taken and placed in sealed and labeled ziploc bags.
- These were transported to the laboratory in a cooler (maintaining a homogeneous temperature), to provide stability in the environmental conditions in which the microorganisms were found.

The samples were delivered the following day to the laboratories of the Pichilingue Tropical Experimental Station of the Instituto Nacional de Investigaciones Agropecuarias [INIAP]. The analyses were carried out on five occasions. The first sample collection was done one day before the start of the trial (before weed control - day 0). Subsequently, samples were taken every 28 days (day 28, 56, 84 and 112), always one day after the application of the weed controls. The variables measured were: population development of rhizospheric microorganisms, morphological identification of the genus of the most representative fungi, and population development of the fungi identified in the samples. The analyses of these variables were related to time (day of observation) and type of weed control (treatment applied).

### *2.3. Laboratory evaluation*

The analyses were carried out by the technicians in charge of the INIAP laboratories. It should be noted that this entity manages its own protocols, which -in this case- used the "Manual for the production of entomopathogenic fungi and quality analysis of bioformulates" as a reference (Hidalgo & Tello, 2022).

Initially, the dilution method was applied. It consisted of weighing 10 g of fresh soil (avoiding lumps, leaf litter, stones, etc.), and mixing it with 90 ml of sterile distilled water (stock sample). From this solution, 1 ml was taken (Dilution Factor [DF]-times the action is repeated) and placed in a tube containing 9 ml of sterile water. This was repeated four times, in order to reach a serial dilution of $10^{-4}$.

The last dilution was seeded in Petri dishes with Papa Dextrose Agar [PDA] based culture media, using 0.1 ml of the dilution distributed by extension. The boxes were incubated at 30 °C for seven days. Finally, to quantify the concentration of rhizospheric microorganisms, we used the formula in which we determined that CFU $ml^{-1}$ is equal to the number of colony per FD divided by the seeding volume.

To identify the genus of the fungi, a macroscopic and microscopic morphological characterization was performed by observing the shape, color, size and shape of the spore colonies. At the same time, the results were contrasted with the dichotomous keys for the identification of isolated fungi of the Sociedad Española de Microbiología (2014).

### *2.4. Data analysis*

The results obtained were analyzed using the Tinn-R statistical tool. An analysis of variance [ANOVA] was performed, and Tukey's 5% test of means was applied to identify statistical differences between treatments, and the relationship between them. In the results of population development, linear trend lines were plotted, and the equations were extracted from the graph and the value of $R^2$.

## 3. Results and Discussion

When the treatments were compared by ANOVA, significant differences were only identified at 28 days of observation, while in the remaining four observations, the populations of rhizospheric microorganisms were similar, as shown in Table 2.

**Table 2.** P-value of the treatments according to days of observation.

| | Days of observation | | | | |
|---|---|---|---|---|---|
| | **0** | **28** | **56** | **84** | **112** |
| **P-valor** | 0.1504 | 0.0269 | 0.2882 | 0.5082 | 0.6798 |

When applying the Tukey's test of means for the treatments applied on day 28 of observation, it was evident that weed control with the contact herbicide paraquat allowed a greater growth of rhizospheric microorganisms in relation to the other treatments. It is possible to confirm that the population of microorganisms present in the soil when applying a contact herbicide is greater, compared to when mechanical (mower) or systemic (glyphosate) weed control is performed. It should be noted that mechanical control has an intermediate population of microorganisms between glyphosate and paraquat (Figure 2).

These results are confirmed by Shan et al. (2021) who argue that the high presence of microorganisms in the soil is a consequence of the fact that contact herbicides, such as paraquat, exert their functions specifically in the plant, since paraquat has limited mobility, which prevents it from exerting its functions in the soil (Klein & McClure, 2022). Similarly, Mookodi et al. (2023) mention that a contact herbicide does not translocate within a plant, therefore, only the specific place where it rests is affected.

On the other hand, it was established that glyphosate, used as a weed control (agrochemical), restricts in greater proportion the growth of microorganisms in the soil (800,167.70 CFU $ml^{-1}$), as shown in Figure 2. In this case, glyphosate when applied as a herbicide has several negative consequences, affecting the concentration of fungi (Mesnage et al., 2020). According to Garcia-Muñoz et al. (2020), the inhibition of the development of fungal species are the result of soil toxicity due to the inappropriate use of this systemic herbicide, which has a free transport throughout the plant. The results of the present investigation are supported by what was reported by Cecilia and Maggi (2020), who state that in order to conserve and protect the agroecosystem it is necessary to be cautious about the toxic effect caused by glyphosate, especially on fungal species associated with the soil, since it has been confirmed that it can cause a reduction of up to 40% of the mycorrhizal population on the soil surface (González-Ortega & Fuentes-Ponce, 2022).

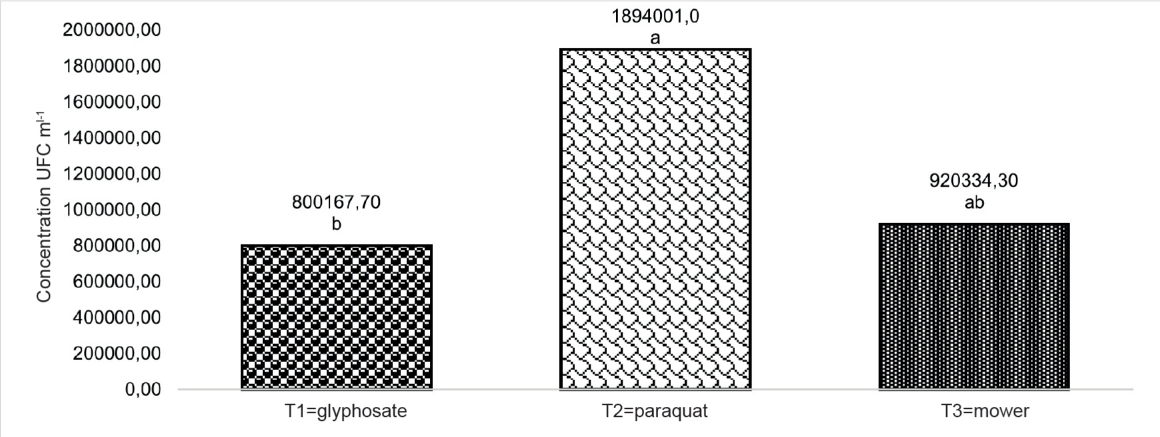


**Figure 2.** Tukey's Test of means over the types of weed control (day 28).*

* Means with a common letter are not significantly different

When evaluating the population behavior or concentration of rhizospheric microorganisms, it was identified that glyphosate (Figure 3a) starts its function with the highest population of microorganisms (3,750,001 CFU ml$^{-1}$) among all treatments. However, it declines drastically (36,168 CFU ml$^{-1}$) on day 28 of observation, and culminates its population development as the treatment with the lowest concentration of microorganisms. There is also a constant growth rate with a negative linear trend in the population of microorganisms associated with glyphosate-treated soils.

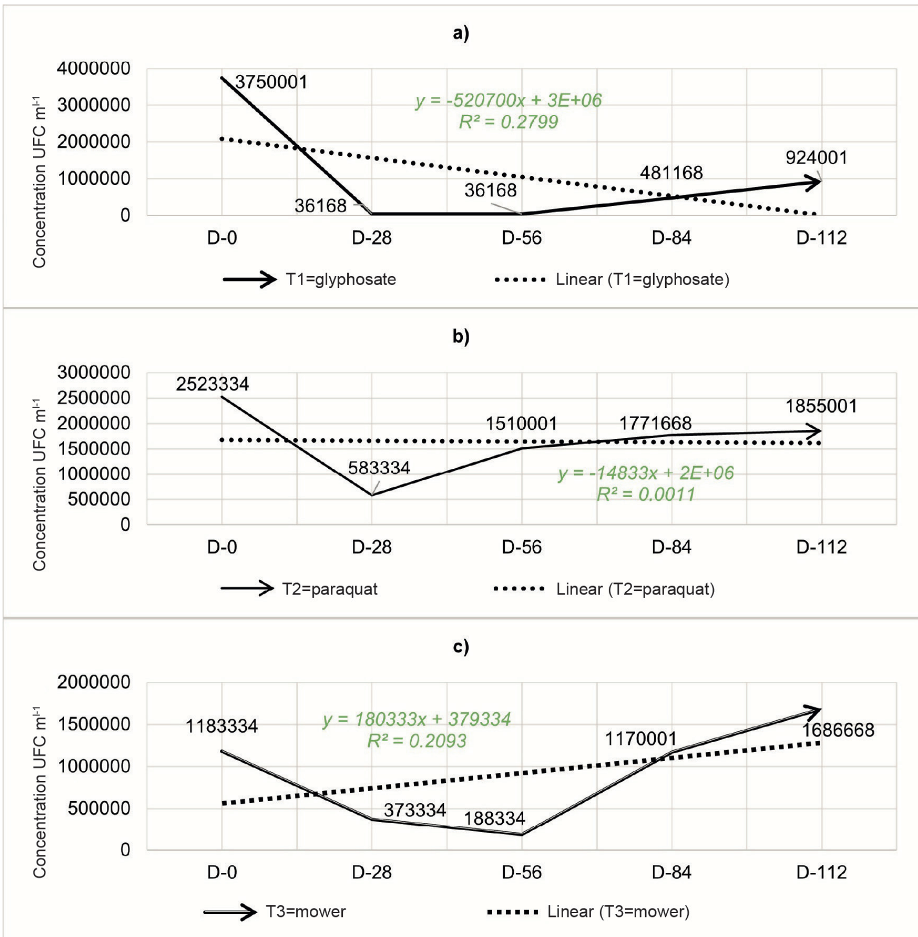


**Figure 3.** Population development of rhizosphere microorganisms during the assessment period, considering the established treatments.

Conversely, in the treatment with the contact herbicide paraquat, a high decrease in the population of microorganisms was evidenced on day 28 of observation (from 2,523,334 to 583,334 CFU $ml^{-1}$). This is contrasted in the following observations, since from day 56 the population of rhizospheric microorganisms increases, although slightly. In this case, a slight trend of linear population growth can be observed, more balanced with a future orientation of population increase, as shown in Figure 3b.

It should be taken into account that the variation of populations in rhizospheric microorganisms can be justified by the efficacy of herbicides. In this aspect, glyphosate and paraquat have an efficacy of 94 and 78% in weed control, respectively (Mookodi et al., 2023). Glyphosate, being non-selective, exerts its effect on a greater number of plants, generating an area with a smaller herbaceous population, resulting in a surface area more exposed to the environment, with a greater probability of having variations in the original properties of the soil, which contrasts with the mode of action of a contact herbicide (Picholi et al., 2024). These results are in agreement with Singh et al. (2024) who indicate that glyphosate, in comparison with paraquat, is the herbicide that has the most damaging effect on crops with subway production, i.e. its harmful effects are high in the soil.

The mechanical weed control (mower) showed a positive trend line, i.e. this treatment retains an orientation to increase proportionally the population of rhizospheric microorganisms in the future. The initial population of microorganisms was 1,183,334 CFU $ml^{-1}$, with the greatest decrease occurring on day 56, when the population was 188,334 CFU $ml^{-1}$. However, the concentration of these microorganisms on the last day of observation was 1,686,668 CFU $ml^{-1}$, increasing the population of microorganisms compared to the initial concentration (Figure 3c). This increase in the population of rhizospheric microorganisms may be a direct consequence of the presence of non-intoxicated stubble, which, being in contact with the soil, and carrying out decomposition and mineralization of the residues, tends to improve the properties and richness of the soil, favoring the presence of microorganisms (Aduov et al., 2020). In turn, the increase in this population could be associated with what is indicated by Aguilar-Bustamante (2013), according to whom after day 56, a total decomposition of weeds is generated, so there is a greater accumulation and use of organic residues to improve soil properties, and therefore its microfauna (Aduov et al., 2020).

When identifying the genus of the microorganisms present in the soil of the cocoa crop, it was obtained the majority presence of *Trichoderma* (beneficial fungus that prevents diseases and strengthens the immune system in the plants) and *Fusarium* (phytopathogenic fungus that causes diseases in the plants), whose population development was analyzed separately.

When evaluating the significance of the fungi in relation to the days of observation, this was positive for the two microorganisms identified (*Trichoderma*, P-value = 0.027, and *Fusarium*, P-value = 0.0139). When a comparison was made between the treatments intended to control weeds, the presence of rhizospheric microorganisms in the cocoa crop showed a significant difference, both for the *Trichoderma* fungus (P-value = 0.0246) and for *Fusarium* (P-value = 0.0001).

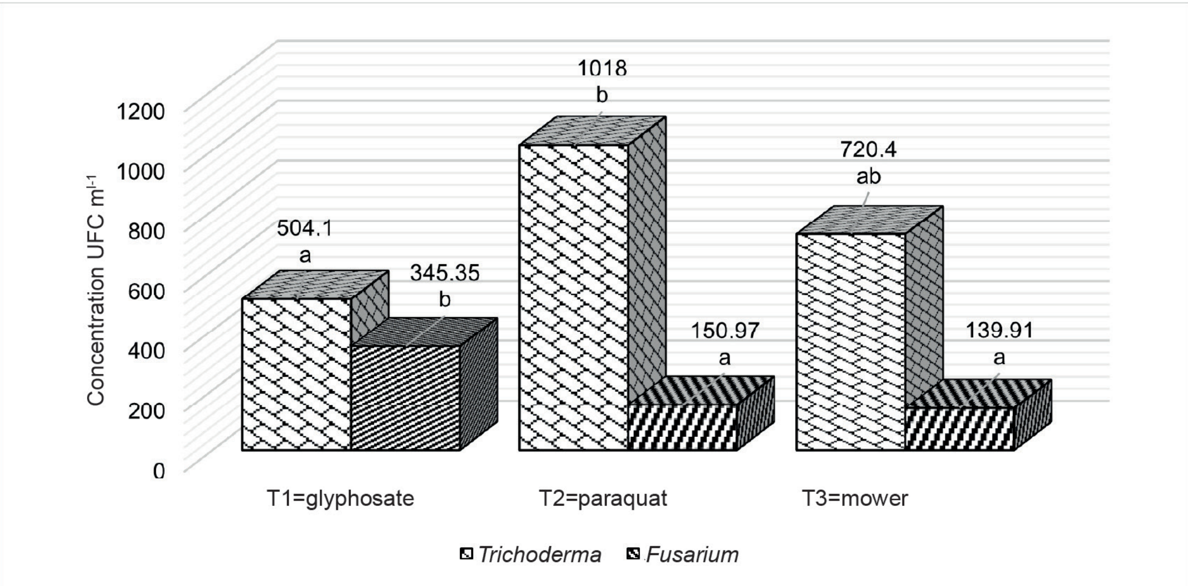


**Figure 4.** Tukey's test of Means comparing the fungi evaluated and the applied weed control (treatments).*

* Means with a common letter are not significantly different.

In the same context, Figure 4 shows that weed control, applying the contact herbicide paraquat, allows a higher concentration of *Trichoderma* in the cocoa crop (1,018 CFU $ml^{-1}$). Despite being an agrochemical, this product allowed a higher population of the beneficial fungus in comparison with the treatment where the mower, recognized as a tool of organic agriculture, was used. In contrast to this result, glyphosate was identified as the treatment that allowed less population development of the fungus.

On the other hand, the phytopathogenic fungus *Fusarium* had a higher concentration (345.35 CFU $ml^{-1}$) in the soils with weed control where glyphosate was used, while in the controls where paraquat-herbicide and mechanical mower were applied, they maintained a lower population, and without significant difference between them (Figure 4).

In general, it is confirmed that the presence of *Trichoderma* in the soil has a protective effect on the root zone of the plant, and at the same time has an isolating aspect of pathogenic fungi, which generates a contradictory behavior of development between this fungus and the infectious agents present in the soil (de Sousa et al., 2021). This action is confirmed in Figure 4, where it is observed that there is a negative proportional growth between Trichoderma and *Fusarium*; that is to say, when one increases its population, the other decreases it; generating the best results in the control by applying paraquat.

In conclusion, the use of herbicides has an effect on the content of beneficial microorganisms in the soil depending on their mode of action. The systemic herbicide inhibits the growth of *Trichoderma* and the contact herbicide strengthens it, acting in the opposite way in the case of *Fusarium*. Chóez-Guaranda et al. (2023) report similar results, and state that high concentrations of the former inhibit the development of the latter. Likewise, Nurlaila et al. (2020) point out that in cocoa cultivation, the presence in large proportions of *Trichoderma* in the roots generates resistance to pathogens such as *Fusarium*, and ensures resistance against diseases, which at the same time increases production levels. This confirms that weed control using paraquat is further beneficial in terms of protection for soil fungal microorganisms.

When taking into account the treatment where glyphosate is used, a drastic fall in the concentration of the *Trichoderma* population is evident on day 28 of evaluation (Figure 5a1), although after day 56 a progressive increase in the concentration began, giving as a final result a negative difference of 378.5 CFU $ml^{-1}$, compared to the initial population. In this case, and more generally, the trend line was negative and close to reflecting an equilibrium of the population with an $R^2$ value of 0.0324. Meanwhile, the concentration of *Fusarium* increased almost twice the initial population (168.13 CFU $ml^{-1}$) (Figure 5a2), maintaining a linear trend with a tendency to increase its proportion ($R^2$ = 0,676).

In the treatment applying the herbicide paraquat, *Trichoderma* initially reduced its population on day 28 (Figure 5b1), and subsequently increased proportionally. The variation of the population between the first and last observation was 555.5 CFU $ml^{-1}$, which caused a negative linear trend ($R^2$ = 0.1747). In contrast, *Fusarium* showed a positive exponential growth (Figure 5b2), increasing its population by 118.10 CFU $ml^{-1}$, i.e. more than double the initial concentration of the fungus. In the treatment using paraquat to control weeds, a tendency to replicate the results was observed ($R^2$ = 0.527).

Finally, when considering the population growth of *Trichoderma* in the treatment using paraquat as weed control there is a reduction of the population until day 56 (Figure 5c1). However, on day 84 the population rises in large proportions (597 CFU $ml^{-1}$), maintaining a final population of 1,120 CFU $ml^{-1}$ (day 112). This generated a positive linear trend of *Trichoderma* population growth. Figure 5c2 shows the growth of *Fusarium* when controlling weeds with mowers, with an increase from the initial to the final observation of 44.4 CFU $ml^{-1}$. Among all the treatments it is the least representative; with a positive linear trend that assures a proportional increase of this population ($R^2$ = 0,4565).

In general, it was identified that the development of *Trichoderma* was positive in the mechanical-ploughing control, generating an increase in the population of 597 CFU $ml^{-1}$. On the contrary, the treatments with glyphosate and paraquat generated a reduction of 378.5 and 555.5 CFU $ml^{-1}$ respectively. This means that, although glyphosate is qualified as the most harmful herbicide, it causes a smaller reduction in the population of the fungus than paraquat. In this context, it can be affirmed that glyphosate is a herbicide that generates short-term benefits within cocoa plantations, since by presenting the lowest decrease of *Trichoderma* (among herbicides), it ensures the translocation of minerals and availability of nutrients for plants, in addition to increasing crop productivity (Silva et al., 2020), as there exists a positive relationship between this fungus and the action of biocontrol and growth promoter (Batool et al., 2020). Within the same aspect, Asad (2022) mentions that among the ecological alternatives, the control that achieves the highest proportion in the *Trichoderma* population is established as the best management alternative, since this fungus is widely used for the sustainability of ecosystems.


**Figure 5.** Population development of rhizosphere microorganisms during the assessment period, considering the identified fungi and the treatment applied.

On the other hand, when evaluating the population of the pathogen *Fusarium*, all treatments tend to encourage the increase of its population. However, the least influential was weed control with a mower (44.4 UFC ml$^{-1}$), while glyphosate and paraquat showed an increase in their populations of, 168.13 and 118.10 UFC ml$^{-1}$, respectively.

Knowing that *Fusarium* is a pathogenic fungus that tends to cause a series of diseases in crops, directly causing great economic losses (Nikitin et al., 2023), it is confirmed that among all the treatments, mower control is the most recommended for weed control. However, when comparing the effect of the herbicides used, it is confirmed that paraquat reduces the development of this fungus to a greater extent, since it acts as a pathogenic agent from the rhizosphere part of the plants and causes several diseases. *Fusarium* is one of the five most representative phytopathogenic fungi of crops worldwide (Crous et al., 2021), so the results of this study indicate that the use of the herbicide paraquat would be the most advisable within the cocoa crop to control this phytopathogenic fungus.

It is worth considering that in-vitro studies conducted by Anderson and Aitken (2021) confirm that when comparing the triple relationship, herbicide (glyphosate)-plant-*Fusarium*, this agrochemical has the ability to minimize the density of the fungus, even with a better effect than when using Imazetapir (herbicide, systemic, selective). On the contrary, Warman and Aitken (2018) mention that the presence of *Fusarium* in banana plants, when treated with herbicides such as glyphosate and paraquat allow easy colonization by this fungus, i.e. the use of herbicides accelerates the spread of the pathogenic fungus. The last criterion was confirmed by the data obtained in the present investigation.

## 4. Conclusions

Regarding the population development of rhizospheric microorganisms, it was found that the 28$^{th}$ day of observation marks a difference between all observations, indicating in general that the concentration of rhizospheric microorganisms increases when the herbicide paraquat is used, in comparison with glyphosate and the mechanical control-mower.

The rhizospheric microorganisms *Trichoderma* (beneficial) and *Fusarium* (pathogenic) were identified as the most abundant fungi in the cocoa crop. The concentration of *Trichoderma* was higher in the treatment using paraquat as weed control, followed by the mechanical control - mower, while the lowest population of this fungus was found when glyphosate was used. On the other hand, the presence of *Fusarium* was higher in glyphosate, while between paraquat and mower there were no significant differences.

Finally, when evaluating the population development of the fungi considering the treatments and the days of observation, it was confirmed that *Trichoderma* increases its population when applying the treatments, especially in the case of mechanical control with mower, when comparing the initial concentration with the final one. However, when analyzing the effect of the herbicides, it was observed that glyphosate decreased the concentration of *Trichoderma* to a lesser extent than paraquat. On the other hand, both herbicides favor the development of *Fusarium*, although paraquat does so to a lesser extent. In addition, the use of paraquat promotes the presence of *Trichoderma*.

## Contributor roles

- Eduardo Patricio Vaca Pazmiño: conceptualization, formal analysis, investigation, methodology, supervision, validation, visualization, writing – review & editing.
- Milton Vinicio Uday Patiño: conceptualization, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing – review & editing.
- Dennis Vinicio Uday Ortega: conceptualization, formal analysis, investigation, project administration, supervision, visualization, validation, visualization, writing – review & editing.
- Rocío Noemí Guamán Guamán: conceptualization, formal analysis, investigation, project administration, resources, supervision, validation, visualization, data curation, writing – review & editing.
- Ángel Fabián Villavicencio Abril: conceptualization, visualization, writing - original draft, writing – review & editing.
- Santiago Miguel Ulloa Cortázar: conceptualization, data curation, software, methodology, resources, writing – review & editing.

## Ethical implications

The authors state that there are no ethical implications.

## Conflict of Interest

The authors declare that there are no financial or non-financial conflicts of interest that could have influenced the work presented in this article.

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