Influence of Glyphosate in the Fungus *Trichoderma Harzianum* Coming from Rhizoctonia Solani in Huancayo 2019

Elsa Xiomara Medrano De La Torre and Verónica Nelly Canales Guerra

***Abstract*—The objective of this research is to determine the influence of glyphosate on the conidia count of the *Trichoderma harzianum* fungus grown under laboratory conditions. This fungus is found in all types of soil, but it is more important in those for agricultural use where they fulfill a beneficial function. Glyphosate is an herbicide widely used in the elimination of weeds and shrubs, its effect can be extended to beneficial fungi and its permanence in the soil exceeds the specifications in the data sheet. For this reason, the culture of T. harzianum is carried out under direct exposure with different concentrations of glyphosate, the average dose (1 ml) in the investigation arises from that recommended by the manufacturer of the product and four more are established, two major doses (1.25 ml and 1.50 ml) and two minor doses (0.50 ml and 0.75 ml). Data was collected using observation tokens. All doses were observed to decrease the number of conidia including the mean 1 ml dose producing a 54% reduction compared to the control sample. The doses of 1.25 ml and 1.50 ml reduce up to 65% and 81% respectively. Therefore, this herbicide negatively influences the growth of the fungus and consequently inhibits its beneficial actions.**

***Index Terms*—Trichoderma harzianum, dosage, glyphosate, test.**

I. Introduction

Glyphosate is a widely used herbicide commercially, it was made with the aim of eliminating herbs and shrubs that have grown in a time and space not desired by humans and for agriculture [1]. This herbicide is absorbed by the leaves and not by the root, there have been cases in which due to time and accumulation factors they have generated derivatives (mixing with other components) or due to high concentrations they have detrimental consequences for the environment and human health [2]. According to the World Health Organization (WHO), this herbicide is considered as a possible carcinogenic compound for humans. The first contact of this herbicide is with the environment, it is where it acts, it accumulates and then, unfortunately they generate reservoirs in the soil [3].

On its microflora and microfauna, this is because for years little importance has been given to the excessive use of herbicides and pesticides on the land cultivation and the consequences of this The soil is interconnected with other components of the environment, from infiltration to groundwater and nearby rivers that end in the sea, it can accumulate substances that become vapors due to a phase change and are released into the atmosphere. Meanwhile, the different living organisms (currently excluding humans) will be in contact with these substances directly or indirectly and will feel the first repercussions of this herbicide. The soil microorganisms are those that have the most contact with these compounds and show the effects, generating the first effects on soil poise and balance [4]. One of these soil microorganisms is the fungus of the genus Trichoderma. It can be found in almost all types of soil with its different species, this is due to its wide temperature range and physicochemical conditions to survive, in addition to presenting rapid growth. One of the main species is *Trichoderma harzianum*, it is found naturally in a significant number of agricultural soils because they require organic matter and plant waste from crops to grow and perform their functions, one of its characteristics is to increase and improve the development of the plant by inhibiting the growth of pathogens, in addition to generating conditions that stimulate the absorption of nutrients for plant growth, making it stand out among the most widely used for biological control of soil fungal pathogens such as *Rhizoctonia solani* [5]. To fulfill with this bio controlling action, it has direct and indirect mechanisms of action, in the case of the first type of mechanism, antibiosis, generation of inhibitory compounds of the pathogen, secretion of enzymes, mycoparasites, competition for nutrients and space with the pathogen stand out; among the indirect mechanisms is the detoxification of the plant from toxins, the stimulation of the plant to generate the capacity of resilience to the infection of pathogens, solubility of nutrients that the plant cannot access under normal conditions and creates a favorable environment for radical development that will help the plant to increase its tolerance to stress [5]. *T. harzianum* produces three types of propagules, that is, it has three forms of reproduction and they are: hyphae, clamisdospores and spores "conidia", the latter being the most used and important. Being found in cultivated soils where glyphosate is applied, the fungus can be affected in its behavior, growth or functions, whether it is found naturally or introduced as biological control.

Interest in investigating these effects or influence of these compounds on soil microorganisms is recent, the Food and Agriculture Organization of the United Nations (FAO) encourages interest in knowing what kind of compounds are used in farmland and what impact does its use have on health and the environment, and recommends avoiding the use of some such as glyphosate. In Peru there are not many studies or investigations that evaluate the effects that glyphosate has on the soil, especially [6]. In the Mantaro Valley it is purchased in two presentations, one-liter bottles or 450 mg / L granules, and they have different names, but it is specified that the main active component is glyphosate; the dose used in the field is L / 200L and it is highly recommended for its efficacy, but the effects it may have on the environment are unknown.

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Taking into account all of the aforementioned, the influence of this herbicide on microorganisms that perform a beneficial function in the soil must be established, *Trichoderma harzianum* fungus is the best option. In order to establish this influence only with this microorganism, one should start from the most visible, such as its growth or reproduction, and for this the best thing would be to perform it under laboratory conditions in order to better control the variables.

II. Materials and Methods

It is acquired from the National Agricultural Health Service (SENASA), the fungus *Trichoderma harzianum* with code CCB-LA101, extracted from its host *Rhizoctonia solani* from cotton cultivation, this is used as a biological controller. Likewise, the herbicide Fuego (1L) is acquired, which has a concentration of 480g / L of Glyphosate and indicates the application in the field of an aqueous solution of 1L / 200L.

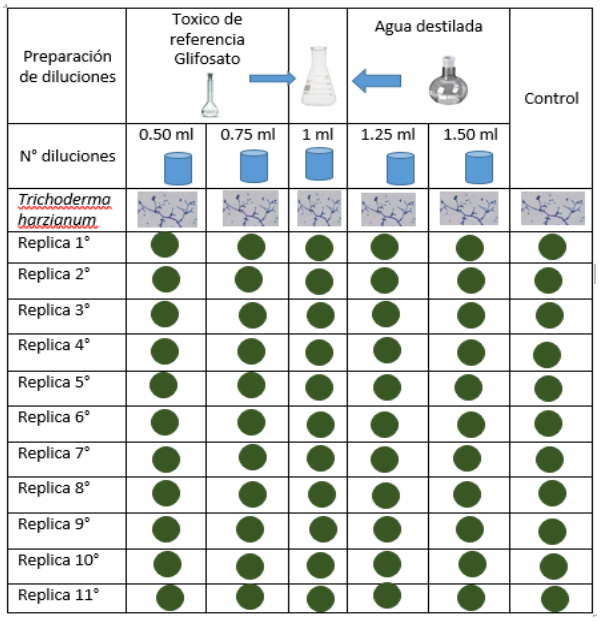

Fig. 1. Procedure model carried out.

To carry out the test as seen in Fig. 1, the range of doses was established based on that recommended by the manufacturer of the product, therefore a preliminary test was carried out with the dose of 1 ml of glyphosate. A lower dose of 0.75 ml and a higher dose of 1.25 ml were established. 8 plates were prepared with potato dextrose agar (APD) culture media, two plates are the control sample and the rest will contain glyphosate doses, having two plates per dose, the fungus was seeded in it and incubated for 5 days; a decrease in the number of conidia of the fungus was observed in the six plates containing glyphosate.

Based on the results of this preliminary test, 1 ml of Glyphosate is established as the average dose, and it is decided to experiment with two minor 0.50 ml and 0.75 ml and two major 1.25 ml and 1.50 ml, with 11 replicates for each doses and control sample, having to a total of 66 plates for the test (See Fig. 1). The fungus strain was activated in a culture medium that favors its growth, which is APD, this medium is simple to prepare, it only requires potato, dextrose, agar, distilled water and an antibacterial, the latter is because it is not a selective medium and therefore an antibacterial is applied so that only the fungus grows on the plaque. To prepare the APD, potato was washed and cut into small squares, boiled in distilled water for 35 minutes, with the intention of releasing all the starch it contained, the potato broth was filtered in a flask and agar, dextrose was added. and the antibacterial, being as homogeneous as possible. A stopper was made and it entered the autoclave, it is necessary to specify that glyphosate was added for those flasks that are for the doses; and it was labeled so as not to confuse them with each other or with the one contained in the agar for the control sample. The flasks were removed from the autoclave after the required time had elapsed and the agar was poured into the sterilized glass plates, the flasks were cooled and the fungus was seeded.

For the activation of the fungus, a small leaf with the fungus from the bottle with silica gel was extracted with a fine-tipped forceps correctly sterilized and cooled with alcohol, it was placed on the plate, with the culture medium, and three drops of distilled water. After 3 minutes, the sowing was sown by dragging. The plate had to be sealed and incubated for 5 days.

With the plates ready and the activated fungus, the sowing was carried out, the plate with the activated fungus was taken and with a correctly sterilized seeding aza a little of the fungus was taken and a point was made in the middle of the plate; The same was done with the 66 plates, after which they were sealed and labeled. The label contained the seeding date, the date the plate was removed from the incubator, the replica number, and the dose of glyphosate it contained. All the plates were taken to the incubator and left for 5 days for the fungus to grow at a temperature of 25 ° C, at the end of this time it was removed from the incubator and counting was carried out with the Neubauer chamber count is performed [7]. See Fig. 2.

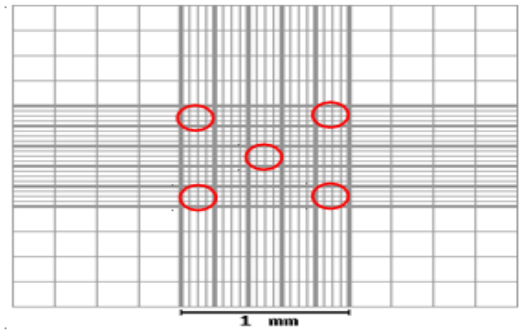

Fig. 2. Hemocytometer.

The number of conidia per ml is determined using the following formula [7]:

$$N°\ of\ conidia/ml\ = X * 5 * 10^4 * ID \quad (1)$$

$$5 = N°\ of\ squares\ contados\ in\ the\ quadrant$$
$$X = Average\ conidia\ counted$$
$$ID = Inverse\ of\ the\ dilution\ used$$

❖ To obtain the N° of total conidia, the following is done:

$$Total\ N°\ of\ C. = N°\ de\ conidias/ml * Orig.conc.vol. \quad (2)$$

A. *Materials*

- Sample (*Trichoderma harzianum*)
- Glyphosate
- APD dextrose potato agar
- Planting and cultivation material in the laboratory
- Petri dishes (66)

B. Equipment

- Optical microscope B100iMSa.
- Electric autoclave 75X-240: 240
- Marienfeld Hemocytometer Brightline CNRB
- Vortex shaker BOE 8055100

To check the research hypothesis, the Kruskal-Wallis test and the Duncan test are used. The research is carried out in the Biology laboratories of "Universidad Continental" at its headquarters in the city of Huancayo.

III. Results

At the conclusion of the experiment and using the data collection instruments, the following results are obtained, each dose and the control sample had 11 replicates each one. Can see a big difference between the control plate and the other plates containing glyphosate in different concentrations (See Fig. 5).

It is observed that the bars decrease as the dose increases, presenting a descending trend line. When comparing the mean of the values obtained from the control sample that represents the number of total conidia of the normal growth of the fungus is 18.3×10^8 with the respective means of the different concentrations of glyphosate as it can be seen in the Fig. 3, the 1.50 ml dose has 3.4×10^8 total conidia that compared to the control shows a very significant decrease, likewise, it can be seen that the other four concentrations have a considerable decrease in the number of total conidia.

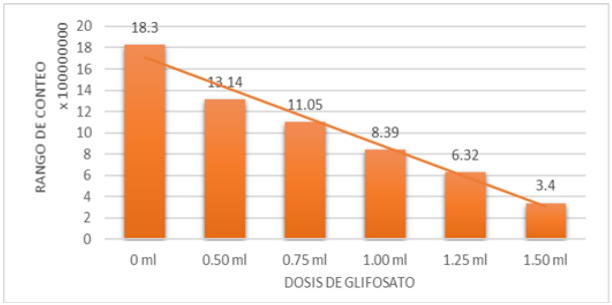

Fig. 3. General results.

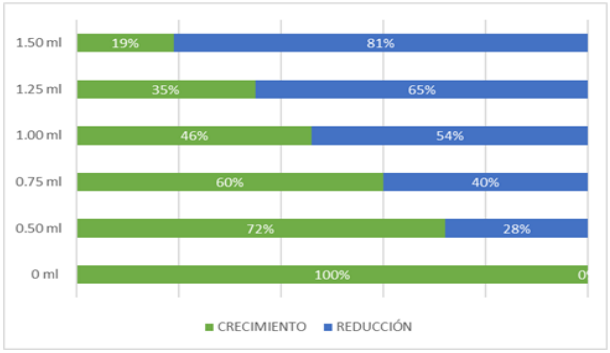

Fig. 4. Growth and reduction of the number of conidia.

The doses used do not cause the death of the fungus; however, they cause a decrease in the number of conidia, being more evident with the control sample. (See Fig. 4), the control sample (0 ml) represents the number of conidia at 100% and when compared to what was obtained in each dose, a continuous decrease is observed. The dose of 1.00 ml, taken from that recommended by the manufacturer, has a 54% reduction in the number of conidia, likewise, the reduction presented by the dose of 1.50 ml is the most worrisome since it decreases by 81% the number of conidia.

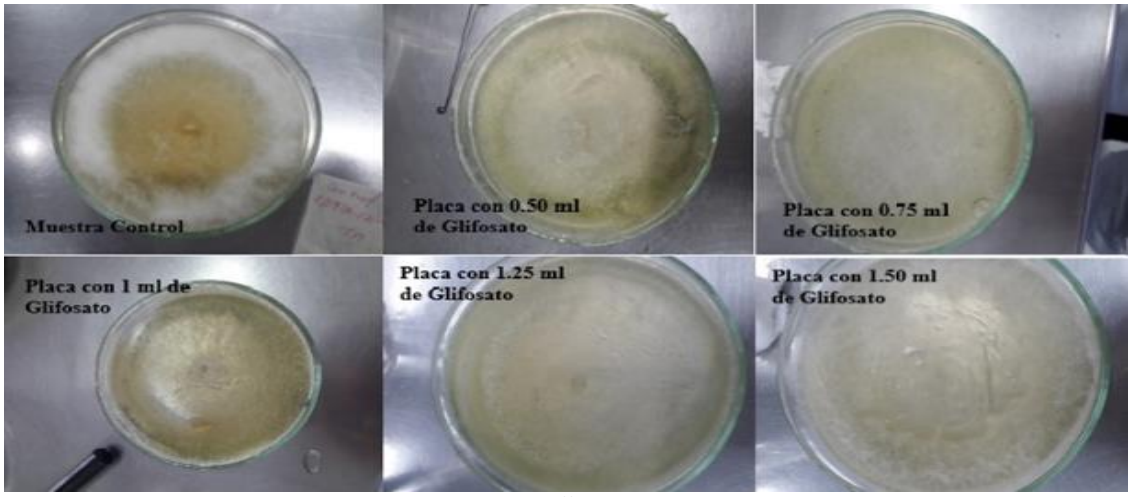

Fig. 5. Plates with different concentrations.

IV. Disscussions

The results obtained in the conidia count show that there is a negative influence of glyphosate on the fungus, which agrees with [8] who evaluates the effect that this herbicide has on native fungi of *Trichoderma sp* in two different concentrations: 4800ppm and 1440 ppm, this compound decreases the number of conidia. Although the time evaluated was only 24 hours compared to the 5 days evaluated in this investigation, time did not play a determining role, because the results show that glyphosate has a negative influence on conidia. Likewise, glyphosate suppresses the ability to generate aromatic amino acids useful for plant growth (2), in addition to inhibiting the production of conidia, which are the most viable means for the propagation or reproduction of the fungus [9].

The effects of glyphosate on bees were evaluated, concluding that the herbicide does not kill them on the spot, but it does have a negative effect on their intestinal microflora,

generating them over time, however, death [10], *Trichoderma harzianum* exhibits similar behavior, that is, the doses proposed in the investigation are not lethal, however, they have a marked influence on the conidia count. In the investigation of [10] as well as in this research, doses used in the field are taken with the aim of simulating what would be happening with glyphosate in the field, they use doses of 5 mg / L and 10 mg / L higher than the environmental levels of glyphosate in their country and range between 1.4 and 7.6 mg / L. In Peru, there is only the dose data that applies to crops L / 200L or 450 mg / L, however, the doses used in the USA are lower compared to Peru and this can be a determining variable for the projections of both investigations, even when comparing them.

The effect of glyphosate on different soil fungi, such as arbuscular mycorrhizal fungi that were directly and indirectly exposed to the compound, resulting in an effect on these [11]; likewise the effect of the herbicide in endophytic fungi of the root of Epidendrum melinanthum having the same result as the previous one [12]. Both investigations worked with different concentrations from the one used in the field, whether three doses were established as such as in the case of the first or only one dose that was diluted three times and said dilutions were applied in 30 replicates. All the doses of glyphosate used in the different investigations mentioned above have a negative influence, being noticeable in some more than others; in a specific case Trichoderma harzianum the impact on the conidia count is verified, something that is detrimental to the long-term subsistence of the fungus. Taking into account only the last two investigations, it is possible to appreciate that glyphosate is generating negative effects on soil fungi, which could also be observed in this investigation and that adds Trichoderma harzianum to the list of fungi affected either directly or indirectly by the compound.

The 1 ml dose of Glyphosate is taken from the recommended dose for field application according to the technical data sheet of the herbicide [13], which reduces the number of conidia by 54%, which is almost half the number of conidia with respect to the control sample and this is observed in all doses, including lower doses (Fig. 4). Therefore, the doses tested in the research inhibit the growth of the fungus in different proportions, which in turn means that the dose recommended by the manufacturer has the same negative effect on the fungus Trichoderma harzianum.

In the microbiological interactions of the rhizospheres of soybean GR1 and GR2, different concentrations of glyphosate were applied and it was obtained as a result that this herbicide has a detrimental impact on the complex interactions that microorganisms have [14] and also on the soil microfauna of coffee growing. It is concluded that this compound does not have a direct effect on the quantity of microorganisms, but that it did indirectly affect its main functions, which are very important for soil health and quality [15]. The aforementioned research and this research show that glyphosate is not lethal, but the detrimental impact it has on the soil and therefore on the crops produced is evident.

V. Conclusion

A decrease in the conidia count of all the repetitions and concentrations used is observed, caused by glyphosate, which means that it has a negative influence on the fungus studied. Conidia are the type of propagule that guarantees the sexual phase of the reproduction of *Trichoderma harzianum* and therefore its viability and beneficial actions on the ground.

The 1.25 ml and 1.50 ml doses have a negative influence on the number of conidia because they reduce the number of conidia by 65% and 81% respectively. Likewise, the doses of 0.50 ml, 0.75 ml and 1 ml also have a negative influence on the number of conidia, so it can be stated that all the doses used in the research, especially the two highest doses, significantly reduce the number of conidia of the fungus and has a negative influence on it.

The doses used in the research arise from the use for crops (1L / 200L) which would mean that this is not good for *Trichoderma harzianum* either in its native form or introduced as biological control and therefore a lower dose is required to 0.50 ml to avoid damaging the fungus.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Elsa Xiomara Medrano De La Torre was in charge of carrying out the investigation, having the responsibility of executing the experiment, as well as treating the data that was later processed.

Verónica Nelly Canales Guerra was the research adviser, in charge of collecting all the information, as well as processing the results. Both wrote the work and have approved the final version.

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