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Original Research Article

Evaluation of the interaction of arbuscular mycorrhizal fungi and *Trichoderma harzianum* in the development and nutrition of potato plants (*Solanum phureja*)

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Abstract

Potato is the fourth most consumed food product around the world and one of the most produced crops worldwide, due to its nutritional and culinary properties. This crop is affected by certain pests such as phytopathogenic fungi, which frequently attack roots and tubers, extracting their nutrients and decreasing their yield. Consequently, our research is focused in finding an environmentally friendly alternative to improve the nutrition of the crop and prevent the attack of pathogens that exist mostly in soils with poor conditions. One of the ways to reduce the use of fertilizers and pesticides, is the application of beneficial microorganisms, among them Arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum*. Arbuscular mycorrhizal fungi create symbiosis with the plant and improve mineral absorption with a significant efficiency. *Trichoderma harzianum* is an effective biological control agent, it induces the defensive response of plants and stimulates plant growing. This investigation evaluated the interaction between *Arbuscular mycorrhizal* fungi (AMF) and *Trichoderma harzianum* in the development and nutrition of potato plants (*Solanum phureja*), showing increasing leaf area, biomass (total, aerial and root) and root length in plants inoculated with each of the microorganisms compared to the plants without inoculation (control treatments) and chemical fertilizer applied plants. Namely, the presence of AMF, *Trichoderma harzianum* and the variation of concentrations of peat at 30% and 50% improved the development, growth and nutrition of potato plants.

Keywords: *Solanum phureja*, *Trichoderma harzianum*, Arbuscular mycorrhizal fungi, Peat

Introduction

Agricultural ecosystems worldwide and in Ecuador are destroyed by several causes such as - monoculture, climatic changes and intensive application of pesticides and chemical fertilizers to the field. These actions result in nutrient deficit that decreases crop yields, deteriorates soil microbiota and turns plants more susceptible to pathogen attack. Potato crop is affected by these causes, it belongs to the Solanaceae family and Solanum genus. Currently potato crop has an economic and social value in different countries, ranking fourth in nutritional importance with wheat, corn and rice, due to its high levels of proteins, minerals, vitamins and other nutrients, indispensable for human life (Tapia et al., 2007).
Potato plant is a perennial herbaceous plant that possesses tubers, which constitute it´s reserve organs and resistance source (Cuesta et al., 2002). *Solanum phureja* is one of the native potato species known as "egg yolk", it has a great importance in the Andean countries for farmers and researchers because of its tubers early germination qualities (without dormancy) that make possible immediate re-sowing and continuous cultivation throughout the entire year. Additionally, it shows resistance to biotic factors as well as to various abiotic factors; this species has not been genetically improved (compared to other species of potato) and its life cycle is short (4 months) compared to other potato species that require nine months to develop (Ochoa, 2001).
In order to mitigate the causes mentioned above, which are responsible for soil deterioration and low production of potato crop, new biotechnological techniques have been investigated to support the quality of life through beneficial microorganism introduction (Alarcón et al., 2000). In this research, the behavior and interaction of two beneficial fungi were studied: Arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* with different doses of peat. Their effects on nutrition and development were evaluated.
Arbuscular mycorrhizal fungi (AMF) belong to endomycorrizas from the Glomales order. These fungi form symbiotic associations with a wide range of plant species. Both organisms establish a specialized connection in which a bidirectional transfer of nutrients occurs, the host supplies carbohydrates to the fungus, while the fungus transport nutrients that the plant requires. This association allows the plant to increase size, mass, seed production, resistance to pests and drought, allowing them to guarantee their establishment and success in disturbed sites (Ramírez et al., 2010).
In soil conditions, mycorrhizae extend their mycelium (hyphae set) (Fig.1) and help the formation of aggregates through particles adhesion, due to a protein called glomalin, which contributes to give structure and stability to the soil. Thereby, erosion is reduced and soil water retention capacity improves. Accordingly, mycorrhizae are potentially useful for dealing with environmental problems such as natural vegetation loss and soil erosion (Montaño et al., 2010).
On the other hand, among the group of beneficial microorganisms, one of the most studied species is *Trichoderma harzianum*. It has several mechanisms of action such as: pathogen antagonist, root colonizer, root system development accelerator, and stress tolerance increaser solubilizing and absorbing inorganic nutrients, stimulating plant growth and inducing resistance (Martínez et al., 2013). In addition, it has effective biological control properties against Rhizoctonia, Fusarium and Pythium (Eldor, 2007) through different mechanisms like mycoparasitism, antibiosis or competition (Beltrán, 2004).
In this research the development and nutritional levels of potato plants (*Solanum phureja*) inoculated with arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* were evaluated.

Material and Methods

In our experiments, two beneficial microorganisms were applied in potato plants: A. mycorrhizal fungi and *T. harzianum*. Potato tubers were selected (60 g to 70 g) and disinfected with sodium hypochlorite (2.5% V/V). They were dried and stored in the dark for 15 days to achieve germination. 65% of sterile black soil and PROMIX PGX peat (sterile) was homogenized with 35% of the mycorrhizal inoculum (propagated in oat trap plants), which finally reached in pot a concentration of 19.19 spores per gram of soil.
In treatments with *T. harzianum*, potato seeds were immersed in a suspension of the fungus ($1.5x10^9$ spores/mL) for 10 minutes. Three Drench applications of this suspension were made at 15, 30 and 45 days.
For the treatments with chemical fertilization, a previous chemical analysis of the soil was carried out (9.17 kg of black soil without sterilization). After one week of planting, 1.28 g of urea (46-0-0), 1.94 g of (10-30-10), 0.83 g of potassium sulphate and 0.105 g

of micro elements were applied. This fertilization was repeated 45 days after sowing. Hewitt nutrient solution (Collados, 2006) was applied (twice a week) for one month. Mycorrhizal analysis was performed using the technique proposed by Gerdemann and Nicholson et al. (1963)

Experimental design

In this research, a Completely Random Design was established with factorial arrangement of 2x2x2 plus an additional. Tukey significance test was applied with a p≤ 0.05%. An analysis of variance (ANOVA) was performed to show the interactions between the microorganisms (AMF and *Trichoderma harzianum*) and the variation of peat concentration (Table 1).

There were 9 treatments with 12 repetitions each one, the experimental unit considered was the potato plant (*Solanum phureja*), there was a total of 108 plants.

The obtained data was evaluated to determine the growth variables of potato plants in the statistical program INFOSTAT applying the Tukey test with a P value of 0.05% for each factor and their interactions.

Results and Discussion

Evaluation of the interaction of AMF and *T. harzianum* on the development of potato plants (*S. phureja*)

Data in Table 2 show that the evaluation of AMF through spore count and percentage of colonization of treatments with simple and combined application of mycorrhizae with *T. harzianum* (Concentration: 1.5 x 10^9 spores/mL) in potato plants after three months of planting. Mattar[12], establishes that the growth promoting effect of mycorrhizae is observed three months after inoculation, consequently this assay was evaluated when the three month period ended (Fig. 2).

In the treatments where both AMF and *T. harzianum* were applied with a concentration of peat of 30% and 50% (according to the Kruskal and Wallis method, p = 0.0024), *T. harzianum* did not influence negatively in the mycorrhizal population increase, the number of spores increased three times from the beginning of the assay (beginning with 19 spores per gram of soil) (Table 2 and Fig. 2). In addition, there was a positive effect on mycorrhizal colonization reaching 95% in all treatments containing mycorrhizae with and without *Trichoderma* (Fig. 2). This shows that the development of mycorrhizae does not decrease with the inoculation of *Trichoderma*.

The behavior of *Trichoderma harzianum* is different (Fig. 3) the concentration decreases when inoculated together with AMF, showing that *T. harzianum* is limited in its growth when interacting with mycorrhizae. This matches with a study about Trichoderma sp. with Glomus intraradices in "root free" soil systems, it was observed that the presence of external mycelium of G. intraradices, suppressed the development of *T. harzianum* population, possibly due to competition for nutrients (Green et al. 1999; Sosa et al. 2006). There were assays in plants of Brachiaria decumbens (Sosa et al. 2006) that showed that the treatment inoculated just with *T. harzianum* presented a statistically higher amount of CFU/g of dry soil compared to the treatment that combined mycorrhizae with *T. harzianum*.

In the treatment where just *T. harzianum* is applied, the concentration of peat affects *T. harzianum* growth, showing that this microorganism develops better with 30% peat than with 50% peat (Fig. 3).

Evaluation of the interaction of AMF and *T. harzianum* on the development of potato plants (*S. phureja*)

After ninety days of the assay, the following parameters of growth variables of potato plants (*Solanum phureja*) were analyzed: lengths (total, aerial and root), biomass (total, aerial and root), perimeter and leaf area through variance analysis (ANOVA) and a Tukey test with a 5% significance. The treatments where simple and combined inoculation of microorganisms (AMF and *T. harzianum*) were applied, presented the highest ranges of significance compared to the control treatments (M0T0Pt30% and M0T0Pt50%) and to the chemical fertilizer treatment (M0T0Pt0F1), the values for M0T0Pt0F1 were the lowest (See Table 3).

Comparison of growth variables between 30% peat vs. 50% peat

Treatments that presented a single microorganism (AMF or *T. harzianum*) with a 30% concentration of peat showed increases in the leaf area, biomass (total, aerial and root) and in the root length compared to the control plants. All interactions of the microorganisms with the two concentrations of peat (Fig. 4, 5, 6, 7 and 8) showed that when a single microorganism is applied, better results were achieved than when the application is combined.

In this sense, the treatments with only mycorrhizal applications that were carried out in our assay, are similar to the research proposed by Calvet et al. (1999) with Tageteserecta (marigold) crop, where it is described that AMF promote root growth of the plant, because fungus hyphae explore a volume of soil that is inaccessible for the root through the mycelium, increasing the surface of absorption, therefore, the uptake of nutrients and water improves. Also, Menge et al. (1980 and 1977) observed in their avocado experiments that treatments inoculated with mycorrhizae had 98% more aerial (dry) biomass and 100% more root biomass than non-mycorrhizal treatments. Azcon and Barea (1997), described that the inoculation of A. mycorrhizal fungi causes stimulation of rooting and growth of seedlings, reducing the external need for phosphate. According to Villegas and Cifuentes (2004), the symbiosis of plants with AMF is more efficient to get nutrients than plants which are not colonized with AMF, consequently the increase in plant growth occurs, due to the increase in the supply of low mobility elements in the environment where they develop.

Our results show that the treatments inoculated only with *T. harzianum* had a positive effect on the total length and the variables mentioned above, increasing their value in biomass and root length of the plants by approximately 30% to 38 % and a 21% increase in overall length compared to control treatments. These results were also verified by Guigón and González (2004), with the application of Trichoderma in chile crop where a 38% increase in aerial and root biomass was obtained. In addition, Chang et al. (1986), found that tomato plants with *T. harzianum* applications had the ability to increase root length of plants. Björkman et al. (1998), observed in their sweet corn plants with the presence of *T. harzianum* increasing root growth and a 66% average in shoot growth.

Treatments with chemical fertilizers without any microorganisms showed poor results in most of the plant development variables (Table 3), although the nutrients were absorbed or assimilated by the plant in an optimal way (see Table 4 and Annex 3), the plants did not increase their development compared to plants treated with microorganisms, showing that microorganisms are more effective than chemical nutrition. This situation is similar to the research held by Hridya et al. (2013), in cassava crops, where higher yields were obtained by using combined Trichoderma and AMF, compared to plants treated with commercial doses of NPK fertilizer.

It was observed that when applying the fungi (AMF and *T. harzianum*) together at a concentration of 50% peat, the increase in total and aerial lengths is activated, probably they act as stabilizers of the nutrient flow, optimizing nutrient absorption (Fig. 9, 10 and 11).

Analysis of foliar and soil nutrient content levels of potato plants (*Solanum phureja*)

In the foliar analysis of the potato crop, the nutrients assimilated by the plants are observed, the different treatment results were submitted to a statistical analysis to obtain the sample mean and the variation coefficient between them. In this study, the results obtained about the concentrations of nitrogen, phosphorus and zinc in leaf tissue were within the normal range in all treatments (see Table 4 and Annexes 1 to 3). Therefore, although all the treatments have normal nutrition, it is necessary to emphasize that the biomasses reached in all the treatments with microorganisms, were higher than those of the controls. For that reason, in relation to this parameter, when microorganisms were applied, plants achieved higher levels of nutrient absorption than the control plants and chemical treatment plants. Tinker (1978), results match with our results, confirming that AMF inoculation increases plant growth by improving the phosphorus absorption surface in soils with deficiency of this element.

Additionally, when applying mycorrhizae with or without *T. harzianum*, plants absorb more phosphorus than when applying only *T. harzianum*, in which case the phosphorus uptake (see Table 4 and Annex 3) decreases. Showing that the plant develops to a high degree and with greater assimilation of nutrients when applying mycorrhizae. It is remarkable that the level of phosphorus in the leaf is related to the level of the nutrient in the soil, it is observed that P in the soil for the treatments with mycorrhizae reaches a higher level probably because these microorganisms solubilize more P than the other treatments (including *T. harzianum*). This is related to what Lascano (1988) and Nielsen et al. (1981), described in their studies, they found that when there was a small amount P in the soil, the level of phospholipids in the membrane of the root cells was low and consequently there was a greater root exudation and this stimulated the colonization of the endophyte. On the other hand, the endophytic activity estimated by the number of spores and the colonization percentage, is depressed when soluble P is added to the soil.

Foliar level results of the analysis of potassium, calcium, sulfur and iron contents of all plants exceeded the normal boundaries. Fe was excessively high and probably due to this high level, plants showed certain symptoms of stress.

In the case of Fe, Albano and Miller (1996), describe that the excess of iron can become toxic causing damages in the foliar tissue, with yellow or brown spots in young leaves finally producing necrosis. They also describe that depending on the crop a delay in growth and a late flowering process may exist. Some of these characteristics were observed in all the treatments of this investigation, presenting an acidic pH in the soil that favors the iron absorption in plants. At 14 days of growth, they presented yellow spots on intermediate leaves and 60 days after their growth, a slight necrosis was observed in some leaves. In addition, the plants showed a delay in flowering, but not in their growth. The possibilities of bacteria, fungi and insects existence were discarded. For that reason, it is deduced that iron concentrations affected some characteristics of the plants, without deteriorating their development.

It is noticeable that K uptake is high in all treatments, probably because the data sets were taken before the tuber was developed. Lazcano-Ferrat (1997) described that almost two thirds of the total absorption of K occurs during the growth of the tubers, because this element is essential for the synthesis of starch and simple sugars. It has also been described that K serves for carbohydrates translocation, vigor, coloration and efficiency in the potato crop.

Table 1: Treatments and doses of AMF, *T. harzianum* and substratum (peat) applied to the potato assay (*Solanum phureja*)

S.No.	Treatments	Treatment Description
1	Healthy control-peat (30%)	0% inoculum of Arbuscular mycorrhizal fungi (AMF) × 0 conidia/mL of *Trichoderma harzianum* × peat (30% of substratum)
2	Healthy control-peat (50%)	0% inoculum of Arbuscular mycorrhizal fungi (AMF) × 0 conidia/mL of *Trichoderma harzianum* × peat (50% of substratum)
3	AMF-peat (30%)	35% inoculum of Arbuscular mycorrhizal fungi (AMF) × 0 conidia/mL of *Trichoderma harzianum* × peat (30% of substratum)
4	AMF-peat (50%)	35% inoculum of Arbuscular mycorrhizal fungi (AMF) × 0 conidia/mL of *Trichoderma harzianum* × peat (50% of substratum)
5	Trichoderma-peat (30%)	0% inoculum of Arbuscular mycorrhizal fungi (AMF) × $1.5x10^9$ conidia/mL of *Trichoderma harzianum* × peat (30% of substratum)
6	Trichoderma-peat (50%)	0% inoculum of Arbuscular mycorrhizal fungi (AMF) × $1.5x10^9$ conidia/mL of *Trichoderma harzianum* × peat (50% of substratum)
7	AMF-Trichoderma-peat	35% inoculum of Arbuscular mycorrhizal fungi (AMF) ×1.5×10^9conidia/mL of *Trichoderma harzianum* × peat (50% of substratum)
8	AMF-Trichoderma-peat	35% inoculum of Arbuscular mycorrhizal fungi (AMF) ×1.5×10^9conidia/mL of *Trichoderma harzianum* × peat (50% of substratum)
9	Positive control: recommended chemical fertilization	0% inoculum of Arbuscular mycorrhizal fungi (AMF) x 0 conidia/mL of *Trichoderma harzianum* × peat (0% of substratum) ×Chemical fertilization (recommended fertilization)

Table 2: Percentage of colonization and number of AMF spores propagated in soils of potato plants (*Solanum phureja*) 90 days after sowing

Treatments	Spores in 1g of soil	% of Colonization
1 M0T0Pt30 %	12.9 bc	16.67 de
2 M0T0Pt50 %	17.80 bc	18.67 d
3 M1T0Pt30 %	44.53 ab	94.67 a
4 M1T0Pt50 %	58.58 a	95.33 a
5 M0T1Pt30 %	8.13 c	33.33 c
6 M0T1Pt50 %	6.97 c	49.33 b
7 M1T1Pt30 %	59.7 a	93.67 a
8 M1T1Pt50 %	60.8 a	95.67 a
9 M0T0Pt0F1	6.43 c	12 e
P	p<0.0001	p<0.0001

Table 3: Interaction of AMF and *T. harzianum* on the development of potato plants (*Solanum phureja*) evaluated in 90 days.

TREATMENTS	PERIMETER (cm)	LEAF AREA (cm^2)	TOTAL LENGHT (cm)	RADICAL LENGHT (cm)	AIR LENGHT (cm)	TOTAL BIOMASS (g)	ROOT BIOMASS (g)	AERIAL BIOMASS (g)
1 M0T0Pt30%	1,22 b	17 c	134 cd	23 c	111 c	224 c	56 c	168 b
2 M0T0Pt50%	1,24 b	18 c	127 d	23 c	104 c	227 c	51 c	176 b
3 M1T0Pt30%	1,39 ab	26 a	154 b	37 a	118 bc	335 a	91 a	244 a
4 M1T0Pt50%	1,51 ab	24 ab	151 b	33 ab	118 bc	284 b	76 b	208 ab
5 M0T1Pt30%	1,63 a	25 ab	170 a	38 a	132 ab	340 a	100 a	240 a
6 M0T1Pt50%	1,36 ab	23 ab	152 b	36 a	116 c	261 bc	78 b	183 b
7 M1T1Pt30%	1,36 ab	25 ab	144 bc	27 bc	117 bc	271 bc	76 b	195 b
8 M1T1Pt50%	1,33 ab	23 b	169 a	26 c	143 a	254 bc	72 b	182 b
9 M0T0Pt0F1	1,25 b	17 c	140 bcd	24 c	116 c	231 c	56 c	175 b
p	0,3155	<0,0001	<0,0001	0,2644	<0,0001	0,0008	<0,0001	0,0119

M0=without AMF, **T0**= without *T. harzianum*, **Pt 30%=** 30%Peat, **Pt50%=** 50%Peat, **M1=**with 19.19AMF spores/pot (V= 10l), **T1**= 1.5 x 10^9 CFU of *T. harzianum*/mL, **F1:** recommended fertilization.

Table 4. Analysis of micro and macronutrients of potato plants at foliar level.

Treatments	K	N	P	Ca	Mg	S	Zn	Cu	Fe	Mn	B
	%	(%)					ppm				
1 M0T0Pt30%	4,58 ab	4,44 ab	0,2 cd	1,87 abc	0,51 ab	0,42 ab	33,13 bc	5,9 bc	413,5 d	71,53ab	58,61ab
2 M0T0Pt50%	4,00 b	4,13 ab	0,29 b	1,69 cd	0,56 a	0,46 a	56,3 a	8,47 bc	1257 a	67,07b	54,68 ab
3 M1T0Pt30%	4,23 ab	3,68 b	0,22 cd	1,99 abc	0,53 ab	0,41 ab	37,37 bc	8,67 bc	1045,67 ab	78 ab	57,55ab
4 M1T0Pt50%	5,21 ab	4,35 ab	0,3 b	1,8 bcd	0,54ab	0,37 b	39,97 bc	7,43 bc	696 bcd	68,53 b	52,44 ab
5 M0T1Pt30%	5,36 ab	3,86 ab	0,17 d	2,05 abc	0,5 ab	0,41 ab	27,03 c	6,37 bc	677 bcd	76,37 ab	60,52 ab
6 M0T1Pt50%	4,34 ab	4,03ab	0,18d	2,01 abc	0,54 ab	0,37 b	34,6 bc	5,03 c	513,67 cd	61,6 b	64,88a
7 M1T1Pt30%	4,92 ab	3,81 b	0,2 cd	2,22 a	0,53 ab	0,4 ab	36,37 bc	7,53 bc	1098,33 ab	78,37ab	49,68 b
8 M1T1Pt50%	4,94 ab	3,06 ab	0,27 bc	2,08 ab	0,56 a	0,35 b	43,47 ab	9,43b	1310,83 a	89,73a	54,89 ab
9 M0T0Pt0F1	4,79 ab	4,76 a	0,4 a	1,44 d	0,46b	0,41 ab	56,1a	20,07 a	884,17 abc	69,67 b	51,91 ab
NORMAL RANGE	>0,99- <3	>3 - <6	>0,2- <0,4	>0,99- <1,5	>0,29- <0,75	>0,15- <0,25	>20- <50	>10 - <20	>60 - <150	>50 - <300	>30 - <100
CV	9,28	8,07%	9,26%	6,95%	6,11%	6,65%	12,72%	15,14%	18,34%	9,19%	8,56%

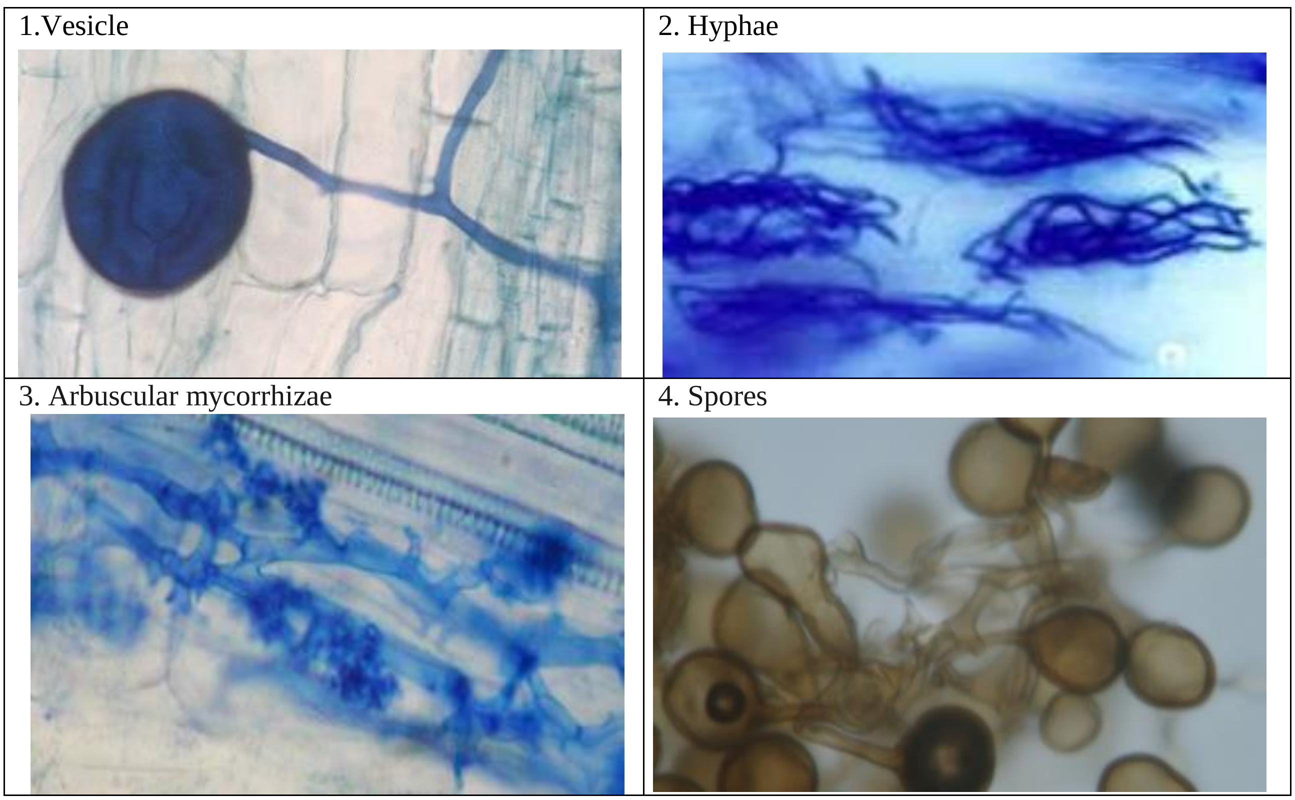

Figure 1. 1) Vesicle 2) Hyphae 3) Arbuscular mycorrhizae 4) Spores of Arbuscular mycorrhizal fungi.

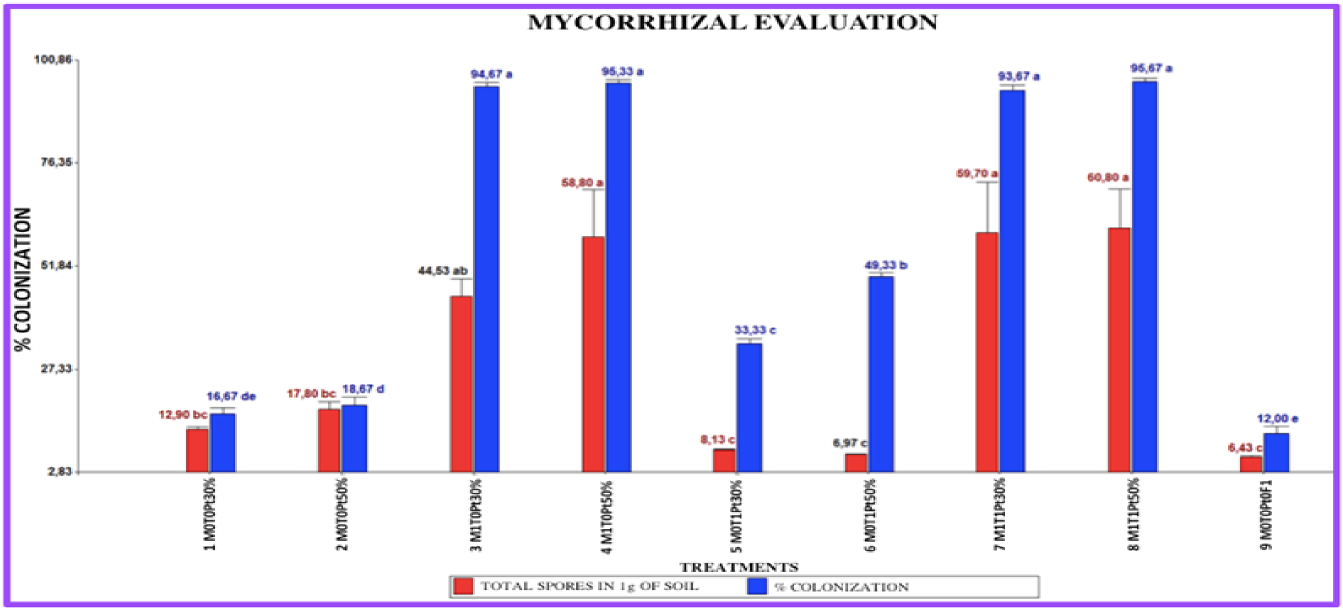

Figure 2. Percentage of colonization and mycorrhizal spores count per gram of soil.

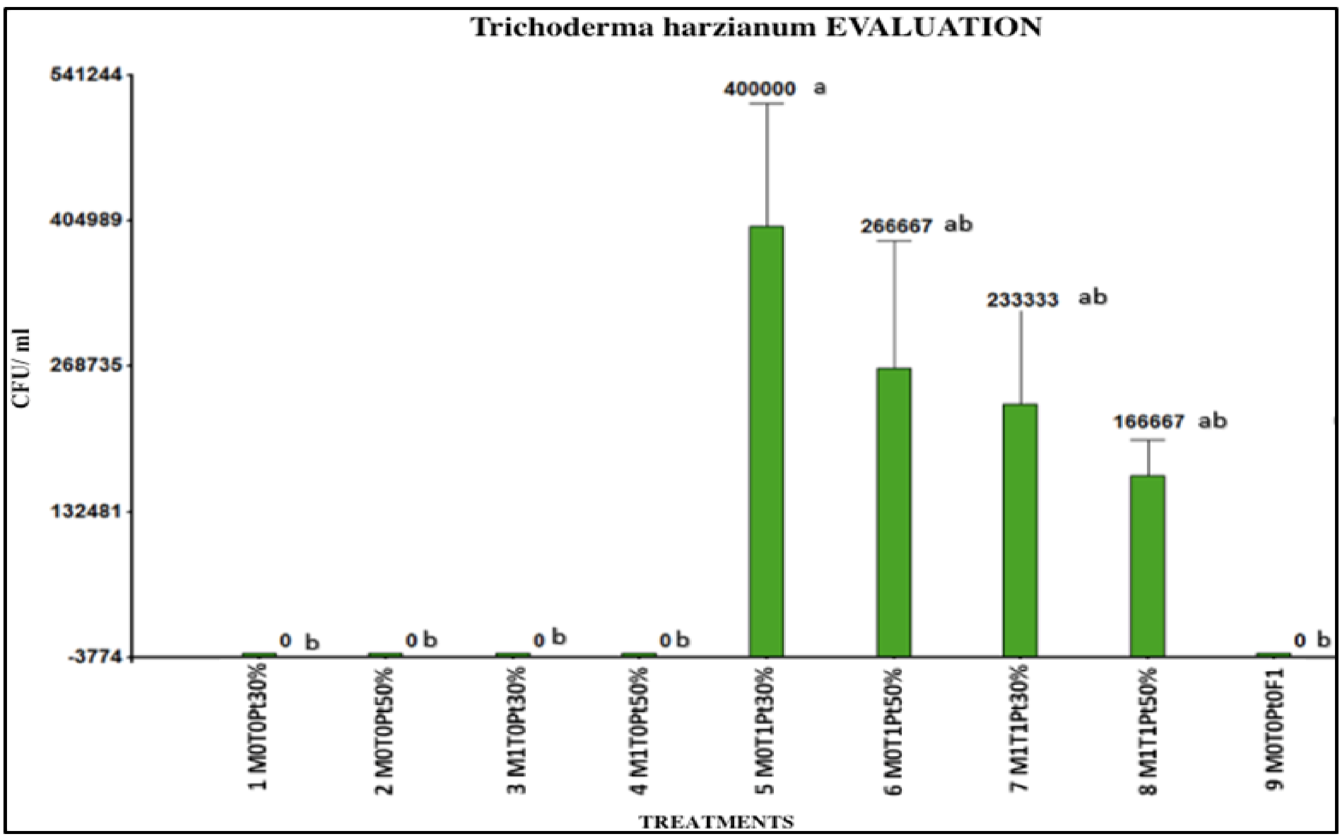

Figure 3. *Trichoderma harzianum* concentration in the different treatments of the assay.

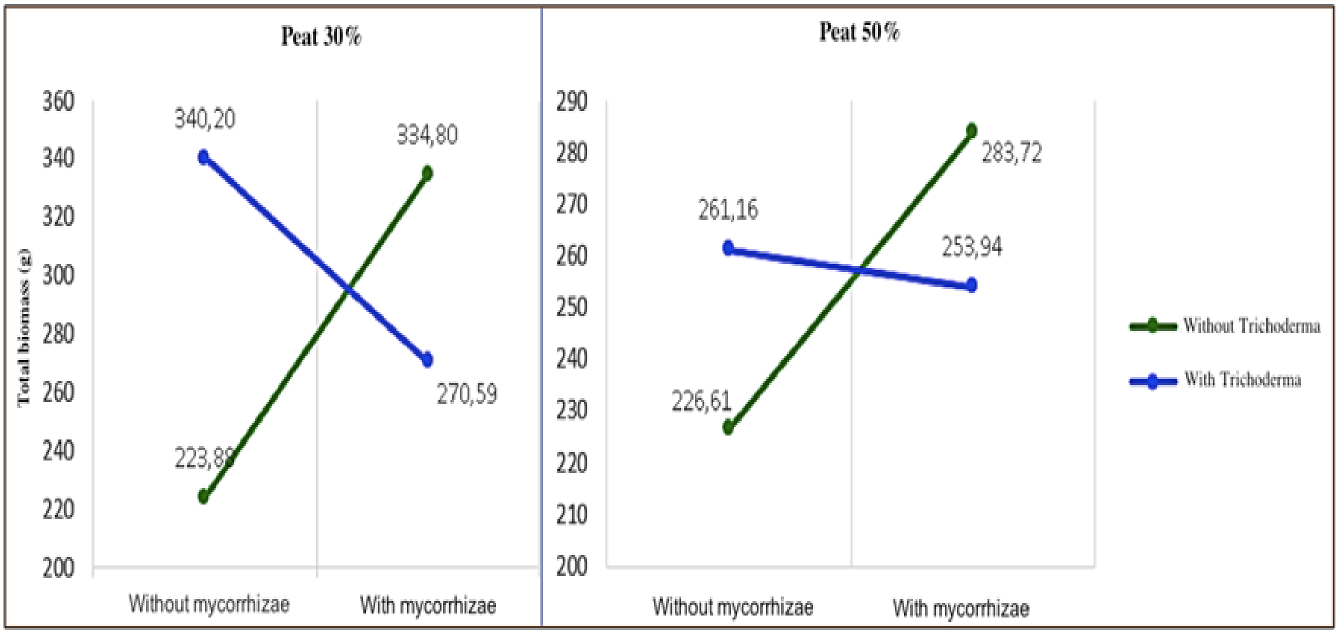

Figure 4: Contingency table of the interaction of microorganisms (AMF and *T. harzianum*) for the development of total biomass with peat at 30% and 50%.

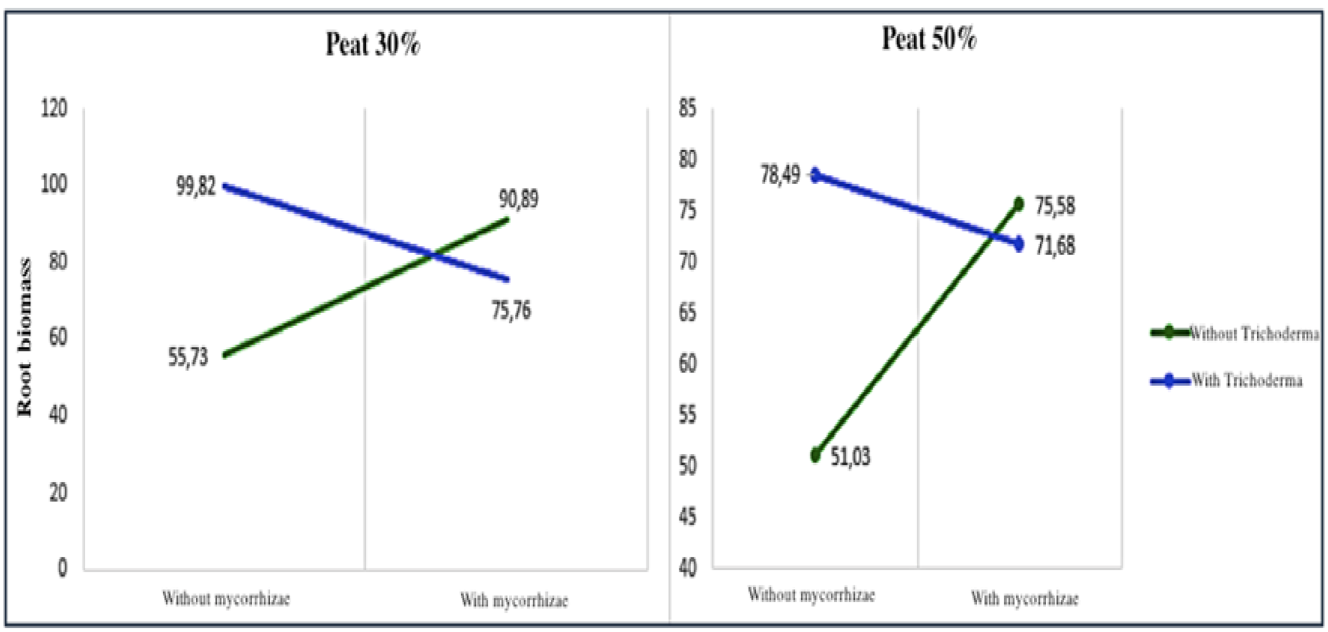

Figure 5: Effect of interaction between AMF, *Trichoderma harzianum* and substratum variation on root biomass of potato plants (*Solanum phureja*) at 90 days of the assay.

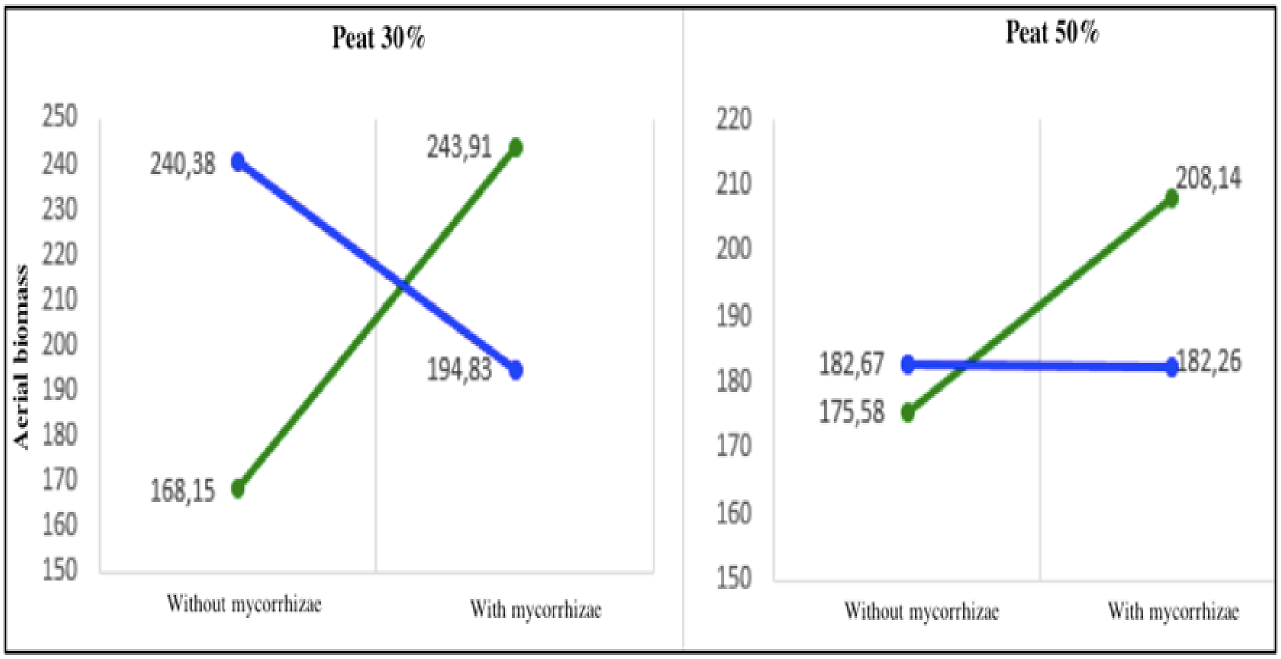

Figure 6: Effect of the interaction between AMF, *Trichoderma harzianum* and substratum variation on aerial biomass of potato plants (*Solanum phureja*) at 90 days of the assay.

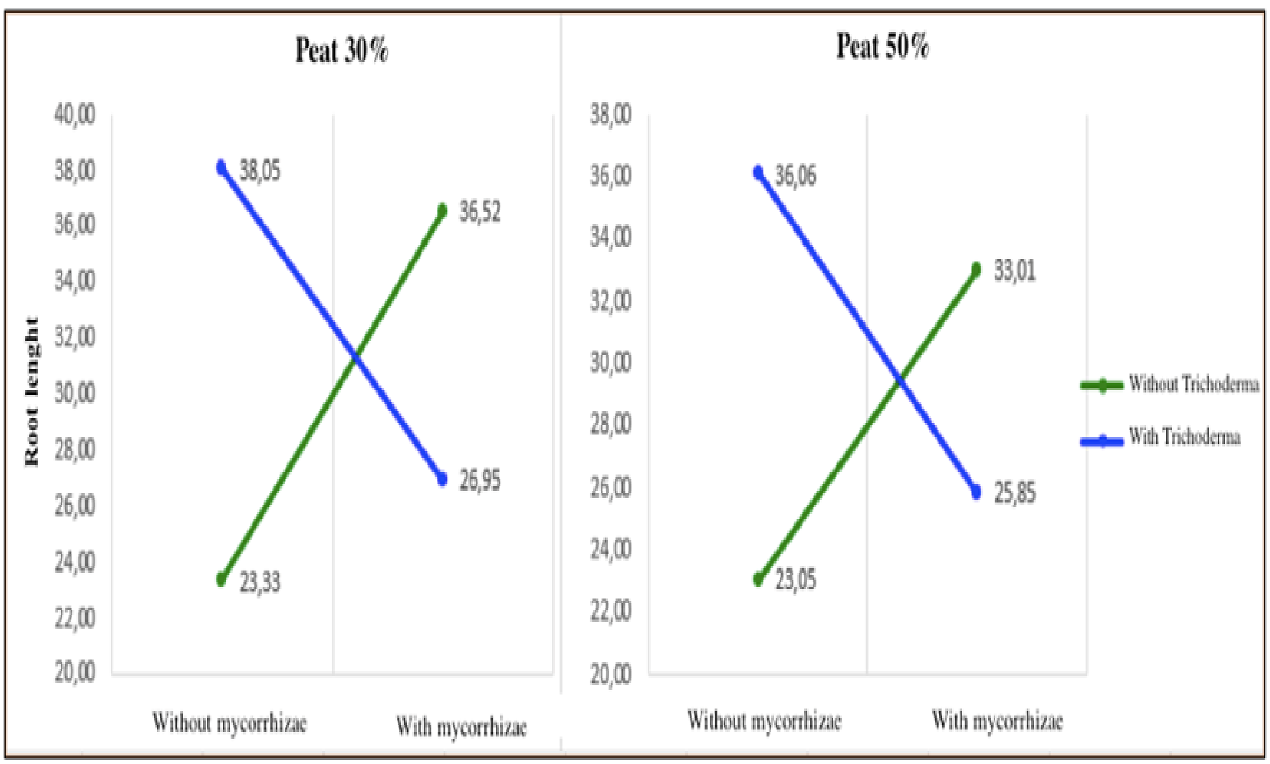

Figure 7: Effect of inoculation of AMF, *Trichoderma harzianum* and substratum variation (peat at 30% and 50%) on root length of potato plants (*Solanum phureja*) at 90 days of assay.

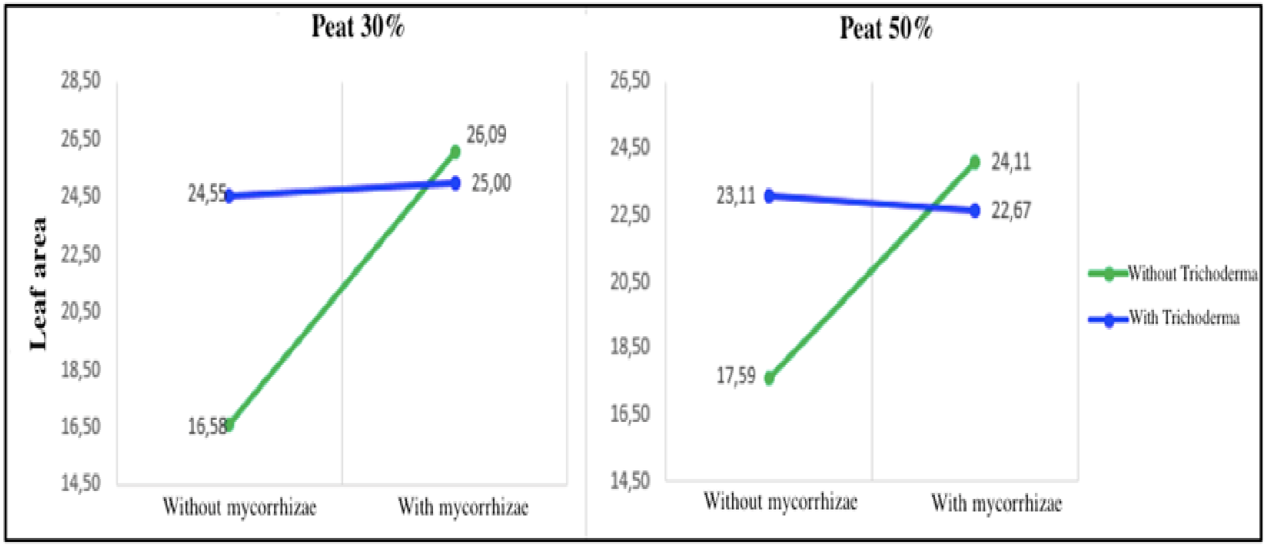

Figure 8: Effect of inoculation of AMF, *Trichoderma harzianum* and substratum variation (30% and 50% peat) on leaf area of potato plants (*Solanum phureja*) at 90 days of assay.

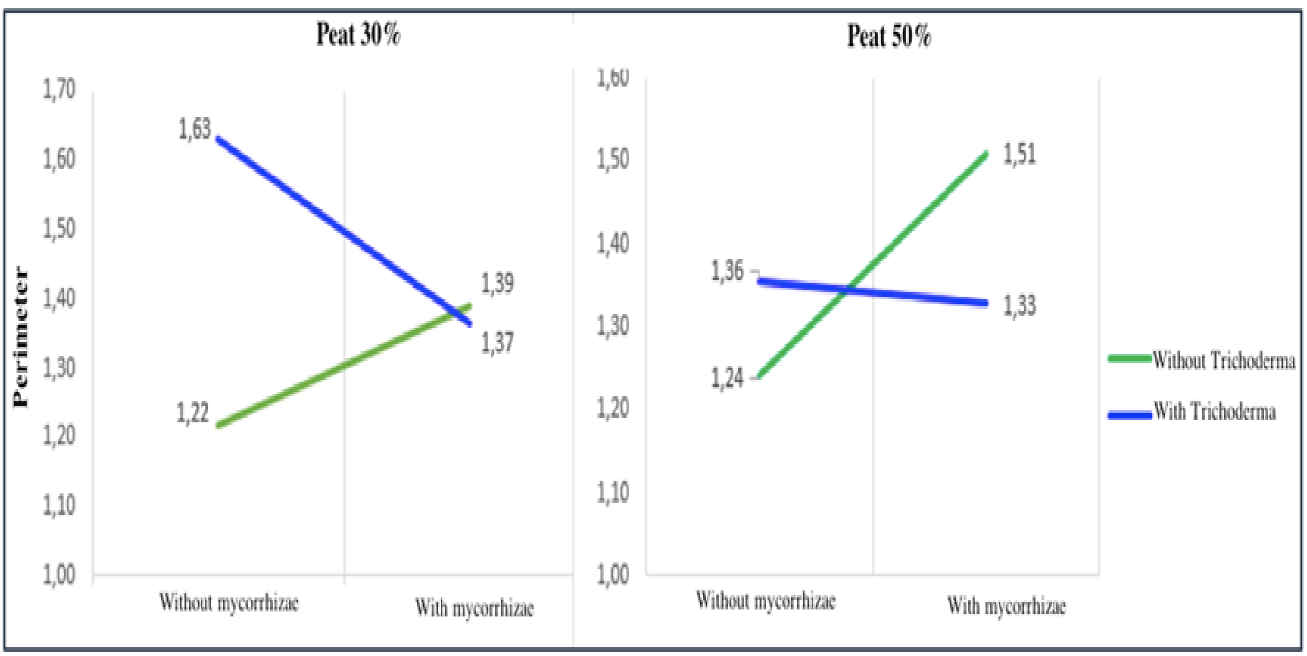

Figure 9: Effect of inoculation of AMF, *Trichoderma harzianum* and substratum variation (peat at 30% and 50%) on the perimeter of the potato plants (*Solanum phureja*) at 90 days of the assay.

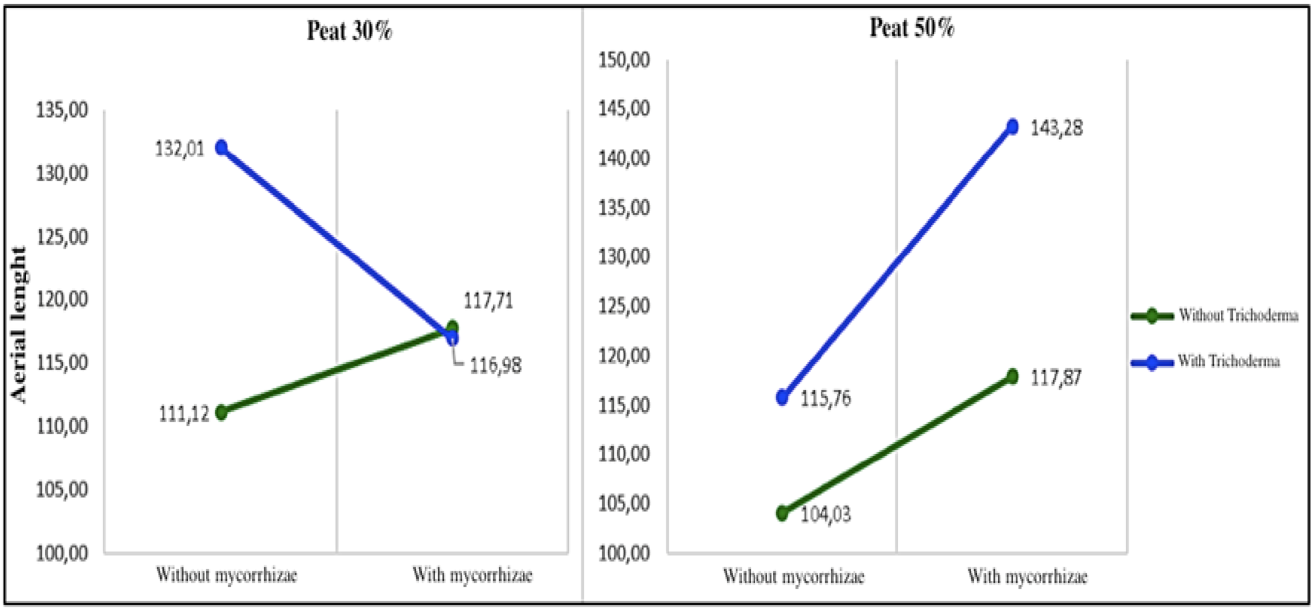

Figure 10: Contingency table of the interaction of microorganisms (AMF and *Trichoderma harzianum*) for the development of aerial length with 30% and 50% peat.

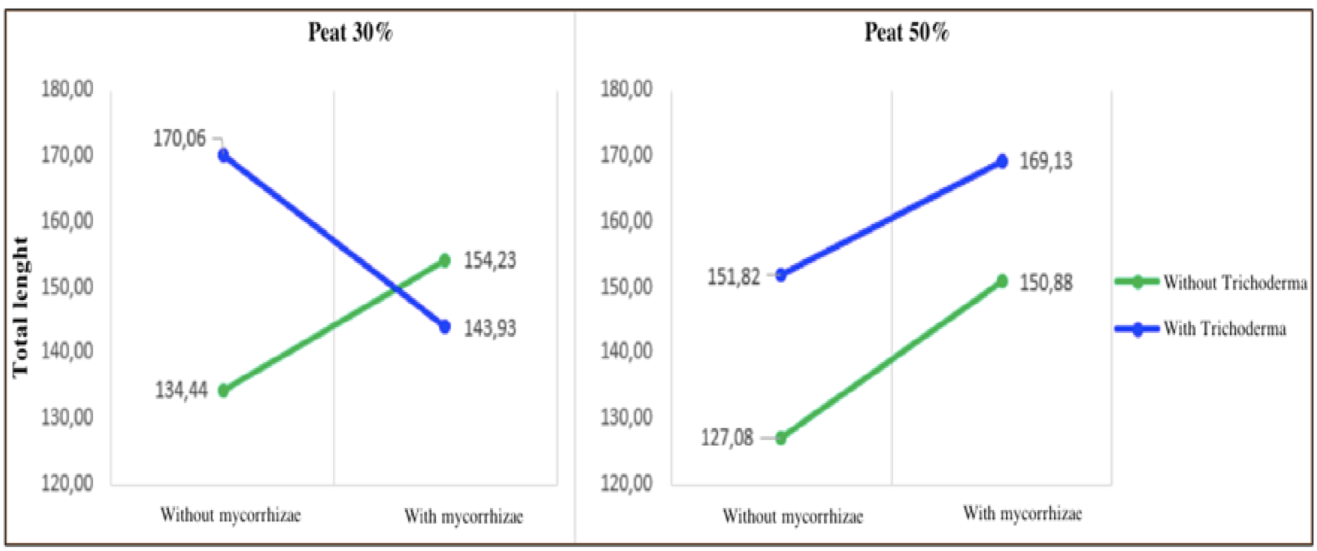

Figure 11: Effect of the interaction between AMF, *Trichoderma harzianum* and substratum variation on the total length in potato plants (*Solanum phureja*) at 90 days of assay.

Conclusion

The individual inoculation of each microorganism (AMF and *T. harzianum*) with 30% peat, improved the biomass (total, root and aerial) as well as the root length of the plant in about 30%, compared to non-inoculated and chemical fertilizer treatments. The co-inoculation of the fungus *T. harzianum* did not present a negative influence in the increase of the mycorrhizal population, the final population AMF spores increased three times compared to the beginning of the assay, reaching additionally a colonization of 95% in all the treatments containing mycorrhizae with and without *T. richoderma*. *T. harzianum* concentration decreases when inoculated together with AMF, showing that *T. harzianum* is limited in its growth when interacting with mycorrhizae.

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