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Indicators of colonization of arbuscular mycorrhizal fungi in "potato" (*Solanum tuberosum* L.)

Indicadores de colonización de hongos micorrícicos arbusculares en “papa” (*Solanum tuberosum* L.)

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Abstract

Arbuscular mycorrhizal fungi (AMF) are soil organisms, they have symbiotic interaction with most plants, they improve the supply of nutrients, growth, and production of host plants, hence their importance as a biofertilizer in agriculture, the objective of this work was to evaluate the effectiveness and infectivity of species of arbuscular mycorrhizal fungi in *Solanum tuberosum* L. "papa" unique variety. The experiment was carried out under greenhouse conditions, with five treatments: control, *Glomus fasciculatum*, *Entrophospora infrequens*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*. 75 spores were inoculated per pot (by species and treatment), evaluating the following indicators: length (cm), number of leaves, fresh and dry weight of the aerial part (g), root length (cm), fresh weight, and root dryness (g), number of tubers and stolons, plant vigor, number of spores and percentage of infection, *G. fasciculatum*, *E. infrequens* and *F. geosporum* were more effective and *C. etunicatum*, *G. fasciculatum* and *F. geosporum* showed higher infectivity.

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Resumen

Los hongos micorrícicos arbusculares (HMA) son organismos del suelo, tienen interacción simbiótica con la mayoría de las plantas, mejoran el suministro de nutrientes, crecimiento y producción de las plantas hospederas, de ahí su importancia como biofertilizante en la agricultura, el objetivo de este trabajo fue evaluar la efectividad e infectividad de especies de hongos micorrícicos arbusculares en *Solanum tuberosum* L. “papa” variedad única. El experimento se realizó en condiciones de invernadero, con cinco tratamientos: testigo, *Glomus fasciculatum*, *Entrophospora infrequens*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*. Se inocularon 75 esporas por maceta (por especie y tratamiento), evaluando los siguientes indicadores: longitud (cm), número de hojas, peso fresco y seco de la parte aérea (g), longitud de la raíz (cm), peso fresco y seco de la raíz (g), número de tubérculos y estolones, vigor de la planta, número de esporas y porcentaje de infección, *G. fasciculatum*, *E. infrequens y F. geosporum* tuvieron mayor efectividad y *C. etunicatum*, *G. fasciculatum* y *F. geosporum* mostraron mayor infectividad

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Introduction

Soil microorganisms play a role important in the agricultural context, contribute to the functioning of terrestrial ecosystems, through the recovery of degraded soils[1]. The study of arbuscular mycorrhizal fungi (AMF) has taken a lot of importance, to understand the symbiotic relationship that occurs between these fungi and the root system of plants[2].

AMF are essential constituents of the microbiota natural soil natural ecosystems, probably colonizing more plant tissues than any other type of organisms, play an important role in soil fertility, because these organisms work associated with their roots[3,4], with a beneficial effect on improving your nutrition, water use, growth, and adaptation to various stressful conditions by biotic as well as abiotic factors[5].

Colonization by AMF does not cause damage, hyphae develop externally, they branch out distributing on the ground, facilitating its absorption both of nutrients as of wáter[6].

The effectiveness of a kind of AMF is manifested by its ability to colonize its host, influence positively in its growth, development, the content of nutrients, yield, favoring the multiplication of the propagules of this species in the soil, as a species has a greater magnitude in these three aspects, the greater its effectiveness[7].

Infectivity refers to the ability of the fungus to penetrate and invade the root intensely and explore the ground, as well as its ability to persist in the productive system[8]. It is influenced by various parameters, such as spore germination, growth of pre-symbiotic mycelia, the formation of appressoria, and intraradical growth[9]. They are currently used in biofertilizers produced from AMF to improve agricultural production, species are especially important native as they present greater possibilities of effectiveness in the field, as they are adapted to conditions of the soil of each region[10].

Currently, in Ayacucho, there are no investigations performed at the level of effectiveness and infectivity with AMF, for this reason, our objective was: to evaluate the effectiveness and infectivity of these species in "Potato" (*Solanum tuberosum* L.), inoculating four AMF species: *Glomus fasciculatum*, *Entrophospora infrequens*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*[11,12].

Materials and methods

The research was conducted from March to October 2016 in the laboratory and greenhouse of Agro-biology from the Professional School of Agronomy of the University National of San Cristóbal de Huamanga, located between geographic coordinates 13º 08'50.92'' south latitude and 74º 13 '09.84'' west longitude, at an altitude of 2750 m.s.n.m. with a temperature average maximum of 18 ºC, average minimum temperature 10 °C, 70-90 % relative humidity, pH between 6.5-7.5. The soil for the extraction of spores It was from farm fields in the Vinchos district, the extraction, and isolation of spores was carried out according to the Gendermann & Nicolson[13] and Daniels methodology & Skipper[14], also used by other researchers[15].

The experimental unit (EU) was made up of a pot with the sterile substrate, host plant, and AMF spores, the extracted substrate was uncultivated soil (virgin soil), collected from non-cultivated areas from the university city of "Pampa del Arco", soil with a sandy clay loam texture (SCL), pH of 7.82 (slightly

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alkaline), the salinity of 0.560 dS/m (very slightly saline), the percentage of matter organic 1.15 % (low) and a percentage of nitrogen total 0.05 %. The soil was sieved and placed in plastic bags in a quantity of 1.5 kg for its automatic autoclave sterilization for three consecutive days, with a duration of 60 min at a 100 °C temperature (1 h/day)[16-18]. The evaluation of effectiveness and infectivity had a completely randomized experimental design, with four AMF species and the control, with a design conformed for five treatments, four repetitions each, with a total of 20 EU and ordered from as follows: 4 uninoculated pots (control) and 16 pots inoculated with AMF spores, each treatment with a different species. (*G. fasciculatum*, *E. infrequens*, *F. geosporum*, *C. etunicatum*)[9,10]. The coding used for the treatments was: T (Control), Fg (*Funneliformis geosporum*), Ce (*C. etunicatum*), Gf (*G. fasciculatum*), Ei (*E. infrequens*). After 110 days, in each EU was evaluated: length of the plant (LP), number of leaves (NL), air fresh weight (AFW), aerial dry weight (ADW) of the plant, root fresh weight (RFW), root dry weight (RDW), root length (RL). The determination of the number of spores/g of the substrate and the percentage of colonization, calculated with the following formula[16].

$$Number\ of\ spores\ in\ 10\ g\ of\ dry\ soil = \frac{(number\ of\ spores\ counted)}{\frac{(g\ of\ dry\ soil)}{10\ g\ of\ moist\ soil}} x100$$

To determine the percentage of colonization, it was used the methodology[18-20], and from Giovanetti & Mosse[21], was calculated with the following equation: [15,16,20].

$$\%\ total\ colonization = \frac{Nº\ of\ colonized\ segments}{N°\ of\ total\ segments}\ x\ 100$$

The data obtained were analyzed with the analysis variance (ANOVA)[16,19], using the Infostat at 95 % confidence, as well as the test of comparison of DUNCAN averages at 5 %, for determining the effectiveness and infectivity in the different treatments.

Results

Table 1 Evaluation of the general characteristics of *S. tuberosum* L. "papa" inoculated with four species of arbuscular mycorrhizal fungi

Treatment	PL (cm)	NL	AFW (g)	ADW (g)	RL (cm)	RFW (g)	DRW (g)	WTS	PV
T	8.25	13	15.5	2.2	26	5.75	3.06	0.97	2.25
Gf	8.25	22.75	16.75	2.59	26.25	8.75	4.8	0.44	2.00
Ei	7.25	28.75	19.5	2.86	25.75	12.75	7.43	0.65	2.25
Fg	6.25	16.75	21.5	2.53	17	8.75	3.08	0.17	1.50
Ce	8.25	22	17.5	2.45	20.5	11.25	4.37	1.16	2.25

C: Control, Gf*: Glomus fasciculatum*, Ei*: Entrophospora infrequens*, Fg*: Funneliformis geosporum*, Ce*: Claroideoglomus etunicatum.* PL plant length, NL number of leaves, AFW air fresh weight, ADW aerial dry weight, RL root length, RFW root fresh weight, RDW root dry weigh, WTS weight of tubers and stolons, PV plant vigor.

Discussion

Mycorrhizal plants need more photosynthates to meet your demand and that of the mycosymbiont in such a way that it allows without affectation stable growth and development of both organisms[22]. In Figure 1, it is shown that the treatment inoculated with *E. infrequens* has a higher quantity in NH compared to the other treatments (table 1 and figure 6), showing its effect positive when obtaining a larger cup diameter and more leaves in mycorrhizal

plants[23,24]. It also increases the amount of minerals, such as phosphorus in the increase aerial biomass[25]. It also increases the amount of minerals, such as phosphorus in the increase of aerial biomass, increasing its root biomass in mycorrhizal plants[26], also indicate fewer photosynthates to root growth when acquiring nutrients mainly through mycelium extra radical of mycorrhizal fungi arbuscular (AMF) associated with their roots[27,28].

Figure 1 Effectiveness of four species of mycorrhizal fungi arbuscular on the number of leaves in *S. tuberosum* L. "potato"

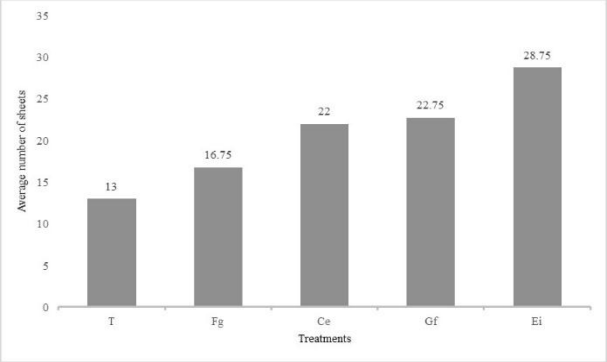
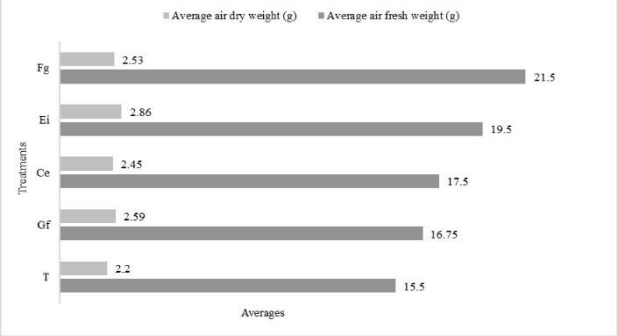


Figure 2 Effectiveness of four species of mycorrhizal fungi arbuscular over aerial fresh weight and aerial dry weight in *S. tuberosum* L. "potato"



Regarding the effectiveness, on the AFW and ADW, in figure 2 it is observed that the treatment inoculated with *F. geosporum*, is greater. AFW increased by training of spores, compared to treatment without inoculating, these differences are the result of the greater the mycorrhization in plants, the development is better. Plants experience a considerable increase in their biomass mainly due to the improvement of your mineral nutrition induced by the fungus[29].

Figura 3 Efectividad de cuatro especies de hongos micorrícicos arbusculares sobre el peso fresco de raíz y peso seco de raíz en *S. tuberosum* L. "papa"

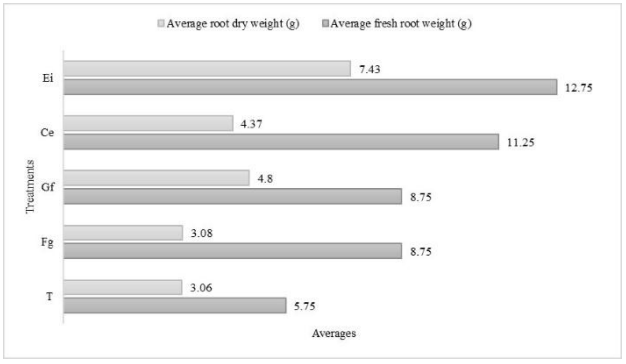


Figura 4 Número de esporas de cuatro especies de hongos micorrícicos arbusculares en *S. tuberosum* L. "papa"

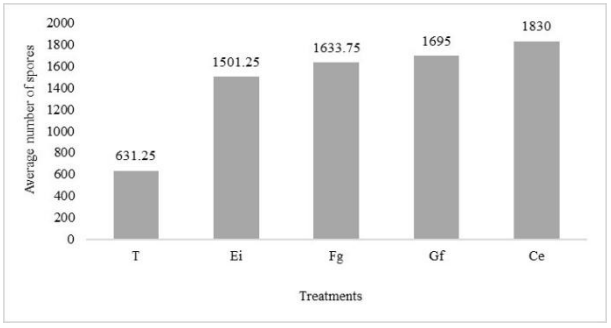


Figure 5 Percentage of infectivity of four species of arbuscular mycorrhizal fungi in *S. tuberosum* L. "potato"

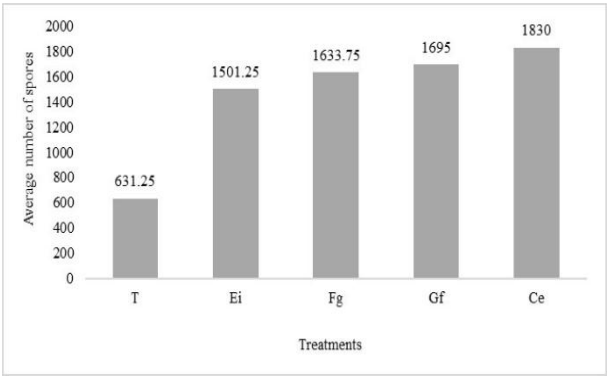


Figure 6 Comparison of the treatments inoculated with different species of AMF versus the control

HFMAs increase the survival and biomass production of host plants and improve the absorption of nutrients[28], likewise, they increase the formation of soil microbiota and a rapid re-establishment of the natural biological balance, allowing their greater and rapid growth, to, a rapid generation of a vegetal cover, formation of a greater mass of roots, a better rooting in the substrate, assimilation of nutritive substances that would not otherwise be available to plants[29].

The ADW is higher with *E. infrequens*, the ADW ratio of the aerial part is normally higher in plants colonized by mycorrhizae, the response of the plant can vary depending on the degree of dependence between the endophytes and the host plant, as well as the degree of colonization. Likewise, fungal activity represents a cost for the plant, which provides carbonated energy sources for the metabolism of the fungus, hence the generation of a system of mutual benefit[8].

It is important to consider that AMF, are microorganisms that represent up to 50 % of the biomass of soil microorganisms, they form a more relevant symbiosis with more than 90 %[30].

The root-mycorrhizal fungus symbiosis stimulates growth in root length density, possibly due to the advantage that mycorrhizal roots possess to explore a greater volume of soil and compensate their nutritional needs, especially capturing those nutrients with little mobility like phosphorus[31].

In figure 3, the effectiveness of four species is observed of AMF on the RFW and RDW in *S. tuberosum* L. "papa". In which it is observed that the treatments inoculated with *E. infrequens* and *C. etunicatum* have higher RFW (table 1), there seems to be a positive relationship between the presence of AMF and the increase in the density of your root system. Mycorrhizae increase the formation of soil microflora, rapid restoration of the natural biological balance, allowing greater and faster growth of plants, a rapid generation of a vegetal cover, formation of a greater mass of roots, a better rooting in the substrate, assimilation of nutritive substances that would not otherwise be available[29].

Table 1 presents the average of the characteristics generals of *S. tuberosum* L. "potato" inoculated with four species of mycorrhizal fungi, results promising in root-fungus symbiosis, due to increased density of the root system, as mycorrhizal roots explore more the soil, received the benefits of the symbiosis of AMF, generally this increase is associated to an increase in the exploration area of the root system, therefore greater availability of nutrients[32,33]. AMFs have a very positive effect on biomass production.

The most important effect that mycorrhizae produce on the hosts is an increase in absorption of mineral nutrients from the soil, which results in further growth and development. The main cause of this effect is the expansion of the outer mycelium of the fungus by the rhizospheric soil, which allows the uptake of nutrients beyond the zone of exhaustion that builds up around the roots[34].

The number of spores has important differences according to where the samples are taken. Various authors have reported variations in the quantification of the number of spores[33-37]. In figure 4, it is observed in the treatment inoculated with C. etunicatum (1830

spores/10 g of soil), statistically similar to the treatments inoculated with *G. fasciculatum* and *F. geosporum*. Differences in the number of AMF spores in the soil may also be related to different survival strategies of AMF species when living in a given ecosystem, that is, the life cycle of AMF presents a high adaptation to the environment. surrounds, especially during the spore formation stage, in addition, its competitive ability can be affected by various factors[38] depending on the inoculated fungal species. By way of it is generally known that variations in the number of AMF spores may be associated with seasonal patterns of sporulation, which may vary according to the AMF species or the host plant[39].

The number of AMF spores in the soil not necessarily is reflected in the colonization capacity of these fungi[32,38], therefore it is important to make observations in annual periods since the association mycorrhizal can vary through time and space. Figure 5 shows the infectivity percentage of four AMF species in *S. tuberosum* L. "papa". under greenhouse conditions. *G. fasciculatum* presents a higher percentage of infection or colonization, being considered more efficient[22], its high infectivity occurs in an early stage and is extensively distributed within the root producing dense infection per entry point[36].

The degree of mycorrhizal colonization may depend on various factors such as humidity, temperature, soil pH, luminosity, the oxygen level in the rhizosphere[39], AMF species and the host plant, together with the AMF biological cycles are synchronized with the phenological cycles and characteristics particular to the plant species to which it is associates increasing the diversity of interactions HMA-plant[40].

It should be noted that the mycorrhizal treatments were statistically similar to each other and superior to the non-mycorrhized[28], and in the case of growth parameters the response was different in each species evaluated[4,41], the HMA consortia state their effectiveness according to their origin.

Several authors affirm that it is necessary to evaluate the effect of some strains and isolated native consortia in these production areas, due to their capacity adaptation and efficiency of these, in addition to reducing economic dependence on commercial biofertilizers[15,42].

We conclude that the most effective species were *Glomus fasciculatum*, *Entrophospora infrequens* and *Funneliformis geosporum*, the most infective *Claroideoglomus etunicatum*, *Glomus fasciculatum* and *Funneliformis geosporum*. In this sense, it would be convenient to use *G. fasciculatum*, *E. infrequens*, *F. geosporum* and *C. etunicatum* as biofertilizers, carrying out inoculations to improve soil quality, plant productivity, and nutrient absorption.

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Conflicts of interest

The research corresponds to a degree thesis carried out at the National University of San Cristóbal de Huamanga, for which the authors express the non-existence of conflicts of interest.

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Ethical considerations

All procedural and experimental aspects were approved by the evaluation commission of the Faculty of Biological Sciences of the National University of San Cristóbal de Huamanga.

Authors' contribution

Silvia Méndez Gálvez, conceptualization, idea, investigation and conduct of the investigation, writing of the manuscript. *Roberta Esquivel Quispe*, development of the research methodology, review, statistical analysis. *Walter Wilfredo Ochoa Yupanqui*, preparation of tables, writing and final revision of the manuscript.

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