



**EFFECTS OF SEVEN DIFFERENT MYCORRHIZAL INOCULUM IN
Persea americana IN STERILE AND NON-STERILE SOIL**

**[EFECTO DE SIETE INÓCULOS MICORRÍZICOS DIFERENTES EN
Persea americana EN SUELO ESTERIL Y NO ESTERIL]**

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SUMMARY

Some mycorrhizal fungal species could have certain compatibility and efficiency in the development of the host. Recently, avocado producers became interested in the use of arbuscular mycorrhizal fungi (AMF) for the production of plants in nurseries; therefore, it is important to revise the inoculants that are more efficient to this crop. The native microbiota could interfere in the establishment of introduced AMF. A 2 factorial experimental design was used. The first factor, AMF, had 8 levels, with 6 AMF species (*Rhizophagus fasciculatum*, *Gigaspora margarita*, *Claroideoglossum etunicatum*, *Pacispora scintillans*, *Rhizophagus intraradices*, *Acaulospora laevis*), a commercial consortia (MTZ-UV1) and a non-mycorrhizal control. The second factor, soil treatment, had 2 levels (sterile and not). Sterile soil treatments had a higher growth and the AMF inoculation increased the height, diameter, fresh and dry weight of the leaves. The inoculant *Rhizophagus fasciculatum* in sterile soil had tendency of the highest growth in most of the variables. On the other hand, *Pacispora scintillans* and *Acaulospora laevis* in not sterile soil decreased the plant growth. The results obtained showed possible plant-AMF compatibility, as well as the importance of the sterilization of the soil before AMF inoculation

Keywords: Desinfection; inoculation; Mycorrhizal; microbiota; compatibility; efficiency.

RESUMEN

Algunas especies de hongos micorrízicos pueden tener cierta compatibilidad y eficiencia en el desarrollo del hospedero. Recientemente, los productores de aguacate se han interesado en el uso de hongos micorrízicos arbusculares (HMA) para la producción de plantas en viveros; por lo que es importante verificar qué inoculantes son más eficientes al usarse en este cultivo. La microbiota nativa puede interferir con el establecimiento de HMA introducidos. Un diseño experimental factorial con 2 niveles se usó. El primer factor, HMA, tuvo 8 niveles, con 6 especies de HMA (*Rhizophagus fasciculatum*, *Gigaspora margarita*, *Claroideoglossum etunicatum*, *Pacispora scintillans*, *Rhizophagus intraradices*, *Acaulospora laevis*), un consorcio comercial (MTZ-UV1) y un control no micorrizado. El segundo factor, esterilización del suelo, tuvo 2 niveles, (esterilizado y no). Las plantas sembradas en suelo mostraron un mayor crecimiento y la inoculación con HMA incrementó la altura, diámetro, peso fresco y seco de las hojas. El inoculante *Rhizophagus fasciculatum* usado en suelo estéril, mostró una tendencia de un mayor crecimiento en la mayoría de las variables. Por otra parte *Pacispora scintillans* y *Acaulospora laevis* en suelo sin esterilizar, mostraron un decremento en el crecimiento de la planta. Los resultados obtenidos muestran una posible compatibilidad hongo-planta, así como la importancia de la esterilización antes de la inoculación con HMA.

Palabras clave: Desinfección; inoculación; micorriza; microbiota; compatibilidad; eficiencia.

INTRODUCCIÓN

Mexico is the avocado center of origin (Gutiérrez-Díez *et al.*, 2009; Gutiérrez-Contreras *et al.*, 2010) and more than 20 different species and three varieties (mexican, antillana, and guatemalteca) are known (Barrientos-Priego and López-López, 2000; Chen *et al.*, 2008). Some studies reports that the avocado is a mycotrophic crop that responds favorably to the mycorrhizal inoculation (Menge *et al.*, 1980; Alarcón, 1997; da Silveira *et al.*, 2003; Bárcenas *et al.*, 2010). This crop generally goes through a nursery phase (da Silveira *et al.*, 2003), but without the proper management during its transplant, it is susceptible to pathogen attack (Graham, 2001; Morales-García, 2009). The soil sterilization and the reintroduction of arbuscular mycorrhizal fungi (AMF) could favor protection, the survival and increase the plant production.

The mechanisms by which AMF improve plant development and protection, could be related to the increase of nutrient absorption, allowing a better nutritional status and a adequate stress response (Smith *et al.*, 2010), as well as allowing a higher survival rate to transplant (Trejo *et al.*, 2011). Although it has not been fully documented, the efficiency of the AMF could depend on the fungal species, host compatibility and biological; physical and chemical soil factors (Klironomos, 2000; González and Cuenca, 2008); as well as the diversity of native fungi or by the introduction of better-adapted fungi (Díaz and Honrubia, 1995; Gustafson and Casper, 2006). Smith and Giaginazzi, (1988) reported that the compatibility between fungal species and the host depends on the genotype, root exudates, root geometry and root hairs, as well as soil pH, humidity, texture, fertility and surrounding microorganisms.

Studies involving avocado plants inoculated with AM fungi have been explored (Haas and Menge, 1990; Jaizme-Vega and Azcón, 1995; da Silveira *et al.*, 2002), where they have proved that the avocado plant is a mycotrophic plant and therefore its interaction is of interest.

Some studies indicate that introduced AMF could be more effective than native ones (González and Cuenca, 2008). But other studies contrast, were the native AMF were more effective than the introduced ones (Requena *et al.*, 2001). Therefore the identification of HMA is a prerequisite for efficient inoculation programs (Caravaca, *et al.*, 2005), because the compatibility level between AMF species and the host depends on the species involved (Smith and Read, 1997).

Although, specificity between fungi and host has not been reported, few researches have mentioned that some AMF could be more efficient in nutrient uptake than others, depending on the host (Klironomos, 2000; González and Cuenca, 2008; Trejo *et al.*, 2011).

Due to the high microorganisms diversity, that interact by different means (Linderman, 1992), the establishment of introduced microorganisms is affected, caused by competition among native populations (Garbaye, 1991)

Considering that Mexico is the origin center of the avocado and that AMF are associated to this plant, this paper therefore seeks to evaluate the response of avocado seedlings to various AMF inoculants in sterile soil and not.

MATERIALS AND METHODS

Experimental conditions

The experiment was developed under greenhouse conditions, at the facilities of the Laboratory of Beneficial Organisms, Faculty of Agricultural Sciences, Universidad Veracruzana. Avocado seeds (Criollo var.) were sown on black plastic bags (2 lt) 45 days after sown. Uniform plants of 15 cm height were selected for the experiment and at 75 days after being sown, the plants were inoculated with the mycorrhizal inoculants, composed mainly by soil containing spores and root fragments. The plants were irrigated to field capacity with tap water and then harvested after 165 days after AMF inoculation (DAI).

Substrate

A mixture of soil, sand and pumice (1:2:1 v/v) was autoclaved for one h for three days and aerated for six h each day. This mixture was characterized by a pH of 6.04, organic matter of 5.93%; N, 22 ppm; P, 4.4 ppm; K⁺, 885 ppm; Ca²⁺, 13,58 ppm; Mg²⁺, 237 ppm; Fe, 6.7 ppm; Cu, 0.5 ppm; Zn, 1.2 ppm and Mn, 3.9 ppm.

AMF inoculation

Six monoxenic species were used, one comercial species, *Rhizophagus fasciculatum* (Micofert); four species provided by the Laboratory of Interaction plant-microorganism-environment of CIEco, UNAM, *Gigaspora margarita* (Gm), *Acaulospora laevis* (Al), *Claroideoglomus etunicatum* (Ce), *Pacispora scintillans* (Ps), *Rhizophagus intraradices* (Ri); and one isolate provided by the Department of Agroecology-Plant Pathology and Entomology, Aarhus University, Denmark. One of the inoculum

used was a consortia (MTZ-UV) that consisted of 12 AMF species, *Acaulospora morrowiae*, *Acaulospora spinosa*, *Acaulospora scrobiculata*, *Funneliformis mosseae*, *Funneliformis geosporus*, *Gigaspora rosea*, *Gigaspora decipiens*, *Glomus macrocarpum*, *Glomus aggregatum*, *Rhizophagus intraradices*, *Scutellospora pellucida*, *Claroideoglossum etunicatum* provided by the Laboratory of Beneficial Organisms, Faculty of Agricultural Sciences, University of Veracruz. They were propagated through the modified Sieverding technique (Sieverding, 1991), using *Brachiaria decumbens* as a host plant in sterile sand as substrate, under greenhouse conditions during 5 months before its use.

Assessment of variables

Plants were harvested 165 DAI and physical variables (height, diameter, fresh and dry weight of root and shoot) and mycorrhizal colonization, by root clearance (Phillips and Hayman, 1970) and the intersection method (Giovannetti and Mosse, 1980) were recorded.

The experiment had a factorial design with two factors, soil sterilization (sterile and not) and mycorrhizal inoculation, with eight levels (with seven mycorrhizal inoculants and a control). Each treatment had three replicates. Data were subjected to a factorial analysis of variance with eight treatments followed by Fisher’s least significant difference (LSD) test, with the statistical software STATGRAPHICS XV for Microsoft Windows.

RESULTS

The sterilization of the soil had significant ($\alpha \leq 0.05$) differences between treatments, expressed in an increased in the growth variables, compared to the not sterile soil (Table 1). The AMF inoculation had a significant effect on most of the plant growth variables, compared to the non-inoculated plants (Table 1). There was an interaction between the sterilization of the soil and the AMF inoculation, mainly on height, leaf area and shoot dry weight (Table 1).

Plants inoculated with *R. fasciculatum* displayed the highest increase on plant growth expressed in leaves number, especially when the soil was sterile (Figure 1a).

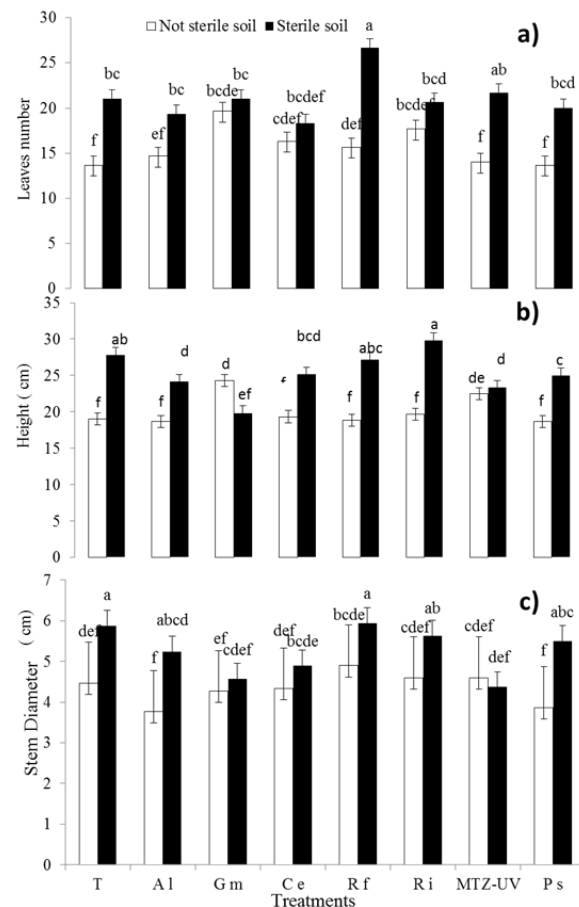


Figure 1. Leaves number a), height b) and stem diameter of avocado plants inoculated and not with seven different mycorrhizal inocula in sterile soil and not. Bars with equal letters are statistically similar (LSD, $\alpha \leq 0.05$); n=3. T, control; A1, *A. leavis*; Gm, *G. mosseae*; Ce, *C. etunicatum*; Rf, *R. fasciculatum*; Ri, *R. intraradices*; MTZ-UV; Ps, *P. scintillans*.

Table 1. ANOVA analysis of each variable between main factors.

| Factor | Height | Diameter | Leaves number | Leaf area | Fresh weight leaf | Shoot dry weight | Fresh weight root | Shoot dry weight |
|----------|--------|----------|---------------|-----------|-------------------|------------------|-------------------|------------------|
| Soil | *** | *** | *** | ** | *** | *** | * | ** |
| AMF | * | * | NS | *** | * | *** | NS | NS |
| Soil+AMF | *** | NS | NS | ** | NS | * | NS | NS |

Fisher ($\alpha \leq 0.05$);***, 0.001;** , 0.01; * , 0.05; NS, not significant; n=3.

Plants inoculated with *R. fasciculatum* and *R. intraradices* exhibit a slight increase in shoot fresh and dry weight, but was not significant (Figure 2a and b). However, plants inoculated with MTZ-UV, had a significant plant growth depression in sterile soil. In not sterile soil, there was not difference among treatments (Figures 2a and b).

The AMF colonization was observed to be higher in sterile soil compared to not sterile only in *A. leavis* and *P. scintillans* treatments (Figure 3). The treatments with the highest AMF root colonization were *A. leavis*, *R. fasciculatum* and *P. scintillans* in sterile soil (Figure 3), and the treatment with the lowest was *C. etunicatum*.

For the not sterile soil, there were not significant differences among treatments (Figure 3).

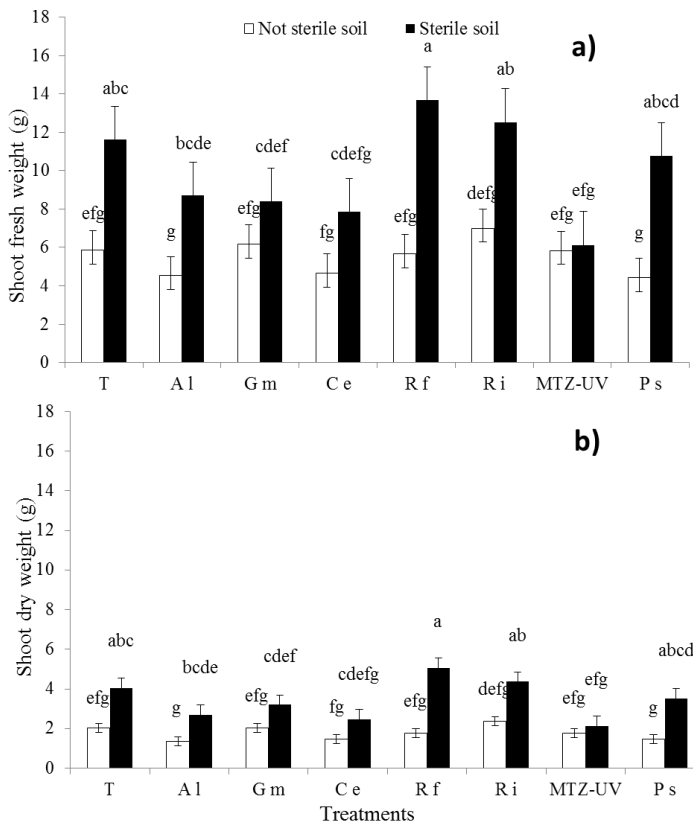


Figure 2. Shoot fresh a) and dry b) weight of avocado plants inoculated and not with 7 different mycorrhizal inocula in sterile soil and not. Bars with equal letters are statistically similar (LSD, $\alpha \leq 0.05$); n=3. T, control; Al, *A. leavis*; Gm, *G. mosseae*; Ce, *C. etunicatum*; Rf, *R. fasciculatum*; Ri, *R. intraradices*; MTZ-UV; Ps, *P. scintillans*.

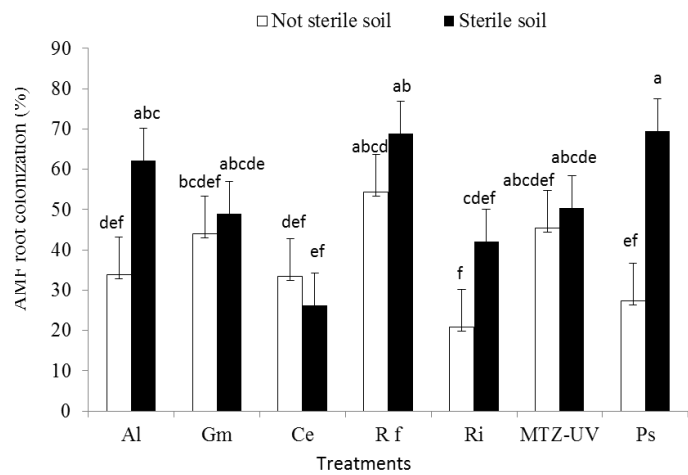


Figure 3. AMF root colonization of avocado plants inoculated with 7 different mycorrhizal inocula in sterile soil and not. Bars with equal letters are statistically similar (LSD, $\alpha \leq 0.05$); n=3. T, control; Al, *A. leavis*; Gm, *G. mosseae*; Ce, *C. etunicatum*; Rf, *R. fasciculatum*; Ri, *R. intraradices*; MTZ-UV; Ps, *P. scintillans*.

DISCUSSION

Some of the species used in the experiments display a better response that could be translated as compatibility with the host, special with *R. fasciculatum*, that presented the highest values among the variables evaluated, but with a very short difference between the other treatments. In the other hand the plants inoculated with *P. scintillans*, exhibited a greater relative difference between the response of the plant under sterile and non-sterile soil conditions in most of the variables.

In the present experiment we found that the soil sterilization promoted an increase on the plant growth, in accordance with De Deyn *et al.* (2004) and Al-Khalil (2010). Such result could be explained by absence of competition for resources in the soil by other microorganisms (Calvet *et al.*, 2002) or, as found by Miransari *et al.* (2009) in wheat, the increase of nutrient uptake in sterile soils by its solubilization. The sterilization promoted a greater colonization percentage, compared with non-sterile soil. Such effect could be explained by the low competition for space and nutrients (Graham, 2001; Calvet *et al.*, 2002).

These findings are different of what Ingham *et al.* (1985), Callaway *et al.* (2004) and He and Cui (2009) found, where they observed a decrease of plant biomass in sterile soil. According to He and Cui (2009), perhaps this could be related to changes in the pH and soil nutrient availability. As some microorganisms still remain unknown; and

therefore, their role in nutrient cycle is not understand, it is complicated to know how they influence plant growth (Crecchio *et al.*, 2004).

The results of this studio could point a functional complementarity between the *R. fasciculatum* and the avocado var. Criollo. Mainly, due to the greater plant growth response of such interaction, especially on sterile soil. Such effect may suggest a better relationship in terms of efficient between avocado plants (var. Criollo) and this inoculum. Similarly, Da Silveria, *et al.* (2003) observed in avocado plants var. Carmen a better plant response of two inoculants (*G. etunicatum* and *S. heterogama*) compared to other inocula tested. Besides, the availability of the nutrients could have been greater due to low competition for resources under sterile conditions, facilitating the disponibility of the nutrients to the AMF.

A few inoculants in his experiment had a detrimental effect on the plant growth (*C. etunicatum*), but some studies have considered the differential effect of the AMF (Gustafson *et al.*, 2006; Toussaint *et al.*, 2007).

The low response of plant growth to the AMF inoculation in non-sterile soil could be due to competition of space (Graham, 2001) and nutrients (Calvet *et al.*, 2002).

Therefore, this result could give us indication of the effectiveness of species like *R. fasciculatum*, above others because it displays a tendency that could be significant if the experiment continued for a few months more. But when comparing the relative difference between the response of an inoculum under sterile and not sterile soil conditions, the AM fungi *P. scintillans* could be recommended. Therefore, it could indicate host compatibility under the conditions expressed previously. Further studios could be performed to unveil the mechanisms involved in this interaction under sterile conditions.

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