World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

MONITORING OF THE EVOLUTION OF A COMPOSITE ENDOMYCORRHIZAL INOCULUM IN THE RHIZOSPHERE OF THREE MYCOTROPHIC SPECIES

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Article Received on 13/04/2016 Article Revised on 02/05/2016 Article Accepted on 23/05/2016

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ABSTRACT

Twenty-one and twenty eight mycorrhizal species were isolated respectively from the rhizosphere of maize and sorghum three months after inoculation with a composite inoculum originating from the rhizosphere of the carob tree (*Ceratonia siliqua*). Similarly, twenty six species were found in the rhizosphere of the carob tree plants ten months after their inoculation with the same inoculum. Analysis of endomycorrhizal fungi spore's communities found in the rhizosphere of these mycotrophic species has shown the dominance of five species: *Acaulospora pustulata* (9,67%), *Glomus etunicatum* (9,67%), *G. clarum* (9,67%), *G. deserticola* (24,5%) et *Scutelospora nigra* (16%). Furthermore, a comparison between AM fungi species of the initial inoculum (30 species) and those isolated has revealed the appearance of twenty six species and the disappearance of eleven others.

KEYWORDS: Composite endomycorrhizal inoculum, evolution, time, rhizosphere, maize, sorghum, carob.

1. INTRODUCTION

The carob tree is considered as a mycotrophic plant species.[1] In 2012, Ouahmane *et al.*[2] reported the presence of *Glomus intraradices*, *G.aggregatum*, *G. constrictum* and other

undetermined species (*Glomus* sp.1, *Glomus* sp.2, *Gigaspora* sp.1 and *Gigaspora* sp.2) in the rhizosphere of the carob tree in Ourika valley (High Atlas).

In their research in the carob tree soils of Oudmine (south west Morocco) Elmaati *et al*.[3] noted the presence of *Glomus aggregatum, G. fasciculatum, G. constrictum*, and others unidentified species: *Glomus* sp.1, *Glomus* sp.4, *Gigaspora* sp.1, *Gigaspora* sp.2 and *Gigaspora* sp.3. In our previous studies[1,4], it has been reported that in the rhizosphere of the carob tree growing in five sites (Nador, Ksibah, Taroudant, Khenifra and Afourar), 30 endomycorrhizal were identified: *Acaulospora laevis* Gerdemann and Trappe **(**1974), *A. gedanensis* Blaszkowski (1988), *A. denticulate* Sieverding and Toro (1987), *Entrophospora infrequens* Ames and Schneider (1979), *Gigaspora decipiens* Hall and Abbott (1984), *G. margarita* Becker and Hall (1976), *Glomus aggregatum* Schenck and Sm (1982), *G. aureum* Oehl and Sieverd (2003), *G. chimonobambusae* Wu and Liu (1995), *G. clarum* Nicolson and Schenck (1979), *G. deserticola* Trappe and Menge (1984), *G. etunicatum* Becker and Gerd (1977), *G. fasciculatum* Gerd and Trappe (1974), *G. geosporum* Nicolson and Gerd (1968), *G. intraradices* Schenck and Sm (1982), *G. macrocarpum* Tul and Tul (1845), *G. monosporum* Gerd and Trappe (1974), *G. mosseae* Nicolson and Gerd (1968), *G. versiforme* Berch (1983), *Pacispora robiginia* Oehl and Sieverding (2004), *Scutellospora castanea* Walker (1993), *S. fulgida* Koske et Walker (1986), *S. heterogamma* Nicolson and Gerd (1968), *S. nigra* Redhead (1979). Others, belonging to different mycorrhizal genera, have not been determined.

These include the cases of *Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, *Glomus* sp.1 and *Glomus* sp.2. All these species have been the subject of composite endomycorrhizal inoculum and have been multiplied using two mycotrophic plant species-namely, maize and sorghum.

In this study, the carob tree plants were inoculated with this inoculum, along with the monitoring of its evolution in the rhizosphere of the plants.

2. MATERIALS AND METHODS

Composite endomycorrhizal inoculum of carob rhizosphere from different Moroccan regions (Taroudant, Khenifra, Afourar, Nador and Ksiba) is multiplied under greenhouse using two mycotrophic plants: maize and sorgho.[5] This inoculum consists of 30 endomycorhizal species.[1,4]

After ten months of carob seedlings inoculation by the composite inoculum, isolation of spores of mycorrhizal fungi is performed according to the wet sieving method described by Gerdemann and Nicolson.[6] In a beaker of 1 L, 100 g of each composite sample of soil is immersed with 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through four superimposed sieves with decreasing mesh (500, 200, 80 and 50 µm).

This operation is repeated twice. The content retained by the sieves of 200, 80 and 50 µm is divided into two tubes and centrifuged for 4 min at 9000 rev/ min. The supernatant is discarded and a viscosity gradient is created by adding 20 ml of sucrose solution, 40% to each tube to centrifugeuse.[7] The mixture is rapidly stirred and the tube is returned again in the centrifuge for 1 min at 9000 rev / min.

Unlike the first centrifugation process, the supernatant is poured onto the sieve with a mesh of 50 µm. The obtained substrate is rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution Streptomycin 10 mg / L. The spores are then recovered in an Erlenmeyer flask with a little distilled water. AM fungi have been identified based on their morphological characteristics. In the end, the spores of mycorrhizal fungi were quantified to estimate their number in 100 g of soil (spore density). The species' frequency of occurrence (F%) is the percentage of a morphotype compared to other species.

$F\% = n_s \times 100 / n_T$.

ns: number of spores of the species X.

nT: Total number of spores.

The statistical treatment of results focused on the analysis of variance to one classification criterion (ANOVA1).

3. RESULTS

After 3 months of culture, microscopic observation reveals the presence of 28 species in the rhizosphere of maize with a spore density of 248 spores / 100 g of soil. *Acaulospora pustulata*, *Glomus etunicatum* and *Glomus clarum* are the dominated species (Figure 1).

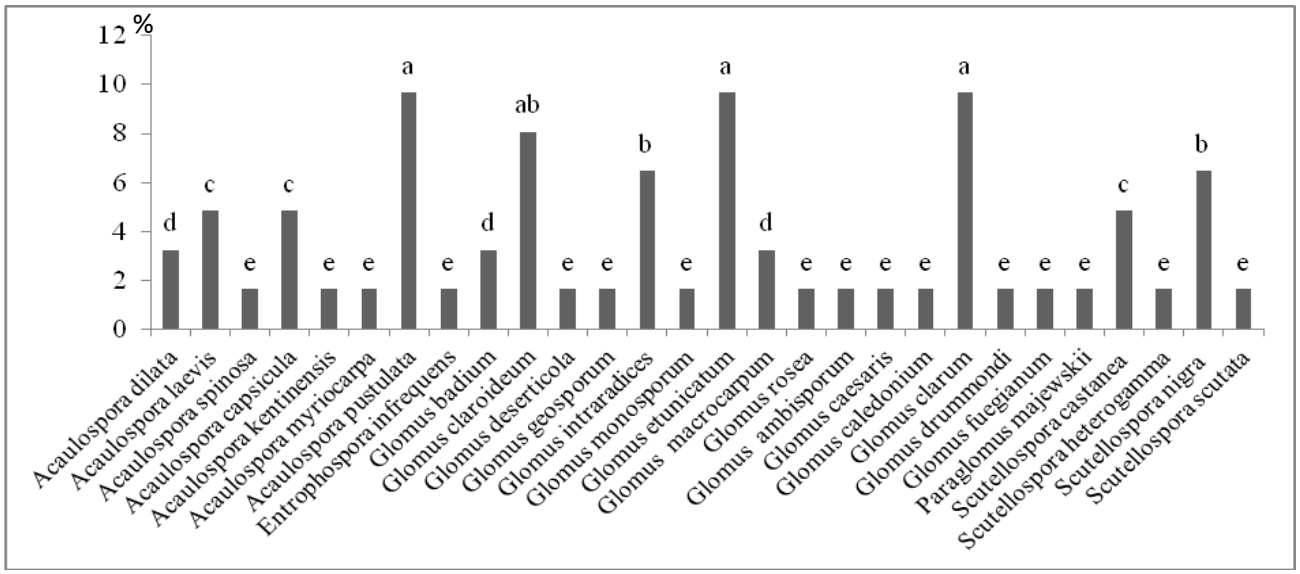

Figure 1. Occurrence frequency of mycorrhizal species isolated from the rhizosphere of maize plants after 3 months of culture with AM fungi.

Two results affected by the same letter are not significantly different at the 5% threshold according to ANOVA test.

Otherwise, 21 species are isolated from the rhizosphere of sorghum with a spore density of 200 spores / 100 g of soil, and *Scutellospora nigra* is the dominated species (Figure 2). Similarly, after 10 months of culture a total of 151 spores / 100 g of soil containing 26 different species are isolated from the rhizosphere of seedlings of the carob tree, with the dominance of *Glomus deserticola* and *G. clarum* (Figure 3).

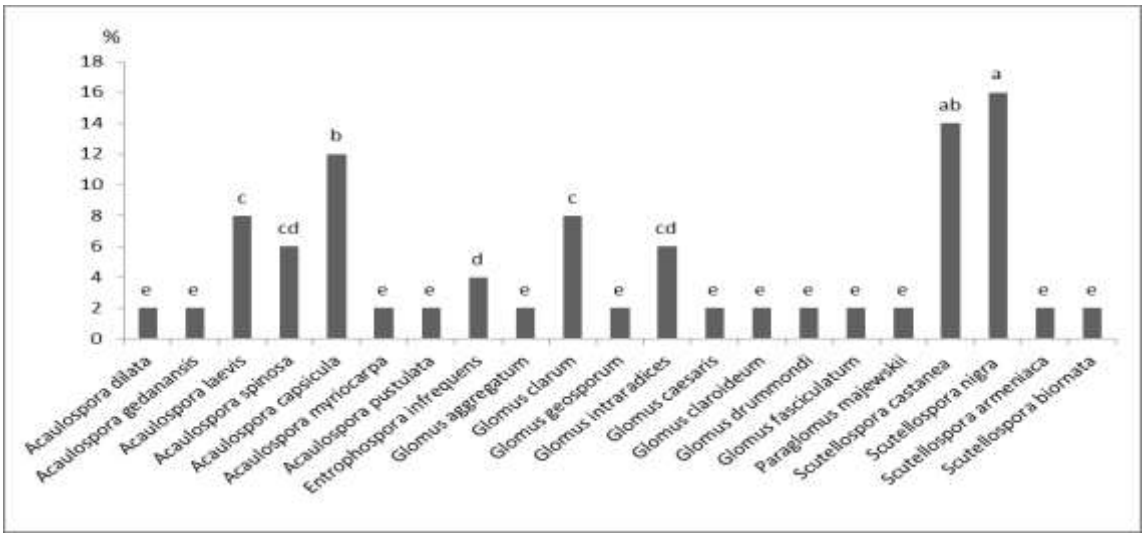

Figure 2. Occurrence frequency of mycorrhizal species isolated from the rhizosphere of sorghum plants after 3 months of culture with AM fungi.

Two results affected by the same letter are not significantly different at the 5% threshold according to ANOVA test.

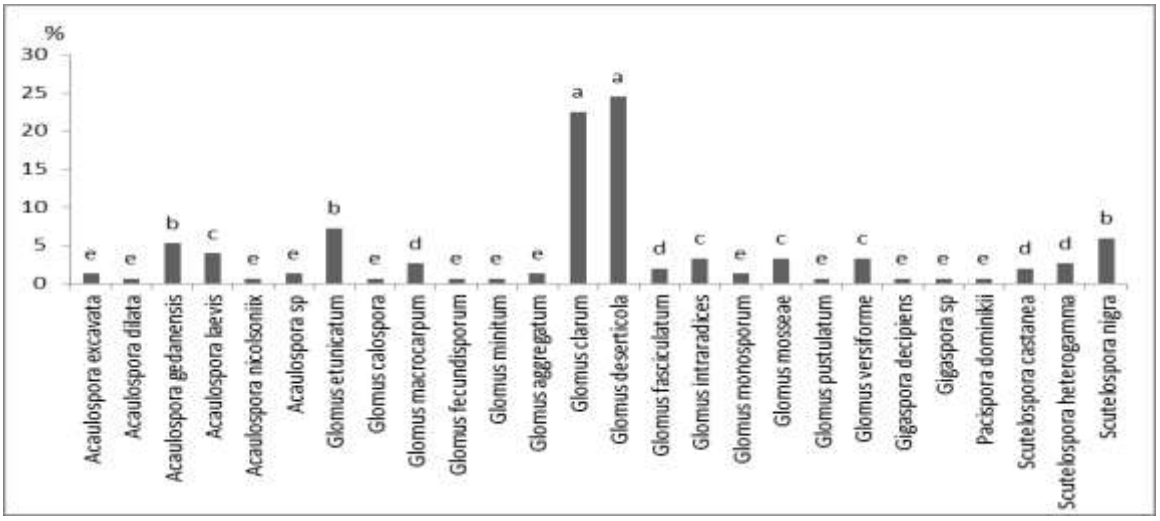

Figure 3: Occurrence frequency of mycorrhizal species isolated from the rhizosphere of carob plants after 10 months of inoculation with AM fungi
Two results affected by the same letter are not significantly different at the 5% threshold according to ANOVA test.

A comparison of different AM fungi species of the original inoculum (Figure 4) with those isolated from the rhizosphere of different mycotrophic plants, reveals the appearance of 26 species (*Acaulospora dilata, A. excavata, A. spinosa, A. capsicula, A. myriocarpa, A. nicolsoniix, A. pustulata, A. kentinensis, Glomus ambisporum, G. badium, G. caesaris, G. claroideum, G. caledonium, G. calospora, G. drummondi, G. fecundisporum, G. fuegianum, G. pustulatum, G. minitum, G. rosea, Gigaspora* sp, *Pacispora majewskii, P. dominikii, Scutelospora armeniaca, S. biornata, S. scutata*) and the disappearance of 11 mycorrhizal species (*Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, *A. denticulate, G. margarita, G. aureum, G. chimonobambusae, Glomus* sp.3, *Glomus* sp.4, *P. robiginia, S. fulgida*).

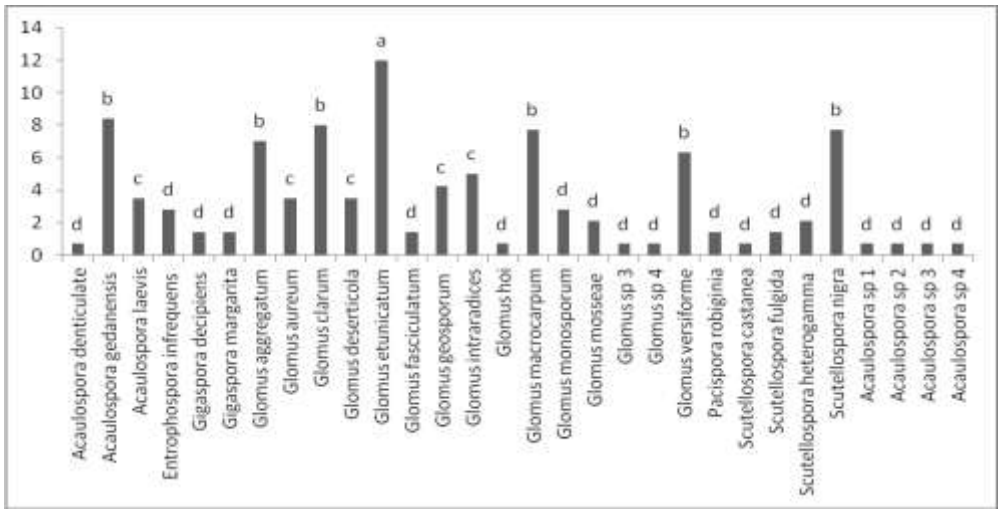

Figure 4: Occurrence frequency of mycorrhizal species, isolated from the rhizosphere of the carob tree.[1]

Figure 5. Some mycorrhizal fungi species isolated from the rhizosphere of maize, sorghum and carob. (A) *Acaulospora kentinensis*, (B) *Glomus macrocarpum*, (C) *Glomus deserticola*, (D) *Glomus geosporum*, (E) *Acaulospora capsicula*, (F) *Gigaspora rosea* (G) *Acaulospora spinosa* (H) *Glomus caesaris* and (I) *Glomus aggregatum*.

4. DISCUSSION

The analysis of mycorrhizal fungi spores communities found in the rhizosphere of mycotrophic plants studied shows the dominance of 5 species *Acaulospora pustulata, Glomus etunicatum, G. clarum, G. deserticola* and *Scutelospora nigra*. Sghir *et al.*[8] reported the dominance of *Glomus clarum* in the rhizosphere of the date palm. Other authors have reported the dominance of *Scutellospora nigra* at the rhizosphere of *Populus alba*[9], *Juncus maritimus*[10] and *Lycium europaeum.*[11] Chliyeh *et al.*[12] noted the dominance of *Glomus etunicatum*, at the rhizosphere of the olive tree. This species dominate also in the rhizosphere of argan tree[13] and plant species of the Brazil Atlantic Forest.[14]

The genus *Acaulospora*, *Gigaspora* and *Glomus* has already been observed in the Sudanese region of Burkina Faso, in the rhizosphere of *Acacia holosericea* and *A. mangion*[15] in the Moroccan coastal dune of Souss Massa[16], at the rhizosphere of *Eryngium maritimum* in Mehdia mobile dunes in Morocco[17], in argan soils[18] and in the rhizosphere of *Casuarina* sp. [19] in Morocco.

A comparison between AM fungi species of the initial inoculum and those isolated from the rhizosphere of different mycotrophic plants studied after multiplication and inoculation shows the appearance of 26 species and the disappearance of 11. Johnson *et al.*[20] found positive correlations between increasing organic matter (including elements such as carbon and nitrogen) and the diversity of Glomales. Recorded differences may be due to the physico-chemical and microbiological properties of soils[21,20,22], microclimate fluctuations[23,24], to covering vegetation[25] and sampling season.[26,27] According to Pearson and Schweiger[28], environmental conditions influence the interactions of fungi with plant roots that also affect indirectly the activity of reproduction.

Moreover, the host plant can genetically control development of mycorrhizal species.[29] This is consistent with the work of Sieverding[30] which reports that the host plant differently affects the development of AM fungi. However, information on specific association between AM fungi and host plants is rare and data are ambiguous. For example, Howeler *et al.*[31] observed that the leguminous plants can produce more AM fungal spores than grasses. On the contrary, Simpson and Daft [32] observed that the sporulation of *Glomus clarum* in the rhizosphere of sorghum and millet is higher than that of the groundnut and chick peas. Furthermore, some AM fungi species have a broad host range, while others were found only in the rhizosphere of a single host plant, as *Acaulospora splendida* at the rhizosphere of *Quercus costaricensis.*[30] It seems that each fungus has its own demand for photoassimilates.[28] Sorghum and maize seem to be less selective of the AM fungi species present in the inoculum, allowing a large proliferation of these organisms in their roots and promoting the development of a rich and varied community.[33] Similarly, sorghum is commonly used for the multiplication of AM fungal spores[34,35,36], many studies have shown that sorghum does not support good sporulation of some species, including *G. fasciculatum*[37] ,*G. macrocarpum, G. claroideum,* and *G. etunicatum*[38], *G. clarum.*[39] Our data contradict previous results concerning *G. fasciculatum, G. claroideum* and *G. etunicatum*, which are present at the rhizosphere of this host plant. Similarly, Carrenho *et*

al.[33] recorded the presence of *Glomus clarum* and *G. claroideum* at the rhizosphere of sorghum. Redmond *et al.*[40] noted that some flavonoids are exclusively produced by leguminous compounds that were often associated with the attraction of germinating tubes of *Glomus margarita* and *Glomus* spp.[41.42] at roots level. These compounds can promote root colonization by AM fungi, influencing the germination of spores and growth of germinating tubes.[33] Saif [43] and Howell *et al.*[31] noted that leguminous tend to favor sporulation of AM fungi more efficiently than grasses.

5. CONCLUSION

It appears that the use of different mycotrophic plant species influences the proliferation of mycorrhizal fungi. Indeed, from a composite inoculum containing 30 species we have observed that some species appear while others disappear in function of time. In the rhizosphere of maize, 28 species were encountered, with the dominance of three species: *Acaulospora pustulata*, *Glomus etunicatum* and *Glomus clarum*. In the rhizosphere of sorghum, 21 species were isolated, with predominance of *Scutellospora nigra*. Inoculation of Carob plants with an inoculum containing the species found in the rhizosphere of maize and sorghum plants was used to isolate, after 10 months of culture, 26 different species, with the dominance of *Glomus deserticola* and *G. clarum*. So it seems that over time each mycotrophic species promotes the growth and dominance of one or several mycorrhizal fungi species.

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