

Production Of Bioinput From Arbuscular Mycorrhiza For Agricultural Sustainability

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ABSTRACT

The objective of this research was to design a process for the production of bio-input from arbuscular mycorrhizal fungi for use in sustainable agricultural production. Practical investigations were developed for the isolation of spores from the soil, determination of the colonizing structures inside the root tissues and the field technique for the production of inoculant for use as a source of biofertilizers. The results show that soil and climatic conditions, plant type and time of year determine spore abundance and colonization effectiveness. A host plant plays a fundamental role for the fungus-plant interaction and the conditions and stages of the colonization process are important for the production of arbuscular mycorrhizal inoculants and their effectiveness in the field. Arbuscular mycorrhizal fungi are a native source of microorganisms as a source for bio-input production and environmentally sustainable agroforestry production.

Key words. Inoculums, microorganisms, biofertilizers, sustainability.

1. INTRODUCTION

According to (Roldán, 1987; Wood, 1992; Barea, 1997; Sánchez, 1999), more than 90% of the plant species on the planet are mycorrhizal when growing under natural conditions, and of these, in 95% of cases, the association corresponds to arbuscular mycorrhizae. It has been found that 97% of phanerogams, including almost all species of agronomic, pastoral and forest interest, have this type of mycorrhizae.

In this sense, as pointed out by (Guerrero, 1996; Van Der Heijden, 1998; Barea, Jeffries, 1999) arbuscular mycorrhizae are an important biological factor in the structure and functioning of soils, and influence the ecological behaviour, productivity and composition of natural plant communities, as well as agricultural crops and forest plantations. Arbuscular mycorrhizal fungi should therefore be considered as part of the biological diversity of soils and should be included both in inventories and in biodiversity analyses at ecosystem and agro-ecosystem level.

A root system can be colonized simultaneously by several species of fungi, and the same fungus can colonize roots of several plant species growing in close proximity. On the other hand, different species and even cultivars of the same species show different degrees of susceptibility to colonization. According to (Azcón and Barea, 1980; Morton, 1996; Sylvia, 1999) mycorrhizal structures are made up of: spores; hyphae (intracellular and extramatric), the former developing inter- or intracellularly in the cortical cells of the root, and the latter emerging from the root and extending through the soil several centimeters, giving rise to the mycelium which constitutes the nutrient absorption system; arbuscules and vesicles.

Similarly, different phases can be distinguished in the process of symbiosis formation: Pre-colonization, initial penetration of the fungus, intraradical colonization and development of the external mycelium and reproductive structures (Sanchez, 1999).

Based on the above, it was proposed to isolate arbuscular mycorrhizae from livestock soils in the department of Sucre, to determine the process of infection and multiplication as an alternative for obtaining bioinputs from these microorganisms.

2. MATERIALS AND METHODS

Sampling. In order to carry out the sampling or zones of interest, the information of the initial diagnosis of the area under study was taken into account, as well as the environmental and edaphic conditions, the diversity of plant species present and the agronomic management of the cultivated soil. Based on the initial diagnosis, samples were collected in the following way: A PVC plastic tube of 3.8 cm in diameter and 25 cm in length was used to take the samples at a depth of 0-20 cm, introducing, rotating and extracting the cylinder with the sample (soil and roots). In each farm, 15-20 samples were taken, these were homogenized per farm to form one sample with a weight of 2000 grams. Two subsamples (1000 g each) were used to determine the percentage of root colonization with arbuscular mycorrhizal fungi, and a subsample of 1000 g for the determination of soil physico-chemical parameters (Pérez and Vertel, 2010).

Spore isolation: Soil samples were sieved to separate the coarse parts of the soil (stones, gravel) and roots. Once sieved, physical-chemical analysis and isolation of spores of arbuscular mycorrhiza-forming fungi was carried out using the technique proposed by (Espitia and Pérez, 2016).

Colonization. This was carried out using the root staining technique. Roots stained by this technique were placed parallel on slides, covered with slides and observed with a 40X objective, and 100 fields were counted in an orderly manner. Negative and positive fields were determined for each field. In the positive fields, the type of structure present (arbuscules, vesicles, hyphae and spores) was taken into account. The percentage of infection in roots was calculated with the following formula proposed by (Pérez and Vertel 2010).

Multiplication of morphospecies. From the isolated morphospecies, those that were present repeatedly and with a higher proportion with respect to the others were selected for multiplication in trap cultures (pot culture) at greenhouse level using *Brachiaria decumbens* (Invam, 2023) as host species.

Assessment of the inoculum. After 120 days of inoculation of the morphospecies in the pot cultures, soil samples were taken to count spores and determine the percentage of colonization in the roots, taking into account the type of colonizing structures.

3. RESULT AND DISCUSSION

Sporulation. For the isolation of spores of arbuscular mycorrhizal fungi, the following scheme was developed as described in figure 1:

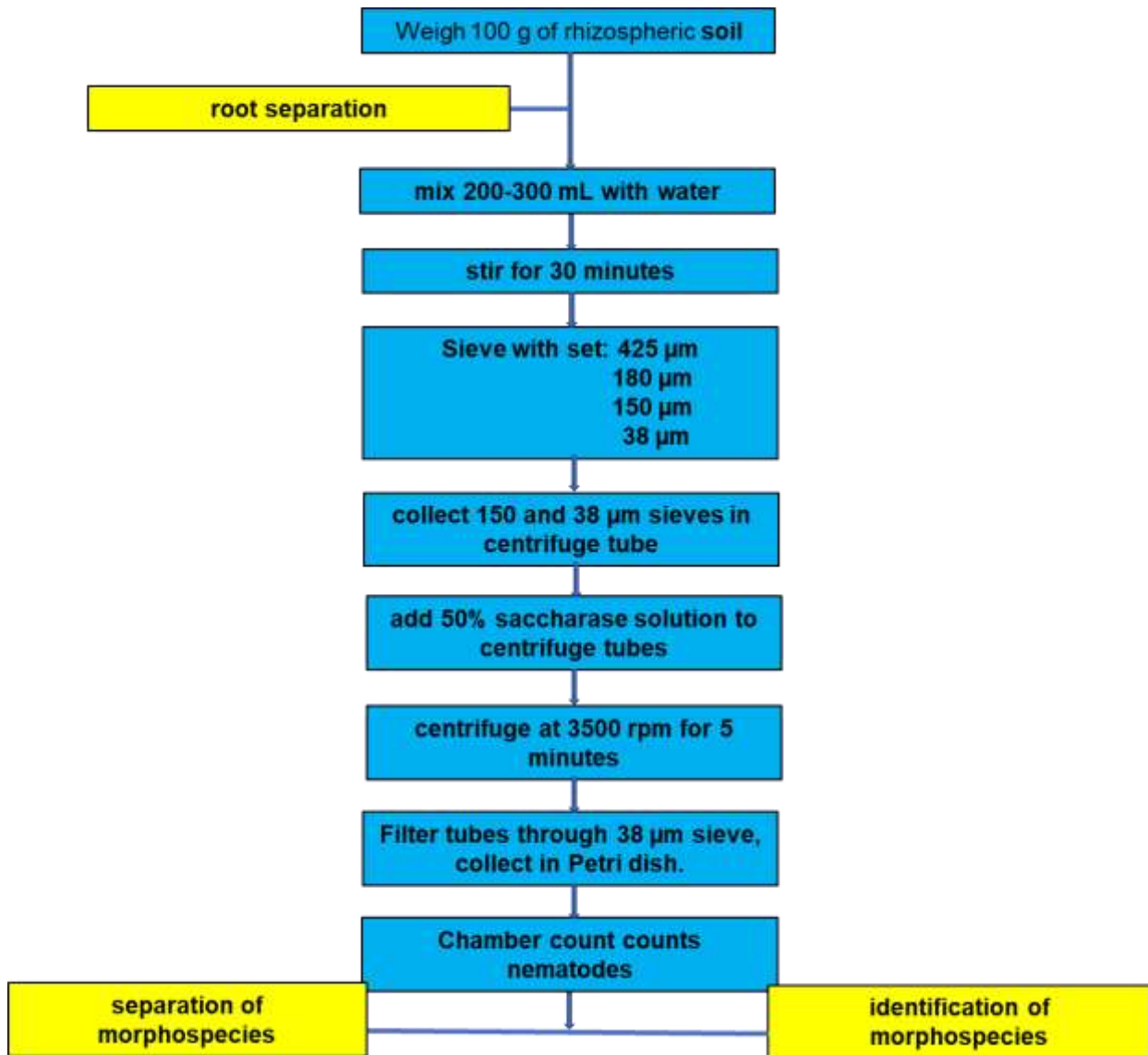


Figure 1. Procedure for isolation of spores of mycorrhiza-forming fungi from rhizosphere sample.

The abundance of spores will depend on the time of year, soil and agroclimatic conditions of the area, type or species of plants and the agronomic management of the crops under study. The effects of agronomic management on arbuscular mycorrhizae have not been extensively studied in the field. Different practices such as crop rotation, intercropping, intercropping, fertilization with composted organic residues, fresh, mulch, can positively affect the presence of native arbuscular mycorrhizal fungi.

There is an inverse relationship between the number of spore/g soil and the percentage of infection, depending on the time of sampling. In summer when plants are under stress conditions, the number of spores increases, while the percentage of infection decreases. Environmental conditions play an important role in the formation of infection-forming units. Water deficit stimulates spore production, which explains the higher number of spores in summer (Bonilla, 2000).

Colonization. The scheme for determining the percentage of colonization is shown in figure 2. There is no exact range of the ideal percentage for the colonization of arbuscular mycorrhizal fungi, but research suggests that a minimum of 25 % of the colonized roots is sufficient for the efficiency and functionality of the interaction between the plant and the fungus. The establishment of the symbiosis will depend on the interactions between the components of the system: the fungus, the plant and the environmental conditions.

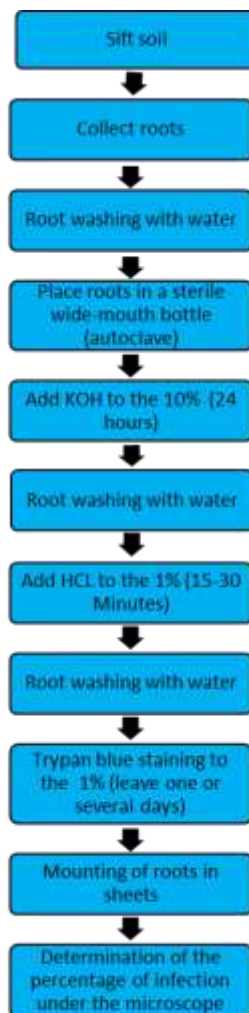


Figure 3. Schematic of the process to determine colonizing structures in roots by arbuscular mycorrhizal fungi.

Excessive soil water levels are said to reduce the growth and infection of arbuscular mycorrhizae. Poorly drained soils saturated for long periods decrease colonization, and low soil water levels decrease infection, stimulation of plant growth and spore production (Whetten and Anne, 1992).

The effectiveness of colonization of arbuscular mycorrhizal fungi under greenhouse or field conditions is determined by: soil physico-chemical conditions (pH, phosphorus content, temperature, aeration, texture and organic matter content), climatic conditions (light intensity and duration, temperatures, humidity, rainy and dry seasons) and by agronomic practices (soil preparation, pesticide application and cultural practices) (Gonzales, 1996).

A colonized root should have the following structures: spores, internal and extramatrix hyphae, vesicles as shown in figure 3.

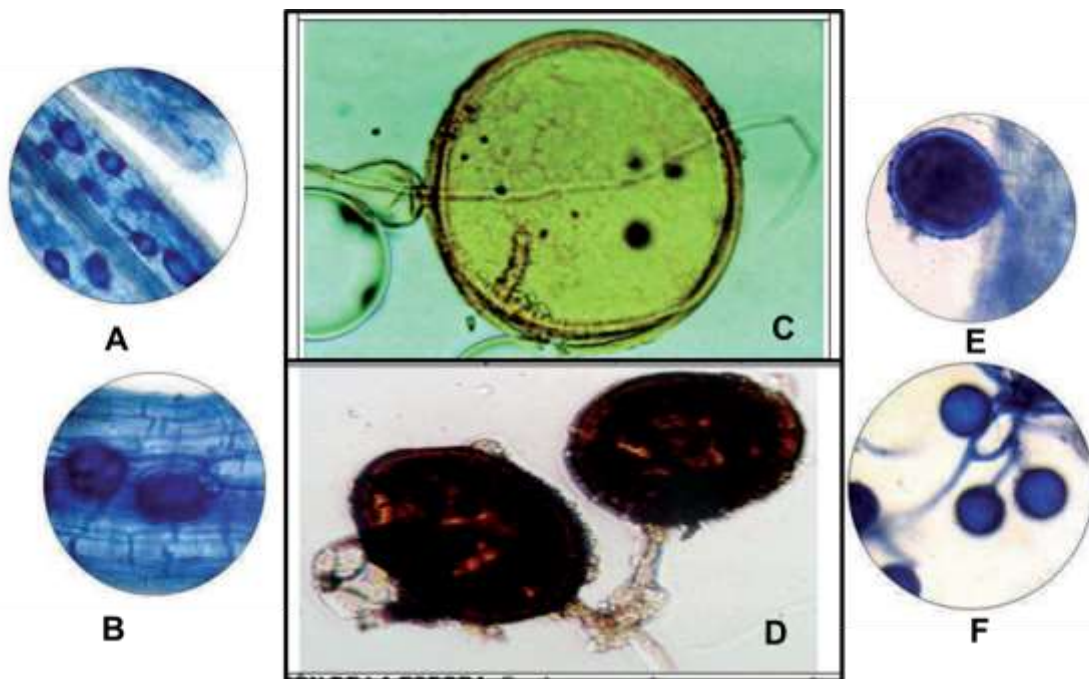


Figure 3. Root-colonizing structures of arbuscular mycorrhizal fungi. A: Vesicles; B: intercellular spores; C: Spores with hyphae; D: Spores with hyphal connections; E: colonizing spores and F: Spore with extramatrix hyphae.

Multiplication of arbuscular mycorrhizal fungi inoculum. For multiplication of a good arbuscular mycorrhizal inoculum, a host plant

(grass or legume) with a good root system, a short life cycle (120-140 days), and high foliage production should be selected. For multiplication, during isolation of the morphospecies, those with the greatest predominance, quantity and a good morphological state (spore + hyphal connections) should be selected. Once selected according to (shape, size, color), they should be kept in sterile water for a maximum of 24 hours, covered in an amber flask and inoculated onto the host plants once the seedlings have developed a root system (10 days after germination of the seedlings). For an excellent inoculum production process, the scheme as presented in figure 4 should be followed.

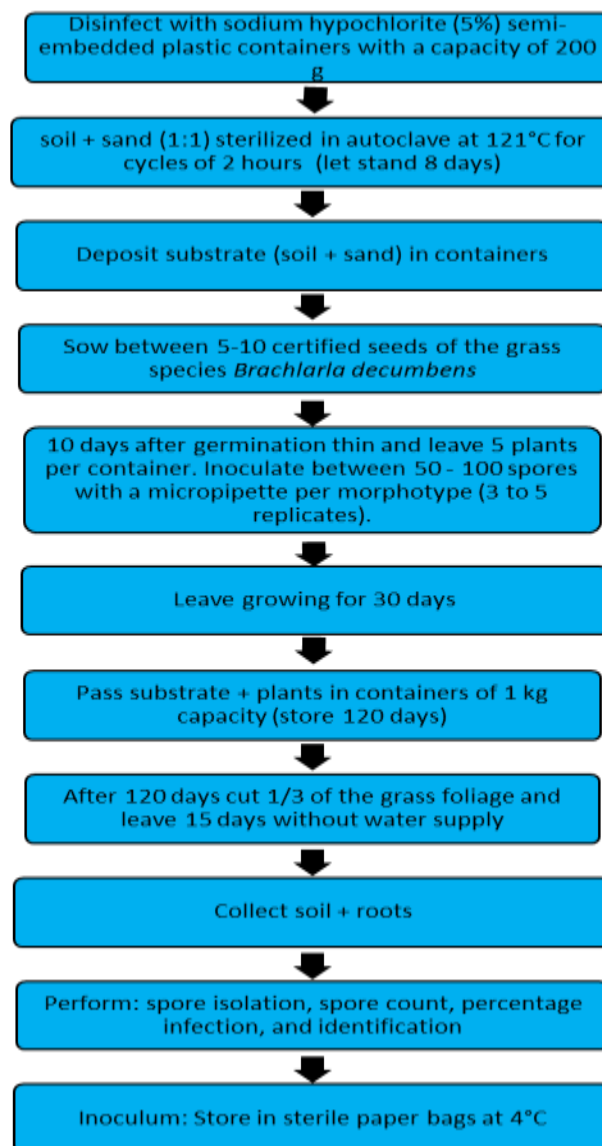


Figure 4. Schematic of the production process of arbuscular mycorrhizal fungi inoculants as a source of bio-input for agro-sustainable production.

A scheme for the bio-augmentation of morphospecies is shown in figure 5. The pot cultures for the multiplication of arbuscular mycorrhizal spores should be funnel-shaped so that the root system of the host plants has reduced space, stress occurs and organic compounds are released by the root system as autoinducers to stimulate the germination of the spores of the arbuscular mycorrhizal fungi and facilitate their plant-fungus interaction process and the respective colonization inside the root.



Figure 5. Bioaugmentation of spores of arbuscular mycorrhizal fungi in pot culture using the grass species *Brachiaria decumbens* as host plant,

"The fungal hyphae grow extensively from the root into the soil, where they develop a three-dimensional network of mycelium specialized in efficiently colonizing and exploring microhabitats to capture minerals and water. The outer hyphae of the fungus act as "bridges" over the nutrient "depletion" zone surrounding the root and reach distances of up to several centimeters from the root surface. The function of the external mycelium is critical for the uptake of poorly mobile nutrients such as phosphates, ammonium and some micronutrients (Azcón and Barea, 1996).

According to Menge and Timmer, 1991; Ferguson and Woodhead, 1991), studies of plant growth and development response in the field, inoculated with arbuscular mycorrhizae, are efficient when mycorrhizal soil is applied as a source of inoculum, because it is more efficient, has spores and infective propagules (external hyphae, fragments of mycorrhizal roots and spores).

The practical importance of arbuscular mycorrhizal fungi, as a microbiological resource of natural ecosystems in the tropics, is known to be an alternative that contributes to the development of sustainable production systems and provides benefits such as: increases the efficiency of the plant for the absorption, translocation and transfer of soil nutrients, especially those that diffuse slowly as phosphorus, ammonium, potassium, calcium and sulphur, also participates in the active transport of micronutrients such as Zn, Cu, B and Mo. Other essential plant micronutrients such as Fe and Mn are generally found in higher concentrations in mycorrhizal plants (Sieverding, 1984).

The quantification of the colonization of arbuscular mycorrhizae in the field depends not only on the location of the inoculum, but also on other aspects such as: the potential inoculum (effectiveness), the density of the inoculum (grams of soil, quantity of spores per unit mass or per area), the type of inoculum and the environmental conditions. There are different methods for inoculum addition in the field, the most commonly used forms being: pelleted seed, mixing the inoculum with the soil, placing the inoculum in a layer next to the seed, under the seed and pre-inoculation in seedbeds (Menge, J. A. and Timmer, 1991).

5. CONCLUSION

Soil micro-organisms such as arbuscular mycorrhizal fungi (AMF) represent the key link between plants and mineral nutrients in the soil. For this reason, in recent years the importance of these types of micro-organisms on farms has increased exponentially. This is radically transforming the fertilizer industry, with a major replacement of chemical products by biological products based on soil micro-organisms that have a beneficial relationship with more than 90% of the world's terrestrial vascular plants.

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7. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

8. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

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