Indian Journal of Science and Technology, *Vol 11(24), DOI: 10.17485/ijst/2018/v11i24/123893,* June *2018*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Effect of Inoculation with Mycorrhizal Fungi on the Quality of Leaves of *Aloe vera* (L) Burm F

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Abstract

Objective: To evaluate the use of mycorrhizal strains as biofertilizers on the quality of the leaves developed by the cultivation of Aloe (*Aloe vera* (L.) Burm. f.) intended as a raw material for the agro-production of gel. **Materials and Methods:** Tillers were selected from plantations located in the village of Capatarida, Falcon State (Venezuela), which were debugged and conditioned during 30 days under controlled conditions. The trial was established in the house of cultivation of Agricultural Microbiology Laboratory of the Faculty of Agronomy of light, the experimental design used was a completely randomized design with three treatments and 10 repetitions. T1: inoculation with *Funneliformis mosseae*, *Rhizophagus intraradices*: T2 and T3: absolute control. **Findings:** The response variables evaluated were the number of stalks (NP), thickness of penca (GP), tiller number (NH) and fresh biomass of stalks (BFP). The inoculation with the mycorrhiza *F. mosseae* strain favored the development and quality of the leaves developed by plants of aloe, appreciating that the inoculated plants showed the highest values for the variables evaluated, presenting itself as an option favorable to the sustainable diversification of sabileros producers, to ensure increase in yields and profitability, obtaining a product free of debris with biotechnology friendly to the environment. **Application/Improvements:** The results obtained in the investigation indicate the cultivation of *A. vera* through inoculation with mycorrhizal fungi on the quality of leaves can be achieved in terms of yields; it helps the cultivation of this species using biotechnology as a sustainable development strategy and improves the processes of clean production.

Keywords: *Aloe vera*, Effect of Inoculation, *Funneliformis mosseae*, Mycorrhizae, *Rhizophagus intraradices*

1. Introduction

The genus Aloe contains between 40 and 80% of resins, a 20% of aloin and 0.003% of protein and 0.003% of protein, and their medicinal value is due to its content of vitamins: A, C, E, B12, carotene, folic acid, niacin, riboflavin, thiamine and minerals: calcium, magnesium, potassium, sodium, iron and aluminum[1,2]. Currently, there is an unmet demand for *A. vera*, especially in the industrially developed countries, which have a high level of buying. However, there is a lack of information on the management and agricultural production to meet the industrial processes and national and international marketing for which we must develop technological packages to ensure crops without problems phytopathological, good crops of stalks and residue-free, ensuring sustainable development of the crop. Because the external market for this crop demand a totally natural product, without chemical additives, such as inorganic fertilizers and pesticides, which means that must occur in an ecological and conservation of the environment, ensuring that they comply with the standards of quality[3].

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In the field of standardization and regulation of the manufacture and trade of *A. vera*, is clearly must meet the requirements set forth by the International Aloe Science Council (IASC), which is the governing body, noting that the handling of the crop should be organic in a sustainable system without the use of toxic products of synthesis. In addition, the culture must undergo a rigorous process of inspection and certification by international entities[4]. To comply with this regulation established it is necessary the implementation of successful strategies of agronomic management, which involves the use of biological products and inputs, formulated on the basis of microorganisms improve the availability of nutrients, protect the environment, and preserve the soil from the point of view of soil fertility and biodiversity[5].

An alternative of organic management for the cultivation of *A. vera* is the use of bioproducts such as mycorrhizal fungi and plant growth-promoting bacteria, these are the mainstays in sustainable agriculture, and at the present time, commercial production has grown considerably at the global level[5]. Due to the above, with the help of technologies these crops can be enhanced as an alternative of sustainable diversification, to ensure increases in yields and profitability of the system, obtaining a product free of waste[4].

2. Materials and Methods

The research was conducted in the house of cultivation of the Plant Technical Unit of the Faculty of Agronomy of the University of Zulia. The town is classified as an area of very dry tropical forest, with a soil moisture regime of aridico[6] with average annual rainfall of 600 mm, average annual temperature of 28.1°C, average annual evapotranspiration of 1800 mm, average relative humidity of 88%. The mechanical Analysis of the physico-chemical characteristics of the soil for the purpose of measuring the fertility are described in the Table 1.

The mycorrhizal inoculum was obtained from the Venezuelan Institute for Scientific Research (IVIC), in a presentation bioinoculante of mycorrhizal fungi (*F. mosseae* and *R. intraradices*). Which were cultured in the laboratory of Microbiology, Faculty of Agriculture, University of the Western Cape, by means of the cultivation technique trap[7]. The material was selected from the production unit AGROCUIMAR, C. A., a member of the consortium marketer of *A. Vera*. The plantations

Table 1. Mechanical analysis of the physico-chemical characteristics of the soil for the purpose of measuring the fertility

Variable	Value
Sand (%)	72,5
Sail (%)	15.0
Clay (%)	12.5
Texture	Fa
Fósphorus(meq/100g)	0.32
Potassium (meq/100g)	0.18
Calcium (meq/100g)	0.61
Nitrogen(meq/100g)	0.27
Organicmatter(%)	0.79
pH 1:2,5 Soil: Water	5.3
C.E. 1:5 $dS{\cdot}m^{-1}$to 25 °C	0.33

of *A. vera* in AGROCUIMAR, C. A., Capatarida, Falcon State (Venezuela) are described in Figure 1, which did not have more than 4 years of established, allowing genetic guarantee the power tillers for its development. Tillers were selected with a height of between 25 to 30 cm from the insertion to the stem toward the apex of the leaves, with 8-9 leaves of firm consistency, without visual symptoms of disease, light green in color, with absence of spots and rotten smell in the root system.

In order to ensure the uniformity of plant material to establish in the field, it is preceded to the conditioning of the cuttings. First of all, we proceeded to the disinfection of the selected material, through the immersion of the cuttings in a solution of sodium hypochlorite at 10%,

Figure 1. Plantations of Aloe vera in AGROCUIMAR, C.A., Capatarida, Falcon State (Venezuela).

Table 2. Definition of the treatments applied in research

Treatment	Description
T1	Micorrhizal plants with *Funneliformismosseae*
T2	Micorrhizal plants with *Rhizophagusintrarradices*
T3	Micorrhizal plants with *Funneliformismosseae* and *Rhizophagusintrarradices*
T4	Uninoculatedplants

for a period of 3 to 5 minutes, by removing the excess and placing it in the open air for 30 minutes. After the disinfection, the cuttings were conditioned by placing them in bags of polystyrene using 2 kg of sterile substrate for a period of one month, so that the cuttings will present a good uniformity at the time of establishing them in field. The cuttings were selected that presented the following parameters: height between 29 to 30 cm established from the insertion to the stem toward the apex of the leaves, with 8-9 leaves, thickness of 1.2-1.5 cm, a length of 24-25 cm with a total fresh weight per plant of 520-530g, they were then transferred to field for your establishment.

Prior to the planting, the cuttings are disinfected through immersion in a solution of sodium hypochlorite at 10%, for 3 to 5 minutes. After removing the excess disinfectant they were airdried for 30 minutes. They were later placed in bags of polystyrene using 3 kg of sterile substrate. The stakes are distributed according to the appropriate treatments for the inoculation. These were placed at the time of planting 30•g plant-1 of the inoculum *F. mosseae* and *R. intraradices*. Were planted and covered in such a way that press and signed on the substrate of the bag, irrigated with a frequency of three days. To carry out the design of the evaluation of a numerical scale of 1 to 5 according to the Pantone color scale, where 1 is the worst color and 5 is the optimum color. Table 3 describes the treatments applied during this investigation, where the scale of Pantone allows you to identify the color of the leaves of *A. vera*.

The indicator variables of development of the crop of *A. vera* are the following: The total number of stems, leaves per plant, thickness of the sheet. Averaged values for each area evaluated, the length of leaves of the three plants representing the experimental unit. The count of the number of suckers, 3 plants were accounted for the experimental unit. The color of leaves was determined through the use of a scale of Pantone colors considering the leaves number 1-5 of three plants that accounted

Table 3. Pantone color reference scale to identify the color of the leaves of *A. vera*

Scale	Compositionof thecolor	%	Color (Pantone)
1	Yellow	4.7	
	Ref. blue	1.6	
	Trans. Wt	93.7	
2	Yellow	9.4	
	Ref. blue	3.1	
	Trans. Wt	87.5	
3	Yellow	18.7	
	Ref. blue	6.3	
	Trans. Wt	75.0	
4	Yellow	75.0	
	Ref. blue	25.0	
5	Yellow	70.6	

for the experimental unit, evaluating from the apex to the base[8]. To carry out the design of the evaluation of a numerical scale of 1 to 5 according to the Pantone color scale, where 1 is the worst color and 5 is the optimum color. Table 3 describes the treatments applied during this research. Fresh biomass of the leaves was determined from the pads 1, 3 and 5 for 3 plants that accounted for the experimental unit, which are weighed individually. To this end, we used a digital scale.

The percent colonization of the roots was determined by the method[9] where the roots free of soil were placed in sterile Petri Dishes, to which added 10% KOH to cover them, warmed during 10 minutes, then the roots were washed with sterile distilled water. Once bleached, the samples were treated with 10% HCl to for five minutes (acidification) and added finally triptanotenirlas with blue to 0.05 per cent in lactoglicerol, heating it for 10 minutes and kept in lactoglicerol for microscopic observation. 10 were selected segments of 1 cm and then placed in the glove compartment to be seen in the light in an optical microscope. Through this procedure, it was determined the number of hyphae, vesicles and/or arbuscules. In the same way two observations by segment and vinyl, with the slide using the equation of the infected was McGonigley segment, on a scale of 1-10, regardless of the intensity of the mycorrhization total of each segment.

$$\text{Percentage of colonization} = \frac{Numberofsegmentscolonized}{Totalnumberofsegments} \times 100$$

The experiment was established through a completely randomized experimental design with six treatments, the experimental unit was represented by a bag of 3 kg with the *A. vera* plant, using 10 plants per treatment. For those factors that were significant tests were performed using the Tukey method for the above procedures were used in the statistical package SAS Statistical Analysis Systems.

3. Results and Discussion

The analysis of variance in the number of stems showed significant differences for treatments of the plants were inoculated with a gain of 21% (2 sheets) on control plants. In relation to the new leaves, which were studied between 45 and 50 days after transplantation (Figure 2). The analysis of variance detected no significant differences in relation to the thickness of the sheets for the treatments involved with the inoculation with the mycorrhizal fungus (T1 and T2), with values of 1.89 and 1.73 cm, Figure 3 describes the thickness of the leaves of plants inoculated with *F. mosseae* and *R. intraradices*.

The analysis of variance detected no significant differences in relation to the variable thickness of leaf of the aloe plants for the analysis of variance detected no significant differences in relation to the variable thickness of leaves of the aloe plants for the treatments involved with the inoculation (T1, T2 and T3), but not with the control plants, being the mycorrhizal plants coinoculated (T3) the plants that developed greater thickness of the stalk with values of 1.73 cm, followed by the plants corresponding to the treatments T1 and T2, with values of 1.79 and 1.65 cm. significant, there are differences with control plants showed values below 1.46 cm, indicating levels of differences of 18% of gain of blade thickness of *A. vera* inoculated with mycorrhizal fungi.

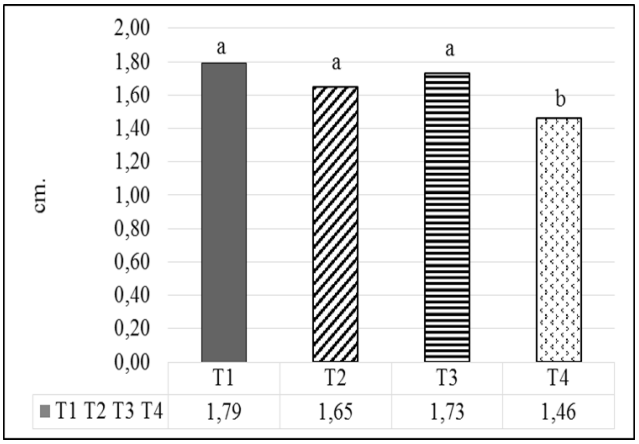

Figure 3. Analysis of the measurements for the thickness of the leaves of plants inoculated with *F. mosseae* and *R. intraradices.*

The increase in the leaf thickness can be attributed to the mycorrhizal symbiosis significantly improves the absorption of minerals from the soil, as well as hormonal stimulation, increasing the metabolic activity of radical and is likely to increase the synthesis of phytohormones in the roots, where it is carried out this process by selecting the cell division and consequently the growth of different organs of the plant, as the leaves[10].With regard to the length of the leaves, there were no significant differences; the plants inoculated with arbuscular mycorrhizal fungi showed the highest values in the length of the stem. These results describe an increase of 12%, Figure 4 describes the means of the variable length of the leaves of *A. vera* inoculated inoculated with *F. mosseae* and *R. intraradices.*

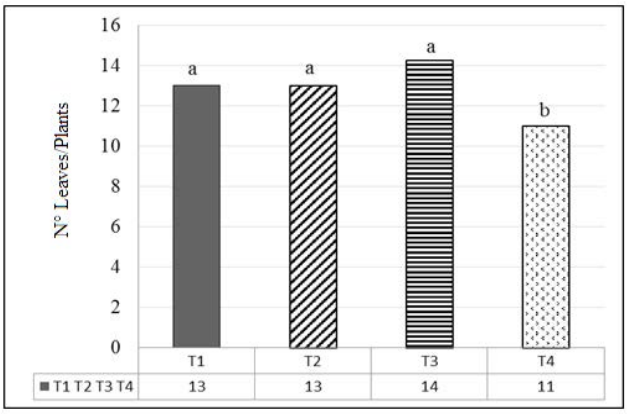

Figure 2. Analysis of the stems developed in plants of *A.vera*inoculated with *F. mosseae* and *R. intraradices.*

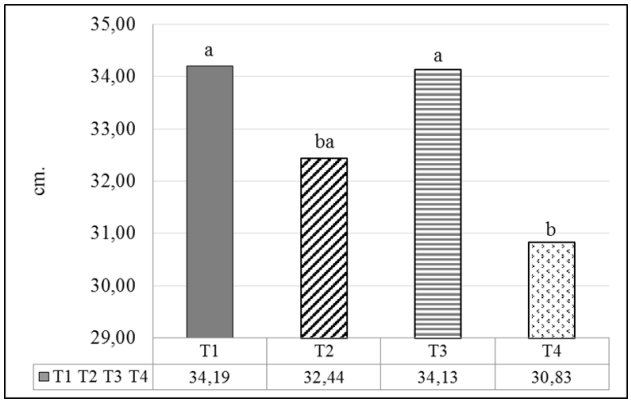

Figure 4. Means of the variable length of the leaves of *A. vera* inoculated inoculated with *F. mosseae* and *R. intraradices.*

Inoculation with arbuscular mycorrhizal fungi with increases in the thickness of the leaves, the most notable being in the parenchyma, and it has also been pointed out that the structural and physiological changes observed in these plants cannot be attributed only to the absorption of water and minerals[11]. These observations indicate that the plant in question presents certain physiological dependence of the mycorrhiza, as occurs in other plant species, mainly in the early stages of development[12].

The length of leaves, there were no significant differences, being the plants corresponding to the Inoculation with arbuscular mycorrhizal fungi and the combination of inocula which showed the highest values of penca length being in the same group of media with values of 34.19 and 34.13 cm respectively, followed by the plants of the treatment T2 with a length of penca of 32.44 cm and the control plants of 30.83 cm. The leaves showed a coloration that ranged from the number 3-5 of the scale you set to estimate this variable. The frequency of analysis revealed that 75% of the leaves of plants with treatment T1 and T3 reached the valuation of 5, 17 per cent in the scale 4 according to Pantone (Table 3), unlike the leaves of the treatment T2 inoculated with R. intrarradices, plants of the control, achieved a rating of 3 and 4, with 75 and 25%, respectively, of the scale of Pantone, the control plants showed a greater inclination to colors with less composition of green in their leaves, reaching 75 per cent in the scale 3. The color of the leaves of Aloe vera inoculated with *F. mosseae* and *R. intraradices* are described in Figure 5.

The analysis of variance was performed at leaves fresh biomass variables, showed no significant differences between the aloe plants inoculated and control plants. For the fresh stems the greatest value is given in the treatments T1 and T3 corresponding to the inoculated plants with a weight between 128.16 and 138.92 gr respectively, and a difference of 7%, followed by the plants of the treatment T2, with values of 114.17 gr, followed by control plants with values of 98.81 gr and a difference of 28% profit in the production of biomass with respect to the inoculated treatments T1 and T3. The averages of the variables of fresh biomass of *A. vera* inoculated with *F. mosseae* and *R. intraradices* described in Figure 6.

The analysis of variance was performed with data on the percentage of mycorrhization, which showed no significant differences. In Figure 7 describes the percentage of colonization of roots of *A. vera* inoculated with *F. mosseae* and *R. intrarradices*. The test showed the high-

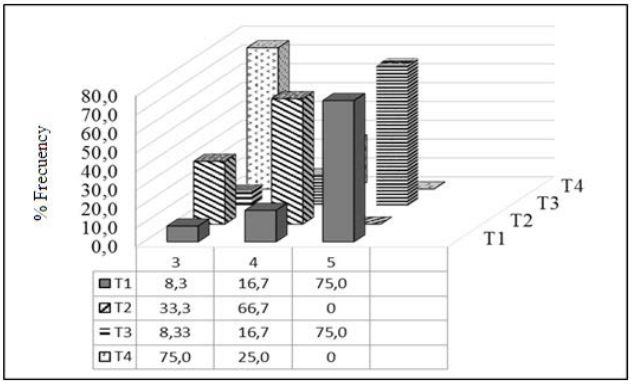

Figure 5. Color of leaves of *Aloe vera* inoculated with *F. mosseae* and *R. intraradices.*

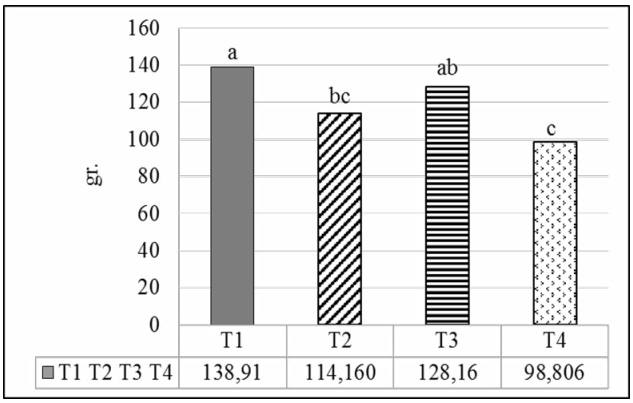

Figure 6. Description of the means for fresh biomass of *A. vera* leaves inoculated with *F. mosseae* and *R. intraradices.*

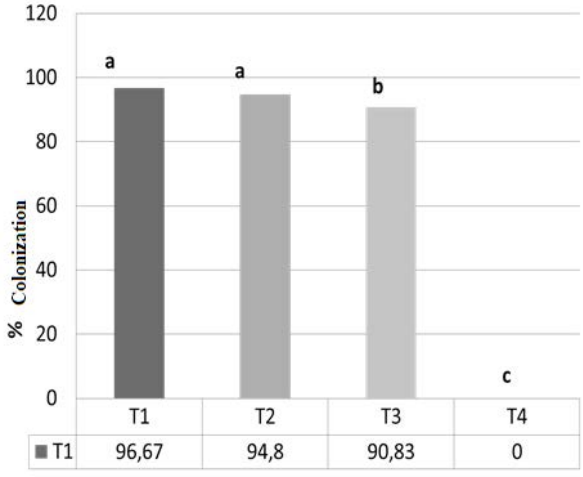

Figure 7. *Percentage of colonization of roots of A. vera inoculated with F.mosseae and R. Intrarradices.*

est levels of infection, with values of 96.67 %, in plants inoculated with the arbuscular *F. mosseae*, followed by those plants with both coinoculated arbuscular mycorrhizal inoculants, where there was a slight decrease in the levels of colonization of the fungus in the roots of *A. vera*, observing the T3 treatment with values of 90.83 %, with a difference of 6% between the two treatments[13]. The combination of inocula did not interfere with the ability infectivity and aggressiveness of mycorrhizal fungi to colonize the tissues of the plant. As well, it has been pointed out that there are groups of mycorrhizae in need of other microorganisms synergistic to potentiate the inoculum applied and favor the mycorrhization, guaranteeing the infectivity and effectiveness at the time of the establishment in the field[14].

Root segments were completely overrun by mycelium inter and intracellular and high percentage vesicular, in a mycelial extension level of 100% of the root segments observed. Despite the large percentage of colonization shown by this fungus; the presence of arbuscules was very low, while the vesicles were presented at a large number per segment, coming to occupy large root volume of hair, 40 vesicles by radical segment observed. Figure 8 shows tissues of plants of *A. vera* mycorrhized after the 90 days established in the field.

4. Conclusion

The *A. Vera* plants responded significantly to the inoculation with the mycorrhiza *F. mosseae* and *R. intraradices*, which favored the development of the plants of *A. vera*, with differences of 12 to 21% in length, thickness and number of stalks with respect to non-inoculated plants. We observed a significant improvement of the multiplication of the aloe plants, stimulating the induction of an 83%. Development of suckers above the values obtained in the sabilas plants witnesses without inoculation.The colonization combined *F.mosseae* and Azotobacter in the root system of the aloe reflected a significant increase of 28 to 40 % in the accumulation of fresh biomass and 12 to 30 % in dry biomass of the aerial part. It is necessary to consider the isolation and characterization of the strains of mycorrhizal fungi native to the areas where it has established plantings of *A. vera*, in order to assess the response of the crop and the ability of colonization and the effectiveness as a possible source of inoculum for the preparation of sabileras biofertilizers for plantations. As well as continue the evaluation of the variables studied up to the stage of crop harvest aloe so as to measure the trend in the behavior of plants with the mycorrhizal biofertil-

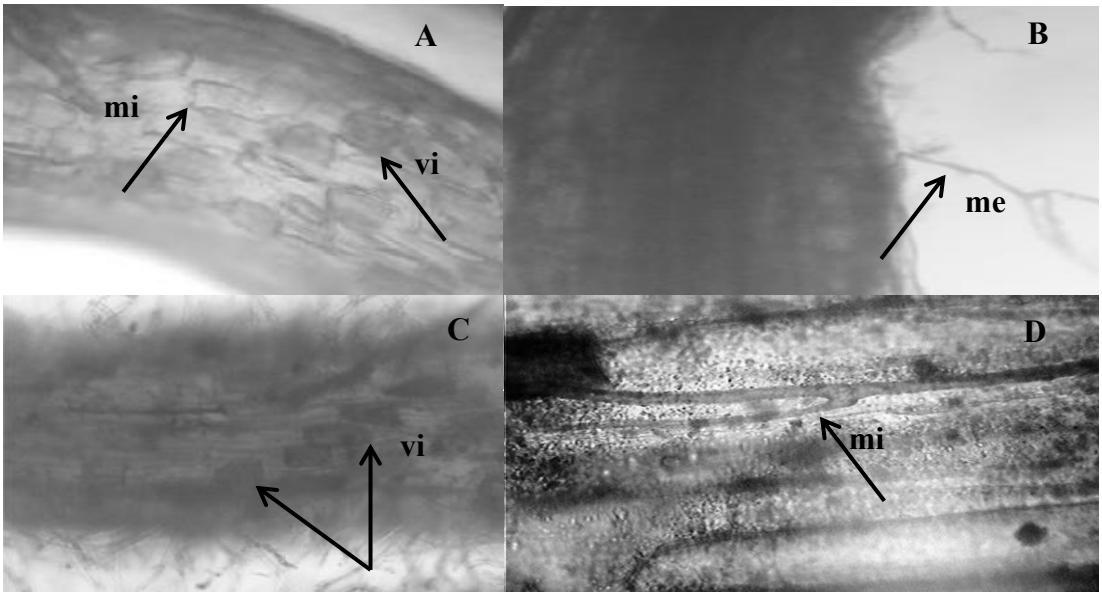

Figure 8. Micorrhizalcolonization of the fungus in the tissues of plants of aloe of 90 days established in the field. **(a)** Presence of vesicles and intracellular mycelium (vi, mi). **(b)** Development of extra-radical mycelium (me). **(c)** and **(d)** Intra-and inter-radical mycelium. 40X

ization on the yield and quality of the gel produced in plants of sabilas inoculated.

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