DETERMINATION OF THE PRESENCE OF MYCORRHIZAL FUNGI IN CHIA (SALVIA HISPÁNICA L.) IN TWO LOCALITIES OF PARAGUAY.

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Manuscript Info

Abstract
As an agricultural crop, Chia (Salvia hispanica L.) has an incipient importance in Paraguay and the presence of mycorrhizal fungi is a symbiotic association of high relevance for the crop. Experiments were carried out in two areas belonging to the departments of Itapúa and Misiones respectively. The study established the objective of determining the presence of mycorrhizal fungi in chia. Sowing was carried out in experimental trials in the above mentioned locations. Root fragments were extracted from the experimental units from 0 to 10 cm deep; they were taken to the Science and Technology Lab of the State University of Itapúa for the determination of the structures. It was possible to confirm the presence of arbuscules and hyphae in the analyzed samples corresponding to the environments involved, the structures were assumed to be similar, taking as variable the vesicles, the averages registered in the departments were statistically similar; indicating that the presence of mycorrhizal structures are the same in both environments.

Introduction:
Chia (Salvia hispanica L.) is an annual and summer herbaceous plant that belongs to the family Lamiaceae. Its origin goes back to 3500 years BC, and was known by the pre-Columbian cultures of Mexico and Guatemala (Rovati et al., S.f).

It is a species that is of fundamental importance because it is intended for human consumption, in Paraguay it is a crop that has been incorporated into agricultural production as an important option for producers in the sector.

Chia seed has a high nutritional value, due to the content of polyunsaturated fatty acids, fibers, proteins and antioxidants, being advantageous when compared to other vegetable sources (Jaramillo 2013).

On the other hand, mycorrhizal fungi are obligatory biotrophs and belong to the phylum Glomeromycota (Schübler et al., 2001). They are characterized by being symbiotic associates with plant roots (Helgason and Fitter 2005). These fungi are functional groups and can contribute to the sustainability of agricultural production ecosystems (Gianinazzi et al., 2010).
The symbiosis between the fungi and the roots of the plants is related to the mineral nutrition of the same, through the potential for the acquisition of soil phosphates by means of the branched extra-radicular hyphae of the fungi (Whipps 2004).

When associated with the roots, Mycorrhizal fungi, form structures that are known as arbuscules and vesicles. The arbuscules are structures of the haustoria type, that are generated inside the cortical cells and whose role is to contribute to the increase of the capacity of absorption and use of nutrients for the plant and for the fungus. On the other hand, the vesicles have the function of storing reserves for the fungus, used in situations of energy limitation for the growth of these. Both structures are originated by intra and intermatrical mycelia, whose characteristics are to translocate the polyphosphate granules to the places where phosphorus is, demanded (Bolan 1991).

The use of arbuscular mycorrhizal fungi (AMF) in agriculture contributes to improve the nutritional level of the plant, however, the condition of monoculture in the agroecosystems, may be causing a decrease in AMF diversity and as a consequence, these microorganisms could be providing an effect, although beneficial, limited to the hosts (Barrer 2009).

Assuming the importance of the association of fungi and plant species, the role played by these in the degradation processes of organic matter is highlighted; facilitating the decomposition of organic waste and the incorporation of nutrients into the soil.

In order to determine the presence of mycorrhizal fungi in chia crops in Paraguay, research work was carried out in two departments belonging to the country; being these Itapúa and Misiones.

Materials and Methods:-
The investigation was carried out in the departments of Itapúa and Misiones, Paraguay in two agricultural years between 2016 and 2017.

Experiments were planted in two locations to obtain the samples for the determination of the presence of mycorrhizal fungi in the chia crop.

The experimental design used was of the complete block type at random, three repetitions.

Sampling of the roots of the chia was carried out in both localities, soil samples were extracted with roots of each experimental unit at a depth of 0 to 10 cm according to the proposal by Sánchez et al. (2010). In the laboratory, the roots of the soil were separated and subsequently washed with distilled water, each sample was taken with 3 g of fresh weight root, cut into segments of 1 to 2 cm, placed in test tubes, covered with a 10% KOH solution and taken to a thermostatic bath at 90 °C for 15 minutes, after which time the KOH was removed and rinsed with water. Then the roots were allowed to stand with an HCl solution for 10 minutes at room temperature. At the end of that time they were washed with water and dried with paper. Dye (mixture of: lactic acid: 325 ml, glycerol: 300 ml, distilled water: 400 ml and trypan blue: 0.65 g) was added, then the roots were placed in the thermostatic bath for 20 minutes at 90 °C, the dye and the excess was washed with plenty of water. Finally, the roots were transferred to a solution of lactic acid and glycerol for conservation until microscopic observation using the method proposed by Phillips and Hayman (1970) modified by Furlan and Fortin (1977) and Macedo and Ferrera (1981).

For microscopic observation, the roots were cut into segments of 1 to 2 cm in length, 10 to 20 segments were placed in a slide between the portal and object cover, three equidistant passages per segment were made, the presence of a mycorrhizal structure was took as a value one, its absence was rated with zero value to obtain the% mycorrhizal colonization of the roots observed, considering the method of Giovannetti and Mosse (1980).

Results and Discussion:-
The results indicate the presence of mycorrhizal fungi in both regions, the amounts being similar to each other as shown (Table 1). It is inferred that the chia culture is colonized in both environments in a similar way p value = 0.96. However, the evaluated structures presented a significant statistical difference, observing that the hyphae and arbuscules were close in presence, showing the difference at the level of the vesicular structure.
Table 1: Average percentage of colonization of mycorrhizal fungal structures in two regions of Paraguay.

| Locality | Structure   | Averages |
|----------|-------------|----------|
|          | Vesícula    | Arbusculos | Hifas |          |
| Misiones | 0.5         | 63       | 62    | 42       |
| Itapúa   | 1           | 63       | 63    | 42       |
| Averages | 0.75 a      | 63 b     | 63 b  | 42       |

Valor p structure <0.0001
Valor p locality 0.9667

Different letters indicate significant differences (p <= 0.05)

According to the experimental results, it was found that mycorrhizal fungi make up a natural population in the evaluated environments, chia is a crop that has a symbiotic association with these fungi in a natural way coinciding with what was expressed by Helgason and Fitter (2005). Also considering the proposal by Barrer (2009) can associate the results obtained in this experiment with the aforementioned author that arbuscular mycorrhizal fungi can improve the nutritional level of plants in this case of chia cultivation and absorption of phosphorus of the floors. From the obtained results, it can be inferred that chia can absorb phosphate, a highly immobile ion in the soil. The presence of mycorrhizal fungi helps crops for this physiological function of plants recognized as absorption of mineral nutrients in this case phosphorus, this agrees with what is expressed by Whipp (2004).

Bolan (1991) mentions that the presence of the arbuscular structure of the mycorrhizal fungi contributes to the increase of the capacity of absorption and utilization of the nutrients, what expressed by the aforementioned author can be related in a direct way to what was obtained in this experiment where found the presence of arbuscular structures of mycorrhizal fungi in Chia in the departments of Itapúa and Misiones of Paraguay.

Conclusion:
Chia is a crop that has an association with mycorrhizal fungi in the departments of Itapúa and Misiones, Paraguay. The arrangement of arbuscular and hyphal structures were confirmed, showing that this crop can naturally absorb the phosphor mineral element from the soil where the experiments were carried out. Therefore, this study indicates that the mycorrhizal fungi found can be constituted as an ecosystemic service of provision of high relevance in Chia crops in Paraguay.

It is important to bear in mind that mycorrhizae are not only recognized for their contribution to phosphorus nutrition, but also provide other benefits such as protection against pathogens, tolerance to water stress, nutrition of micronutrients, tolerance to toxicity with heavy metals and secretion of substances that contribute to the maintenance of the soil structure.

Gratitude
To the Consejo Nacional de Ciencias y Tecnologías (CONACYT) for the scholarship

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