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Full Length Research Paper

Biofertilization With *Glomus intraradices* and *Azospirillum brasilense* to Improve the Quality of Papayo Plantule.

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The replacement of mineral fertilizer in papaya crop with biofertilizer based on *Glomus* spp. and *Azospirillum* spp., can to increase the productivity in this crop, moreover to improve soil conditions. The aim of the study was to determine the quality of papayas seedling, variety "Red Maradol", obtained with the use of *Glomus intraradices* and *Azospirillum brasilense*, as biofertilizers for production of plants.The experiment was developed at greenhouse of High Study School of Xalostoc, Morelos. Was used a completely randomized design, with eight treatment and five repetitions: *G. intraradices* along (*GI*), *A. brasilense* along (*Az*), Fertilizer 8-24-0 along (F), mixture of *GI*, *Az* and fertilizer 8-24-0 (*GI*+ *Az* + F), mixture of *Az* and F (*Az* + F), mixture of *GI* and F (*GI* + F), mixture of *GI* and *Az* (*GI* + *Az*); and the witness (T), without fertilization. These were applicated twice, on papaya seeds and in the sowing over the seedling. Such effect was evaluated using the variables: plant height, stem diameter, radicular volume, fresh total weight, dry total weight, foliage fresh weight, root fresh weight, as well as the seedling quality index by the Dickson method. The results shown that the biofertilizer *G. intraradices* and *A. brasilense* improved the nutrient uptake and stimulated the growth of papaya plants, when were applied together and with mineral fertilizer (8:24:0). The applications of *G. intraradices* by separated, get the best quality of the plantule, getting better radicular develop of it.

Keywords: Biofertilization, *Glomus intraradices*, *Azospirillum brasilense*, *Carica papaya.*

INTRODUCTION

Chemical fertilizers are important in plant nutrition, however their irrational use has as a consequence negative effects on the health of man and our environment, besides the excess or deficiency of an element, can increase the succulence of the crop, early maturation and reduction in post-harvest . Agricultural systems should be sustained by maintaining soil fertility and soil structure by

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Table 1. Treatments applied during the trial and abbreviations that identify it.

Trataments	Description	Abbreviations
1	*Glomus intraradices*	Gl
2	*Azospirillum brasilense*	Az
3	Mineral fertilizer (8-24-0)	F
4	*Glomus +Azospirillum*	Gl + Az
5	*Glomus*+ Fertilizer	Gl + F
6	*Azospirillum*+ Fertilizer	Az + F
7	*Glomus + Azospirillum* + Fertilizer	Gl + Az + F
8	Control	T

effective use of fertilizers with increased profitability and reduced harm to the environment. Other strategies involve application of ecological concepts and principles to the design, development, and management of sustainable agricultural systems. Microbial inoculants, including arbuscular mycorrhizal (AM) fungi and plant growth-promoting rhizobacteria, for increasing the efficient use of fertilizers are potential components of such management. AM fungal inoculants have been marketed as an important biological component to the commercial horticulture and agriculture (Weber, 2014).

The application of fertilizers, particularly phosphates, can be reduced when inoculations of mycorrhizal fungi, because the fungi favor the more efficient use of the inorganic nutrients contained in the substrate. In addition, this benefit of fungi and bacteria allows a more rationed use of fertilizers and there by reduce the costs of plant production. Biofertilizer could be an economically viable alternative to mitigate the effects of irrigation water salinity and may raise productivity in yellow passion fruit cultivation systems (Nascimento et al., 2016).

Colonisation prevalence for plants inoculated with *G. intraradices* ranged from 18.53% to 26.67%, the highest values being recorded for the treatment involving combined inoculation with *A. brasilense*. The methodology improved seed germination rate, obtained greater homogeneity in plant emergence and thus reduced time spent in the nursery.

The double inoculation (seed and seedling) promoted higher growth and biomass of the crop than single inoculation (only seedlings), when organic matter was added at an intermediate dose (25 or 35%) and *G. intraradices* was applied as a single inoculant. However, the single or double inoculation did not modify the nutrient content in papaya seedlings (Constantino et al., 2011).

The ecological impact of Arbuscular mycorrhizal fungi (AMF) interactions with microorganisms involved in potassium solubilization is not well resolved compared to those involved in phosphate solubilization. Although direct studies on the interactions between AMF and potassium-solubilizing microorganisms (KSMs) on plant growth are limited, studies on plant growth-promoting microorganisms (PGPMs) and AMF do involve organisms with K-solubilizing capabilities. Evidence does exist on the influence of KSMs on mycorrhizal formation and function. Interactions between AMF and KSMs are vital in sustainable low-input crop production systems that rely on biological processes to achieve improved plant growth and yield in addition to maintaining soil fertility (Meena et al. 2016).

In countries with sustainable agriculture and long-cycle tropical crops, it is very important to have some nutritional alternatives that allow the partial or total substitution of mineral fertilizers to be improved. For this reason, the aim of the study was to determine the quality of papayas seedling, variety "Red Maradol", obtained with the use of *Glomus intraradices* and *Azospirillum brasilense*, as biofertilizers for production in Xalostoc, Morelos.

MATERIALS AND METHODS

The research was conducted in a greenhouse at the Higher School of Studies Xalostoc (HSSX). Autonomun University State of Morelos (UAEM), Ciudad Ayala, Morelos. Located at 18 ° 43 '00 "north latitude and 98 ° 54' 00" west longitude, at an altitude of 1250.

An experiment was performed using a completely randomized design, with eight treatments and five replicates: 1. *Glomus intraradices (Gl);* 2. *Azospirillum brasilense (Az); 3.*Fertlizer (F); 4. *Glomus +Azospirillum (Gl+Az); 5. Glomus* + Fertilizer (Gl + F); 6. *Azospirillum* + Fertilizer (Az + F) ; 7. *Glomus + Azospirillum* + Fertilizer (Gl + Az + F); 8. Control (T). The treatments were applied on seeds of *Carica papaya* L. cv. 'Maradol roja' and then on the seedlings, at the time of sowing. Each experimental unit consisted of five seeds treated at two times, when pregerminating and at the time of planting (González et al, 2012) (Table 1 above).

The fertilizer (8-24-0) was applied in a dose of 1.0 $mL.L^{-1}$ of water and formulated from ammonium sulfate as a source of nitrogen (20.5%) and triple superphosphate as source of phosphates (46%).

For the treatments with biofertilizers, two microorganisms were used: *Azospirillum brasilense* (5·108 CFU per g) and *Glomus intraradices* Schenck & Sm. (200 spores per g). The first, a nitrogen fixing bacterium, in doses of 1.14 $kg \cdot ha^{-1}$ dissolved in 200 liters of water; The second a fungus that facilitates the absorption and assimilation of phosphorus, applied in doses of 3.0 $kg \cdot ha^{-1}$, diluted in 200 liters of water.

Seeds were pregerminated, soaking for 48 hours, with water change every 12 hours; And placing them in a humid chamber between chiffon flannels, as a sandwich, at an average temperature of 25 ºC.

When the radicle was visualized in more than 60% of the seeds, they were planted in trays of 200 alveoli, on substrate composed of peat moss and coconut fiber (1:1). The third part of each well was filled with substrate, 1.0 g of the corresponding treatment was added, the pregerminated seed was placed and covered with the substrate. It was moistened to 85% of field capacity using a watering can, to avoid removing the seeds from the cavity of the tray. At 30 to 35 days of planting, the seedlings were extracted and their roots were submerged in the corresponding treatment for 20 minutes (Constantino et al., 2011). Four seedlings were then planted per pots, on a substrate composed of soil mud, peat moss and tezontle in a 1: 1: 1 ratio. (González et al, 2012).

Between 7-45 days of sowing, they were measured every eight days, plant height (cm) and stem diameter (mm). At 60 days the plants were collected, washed with plenty of water, leaving no residue on the substrate and dried with adsorbent paper. Todos las actividades ejecutadas para el manejo y obtencion de plantulas de papaya, fueron aplicadas, segun el Instructivo Técnico del Cultivo de la Papaya (MINAG, 2011; ACTAF, 2012).

Using an analytical balance, the total fresh weight of each seedling (g), fresh weight of the aerial part, fresh weight of the root were measured. The root of the plant was separated, measured with the aid of a ruler and immersed in a graduated specimen with 50 mL of water, recording the displaced volume, which coincided with the root volume (Córdoba *et al.*, 2011).

The parts of the seedlings were placed in a stove (Memmert, Germany) at 60 ° C for seven days and then the total dry weight of the aerial and radicular parts were determined (Ballesteros *et al.*, 2011).

With the Dickson method, seedling quality was determined according to the ratio of dry weight (shoot: root), length and diameter of the stem of the seedling, according to the equation:

$$IC = \frac{Ps_T}{\left(\frac{lT}{dT}\right)\cdot\left(\frac{Ps_A}{Ps_R}\right)} \qquad \text{(Mohedano, 1999)}$$

Where: IC is quality index; PsT is Total dry weight (g); LT is stem length (cm); DT is stem diameter at its base (mm); PsA is Dry weight of the aerial part (g); PsR is dry weight of the root (g).

According to the value obtained with this equation, the closer value of the quality index (CI) to unit (1), the higher the quality of seedlings produced in the nursery will be estimated (Mohedano, 1999).

The simple variance analyzes were performed, after verification of the parametric assumptions of normality (Shapiro Wills test) and homogeneity (Levene test). The differences between the means of the treatments were verified through Tukey test, with a probability of error $P \leq 0{,}01$. Data were processed using the SPSS ver. 19, for Window.

RESULTS

At 45 days after seeding, greater height of papaya plants was obtained by applying chemical fertilizers (8: 24: 0), *A. brasilenses* and *G. intraradices* (Gl + Az + F), with significant differences to control and the rest of the treatments.

However, when was applicated the fertilizer alone, had to the largest diameter in the plants were obtained, similar to that verified with the mixtures (Az + F); (Gl + Az + F); (Gl + Az and Gl), but with significant differences with the control and the others treatments (Figure 1 below).

The results of using microorganisms (biofertilizers) mixed with inorganic fertilizers (chemicals), represent increase the height of the seedlings by 70%, which proof a better nutritional status. This may be related by a higher uptake of N-P-K given by the stimulation of mycorrhizal populations present in the substrate.

Seedling growth after 60 days of sowing was higher when both biofertilizers mixed with chemical fertilizer (Gl + Az + F) were used. This was evident in total fresh weight, aerial part, root, root volume and total dry weight of the seedling, where significant differences were found with the control and the other treatments.

The treatment with *G. intraradices* reached greater length of the main root and consequently greater root volume, however its mixture with fertilizers, significantly reduced the fresh weight of the roots. But, its effect generated the lowest cost and highest quality plants, with no difference between the (Gl + Az) and (Gl + Az + F), significantly higher than with fertilizer treatment (Table 2 below).

Comparing the values of total dry weight and seedling quality obtained in the treatments (Gl + Az), (Az) and (Gl), suggests a greater stimulating activity of *G. intraradices*, specifically in the elongation and volume of the root, which allows a greater absorption surface and accumulation of dry mass in the seedlings, which was verified when assessing the quality parameter of the plants.

The application of the biofertilizers together with the mineral fertilizers (8: 24: 0), compared to these separately, promoted fresh and dry weight, however root length and

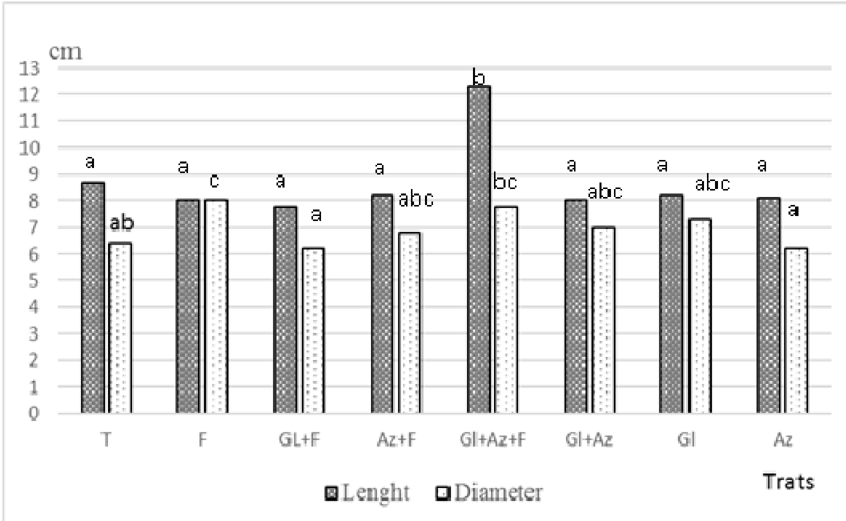

Figure 1. Biofertilizers effect on stem height and the papaya plants diameter up to 45 days after planted. Means with different letters per bars differ for Tukey (P≤0,01).

Table 2. Biofertilizers efect on the seeling growth at to 60 days of planted. Trataments: (T) = Control; (F)= Fertlizer; (*Gl* + F) = *Glomus* + Fertilizer; (*Az* + F) = *Azospirillum*+ Fertilizer; (Gl + Az + F) = *Glomus + Azospirillum* + Fertilizer; (*Gl + Az) = Glomus +Azospirillum; (Gl) = Glomus intraradices; (Az) = Azospirillum brasilense.*

Trataments	**Fresh weight. (g)**		**Dry weight aereal (g)**		**Weight root (g)**		**Rooting Volums (mL)**		**Lenght of roots (cm)**		**Tot.Fresh weight of plants (g)**		**Plants quality -**	
T	15,32	**ab**	8,04	**a**	7,28	**bc**	14,27	**cd**	19,26	**bc**	1,62	**a**	0,54	**a**
F	17,02	**ab**	9,23	**a**	7,77	**bc**	9,3	**ab**	14,71	**a**	1,71	**ab**	0,82	**b**
Gl + F	14,04	**a**	8,61	**a**	5,42	**a**	8,08	**a**	15,16	**b**	1,86	**abc**	0,62	**a**
Az + F	15,29	**ab**	8,09	**a**	7,2	**b**	9,07	**ab**	17,95	**bc**	1,66	**a**	0,56	**a**
Gl + *Az* + F	26,19	**c**	13,52	**b**	12,66	**d**	16,58	**de**	23,55	**bc**	3,24	**d**	0,91	**bc**
Gl + *Az*	17,80	**b**	9,28	**a**	8,52	**bc**	11,4	**bc**	15,84	**b**	2,23	**c**	0,87	**bc**
Gl	18,46	**b**	9,48	**a**	8,97	**c**	17,61	**e**	28,67	**d**	2,16	**bc**	0,96	**c**
Az	16,84	**ab**	8,98	**a**	7,85	**bc**	13,4	**c**	27,2	**c**	1,73	**ab**	0,62	**a**

F.w (Fresh weight); D.w (Dry weight); R.w (Rood weight). Means with different letters per column differ by Tukey (P≤0,01).

root volume was higher with *G. intraradices*, for which resulted in better quality seedlings.

DISCUSSION

The nursery is the initial stage of crop development and quality of the positions is a key component of the subsequent performance of the production areas. The fertility of the substrates used in this stage defines the results to be obtained (González et al, 2012). Several researchers have reported on the substitution of mineral fertilization (N-P-K) or the alternative use of biofertilization with soil-improving microorganisms, especially to reduce the nursery stage in tropical fruit trees, as were indicated in other investigations, in related to agreater absorption of nitrogen, phosphorus, potassium and also a stimulation of native the mycorrhizal populations (Köhl *et al.,* 2016).

Similar reports, explained the use of mycorrhizae may substitute mineral fertilizer by more than 20%, increasing the absorption and assimilation of essential ions for the radish and coffee plants in the nursery phase (Barroso et al., 2013; Barroso and Abad, 2015). This results could explaine the efecte obtanied in the nursery phase with papaya seedlings.

On the other hand, have to explained tha nutrients applied with the mineral fertilizers, increased their availability in the soil, which is an essential factor in the nutrition of all, these can be used to a greater extent when applying these biofertilizers (Constantino et al., 2011), although its symbiotic effects acquire greater relevance when there are deficiencies of phosphorus and low pH in the soil (León, 2004).

Could be important for this assay, the results obtanied by Constantino et al. (2011) referent to that mycorrhiza *G. intraradices* may hinder the stablishment and development of other beneficial microorganisms when combined in the application.

The transplanting of pregerminated seeds can cause some water stress in the tissues and thus reduce the growth of the radicle and hypocotyl of papaya seedlings, which has been counteracted by the effect of arbuscular mycorrhizae, specifically *G. intraradices*, where activates the defenses for oxidative stress, especially the uptake of reactive oxygen species (Bothe, 2012).

CONCLUSIONS

Biofertilizers with the incorporation of *G. intraradices* and *A. brasilense,* applied in mixtures with mineral fertilizers (8: 24: 0), improved nutrient utilization and growth of papaya seedlings, however the application of *G. intraradices* separately, produced higher quality seedlings with better root development.

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