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Mycorrhizae Effect on Growth of Guava Plants (*Psidium guajava*) Under Nursery Conditions

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Abstract: Guava (*Psidium guajava* L.) is the main fruit crop in the Calvillo-Cañones region, with a cultivated area of 6,000 hectares in 2024 and a production of 90,400 tons. Plants for establishing new orchards are obtained primarily through asexual propagation by air layering and cuttings. Both propagation methods use soil as a substrate, and nursery growers do not use products that promote root development to avoid transplant shock. The objective of the present study was to evaluate the effect of mycorrhizae on the development of guava plants. Two experiments were conducted under nursery conditions and the following treatments were evaluated. In Exp.1-2018) 0.0, 3.0, 6.0 and 9.0 g/plant of the mycorrhizae INIFAP^{MR} (*Glomus intraradices*) and the rooting agent RADIX® 10000 (Indole-3-Butyric acid) at a dose of 2.0 g/plant; whereas in Exp.2-2019) the following treatments were evaluated: 0.0, 3.0, 6.0 and 9.0 g/plant of the mycorrhiza MicorrizaFer (*Glomus* spp) and the plant growth-promoting bacterium *Azospirillum* at a dose of 6.0 ml/liter. Guava plants from selection 20, obtained by air layering in 2017 and established in the nursery of the “Los Cañones” Experimental Site of INIFAP, were used. The air layering guava plants were placed under nursery conditions in black plastic pots 15 cm in diameter and 30 cm high, containing composted soil with bovine manure as a substrate. The experimental design was a randomized complete block design with five replications, with one plant as the experimental unit. After 97 and 126 days of applying the treatments in Exp.1 and Exp.2, respectively, the following variables were recorded: plant height (PH), number of leaves (NL), basal stem diameter (BSD), fresh weight of leaves (FWL) and roots (FWR), dry weight of leaves (DWL) and roots (DWR), total dry weight (TDW = DWL + DWR) and the DWL/DWR ratio. Results indicated trends toward greater fresh biomass with Radix® and *Azospirillum*. However, the DWL/DWR ratio of all mycorrhizal treatments showed lower values than the control without mycorrhizae in Exp. 1, and with treatment Mic. 3.0 g in Exp. 2. These results suggest that there was a greater dry biomass in roots than in the aerial parts, which may contribute to reducing transplant shock and improving establishment of plants in the field.

Keywords: *Psidium guajava*, nursery, aerial biomass, root biomass.

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the 15 most important fruit crops cultivated in Mexico. In 2024, the national planted area reported was of 21,147 hectares, with an annual production of 302,000 tons. The states that contribute most to guava production are Michoacán, Aguascalientes, and Zacatecas, which together accounted for 89.5% of the planted area and contributed 93.6% of the total production volume. In the Calvillo-Cañones region, which includes the municipalities of Calvillo in the state of Aguascalientes and the municipalities of Tabasco, Huanusco, Jalpa, Apozol, and Juchipila in Zacatecas, approximately 6,017 hectares were cultivated, with an average fruit yield of 15.0 tons/hectare. It is important to point out that, during the period 2017-2024 in Calvillo, Aguascalientes, an average area of 859 hectares has been dedicated to the production of guava for exportation mainly to the United States of America, with an annual average of 12,000 tons. (SIAP, 2026).

In Calvillo, Aguascalientes, there are numerous nurseries dedicated to the production of guava plants, which are obtained primarily through vegetative or asexual propagation, since this type of propagation maintains the characteristics of the "mother" plant. In contrast, sexual or seed propagation produces great genetic variability, which is manifested in productivity and characteristics of the fruit and the plant because of the high heterozygosity (Albany et al. 2004; Marak and Mukunda, 2005). This is because the high level of cross-pollination in guava, which ranges from 25.7 to 41.3%, with honeybees (*Apis mellifera*) being the chief pollinators (Morton, 1985).

Thus, the most commonly used asexual propagation methods for obtaining guava plants are air layering and cuttings, where the former is considered fast and low cost, and also avoids the possible transmission of root diseases or root-knot nematodes (Albany et al. 2004; Saroj and Kumar, 2020).

Even though this propagation method has good rooting percentages for the production of new plants, it is necessary to separate the air layering from the mother plant and transplant them into black plastic bags of approximately 2.0 to 3.0 kg, using nematode-free soil with good organic matter content and medium texture as a substrate, and keep them in nursery conditions until these new plants produce more roots and shoots before being planted in the field. This “acclimation” period may take up to several months. It is important to note that currently, during this stage, nursery growers do not use any products that promote the development of a greater number of roots to ensure good establishment and development of the plants in the field, reducing transplant stress and consequently the loss of plants.

In this regard, the benefits of using mycorrhizae have been widely documented in several fruit tree species. These benefits include: increased photosynthetic efficiency, water and nutrient absorption, tolerance to biotic and abiotic stress, greater development, and increased survival during transplanting of nursery-grown plants (Kumari et al. 2017).

Vieira et al. (2024) based on an extensive review of several studies on the effects of arbuscular mycorrhizal fungi (AMF) on guava (*Psidium guajava* L.) concluded that AMF (native or inoculated) play an important role in their association with guava, leading to various beneficial effects, such as: improving water acquisition and nutrients, plant growth, defense against fungal, bacterial and nematoid pathogens, with different levels according to the AMF inoculum used, management practices, the environment, nutrients, and other biofertilizing microorganisms involved. Estrada-Luna et al. (2000) evaluated the application of mycorrhizae on the growth of guava seedlings during the acclimatization period after *in vitro* propagation. After 18 weeks, the mycorrhizal seedlings showed greater values for stem length, leaf area, number of leaves, and dry weight of stems and roots. They also observed increased levels of P, Mg, Cu, and Mo in the mycorrhizal seedlings.

Dey et al. (2005) reported that the application of mycorrhizae to guava plants under field conditions improved fruit size and its physicochemical characteristics, specifically vitamin C content and the °Brix/acidity ratio. In a previous study, Padilla et al. (2019) reported the application of mycorrhizae (*Glomus* spp.) to guava plants under nursery conditions, observing that the root dry weight/leaf dry weight ratio was higher with the mycorrhizae compared to the control, suggesting greater biomass in the roots than in the aerial parts, which may contribute to reducing transplant shock.

Regarding studies with other species, Sotomayor et al. (2018) reported the application of biostimulants based on mycorrhizal fungi in the propagation of avocado rootstocks, observing that the treatments with mycorrhizae and *Trichoderma* increased 35% more in root and 13% in leaf area compared to the control plants, as well as greater assimilation of some mineral nutrients such as phosphorus.

Bautista et al. (2017) evaluated mycorrhizal colonization in the nursery production of blueberry (*Vaccinium* spp.) variety Biloxi. Treatments were studied using Endospor®, a commercial mycorrhizal complex, and native mycorrhizae from a species of *Gaultheria* sp. and the wild blueberry *V. confertum*. Colonization by *Gaultheria* sp. showed a positive effect on plant height, number of leaves, and the frequency and intensity of root colonization, while the commercial product and the mycorrhizae from *V. confertum* did not affect growth or colonization variables.

The beneficial effects on the development of some vegetable species inoculated with mycorrhizae have also been reported. Alvarado et al. (2014) conducted a study of mycorrhizal inoculation in transplanted tomatoes grown under shade house conditions, observing a significant increase of 12% in height, 18% in biomass, chlorophyll content, and a 30% increase in fruit production in the mycorrhizal plants, compared to the non-mycorrhizal plants. Manjarrez et al. (1999) evaluated the effects of vermicompost and arbuscular mycorrhizae on the development and photosynthetic rate of chili pepper plants, reporting no positive effects when the treatments were applied individually. However, when vermicompost and mycorrhizae were applied together, greater vegetative development and yield were observed.

Therefore, the objective of this study was to evaluate the effect of mycorrhizae application on the growth of guava plants (*Psidium guajava* L.) established under nursery conditions.

MATERIALS AND METHODS

Two experiments were conducted: Exp.1) in the summer of 2018 and Exp.2) in the spring of 2019. Both studies were carried out in the nursery of the "Los Cañones" Experimental Site of INIFAP, located in the Municipality of Huanusco, Zacatecas, (21° 44.7´ N; 102° 58.0´ W; 1,508 masl), which has shade netting (60%), where the following treatments were evaluated: Exp.1) Control without mycorrhizae; Mycorrhizae at doses of 3.0, 6.0 and 9.0 g/plant and the commercial rooting agent RADIX® 10000 (Indole-3-Butyric acid) at a dose of 2.0 g/plant. In Exp.2 the same four treatments as in Exp.1 were evaluated, but a different source of mycorrhizae was used, and a commercial product based on the plant growth-promoting bacteria *Azospirillum* at a dose of 6.0 ml/plant. *Azospirillum* is free-living nitrogen-fixer commonly used as a biofertilizer (Dey et al. 2005). The mycorrhizae applied in 2018 was the

INIFAP[MR] Mycorrhizae (*Glomus intraradices*), and the mycorrhizae applied in 2019 was obtained from the commercial product "MicorrizaFer" (*Glomus* spp) from Biofábrica Siglo XXI (Table 1). In both experiments, guava plants from the genotype "Sel-20" were used, which were propagated in 2017 by air layering from the mother trees of the Guava Germplasm Bank established at "Los Cañones" Experimental Site of INIFAP.

The air layering plants were established in black plastic pots, 15 cm in diameter and 30 cm high, containing local soil composted with bovine manure as a substrate and maintained under nursery conditions. Treatments were applied on May 16, 2018 in Exp. 1 and on February 19, 2019 in Exp. 2, according to the specified doses on plants randomly chosen from the air layering plants kept on the nursery. The product was applied around the stem, making a small trench approximately 2.0 cm deep where the mycorrhizae were placed. The area was then immediately covered with soil, and the plants were watered every three days. The rooting hormone RADIX® 10000 and *Azospirillum* were applied following the same procedure as for the mycorrhizae application. During the study period, a nutrient solution was applied weekly with 100 g of MAP (12-61-00), plus 100 g of potassium sulfate (00-00-50) and 20 g of Bayfolan (micronutrients) in 10 liters of water, applying 400 ml to each plant.

Table 1: Treatments evaluated in the growth of guava plants (*P. guajava*) under nursery conditions.

Exp. 1 2018	Exp. 2 2019
Control without mycorrhiza	Control without mycorrhiza
[a] Mycorrhiza 3.0 g/plant	[b] Mycorrhiza 3.0 g/plant
Mycorrhiza 6.0 g/plant	Mycorrhiza 6.0 g/plant
Mycorrhiza 9.0 g/plant	Mycorrhiza 9.0 g/plant
RADIX® 10000 2.0 g/plant	*Azospirillum* 6.0 ml/plant

[a] Mycorrhiza (*Glomus intraradices*) from INIFAP[MR]; [b]Mycorrhiza (*Glomus* spp) MicorrizaFer.

After 97 and 126 days of applying the treatments in Exp. 1 and Exp. 2, respectively, the following variables were recorded: plant height (PH), basal stem diameter (BSD), number of leaves (NL), fresh leaf weight (FLW), fresh root weight (FRW), dry weight of leaves (DWL), dry weight of roots (DWR), total dry weight (TDW = DWL + DWR) and the DWL/DWR ratio was also obtained.

The ratio has been commonly used as indicator of seedling quality (Dickson et al. 1960) and expresses the top growth relative to the root system.

The procedure to obtain the aforementioned variables was as follows: For each plant, the height from the base of the pot to the tips of the shoots was recorded. Then, the basal diameter of the stem was measured with a Mitutuyo® caliper to the nearest 0.001 mm. Subsequently, the leaves were separated, counted, and placed in perforated and labeled paper bags. To obtain the guava plant roots, they were washed, all soil was removed, and the roots were separated from the stem and placed in paper bags similar to those used for the leaves (Figure 1). The fresh weight of the leaves and roots was recorded using a precision scale (0.001 g), and the dry weight was obtained after eight days of drying the bags of leaves and roots in a solar dehydrator until a constant weight was reached.

In both experiments, a randomized block design with five replicates was used, with one plant as the experimental unit. The data obtained were analyzed independently in each experiment.

Figure 1: General view of the layout of experiments on the effect of mycorrhiza on the growth of guava plants under nursery conditions, application of mycorrhiza and recording of variables.

RESULTS AND DISCUSSION

The average values of the variables plant height (PH), basal stem diameter (BSD), number of leaves NL), fresh leaf weight (FLW) and fresh root weight (FRW), obtained in guava plants evaluated with the application of mycorrhizae in both experiments are shown in Table 2. Although no significant differences ($p \leq 0.05$) were observed in the variables analyzed, some trends in the development of the guava plants could be detected.

Table 2: Means of the variables recorded in guava plants evaluated with the application of mycorrhizae in Exp. 1 2018 and Exp. 2 2019.

Treatments	[1]PH (cm)	BSD (mm)	NL	FLW (g)	FRW (g)
--------------------- Exp. 1 2018 (97 days after treatments application) ------------------					
Control	74.4	20.3	87.0	51.5	10.1
[2]Mic. 3.0 g	85.4	13.4	57.4	39.0	13.5
Mic. 6.0 g	74.0	14.9	62.0	37.0	10.2
Mic. 9.0 g	82.0	14.8	66.0	39.2	12.5
Radix® 2.0 g	73.4	19.9	95.4	53.9	15.2
--------------------- Exp. 1 2019 (126 days after treatments application) ------------------					
Control	76.3	12.1	117.5	51.8	22.9
[3]Mic. 3.0 g	106.3	18.3	136.5	67.0	27.3
Mic. 6.0 g	95.3	14.2	117.8	59.0	21.6
Mic. 9.0 g	91.5	11.4	103.0	54.5	17.5
Azospirillum 6.0 ml	78.3	14.0	117.8	58.0	38.6

[1]PH= Plant height; BSD= Basal stem diameter; NL= number of leaves; FLW= Fresh leaf weight; FRW= Fresh root weight; [2]Mycorrhiza (*Glomus intraradices*) from INIFAP[MR]; [3]Mycorrhiza (*Glomus* spp) MicorrizaFer.

Plant height (PH) was slightly higher in the mycorrhizal treatment Mic. 3.0 g, which had an average of 83.7 and 106.3 cm in Exp. 1 and Exp. 2, respectively, compared to the control (74.4 and 76.3 cm), although the basal stem diameter (BSD) of the control and Radix® treatments exceeded that of the mycorrhizal plants with an averages of 20.1 mm vs 14.4 mm in Exp.1, while in Exp.2, Mic. 3.0 g had the highest BSD value (18.3 mm) as compared to the average of the rest of treatments (12.9 mm). The number of leaves/plant (NL) was higher in the control and Radix® treatments with an average of 91.2 NL, compared to the mycorrhizal treatments that had an average of 61.8 NL in Exp. 1, while in Exp. 2 the treatment Mic. 3.0 g showed a higher number of leaves (136.5) and the rest of the treatments had an average of only 114.0 NL.

Regarding fresh leaf weight (FLW), in Exp. 1, Radix® and control treatments showed the highest values with an average of 52.7 g, while the rest of the treatments had an average of 38.4 g. In Exp. 2, the Mic. 3.0 g treatment stood out with a FLW of 67.0 g, while the average in the rest of the treatments was 55.8 g. The fresh root weight (FRW) in Exp. 1 was highest in plants treated with Radix® (15.2 g), followed by the Mic. 3.0 g with a FRW of 13.5 g, while all other treatments had an average of 10.9 g. In Exp. 2, the outstanding treatments were *Azospirillum* and Mic. 3.0 g with an average of 32.9 g, compared to a FRW average of 20.7 g in the rest of treatments. Regarding root biomass, only a slight increase was observed in treatment Mic 3.0 g, although Radix® and *Azospirillum* showed higher values.

These results contrast with those reported by Estrada-Luna et al. (2000), who noted significant effects on biomass accumulation and number of leaves in guava plants under greenhouse conditions when mycorrhizae were applied. Meanwhile, Ibrahim et al. (2010) reported greater shoot length, leaf area, weight, and number of fruits in guava trees treated with mycorrhizae plus phosphate-solubilizing bacteria, possibly due to a synergistic effect of both microorganisms. Similar results were mentioned by Manjarrez et al. (1999) reporting no effects when the vermicompost and arbuscular mycorrhizae on the development and photosynthetic rate of chili pepper plants were applied individually, but when both treatments were applied together, greater vegetative development and yield were observed. This highlights the importance of the combined application of several plant growth-promoting microorganisms.

The average dry weight of leaves (DWL), roots (DWR), and total dry weight (TDW) obtained in Experiments 1 and 2 are shown in Figure 2. These three variables (DWL, DWR and TDW) were highest in the guava plants with Radix® followed by the control, while mycorrhizae plants showed lower values in Exp. 1. However, in Exp. 2 the highest DWL, DWR and TDW values were recorded with Mic. 3.0 g and *Azospirillum*, surpassing those of control plants without mycorrhizae. This could be attributed to the longer time that plants were exposed before to evaluate the effect of mycorrhizal application in Exp. 2 compared to Exp. 1 (97 vs. 126 days), which allowed for greater root biomass development. On the other hand, the effect of Radix® and *Azospirillum* on increasing TDW was observed in both experiments. Quiñones-Aguilar et al. (2020), reported a differential effect on growth of guava plants under greenhouse conditions when inoculated with different native consortium of arbuscular mycorrhizal fungi (AMF). Only one of the consortia evaluated promoted the best plant development and quality, while a higher mortality rate was observed in plants without mycorrhizae. Thus, authors suggest that the use of AMF could be a recommended practice for the sustainable cultivation of guava in greenhouses.

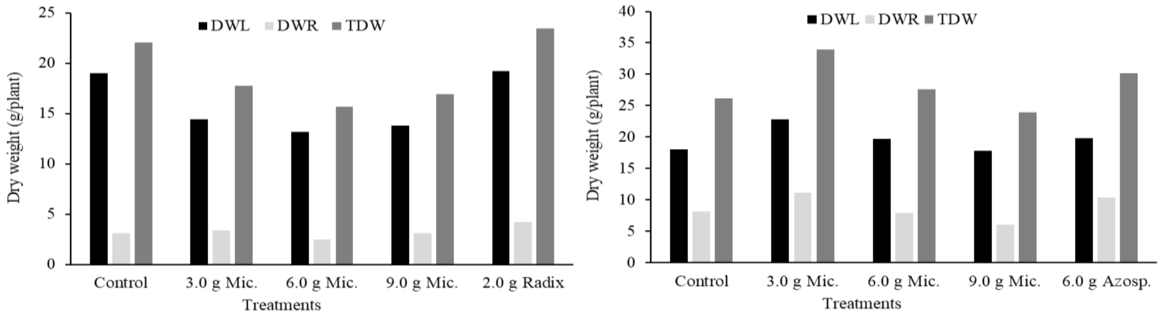

Figure 2: Averages of dry weight of leaves (DWL), dry weight of roots (DWR) and total dry weight (TDW) in Exp. 1 2018 (left) and in Exp. 2 2019 (right) of guava plants evaluated with the application of mycorrhizae.

The DWL/DWR ratio of guava plants is shown in Figure 3. This ratio ranged from 4.2 to 6.1 in Exp. 1, and from 1.9 to 2.9 in Exp. 2, meaning that in general guava plants improved root growth over time in the nursery. By looking the effect of treatments on each experiment, it was observed that in Exp. 1, lower values were obtained in all treatments with mycorrhizae and Radix® (4.6) compared to the control (6.1), while in Exp. 2, only the Mic. 3.0 g and *Azospirillum* treatments had values close to 2.0 and the other treatments had an average of 2.6. This suggests that biomass accumulation in the roots is more important than in the shoot, which may contribute to greater plant survival in the field. Quiñones-Aguilar et al. (2020) mentioned that according DWL/DWR ratio they classified two types of guava plant quality: low quality when the DWL/DWR was > 2.0 observed in plants without arbuscular mycorrhizal fungus (AMF) or with the mycorrhizae INIFAP^{MR} and high quality when the DWL/DWR ratio was < 2.0, obtained when guava plants were inoculated with any of the AMF consortium evaluated. The greater allocation of the photosynthesis products on the roots could be attributed to a greater photosynthetic activity in mycorrhizal plants and/or to a more efficient translocation of photoassimilates toward the roots rather than to a larger leaf area.

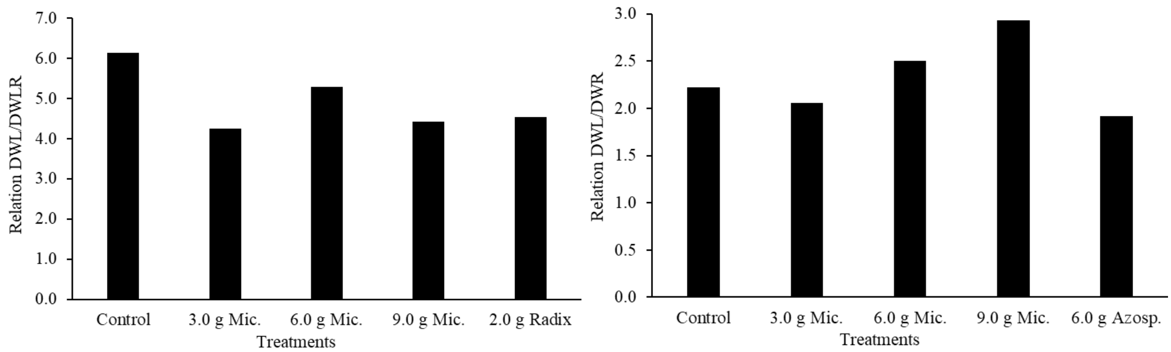

Figure 3: Relation dry weight of leaves/dry weight of roots (DWL/DWR) of guava plants evaluated with the application of mycorrhizae in Exp. 1 2018 (left) and in Exp. 2 2019 (right).

CONCLUSIONS

Guava plants treated with mycorrhizae and the rooting hormone and/or *Azospirillum* showed a lower DWL/DWR ratio compared to the control plants, although this trend was more pronounced in Exp. 1. The treatment Mic. 3.0 g in both experiments had a low DWL/DWR ratio. This suggests that applying these products may help reduce transplant shock in the field and increase survival rates.

It is recommended to evaluate mycorrhizae in conjunction with other plant growth-promoting microorganisms and/or root promoting products that may exhibit synergistic activity in producing greater root biomass.

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