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Influence of *Funneliformis mosseae* in the growth and accumulation of dry biomass in Dalia plants

García-Sánchez, Claudia[1]; Becerril-Román, A. Enrique[1]; Jaén-Contreras, David[1]; Hernández-Melchor, Dulce J.[1]; López-Morales, Fernando[2*]; Gaytán-Acuña, E. Araceli[1]

[1] Colegio de Postgraduados en Ciencias Agrícolas, Campus Montecillo. Carretera México-Texcoco Km 36.5, Montecillo, Texcoco, estado de México, México. C.P. 56264.

[2] Benemérita Universidad Autónoma de Puebla, EcoCampus Valsequillo. Carretera a San Baltazar Tetela Km 1.7, C.P. 72960, San Pedro Zacachimalpa, Puebla, México.

* Correspondence: fernando.lopez@colpos.mx

ABSTRACT

Objetive: in the production of *Dahlia* spp., only chemical fertilization has been used, and an option that has not yet been explored is the implementation of a microbial inoculant. Therefore, the objective of this study was to evaluate the effect of the mycorrhizal fungus *Funneliformis mosseae* on the growth and development of the dahlia (*Dahlia variabilis* var. Variegated dwarf).

Design/methodology/approach: the seeds were sown in polyethylene bags containing a mixture of black soil, peat moss, and agrolite. A completely randomized design was used, and the treatment structure was 2×3 factorial. The study factors were *F. mosseae*, chemical fertilization, and substrate sterilization.

Results: an analysis of variance was performed, and the mean values of the treatments were compared with Tukey's test (α=0.05).

Conclusions: with the inoculation of *F. mosseae*, a significant increase was obtained in the study variables: plant height, stem diameter, number of buds and flowers per plant; leaf + stem, flower, root, and total biomass, compared to non-inoculated plants. A colonization of 89% in the roots was recorded. A limitation of the study is that the effect of the inoculum on plant growth can vary according to the mycorrhiza species used. In conclusion, inoculation with *Funneliformis mosseae* increased growth and biomass accumulation in Dahlia plants.

Keywords: *Dahlia variabilis* var. Variegated dwarf; biomass; plant quality; mycorrhizal colonization.

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INTRODUCTION

Mexico is the center of biological diversity of the genus *Dahlia*, and its cultivation began in the times of the Aztec empire. Due to its importance, in the year 1963 it was declared the symbol of National Floriculture (Jiménez-Mariña, 2015). Despite this, its production does not occupy a predominant place in national floriculture, while in countries such as the Netherlands, Japan, England, and the United States its production is higher (Vidalie, 2001). Dahlias belong to the Asteraceae family and can be herbaceous or shrub plants, their leaves are simple or tripinned-compound, with hollow stems between the internodes. Their inflorescences are arranged in chapters made up by ligulated and tubular flowers. Their fruit is an achene and their roots are tuberous (Bye and Linares, 2008).

In the cultivation of dahlia, it is recognized that applying chemical fertilizers has increased their

development (Arenas-Julio *et al.*, 2011), although their excessive use has caused problems of environmental contamination, such as the acidification of soils which affects fertility and the eutrophication of bodies of water (Bertani and Lu, 2021); because of this, the use of fertilizers that are not damaging to the environment, such as biofertilizers and microbial inoculants, has been promoted.

Arbuscular Mycorrhizal Fungi (AMFs) are members of the *Glomeromycota phylum* and they form symbiosis with 80 to 95% of plant species. The fungus colonizes biotrophically the root crust, without damaging the plant and becoming part of it, physiologically and morphologically (Koltai, 2010). In ornamental species of the Asteraceae family, inoculation with AMF has been found to increase the height, number of flowers per plant, and biomass (Vaingankar and Rodrigues, 2012; Uc-Ku *et al.*, 2019).

It is necessary to continue with the implementation of biofertilizers through the use of AMFs, since they promote growth and flowering in various species, in addition to representing a sustainable alternative in flower production (De Pascale *et al.*, 2020). Dahlia is appreciated internationally because of the large variety of shapes and colors that it presents, which is why it is necessary to rescue its cultivation in Mexico, in addition to the application of AMFs as an organic fertilization alternative, which has not been explored in its growth. Therefore, the research objective was to evaluate the effect of *Funneliformis mosseae* on the growth and development of dahlia (*Dalia variabilis* var. Enana variada) in pots under greenhouse conditions.

MATERIALS AND METHODS

The experiment was established in a greenhouse with milky plastic cover, in Colegio de Postgraduados (CP), Campus Montecillo, Texcoco, Estado de México. Commercial Hortaflor® dahlia seeds were used, which were sown in germination trays that contained peat moss and vermiculite (1:1); the substrate was sterilized in an autoclave at 120 °C during three hours. Germination began in February and seed emergence was observed 10 days after sowing. The transplant was carried out 15 days after emergence, for which black polyethylene bags of 35×35 cm were used, which contained a mixture of black soil, peat moss and agrolite (1:1:1), which were sterilized in autoclave at 120 °C during three hours. At the time of transplant, each of the seedlings was inoculted with 10 g of inoculant that contained 350 spores of the mychorrhizal fungus *Funneliformis moseae*. An orifice with the size of the ball of the seedling was made in the center of the pot where the inoculum and the seedling were placed. Chemical fertilization was carried out 30 days after transplant, 1 g L^{-1} of chemical fertilizer of continuous liberation 11-7-7 (N-P-K) was placed in each of the seedlings, which was distributed to the maximum possible, since dahlia is sensitive to excess salts. The variables of study were plant height (cm) which was considered from the basal area to the apex of the main stem, the stem diameter (mm) was measured at the base, the number of buds and of flowers per plant were counted, and the dry matter weight (g) of each of the plant structures was recorded: leaf + stem, flower buds, flower, root and total.

The mychorrizal colonization in the dahlia roots was determined, for which the clearing and dyeing method by Phillips and Hayman (1970) was used. And the observation of roots was carried out in an American optical® optical microscope.

The experimental design was completely random and the treatment structure was a complete 2×3 factorial arrangement; the variation factors, and the levels of application were: 1) Inoculation with the arbuscular mychorrizal fungus *F. mosseae* (M): 0 and 10 g of inoculant; 2) Chemical fertilization (F) with 11-7-7: 0 and 1 g L^{-1}; and 3) The sterilized and non-sterilized substrate (S). With that, the combination of eight treatments was obtained: M0F0S0, M1F0S0, M0F1S0, M1F1S0, M0F0S1, M1FIS1, M1F1S1 and M1F1S1.

Fifteen plants were established per treatment; one plant was considered as experimental unit, and three repetitions per treatment were selected, for each of the variables evaluated. The sampling was done in the flowering period, in the month of June.

The results obtained from each of the factors and their interactions were examined in the statistical software Statistical Analisis System (SAS®), version 9.0, with which an analysis of variance (ANOVA) was conducted, and the mean values of the factors and their interactions were compared with Tukey's test (α=0.05).

RESULTS AND DISCUSSION

In the analysis of factors in study, it was observed that the inoculation with *F. mosseae* (M1) promoted and increased height, stem diameter, number of buds, and number of flowers per plant, compared to the non-inoculated plants (M0) (Table 1). When analyzing the interaction of the three factors in study, mychorrizal fungus, chemical fertilization and substrate (M*F*S), the greatest height was obtained with the treatment (M1F1S0), without

Table 1. Mean values of height, stem diameter, number of buds and flowers in dahlia plants (*Dahlia variabilis* var. Variegated dwarf).

Treatments	Plant height (cm)	Stem diameter (mm)	Buds	Flowers
			Number per plant	
M				
M0	46.7 b	7.7 b	15.5 b	9.2 b
M1	55.8 a	11.2 a	41.3 a	20.2 a
DMS	7.8	1.5	11.8	6.1
M*F*S				
M0F0S0	50.6 a	6.8 b	17.3 bc	10.0 c
M1F0S0	68.5 a	10.7 ab	25.3 abc	32.7 ab
M0F1S0	45.0 a	7.6 ab	13.7 c	12.0 c
M1F1S0	46.6 a	12.6 a	56.3 a	10.0 c
M0F0S1	43.8 a	7.7 ab	17.7 abc	1.7 c
M1F0S1	61.2 a	11.6 ab	28.3 abc	33.7 a
M0F1S1	47.2 a	8.7 ab	13.3 c	13.3 bc
M1F1S1	47.2 a	9.9 ab	55.3 ab	4.7 c
DMS	25.5	5.0	38.8	19.9

M=Inoculation factor with *Funneliformis mosseae*; M*F*S=Interaction of the inoculation with *F. mosseae*, with chemical fertilization and the substrate; M0=Without *F. mosseae*; M1=With *F. mosseae*; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate; DMS=Minimum Significant Difference. Means with the same letter in each column are equal according to Tukey's test ($\alpha \leq 0.05$).

statistically differing from the other treatments (Table 1). Regarding the stem diameter, the best results were found with the treatment (M1F1S0) and the smallest diameter with the control (M0F0S0). With the treatment (M1F1S0), the highest number of buds was obtained with 56.3 and the lowest number of buds was 13.3 with the interaction (M0F1S1). The highest number of flowers per plant was 33.7 with the treatment (M1F0S1) and the lowest number was 1.7 with the interaction (M0F0S1) (Table 1).

Concerning the dry biomass, the greatest accumulation of biome was observed in leaf + stem, flower, root and total with inoculation of the mycorrhizal fungus (M1), compared to the control (M0) (Table 2). And in the interaction of the three factors (M*F*S) a similar behavior was observed, since with the M1*F0*S0 interaction the greatest accumulation of dry biomass was found in leaf + stem (37.4 g), flower (13.5 g), root (20.5 g) and total (71.4 g), compared to the other treatments (Table 2).

The increase in plant height, stem diameter, number of buds and flowers, as well as the biomass in each of the structures and total, can be attributed to the fact that the mycorrhized roots can explore a greater volume of soil, due to the presence of external hyphae that mycorrhizal fungi develop, and these results are associated to a higher absorption of nutrients from the less soluble sources (Sohn *et al.*, 2003); it is considered that the fungi hyphae are the ones responsible for transporting more photosynthates to the plant, which contributes to the increase in growth, in the development and in the production of biomass of the plant species (Ibarra-Puón *et al.*, 2014).

Table 2. Mean values of dry biomass in each one of the dahlia plant structures (*Dahlia variabilis* var. Variegated dwarf).

Treatments	Dry weight (g)			
	Leaf + Stem	Flower	Root	Total
M				
M0	9.2 b	3.7 b	6.5 b	19.4 b
M1	30.3 a	9.4 a	11.8 a	51.6 a
DMS	4.5	1.5	4.6	8.5
M*F*S				
M0F0S0	10.1 bc	3.9 cd	9.1 abc	23.1 cd
M1F0S0	37.4 a	13.5 a	20.5 a	71.4 a
M0F1S0	10.2 bc	4.7 cd	7.7 abc	22.6 cd
M1F1S0	27.7 a	7.4 bc	6.9 abc	41.9 bc
M0F0S1	4.8 c	1.4 d	1.2 c	7.4 d
M1F0S1	32.8 a	11.7 ab	16.4 ab	60.8 ab
M0F1S1	11.8 bc	4.7 cd	7.9 abc	24.5 cd
M1F1S1	23.4 ab	5.0 cd	3.6 bc	32.1 cd
DMS	14.8	5.0	14.9	27.8

M=Inoculation factor with *Funneliformis mosseae*; M*F*S=Interaction of the inoculation with *F. mosseae*, with chemical fertilization and the substrate; M0=Without *F. mosseae*; M1= With *F. mosseae*; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate; DMS=Minimum Significant Difference. Means with the same letter in each column are equal according to Tukey's test ($\alpha \leq 0.05$).

With the inoculation of AMFs, ornamental species can acquire the nutrients efficiently, which is reflected in a higher growth and development of the plant (Jiménez-Moreno *et al.*, 2018).

However, in this case no significant differences were found in the plant height between treatments, and this can be influenced by the plant's genetics, the soil and the climate conditions, as well as the species of inoculant used (Linderman and Davis, 2004).

In the roots of dahlia plants, a colonization of 89.36% was found with the M1F0S0 treatment. The highest mycorrhizal colonization was found when the dahlia plants were only inoculated with *F. mosseae*, while the addition of chemical fertilizer caused a decrease in the percentage of colonization in the roots observed (Table 3, Figure 1C), which is because the addition of chemical fertilizers affects the establishment of AMFs (Rouphael *et al.*, 2015). Meanwhile, the substrate sterilization did not influence this result.

The M0F0S0, M0F0S1 and M0F1S1 interactions, where the mycorrhizal inoculant was not applied are not shown, since the presence of fungal structures was not found.

The level of mycorrhizal colonization among ornamental species is variable, it depends on the species, the inoculum used, and the conditions in which the experiment is developed (Vaingankar and Rodrigues, 2012). To consider that the mycorrhizal symbiosis with the

Table 3. Percentage of mycorrhizal colonization in the dahlia roots (*Dahlia variabilis* var. Variegate dwarf).

Treatments	Mycorrhizal colonization (%)
M1F0S0	89.36 ± 2.30 a
M1F1S0	70.31 ± 8.50 bc
M1F0S1	87.61 ± 2.62 ab
M1F1S1	69.20 ± 4.13 c
DMS	17.44

M1=With *F. mosseae*; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate. The values are means ± standard error. Means with the same letter in each column are equal according to Tukey's test ($\alpha \leq 0.05$). DMS=Minimum Significant Difference.

Figure 1. A-B: Dahlia flower. C: Arbuscule, fungal structure of the arbuscular mycorrhizal fungus *Funneliformis mosseae*, on the roots of the dahlia.

plant roots is helpful, the plants inoculated with the AMF must be capable of producing more dry matter in comparison to non-inoculated plants (Asrar *et al*., 2012). In this case, it can be stated that inoculation of the dahlia plants with *F. mosseae* was beneficial since it fostered the highest accumulation of dry biomass in each of the structures and in total (Table 2). The implementation of microbial inoculants such as mycorrhizal fungi represents an option that is environmentally friendly, where a representative increase in the production of ornamental crops is achieved, and in addition, they can provide savings in the application of chemical inputs (Zulueta-Rodríguez *et al*., 2013). In this case, the inoculation with *F. mosseae* promoted the growth and the accumulation of biomass in dahlia plants.

CONCLUSION

The inoculation with *F. mosseae* promoted a significant increase in plant height, stem diameter, number of buds and flowers, and dry biomass accumulation; because the AMF helped the absorption of nutrients, it represents a viable option for the production of dahlia in pots under greenhouse conditions.

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