Revista Iberoamericana de las Ciencias Biológicas y Agropecuarias

Ciba

ISSN: 2007 - 9990

https://doi.org/10.23913/ciba.v12i23.116

Artículos científicos

Efecto de microorganismos promotores de crecimiento vegetal y yeso agrícola en el cultivo de higo

Effect of Plant Growth-Promoting Microorganisms and Agricultural Gypsum on Fig Cultivation

Efeito de microrganismos promotores de crescimento vegetal e gesso agrícola no cultivo de figos

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Resumen

La demanda de higo (*Ficus carica* L.) está creciendo mundialmente por lo que se busca mayor rendimiento del cultivo. Una de las estrategias más promisorias es la inoculación de las plantas con microorganismos promotores de crecimiento vegetal (MPCV). También se emplean enmiendas para mejora de las condiciones del suelo, una de las más utilizadas es el yeso agrícola. El objetivo del presente estudio fue evaluar la aplicación con un consorcio bacteriano (*Bacillus subtilis, Bacillus cereus, Pseudomonas fluorescens;* 10^8 UFC m^{-2} para cada cepa) y *Trichoderma harzianum* combinado con yeso agrícola (fuente de Ca) como promotores de crecimiento en plantas de higo. La inoculación se realizó en tres tratamientos: testigo (T1), consorcio bacteriano y *T. harzianum* con yeso agrícola (T2) y *T. harzianum* con yeso agrícola (T3), bajo un diseño experimental en bloques completos al azar. La inoculación con T2 y T3 ayudó a mejorar las variables de fotosíntesis, número de fruto, peso de fruto

(30.5 %) y rendimiento (T2 38.35 % y T3 41.98 %). Además, la inoculación con T3 ayudó a la resistencia a la penetración (T3 7.2 lbF- T1 4.6 lbF) y el aumento en grados Brix (T3 22.5-T1 21.9) en los frutos.

Palabras clave: biofertilizante, bioinoculante, *Ficus carica,* promotor de crecimiento, rendimiento.

Abstract

The demand for fig (*Ficus carica* L.) is growing worldwide, leading to the search for higher crop yield. One of the most promising strategies is the application of plant growth promoter microorganisms (PGPM), which are used as inoculants in plants. Amendments are also used to improve soil conditions, one of the most widely used in agricultural gypsum. The objective of this study was to evaluate the combined application of PGPM (*Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens,* and *Trichoderma harzianum*) with agricultural gypsum (AG) in the soil, through microbiological, nutritional, and physiological analysis in fig plants. The inoculation of PGPM (108 CFU m^{-2}) with AG (calcium 40 kg ha^{-1}) was performed in three treatments: T1: Control, T2: Bacterial consortium, and *T. harzianum* with AG and T3: *T. harzianum* with AG under a randomized complete blocks experimental design. The inoculation with T2 and T3 helped improve the photosynthesis variables, fruit number, fruit weight (30.5 %), and yield (T2 38.35 % and T3 41.98 %). Furthermore, the inoculation with T3 helped the resistance to penetration (T3 7.2 lbF-T1 4.6 lbF) and the increase in Brix degrees (T3 22.5-T1 21.9) in the fruits.

Keywords: biofertilizer, bioinoculant, *Ficus carica*, growth promoter, yield.

Resumo

A demanda por figos (Ficus carica L.) está crescendo em todo o mundo, razão pela qual se busca maior produtividade. Uma das estratégias mais promissoras é a inoculação de plantas com microrganismos promotores de crescimento vegetal (MPCV). As emendas também são utilizadas para melhorar as condições do solo, uma das mais utilizadas é o gesso agrícola. O objetivo deste estudo foi avaliar a aplicação com um consórcio bacteriano (Bacillus subtilis, Bacillus cereus, Pseudomonas fluorescens; 108 UFC m-2 para cada cepa) e Trichoderma harzianum combinado com gesso agrícola (fonte de Ca) como promotores de crescimento em plantas da fig. A inoculação foi realizada em três tratamentos: controle (T1), consórcio bacteriano e T. harzianum com gesso agrícola (T2) e T. harzianum com gesso agrícola (T3), em delineamento experimental em blocos ao acaso. A inoculação com T2 e T3 ajudou a melhorar as variáveis de fotossíntese, número de frutos, peso de frutos (30,5%) e rendimento (T2 38,35% e T3 41,98%). Além disso, a inoculação com T3 auxiliou na resistência à penetração (T3 7,2 lbF- T1 4,6 lbF) e no aumento dos graus Brix (T3 22,5-T1 21,9) nos frutos.

Palavras-chave: biofertilizante, bioinoculante, Ficus carica, promotor de crescimento, produtividade.

Fecha recepción: Julio 2022 **Fecha aceptación:** Enero 2023

Introduction

The fig tree (Ficus carica L.) is native to Central Asia, from where it spread to the entire Mediterranean and later to the American continent (Pereira et al., 2015). Deciduous tree belonging to the Moraceae (mulberries), its color varies from dark purple to green. The fruit is consumed raw and processed in different presentations (Takahashi, Okiura, Saito and Kohno, 2014). It is a source of vitamins, minerals, antioxidants and amino acids (Garza, Olivares, Gutiérrez, Vázquez and López, 2019).

The economic importance of the fig has increased worldwide, along with the interest in improving production methods, processing, pest and disease control, packaging and conservation (Mendoza, Vargas, Calderón, Mendoza and Santacruz, 2017). Currently, Mexico is positioned in 19th place out of 51 fig-producing countries (Fernández, García, Fernández and Muratalla, 2020; Food and Agriculture Organization of the United Nations [FAO], 2018). The state of Sonora registers 105 cultivated ha, 100 of them for export and only five for national consumption (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2021).

Intensive production usually uses large amounts of chemicals that could degrade the soil and alter its microbial communities (Villarreal et al. 2018). Among the alternatives to the use of pesticides, the application of microorganisms known as plant growth promoters (MPCV) stands out, which increase plant growth (Leal et al., 2018) by increasing the availability of nutrients, protection against phytopathogens and other types of growth stimulation (Morales et al., 2021; Sherathia et al., 2016). These microorganisms are found in the soil and constitute excellent biotechnological alternatives for improving the yield of some crops (Gómez et al., 2012). It has been found that the application of MPCV in a consortium can be more favorable than in single strains, since acting synergistically enhances the benefit for the plant (Shah et al., 2021).

On the other hand, agricultural gypsum is used as a soil amendment (Fisk, February 6, 2019; Trasviña et al., 2018). Helps regulate the potential of hydrogen (pH) by providing calcium and sulfur in the form of sulfates. Applied directly to the soil, it allows pH values close to 6.0, which are favorable for the development of MPCVs. (Shah *et al*., 2021).

The objective of this study was to evaluate the effect of the combined application of MPCV (Bacillus subtilis, Bacillus cereus, Pseudomonas fluorescens and Trichoderma harzianum) with agricultural plaster, as well as Trichoderma harzianum with agricultural plaster on the fig crop through microbiological analysis, nutritional and physiological.

Materials and methods

The research was carried out during the 2021-2022 cycle, in an experimental plot of a fig orchard, located in the Esperanza ejido, Cajeme, Sonora, Mexico, within plot number 98 ZP-1 P 1/1 (27.610063 N- 109.910701 O). The plantation was in a 3 × 3 m frame with the Black Mission variety, in soil with a loamy-sandy-clay texture, with 1.26 % organic matter, pH of 7.6 and electrical conductivity of 0.99 dS m-1, determined following the methodology of the official Mexican standard NOM-021-Recnat-2000 (Secretaría de Medio Ambiente y Recursos Naturales [Semarnat], December 31, 2002). A randomized complete block design with four replicates per treatment was used.

Treatments

Three treatments were applied: 1) control (T1), 2) bacterial consortium (Bacillus subtilis, Bacillus cereus and Pseudomona fluorescens) and Trichoderma harzianum with agricultural plaster (T2) and 3) T. harzianum with agricultural plaster (T3). Treatments T2 and T3 contained 40 kg ha-1 of calcium contributed by agricultural gypsum. The MPCV were inoculated in suspension in distilled water with a concentration of 108 CFU ml-1 per square meter for each species. The application was direct on each tree and 16 biweekly applications were made from March 8, 2021 to October 4, 2021.

Measured variables

Quantification of UFC g^{-1} floor

A count of viable microorganisms in the soil was carried out to monitor the population density for each microorganism prior to the applications and 15 days after the start of the application. The samples were taken following the methodology of NOM-021-Recnat-2000 (Semarnat, December 31, 2002).

The technique of serial dilutions (10-4, 10-5 and 10-6) and plate pouring was used, in triplicate on mannitol-egg yolk-polymyxin agar medium (MYP agar), for the morphological identification of B. subtilis. and B. cereus; P. fluorescens isolation agar was used for P. fluorescens and potato-glucose agar for T. harzianum. The bacteria were incubated at 30 °C and the fungus at 25 °C. The microbiological count was carried out at 24, 48 and 120 h; the results were recorded as CFU g-1 of soil (Pepper & Gerba, 2004).

Foliar nutritional analysis

The concentration of nutrients (N, P, K, Ca, Mg, Fe, Cu and Zn) was determined in a composite sample of plant tissue for each treatment, two months after the start of applications and at the end of applications (seven months later). . The analysis was carried out with a spectrophotometer (DR3900 Hach), following the methodology established by Alcántar and Sandoval (1999). Modifications adjusted to the nature of the samples were made..

Chlorophyll

Chlorophyll content was estimated following the Soil Plant Analysis Development (SPAD) method (with a SPAD 502 model meter), taking the average of three physiologically mature leaves (three readings from each leaf) per tree. Biweekly measurements were made from the start of the application.

Photosynthesis

Photosynthetic activity (μmol CO2 cm-2 s -1) was measured with the IRGA LI-6400-XT equipment, between 11:00 a.m. and 2:00 p.m. (with light saturation), following the manufacturer's instructions. , in two phenological stages of the crop: vegetative development in July 2021 and harvest in December 2021.

Plant height

Measurement of the length (cm) from the base of the stem to the apex of the plant. The measurements were made weekly from the first application of the treatments.

Yield, number and weight of fruit

It was determined by counting the number and weight of figs in total kilograms for each tree (experimental unit). The result was extrapolated in terms of tons per hectare (t ha^{-1}).

Weight loss in fruit

The weight of the fruit (g) was taken every five days during a period of 20 days, and the calculation was made regarding the initial weight after harvest.

Penetration resistance

Fruit firmness was measured using a penetrometer (FT 10 Wagner Instruments). The force required (lbF) to penetrate the equatorial epidermis of the fruit was recorded. (Baldoni *et al.*, 2016).

Grados Brix (°Bx)

The total soluble solids content (°Bx) was measured by refractometry, using a digital refractometer (HANNA 96801) and following the methodology used by Soberanes, Calderón, López y Alvarado (2020).

Statistic analysis

An analysis of variance and comparison of means was carried out by means of the Tukey test ($P \leq 0.05$), with the statistical program IBM-SPSS Statistics 22, for the variables of height, chlorophyll and photosynthesis.

Results

Soil microbiological analysis

A count of microorganisms (CFU g-1 of soil) was carried out by morphological identification prior to the applications and 15 days after the start of applications, where the highest concentration of inoculated microorganisms was found (table 1).

Table 1. Viable microorganisms in fig orchard soil (UFC g-1 of soil). Before and 15 days after the start of the applications

Count of viable microorganisms (UFC g-1 of soil)					
		B. subtilis	*B. cereus*	*P. fluorescens*	*T. harzianum*
Prior to application of treatments		3.6×10^2	ND	2.3×10^2	ND
15 days after the application of treatments	T1	3.4×10^3	ND	2.8×10^2	ND
	T2	5.3×10^7	5.2×10^5	1.47×10^8	2.1×10^6
	T3	2.6×10^3	ND	2.8×10^2	2.3×10^6
ND: not detected					

Source: self made

Chlorophyll

Trees treated with T2 and T3 present higher values of chlorophyll 3.5 UC (9.9 %) and 3.11 UC (8.8 %), respectively, compared to T1, although no significant differences were shown in the increase in chlorophyll units (UC).

Height

The plants treated with T3 showed higher values in height increase (6.5 %) compared to the plants treated with T1, without presenting a significant difference.

Photosynthesis

Photosynthetic activity (µmol CO2 cm-2 s-1) was evaluated using the IRGA LI-6400-XT equipment, taking measurements between 11:00 a.m. and 2:00 p.m. (with light saturation), following the manufacturer's instructions. in two phenological stages of the crop: vegetative development in July 2021 and harvest in December 2021. The plants treated with

T3 presented a significant difference with T1 in the month of July; for the measurements made in December, both T2 and T3 presented a significant difference with T1 (table 2).

Table 2. Effect of the inoculation of growth promoting microorganisms and agricultural plaster on the photosynthetic activity of fig trees

Treatment	Photosynthesis (µmol CO_2 cm^{-2} s^{-1})	
	July 2021	December 2021
T1	11.08 b	11.13 b
T2	12.57 ab	13.75 a
T3	12.74 a	13.59 a
Values with a different letter in a column are significantly different ($P \leq 0.05$).		

Source: self made

Foliar nutritional analysis

Table 3 shows the mineral content determined in the fig leaves after two periods of application. The results reveal that most of the values were within or above the sufficiency values established by Moreno, Pulgar, Víllora and Romero (1998) and Brown (1994). According to the criteria of Moreno et al. (1998), deficiencies were observed in the content of N, Cu and Zn. Specifically, there was N deficiency at two and seven months of application in T1 and T2; there were also insufficient copper values two months after application in T1 and T2; and Zn deficiency was found at seven months in T1, and at two and seven months in T3; however, considering the reference values of Brown (1994), deficiencies of Ca and Mg occurred. Particularly, Ca deficiency was found at two months in the three treatments; and an insufficient concentration of Mg was also observed at two months in the three treatments, and at seven months in T1 and T3.

Table 3. Foliar nutritional analysis in fig trees

		Macronutrients				Micronutrients			
Time after treatment application (months)	Treatment	N	P	K	Ca	Mg	Fe	Cu	Zn
		(%)				(ppm)			
2	T1	1.8	0.2	2.3	1.66	0.39	300	7	30
	T2	2.2	0.31	3.7	1.68	0.3	600	14	40
	T3	1.92	0.29	2.3	1.6	0.5	450	34	10
7	T1	1.6	0.71	2.62	2.89	0.4	208	27	29
	T2	1.62	0.71	3.4	2.94	0.74	223	34	31
	T3	2.94	0.64	3.15	3.5	0.61	218	30	20
	*	2.64-2.66	0.14-0.15	1.1-1.7	0.34-0.35	0.04-0.05	183-189	15-21	30-35
	**	1.5-2.3	0.08-0.14	0.25-1.4	2.8-3.5	0.7-0.9	78-165	4-8	9-14

* Moreno *et al.* (1998)

** Brown (1994)

Source: self made

Postharvest evaluations

Yield, number and weight of fruits

The plants treated with T2 (38.35 %) and T3 (41.98 %) obtained higher yields compared to those treated with T1 (table 4). Also, the trees treated with T2 (60 %) and T3 (100 %) presented a greater number of fruits compared to T1 (table 4). Regarding the weight variable, in the treatments T2 (273 g) and T3 (299.8 g) a significant difference was observed in comparison with the control (table 4).

Table 4. Number, weight and yield in fig fruits

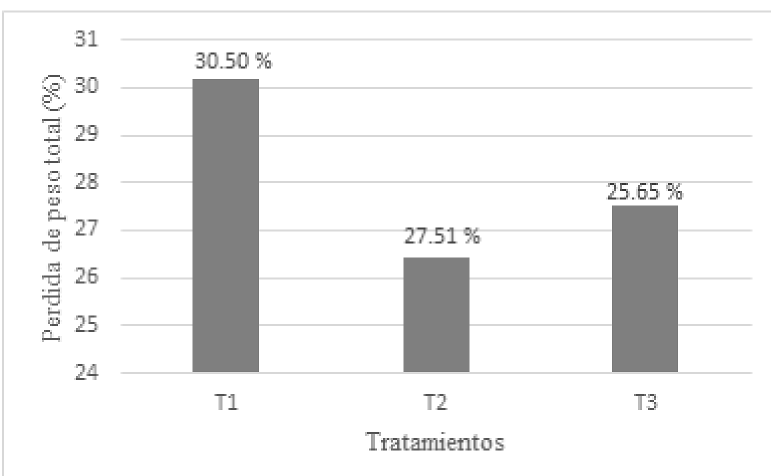
Treatment	Total number of fruits per treatment	Fruit weight (g) total per treatment	Yield (t ha-1)
T1	212	415.5 b	12.8 b
T2	340	689.3 a	21.26 a
T3	424	715.3 a	22.06 a
T1:Control, T2: Bacterial consortium (*Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas fluorescens*), *Trichoderma harzianum* and agricultural plaster, T3: *Trichoderma harzianum* and agricultural plaster. Values with a different letter in a column are significantly different (Tukey, P ≤ 0.05).			

Source: self made

Fruit weight loss

Regarding the percentage of weight loss accumulated for 20 days in fig fruit, the plants treated with T1 lost a higher percentage of moisture (30.50 %) compared to T2 (27.51 %) and T3 (25.65 %) (figure 1).

Figure 1. Weight loss in fruit of trees treated with bacterial consortium and *T. harzianum* with agricultural plaster (T2) and *T. harzianum* with agricultural plaster (T3) and control (T1)

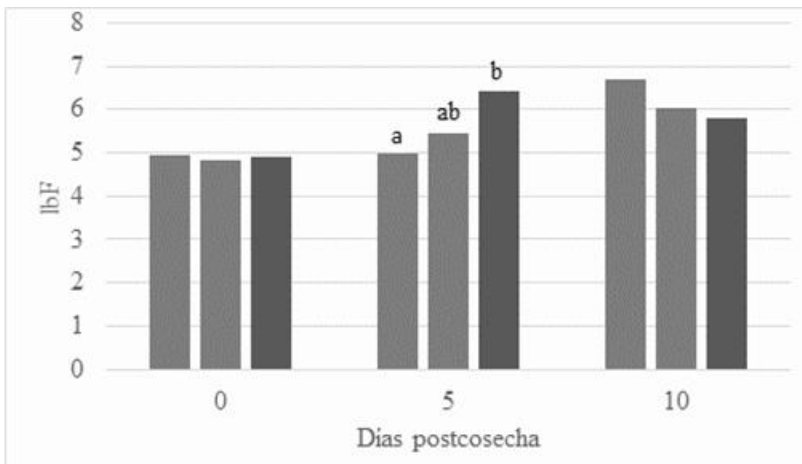


Source: self made

Penetration resistance

The fruits treated with T3 (7.2 lbF) maintained greater firmness, presenting a significant difference on the fifth day postharvest with values higher than T1 (4.6 lbF) (figure 2).

Figure 2. Resistance to penetration (lbF) of fig fruits at 0, 5 and 10 days postharvest; a, b different letters indicate significant differences between treatments (Tukey, P ≤ 0.05)

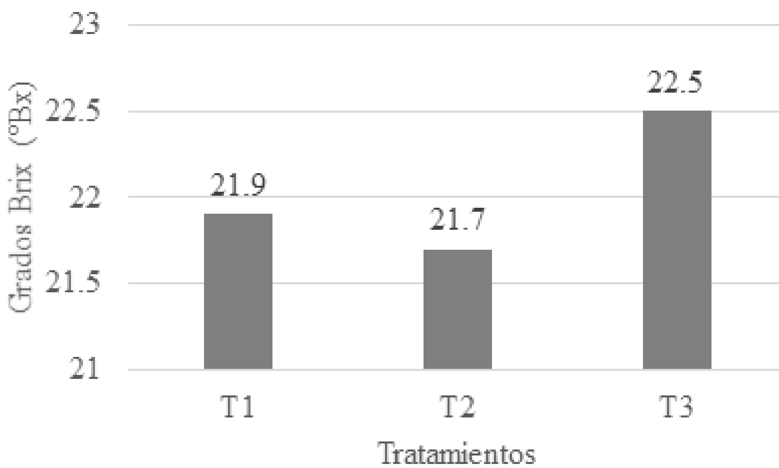


Source: self made

Degrees Brix (°Bx)

The fruits of T3 (22.5 °Bx) presented a higher sweetness value compared to T1 (21.9 °Bx) and T2 (21.7 °Bx) (figure 3).

Figure 3. Brix degrees (°Bx) in fruits treated with bacterial consortium and *T. harzianum* with agricultural plaster (T2) and *T. harzianum* with agricultural plaster (T3) and control (T1)



Source: self made

Discussion

The results obtained indicate that the inoculated microorganisms were able to establish themselves in the rhizosphere. For the inoculation with MPCV to be successful, they must be established and interact with the host plant, as well as maintain biological activity (Restrepo et al., 2015, Villarreal et al., 2018). Díaz, Ferrera, Almaraz and Alcantar (2001) found that after several inoculations the MPCV are able to establish themselves and multiply. The strains inoculated in the present study have been cited as promising MPCV in studies in liquid formulation (Cantú et al., 2021; Leal et al., 2018).

On the other hand, the application of agricultural gypsum helps the establishment of the MPCVs (Leal et al., 2018), by favoring a temporary acidification in the soil, since the optimal pH of these microorganisms is slightly acid (Shah et al., 2021).

The increase in chlorophyll in plants treated with T2 and T3 agrees with what was observed by Pandey et al. (2016) in rice plants treated with T. harzianum, which showed a higher chlorophyll content compared to control plants, due to the increase in nitrogen available to the plants due to the action of the fungus. Likewise, Thakur and Niranjan (2018) obtained an increase in chlorophyll in strawberry plants and attribute it to the balanced nutritional environment in the soil due to inoculation with MPCV.

Regarding the height, the plants treated with T3 presented higher values. Among the main properties of Trichoderma are its ability to protect plants and contain populations of pathogens under different soil conditions (Bhale, 2020; Vinale et al., 2008), the increase in mineral solubilization/absorption (Li et al., 2015) and the production of substances that stimulate plant growth (Bécquer et al., 2015). Przybyłko, Kowalczyk and Wrona (2021) pointed out that fungi help plants to take up N and Mg.

The N values found coincide with those found by Brown (1994) for orchards with low-vigor fig trees, as in the present study, since this was the orchard's first productive year. The increase presented in N could be due to the fact that the inoculated MPVC make it more available and easy to be absorbed by plants (Rojas et al., 2022; Rubio et al., 2017). Regarding the concentration of P, K, Ca, Mg and Fe, it is above the optimal values in fig according to Moreno et al. (1998). High phosphorus values may be due to the action of bacteria of the genus Pseudomonas, which have been widely studied as phosphate solubilizers (FAO, 2018).

Regarding photosynthesis, it could be observed that the inoculation with T2 and T3 helped the photosynthetic rate, this may be due to the increase in the availability of nutrients due to the inoculation with MPCV, as Arıkan and Pırlak (2016), who found an increase in the photosynthetic rate in apple trees due to inoculation with salinity-resistant bacteria.

The combined inoculation of MPCV and agricultural plaster favored the increase in fruit production. This agrees with what was found by Sánchez, Gómez, Garrido and Bonilla (2018) in tomato fruit production, in plants inoculated with Enterobacter and Pseudomonas putida, which exceeded the chemical control (29 %) and the absolute control (17 %). . In addition, in studies with sour cherry (Prunus cerasus L.) treated with Bacillus, the yield in number and weight of the fruit increased (Arikan and Pirlak, 2016). The weight of the fruits is related to the content of Ca, an element that confers firmness to the wall and membrane (Soberanes et al., 2020), in this sense it has been documented that bacteria such as P. fluorescens and the fungus T. harzianum release P and Ca present in the form of calcium phosphate, so that the T2 and T3 treatments may be favoring the fruits to present greater weight by maintaining integrity for a longer time (Otieno et al., 2015). Likewise, works carried out on citrus with MPCV inoculation showed an increase in the size and weight of the fruit (Abobatta and El-Azazy, 2020).

Work carried out on quince plants inoculated with Bacillus showed an increase in yield per tree, specifically 35.6 % in plants treated with Bacillus OSU-142 and 44 % in plants treated with Bacillus T8, compared to the control (Arıkan, İpek and Pırlak, 2013), as well as the inoculation of T. asperellum in the soil of the blackberry crop (Rubus glaucus, Benth) positively affected productivity (17 %) compared to the control (Viera et al., 2019). The change in firmness is one of the main indicators of postharvest quality in the fruits (Baldoni et al., 2016). Our results coincide with the values obtained by Sozzi, Abraján, Trinchero and Fraschina (2005) on loss of firmness in the fruit of the Brown Turkey variety fig, who documented loss of firmness on the third day of storage. Regarding weight loss, the fruits of the trees treated with MPCV and agricultural plaster maintained their weight for longer, this is very important since softening and weight loss are the factors responsible for the decrease in postharvest life. (Allegra et al., 2017).

Regarding the result obtained in °Bx, the fruits of the plants treated with T3 surpassed T2 and T1. This result coincides with the work carried out by Lombardi et al. (2020) in strawberries, with the application of metabolites from T. harzianum, where the percentage of °Bx increased compared to the control. In the present investigation, higher °Bx values were obtained than those found by Soberanes et al. (2020) in figs of the Netzahualcóyotl variety (14.86-17.3 °Bx) but they coincide with the intervals mentioned by Villalobos et al. (2016) in three fig cultivars, who also argue that these higher values in °Bx are due to high levels of radiation reached in the area of Guadajira, Badajoz, Spain. In the present study we can infer that the high values of °Bx are partly due to the high radiation reached in the Yaqui Valley. (Pinto, Lopes, Collins y Reynolds, 2016).

Conclusions

With the application of treatments T2 and T3, an improvement was obtained in the variables of photosynthesis, fruit number, fruit weight and yield.

Likewise, the inoculation with T3 helped the resistance to penetration and the increase in Brix degrees in the fig fruits.

Future lines of research

For future research, it is proposed to continue with the application of the treatments for at least two production cycles. In addition, try different combinations of the MPCV with and without agricultural plaster, in order to verify that this helps the establishment of the MPCV. Finally, it is recommended to evaluate different methods of reproduction of microorganisms that are more efficient and less costly.

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