Investigation note

Pseudomonas fluorescens UM270 promotes growth and production in husk tomato

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Abstract

One of the agroecological strategies that increases agricultural production is the use of bacterial inoculants, which lack the toxic effects that agrochemicals have. This work evaluated the effect of inoculation of the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* UM270 on the cultivation cycle of husk tomato (*Physalis ixocarpa*) plants under field conditions (irrigation) in 2019. The results showed that plants inoculated with the rhizobacterium UM270 exhibited significant effects on plant height (14.64%), stem diameter (17.74%), biovolume index (35.14%) and fruit set production by 65.54%. This suggests that the strain UM270 of *P. fluorescens* is an excellent bioinoculant that improves the production of the husk tomato crop under field conditions.

Keywords: *Physalis ixocarpa*, *Pseudomonas fluorescens*, agrochemicals, rhizobacteria.

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Husk tomato (*Physalis* spp.) is a crop of forage, medicinal, ornamental, industrial and human consumption importance, and includes about 100 species distributed in the American continent (Santiaguillo-Hernández and Blas-Yáñez, 2009). Mexico is the country considered the center of domestication of this genus (Engels *et al*., 2006), where about 70 wild species are recognized, although *Physalis ixocarpa* Brot. Ex Horm (= *P. philadelphica* Lam.) and *P. angulata* are grown for edible purposes. The cultivation of *P. angulata* is restricted to the state of Jalisco and that of *P. ixocarpa* is widespread in various regions of the country (Caballero-Salinas *et al*., 2020).

The husk tomato crop has great economic importance in our country; however, it is seriously affected by various pathogens (Ayala-Armenta *et al*., 2020). For these reasons, it is important to explore other ecological options that do not involve the use and application of mineral fertilizers or synthetic pesticides. One option is the use of plant growth-promoting bacteria (PGPB). PGPB are capable of forming close associations with plants, either through the colonization of roots (rhizosphere), internal tissues (endosphere) or aerial tissue surfaces (phyllosphere) (Orozco-Mosqueda and Santoyo, 2021).

Being part of the plant microbiome, PGPB can produce antibiotics, lytic enzymes, among other metabolites such as siderophores, volatile organic compounds, among others, to inhibit the growth of potential pathogens. (Orozco-Mosqueda *et al*., 2023). Other beneficial activities of PGPB include improving plant nutrition through nutrient solubilization, as well as inducing the plant's immune system. One of the most widely used species in pathogen biocontrol and plant growth promotion is *Pseudomonas fluorescens* (Garrido-Sanz *et al*., 2016).

For example, the strain UM270 of *P. fluorescens* has been widely characterized as a biocontroller of pathogenic fungi and a promoter of plant growth, including *M. truncatula* and *P. ixocarpa* (Hernández-León *et al*., 2015; Rojas-Solis *et al*., 2016). However, previous works have been done *in vitro*, not in the field. Thus, in this study it is hypothesized that the inoculation of *P. fluorescens* UM270 during a growth cycle of husk tomato plants significantly improves various phytometric and production parameters under field conditions.

A total of 120 seeds of husk tomato (*Physalis ixocarpa* Brot. ex Horm) were placed on gemination trays and irrigated with running water. Transplantation of seedlings was carried out 22 days later, planting was carried out in a loamy-sandy soil. The experiment was carried out from September to December 2019, in a plot located in the locality of Uruapan, Michoacán, whose geographical location is as follows: 19° 25' 37.9" north latitude 102° 01' 24.3" west longitude. The physicochemical properties of the soil were analyzed at INIFAP-Celaya, with the following characteristics: the soil is loamy, with 53.48% sand, 19.60% clay and 26.92% silt, and a pH of 6.32. It should be noted that during the experiment no mineral fertilizers were added. The design of the experiment consisted of randomized blocks with n= 24 plants (experimental units) per each treatment (control plants without bacterial inoculation and plants + inoculated with the strain UM270).

The strain was grown in Petri dishes with nutritive agar at 28 °C for 24 h. Subsequently, a colony was isolated and grown in a flask with 250 ml of nutrient broth with constant stirring (250 rpm) until having an optical density of 1×10^6 colony-forming units (CFU ml^{-1}). The supernatant was removed with the help of a centrifuge (at 5 000 rpm for 5 min) and the bacterial pellet was diluted in a sterile solution of $MgSO_4$ (10 mM). The prepared inocula were evaluated again for cell viability. For field inoculation, each plant was inoculated at the root periphery with a 50 ml bacterial inoculum with approximately 1 $\times 10^6$ CFU ml^{-1}.

Bioinoculation began on the day of transplantation and was repeated weekly with a total of 11 applications. The control plants without inoculation, as well as those inoculated with the strain UM270 were additionally spray-irrigated with running water every week. The data presented for analysis correspond to measurements of week 12 after transplantation. All data were processed in the social science statistics program with a *t*-test for independent means ($p< 0.05$) (Social Science Statistics, 2018).

Table 1 shows the significant differences, with an average height of 70.7 cm in the control plants and 81.16 cm for those inoculated with the strain UM270, which corresponds to an increase of 14.64% in the height of the inoculated tomato plants. These data are consistent with those published by Rojas *et al*. (2016), where they observe an increase in the size of tomato plants inoculated with the strain UM270 in *in vitro* experiments. However, they differ from the results obtained by Rocha-Granados *et al*. (2019) carried out in *Casuarina equisetifolia* plants inoculated with several strains (for example, *Bacillus* spp.), including UM270, which showed no growth promotion activity in that plant species.

The application of *Pseudomonas fluorescens* UM270 modified the thickness of the stem in the plants, at the end of the experiment significant statistical differences were observed, with averages of 5.9 mm in thickness in the control plants and 6.9 mm in those inoculated with UM270 for week 11. While for week 12, the data were 6.2 mm for control plants and 7.3 mm for those inoculated with UM270 (17.74%) (Table 1). Similar results have been reported by Patel *et al*. (2019), where they observe greater stem thickness in sugarcane plants inoculated with strains of *Ochrobactrum intermedium* (TRD14), *Acinetobacter* sp. (PK9) and *Bacillus* sp. (RSC29 and KR91), compared to uninoculated plants. This indicates that in some way the PGPB induces greater stem thickness, since the plants, being larger, develop a more vigorous stem.

Table 1. Phytometric parameters evaluated in husk tomato plants inoculated with the strain UM270 of *Pseudomonas fluorescens* compared with the control without inoculation.

Phytometric parameters evaluated	Control plants	Plants + UM270	Increase (%)
Plant height (cm)	70.7	81.16*	14.64
Stem diameter (mm)	6.2	7.3*	17.74
Biovolume index	44	59.6*	35.14
Total fresh weight of plant (g)	76.75	196.25*	155.7

The asterisk (*) indicates a statistically significant difference $p< 0.05$, *t*-test.

The biovolume index is a measure of plant growth promotion, which relates plant height and stem thickness (Flores *et al*., 2020). The inoculation of *P. fluorescens* UM270 in husk tomato plants showed statistically significant differences in the variable biovolume index, with an average of 44.1 for control plants compared to 59.62 for plants inoculated with UM270. In percentage, the biovolume index increased by 35.14% in inoculated plants.

Figure 1 shows representative plants without inoculation and inoculated with the strain UM270. The statistical test shows significant differences in fresh plant weight. The values for control plants (without inoculation) were on average 76.75 g, while for plants inoculated with UM270, they reached an average of 196.25 g. These data are related to those previously presented by Rojas-Solis *et al*. (2016) for UM270, where growth promotion capacities are observed in tomato plants. The authors conducted these experiments in greenhouse and in co-inoculation with the strain UM96 of *Bacillus thuringiensis*. However, when they inoculated only the strain UM270, they identified higher fresh and dry weight in the plants.

Figure 1. Representative husk tomato plants inoculated with the strain UM270 of *Pseudomonas fluorescens* compared to the control (without inoculation).

Based on the records of the strain and the results reported in this work, it is proposed that the effect of the volatile organic compounds produced by the strain, the ability to synthesize siderophores or even the activity of the enzyme ACC deaminase to lower ethylene levels in plants (Hernández-León *et al*., 2015) could be stimulating the growth of husk tomato plants in the field.

The last parameter evaluated was to know if the inoculation of UM270 represents an advantage to increase tomato production in the field, so the number of fruits produced in tomato plants was counted. Thus, the application of *P. fluorescens* UM270 showed a significant increase in the production of fruit set per plant, with a total of 2 205, compared to the control plants, whose production was 1 332 fruits (Figure 2).

This result means an increase in production of 65.54% in plants inoculated with the strain UM270. It should be clarified that in these experiments no chemical fertilizers were added, only the nutrients that were in the soil and the nutritional effect that improved in the plants by inoculation with the strain UM270. It should be noted that other beneficial strains of bacteria such as *Atlantibacter* sp. have also shown beneficial effects in improving the nutrition of seedlings of tomato (*Physalis ixocarpa*), increasing some elements such as K, Ca and Mg (Ramírez-Cariño *et al*., 2023).

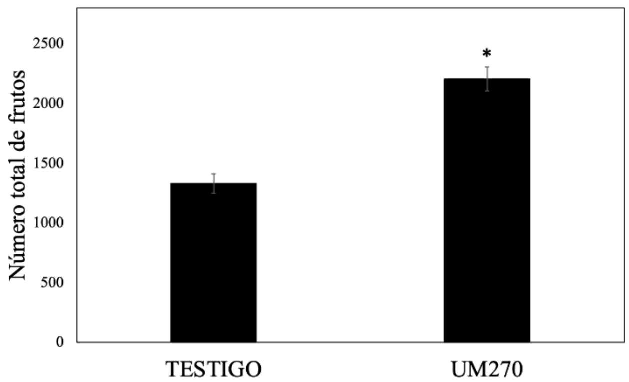

Figure 2. Total fruits harvested from plants inoculated with the strain UM270 of *Pseudomonas fluorescens* and compared with those harvested from control plants without inoculation. The asterisk (*) indicates a statistically significant difference $p < 0.05$, *t*-test.

Recently, Aguirre-Medina and Espinosa (2016) mention that the inoculation of a strain of *Pseudomonas fluorescens* increases the number of fruits produced in *Capsicum annuum* L. compared to the controls without inoculation. Castro-Barquero *et al*. (2015) also report that inoculation of a strain of *Pseudomonas* managed to increase the biomass in the tomato crop compared to the control without inoculation.

Conclusions

The inoculation of the strain UM270 of *P. fluorescens* stimulated the growth of *Physalis ixocarpa* and the production of fruits in the field, suggesting that this strain could be used as a sustainable alternative in the cultivation of this vegetable.

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