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Mitigating Chili Plant Wilt: Synergy of Arbuscular Mycorrhizal Fungi and Silver Nanoparticles

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ABSTRACT

Background/Objective. The chilaca pepper (*Capsicum annuum*) is significantly impacted by the attack of the oomycete *Phytophthora capsici*, which causes chili wilt. Current methods for controlling this disease have been inefficient. Therefore, the search for more environmentally friendly alternatives is of great importance. In pursuit of this objective, we assessed the potential of arbuscular mycorrhizal fungi (AMF) and silver nanoparticles (AgNPs) to try to reduce or postpone chili pepper wilt.

Materials and Methods. Growth parameters were measured in inoculated and non-inoculated chili pepper plants with AMF from a commercil consortium TM-73 (Biotecnología Microbiana) and the protective effects of AMF and AgNPs (NanoID®) against *P. capsici* as evaluated using a severity scale for wilt symptoms. Plant response to pathogen infection was assessed by measuring the activities of antioxidant enzymes: PER, SOD, CAT and H_2O_2.

Results. The results indicated that AMF application improved the growth parameters of *C. annuum*, while the plant-pathogen interaction induced an antioxidant enzymatic response. AMF maintained wilt symptoms at or

below 80%, preventing plant death. Meanwhile, AgNPs (50ppm) delayed plant mortality compared to the control treatment.

Conclusion. The combined use of AMF and AgNPs offer options for future research in the disease management for chili peppers.

Keywords: Antifungal activity, enzymatic activity, oxidative stress, wilting, nanotechnology.

Introduction

The chili pepper (*Capsicum annum*) is one of the largely important crops worldwide (Hasan *et al*., 2020). This vegetable is one of the most important in Mexico, since it is the country with the greatest variety of chili peppers planted and it is the world's largest exporter of green peppers. A total of 165,226 hectares of peppers was planted in Mexico in 2023, with Chihuahua being the second largest chili pepper producing state (SIAP, 2023). The crop is affected by diverse diseases out of those caused by root pathogens. These are very difficult to control and they represent one of the main factors that reduce yield, making it a threat to food security (Rizzo *et al*., 2021). In this context, the chilaca pepper is one of the most affected by these diseases. Pathogens of the *Fusarium, Rhizoctonia* and *Phytophthora* genera cause reductions of up to 50% in the production (Velarde-Félix *et al*., 2018). In particular, studies on *Phytophthora capsici* have proven this pathogen to be one of the most devastating, leading the crop to complete losses (Sánchez-Gurrola *et al*., 2019).

Fast-acting chemical fumigants and fungicides, such as mefenoxam (active enantiomer of metalaxyl), have been used against these pathogens (Sánchez-Gurrola *et al*., 2019; Shi *et al*., 2022). However, its use has led to the development of resistance in pathogens and has been harmful for the soil ecosystem and human health (Ma *et al*., 2023). Consequently, the current tendency in research in the world is directed towards more eco-friendly approach, in an attempt to reduce the use of organosynthetic products for the control of diseases (Eke *et al*., 2019).

Thus, the use of arbuscular mycorrhizal fungi (AMF) is a promising option within the integrated management of *P. capsici*. These fungi have been proven to act as antagonists of the pathogens (Hashem *et al*., 2021), as well as to increase the growth and yield of plants. Likewise, they provide tolerance to diverse stress and toxicity factors by heavy metals (Dowarah *et al*., 2022; Liu *et al*., 2023).

On the other hand, the metallic nanoparticles have attracted great attention due to their biocidal effect and their application as antimicrobials in crops (Bawskar *et al*., 2021; Ávila-Quezada *et al*., 2023). The impact of these nanoparticles on phytopathogenic fungi is due mainly to the damage they cause in the dynamics of the fungal membrane, which may compromise their integrity (Athie-García *et al*., 2018). Among the metallic nanoparticles, silver nanoparticles stand out for their antifungal potential, being widely studied in the agricultural context against diverse root pathogens, including *P. capsici* (Shen *et al*., 2020; Li *et al*., 2022; Ávila-Quezada and Rai, 2023a).

Both AMF (Hashem *et al*., 2021) and metallic nanoparticles (El-Shetehy *et al*., 2021) promote the systemic defense of plants. AMF induce the activity of defense enzymes such as glutathione reductase and catalase (CAT), which play a crucial role in the elimination of reactive oxygen species (ROS) (Hashem *et al*., 2021). In addition, they exert an influence on the activities of CAT, superoxide dismutase (SOD) and peroxidase (POD) (Wang *et al*., 2022).

Due to the worldwide importance of the pepper and to the susceptibility of the crop to the attack of *P. capsici*, the aim of this study was to analyze the effect of arbuscular mycorrhizal fungi and silver nanoparticles in the reduction of the wilting of chilaca pepper plants, as well as to determine the activity of SOD, CAT, PER enzymes and H_2O_2 concentration.

Materials and Methods

This investigation was carried out under uncontrolled weather conditions, using a shade mesh; the preparation of solutions, the production of zoospores and the percentage of mycorrhizal colonization were performed in the Faculty

of Agrotechnological Science of the Universidad Autónoma de Chihuahua. The analysis of enzyme activities was performed in the plant nutrition laboratory of the Centro de Investigación en Alimentación y Desarrollo in Delicias, Chihuahua.

Chili pepper seedling production. Colegio 64 chilaca pepper seeds (Semillas Western, S.A. de C.V.) were placed in a polystyrene tray with 200 wells, using a sterile mixture of vermiculite-perlite (2:1) as a substrate. Twelve days after germination, a nutrient solution was applied, based on the formula proposed by Steiner (1961), adjusted to a final pH of 5.5 and with a phosphorous concentration of 22 ppm. The composition of the nutrient solution is as follows: 6 mM of NH_4NO_3, 0.71 mM of K_2HPO_4, 0.3 mM of K_2SO_4, 4.0 mM of $CaCl_2$ •$2H_2O$, 1.4 mM of $MgSO_4$ •$7H_2O$, 2 µM of $MnSO_4$ •H_2O, 1.0 µM of $ZnSO_4$ •$7H_2O$, 0.25 µM of $CuSO_4$ •$5H_2O$, 0.3 µM of $(NH_4)6Mo_7O_{24}$ •$4H_2O$ and 0.5 µM of H_3BO_3 (J.T. Baker, Mexico). The seedlings were transplanted into 13x12 cm pots, using the same substrate mixture, 30 days after germination.

Inoculation with AMF. During the transplant, carried out 30 days after germination, 1000 spores of the commercial consortium TM-73 (Biotecnología Microbiana, Mexico), containing the arbuscular mycorrhizal fungi (*Gigaspora margarita, Glomus fascilatum, Glomus constrictum, Glomus tortuosum, Glomus geosporum* and *Acaluspora scrubicurata,* were incorporated into 20 chilaca pepper plants. AMF inoculation was carried out to determine its effect on growth, comparing them with 40 control plants. The growth variables evaluated 30 days after AMF application (60 days after the seed germination) were plant height, stem diameter and number of leaves. The stem diameter was measured at the base (mm) using a digital caliper. Plant height was determined from the base of the stem to the tallest point of growth (cm). The number of leaves was counted manually for each plant (number of pieces).

Mycorrhizal colonization. The percentage of mycorrhizal colonization was determined in three chilaca pepper plants 30 days after AMF inoculation. To confirm root colonization, the roots were dyed with trypan blue, according to the methodology described by Phillips y Hayman (1970) and Muñoz-Márquez *et al*. (2009). The roots were rinsed with tap water and were then soaked in KOH at 10%

(J.T. Baker, CTR, México), followed by a hot wet treatment in an autoclave at 1 atmosphere pressure for 10 min. Subsequently, the KOH was removed and three washes were performed with distilled water. Later, the roots were exposed to H_2O_2 cover and allowed to stand for 30 min, followed by rinsing and addition of ClH at 10% (J.T. Baker, CTR, Mexico), with a resting time of 15 min. After removing the excess ClH, a trypan blue solution (J.T. Baker, CTR, Mexico) was added and the roots were placed under hot wet treatment once again, for 15 min at 1 atmosphere pressure.

Subsequently, the 1 cm roots were placed on a slide, obtaining a total of 100 roots per treatment. These were observed in a binocular microscope (ZEISS model Axiostar, Germany). The percentage of total mycorrhizal colonization (including mycelia, arbuscules, spores, vesicles) was calculated using the following formula:

$$\% \text{ colonization} = \frac{\text{Number of colonized segments x } 100}{\text{Total number of segments}}$$

Application of AgNPs. Sixty days after the seed germination, the treatment with NanoID® (Investigación y Desarrollo de nanomateriales S.A de C.V.), which contains AgNPs of 20 nm and a concentration of 3400 mg/L. A total of 0.5 mL of AgNPs were applied in a colloidal solution at a concentration of 50 ppm at a depth of 1 cm under the soil surface, directly on the secondary roots of the plants.

Production of zoospores and inoculation of *P. capsici*. Growth of *P. capsici* previously isolated from diseased bell pepper roots in Delicias, Chihuahua (NCBI: KM369965) was performed on oat agar at 28 °C for 7 days of growth. A portion of this growth was transferred to a new Petri dish, covered with a sterile phosphate buffer solution and incubated at 28 °C for 72 h. Subsequently, it was incubated at 4 °C for 1 h, followed by an hour of incubation at 25-28 °C. The zoospores emerged from the sporangia, which was confirmed by observation under an optical microscope (ZEISS Axiostar model, Germany).

The pathogen was inoculated by adding 1 mL of a *P. capsici* zoospore solution at a concentration of $1x10^6$, along with the root of each plant at the same place where AgNPs were applied, at a depth of 2 cm below the soil surface, 61 days after the seed germination, 24 hours after AgNPs application.

Disease severity. The disease severity was evaluated every day after the inoculation of *P. capsici* using the scale by Sunwoo *et al*. (1996) with slight modifications (Table 1).

Table 1. Scale of severity of wilting by *P. capsici* in *C. annuum*.

Scale	Symptoms	Percentage
0	Healthy plant	0
1	Leaves slightly withered, spread drooping downwards	1-20
2	Plant with leaves extended on the surface and those below curled	21-40
3	Plant with all the leaves curled up	41-60
4	Defoliated plant, green living stem	61-80
5	Living stem brown to dead plant	81-100

Experimental design and treatments. Each treatment included 10 plants.

I. AgNPs + *P. capsici*
II. AMF + *P. capsici*
III. Positive control with *P. capsici*
IV. Negative control (absolute control)
V. AMF
VI. AgNPs

A completely randomized design with different replicates per treatment was established to evaluate the disease severity, which was measured every day from the *P. capsici* inoculation until day 12.

Enzyme activity. Enzyme activity was measured in young leaves three days after the infection with the pathogen, which were frozen for their subsequent processing.

Four biochemical parameters were measured to investigate the plant response to mycorrhizal colonization and *P. capsici,* including the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (PER) and the concentration of hydrogen peroxide (H_2O_2).

Superoxide Dismutase (SOD). Between 0.3 and 0.5 g of plant tissue from each treatment was used. The sample was homogenized in 5 mL of Heppes-ClH 50 mM buffer at pH 7.6 and centrifuged at 11000 rpm for 10 min. Out of the enzyme extract (supernatant) 100 µL were used, along with 5 mL of reaction buffer to determine enzyme activity. Readings were taken at 560 nm in a spectrophotometer equipped

with a blue daylight lamp with a light intensity of 380 µmol m^{-2} s^{-1} (Giannopolitis and Ries, 1977). The enzyme activity was calculated using the following formula:

SOD Activity = SOD Units / mg Protein

Concentration of hydrogen peroxide (H_2O_2). Between 0.5 and 1 g of frozen plant tissue from each treatment was used. The sample was homogenized in 5 mL of cold acetone, filtered and two additional washes were carried out with cold acetone. Subsequently, it was centrifuged at 3500 rpm for 5 min. From the filtrate, 2.5mL were taken and 0.5mL of 20% $TiCl_4$ was added and vigorously shaken. Then, 2.5mL of 20% NH_4OH was added to the mixture and centrifuged at 3500 rpm for 5 min. The supernatant was discarded and the precipitate was washed with cold acetone. The precipitate was resuspended in 7.5mL of 2N H_2SO_4 and diluted to 12.5mL with distilled water. The H_2O_2 concentration was determined using a calibration curve over a range of 0.1-0.75nM H_2O_2 with absorbance measured at 485 nm (Brennan and Frenkel, 1977). The H_2O_2 concentration was expressed as:

H_2O_2 = μmol of H_2O_2 / mg

Catalase (CAT). Between 0.5 and 1 g of frozen plant tissue from each treatment was weighed. The sample was homogenized in 5 mL of 25mM Heppes-ClH buffer (pH 7.8). Subsequently, it was filtered through four layers of gauze and centrifuged at 11500 rpm for 20 min. A total of 500 µL of the enzyme extract (supernatant) was used and 0.75 mL of 25 mM sodium phosphate buffer, 0.75 mL 0.8 EDTA-Na and 1 mL of 20 mM H_2O_2 were added for the enzyme activity determination. CAT activity was measured by the amount of oxidized H_2O_2, using absorbance measured at 240 nm (Buturi *et al*., 2022).
Enzyme activity was calculated using the following formula:

CAT Activity = μmol of H_2O_2 / mg Protein x min

Peroxidase (PER). Peroxidase activity was determined by measuring the change in absorbance at 485 nm due to the oxidation of guaiacol, according to the following procedure. Between 0.5 and 1 g of frozen plant tissue from each treatment was used. The sample was homogenized in 5 mL of 25 mM Heppes-ClH 25 buffer (pH 7.8) and PVPP. Subsequently, it was filtered and centrifuged at 11500 rpm for 10 min. Of the enzyme extract (supernatant) 500 µL were used, to which 0. 5 mL of

25 mM sodium phosphate buffer, 0.5 mL of 1 µmol EDTA-Na, 0.75 mL of 0.05% guaiacol and 0.75 mL of 10 mM H_2O_2 were added. The activity of the enzyme guaiacol peroxidase was quantified measuring the absorbance of 485 nm (Kalir *et al*., 1984). This was expressed as PER units, defined as the change in an absorbance unit per min and calculated using the following formula:

$$PER = \mu mol\ guaiacol\ /\ mg\ prot\ x\ min$$

Statistical analysis. To compare the effects of AMF treatments on growth parameters, a mean difference analysis was performed using the t-Student test ($p < 0.05$), using a total of 60 chilaca pepper plants. This analysis was carried out with the SAS statistical package.

The severity analysis was based on a variable number of pepper plants per treatment, each plant being a replicate. The curves of each treatment were plotted with the daily averages and the area under the curve was calculated for each. These data underwent an analysis of variance ($p \leq 0.05$) and a means comparison using Tukey's test (95%) with the SAS statistical package.

For the analysis of enzyme activity, a total of three chili plants per treatment were used, considering each plant as a replicate. The data obtained for CAT, SOD, PER and H_2O_2 concentration were analyzed with an analysis of variance ($p \leq 0.05$) and a Tukey multiple mean comparison using the SAS statistical package.

Results

Mycorrhizal colonization. In this study it was found that the roots of chilaca pepper inoculated with the AMF consortium (*G. margarita, G. fascilatum, G. constrictum, G. tortuosum, G. geosporum* and *A. scrubicurata*), presented a mycorrhizal colonization of 36%. This colonization was distributed with 71% hyphae, 18% vesicles and 11% arbuscules.

Confirmation of mycorrhizal colonization was carried out by staining with blue trypan, which allowed us to observe a diversity of AMF structures (Figure 1). Additionally, cross-contamination was ruled out in treatments

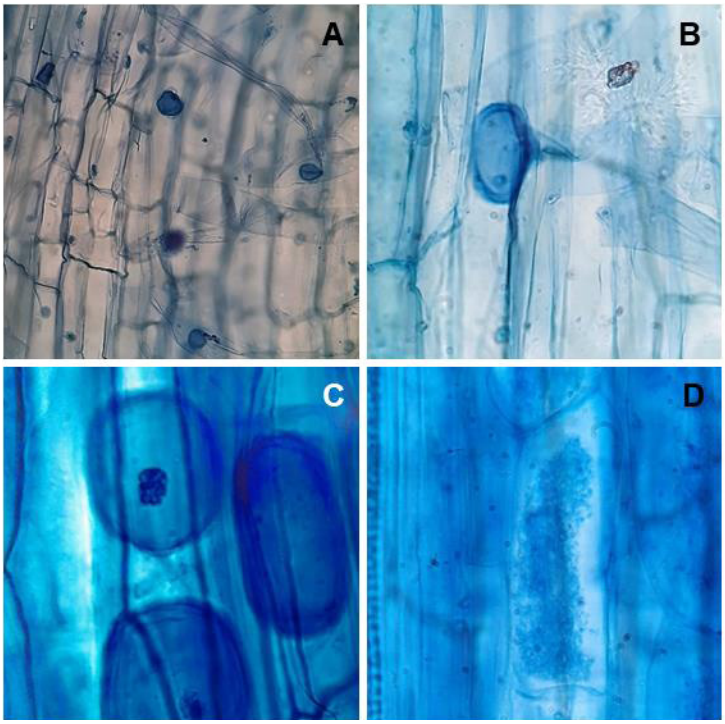

Figure 1. Mycorrhizal structures in chilaca pepper roots inoculated with AMF: A, B, C) Hyphae (H) and Vesicles (V), d) Arbuscules (A).

(I, III, IV and VI) that did not include AMF, since no mycorrhizal structures were found in the roots of these plants.

Growth parameters. The effect of the AMF on chilaca pepper plant development is presented in Table 2. AMF inoculated plants showed increasingly significant increases ($p \leq 0.05$) in all growth parameters evaluated. Specifically, an increase of 25.4, 32.67 and 17.2% was recorded in plant height, stem diameter and number of leaves, respectively, compared to plants without AMF.

Table 2. Effect of AMF in the development of the chilaca pepper (*Capsicum annuum*).

Treatment[x]	Number of leaves	Plant height (cm)	Stem diameter (mm)
With AMF	16.8±2.1[a]	19.9±3.9[a]	3.4±0.5[a]
Without AMF	13.4±2.7[b]	15.0±2.9[b]	2.9±0.6[b]

[x]Treatments: with the inoculation of arbuscular mycorrhizal fungi (AMF), without the inoculation of AMF. †Difference of means obtained with Student's t test. Different letters in each column indicate a significant difference ($p \leq 0.05$).

Severity of the disease. The first symptoms of the disease caused by *P. capsici* appeared four days after inoculation in treatments I (AgNPs + *P. capsici*), II (AMF + *P. capsici*) and III (positive control with the pathogen). On the seventh day, the three treatments showed a symptom severity of 60%. However, on the ninth day of inoculation, all plants of the control treatment (*P. capsici)* displayed a severity higher than 96%, due to the aggressiveness of the pathogen. In contrast, the plants treated with AMF maintained the same severity symptoms (60%), while the AgNPs treatment showed less effective control than AMF, with an average severity of 80% in the same period. Despite this, the AgPNs reduced wilt symptoms by 16.4% compared to the control 9 days after inoculation. Figure 2 illustrates the increase in disease symptoms according to the daily mean of the severity scale described above.

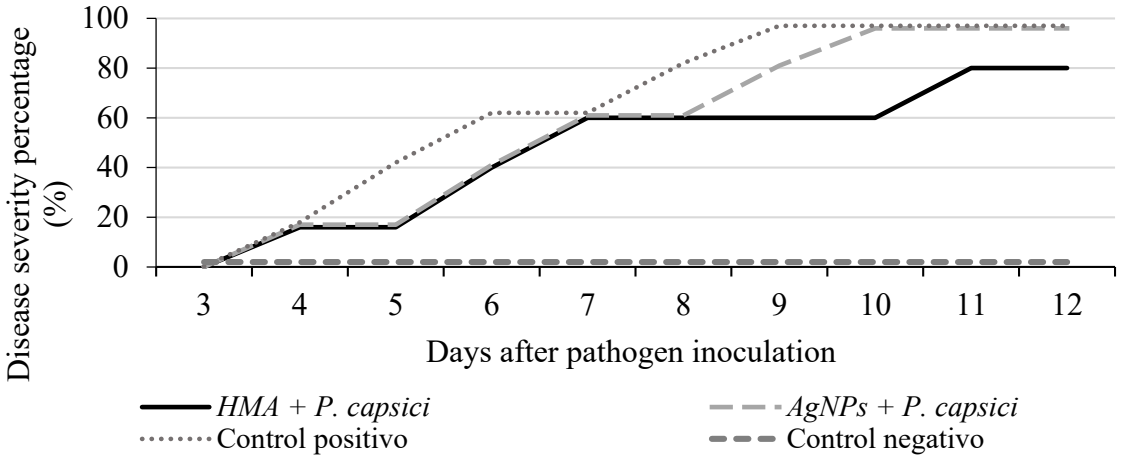

Figure 2. Percentage of severity of the disease caused by *P. capsici* in chilaca peppers. Severity was evaluated with a scale for 12 days after the inoculation of the pathogen.

The results of the area under the curve of this experiment displayed a significant statistical difference between the AMF treatment and the pathogen control. The arbuscular mycorrhizae delayed and reduced the disease severity (Figure 3). At the end of the experiment, the mycorrhizal plants did not exceed 80% severity, compared to the positive control plants, which showed 100% severity, reducing severity by 17.5% in the total number of plants tested 12 days after inoculation.

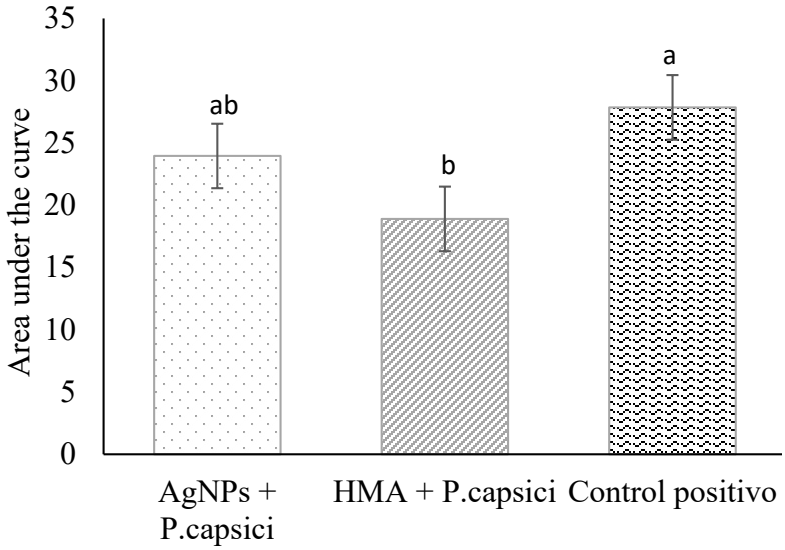

Figure 3. Comparison of averages of the area under the curve of the scale of severe diseases caused by *P. capsici* in chilaca peppers. Standard error 5%. Different letters in each column indicate a significant difference ($p \leq 0.05$).

Enzyme activity

Superoxide dismutase (SOD). The analysis of variance revealed no significant differences ($p > 0.05$) between treatments for SOD enzyme activity. The treatments corresponding to AgNPs+*P. capsici*, AMF+*P. capsici* and the positive control presented higher enzyme activity with (214.8, 199.8 and 181.8 U/mg prot) respectively. This represented an increase of 84%, 70% and 54% with regard to the negative control in SOD activity 3 days after inoculation with *P. capsici* (Figure 4).

Hydrogen peroxide (H_2O_2). The highest H_2O_2 concentration was observed in the treatment inoculated with the pathogen (AgNPs+*P. capsici*, AMF+*P. capsici* and positive control) with a concentration of 14.9,13.8 and 12.5 $\mu molg^{-1}$ respectively, attributable to a high SOD activity as a plant defense mechanism. In contrast, treatments with AMF and pathogen-free AgNPs displayed a reduction in H_2O_2 accumulation compared to healthy plants (Figure 5).

Catalase (CAT). The statistical analysis displayed no significant statistical differences ($p \leq 0.05$) in the CAT enzyme activity between treatments.

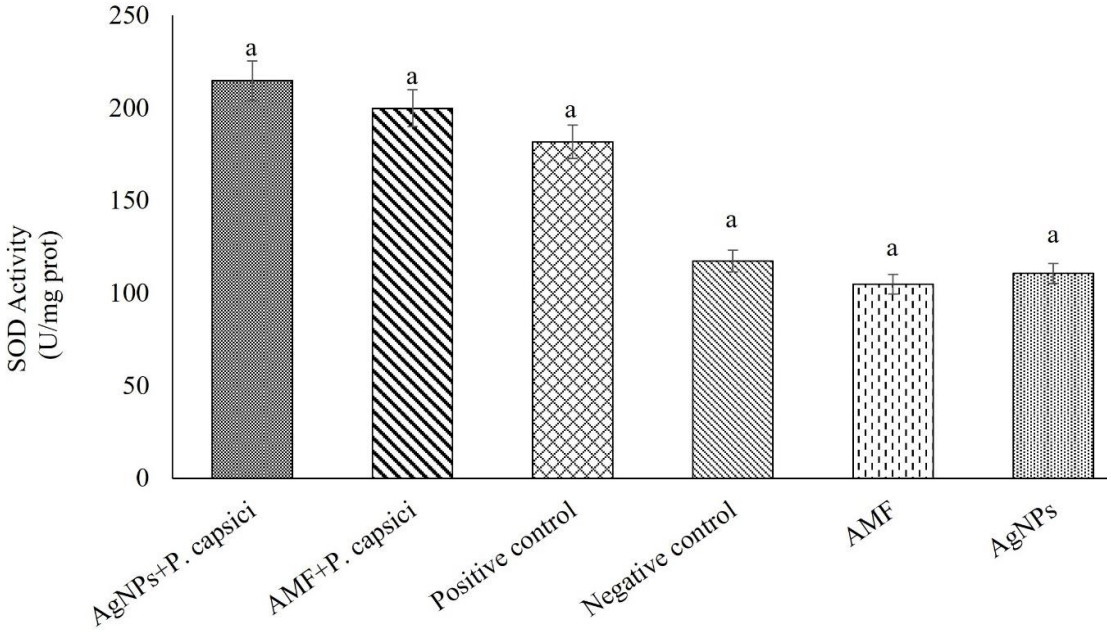

Figure 4. Superoxide dismutase (SOD) activity in chilaca pepper plants inoculated with *P. capsici* treated with AMF and AgNPs. Same letters in each column indicate there were no significant differences (p >0.05).

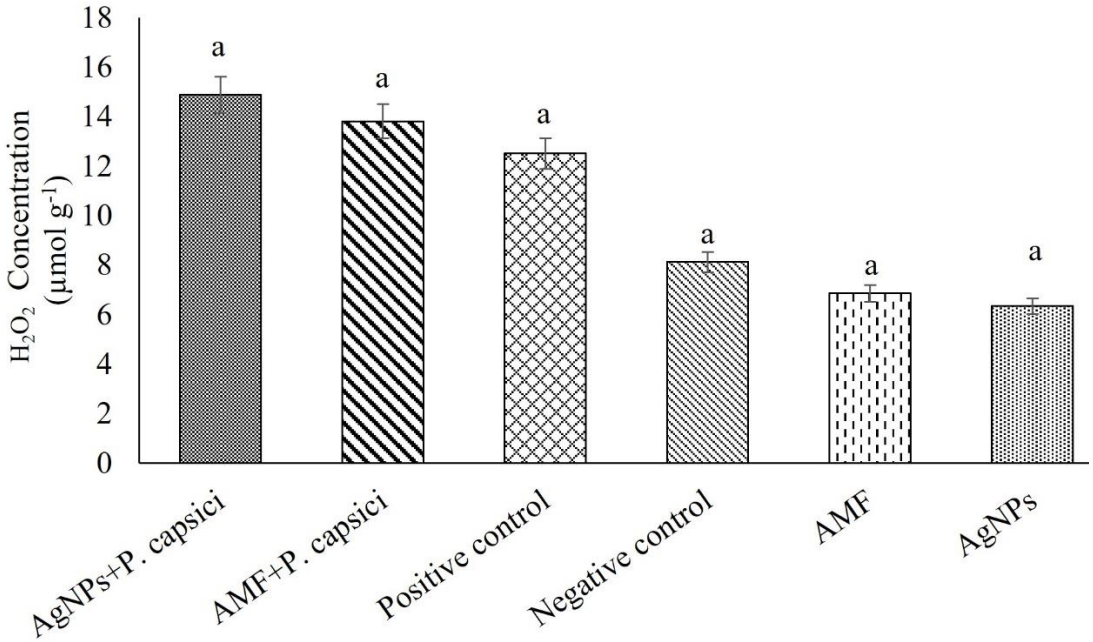

Figure 5. Concentration of H_2O_2 in chilaca pepper plants inoculated with *P. capsici* treated with AMF and AgNPs. Same letters in each column indicate there were no significant differences (p >0.05).

However, a significant reduction in the enzyme activity was observed in the treatments inoculated with *P. capsici* four days after inoculation. The highest CAT activity in this study was observed in the negative control treatment with 0.28 µM H_2O_2 mg $prot^{-1}min^{-1}$, followed by the AgNPs and AMF treatments, both with 0.27 µM H_2O_2 mg $prot^{-1}min^{-1}$ (Figure 6).

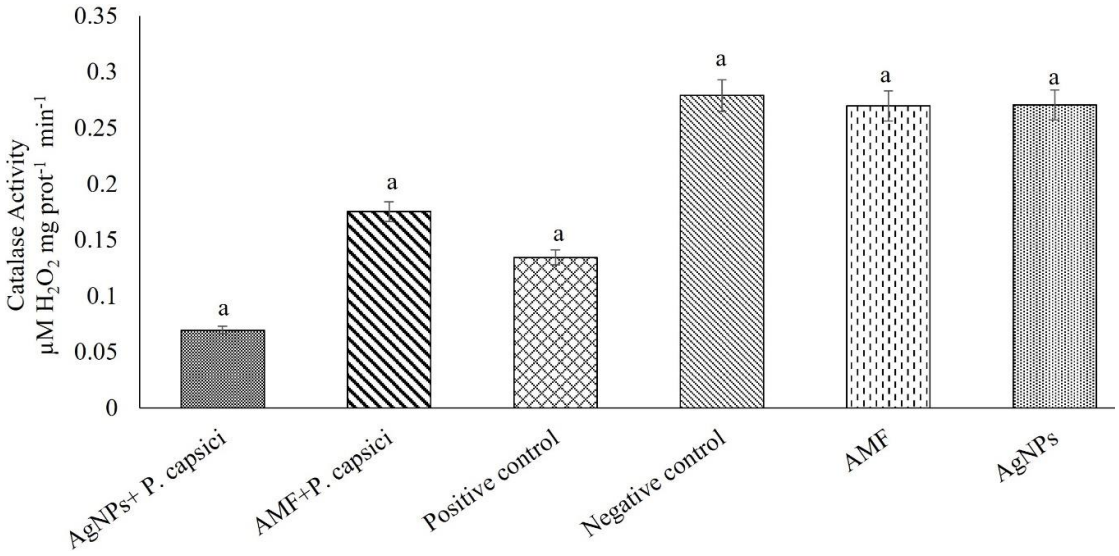

Figure 6. Catalase (CAT) activity in chilaca peppers inoculated with *P. capsici* treated with AMF and AgNPs. Same letters in each column indicate there were no significant differences (p >0.05).

Peroxidase (PER). Although peroxidase activity did not showed significant statistical differences between treatments (p> 0.05), a trend towards higher PER production can be observed in the AgNPs+*P. capsici*, AMF+*P. capsici* and positive control treatments with a concentration of 0.024, 0.024 and 0.025 µMol guaiacol mg $prot^{-1}$ min^{-1} respectively (Figure7).

Our overall results indicate that in response to pathogen-induced stress, the plant uses peroxidase enzymatic activity to break H_2O_2 down into H_2O, since CAT activity is inhibited in the pathogen treatments (Figure 8).

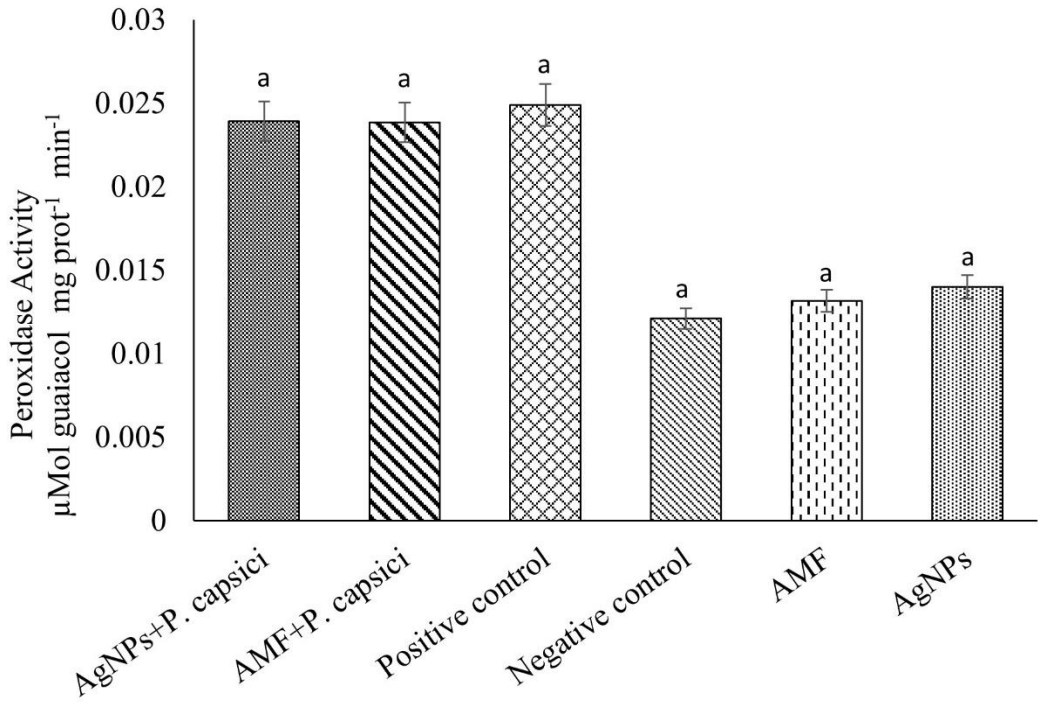

Figure 7. Peroxidase (PER) activity in chilaca pepper plants inoculated with *P. capsici* treated with AMF and AgNPs. Same letters in each column indicate there were no significant differences (p >0.05).

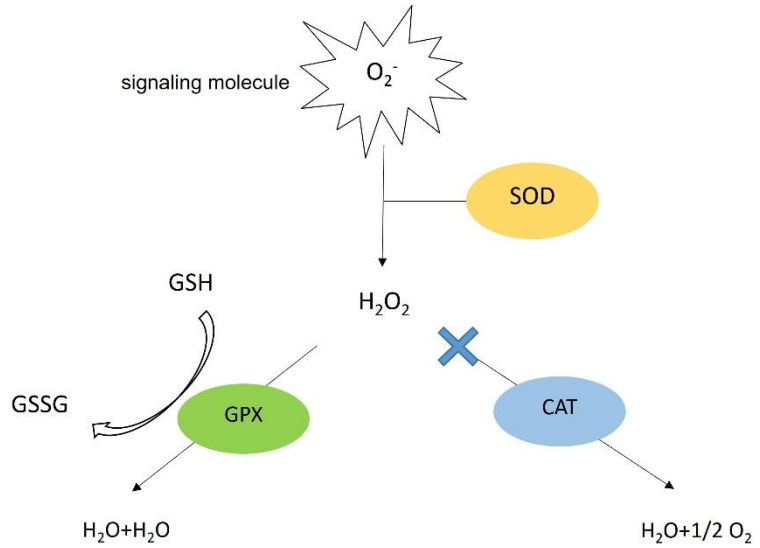

Figure 8. Effect on the enzyme activity in chilaca pepper plants inoculated with *P. capsici.* Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (PER).

Discussion

In this study, the presence of hyphae, mycelia and arbuscules in discontinuous areas of the root system was observed. Arbuscules play a very important role, since they facilitate the exchange of inorganic minerals and carbon and phosphorous compounds within the cells, providing essential nutrients to the host plant (Prasad *et al*., 2017). The proven ability of mycorrhizal plants to mitigate diseases is attributed to various mechanisms such as the improved plant nutrition, competition for penetration sites, alterations in the morphology and structure of roots, modifications in the rhizosphere microbiota, the release of antimicrobial compounds and the induction of resistance in plants (Huang *et al*., 2003).

The effective symbiosis observed in our study is notably reflected in the improvement of development, as demonstrated by the measured growth parameters: stem diameter, plant height and number of leaves. This finding is consistent with previous research that has documented the positive effects of mycorrhizae on plant development (Fauziyah *et al*., 2017; Malik *et al*., 2022). It has been suggested that these improvements may be partly attributed to the increased surface area for nutrient uptake in the soil by fungal hyphae, which extend their exploration in the soil and express proteins with a specific affinity for phosphorus. Additionally, AMF improve the uptake and transport of other essential elements such as N, K, Mg and Zn, as well as the photosynthetic rate and water use efficiency (Begum *et al*., 2019; Olalde-Portugal *et al*., 2020; Madrid-Delgado *et al*., 2021).

In this study, the beneficial effect of applying the AMF consortium on the growth of chilaca pepper plants was demonstrated, a finding that coincides with previous research on agave inoculated with AMF (Carballar-Hernández *et al*., 2018). Furthermore, the AMF in this investigation were able to reduce the severity of the disease caused by *P. capsici,* and our results are similar to those reported by Reyes-Tena *et al*. (2017), who observed that the greater the mycorrhizal colonization, the lower the percentage of *P. capsici* infection in the pepper roots. These authors suggest a possible mechanism of competition for penetration sites in the pepper roots.

The protective effect of AMF has also been proven in other crops such as beans and tomatoes, where the AMF *R. irregularis* induced protection against *S. sclerotiorum* by reducing the size of the lesions (Mora-Romero *et al*., 2015). Additionally, other protection mechanisms have been found in AMF, including

increased plant resistance to pathogens by inducing the expression of pathog-related genes such as cAPX, Osmotin, β-1,3-glucanase, phenylalanine ammonia-lyase and NPR 1 (Sarathambal *et al*., 2023).

Additionally, AMF stimulate Induced Systemic Resistance (ISR), thus activating the plant's defense mechanisms, promoting the establishment of beneficial microorganisms and modifying root exudation levels (Reyes-Tena *et al.* 2017).

Among the compounds of AMF-induced root exudation is the synthesis of the phytoalexin capsidiol in *C. annuum,* which delays wilting symptoms and balances oxidative stress (Reyes-Tena *et al.* 2017). Fatty acids such as tetradecanoic acid, n-hexadecanoic acid, octadecanoic acid and nonacos-1-ene have also been identified in the roots of black pepper plants (Sarathambal *et al*. 2023), which have antifungal properties against *P. nicotianae* (Zhang *et al*., 2020).

By contrast, in roots infected by *P. capsici,* other compounds have been identified in the exudates such as alkane hydrocarbons, including (z)-3-tetradecene, octylcyclohexane, 4-cyclohexylundecane and decylcyclohexane (Sarathambal *et al*., 2023). The presence of these hydrocarbons stimulates the mycelial growth of this oomycete (Zhang *et al*., 2020). Nevertheless, the relationship between root exudates and plant resistance remains complex and not yet fully understood.

Regarding the recent term "Nanophytopathology" coined by Ávila-Quezada and Rai (2023b), which refers to the use of nanomaterials to reduce or eradicate plant pathogens, this study displayed a reduction in disease severity with the use of AgNPs compared to the positive control treatment (plant+*P. capsici*). Only on day five after pathogen inoculation did the treatment (plant+*P. capsici*+AgNPs) reduce disease development by 50%, suggesting that additional applications of AgNPs could further delay wilting symptoms in pepper plants.

After 9 days, the plant reached a wilt severity of more than 90%, while the AgNPs treatment stopped the pathogen, keeping the stem green at 80%. This treatment helped to extend the lifespan of the pepper plants. Ali *et al*. (2015) conducted a study on tobacco plants to demonstrate the effect of AgNPs against *P. parasitica*, observing survival rates of 96.3 and 77.8% for plants treated with 100 and 10 µg ml^{-1} of AgNPs, respectively, compared to the control, 5 days after inoculation. Another study showed the protective effect of AgNPs in pepper plants and found a significant reduction of the disease symptoms (53.3 and 95%) in *P. capsici* infection in treatments with 1 and 10ppm (Luan and Xo, 2018).

The antifungal activity of AgNPs has been previously reported and their protective effect on plants against pathogens is attributed to their impact on the fungal cell membrane (Abdel-Azi *et al*., 2018; Ávila-Quezada *et al*., 2022; Ávila-

Quezada *et al*., 2023). However, the antifungal effectiveness of AgNPs may vary depending on the shape and size of the particle, the concentration used and the method of application on the plant (Le *et al*., 2019), therefore it is necessary to consider these characteristics and explore them in future studies.

The results of this investigation show that both AMF and AgNPs treatments are promising. Although the damage caused by *P. capsici* in the chilaca pepper plant was unavoidable, the appearance of the disease symptoms was delayed. To improve these results, it is suggested to apply multiple treatments in the future, at time intervals to further protect the symptomatic phase of wilting in the plant.

Understanding plant enzymatic activity in response to biotic stress remains complex, although previous studies have proven that genes related to plant defense are overexpressed in the presence of *P. capsici*, leading to the production of defense enzymes and root exudates (Sarathambal *et al*., 2023).

On the one hand, SOD expression against *P. capsici* may be related to the induction of signal transduction pathways (Karipçin *et al*., 2018) to mitigate the effects of oxidative stress caused by the pathogen (Ingle *et al*., 2017). SOD is known to be a part of the first line of defense against ROS, and its overexpression takes place in plants that display some tolerance to the oomycete (Mohammadbagheri *et al*., 2021). Therefore, an increase in the SOD enzymatic activity can benefit the plant by counteracting the harmful effects of ROS (Kapoor *et al*. 2019).

Our results on SOD activity are consistent with the findings by Hashem *et al*. (2021) in tomato inoculated with AMF and *Fusarium oxysporum*, where infected plants displayed an increase in SOD and other enzymes activity compared to the control treatment and AMF-treated plants. Similarly, our study revealed a significant SOD accumulation in *P. capsici*-affected plants compared to healthy plants.

In contrast, our results show that plants inoculated with *P. capsici* exhibited a reduction in CAT enzymatic activity. This reduction in plants treated with microorganisms has been previously documented (Duc *et al*., 2018), since CAT favors pathogen survival by inhibiting host-induced oxidative stress (Sharma *et al*., 2019). Although the balance between SOD and CAT activities is crucial to mitigate toxic levels of ROS in the cell, low CAT activity may indicate that the plant induces other compensatory mechanisms involving enzymes such as ascorbate peroxidase and glutathione peroxidase to reduce SOD levels (Banerjee and Roychoudhury, 2019).

ROS production is known to be induced in plants in response to metabolic imbalances caused by stress, including the first contact with mycorrhizae, which is necessary for the establishment of the mycorrhizal symbiosis (Branco *et al*., 2022). However, in this study, AMF-induced chilaca pepper, although not infected by *P. capsici*, displayed PER, CAT and SOD activity similar to healthy plants, suggesting that the stress generated by contact with mycorrhizae had already been regulated by the plant.

Regarding the interaction of AgNPs at 50 ppm in the chilaca pepper plants, it was observed that they do not induce an imbalance in the activity of the antioxidant enzymes analyzed. On the contrary, AgNPs have antioxidant capacity by splitting H_2O_2 into water and oxygen (Bharathi *et al*., 2018).

To understand the plant response, further studies are needed that include measurement of gene expression and enzyme activity in chilaca peppers at various intervals following AMF and pathogen inoculation, preferably every 24 hours for a period of 5 days or more. This time focus will allow a detailed understanding of the dynamic responses of the plant in its interaction with AMF and pathogens.

Conclusions

The application of arbuscular mycorrhizal fungi significantly increased the number of leaves, stem diameter and height of chilaca pepper plants. In addition, these microorganisms provided some protection against *P. capsici.* Likewise, AgNPs provided protection against *P. capsici*, delaying disease progression.

The pant-pathogen interaction induced an enzymatic antioxidant response. This was reflected in the increase in SOD and PER activity, along with an inhibition of CAT enzyme activity and an accumulation of H_2O_2. These enzymatic mechanisms behaved oppositely compared to the application of AMF and AgNPs. The combination of AMF and AgNPs emerged as a promising strategy, providing an enhanced defense mechanism against plant pathogens.

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