

Fungicides, bio-controllers and resistance inducers affect bioactive compounds and oxidative metabolism in bell pepper plants inoculated with *Phytophthora capsici*

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ABSTRACT

The rational use of chemical fungicides in chilli pepper (*Capsicum annum* L.) for controlling *Phytophthora capsici* Leo. should include molecules that promote induced systemic resistance in the plant. The study objective was to assess how the application of fungicides, bio-controllers and resistance inducers affects bioactive compounds production and oxidative metabolism in pepper plants when inoculated with *P. capsici* zoospores. Statistical analyses show significant differences in total flavonoids, total phenols and salicylic acid concentrations in plants treated with *Trichoderma harzianum*, Acibenzolar-S-Methyl (ASM) and phosphonic acid. In addition, the application of ASM and *Bacillus subtilis* showed the highest values of antioxidant capacity. On the other hand, ASM application favoured increases in superoxide dismutase and phenyl ammonium lyase activity with values of 4.42 ± 0.016 (units $\cdot$ min⁻¹ $\cdot$ g⁻¹) and 3.90 ± 0.22 U $\cdot$ g⁻¹, respectively. However, hydrogen peroxide production was similar to that of phosphonic acid treatments. Similarly, plants treated with *B. subtilis* and Metalaxyl-M showed increases in peroxidase enzyme activity with values ranging from 2.86 ± 0.05 to 4.52 ± 0.02 (μmol $\cdot$ [mg protein]⁻¹ $\cdot$ min⁻¹). Likewise, phosphonic acid-treated plants exhibited high catalase enzyme activity with values ranging from 2.38 ± 0.032 μmol H₂O₂ $\cdot$ min⁻¹ $\cdot$ g⁻¹ to 4.30 ± 0.024 μmol H₂O₂ $\cdot$ min⁻¹ $\cdot$ g⁻¹. The complementary use of products with biological and resistance-inducing functions could help to mitigate the environmental impacts that occur due to the use of chemical fungicides for controlling *P. capsici* on chilli pepper.

Keywords: *Bacillus subtilis*, *Capsicum annum* L., phenylalanine ammonia lyase, phosphonic acid, superoxide dismutase, *Trichoderma harzianum*, wilting

Abbreviations: AC, antioxidant capacity; ASM, acibenzolar-S-methyl; Bc, bio-controllers; BSA, bovine serum albumin; CFU, colony forming units; CAT, catalase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenedinitrilotetraacetic acid; H₂O₂, hydrogen peroxide; Na₂EDTA·2H₂O, ethylenediaminetetraacetic acid disodium salt dihydrate; NBT, nitroblue tetrazolium; PAL, phenylalanine ammonia lyase; POX, peroxidase; RI, resistance inducer; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; TFI, total flavonoids; TP, total phenols.

INTRODUCTION

Chilli belongs to the genus *Capsicum* (Solanaceae: Solanaceae) and together with maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), bean (*Phaseolus vulgaris* L.) and pumpkin (*Cucurbita* sp.) is one of the most important domesticated plants, being of high

nutritional value, cultural significance and economic importance across the world (Aguilar-Carpio et al., 2018; Marín-Montes et al., 2019; Hernández-Huerta et al., 2021). There are five cultivated species of chilli: *C. chinense*, *C. pubescens*, *C. baccatum*, *C. frutescens*

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and *C. annuum* with fruits whose taste varies widely in hotness and sweetness (Pérez-Grajales et al., 2021). Of these, *C. annuum* L. is of the widest geographical distribution and morphological diversity going under the common names of ‘jalapeño’, ‘guajillo’, ‘chiltepin’, ‘poblano’, ‘costeño’, ‘agua’, amongst others (Figueroa-Cares et al., 2015). Among these chilli types, the sweet pepper stands out as having fruits with an exciting sweet taste, bright colours (red, yellow and orange) and can be consumed either raw or cooked (García-Bañuelos et al., 2017).

Among the many reasons for the high consumer demand for pepper fruit, and thus for its widespread cultivation both in the open field and the greenhouse, is that it is an important source of biologically active compounds associated with human health, including vitamins (provitamin A, E and C) and a range of phenolics and carotenoids (Anaya-Esparza et al., 2021). However, the yield and quality of peppers can be affected severely by attack from the oomycete *Phytophthora capsici* (Sánchez-Gurrola et al., 2019; Pérez-Grajales et al., 2021). This widely distributed pathogen causes root rot, chlorosis and leaf drop (chilli wilt) with infection levels often ranging between 10% and 100% of plants (Palma-Martínez et al., 2017; Jiménez-Camargo et al., 2018).

The use of conventional fungicides minimised the damage caused by *P. capsici* over the years but also led to significant increases in fungicide resistance. Their use has also raised production costs and caused irreversible damage to the ecosystem—soil, water, air and microorganisms (Piccini et al., 2019). Thus, integrated management strategies have been developed which include the use of pathogen-resistant rootstocks and scion varieties (Hernández-González et al., 2014). However, their efficiency is compromised by the type of rootstock, soil and climatic conditions and the species or variety used as a scion (García-Bañuelos et al., 2017).

A better and more practicable alternative to fungicide use may be taking the advantage of or stimulating the plant's own natural defence mechanisms. This can involve the application of resistance-inducer or bio-controller compounds of either natural or synthetic origin. These can function both before or after the pathogen penetrates the plant (Huallanca and Cadenas, 2014). Among the passive defence mechanisms associated with natural plant resistance, are the presence of waxes, lignified cell walls and the pre-existence of chemical compounds, including ethylene, jasmonic acid, polyphenolic compounds and salicylic acid (SA). Meanwhile, natural openings (stomata, lenticles) present are gateways to infection, along with failure to maintain sufficient essential mineral nutrients and light (in glasshouse or semi-sheltered production) (Lozoya-Saldaña et al., 2007).

On the other hand, in addition, the resulting wounds (holes) are easily penetrated by spores of the pathogen, caused by exposure to unfavourable biotic and abiotic factors (pests, diseases, wind-rub, frost, etc) which can increase oxidative damage and compromise the

effectiveness of the enzymatic antioxidant defence apparatus (including catalase [CAT], peroxidase [POX], ascorbate peroxidase, superoxide dismutase [SOD] and glutathione reductase) and of the non-enzymatic ones (including ascorbate, glutathione, flavonoids and phenolic compounds), accelerating the onset of symptoms and premature tissue death due to the accumulation of reactive oxygen species (ROS) (Frías-Moreno et al., 2019; Piccini et al., 2019). Under these conditions, an increase in the synthesis of phenolic compounds associated with phenylalanine ammonium lyase enzyme activity has been reported (Lozoya-Saldaña et al., 2007).

Phenolic compounds are the most abundant secondary metabolites in plants. Their functions in a food plant include organoleptic characteristics (taste, astringency), nutritional characteristics and antioxidant properties (Terán-Erazo et al., 2019). Hydroxyl and carboxyl groups' presence in these compounds inhibits lipid peroxidation and protects plant tissues from ROS damage (Avendaño-Arrazate et al., 2021). These functions may explain the modulation of their concentrations depending on stress levels caused by abiotic (salinity, heavy metals, drought) and biotic factors (pests and diseases) (Palma-Martínez et al., 2017). They are involved in multiple processes including nutrient uptake, protein synthesis, enzymatic activity, photosynthesis and allelopathy (Tucuch-Haas et al., 2017).

With climate change, water scarcity, contamination of soil and air pollution from fungicide overuse in agriculture, a viable solution would involve using of products that enhance the plant's response to biotic and abiotic stress factors, such as resistance inducers and bio-controllers. These have indirect effects on pathogenic organisms and cause much less environmental damage than many conventional management tools such as fungicides. Our study aimed to evaluate changes in the concentrations of the plant's natural bioactive compounds (total flavonoids [TFI], total phenols [TP], SA and antioxidant capacity [AC]) and oxidative metabolism (SOD, hydrogen peroxide, CAT, POX and phenyl ammonium lyase) associated with applications of fungicides, bio-controllers and resistance inducers in pepper plants inoculated with *P. capsici*.

MATERIALS AND METHODS

Experimental site, plant material and growth conditions

This research was carried out in a P-5 tunnel-type greenhouse with passive temperature control, located in the Experimental Agricultural Field of the Department of Plant Science at the Universidad Autónoma Chapingo (UACH), State of Mexico, Mexico (19°29'33"N, 98°52'21"W), at an altitude 2,267 m, with mean annual temperature 15.9°C, relative humidity between 50% and 60% and natural irradiation of 65% and 410 ppm carbon dioxide. Plants of the commercial pepper hybrid

PS16364212 (*C. annuum* L.) (Seminis, St. Louis, USA) were used. Four weeks after germination, seedlings were transplanted into white polystyrene bags filled with volcanic rock (red tezontle Ø ≈ 4 mm). Nutrients were supplied in solution starting the day after transplanting ($\text{mg} \cdot \text{L}^{-1}$): N(250), P(60), Ca^{2+} (250), K^+ (250), Mg^{2+} (60), S(205), Fe^{2+} (3), Mn^{2+} (1), B(0.5), Cu^{2+} (0.1) and Zn^{2+} (0.5). The nutrient solution was applied daily through an automatised system in irrigations of 250 mL for 5 min, four times a day. The nutrient solution was maintained at pH 6.3 and electrical conductivity $1.7 \text{ dS} \cdot \text{m}^{-1}$.

Experimental setup

Isolation, inoculum preparation and pathogenicity test with *Phytophthora capsica*

P. capsici strain 6143 was obtained from the special collection of plant health pathology of the Colegio de Postgraduados-Campus Montecillo, State of Mexico, Mexico. Multiplication was carried out by sowing $0.5 \times 0.5 \text{ cm}$ squares in Petri dishes with V8-agar juice culture medium, left to stand for 24 h at room temperature ($18 \pm 1^\circ\text{C}$). After this, they were placed in an incubator at 28°C for 6 days. After sporangia became visible, the production of motile zoospores was encouraged by adding 10 mL of sterile distilled water and incubation was done for 8 days further at 28°C .

The experimental plants were inoculated at the base of the stem with 50 mL of a solution of motile zoospores ($1 \times 10^6 \text{ zoospores} \cdot \text{mL}^{-1}$) and from those that developed consistent isolates (cottony white mycelium), a portion of the mycelium was taken for morphological characterisation. Once the presence of the oomycete was confirmed in the samples, it was isolated and sown in Petri dishes with V8-agar juice

culture medium. Subsequently, to form sporangia, 10 mL of sterile distilled water was added and incubated at 28°C for 6 days. Finally, to verify Koch's postulates, 50 mL of zoospore solution ($1 \times 10^6 \text{ zoospores} \cdot \text{mL}^{-1}$) was prepared and applied directly to the root base of 10 untreated plants 60 days after transplanting, with three uninoculated plants used as controls.

Inoculation with *P. capsici* and application of resistance inducers, bio-controllers and chemical fungicides

Sixty-five-day-old plants were selected as experimental units and inoculated with 50 mL of a solution of motile zoospores ($1 \times 10^6 \text{ zoospores} \cdot \text{mL}^{-1}$). There were six treatments, which are described in Table 1. Likewise, untreated controls were used. All proprietary products were applied according to the manufacturer's instructions ($\text{dose} \cdot \text{ha}^{-1}$) at 15 days and 25 days after transplanting, at a density of $2.5 \text{ plants} \cdot \text{m}^{-2}$. Actigard 50 GS® and Alliete® WG were sprayed with a 250 mL atomiser and the other products were injected into the substrate (stem base area). The preparation of the treatment solutions was carried out in 20 L containers. Volume determination was carried out with graduated cylinders, while for powders and granules an Ohaus Scout® digital balance was used (Ohaus, Parsippany, USA) with 0.01 g accuracy and a maximum capacity of 650 g.

Sampling

After 90 days of cultivation, around 700 leaves of the selected plants were collected in paper bags and transported in polystyrene foam containers ($20 \text{ cm} \times 15 \text{ cm} \times 11 \text{ cm}$) to the Multipurpose Laboratory of the Department of Plant Science (UACH), where

Table 1. Trade name, active ingredient and applied doses of fungicides, biocontrol agents and resistance inducers in bell pepper plants inoculated with *P. capsici*.

Trade name	Active ingredient	Doses $\cdot \text{ha}^{-1}$
Ridomil® Gold 480 SL ^x (Syngenta, Greensboro, USA)	45.28% by weight, equivalent to $480 \text{ g a.i.} \cdot \text{L}^{-1}$ at 20°C (methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate)	0.5 L (1 st application) and 1.25 L (2 nd application)
Alliete® WDG ^x (Bayer Crop Science, St. Louis, USA)	80% by weight, equivalent to $800 \text{ g a.i.} \cdot \text{kg}^{-1}$ aluminium tris (ethyl phosphonate)	2.5 kg
Serenade® ASO ^y (Bayer Crop Science, St. Louis, USA)	<i>Bacillus subtilis</i> strain QST 713, 1.34%	1.5 kg
Spectrum® Trico-Bio ^y (Promotora Técnica Industrial, S.A. de C.V., Benito Juárez, México)	<i>Trichoderma harzianum</i> , 0.45% equivalent to $4.653 \text{ g active ingredient} \cdot \text{L}^{-1}$ ($1.0 \times 10^{11} \text{ CFU} \cdot \text{L}^{-1}$)	2 L
Actigard 50® GS ^z (Syngenta, Greensboro, USA)	50% by weight, equivalent to $500 \text{ g a.i.} \cdot \text{kg}^{-1}$ (S-methylbenzo [1,2,3]thiadiazole-7-carbothioate)	50 g
Fitopron® (Probelte México, S.A. de C.V., Guadalajara, México)	70% (w/v), equivalent to $475 \text{ g} \cdot \text{L}^{-1}$ phosphonic acid (containing phosphoric anhydride, potassium oxide and potassium phosphonate)	2 L
Untreated control	50 mL of a solution of motile zoospores ($1 \times 10^6 \text{ zoospores} \cdot \text{mL}^{-1}$) but without application of any products	-

^xFungicides.

^yBiocontrol agents.

^zResistance inducers.

QST, corresponds to the identification of the strain of *Bacillus subtilis* equivalent to 14.6%, equivalent to $8 \times 10^9 \text{ CFU/g}$.

they were wrapped in aluminium foil and frozen in liquid nitrogen. The frozen samples were ground into fine powder in liquid nitrogen and stored at -80°C for further analysis.

Extract preparation and determination of bioactive compounds and AC

Extraction of bioactive compounds was carried out according to the method of Cera-Campos et al. (2019) with some modifications. Fresh samples (0.5 g) were homogenised with 5 mL of 80% (v:v) methanol. The homogenised sample was centrifuged at 17000 g for 10 min at 4°C . The supernatant was used to quantify TP and TFI. TFI were quantified according to the method of Chang et al. (2002). Here, 0.250 mL of supernatant was added to 0.075 mL of NaNO_2 , the mixture was shaken vigorously for 5 min, then 0.150 mL of AlCl_3 , 0.500 mL of NaOH and 2.025 mL of deionised water were added, and the mixture left to stand for 40 min, after which the absorbance was determined at 510 nm. The results are expressed as equivalent mg of quercetin $\cdot \text{g}^{-1}$ (mg QE $\cdot \text{g}^{-1}$). TP were quantified according to the Folin-Ciocalteu method described by Waterman and Mole (1994). Two samples were taken with 0.250 mL of the supernatant and 0.750 mL and 0.250 mL of 2% and 50% (v:v) Na_2CO_3 and Folin-Ciocalteu were added, respectively. The mixture was incubated for 1 h and the absorbance was measured at 725 nm. The results are expressed in mg GAE $\cdot 100 \text{ g}^{-1}$. Finally, the AC was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH $^{\cdot}$) method. Aqueous ethanol (80%) was used for sample preparation. The DPPH $^{\cdot}$ assay was carried out in accordance with Brand-Williams et al. (1995). Briefly, 0.3 mL of extract and 5.7 mL of the compound DPPH were mixed at a concentration of 0.0375 g $\cdot \text{L}^{-1}$. The mixture was kept in the dark conditions for 35 min. The decrease in the DPPH radical was measured at 515 nm. The results are expressed as inhibition of DPPH.

Determination of SA

The extraction and quantification of SA content were performed using the spectrophotometric method reported by Warrier et al. (2013) with slight modifications. Briefly, 100 mL of distilled water was added to 0.1 g of sample and left to stand for 72 h at 4°C . The mixture was centrifuged at 10000 g for 10 min at 4°C . To 100 μL of the supernatant, 2.99 mL of FeCl_3 (0.1%) was added and after the appearance of the violet tone, the absorbance reading was taken at 540 nm. Concentrations of 0, 200, 300, 400, 400 and 500 $\mu\text{g} \cdot \text{mL}^{-1}$ of SA were used to construct a standard curve. Results are expressed in mg $\cdot \text{g}^{-1}$.

Oxidative metabolism (enzymatic activity)

SOD (EC 1.15.1.1)

The extraction and activity of SOD (EC 1.15.1.1) was determined by the nitroblue tetrazolium (NBT)

photochemical reduction inhibition control method (Sanchez et al., 2000). Briefly, 5 g of sample was homogenised for 2 min with 1.5 g of quartz sand and 5 mL of a solution composed of 50 mM HEPES (4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid) buffer (pH 7.6) and 0.1 mM $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$. The mixture was centrifuged at 17000 g for 15 min at 4°C , and then filtered with qualitative filter paper 1004 (Whatman, Marlborough, USA). The extract was used for SOD and protein analysis, the latter by the Bradford (1976) method and BSA as standard.

Total SOD activity assay was performed with a 2.5 mL reaction mixture composed of HEPES 50 mM (pH 7.6), Ethylenedinitrilotetraacetic acid (EDTA) 0.1 mM, Na_2CO_3 50 mM (pH 10) methionine 13 mM, Triton X-100 0.25% (w/v), NBT 63 μM , riboflavin 1.3 μM and an aliquot of enzyme extract. The reaction mixtures were illuminated for 15 min at a photosynthetic photon flux density of 380 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Background absorbance correction was performed using unilluminated reaction mixtures. Enzyme activity is reported in SOD units $\cdot \text{mg} \cdot \text{protein}^{-1}$. One unit of SOD activity corresponds to the amount of enzyme required to cause 50% inhibition of NBT reduction assessed at 560 nm.

Hydrogen peroxide (H_2O_2 total)

The extraction and quantification of hydrogen peroxide (H_2O_2 total) was performed by the spectrophotometric method described by Brennan and Frenkel (1977). This method is based on the formation of a specific complex (hydroperoxides [ROOH] and titanium [Ti^{4+}]) and can be determined at 415 nm. The concentration of total H_2O_2 in the extracts was determined by the ratio of their absorbance values against a standard curve (0.1–1 mM) representing a Ti^{4+} and H_2O_2 complex. The hydroperoxides produced by the reaction (total peroxides) are expressed in $\mu\text{mol} \text{H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$.

POX and CAT

The assay of POX (EC 1.11.1.7) and CAT (EC 1.11.1.6) activities were performed according to Sanchez et al. (2000). The sample was macerated with a solution composed of 50 mM Tris-acetate buffer (pH 7.5), 5 mM 2-mercaptoethanol, 2 mM 2-mercaptoethanol, 2 mM 1,4-dithio-DL-threitol (DTT), 2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w:v) polyvinylpyrrolidone (PVP). The mixture was placed on a two-layer filter (Miracloth, Mendota Heights, USA) and centrifuged at 30000 g for 30 min at 4°C . The supernatant was taken for filtration and further for POX and CAT analyses, where BSA was used as standard.

The oxidation reaction of guaiacol and the consumption of H_2O_2 (A485 and A240, respectively) allowed the determination of POX and CAT activities (Sanchez et al., 2000). The processing and analysis of the control extracts allowed us to confirm whether

the reactions were due to enzyme activity. POX and CAT results are expressed as oxidised guaiacol ($\mu\text{mol} \cdot [\text{mg protein}]^{-1} \cdot \text{min}^{-1}$) and oxidised H_2O_2 ($\mu\text{mol} \text{H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), respectively.

Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) extraction and activity (PAL, EC 4.3.1.25) was carried out by the method described by Lozoya-Saldaña et al. (2007) with some modifications. Briefly, 0.1 g of the sample was homogenised with 5 mL of 0.1 M sodium borate (pH 8.8) and 20 mM b-mercaptoethanol. The mixture was gently shaken for 20 min on ice (4°C), filtered through a mesh cloth and centrifuged at 15000 g for 20 min at 4°C . The enzyme was precipitated by adding 0.46 g of $(\text{NH}_4)_2\text{SO}_4 \cdot \text{mL}^{-1}$ of recovered supernatant. This mixture was gently agitated for 30 min at 4°C , then centrifuged at 15000 g for 30 min at 4°C . Further, 5 mL of 0.1 M sodium borate (pH 8.8) was added to the supernatant. The reaction mixture consisted of 1.8 mL of 0.1 M sodium borate (pH 8.8) and 0.9 mL of the final extract pre-incubated at 40°C for 5 min, to which 0.3 mL of 100 mM L-phenylalanine was added and the absorbance value at 290 nm was recorded. Simultaneously, the assay was performed with 2.1 mL of 0.1 M sodium borate (pH 8.8) and 0.9 mL of the final extract. The change in absorbance was evaluated over 2 h at 290 nm, keeping the sample in a water bath at 40°C . Enzyme activity is reported as $\text{U} \cdot \text{g}^{-1}$ fresh weight, where $\text{U} = \Delta$ (absorbance) at 290 nm $\cdot \text{h}^{-1}$.

Experimental design and statistical analyses

The experiment was set up in a completely randomised design, where the experimental unit consisted of one plant with 10 replicates per treatment. The normality and homogeneity of variances of the data were verified respectively with the Kolmogorov–Smirnov and Bartlett tests (Hanusz and Tarasińska, 2015). Subsequently, simple rank analysis of variance and multiple comparisons of means were performed using Tukey–Kramer test ($p \leq 0.05$). The data were analysed with the statistical package Statistical Analysis Software (SAS/STAT|SAS), version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Plants inoculated with motile zoospores of *P. capsici* showed leaf drop, chlorosis, loss of turgor and root rot, while the control plants remained healthy, confirming Koch's postulates.

Bioactive compounds

Data associated with bioactive compounds (TfI, TP and SA) and AC are shown in Table 2. Our pepper leaves inoculated with *P. capsici* and subjected to untreated controls or *Trichoderma harzianum* showed the highest TfI concentrations with values of $18.29 \pm 0.16 \text{ mg QE} \cdot \text{g}^{-1}$ and $16.06 \pm 0.38 \text{ mg QE} \cdot \text{g}^{-1}$, respectively. Likewise, there is a significant effect on TP concentration in untreated controls and phosphonic acid. Our data for SA concentration in plants inoculated with *P. capsici* showed a significant change between treatments, with values ranging from $3.21 \pm 0.18 \mu\text{g} \cdot \text{mL}^{-1}$ to $4.85 \pm 0.28 \mu\text{g} \cdot \text{mL}^{-1}$, where the use of phosphonic acid and Acibenzolar-S-Methyl (ASM) showed the best results compared to the untreated controls. On the other hand, plants treated with ASM and *Bacillus subtilis* showed AC values between $88.20 \pm 0.078\%$ and $86.42 \pm 0.070\%$ DPPH inhibition, respectively.

Oxidative metabolism

Data associated with oxidative metabolism (SOD, H_2O_2 , POX, CAT and PAL) are shown in Table 3. In this respect, the application of the resistance inducer ASM favoured a significant increase in the activity of SOD, the main component of the antioxidant enzyme system but the production of H_2O_2 was similar to the treatments with phosphonic acid. Plants treated with *B. subtilis* and Metalaxyl-M showed increases in POX enzyme activity with values ranging from 2.86 ± 0.05 to $4.52 \pm 0.02 (\mu\text{mol} \cdot [\text{mg protein}]^{-1} \cdot \text{min}^{-1})$. On the other hand, CAT is another important enzyme for its role in the antioxidant system and the reduction of the impact of oxidative stress in plants induced by biotic factors such as fungal disease, whose enzyme activity values fluctuated between $2.38 \pm 0.032 \mu\text{mol H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ and $4.30 \pm 0.024 \mu\text{mol H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ in which plants sprayed with potassium phosphonate stand

Table 2. Concentration of flavonoids, TP, SA and AC in bell pepper plants inoculated with *P. capsici* and treated with fungicides, biocontrol agents and resistance inducers.

Treatment	TfI (mg QE $\cdot \text{g}^{-1}$)	TP (mg GAE $\cdot \text{g}^{-1}$)	SA ($\mu\text{g} \cdot \text{mL}^{-1}$)	AC (% DPPH inhibition)
Metalaxyl-M	$4.66 \pm 0.23 \text{ d}^*$	$5.02 \pm 0.11 \text{ c}$	$3.21 \pm 0.18 \text{ c}$	$77.56 \pm 0.045 \text{ c}$
Fosetyl-Al	$10.98 \pm 0.23 \text{ c}$	$5.90 \pm 0.76 \text{ c}$	$3.62 \pm 0.34 \text{ bc}$	$79.93 \pm 0.034 \text{ bc}$
<i>B. subtilis</i> strain QAT 713	$14.60 \pm 0.25 \text{ b}$	$4.03 \pm 0.17 \text{ d}$	$3.48 \pm 0.16 \text{ c}$	$86.42 \pm 0.070 \text{ a}$
<i>Trichoderma harzianum</i>	$16.06 \pm 0.38 \text{ ab}$	$11.29 \pm 0.26 \text{ b}$	$3.28 \pm 0.09 \text{ c}$	$83.05 \pm 0.054 \text{ b}$
Acibenzolar-S-methyl	$15.81 \pm 0.45 \text{ bz}$	$5.82 \pm 0.09 \text{ c}$	$4.58 \pm 0.11 \text{ ab}$	$88.20 \pm 0.078 \text{ a}$
Phosphonic acid	$13.81 \pm 0.14 \text{ b}$	$11.82 \pm 0.34 \text{ ab}$	$4.85 \pm 0.28 \text{ a}$	$80.63 \pm 0.029 \text{ bc}$
Untreated control	$18.29 \pm 0.16 \text{ a}$	$12.15 \pm 0.15 \text{ a}$	$3.61 \pm 0.23 \text{ b}$	$78.90 \pm 0.068 \text{ c}$

*Values with the same letter within a column are not significantly different according to Tukey–Kramer's test ($p \leq 0.05$).

TfI, total flavonoids, quercetin equivalents (QE); TP, total phenols, galic acid equivalents (GAE); SA, salicylic acid; AC, antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Table 3. Oxidative metabolism in bell pepper plants inoculated with *P. capsici* and treated with fungicides, biocontrol agents and resistance inducers.

Treatment	SOD	H ₂ O ₂	POX	CAT	PAL
Metalaxyl-M	3.18 ± 0.027 c*	1.18 ± 0.017 bc	4.78 ± 0.02 a	2.38 ± 0.032 c	2.84 ± 0.12 c
Fosetyl-Al	3.29 ± 0.031 c	1.36 ± 0.034 b	3.87 ± 0.02 c	2.98 ± 0.001 c	3.09 ± 0.29 bc
<i>B. subtilis</i> strain QAT 713	3.34 ± 0.019 b	1.29 ± 0.0065 b	5.02 ± 0.03 a	3.09 ± 0.001 bc	3.26 ± 0.16 bc
<i>Trichoderma harzianum</i>	3.32 ± 0.022 c	1.32 ± 0.054 b	2.86 ± 0.05 e	3.54 ± 0.003 b	3.67 ± 0.09 b
Acibenzolar-S-methyl	4.42 ± 0.016 a	1.42 ± 0.007 ab	4.52 ± 0.02 b	3.06 ± 0.002 bc	3.90 ± 0.22 a
Phosphonic acid	3.34 ± 0.023 c	1.41 ± 0.009 ab	3.65 ± 0.02 c	4.02 ± 0.018 a	2.90 ± 0.34 c
Untreated control	4.21 ± 0.034 a	1.67 ± 0.043 a	3.05 ± 0.04 d	4.30 ± 0.024 a	4.18 ± 0.29 a

*Values with the same letter within a column are not significantly different according to Tukey-Kramer's test ($p \leq 0.05$).

CAT, catalase ($\mu\text{mol H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$); H₂O₂, hydrogen peroxide ($\mu\text{mol g}^{-1}$); PAL, phenylalanine ammonia lyase ($\text{U} \cdot \text{g}^{-1}$); POX, peroxidase [$\mu\text{mol} \cdot (\text{mg protein})^{-1} \cdot \text{min}^{-1}$]; SOD, superoxide dismutase ($\text{units} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$).

out. The application of ASM increased PAL activity, whose values fluctuated between $2.30 \pm 0.09 \text{ U} \cdot \text{g}^{-1}$ and $4.18 \pm 0.29 \text{ U} \cdot \text{g}^{-1}$, however, these plants showed similar behaviours with respect to inoculated plants treated only with distilled water.

DISCUSSION

In general, the interaction of a pathogen with its plant host manifests itself in complex associations characterised by the recognition of molecular patterns in the host's immune system, which may result in the activation of defence mechanisms. These mechanisms may include synthesis and accumulation of phenolic compounds, lignin, phytoalexins and increased enzyme activity, including phenylammonium lyase, and POX (Ji and Csinos, 2015). In this respect, when applying *T. asperellum* strain T34 (10^4 conidia $\cdot \text{mL}^{-1}$) on green chilli (*C. annuum* L.) 'Dulce Italiano' seedlings, it is reported that a significant reduction in the incidence of *P. capsici* of around 71% compared with the application of etridiazole (Terrazole® 35% WP) (Arysta LifeScience, Cary, USA) ($2 \text{ L} \cdot \text{ha}^{-1}$) (Segarra et al., 2013). Thus, the use of bio-controllers, such as *Trichoderma*, can help to generate a 'priming' effect in the plant and hence help to improve its defence mechanisms. However, the efficiency varies with the fungal strain, the number of applications and the nutritional status of the host plant (Ramírez-Prado et al., 2018). Multiple defence mechanisms in plants include the rapid synthesis and early accumulation of phenolic compounds in tissues in the presence of the pathogen (Kisa et al., 2016). However, a previous study by Godínez-Paoli et al. (2020) on several commercial pepper hybrids, including 'PS16364212' reported a 14.9% incidence and an area under the disease progress curve of $15.7\% \cdot \text{day}^{-1}$ *P. capsici*, where phosphonic acid showed a limited effect for the control of this disease.

On the other hand, SA has been reported as a regulatory compound in several physiological and biochemical processes, including in the synthesis and accumulation of phenols, flavonoids, anthocyanins, etc (Ramírez-Prado et al., 2018; Vázquez et al., 2022), compounds associated with endogenous defence mechanisms and tolerance to stress induced by abiotic

and biotic factors (pests and diseases) (Tucuch-Haas et al., 2017). The manifestation of systemic acquired resistance in plants, including in chilli, requires the accumulation of endogenous signals induced by SA, which in turn must mediate the activation of multiple genes related to pathogenesis (Guarnizo et al., 2020). Likewise, several investigations have shown that hormone signaling can participate in the activation of defence genes against pathogenesis or indirectly prepare the tissue for increased expression of defence-associated genes, even though the level of success depends on the initial condition of the plant, including its nutritional status, phenological stage (Tripathi et al., 2019).

It is well known that a chilli plant when attacked by *P. capsici* shows a range of symptoms, including necrosis, chlorosis and wilting. However, one of the consequences that has a severe impact is genome damage caused by ROS synthesis and accumulation, where enzymatic and non-enzymatic antioxidant mechanisms lose their efficiency as the pathology worsens (Teran-Erazo et al., 2019). On the other hand, *Bacillus* strains showed good adaption to biotic and abiotic stresses (heat, salinity and drought), which when sprayed on leaves can induce resistance in the plant and prove to be an excellent alternative tool for integrated management of *P. capsici* (Liu et al., 2014; Moradi et al., 2018). Likewise, ASM has proved to be an excellent product for resistance induction and significant reduction of the area under the disease progress curve, however, it does not inhibit the infection process by the pathogen caused by *P. capsici* and *Phyllachora maydis* in pepper (*Capsicum annuum* L.) and maize (*Z. mays* L.), respectively (Díaz-Morales et al., 2019; Godínez-Paoli et al., 2020). In this sense, efficiency in the control of *P. capsici* in terms of resistance induction should consider aspects such as the number of applications and the phenological stage of the plant (Cosme-Velázquez et al., 2015), otherwise a combination of strategies is recommended, including crop rotation, application of systemic fungicides, subsoiling and elevation of planting beds.

In general, plants have developed a series of complex defence mechanisms to protect themselves against pathogen attacks (fungi, viruses and bacteria), where one of the most efficient and rapidly activated mechanisms

is linked to the accumulation of ROS, such as H_2O_2 , superoxide anion (O_2^-) and radical ($\text{OH}^\cdot$) (Fortunato et al., 2015). Previous studies have demonstrated the involvement of these molecules as antimicrobial agents, promoters for protein synthesis in cell walls and may even play key roles as secondary messengers for the synthesis of other secondary compounds such as SA (Malenčić et al., 2010; Tripathi et al., 2019). However, an imbalance between antioxidant systems during pathogen-host interaction can cause cell death. Thus, all microorganisms, including *P. capsici* generate ROS as part of the by-products of their aerobic metabolism. Nevertheless, the concentration of these molecules increases significantly with pathogen-host interaction, where as part of the defence and attack mechanisms it is associated with SOD activity (Cu/Zn SOD1 and Mn-containing SOD2) (Broxton and Culotta, 2016). On the other hand, Ali et al. (2020) demonstrated that silencing of the *CaChiVI2* gene (Capana08g001237) in chilli (*C. annuum* L.) increased susceptibility to *P. capsici* with severe leaf symptoms and increased superoxide (O_2^-) and hydrogen H_2O_2 concentration compared with the control.

One of the mechanisms plants include in the attack of pathogens is the production of various secondary metabolites including polyphenolic compounds, antibiotics and enzymes, which have a wide range of activities and overlapping effects that act simultaneously as antagonistic factors (Kisa et al., 2016). Thus, one of the best strategies for biological control of fungal diseases is determined by the use of antagonistic microorganisms, including other fungi such as *Trichoderma* or bacteria (e.g. *Pseudomonas*, *Streptomyces*, *Bacillus*, among others). In this regard, *in vitro* trials with various *Bacillus* strains (BSIPR35, BSIPR22 and BSIPR7) have shown efficacy (between 54% and 80%) in controlling *P. pistaciae* inoculated on pistachio (*Pistacia vera* L.) seedlings (Moradi et al., 2018). In contrast, the mode of action of the traditional chemical method (metalaxyl-M) for the control of the oomycete *Phytophthora* sp. involves the blocking of protein synthesis by interfering with ribosomal RNA synthesis, however, its use should be complemented with other control strategies, including biological control, because its excessive use can induce pathogens to generate resistance (Álvarez-Rodríguez et al., 2018).

Previous studies have demonstrated the antifungal effects of phosphonates (potassium phosphonate) by limiting the growth of oomycete mycelium by blocking essential enzymes of the oxidative phosphorylation process (Kromann et al., 2012) and with low risk to human health and the environment, compared to proprietary products registered as contact fungicides. Likewise, it has recently been observed that it participates in the stimulation of natural defence mechanisms (induced resistance) in plants, by increasing the proteins associated with pathogenesis in chilli after applying the product (Godínez-Paoli et al., 2020).

However, their efficiency and potential of inducing resistance in the control of *P. capsici* are a function of genotypic interactions (disease severity) and the level of susceptibility of the host. Thus, the application of this type of product could be managed as an alternative for prevention, i.e. prior to the development of disease symptoms. In this regard, the application of defence activators (potassium phosphonate) on 'Papri King' and 'Caoba rojo' pepper plants with symptoms or previously inoculated with *P. capsici* has shown limited efficiency in controlling this pathogen (Huallanca and Cadenas, 2014; Godínez-Paoli et al., 2020). Nevertheless, in the case of resistant genotypes such as PS16364212, the processes of infection, colonisation and reproduction of *P. capsici* are delayed or do not occur at all due to a physical, biochemical and molecular defence response (Quispe-Quispe et al., 2022).

Plants in general, including chilli, have several metabolic pathways for the synthesis of new molecules with specific functions. One of these pathways is the phenylpropanoid pathway, where the enzyme phenylalanine ammonia lyase (PAL) is involved in the synthesis of several secondary compounds related to plant defence, including phenols and lignin, and is therefore considered as an indicator of resistance (Ashfaq et al., 2021). In this regard, the literature suggests that resistance is related to chemical mechanisms that include the prior synthesis of enzymes such as PAL, POX and SOD which interact indirectly with the pathogen, minimising the impact of induced oxidative stress and participating in the synthesis of phenols, flavonoids, lignin, SA, among others (Baysal et al., 2005; Koç et al., 2011; Ali et al., 2020). Nevertheless, the efficiency of these mechanisms may weaken or disappear depending on plant age, conditions (physiological, sanitary and physical) and the particularities of the pathogen. Previous studies conducted with foliar application of $200 \text{ mg} \cdot \text{dm}^{-3}$ of ASM by Baysal et al. (2005) on 'Arikanda' and 'Charlston' chilli seedlings inoculated with *P. capsici* report a significant increase in PAL, kinase, chitinase and β -1,3-glucanase activity, as well as accumulation and polymerisation of phenolic compounds in the cell wall. In other crops such as papaya (*Carica papaya* L.) 'Sunrise Solo' with pre- and postharvest applications of acibenzolar-S-methyl ($0.15 \text{ mg} \cdot \text{L}^{-1}$ and $0.30 \text{ mg} \cdot \text{L}^{-1}$, respectively), a significant reduction in disease severity caused by *P. palmivora* is reported in pre-harvest treated plants (de Oliveira et al., 2018). This behaviour can be linked to the overexpression of systemic acquired resistance genes and the simultaneous synthesis of phytoalexins in response to chemical, physical and biological stress caused by the pathogen.

CONCLUSIONS

In this study, the complementary use of biocontrollers (i.e. *T. harzianum*) and resistance inducers

(i.e. phosphonic acid) could help to minimise the environmental impact of the excessive use of chemical fungicides for the control of *P. capsici* on chilli pepper. These products promote the synthesis of diverse secondary metabolites (flavonoids, phenols and SA) and the activities of the antioxidant enzyme system (SOD, CAT, POX and PAL), a range of effects that help to increase the systemic resistance of chilli pepper plants to pathogen attack, including by *P. capsici*. Furthermore, these findings allow us to visualise the impact on the improvement of systemic resistance to *P. capsici*. Nevertheless, future studies must evaluate the genes in detail that are linked to defence mechanisms in chilli plants.

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AUTHOR CONTRIBUTIONS

M.T.M.D. designed experiments and performed analytical measurements. O.C.A. performed statistical analysis. O.C.A. and D.L.O.B. equally contributed to manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- AGUILAR-CARPIO, C., JUÁREZ-LÓPEZ, P., CAMPOS-AGUILAR, I. H., ALIA-TEJACAL, I., SANDOVAL-VILLA, M., AND LÓPEZ-MARTÍNEZ, V. (2018). Analysis of growth and yield of cape gooseberry (*Physalis peruviana* L.) grown hydroponically under greenhouse conditions. *Revista Chapingo, Serie Horticultura*, 24(3), 191–202.
- ALI, M., MUHAMMAD, I., UL HAQ, S., ALAM, M., KHATTAK, A. M., AKHTAR, K., ULLAH, H., KHAN, A., LU, G., AND GONG, Z. H. (2020). The CaChiVI2 gene of *Capsicum annuum* L. confers resistance against heat stress and infection of *Phytophthora capsici*. *Frontiers in Plant Science*, 11, 219, <https://doi.org/10.3389/fpls.2020.00219>.
- ÁLVAREZ-RODRÍGUEZ, B., GARCÍA-ESTRADA, R. S., VALDEZ-TORRES, J. B., LEÓN-FÉLIX, J., AND ALLENDE-MOLAR, R. (2018). Sensitivity to metalaxyl and pathogenic potential of *Phytophthora hydropathica* isolated from irrigation canals of the Valley of Culiacán. *Mexican Journal of Agricultural Sciences*, 9(6), 1149–1157.
- ANAYA-ESPARZA, L. M., MORA, Z. V. D. L., VÁZQUEZ-PAULINO, O., ASCENCIO, F., AND VILLARRUEL-LÓPEZ, A. (2021). Bell peppers (*Capsicum annum* L.) losses and wastes: Source for food and pharmaceutical applications. *Molecules*, 26(17), 5341, <https://doi.org/10.3390/molecules26175341>.
- ASHFAQ, B., ARSHAD, H. M. I., ATIQ, M., YOUSAF, S., SALEEM, K., AND ARSHAD, A. (2021). Biochemical profiling of resistant phenotypes against *Bipolaris oryzae* causing brown spot disease in rice. *Frontiers in Agronomy*, 3, 675895, <https://doi.org/10.3389/fagro.2021.675895>.
- AVENDAÑO-ARRAZATE, C. H., CAMPOS-ROJAS, E., LÓPEZ-PALESTINA, C. U., MARTÍNEZ-BOLAÑOS, M., CABALLERO-PÉREZ, J. F., BÁEZ-ALONSO, M., ARIZA-ALONSO, R., AND CADENA-IÑIGUEZ, J. (2021). Antioxidant activity in genotypes of *Theobroma* spp. (Malvaceae) in Mexico. *Revista de Biología Tropical*, 69(2), 507–523.
- BAYSAL, O., TURGUT, C., AND MAO, G. (2005). Acibenzolar-S-methyl induced resistance to *Phytophthora capsici* in pepper leaves. *Biologia Plantarum*, 49, 599–604.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254.
- BRAND-WILLIAMS, W., CUVELIER, M. E., AND BERSET, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
- BRENNAN, T., AND FRENKEL, C. (1977). Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiology*, 59, 411–416.
- BROXTON, C. N., AND CULOTTA, V. C. (2016). SOD enzymes and microbial pathogens: Surviving the oxidative storm of infection. *PLoS Pathogens*, 12(1), e1005295, <https://doi.org/10.1371/journal.ppat.1005295>.
- CERA-CAMPOS, J. I., JACOBO-CUELLAR, J. L., RODRÍGUEZ-ROQUE, M. J., PARRA-QUEZADA, R. A., SOTO-CABALLERO, M. C., PÉREZ-LEAL, R., AND CRUZ-ÁLVAREZ, O. (2019). Vegetative growth and quality of blueberry fruit cultivated in Chihuahua, Mexico. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(2), 450–457.
- CHANG, C. C., YANG, M. H., WEN, H. M., AND CHERN, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182.
- COSME-VELÁZQUEZ, Y., GUZMÁN-PLAZOLA, R. A., SANDOVAL-ISLAS, S., CORONA-TORRES, T., AND MENDOZA-PÉREZ, D. (2015). Effect of acibenzolar S-methyl on serrano pepper plants (*Capsicum annuum*) infected with *Phytophthora capsici* at

- different ages. *Mexican Journal of Phytopathology*, 33(2), 156–172.
- DE OLIVEIRA, T. A. S., BLUM, L. E. B., DUARTE, E. A. A., AND LUZ, E. D. M. N. (2018). Reduction of papaya rot (*Phytophthora palmivora*) with phosphite and acibenzolar-S-methyl in preharvest and postharvest. *Bioscience Journal*, 34(6), 1522–1531.
- DÍAZ-MORALES, F., LEÓN-GARCÍA DE ALBA, C. D., NAVA-DÍAZ, C., AND MENDOZA-CASTILLO, M. D. C. (2019). Induced resistance to *Puccinia sorghi* and tar spot complex (*Phyllachora maydis* and others) in maize (*Zea mays*). *Mexican Journal of Phytopathology*, 37(1), 1–15.
- FIGUEROA CARES, I. E., MARTÍNEZ-DAMIÁN, M. T., RODRÍGUEZ-PÉREZ, J. E., CRUZ-ÁLVAREZ, O., COLINAS-LEÓN, M. T. B., VALLE GUADARRAMA, S., AND RAMÍREZ-RAMÍREZ, S. P. (2015). Antioxidant capacity in sweet pepper (*Capsicum annuum* L.) varieties. *Interciencia*, 40(10), 696–703.
- FORTUNATO, A. A., DEBONA, D., BERNARDELLI, A. M. A., AND RODRIGUES, F. Á. (2015). Changes in the antioxidant system in soybean leaves infected by *Corynespora cassiicola*. *Phytopathology*, 105(8), 1050–1058.
- FRÍAS-MORENO, M. N., OLIVAS-OROZCO, G. I., GONZÁLEZ-AGUILAR, G. A., BENITEZ-ENRIQUEZ, Y. E., PAREDES-ALONSO, A., JACOBO-CUELLAR, J. L., SALAS-SALAZAR, N. A., OJEDA-BARRIOS, D. L., AND PARRA-QUEZADA, R. A. (2019). Yield, quality and phytochemicals of organic and conventional raspberry cultivated in Chihuahua, Mexico. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(2), 522–530.
- GARCÍA-BAÑUELOS, M. L., SÁNCHEZ, E., GARDEA-BÉJAR, A. A., MUÑOZ-MÁRQUEZ, E., SOTO-PARRA, J. M., AND OJEDA-BARRIOS, D. L. (2017). Nitrogen use efficiency and yield in response graft bell pepper cultivars. *Emirates Journal of Food and Agriculture*, 29(6), 420–428.
- GODÍNEZ-PAOLI, R., LEYVA-MIR, S. G., GARCÍA-MATEOS, M. D. R., MAGDALENO-VILLAR, J., CRUZ-ÁLVAREZ, O., AND MARTÍNEZ-DAMIÁN, M. T. (2020). Fungicides, biocontrollers and resistance inducers in commercial bell pepper hybrids inoculated with *Phytophthora capsici*. *Mexican Journal of Phytopathology*, 38(2), 258–268.
- GUARNIZO, N., OLIVEROS, D., MURILLO-ARANGO, W., AND BERMÚDEZ-CARDONA, M. B. (2020). Oligosaccharides: Defense inducers, their recognition in plants, commercial uses and perspectives. *Molecules*, 25(24), 5972, <https://doi.org/10.3390/molecules25245972>.
- HANUSZ, Z., AND TARASIŃSKA, J. (2015). Normalization of the Kolmogorov–Smirnov and Shapiro–Wilk tests of normality. *Biometrical Letters*, 52(2), 85–93.
- HERNÁNDEZ-GONZÁLEZ, Z., SAHAGÚN-CASTELLANOS, J., ESPINOSA-ROBLES, P., COLINAS-LEÓN, M. T., AND RODRÍGUEZ-PÉREZ, J. E. (2014). Effect of rootstock on yield and fruit size in grafted cucumber. *Revista Fitotecnia Mexicana*, 37(1), 41–47.
- HERNÁNDEZ-HUERTA, J., TAMEZ-GUERRA, P., GÓMEZ-FLORES, R., DELGADO-GARDEA, M. C. E., GARCÍA-MADRID, M. S., ROBLES-HERNÁNDEZ, L., AND INFANTE-RAMÍREZ, R. (2021). Prevalence of *Xanthomonas euvesicatoria* (formally *X. perforans*) associated with bacterial spot severity in *Capsicum annuum* crops in South Central Chihuahua, Mexico. *PeerJ*, 9, e10913, <https://dx.doi.org/10.7717/peerj.10913>.
- HUALLANCA, V. C. A., AND CADENAS, G. C. A. (2014). Control of *Phytophthora capsici* Leonian in *Capsicum annuum* cv. king papri with fungicides, fertilizers and biocontrol agents. *Anales Científicos*, 75(1), 130–137.
- Ji, P., AND CSINOS, A. S. (2015). Effect of oxathiapiprolin on asexual life stages of *Phytophthora capsici* and disease development on vegetables. *Annals of Applied Biology*, 166(2), 229–235.
- JIMÉNEZ-CAMARGO, A., VALADEZ-MOCTEZUMA, E., AND LOZOYA-SALDAÑA, H. (2018). Antagonism by *Penicillium* sp. against *Phytophthora capsici* (Leonian). *Revista Fitotecnia Mexicana*, 41(2), 137–148.
- KISA, D., ELMASTAŞ, M., ÖZTÜRK, L., AND KAYIR, Ö. (2016). Responses of the phenolic compounds of *Zea mays* under heavy metal stress. *Applied Biological Chemistry*, 59, 813–820.
- KOÇ, E., ÜSTÜN, A. S., İŞLEK, C., AND ARICI, Y. K. (2011). Defence responses in leaves of resistant and susceptible pepper (*Capsicum annuum* L.) cultivars infected with different inoculum concentrations of *Phytophthora capsici* Leon. *Scientia Horticulturae*, 128(4), 434–442.
- KROMANN, P., PÉREZ, W. G., TAIPE, A., SCHULTE-GELDERMANN, E., SHARMA, B. P., ANDRADE-PIEDRA, J. L., AND FORBES, G. A. (2012). Use of phosphonate to manage foliar potato late blight in developing countries. *Plant Disease*, 96(7), 1008–1015.
- LIU, H. X., LI, S. M., LUO, Y. M., LUO, L. X., LI, J. Q., AND GUO, J. H. (2014). Biological control of Ralstonia wilt, Phytophthora blight, Meloidogyne root-knot on bell pepper by the combination of *Bacillus subtilis* AR12, *Bacillus subtilis* SM21 and *Chryseobacterium* sp. R89. *European Journal of Plant Pathology*, 139, 107–116.
- LOZOYA-SALDAÑA, H., RIVERA-HINOJOSA, R., AND COLINAS-LEÓN, M. T. (2007). Phenols, peroxidase and phenylalanine ammonia-lyase: Their relationship to the genetic resistance against late blight (*Phytophthora infestans* Mont. De Bary) in potato (*Solanum tuberosum* L.) clones. *Agrociencia*, 41(4), 479–489.
- MALENČIĆ, D., KIPROVSKI, B., POPOVIĆ, M., PRVULOVIĆ, D., MILADINOVIĆ, J., AND DJORDJEVIĆ, V. (2010). Changes in antioxidant systems in soybean as affected by *Sclerotinia sclerotiorum* (Lib.) of Bary.

- Plant Physiology and Biochemistry*, 48(10-11), 903–908.
- MARÍN-MONTES, I. M., LOBATO-ORTIZ, R., CARRILLO-CASTAÑEDA, G., RODRÍGUEZ-PÉREZ, J. E., GARCÍA-ZAVALA, J. J., AND VELASCO-GARCÍA, Á. M. (2019). Allelic richness of native tomato populations (*Solanum lycopersicum* L.) for plant breeding. *Agrociencia*, 53(3), 355–370.
- MORADI, M., NEJAD, F. J., BONJAR, G. H. S., FANI, S. R., MIMAND, B. M., PROBST, C., AND MADANI, M. (2018). Efficacy of *Bacillus subtilis* native strains for biocontrol of *Phytophthora* crown and root rot of pistachio in Iran. *Tropical Plant Pathology*, 43(4), 306–313.
- PALMA-MARTÍNEZ, E., AGUILAR-RINCÓN, V. H., CORONA-TORRES, T., AND GÓMEZ-RODRÍGUEZ, O. (2017). Resistance to *Phytophthora capsici* Leo in Huacle pepper (*Capsicum annuum* L.) lines. *Revista Fitotecnica Mexicana*, 40(3), 359–363.
- PÉREZ-GRAJALES, M., PÉREZ-REYES, T. Q., CRUZ-ÁLVAREZ, O., CASTRO-BRINDIS, R., AND MARTÍNEZ-DAMIÁN, M. T. (2021). Compatibility of the rootstock CM-334 and its response on the yield, physicochemical quality and content of capsaicinoids in *Capsicum pubescens*. *ITEAInformación Técnica Económica Agraria*, 117(4), 332–346.
- PICCINI, C., PARROTTA, L., FALERI, C., ROMI, M., DEL DUCA, S., AND CAI, G. (2019). Histomolecular responses in susceptible and resistant phenotypes of *Capsicum annuum* L. infected with *Phytophthora capsici*. *Scientia Horticulturae*, 244, 122–133.
- QUISPE-QUISPE, E., MOREIRA-MORRILLO, A. A., AND GARCÉS-FIALLOS, F. R. (2022). A review about biocontrollers of *Phytophthora capsici* and its impact on *Capsicum* plants: A perspective from outside to inside the plant. *Scientia Agropecuaria*, 13(3), 275–289.
- RAMÍREZ-PRADO, J. S., ABULFARAJ, A. A., RAYAPURAM, N., BENHAMED, M., AND HIRT, H. (2018). Plant immunity: From signaling to epigenetic control of defense. *Trends in Plant Science*, 23(9), 833–844.
- SÁNCHEZ, E., SOTO, J. M., GARCÍA, P. C., LÓPEZ-LEFEBRE, L. R., RIVERO, R. M., RUIZ, M. J., AND ROMERO, L. (2000). Phenolic compounds and oxidative metabolism in green bean plants under nitrogen toxicity. *Australian Journal of Plant Physiology*, 27(10), 973–978.
- SÁNCHEZ-GURROLA, C., GÓMEZ-DORANTES, N., RODRÍGUEZ-ALVARADO, G., FERNÁNDEZ-PAVÍA, S. P., AND ÁVILA-QUEZADA, G. (2019). Morphological variability and sensitivity of *Phytophthora capsici* causing wilt in bell pepper in Chihuahua, Mexico. *Mexican Journal of Phytopathology*, 37(1), 65–71.
- SEGARRA, G., AVILES, M., CASANOVA, E., BORRERO, C., AND TRILLAS, I. (2013). Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34. *Phytopathologia Mediterranea*, 52(1), 77–83.
- TERÁN-ERAZO, B., ALIA-TEJACAL, I., BALOIS-MORALES, R., JUÁREZ-LÓPEZ, P., LÓPEZ-GUZMÁN, G. G., PÉREZ-ARIAS, G. A., AND NÚÑEZ-COLÍN, C. A. (2019). Physical, chemical and morphological fruit characterization of soursoup (*Annona muricata* L.). *Agrociencia*, 53(7), 1013–1027.
- TRIPATHI, L., TRIPATHI, J. N., SHAH, T., MUIRURI, K. S., AND KATARI, M. (2019). Molecular basis of disease resistance in banana progenitor *Musa balbisiana* against *Xanthomonas campestris* pv. *musacearum*. *Scientific Reports*, 9(1), 7007, <https://doi.org/10.1038/s41598-019-43421-1>.
- TUCUCH-HAAS, C., ALCÁNTAR-GONZÁLEZ, G., SALINAS-MORENO, Y., TREJO-TÉLLEZ, L. I., AND LARQUÉ-SAAVEDRA, A. (2017). Leaf spraying of salicylic acid increases the concentration of phenols in maize grain. *Revista Fitotecnica Mexicana*, 40(2), 235–238.
- VÁZQUEZ, K. E., FLORES-CÓRDOVA, M. A., SOTO-PARRA, J. M., SÁNCHEZ, E., SOTO-CABALLERO, M. C., SALAS-SALAZAR, N. A., RODRÍGUEZ-ROQUE, M. J., AND PÉREZ-ÁLVAREZ, S. (2022). Antioxidant activity and bio compounds induced by salicylic acid and potassium from 'Flame' grapes. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(2), 12756–12756.
- WARRIER, R. R., PAUL, M., AND VINEETHA, M. V. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology*, 3(1–2), 90–97.
- WATERMAN, P. G., AND MOLE, S. (1994). *Analysis of Phenolic Plant Metabolites* (pp. 238). Oxford, UK: Blackwell Scientific Publications.

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