Impact of *Argemone mexicana* L. on tomato plants infected with *Phytophthora infestans*

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Abstract

Background. Fungal diseases cause great losses in the tomato crop. *Phytophthora infestans* causes the late blight disease, which considerably affects tomato production worldwide. Weed-based plant extracts are a promising ecological alternative for disease control.
Methods. In the present study, the plant extract of *Argemone mexicana* L. was evaluated on the severity of *P. infestans* and the components of the antioxidant defense system in tomato plants.
Results. The results showed that the application of *A. mexicana* extract reduced the severity of *P. infestans*, increased the yield of tomato fruits, the content of photosynthetic pigments, ascorbic acid, phenols, flavonoids and reduced the biosynthesis of H_2O_2, MDA and superoxide anion in the leaves of plants inoculated with this pathogen. These results suggest that the application of *A. mexicana* extract may be a viable option for the control of diseases such as *P. infestans* in tomato crops.

Keywords: Antioxidants; biofungicide; biostimulant; defense compounds; biotic stress

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important crops produced and consumed worldwide (Nkongho et al., 2022) due to its high content of vitamins A and C, phosphorus, iron, beta-carotene, anthocyanins, and lycopene (Saffan et al., 2022). According to the latest data from FAOSTAT, 177 million tons of tomatoes were produced worldwide (Nkongho et al., 2022). However, diseases in crops caused by phytopathogenic fungi cause losses of between 20% and 40% of production (Ali et al., 2020). One of the diseases that affects tomato production is *Phytophthora infestans.*

P. infestans is a heterothallic, hemibiotrophic oomycete responsible for late blight disease (Kumar et al., 2022; Dong & Zhou, 2022). The infection spreads by air to other plant tissues, reaching total necrosis of infected plants within 5 to 10 days (Nowicki et al., 2012). Control of late blight can be achieved through a single procedure, but in most cases, it requires the use of integrated pest and disease management (IPM) (Ramasamy & Ravishankar, 2018) to minimize the use of pesticides (Zhang, Islam & Liu, 2022). Although chemical fungicides remain the most effective strategy to control this disease, however, excessive use can generate health problems, pathogen resistance, and environmental contamination (Bouket et al., 2022). Therefore, there is a growing need to explore and generate organic products to control plant pathogens.

Weed-based fungicides are gaining popularity in organic agriculture because they are safe to use on crops grown for human consumption and there is currently a lucrative market among consumers willing to pay more for organically produced foods (Ngegba et al., 2022). The use of plant extracts represents a great opportunity to obtain new biofungicides (Borges et al., 2018), in addition, foliar applications of botanical extracts are used to increase yield and quality in crop production (Osman et al., 2021).

A weed plant is any species that compete for space, nutrients, and solar energy with a crop of commercial interest (Horvath et al., 2023), one of them is *Argemone mexicana* L. commonly known as chicalote, this species contains berberine, dehydrocorydalmine, allocryptopin, oxyberberine, cysteine, phenylalanine, to which different biological activities are attributed (Brahmachari et al., 2013).

There are some reports of the use of plant extracts based on weed plants as biofungicides and/or biostimulants. (Hanaa et al., 2011), used extracts of neem (*Azardiachta indica*) in tomato seedlings infected with *Fusarium oxysporum* and reported a significant increase in the growth of shoots and roots of tomato seedlings, in addition to inhibiting the growth of this phytopathogen. (Tighe Neira et al., 2013) evaluated the effect of nettle extract (*Urtica dioica* L.) and thorny broom (*Ulex europaeus* L.), on chili seedlings (*Capsicum annuum* L.), both extracts generated an effect on the production of biomass and concentration of phenolic compounds. (Jasso de Rodríguez et al., 2020) report that sumac (*Rhus muelleri*) extract increased stem length and diameter, dry weight of leaves, number, and weight of fruits, and fruit production in tomato plants.

Therefore, the~~ aim of this work was~~is work aimed to evaluate the antifungal activity of the extract of *Argemone mexicana* L. and research its role in improving the physiological and biochemical effects in tomato plants infected with *Phytophthora infestans*.

Materials & Methods

Plant material

Random samples were collected from stems with leaves of *Argemone mexicana* in the vegetative development stage, in the winter period in the Cuautepec region of Hinojosa, Hidalgo, Mexico, located at 20 ° 09' 00"N, 98'00" W., at an altitude between 2,200-2,900 meters above sea level with an average annual temperature of 20 ºC. The collected samples were deposited in plastic bags and transported to the Postharvest laboratory of the Universidad Autonoma del Estado de Hidalgo (UAEH). Immediately the leaves separated from the stems. Leaves were stored at −70 °C (Thermo Scientific 703 Ultra-Low Freezer, Grand Island, NY, USA), and then preserved in a freeze dryer (Model 79480 LABCONCO, Kansas City, MO, USA), were subsequently ground in a blade mill (GM 200, Grindomix, Glen Mills Inc, Clifton, New Jersey, USA). Samples were stored at 5 ºC until further analysis. A complete plant was conserved for the identification of the species; This was carried out in the botany laboratory of the Institute of Biological Sciences of the Universidad Autonoma del Estado de Hidalgo.

Obtaining the plant extract

The leaf extract was obtained by maceration with ethyl acetate (200 mL) and 20 g of plant sample (1:10). The maceration was maintained for 7 days, after which the extract was filtered twice through Whatman no. 1. The solvent in the extract was removed under vacuum, using a rotary evaporator (Büchi R-215, Flawil, Switzerland) for 4 hours at a temperature of 40 ºC and a pressure of 60 mbar, as indicated by the instrument. The extract was stored in a desiccator at 26 °C and 0% relative humidity (RH) until its use in bioassays; ~~It~~ following the methodology described by (Jasso de Rodríguez et al., 2011).

Crop development and management

The culture was established in a greenhouse with a polyethylene cover. Seeds of tomato Saladette 'El Cid F1' (Harris Moran, Davis, CA, USA) with indeterminate growth, were transplanted into 12-L black polyethylene bags using a mixture of peat and perlite as substrate in a 1:1 ratio (v/v). The tomato plant was grown on a single stem. For crop nutrition, an irrigation system directed at different concentrations was used: 25% in the vegetative stage, 50% in flowering, 75% in fruit setting, and 100% in fruit filling and harvesting, according to the methodology described by (Steiner, 1961).

Preparation of the inoculation and evaluation of the severity of the disease

The inoculation was prepared following the method described by (Smith, Hammerschmidt & Fulbright, 1991) with some modifications. *P. infestans* was propagated on potato dextrose agar (PDA) and incubated for 18 days at 27 °C. The fungal growth, together with the PDA and sterile distilled water, was mixed, placed in a flask, and shaken, then the mixture was filtered through sterile gauze and the mycelium was collected. The liquid from the Petri dishes was concentrated

and a spore count was performed in the Neubauer chamber to adjust to a concentration of (1×10^6 conidia mL^{-1}). Tomato plants with young and developed leaves were inoculated with the conidia suspension (2 mL per plant), 30 days after transplanting using a camel-hair brush. Likewise, the plants were covered with a perforated plastic bag to achieve a relative humidity of ≥70% around the foliage, conditions proposed by the methodology of (Ortiz et al., 2016). The severity scale of *P. infestans* was determined following the method described by (Zárate-Martínez et al., 2018) with some modifications.

Application of treatments

Five treatments were considered: Plants without inoculation and the application of chicalote extract (T1), Inoculated plants and application of extract (T2), Plants inoculated with the pathogen and commercial fungicide captan (T3), Plants inoculated with the pathogen without additional treatment. (T4) and plants without inoculation and any additional treatment (T5). Tomato plants were sprayed with a multipurpose manual spray pump with an extract solution at a concentration of 3500 mg L^{-1} (100 mL^{-1} plant in each application) after one week of inoculation; ~~It~~ following the methodology described by (El-Nagar et al., 2020). Five applications of the treatments with the mentioned concentration were made, one week after the inoculation for the first application, then two weeks between each application, starting 45 days after the transplant.

Agronomic analysis

To evaluate the effect of the treatments on the agronomic variables of the tomato plants (average weight of the fruits, number of fruits per plant, average weight of the fruit per plant, stem diameter, dry weight of the aerial and root biomass) measurements were made every two weeks from 45 ddt. When the plants had 6 clusters, the growth apex of the plants was removed to facilitate crop management. Stem diameter was measured with a digital Vernier between the first and second leaves at the base of the plant. The average weight of the fruits and the number of fruits harvested consider the data of the five samplings during the experimentation time. In addition, 105 days after the transplant, the plants were cut on the surface of the substrate; The dry weight of the roots and shoots (stems and leaves) was obtained after they were dried in a drying oven (Model HFA-1000DP CRAFT, CDMX, Mexico) for 72 hours at a constant temperature of 80 °C, according to the methodology described by (Hernández -Hernández et al., 2018).

A sampling of leaves and fruits

Sampling was carried out one week after the first application, then every two weeks from 45 ddt to one week after each respective application; the samples were composed of three plants per treatment for each block; and four fully expanded young leaves from each plant (2nd and 3rd leaves). In the case of the fruits, the harvest was carried out every week ~~after~~ 60 days after transplanting, when the fruits had a commercial maturity index (completely colored) at ripening stage 6 according to the scale of the United States Department of Agriculture. States (USDA, 2021). Samples were stored at -70 °C (Thermo Scientific 703 Ultra-Low Freezer, Grand Island, NY, USA) and subsequently lyophilized (Freeze Dryer (Model 79480 LABCONCO, Kansas City, MO, USA), and macerated until a fine powder was obtained; using this sample,

photosynthetic pigments, stress biomarkers, and non-enzymatic antioxidant compounds were determined.

Photosynthetic Pigments Measurement

The concentrations of chlorophyll a, b, and total, were analyzed in lyophilized leaves. A mix of 10 mg of lyophilized leaves and 2 mL of hexane: acetone (3:2) was centrifuged (12000 rpm, 10 min, 4 °C). The ~~resulted~~ resulting extract was read in a spectrophotometer at different wavelengths (645, and 663 nm). The ~~resulted~~ resulting absorbances were used for later calculation with equations proposed by (Nagata & Yamashita, 1992).

Stress Biomarkers Test

Hydrogen peroxide (H_2O_2) was assessed according to the methodology described by (Service, Alexieva & Karanov, 1997) with some modifications. 10 mg of lyophilized sample was homogenized in an ice bath with 1000 µL of cold trichloroacetic acid (0.1%). The homogenate was centrifuged (12000 rpm, 15 min, 4 ºC) and 250 µL of the supernatant was added to 750 µL of potassium phosphate buffer 10 mM (pH 7.0) and 1000 µL of potassium iodide (1 M). The absorbance of the supernatant was read at 390 nm.

For the measurement of lipid peroxidation in leaves, the thiobarbituric acid (TBA) test, which determines the malondialdehyde (MDA) as an end product of lipid peroxidation was used; MDA was determined according to the methodology described by (Heath & Packer, 1968) with some modifications. In total, 50 mg of sample was mixed with 1000 µL of thiobarbituric acid (TBA) (0.1%) and was centrifuged (10000 rpm, 20 min, 4 ºC) and 500 µL of the supernatant was added to 1000 µL of TBA (0.5%) in trichloroacetic acid (20%). The mixture was incubated in water at 90 ºC for 30 minutes, the reaction was quenched with ice, and the sample was centrifuged (10000 rpm, 5 min, 4 °C). Then, the absorbance of the supernatant was measured at 532 nm to calculate the amount of MDA-TBA complex using an extinction coefficient of 155 mM-1 cm-1.

The superoxide anion ($O_2^{\bullet-}$) content was determined according to the methodology described by (Wang & Luo, 1990) with some modifications. 20 mg of lyophilized sample was added with 5 mg of PVP and homogenized with 1000 µL of cold 50 mM phosphate buffer (pH 7.8). The mix was centrifuged (5000 rpm,15 min, 4 °C) and 600 µL of the supernatant was added to 550 µL of potassium phosphate buffer (50 mM) (pH 7.8) and 60 µL of hydroxylamine hydrochloride (10 mM). The mixture was incubated (30 min, 25 °C). In total, 650 µL of the incubated solution was mixed with 650 µL of 3 Aminobenzenesulphonic acid (17 mM) and 650 µL of 1-Naphtylamine (7 mM). The absorbance was read at 530 nm.

Non-enzymatic antioxidant compounds Measurement

The content of total phenols was obtained according to (Singleton & Rossi, 1965) with some modifications.100 mg of lyophilized sample more1 mL of a water/acetone solution (1:1) was homogenized for 30 s. The sample tubes were centrifuged (17500 rpm, 10 min, 4 °C). Then, 18 µL of the supernatant, 70 µL of the Folin–Ciocalteu reagent, and 175 µL of 20% sodium carbonate (Na_2CO_3) were placed in a test tube, and 1750 µL of distilled water was added. The samples were placed in a water bath (30 min, 45 °C). Finally, the reading was taken at a wavelength of 750 nm.

The concentration of total flavonoids was determined by mixing 20 mg of lyophilized tissue with more than 2 mL of methanol and subsequently filtered with a Whatman Filter No. 1. For the quantification, a mix of 1 mL of solution and 1 mL of $AlCl_3$ (2%) was incubated in dark conditions for 20 min. After this time, the sample was read at 415 nm, according to the methodology described by (Arvouet-Grand et al., 1994).

The vitamin C content was determined according to the methodology described by (Klein & Perry, 1982), which was read at 515 nm, and the results were expressed in mg 100 g^{-1} of DW.

The β-carotene content was determined according to the method of (Zscheile, Comar & Mackinney, 1942; Nagata & Yamashita, 1992). The absorbances were read at 453, 505, 645, and 663 nm, and the results were expressed in mg 100 g^{-1} of DW.

Yellow carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin) were evaluated according to the method reported by (Hornero-Mendez & Minguez-Mosquera, 2001). The measurements of yellow carotenoids were expressed as milligrams per 100 g of dry weight (mg 100 g^{-1} DW).

The quantification of proteins was determined using Bradford's colorimetric technique, a method reported by (Bradford, 1976). The samples were read at a wavelength of 630 nm on a microplate reader. The total proteins were expressed in mg g^{-1} of DW.

Statistical analysis

Five replicates with three plants per experimental unit were considered for each of the treatments, in a randomized complete block design. An analysis of variance and Fisher's least significant difference (LSD) test of means ($\alpha = 0.05$) were performed to analyze the agronomic and biochemical variables of tomato. To determine the differences between treatments in the severity of *P. infestans*, a repeated measures multivariate analysis of variance and Hotteling test ($\alpha = 0.05$) were performed. All Statistical procedures were performed with the Infostat 2020 software.

Results

***P. infestans* severity and crop development**

The severity of the disease decreased with the application of *A. mexicana* extract and captan. Late blight reached a severity of 90% in the Infes treatment (Fig. 1A). However, the Infes+EXAm treatment generated a 48% decrease while the captan reduced the disease by 69% during the entire vegetative cycle of the crop; It should be mentioned that during the first weeks after inoculation, when *P. infestans* was most infective, the Infes+ EXAm and Infes+Captan treatments reduced severity by 57.6% and 71%, respectively, compared to the Infes treatment. The rest of the treatments (EXAm and control) remained free of the disease throughout the evaluation, which was expected (Fig. 1B).

For the agronomic parameters (Fig. 2), the results indicate that the EXAm treatment was the best, this increased the average weight of the fruits by 138% compared to the control treatment and 167% compared to the Infes treatment (Fig. 2A). The number of fruits per plant was reduced by

20% with the Infes treatment and 12% with the treatments: Infes+EXAm, Infes+Captan, and control treatment ~~with respect to~~for the EXAm treatment (Fig. 2B). Similar behavior was presented for the average weight of the fruits per plant (Fig. 2C). The Infes treatment reduced the average weight of the fruits by 28% compared to the EXAm treatment, but this same treatment increased the weight by 127% ~~with respect~~ to the treatment control; even in diseased plants with the application of extract (Infes+EXAm) and application of commercial fungicide (Infes+Captan), the weight increased on average 110% in the same sense of comparison. Throughout the vegetative cycle, stem diameter was reduced by an average of 15% in the Infes+EXAm and Infes+Captan treatments, although this increased by 110% with the EXAm treatment compared to the control treatment and was reduced by 23% in the Infes treatment compared to the EXAm treatment (Fig. 2D). Although the foliar application of chicalote extract ~~had an effect on~~affected crop yield and development, there were no significant differences in aerial (Fig. 2E) and root (Fig. 2F) dry weight.

Photosynthetic pigments content in leaves

The content of photosynthetic pigments was variable throughout the different evaluations in the vegetative cycle of the crop (Fig. 3). The results indicate that the chlorophyll a content (Fig. 3A) increased by 157% with Infes+EXAm compared to the control 45 ddt (in the vegetative development stage); During the following three evaluations, 60 and 75 ddt (flowering stage and fruit set) EXAm and Infes+EXAm increased the content by 129% and 121% ~~with respect~~ to the control. But this was reduced by 35% with the Infes+Captan treatment in the same sense of comparison; On days 90 and 105 after transplanting (fruiting stage) the chlorophyll content was reduced by 52% with the Infes treatment, however, it increased by 150% with the EXAm treatment compared to the control treatment.

The chlorophyll b content (Fig. 3B) decreased by an average of 47% with the Infes+Captan treatment compared to the control treatment throughout the different evaluations of the vegetative cycle; for example, between 60 and 75 ddt (flowering stage and fruit set) the content was reduced by 78%. In these same evaluations, the EXAm and Infes+EXAm treatments increased the content by 117% and 133% respectively ~~with reference to~~about the control treatment. 90 and 105 ddt (fruit set and fructification stage) the content was reduced by 48% with the Infes treatment, but it increased by 138% with the EXAm treatment compared to the control treatment.

In general, the highest values in total chlorophyll content (Fig. 3C) were presented by the control treatment except for 45 ddt (vegetative growth stage), as well as for days 60, 75, and 90 after transplantation (flowering and fruit set stage). fruit), for these stages the EXAm and Infes+EXAm treatments increased 129%, 154%, 110%, and 124% respectively compared to the control treatment. Throughout the different evaluations of the vegetative cycle, the total chlorophyll content decreased considerably with the Infes+Captan treatment, for example, at 60 and 75 ddt (flowering and fruit set), this content was reduced by 68% compared to the control treatment. For days 90 and 105 after the transplant (fruiting) the content was reduced with the treatments: Infes+EXAm, Infes+Captan, and Infes by 48%, 47%, and 52% respectively ~~in~~

~~reference to~~about the control treatment, although this was increased with the EXAm treatment 149% compared to the Infes treatment.

Stress biomarkers in tomato leaves

The hydrogen peroxide (H_2O_2) content in tomato leaves showed significant differences between treatments in some evaluations (Fig. 4A). Although 45 ddt (vegetative development stage) there were no significant differences between treatments, in 60 and 75 ddt (flowering stage and fruit set) the behavior was different, the H_2O_2 content increased with the Infes+EXAm and Infes treatments in a 151% and 200% ~~with respect~~ to the EXAm treatment, which was the treatment with the lowest H_2O_2 content, even with 10% less than the control treatment. On days 90 and 105 ddt (fruiting), the highest values of H_2O_2 occurred with the Infes treatment; At this same stage, it is important to mention that the H_2O_2 content was reduced by 58% with the EXAm treatment and 60% with the Infes+EXAm treatment, as well as the control treatment ~~with respect~~ to the Infes treatment.

The results of malondialdehyde (MDA) (Fig. 4B) did not present significant differences between treatments. However, it is important to highlight that at 45 ddt (vegetative development stage) the MDA content was reduced with the EXAm and Infes+EXAm treatments by 35% and 28% respectively compared to the control treatment. During days 60 and 75 after transplanting (phenological flowering and fruit set), as well as 90 and 105 ddt (fruiting), MDA synthesis increased with the treatments previously inoculated with *P. infestans*, while EXAm and the control treatment reported the lowest concentrations as expected; for example, the Infes+EXAm, Infes+Captan and Infes treatments increased the MDA content by 133%, 120% and 145% respectively compared to the control treatment 60 and 75 ddt (flowering stage and fruit set); although 90 and 105 ddt (fruiting) Infes+Captan increased the MDA content even more by 17% and 18% compared to the Infes+EXAm and Infes treatment respectively.

In most evaluations of the vegetative cycle of this crop, the superoxide anion concentration (Fig. 4C) increased with the Infes treatment; for example, 45 ddt (vegetative development stage), as well as 60 and 75 ddt (flowering stage and fruit set) this increase on average 132% compared to the control treatment. On the 45 ddt (vegetative development stage) the superoxide anion content was reduced with the EXAm and Infes+EXAm treatments by 26% and 6% respectively compared to the control and 41% and 28% ~~with respect~~ to the Infes treatment. For days 60 and 75 ddt (flowering and fruit set stage), as well as 90 and 105 ddt (fruiting), the EXAm and/or Infes+EXAm treatment reduced the concentration of superoxide anion by an average of 5% and 8%, respectively. compared to the control treatment. For the last two evaluations, 90 and 105 ddt (fruiting stage), the control treatment increased superoxide synthesis by 114% compared to the EXAm treatment.

Non-enzymatic antioxidant compounds

The phenol content was reduced by an average of 21% with the Infes treatment compared to the control treatment throughout the evaluations of the vegetative cycle (Fig. 5A), in some evaluations the difference was considerable, for example, 45 ddt (vegetative development) the

content was reduced by 43% in the same sense of comparison, although the phenol content was affected with the Infes treatment, this increased an average of 127% with the EXAm treatment ~~with respect~~ to the control treatment 60 and 75 ddt (stage flowering and fruit set).
The flavonoid results present significant differences between the treatments (Fig. 5B), the flavonoid content was reduced with the Infes+Captan and Infes treatments by 22% and 25% respectively ~~with respect~~ to the control treatment, and this increased with the EXAm treatment by 113% compared to the control treatment at the different evaluations. The vitamin C content did not present changes with the presence of *P. infestans* (Fig. 5C).
On days 60 and 75 after transplanting (phenological stage of flowering and fruit set) the β-carotene content increased by 106% with the Infes+EXAm treatment compared to the control treatment (Fig. 5D). The Infes+Captan treatment reduced an average of 38% compared to the control treatment. In general, yellow carotenoids (Fig. 5E) were reduced by an average of 30% with the Infes+Captan treatment compared to the control treatment; In these same evaluations, 60 and 75 ddt (phenological stage of flowering and fruit set) the content of yellow carotenoids increased with the EXAm treatment by 113% compared to the control treatment. The protein content (Fig. 5F) increased with the EXAm and Infes+EXAm treatments by 127% and 124% respectively compared to the control treatment 60 and 75 ddt (phenological stage of flowering and fruit set); 90 and 105 ddt (fruiting stage) the Infes treatment reduced the protein content by 30% ~~with respect~~ to the control treatment.

Discussion

P. infestans severity and crop development

In this investigation, the severity of the disease of 90% in tomato plants inoculated with *Phytophthora infestans* can be explained by a variety of effector proteins, both apoplastic and cytoplasmic, to manipulate the physiology of the host plant and facilitate its colonization. Apoplastic effects include hydrolytic enzymes such as proteases, lipases, and glycosylases that degrade plant tissues to inhibit defense enzymes such as peptidases and proteases (Whisson et al., 2007; Haas et al., 2009). When pathogenesis molecules produced by *P. infestans* are recognized within plant cells by plant receptors containing leucine-rich repeat domains and the intracellular nucleotide-binding domain they can trigger a localized programmed cell death process. This mechanism is aimed at limiting the spread of the pathogen and limiting its further expansion. (Jones & Dang, 2006). The programmed cell death located in the plant to restrict a further expansion of the phytopathogen influenced the severity of the late blight disease in tomato plants inoculated with *P. infestans* (Fig. 1B), and this is related to the fact that *P. infestans* is hemibiotrophic, that is, it shows a biotrophic phase where the fungus feeds on living tissues and is later followed by a necrotrophic phase where it feeds on dead cells (Zuluaga et al., 2016).
In this study, the foliar application of *A. mexicana* extract on inoculated plants reduced severity by 48% (Fig. 1A), and this can be explained because the plant extracts contain a multitude of secondary metabolites with antifungal activity, including alkaloids, cyanogenic glycosides,

glucosinolates, lipids, phenolic compounds, terpenes, polyacetylenes and polythienyls, and even some of them stimulate the ability of infected plants to cause an increase in VOCs in neighboring uninfected plants (Du Fall & Solomon, 2011; Gurjar et al., 2012). Constituents of plant origin can exhibit different modes of action against phytopathogens, including interference with phospholipid cell membranes, which results in increased permeability profile and loss of cellular constituents (Omojate Godstime et al., 2014); inhibition of cellulase synthesis, chelation of metals necessary for the activity of microbial enzymes, polymerization into crystalline structures that can act as a physical barrier during pathogen attack (Skadhauge, Thomsen & Von Wettstein, 1997); disruption of the electron transport chain and consequently slowing down of all ATP-dependent functions, inhibition of DNA synthesis and helicase activity, compromising cell division and termination of chromosome replication resulting in inhibition of the growth (Fontana et al., 2022).

The presence of *P. infestans* in crops affects the development and yield, as well as the quality of the fruit, the total yield losses in tomatoes reach up to $112 million annually (Nowicki et al., 2012) and it is estimated that the costs of late blight control in tomato exceed $5 billion annually worldwide (Galeano-Garcia et al., 2018). In this investigation, the extract of *A. mexicana* increased the yield of tomato plants by 167% compared to the yield of plants inoculated with *P. infestans* (Fig. 2A). (Turóczi et al., 2020) evaluated the extract of *Populus nigra* in potato plants previously inoculated with *P. infestans* and report a decrease in severity using foliar applications of *P. nigra.* (Islam et al., 2013) obtained a yield reduction of 48.2% in tomato plants with the presence of *P. infestans* and an increase of 126% with the foliar application of botanical extracts ~~with respect~~ to the control treatment, these results coincide with what was observed in this research. And this is related to the fact that plant extracts improve the availability, absorption, and assimilation of nutrients in plants, which helps to tolerate stress by pathogens, increase product quality and finally minimize the use of fertilizers and fungicides (Caruso et al., 2019).

Photosynthetic pigments content in leaves

On average, the content of chlorophyll a, b, and total chlorophyll was reduced by 50% in inoculated plants (Fig. 3), this is ~~due to the fact that~~because *P. infestans* causes notable changes at the physiological level, such as a reduction in the photosynthetic rate, modification in the transpiration, changes in membrane permeability, increased respiratory rate, alterations in tissue expression profiles, among others (Arévalo-Marín et al., 2021). The application of *A. mexicana* extract increased the content of photosynthetic pigments by an average of 133% (Fig. 3). (Naboulsi et al., 2022) reported an increase of 114% in chlorophyll a and 150% in chlorophyll b in tomato plants subjected to abiotic stress and treated with foliar applications of the plant extract based on *Crataegus oxyacantha*, which coincides with what is reported here observed and this may be related to the fact that the application of plant extracts contributes to a greater synthesis of photosynthetic pigments in leaves, mainly to the large amount of natural nitrogenous compounds that are important for the synthesis of chlorophyll pigments, therefore, improves plant survival under stress situations (González et al., 2013). In addition, biostimulants of botanical origin are involved in signaling events and gene expression such as DtDREB2A, DtMYB30, DtNAC019, DtNAC72, DtNAC19, DtNAC69, DtZIP63, DtABF3, DtHB12, GRMZM2G439784,

GRMZM2G324221, GRMZM2G164 129, GRMZM2G163866 to mention a few; which have reports of transmitting signals during development, activating defenses against stimuli against pathogens, regulating the response to infections, improving nitrogen metabolism, and some others respond to abscisic acid and promote the antioxidant system (González-Morales et al., 2021).

Stress biomarkers in tomato leaves

When optimal growth conditions are provided, the levels of reactive oxygen species such as superoxide radicals, hydrogen peroxide (H_2O_2), hydroxyl radicals (OH •), and singlet oxygen ($^1O_2$), are low within the organelles (Nadarajah et al., 2020). However, in periods of stress, these levels rise, which hinders the natural physiological or metabolic state of plants because they affect proteins and lipids, causing cell damage and death. This can be quantified with malondialdehyde (MDA) (Verma et al., 2021). In the context of plant-pathogen interactions, elevated MDA levels are indicative of severe oxidative stress in plants. When pathogens attack plants, MDA levels can be an excellent indicator of membrane damage (Behiry et al., 2022). The overproduction of reactive oxygen species and the derived lipid peroxidation is closely related to a decrease in plant growth, fruit quality, and low content of secondary metabolites in the crop of commercial interest (Ren et al., 2022). In this study, MDA levels increased by 120% when plants were sprayed with the commercial fungicide Captan, but overall reactive oxygen species and malondialdehyde content were reduced by 31% when plants were sprayed with extract of *A. mexicana* (Fig. 4B). (Naboulsi et al., 2022) report a decrease in the MDA content of 44.78% in tomato plants subjected to abiotic stress and with applications of hawthorn extract (*Crataegus oxyacantha*).

(Behiry et al., 2022) report that the levels of MDA and H_2O_2 did not present statistical differences with foliar applications of bottle tree extract (*Chorisia speciosa*) in tomato plants under stress by *Rhizoctonia solani* compared to the control, these results coincide with what was observed in this research. ~~And t~~This can be explained because the extracts used as a biostimulant are complex matrices that contain substances of natural origin with different useful active compounds (Campobenedetto et al., 2021), where minerals and secondary metabolites are included (~~ege.g.~~, proline, simple sugars, alcohols, abscisic acid, and antioxidant compounds) that mitigate the concentration of reactive oxygen species and at the same time improve photosynthetic activity, transpiration rate, stomatal conductance, and antioxidant activity, which are strongly and positively correlated with optimal plant development (Hernández-Herrera et al., 2022). The increase in MDA in plants that received foliar sprays of Captan is related to the fact that chemical fungicides do not discriminate between plant and fungal cells, in other words, fungicides contribute to membrane lipid peroxidation and mitochondrial dysfunction in plant cells (Gorshkov et al., 2020).

Non-enzymatic antioxidant compounds

Among the defense-related secondary metabolites, phenols play an essential role due to their chemical structure that acts as antioxidants. Various internal and external factors, including stages of growth, development, and pathogen attack, affect the synthesis and accumulation of phenols (Dadáková et al., 2020). In this investigation, the phenol content was reduced by an

average of 32% in inoculated plants and increased by 69% with the application of extract (Fig. 5A); this is because the presence of phytopathogens causes unfavorable changes that compromise the synthesis of non-enzymatic antioxidant compounds, which include phenolic compounds (Aina et al., 2022). The results of this research coincide with what was reported by (Ertani et al., 2014) who reported that the phenol content increased in pepper leaves (*Capsicum chinensis* L.) after the application of extracts of alfalfa (*Medicago sativa*) and grape (*Vitis vinifera*). According to the results obtained in this work, as well as those reported in the literature, plant extracts can modify the synthesis of non-enzymatic and enzymatic antioxidant compounds as a result of increased expression of the DET2 gene (Taha et al., 2020). which benefits the defense of the plant in the presence of pathogens (Aitouguinane et al., 2020).

The flavonoid content increased by 113% in tomato plants sprayed with extracts of *A. mexicana* (Fig. 5B), results similar to those reported by (Giordano et al., 2022) where they reported an increase of 141% in the flavonoid content with the application of extracts of tropical plants in lettuce (*Lactuca sativa)* cultivation. This effect is ~~due to the fact that~~because the application of plant extracts in crops activates secondary metabolism through an increase in the expression of genes that code for phenylalanine (tyrosine), an ammonia-lyase enzyme. Cinnamic acid and later coumaric acid which originates from phenylalanine, are transformed by the PAL enzyme. From coumaric acid begins the synthesis of flavonoids (Schiavon et al., 2010; Ertani et al., 2011). Flavonoids are synthesized in all parts of the plant and are directly involved in the inhibition of the pathogen's enzymes, especially those that digest the cell wall of the plant, by chelating the metals necessary for their activity, likewise, they can alter the membranes of bacteria, change their fluidity and alter the respiratory chain (Mierziak et al., 2014), which is also related to the decrease in the severity of the disease in tomato plants inoculated with *P. infestans* and sprayed with *A. mexicana* extract.

Vitamin C or ascorbic acid is one of the antioxidant molecules present in most living organisms including plants. In plant tissues, it is abundant in almost all subcellular compartments and the apoplast, as well as in both photosynthetic and non-photosynthetic tissues (Egea et al., 2022). In turn, it is responsible for the remarkable diversity of its function in plants, which includes ROS detoxification, plant development and hormonal signaling, cell cycle and expansion, flowering, seed germination and viability, regeneration of other antioxidants, plant responses to pathogen attack, cellular redox system, as well as an enzyme cofactor (Mellidou et al., 2021). In this study, the vitamin C content increased at days 45, 60, and 75 after transplant when the plant was in vegetative development and flowering (Fig. 5C); although the spraying of *A. mexicana* extract did not generate differences in the vitamin C content, it is important to mention that at day 75 ddt the vitamin C content increased by an average of 133% when the plants were sprayed with *A. mexicana* compared to the control treatment and this coincides with what was reported by (Abd El-Hamied et al., 2015) where the spraying of extracts of *Moringa oleifera* in pear trees increased the content of vitamin C.

In this study, the variation in the vitamin C content throughout the investigation can be explained because the content is influenced by the development stage of the plant and by stress (Kukavica et al., 2004); in addition, when there is the presence of a phytopathogenic fungus, genes such as

MDHAR and DHAR are expressed (Paciolla et al., 2019); and specifically in tomato plants, HZ24 is overexpressed, a transcriptional factor that binds to the GMP, GME2, and GGP promoters, which raise vitamin C levels and reduce oxidative stress in plants (Hu et al., 2016). Carotenoids are important natural pigments found in all plants and carry out important biological functions such as stabilization of lipid membranes, assembly of lipoprotein structures, photosynthetic light harvesting, and protection of the photosystem from cell-mediated damage of reactive oxygen species (ROS) (Giuliano, 2017; Simkin, 2021). In this study, the β-carotene content increased by an average of 84% in tomato plants that received the foliar application of the *A. mexicana* extract compared to the Infes treatment. (Fig. 5D) These results coincide with what was reported by (Giordano et al., 2022) where an increase in β-carotene was reported in lettuce plants (*Lactuca sativa*) when they received foliar application of extract from tropical plants. In this study, the increase in the β-carotene content can be justified because the plant extracts contain phytohormones (auxins and cytokinins), carbohydrates, amino acids, and proteins, which delay the oxidation of b-carotene and stimulate the expression of genes such as GmNAC018, GmNAC030, GmNAC039, GmNAC043 which delay the senescence of the leaves (Fraga et al., 2021; Yuniati et al., 2022). Leaf senescence is a crucial developmental stage in plant life, therefore, delaying it plays a vital role in the biosynthesis of carotenoids, as well as other natural pigments (Azaizeh et al., 2005). Therefore, plant extracts are a promising and sustainable approach that farmers can incorporate into their farming systems (Ali et al., 2021).

Yellow carotenoids are located in subcellular organelles (plastids), that is, chloroplasts and chromoplasts. In chloroplasts, carotenoids are mainly associated with proteins and serve as accessory pigments in photosynthesis, while in chromoplasts they are deposited in crystalline form or as oily droplets (Khoo et al., 2011). The colors of fruits and vegetables depend on the conjugated double bonds and the various functional groups contained in the carotenoid molecule, generally the carotenoid content increases in mature plants and fruits (Tanaka et al., 2008). In this study, the values of yellow carotenoids increased in plants that received the foliar application of *A. mexicana* extract compared to the other treatments evaluated (Fig. 5E). This coincides with what was reported by (Khan et al., 2019) where the carotenoid content in carrot plants (*Daucus carota* L.) increased under biotic stress and with the application of *Phyllanthus amarus* plant extract. In this investigation, the increase in the content of yellow carotenoids can be explained because the botanical extracts induce the biosynthesis of enzymes such as 1-deoxy-D-xylulose 5-phosphate synthase (DXS); 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR); 2-Cmethyl-D-erythritol 4-phosphate cytidyltransferase (MCT); acetoacetyl-CoA thiolase, HMG-CoA synthase; MVPP decarboxylase, to mention a few, which regulate the mevalonic acid (MVA) pathway and the methylerythritol 4-phosphate (MEP) pathway, pathways responsible for the synthesis of yellow carotenoids; increased carotenoid biosynthesis is also associated with the presence of important plant hormone precursors, including abscisic acid, strigolactones, and different signaling molecules β-cyclocitral, zaxinone, and anchorene, which moderate plant development and help to tolerate stress. stress by phytopathogens (Godlewska et al., 2019; Zheng et al., 2020; Godlewska et al., 2020). In addition, it is important to emphasize that carotenoid-rich

fruits and vegetables play an important role in the prevention of chronic-degenerative diseases in humans (Saini et al., 2015).

Proteins are of great importance in the metabolism of plants. There are catalytic, transport, structural, defense, and reserve proteins, which are involved in all metabolic processes in plants (Sariñana-Aldaco et al., 2022). In this investigation, the protein content did not present differences in the different treatments; however, it decreased in tomato plants inoculated with *P. infestans* and increased in plants that received foliar sprays of extract (Fig. 5F). These results coincide with what was reported by (Ertani et al., 2016) where the application of plant extracts of hawthorn and skin of the red grape and blueberry increased the protein content in corn plants (*Zea mays* L.) by 115% on average. The reduction in the protein content can be justified because when the stress in the crop is high, the generation of ROS increases and, therefore, greater oxidative stress is caused, which consequently can decrease the production of proteins (González-Moscoso et al., 2019).

On the other hand, the increase in protein is related to the fact that biostimulants contain plant hormones, such as cytokinins, which reduce the mRNA and protein levels of proteases, thus preventing the increase in proteolytic activity (Veerasamy & Huang, 2007). Any circumstance that promotes the synthesis of cytokinins is beneficial for the crop, however, it is important to consider the type of biostimulant, the dose used, and the moment of application (Del Buono et al., 2023).

Conclusions

In the present study, tomato plants inoculated with *Phytophthora infestans* reduced crop development and yield; however, foliar application of *Argemone mexicana* extract controlled disease severity and increased crop yield.

In the same way, with the application of *A. mexicana* extract, an increase in chlorophyll, non-enzymatic antioxidant compounds such as phenols, flavonoids, β-carotenoids, yellow carotenoids, and proteins was generated, which helped the plants to tolerate the stress caused by *P. infestans*. This indicates that *A. mexicana* extracts can be an ecological alternative to mitigate the negative effects of phytopathogens in tomato crops by inducing growth and activating the antioxidant system.

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