Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Leandris Argentel Martínez
Ofelda Peñuelas Rubio
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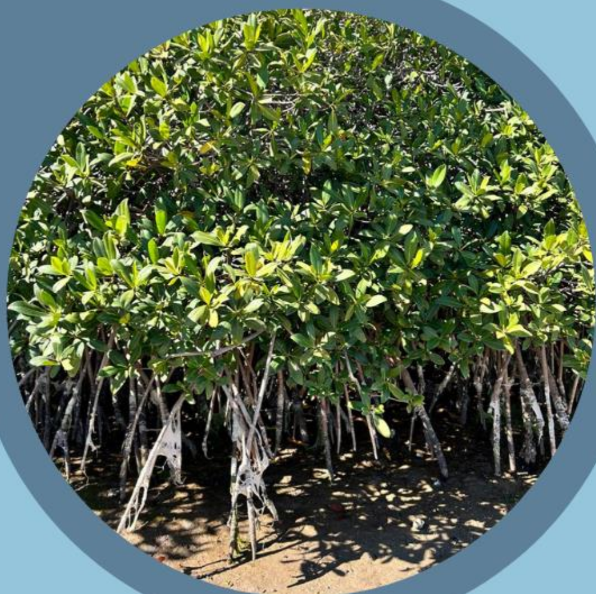

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Prologue

Advances in biology through agronomy, aquaculture, coastal and environmental sciences is an electronic book, edited by Pantanal Editora, based on the compilation of research papers where the authors of the different chapters have used highly current scientific methodologies and research equipment.

The biological sciences as the main object of research in agriculture, aquaculture, coastal and environmental sciences generate every day an understanding of knowledge that allows raising the scientific level of society as part of universal access to knowledge.

This book mainly addresses issues related to the use of plants extracts as sustainable alternatives for biocontrol of pests and bacterial diseases. It also brings together information on viruses and other diseases in aquatic organisms. In addition, studies of mangroves structure and their contribution to carbon sinks in experimental sites in northwestern Mexico are presented. Finally, an analysis on educational strategies for environmental education based on plant biology is carried out.

Editors appreciate the participation of the authors who have come from higher education institutions and research centers of great scientific prestige in Mexico. The majority of them are members of the National Research System of CONACyT, Mexico.

The authors

Summary

Chapter 1

Phytotoxicity of hydroalcoholic extracts of *Parkinsonia aculeata* L. sp. Pl., in tomato plants. Polyphenol and flavonoid content

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ABSTRACT

This research aimed to determine the content of polyphenols and flavonoids in hydroalcoholic extracts obtained from the stems and leaves of *Parkinsonia aculeata* and their phytotoxicity in tomato seedlings applied at the early stages of growth. The extracts were applied at 15, 25 and 35 days after emergence (DAE). The highest content of polyphenols and flavonoids was obtained in the leaves, and the polyphenol concentration exceeded that of flavonoids. The hydroalcoholic extracts of both stems and leaves presented level 5 phytotoxicity in tomato plants at 15 DAE. However, from 25 DAE, there was no phytotoxicity. At 35 DAE, there was only phytotoxicity when the volume of both organs was 5 mL plant^{-1}. There was a significant interaction between organ and volume factors. The study shows that leaf and stem extracts can be used for biocontrol without causing phytotoxicity in tomato plants from 25 days, using volumes between 1 and 3 mL plant^{-1}.

Keywords: antioxidants, biocontrol, palo verde.

INTRODUCTION

Phytopathologists and producers, with an organic approach, employ various agrotechnical alternatives with the aim of reducing the contaminant load from the excessive application of broad-spectrum fungicides. The use of plant extracts to control pests and diseases is one of these agrotechnical alternatives (Báez et al., 2021). Among them, extracts of species such as creosote bush (*Larrea tridentate* (DC.) Coville) and oregano (*Origanum vulgare* L.) have been used to control phytopathogenic fungi of the

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genus Fusarium, which produces significant losses in the yield of crops of economic interest (Figueroa et al., 2019).

Plants sometimes produce a significant amount of primary and secondary metabolites as a regulatory action on a large number of pests and diseases; for this reason, the possibility of being used with a focus on environmental protection through integrated management is being studied (Zoppolo et al., 2008). Some of these metabolites are synthesized as defenses (repellents), and others intoxicate and directly eliminate microorganisms and pests. For example, saponins, amygdalins, and norhydroguaiaretic acid are used for the control of fungi and bacteria (Martínez-Olivo et al., 2020). Protocatechotic acid has a significant effect on the control of pathogens in general and in particular on preventing basal rot in tomatoes (El-Nagar et al., 2020). Among the metabolites that plants synthesize are polyphenols (PP), which are obtained from the shikimic acid biosynthetic pathway (Santos-Sánchez et al., 2019). PP is normally an antioxidant whose main function is to prevent damage to foliar organs. The damage can be due to biological oxidation induced by abiotic stress or caused by insects and microorganisms (Lee et al., 2020), including bacteria and fungi (Rivera-Solís et al., 2021).

Many species of the semidesert also show characteristics of tolerance to pests and diseases, subsisting in addition to prevailing adverse conditions such as salinity and drought (Gonzáles et al., 2021), as is the case for *Parkinsonia aculeata* L. Sp. Pl., commonly known as “palo verde or bacaporo” (Adhikari, White, 2014). This species has been studied in various regions of the world mainly for clinical purposes (Divya et al., 2011; Franco et al., 2022), and phytochemical studies have been developed to determine the presence of various metabolites that can be used for the biocontrol of pathogens, such as *Fusarium oxysporum* (Arvizu-Quintana et al., 2021). These studies are of great importance in the prevention of environmental contamination after having proven that they present minimal or no phytotoxicity in plants of economic interest. An important step is to evaluate whether these extracts obtained from model plants, such as “palo verde”, affect the physiological, biochemical and/or agronomic performance of crops. Another important point is to substitute some chemical pesticides with these products. This substitution may reduce the amounts of pollutants in agricultural areas. Taking these elements into account, a study was carried out with the objective of determining the content of polyphenols and flavonoids in hydroalcoholic extracts obtained from stems and leaves of P. aculeata and evaluating the phytotoxicity in tomato seedlings applied in the initial stages of development.

MATERIALS AND METHODS

Study site

Location of the experimental area. The research was developed in the Biotechnology Laboratory of the National Institute of Technology of Mexico, Valle del Yaqui Campus, in the municipality of Bácum, Sonora, Mexico. Leaf and stem samples of *P. aculeata* plants were taken from the semidesert of

Sonora to obtain hydroalcoholic extracts by percolation according to the process described by Fecker et al. (2020).

Extract preparation

The samples were separated at a rate of 100 g and then remained in 1 L of 76% alcohol for 10 days (Figure. 1a-b, leaves and stems, respectively). After this period, the alcohol was separated using a rotary evaporator at 30 revolutions per minute and at a temperature of 65°C (BUCHI® R215, USA), with an extraction efficiency of 75%. The extracts were kept at 4°C until they were used (Figure 1c).

Figure 1. Sample maceration from (a) stem (b) and hydroalcoholic extracts from leaves (c).

Tomato variety used to evaluate phytotoxicity

As an experimental model, tomato seeds of the Río Grande® variety were used, with determined growth, cataloged as susceptible to fusarium wilt (Arellano-Aburto et al., 2021). The seeds were sown in 200-well polyfoam trays under semicontrolled conditions in a growth chamber. The conditions inside the chamber were adjusted to 10 hours of light, a temperature of 25°C and a relative humidity of 75%. Peat moss-type substrate (PROMIX®) was used for seed germination. At 15 days after emergence (DAE), the seedlings were selected and subjected to the established treatments.

Treatments and experimental design

The treatments consisted of the combination of two sources of variation: A) plant organs, with two levels (stems and leaves); and B) volumes of extracts applied, with four levels (0, 1, 3 and 5 mL $plant^{-1}$). The level of zero applications of the extract was taken as the control treatment. Each treatment of the eight conformed had a sample size of 30 plants. The application of the extracts was carried out on the roots and foliar route in unison three times after emergence: at 15, 25 and 35 DAE. This last factor was not included as a source of variation, and the results were compared separately in the respective statistical analyses.

The treatments were distributed under semicontrolled conditions, following a completely randomized experimental design with a bifactorial arrangement. In all treatments, the edge effect and neighboring variants were taken into account for phytotoxicity variable measurement. These plants were dispensed with to reduce the possible experimental error.

Evaluated variables

Polyphenol content was determined by the Folin-Ciocalteu method developed by Anesini et al. (2008). For the test, 125 µL of the gallic acid standard solution was prepared, and 0.5 mL of distilled H2O and 125 µL of Folin-Ciocalteu reagent were added. These reagents remained in the reaction for 6 min, and 1.25 mL of a 7% Na_2CO_3 solution was added. Finally, 1 mL of distilled H_2O was added and left to stand for 90 min at a temperature of 17°C and 65% relative humidity.

An absorbance reading was performed on the obtained solution in a UV Vis Genesys 10S spectrophotometer (Thermo Scientific®) at a wavelength (λ) of 760 nm. Then, both stem and leaf extracts were diluted at a 1:5 ratio with 50% methanolic solution, and the total polyphenol content was determined in the same way as gallic acid standards. Then, by interpolating the absorbance of the extracts in the gallic acid curve, the content of total polyphenols expressed in mg L^{-1} of extract was determined in triplicate samples.

Flavonoid content was determined by the method described by Muñoz et al. (2007). Samples of 250 µL of the extracts of *Parkinsonia aculeata* L. Sp. were dissolved in 1000 µL of deionized water. Then, 75 µL of $NaNO_2$ was added and allowed to react for 5 minutes. Subsequently, 75 µL of 10% $AlCl_3$ and 500 µL of 1 M NaOH were added. The mixture was centrifuged at 3500 r.p.m. for 5 minutes. Finally, the absorbance was measured at a wavelength of 510 nm. The final concentrations of total flavonoids were expressed in mg L^{-1} of stem and leaf extracts (Herrera et al., 2017).

Table 1. A phytotoxicity scale was established when evaluating extracts from leaves and stems of *P. aculeate* L. Sp.Pl.

Value	Description	Phytotoxycity
1	No foliar damage or death	Null
2	Foliar damage or death of at least 2 plants	mild
3	30% of leaf area damaged and 5 plants dead	half
4	More than 30% of the leaf area damaged and more than 10% dead plants	moderate
5	More than 50% of the leaf area damaged and more than 15% dead plants	high

Evaluation of the phytotoxicity of the extracts

The evaluation of the phytotoxicity of hydroalcoholic extracts from leaves and stems of *P. aculeata* was carried out in the initial stages of the Rio Grande tomato variety, following the scale described in Table 1, taking the scale proposed by Esparza-Díaz et al. (2010) as a reference. This evaluation was carried out twice (August-December 2020 and the same period of 2022).

Statistical analysis

To compare the concentrations of polyphenols and flavonoids, the theoretical distribution of student probabilities proposed by Gosset (1917) was used, establishing the differences between the organs where they were determined.

For the evaluation of phytotoxicity, a double classification analysis of variance was carried out based on a linear model of fixed effects (Fisher, 1937). The number of damaged or dead plants during each treatment (discrete quantitative variable) was taken as a variable response. When there were differences between the means of the treatments, Tukey's multiple comparison test was used for a significance level of 5% (Tukey, 1960). The statistical indicators of the coefficient of variation (CV), standard error of the mean of the treatments (ESx) and the coefficients of determination (R^2) were determined without adjusting for the isolated factors (organs and volume) and for the interaction between these two factors. STATISTICA professional software, version 14.1 for Windows (Statsoft, 2014), was used for statistical processing.

RESULTS

Polyphenol and flavonoid contents in the hydroalcoholic extracts of stems and leaves.

Polyphenol concentrations and total flavonoids showed highly significant differences between the organs from which the extracts were obtained. The concentration was in both parts, but it was higher in the leaves (Table 2).

Table 2. Total polyphenol and flavonoid contents in extracts of *P. aculeata* L. Sp.Pl. [A1.3: absorbance, Ax: average absorbance; F: dilution factor].

Organ	Absorbance for polyphenols					Dilution factor	
	A_1	A_2	A_3	A_x	Without Factor	F=5	F=20
Stem	0.75	0.76	0.76	0.76	74.88	374.44	
Leaves	0.89	0.90	0.92	0.91	91.52		1830.34**
						Y = 0.0089x + 0.0925	
			Absorbance for flavonoids				
Stem	0.09	0.09	0.089	0.09	1.53	7.63	
Leaves	0.11	0.11	0.10	0.10	1.75		35.04**
						y = 0.0576x + 0.0038	

The results demonstrate the plant's ability to store these compounds to be protected from pests and diseases. The storage is more in the leaves than in the stem. It was verified that hydroalcoholic extracts of leaves and stems of *P. aculeata* at concentrations of 10% were effective in promoting a low severity of the disease (3.7 and 3.3, respectively). These results may explain the low abundance of reports showing the presence of pests and diseases in this species. This was attributed to the synthesis and accumulation of these compounds as main biocontrol agents.

Phytotoxicity of extracts in tomato seedlings

The application at 15 DAE of treatments T2 to T8 showed highly significant phytotoxicity ($p=0.0017$) in the Rio Grande tomato variety, causing damage to 77% of the plants of each mentioned treatment (value of 5). For this reason, the hydroalcoholic extract application of *P. aculeata* at 15 DAE is not recommended (Table 3). In the statistical analysis, at this moment of application (15 DAE), although there was a significant interaction between the organ*volume factors ($p=0.01796$), it was observed that the effect of the applied volume explained 98% of the total variability obtained (R^2 (volumes)=0.98). These findings show that any volume used close to emergence can generate phytotoxicity and that this increases significantly as the volume of extract applied increases (Table 3).

Table 3. Evaluation of the phytotoxicity of the hydroalcoholic extracts of *Parkinsonia aculeata* L. Sp. Pl. in tomato seedlings, Rio Grande variety.

Treatments	Number of dead plants (%)			Phytotoxicity (1-5)		
	15 DAE[1]	25 DAE	35 DAE	15 DAE	25 DAE	35 DAE
T1	0.33a	0.3a	0.2a	1	1	1
T2	24.3b	2.3b	1.7ab	5	2	1
T3	25.6b	3.0 cb	2.3ab	5	2	2
T4	28.6c	3.3b	3b	5	2	1
T5	0.35a	2.3b	0.3a	5	2	1
T6	25.6b	3.6b	3.3b	5	2	2
T7	28c	3.3b	1.6ab	5	2	1
T8	29c	3.3b	1.6b	5	2	1
$R^2_{(organs)}$	0.03	0.14	0.01			
$R^2_{(volume)}$	0.98	0.64	0.79			
$R^2_{(interaction)}$	0.02	0.15	0.12			
ES	0.11	0.33	0.21			
CV	26.2	6.04	5.1			

[1]DAE: days after emergence; R^2: unadjusted coefficients of determination for the isolated factors and their interaction. ES: standard error of the mean of the treatments; CV: coefficient of variation of the treatments. Means with equal superscripts in the columns of number of dead plants do not differ statistically by Tukey's test, $p<0.005$.

When the treatments were applied at 25 DAE, there were also significant differences between the organs used to obtain the extracts ($p=0.00274$), as well as between the volumes ($p=0.0003$), with a significant interaction ($p=0.0179$). When the extracts were applied during 25 DAE, a total variability found in phytotoxicity was explained by 64%, and the average phytotoxicity between treatments was 2. A similar result was found at 35 DAE, where the source of volume variation contributed 79% to the total variability found in phytotoxicity, although the average value of phytotoxicity was 1 (Table 3). The results obtained indicate that high volumes of the extract can cause phytotoxicity in plants. Because of this, these studies must be carried out to recommend its use. This can be useful to producers to control diseases in seedlings without causing damage to the initial morphological and physiological characteristics of plants.

DISCUSSION

Various investigations on the use of crude plant extracts have revealed their inhibitory activities on microorganisms. For example, the antimicrobial activity of *Pinus wallichiana* A.B. Jack leaf extracts against *Fusarium oxysporum* f. sp. *cubense* (Foc), attributed to the significant presence of flavonoids and polyphenols (Ain et al., 2022). These results confirm that the palo verde extract has important potential as a biocontrol agent because it comes from a plant. This product easily mitigates into the plant (Stefanovic; Comic, 2012), so it would generate little or no phytotoxicity. This characteristic confirms the importance of this study.

Many of these extracts contain terpenoids, alkaloids, tannins, saponins, phenylpropanoids, and flavonoids, which are used to manufacture fungicides and pesticides at high concentrations (Nxumalo et al., 2021).

In Mexico, various plant species extracts have also been obtained. They have a significant concentration of flavonoids and polyphenols used as biocontrol agents of insects. A major control of 48-hour-old larvae with seven concentrations was obtained when the insecticidal activity of mistletoe dust (*Phoradendron densum* Torr. ex Trel.) on *Spodoptera frugiperda* was evaluated by Hernández et al. (2018).

In general, flavonoids play an important role in protection against biological oxidation induced by biotic and abiotic stresses (Sun et al., 2022). The content of polyphenols in plants and fruits varies depending on the genotype, species, environmental conditions, degree of maturity, soil composition, geographic location, and storage conditions (Shen et al., 2022). Flavonoids are also a frequent object of research due to their diverse functions, such as nutrient assimilation, protein synthesis, enzymatic activity, photosynthesis, formation of structural components, and defense against adverse environmental factors such as aggression of pathogens and insects (Figueirinha et al., 2008; Vélez-Terranova et al., 2014).

Multiple extracts have been obtained from semidesert plants for agronomic purposes, with an organic and integrated management approach for pest and disease control (Heikal et al., 2021). The governor species (*Larrea tridentata* (DC.) Coville) has been used for the biocontrol of Fusarium sp., with reductions in radial growth of 10% (Martínez-Olivo et al., 2020). Neem extract (*Azadirachta indica* A. Juss) has been used to evaluate antifungal activity against tomato vascular wilt and showed high control efficiency and minimal phytotoxicity in seedlings (Ayvar-Serna et al., 2021).

For fungal diseases such as vascular wilt, the hydroalcoholic extract of *Acacia farnesiana* was tested for a decade (Rodríguez et al., 2012) under in vitro culture conditions, with significant decreases in the mycelial growth of the fungus and minimal phytotoxicity in plants. Rivera-Solis et al. (2021) also tested extracts of *Sargassum* spp. as inducers of tolerance to *Fusarium oxysporum* in tomato seedlings without finding significant phytotoxicity. These results demonstrate the practical value of plant extracts in controlling diseases that affect agricultural crops and their contribution to caring for the environment by reducing the application of chemical products.

Plant extracts in pest and disease management are currently recognized as environmentally safe, less hazardous and cheaper. In their most natural form, many plant species have insecticidal characteristics (Tavares et al., 2021). Its use constitutes an alternative to mitigate contaminant loads due to concentrated chemical products that sometimes generate resistance in organisms.

The production of plant extracts is still important in the discovery of innovative and environmentally safe antimicrobials to overcome problems with resistance to multiple pesticides. The use of extracts to minimize or eliminate the damage caused by pests and diseases can contribute to the national and international scientific community. This could have great economic and ecological significance.

CONCLUSIONS

The hydroalcoholic extracts of *Parkinsonia aculeata* L. Sp.Pl. present a higher concentration of flavonoids and polyphenols in the leaves than in the stems. The Río Grande tomato variety presents high phytotoxicity (f=5) at 15 DAE. Therefore, the application of hydroalcoholic extracts of *P. aculeata* at this time is not recommended. The safe application of the hydroalcoholic extract of *Parkinsonia aculeata* L. Sp.Pl. without symptoms of phytotoxicity appearing, must be carried out from 25 to 35 DAE of the seedlings.

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