Article

Antimicrobial activity of extracts of *Zingiber officinale* and *Maclura pomifera* on *Pseudomona syringae*

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Abstract

Bean and tomato in Mexico are two of the products that are an essential part of the diet of the population of this country, they are of great economic, cultural and gastronomic importance. The presence of pests and diseases in crops increases production costs, in addition to the environmental impact and the residuality of pesticides, they can affect human health. The main objective of the research was to study a biocontrol alternative for bacterial diseases, effective, low cost and lower environmental impact. It was developed in the city of Aguascalientes, Mexico in 2019. Natural extracts (ethanol, methanol and water) of *Maclura pomifera* and *Zingiber officinale* were produced in raw form by maceration and concentrates by rotary-evaporation, using bases of ethanol, methanol and water. Strains of *Pseudomona syringae* of pathologies in tomato and green bean were isolated and identified. 14 treatments with three repeats of raw and concentrated extracts were used. Bactericidal activity was evaluated by antibiograms with intervals of 24 h for seven days. Showing the greatest inhibitory effect, the extracts of *M. pomifera* base ethanol of 100 mg ml^{-1} and *Z. officinale* of 100 mg ml^{-1} base ethanol. For the second test in the decrease in concentrations, the effectiveness of *M. pomifera* extracts at low concentrations was reiterated, where 10 mg ml^{-1} was the lowest and most effective concentration in bacterial inhibition. This positions *M. pomifera* plant extracts as a viable, efficient and economical option in the biocontrol of agricultural bacterial diseases.

Keywords: *Maclura pomifera*, *Pseudomona syringae*, *Zingiber officinale*, biocontrol.

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Introduction

In Mexico, beans and tomatoes form an important part of the basic diet of the population. For its part, the production of beans in our country stands out due to the area sown and because it is the fifth country in legume production worldwide with 5.8%, below Brazil with 16% of total production, India 15.9%, Myanmar 10.5% and China 8.9% (Lépiz *et al*., 2015; Lépiz *et al*., 2016). Mexico ranks second in bean harvested area, after maize (Lépiz *et al*., 2015; Lépiz *et al*., 2016; SIAP, 2019).

The total area harvested in red tomato recorded an increase of 2.8% for the 2019 cycle with 21 246 ha, compared to 2018 with 20 659 ha. Registering a cumulative production to November 2019 of 52 646 t, for this case, unlike bean, production increased by 822 t compared to that obtained in the same period of the previous year (one million 524 thousand 826 t), being the states of Baja California (30 768 t), Durango (17 765 t), Aguascalientes (9 815 t) and Zacatecas (9 126 t), entities with the largest increases in production in the last year, compared to 2018 (SIAP, 2019).

Globally, agricultural crop production is limited or decreased mainly by disease-causing pathogens which are traditionally attacked by chemical methods that, in addition to presenting residuality and toxicity in the environment, affect human health, while the generation of resistance of pathogens is a common consequence of their overuse. This resistance causes the increase in dosage and therefore the gradual increase in food production costs (Reyes *et al*., 2015; Cúndom *et al.*, 2016).

There is a projection for the year 2030 of twice the current consumption of chemicals products. Long term use of antimicrobials in the control of animal and plant diseases can cause bacterial resistance. Therefore, the appropriate, effective and controlled use of these will help to keep them effective, moderating their prevalence in the field (FAO, 2017).

Concerned about the care of the planet, toxicity, residuality and production costs, today new alternatives are investigated in the management of diseases in crops, giving way to biological control, which involves the use of living organisms capable of fighting phytopathogens, as well as metabolites of these or some plants that help control diseases through the production of bioactive compounds (Reyes *et al*., 2015; Solís y Armas, 2017; Pérez *et al*., 2018).

In recent years, the use of natural resources and their products or subproducts has been increasing. This could be due to publications of new knowledge about their chemical composition and the performance of various tests that support their effectiveness (Abadie *et al*., 2014; Perez *et al*., 2017). Among their main effects are attributed: antibacterial activity, antifungal, antiviral, antioxidant, anticancer, among others. So, research has focused on the study and search for new substances of plant origin, to check their effectiveness against pathogens and may be the new source of antibacterial (Rojas *et al*., 2016).

In support of this global initiative, this work focuses on the investigation of new alternatives with the use of vegetable products, such as plant extracts, for the control of bacterial diseases caused by *Pseudomona syringae* in vegetables of economic importance in Mexico and the world such as tomatoes and beans.

Materials and methods

Experimental location

The experimental work was carried out in 2019, in the Agricultural Parasitology Laboratory of the Center for Agricultural Sciences, of the Autonomous University of Aguascalientes, where the *in vitro* tests and isolation of phytopathogens were carried out.

Obtaining vegetable extracts

In the city of Parras de la Fuente, Coahuila, with coordinates 25.4949609-102.1836501, were collected fruits of *Maclura pomaifera* and transported in wooden boxes to the city of Aguascalientes. The fruits were disinfected using 3% sodium hypochlorite, cutting them into pieces between two and three centimeters, letting them dry on trays with absorbent paper at 35 °C for five to seven days, until the fruits lost their moisture. The ginger roots (*Zingiber officinale*) obtained in commercial premises in the city of Aguascalientes were treated in the same way, fractionated and dried under the conditions described for *Maclura pomifera* fruits.

The dry materials were pulverized using a COSUAI brand electromechanical mill, model number: CS-700. The pulverized material was stored until its maceration. Using ethanol, methanol and water as extraction vehicles, maceration was carried out at room temperature, in constant agitation on mechanical magneto grill, for 72 h at a proportion of 70 g L^{-1} of the extraction solvent.

Using Whatman filter paper grade 1:11µm the product of maceration was filtered separating the aqueous phase into two parts of equal volume. The first part was stored at 4 °C in absence of light, the second part of the alcoholic extracts was processed in rotavapor Büchii B490® at 58 °C, 75 rpm and 35 psi. Bringing to total dryness the last 10 ml on open glass Petri box in a Felisa stove model FE-131 at 39 °C, drying in the same way the aqueous extracts until get a dry or rubbery layer losing all the solvent. The dry extracts were identified and stored at 4 °C in the absence of light.

Established concentrations for treatments

The concentration established for each treatment was calculated in milligrams per milliliter. A total of 14 treatments were established. Three treatments with raw extracts of ethanol, methanol and water, in addition to this, three different concentrations for each of these extracts, using in a first test, 500, 300 and 100 mg ml for each extract mentioned, for a total of nine treatments plus one absolute witness (in proportion 1:1:1 sterile distilled water, ethanol and methanol) and a commercial witness using a product released according to the indications and concentration recommended by the manufacturer, product based on three plant extracts (Gamma of Prograngic: garlic extract (*Allium sativum* 25%), spicy chili extract (*Capsicum frutescens* 25%), cinnamon extract (*Cinnamomum zeylanicum* 10%) 1.5-2 L ha^{-1} in 200 L of water).

Establishing a second bioassay with a reduction in concentrations for the extracts of *Maclura pomifera* evaluating 14 total treatments in the previous distribution with the variation of concentrations of 80, 50, 30 and 10 mg ml^{-1}, for alcoholic extracts that had the best result in the first experimental stage.

Isolation and preliminary identification of bacterial strains

Pseudomona syringae was isolated from green bean pods with the symptoms of greasy spots, while in tomato fruits were selected with small black freckles, 1 to 3 mm in diameter, characteristic signs of the respective bacterial diseases for each crop. Both green beans and tomatoes were collected from commercial crops in the city of Aguascalientes.

The isolation was carried out the laboratory of Agricultural Parasitology of the Autonomous University of Aguascalientes according to the methodology described by Agrios (2005): Zhang *et al*. (2017); *P. syringae* was purified by the quadrant stria technique, performing preliminary tests such as gram stain, oxidase, ryu test, catalase and fluorescence in king B medium, corroborating its molecular identity through the sequencing of genetic material extracted from pure crops.

Molecular identity of phytopathogenic bacteria by 16S rDNA gene

The genomic DNA of presumptive strains of *P. syringae*, initially characterized by preliminary tests and Gram, were processed from isolates in LB broth tubes (Luria Bertani LB brand Dibico®, sodium chloride 5, yeast extract 5, casein peptone 10 (pH 7.2 ±0.2) incubated at 26 °C for 48 h in rotary agitator, performing the isolation of genetic material according to the methods described by Ríos *et al*. (2016). Using this as a mold in the amplification process in PCR reactions (Polymerase Chain Reaction), 20 µl reactions were prepared.

Amplification of the 16S-23S DNAr gene was carried out by the F1624 (3'-CCTTTGTACACACCGCCCGTCG-5') and R1494 (5'-CTACGGRTACCTTGTTACGAC-3') primers. Each reaction mixture (20µl) contained 0.2 µl of Taq DNA polymerase (1U μl^{-1}), 2 µl of 10x PCR Buffer QIA, 0.5µl of DMSO, 0.4 µl dNTPs (10 mmol L^{-1}), 0.8 µl (5 µmol L^{-1}) of each primer, 14.3 µl of Milli-Q water and 1 µL of DNA mold to 20 ng μl^{-1}. Using a Bio-Rad T100® DNA thermal cycler, a denaturation program was established at 95 °C for 2 min, followed by 35 temperature cycles of 95 °C for 40s, 55 °C for 30s and 72 °C for 1 min and 30 s, ending with an extension to 72 °C for 7 min. Amplification was observed on 1% agarose gel by electrophoresis at 60 V. The PCR products were increased and purified using a GeneAll PCR® ExpinTM SV purification kit.

The sequencing of the 16S rDNA partial regions was performed by the Macrogen Laboratory (Rockville Maryland, USA). The sequences obtained were manually edited, eliminating the ends with bases omissions, to increase the sensitivity of the analysis, these purified sequences, were analyzed in the database of the National Center for Biotechnology Information (NCBI)-GenBank.

Antimicrobial activity of vegetable extracts

Using the antibiogram protocol, the identified axenic strains of the characteristic symptoms of the disease were inoculated two tubes of Trypticase BD Bioxon® soy broth transferring with bacteriological handle a colony with 24 h growth in Petri box, incubating for 8 h to 28 °C. In aseptic conditions under a laminar flow hood Eseve® model CFL102, shaking the tube with bacterial growth the sterile swab is introduced and the excess broth is removed by pressing against the walls of the tube. On Petri dishes with Mueller Hilton agar, it was spread a layer of the bacterial broth with the help of the swab across the length and width of the plate, allowing it to dry for one minute. 6 mm discs of Whatman filter paper grade 1:11µm were drilled and sterilized to be impregnated by extracts at different concentrations, these were placed at four points distant from each other and one at the center for each experimental unit. Petri dishes were identified and incubated at 28 °C for 24 h.

Antimicrobial activity was determined every 24 hours for seven days, measuring the inhibition halos produced by contact and for the active substances in the discs, using a plastic rule graduated in mm. The results recorded every 24 hours on halos produced and measured in cm, were analyzed by R statistical program version 3.6.3 (R Core Team, 2020). The data were analyzed under a factorial design and grouping of means, with 14 treatments, three repetitions per treatment, and five units of measurement per experimental unit (number of discs per box).

Results and discussion

Obtaining vegetable extracts

The macerated extracts obtained from the ginger roots (*Zingiber officinale*) and the fruits of *Maclura pomifera* in raw form had a recovery of 96 and 95%. While by rotary evaporation the concentration of concentrated pure extracts reduced their yield up to 2.7% and 3.4% p/p respectively according to their weight ratio and that obtained at the end of the process.

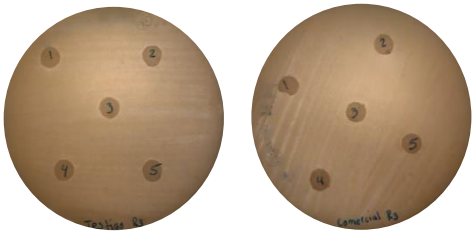
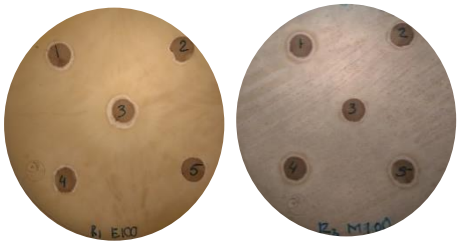
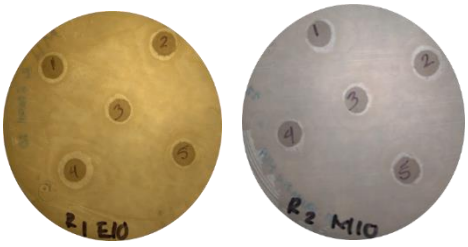
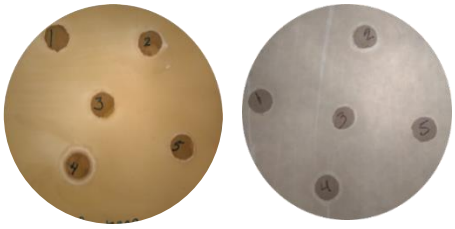
Isolation and identification of bacterial strains

Preliminary tests for axenic strains isolated from tomato and green bean symptoms showed on King's B plates, round cream-colored colonies, round edges, raised, creamy and shiny, with the characteristic fluorescence under ultraviolet light shown by colonies of *Pseudomona syringae* the fluorescent group. Microscopic morphology shows red bacilli, for gram staining is negative, oxidase negative, catalase positive and ryu positive. The molecular identity of the bacteria was corroborated by the sequencing the genetic material and in the National Center for Biotechnology Information (NCBI Blast) with access number JX876900.1 for *Pseudomonas syringae* pv. *phaseolicola* and AE016853.1 *Pseudomonas syringae* pv. *tomato.*

Antimicrobial activity of vegetable extracts

The effect of bactericidal activity of the different extracts on the growth of *P. syringae* both pathovars (pv. *phaseolicola* and pv. *tomato*) determined every 24 hours for seven days, showed (Table 1), the presence of active substances in the discs impregnated by mainly ethanolic and methanolic extracts.

Table 1. Antibacterial effect of vegetable extracts on *Pseudomona syringae* both pathovars (pv. *phaseolicola* and pv. *tomato*) isolated from the symptomatology of bacterial freckle in tomato (*Solanum lycopersicum*) and greasy spot in green bean (*Phaseolus vulgaris*).

Picture	Concentration	Description
	Absolute witness and commercial witness 5 µL ml^{-1}	Absolute and commercial controls No presence of inhibition halos of *Pseudomona syringae* both pathovars (pv. *phaseolicola* and pv. *tomato*) were observed in the absolute witness and almost imperceptible (1 mm or less) in the use of the commercial product Gamma from Prograniс.
	Base extracts Ethanol and methanol raw 100 mg ml^{-1} 300 mg ml^{-1} 500 mg ml^{-1}	First essay *M. pomifera* Ethanol and methanol extracts had bacterial inhibitory activity. Where 100 mg ml^{-1} ethanol was the superior treatment with an average of 0.8219.
	Base extracts Ethanol and methanol 10 mg ml^{-1} 30 mg ml^{-1} 50 mg ml^{-1} 80 mg ml^{-1}	Second essay *M. pomifera* Ethanol and methanol extracts had bacterial inhibitory activity. Where the most effective treatment was ethanol of 10 mg ml in inhibition of *P. syringae* with an average of 0.9838.
	Base extracts Ethanol and methanol raw 100 mg ml^{-1} 300 mg ml^{-1} 500 mg ml^{-1}	Essay *Z. officinale* The most outstanding treatment was 100 mg ml ethanol. The 100 mg ml Methanol-based extracts also showed bacterial inhibitory activity to a lesser degree, with an average of 0.4166.

Comparisons of means of the solvents used as the extraction base in which the extracts were prepared are presented in Table 2a, while Table 2b shows the comparison of means between the concentrations used (500, 300, 100 mg ml^{-1}). Highlighting as the best extraction base to ethanol and 100 mg ml at the most effective concentration by presenting the highest mean before the inhibitory effect produced by the extract on *P. syringae* with an average of 0.8219.

Table 2a. First assay *M. pomifera*, high concentrations of aqueous and alcoholic extracts. Comparison of means between solvent extraction analysis factors and concentrations. Comparison of means between the extraction bases according to the inhibitory halo given in cm (a).

Extraction base	Average	sd	G
Ethanol	0.702	0.363	A
Methanol	0.6207	0.321	B
Water	0.576	0.291	C

Table 2b. Comparison of means between concentrations used in the presence and size of inhibitory halo in cm (b). With 210 evaluations, three raw extract treatments, three extraction solvents (ethanol, methane and water), each with three respective concentrations (100, 300 and 500 mg ml), each treatment with three repetitions (Petri box unit) and each repetition with five paper discs inside, impregnated with the treatment, thus determining five units of measurement within each repetition, for all cases.

Extraction base	Average	sd	G
100	0.8219	0.8219	A
300	0.7952	0.121	B
500	0.772	0.0898	C
Raw	0.7755	0.1025	C
Witness	0	0	D

Sd= standard deviation; G= group.

In this analysis, significant differences were found in the extraction base factors where ethanol was the superior treatment, and in the second factor, concentration, the treatment of 100 mg ml was the one with the highest diameters in the inhibition halos recorded with an average of 0.8219, being the minimum concentration tested, the most effective for the control of *P. syringae.* This indicate that not in all cases high concentrations are the best in the expected control, as is thought in most cases. Supporting this, studies that used extracts of Ka'a He'ẽ (*Stevia rebaudiana* (Bertoni) Bertoni) for the control of Septoria and bacterial spot of the tomato (*Solanum lycopersicum* L.), showed at their lowest dose of 50 ml L^{-1} reduces the severity of Septoria in tomato, in addition to increasing the foliar area, height, root length and dry mass. As well as its average dose of 150 ml L^{-1} reduced the severity of the bacterial spot (Lesme *et al*., 2017).

Otherwise, other research with governor extracts, it was observed that the higher the concentration, the greater the inhibition, since concentrations of 0.7 and 0.35 ppm totally inhibited the growth of the four bacteria (*Pectobacterium carotovora* subsp. *carotovora*, *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas axonopodis* pv. v*esicatoria* and *Pseudomonas cichorii*), while with the witness there was noinhibition of growth (Osorio *et al*., 2009).

The effect of both factors ($F_{8, 1560}$), $p< 0.001$, is also significant, this is represented graphically in Figure 1. Where treatment with ethanol-based extracts showed superior behavior. Ramírez *et al*. (2015) studied raw ethanol extracts with different tissues of *Magnolia schiedeana* on phytopathogenic bacteria *Pectobacterium carotovorum* and *Pseudomonas cichorii*. Their results indicate that the floral ercholic extract, as in this study, inhibited *P. carotovorum* growth in the same proportion as the antibiotic tested ($p= 0.079$).

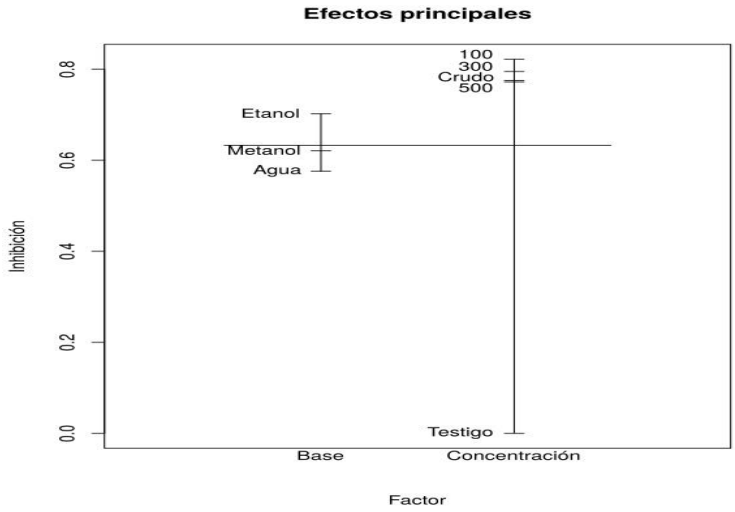


Figure 1. Comparison of the main effects of extraction base and concentrations of treatments *Maclura pomifera* against *P. syringae*.

The main effects represented in Figure 1, schematize the separation of factors. The extracts stand out, over the absolute witness, being the ethanol-based treatments and the concentration 100 mg ml^{-1} superior, presenting the best behavior in inhibiting of bacterial growth. In opposite way water-based extracts and concentrations of 500 mg ml^{-1} were treatments where the inhibitory response was lower, even if it is above the absolute witness.

The concentration factor is represented graphically in Figure 2, where the highest inhibition is observed in the concentrations of 300 and 100 mg ml^{-1}, marking a peak in extracts made from ethanol in all cases studied. Therefore, it is reiterated that low concentrations produce a greater inhibitory effect. And so, based on the results analyzed, a second test with minimum concentrations was designed to evaluate better behavior and control produced by vegetable extracts and their dissolved components.

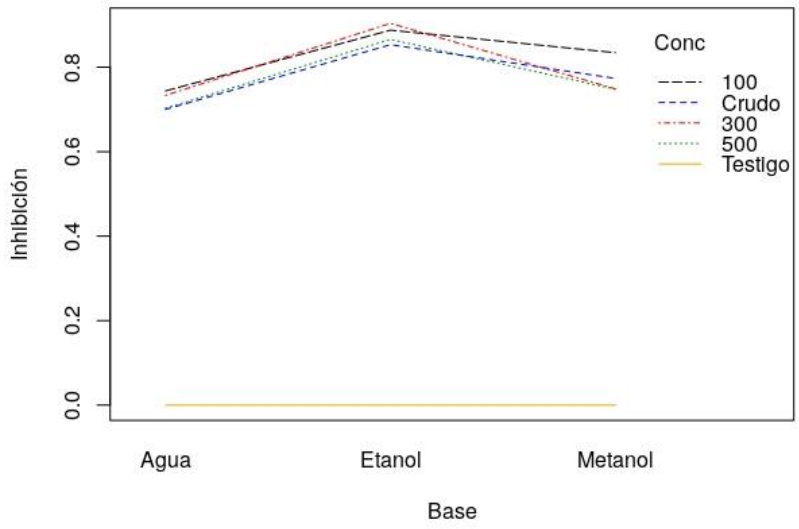


Figure 2. Comparison of the inhibitory effect of concentrations in the treatments of *Maclura pomifera* in three extraction bases, against *P. syringae*.

The dissolution of the active compounds in vegetable extracts depends on the extraction base used, in which dissolved compounds are found, depending on their affinity with the solvent, so the nature of this extraction base can increase or decrease the effects of the extract depending on the dissolved components in it or its dispersion on the applied surface. The presence of secondary metabolites in vegetable extracts is a characteristic of the defensive strategies of plants (Celis *et al*., 2009).

Given these conditions, alcoholic solvents for this research presented the best performance against bacterial inhibition, possibly due to the nature of the plant metabolites present. Unlike other species such as *Citrus* x *paradisi*, *Pimienta dioica*, *Origanum majorana*, *Tilia platyphyllos*, which in aqueous extracts at concentration of 25%, exhibited inhibition in the mycelial growth of 100% on the fungus *Moniliophthora roreri*, isolated from sick cocoa fruits, unlike the witness that presented a growth radius of 25 mm (Arcos *et al*., 2019).

The data for the second *M. pomifera* assay were analyzed in a factorial method, where again the concentration factors and extraction base factors showed, significant differences in Table 3, for the case of the base of the extracts, ($F_{1,\ 832}$)= 116.8, $p< 0.001$, while the difference between concentrations is ($F_{3,\ 832}$) = 40.492, $p< 0.001$.

Based on the results of the first assay, ethanolic and methanolic extracts were chosen as the best treatments in bacterial inhibition of *P. syringae.* Similarly, taking into account that the lower concentration (100 mg ml^{-1}) of the previous assay was the most effective with an average of 0.8219, concentrations below this value were selected to establish the new design. The antibacterial inhibitory effect was presented in all cases with ethanol and methanol extracts.

Table 3. Second *M. pomifera* assay, decreased in concentrations of Ethanolic and Methanolic extracts and their inhibitory effect on *P. syringae*. Comparison of means between analysis factors extraction bases and concentrations. Comparison of means of the ethanol and methanol extraction base (a), comparison of means between the concentrations used for each treatment 10, 30, 50 and 80 mg ml^{-1} (b), with 210 total evaluations, comparing the size in cm of the inhibitory halos produced.

a)

Extraction base	Average	sd	G
Ethanol	0.9616	0.1729	a
Methanol	0.868	0.0807	b

b)

Extraction base	Average	sd	G
10	0.9838	0.1729	a
30	0.8938	0.0807	b
50	0.928	0.1622	c
80	0.8538	0.1012	d

Table 4a shows the difference between the means treatments, locating the treatment of ethanol with a superior bactericidal effect, while Table 4b shows the concentration of 10 mg ml^{-1} as the most effective treatment for the control of the pathogen *P. syringae*.

Inhibition of bacterial growth by plant extracts has been documented by researchers in recent years, looking for the scientific support of the active components in the extracts, which are lower cost and broad effectiveness. As evidenced by studies of C*itrus paradisi* seed extracts (grapefruit) inhibiting bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and the fungus *Candida albicans* (Cabrera, 2019).

Table 4. First *Z. officinale* assay, high concentrations of aqueous and alcoholic extracts. Comparison of means between two factors, the extraction bases (a). Comparison of means between the concentrations used; and (b). With 210 total evaluations, three raw extract treatments, three extraction solvents (ethanol, methanol and water), each with three respective concentrations (100, 300 and 500 mg ml^{-1}), each treatment with three repetitions (Petri box unit) and each repetition with five paper discs inside, impregnated with the treatment, thus determining five units of measure within each repetition, for all cases, evaluating the size in cm of the inhibitory halos produced.

a)

Extraction base	Average	sd	G
Ethanol	0.1951	0.3369	a
Methanol	0.1081	0.2679	b
Water	0	0	c

b)

Extraction base	Average	sd	G
100	0.4166	0.3833	a
300	0.0888	0.2518	b
500	0	0	c
Raw	0	0	c
Witness	0	0	c

sd= standard deviation; G= group.

As the effect between base and concentrations is significant $F_{3, 832}) = 8.612$, $p< 0.001$, the figure of main effects is presented (Figure 3), where the efficacy of the ethanol treatments of 10 mg ml^{-1} is reflected.

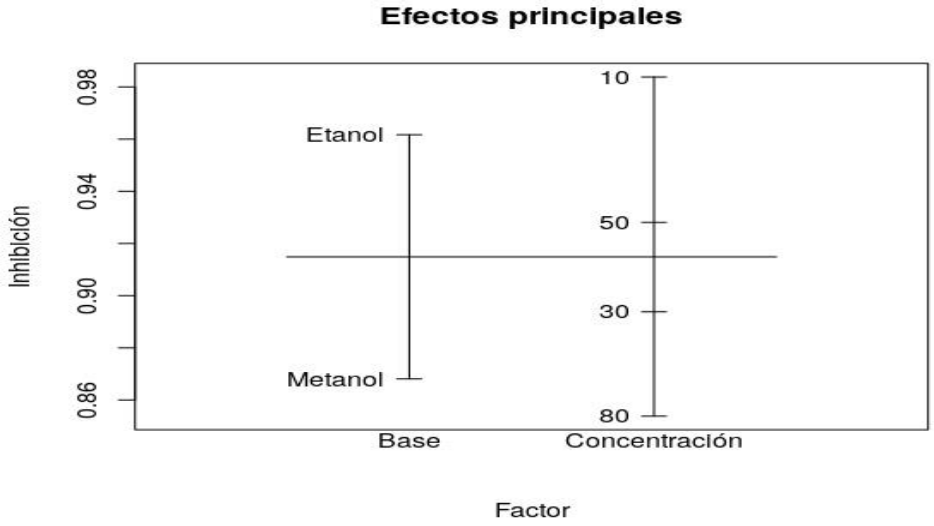


Figure 3. Second assay decrease in concentrations, extract of *M. pomifera*. Main effects of bacterial inhibition of extraction bases and concentrations on *P. syringae*.

The behaviour of the extracts in the second *M. pomifera* assay depicted in Figure 4, reiterates, to ethanol-based extracts with a superior effect on the inhibition of *P. syringae* and the second factor, concentration, in the same way highlighting the lower concentration treatment (10 mg ml^{-1}) as the most effective in this same evaluation.

The comparison of plant extract against commercial product was analyzed by non-parametric statistics. The commercial product was compared with the best concentration (10 mg ml^{-1}), based on ethanol, this being the highest inhibition mean. The data were analyzed using Wilcoxon's ranks sum test (Wilcoxon rank sum test: W=11025, p-value <2.2 e-16), the medians are statistically different.

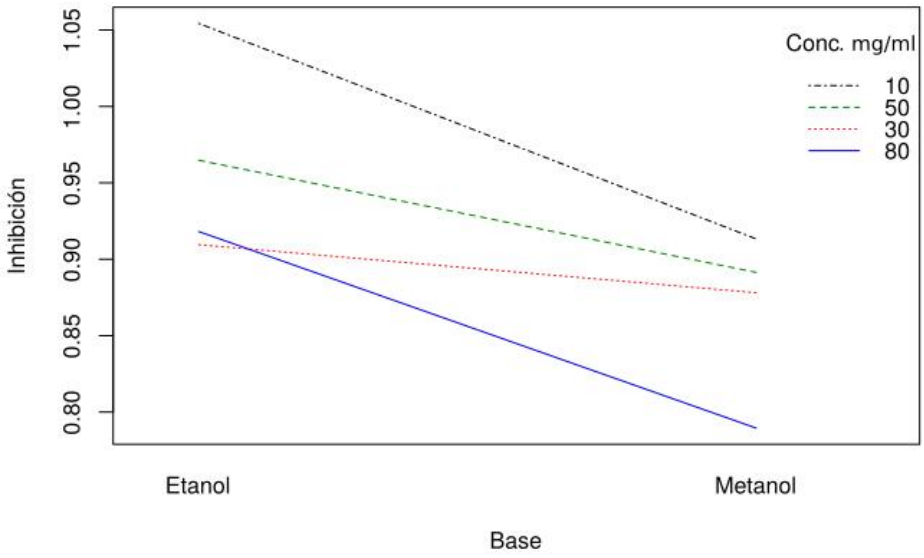


Figure 4. Second assay decrease in concentrations extract of *M. pomifera*. Interaction bacterial inhibition factor of extraction base and concentration, on *P. syringae*.

In Figure 5 shows the superiority of the antimicrobial effect of ethanol extract 10 mg ml^{-1}, over inhibition of the commercial product Gamma of Progranic (garlic extract (*Allium sativum* 25%), spicy chili extract (*Capsicum frutescens* 25%), cinnamon extract (*Cinnamomum zeylanicum* 10%) of 1.5-2 L ha^{-1} in 200 L of water).

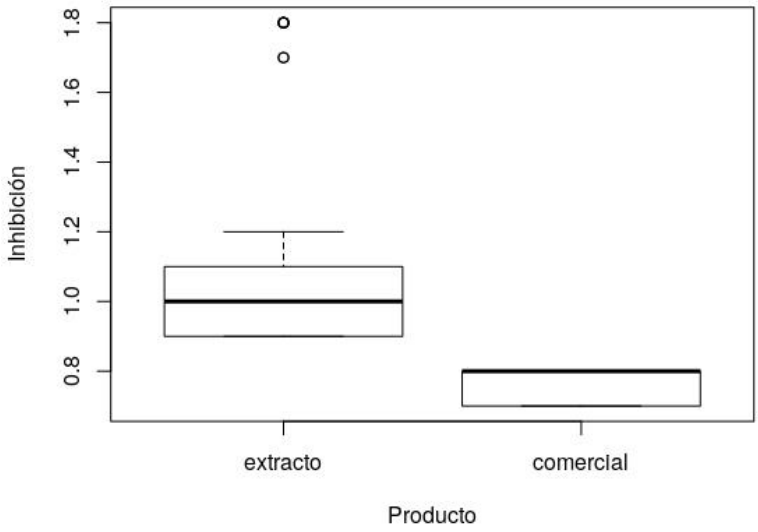


Figure 5. Second assay decrease in concentrations extract of *M. pomifera*. Comparison of plant extracts against commercial product evaluating the effect of bacterial inhibition, on *P. syringae*.

For the assay with *Z. officinale* extracts, the data recorded in Table 4 were analyzed under a factorial design and grouping of means, with 14 treatments, three repetitions for each treatment and each experimental unit with five measurement elements, which were the number of discs per box.

The Anova demonstrated significant differences between the treatments and the data were analyzed in a factorial arrangement, from which two main factors were separated, as extraction base and concentration, as shown in Table 4a and 4b respectively. Highlighting as main groups with the greatest inhibitory effect the ethanol extraction base and the concentration of 100 mg ml^{-1} corresponding to each of the analyzed factors shown in Figure 6.

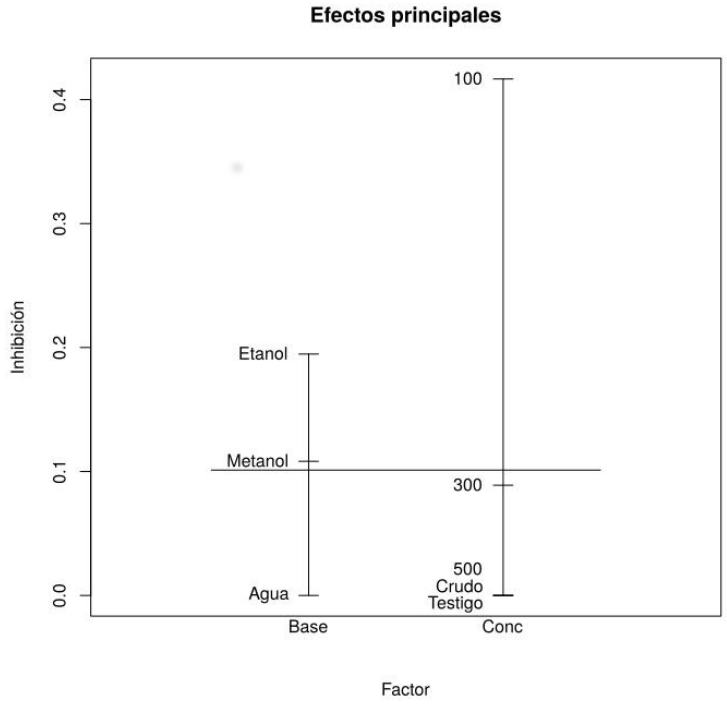


Figure 6. Main effects of bacterial inhibition of extraction base and concentration of *Z. officinale* extracts on *P. syringae*.

The main effects were separated into two groups, extraction base and concentration. As shown in Figure 6, in the first group of extraction bases, ethanol-based extracts showed a superior effect on inhibition of *P. syringae*. While for the concentration effect, the concentration of 100 mg ml^{-1} showed a wide separation of the group, when registering the largest inhibitory halos.

Figure 7 shows the behavior, as depending on the type of base, as the concentration of the extract decreases, the inhibitory effect on the bacteria increases. Presenting marked peaks in the ethanol base and the inhibitory increase represented in the concentration of 100 mg ml^{-1}.

Currently, as well as this research, there are sources and studies on the active components of plants with various therapeutic uses, fungicide, insecticides and bactericides. *Z. officinale* demonstrated bactericidal effects on ethanol-based extracts as an alternative in biocontrol. In Argentina, studies of hydroalcoholic extracts (ethanol: water, 1:1) of 80 wild species from various regions of the country, showed inhibition against *Staphylococcus aureus* in 41.25% of the species studied. Thus, demonstrating the value of plant extracts in biological control (Toribio *et al.*, 2009).

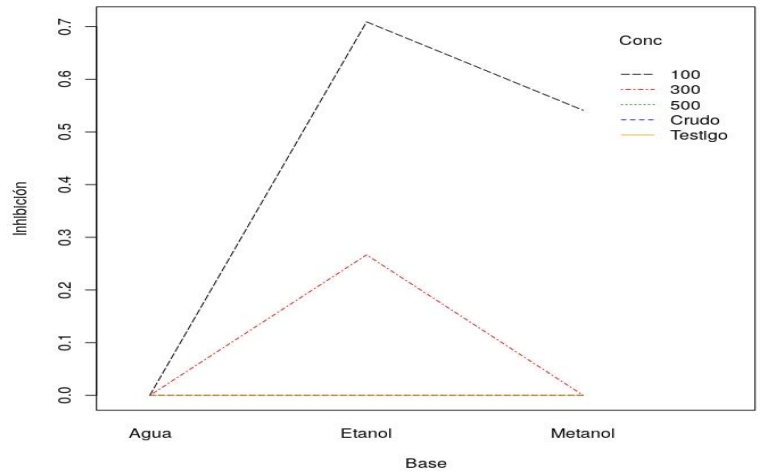


Figure 7. Inhibitory effect of bacterial growth produced by *Z. officinale* extracts on *P. syringae*. In function of the extraction base and its concentration.

Recently Celaya *et al*. (2019) confirms the efficiency of hydroethanolic extracts, they showed in their research that *Eucalyptus globulus* extract and the mixture of 12 extracts had antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Vibrio cholerae* and *Staphylococcus aureus* ($p< 0.05$).

Conclusions

Ethanol and methanol extracts inhibited the growth and development of *Pseudomona syringae* of tomato and green bean under *in vitro* conditions. In particular, the extracts of *M. pomifera* in their first and second assays showed the maximum inhibitory effect on ethanolic treatments with the lowest concentrations, which in the case of the first assay was 100 mg ml and for the second test in the reduction of concentrations the best concentration was 10 mg ml, on *P. syringae* pv *tomato.*

The greatest inhibitory effect of *P. syringae* pv *phaseolicola* with *Z. officinale* extracts was recorded by ethanol-based extracts and at the concentration of 100 mg ml^{-1}. The results obtained give guideline for these extracts to be considered in a biological control program, in the next stage *in vivo*, they can be a biocontrol alternative for bacterial diseases, effective, low cost and less or no environmental impact.

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