Ab Intus Revista Ab Intus FAV-UNRC
2025, 16(8) ISSN 2618-2734

# Study of the antimicrobial activity of phytochemical preparations obtained from *Thymus vulgaris* and *Origanum vulgare* against phytopathogenic *Pseudomonas* isolated from wheat

Estudio de la actividad antimicrobiana de preparaciones fitoquímicas obtenidas de *Thymus vulgaris* y *Origanum vulgare* contra *Pseudomonas* fitopatógenas aisladas de trigo

DOI: https://doi.org/10.63207/ai.v8i16.180

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**Abstract.** Wheat (*Triticum aestivum* L.) is one of the most important cereals worldwide, but is affected by various bacterial diseases, including bacterial leaf blight caused by *Pseudomonas syringae,* that decrease productivity significantly. The indiscriminate use of synthetic pesticides for disease management has led to environmental contamination, development of resistance and health risks, highlighting the need for alternative and environmentally friendly solutions. In this study, phytopathogenic *Pseudomonas* were isolated from symptomatic wheat leaves collected in Córdoba province (Argentina). The isolates were phenotypically identified using biochemical tests and the LOPAT scheme, confirming their classification within Group I of *P. syringae*. Pathogenicity tests verified their ability to induce typical leaf blight symptoms, and nine isolates were positive for the production of syringomycin, a phytotoxin associated with host tissue necrosis. The antimicrobial activity of different phytochemical preparations obtained from *Thymus vulgaris* and *Origanum vulgare* (decoctions, alcoholic, hexanic, chloroformic and propanolic extracts, as well as essential oils (EOs)) against the isolated and reference strains of *P. syringae* were evaluated. Disk diffusion assays revealed that thyme and oregano EOs exhibited the strongest inhibitory effects compared to the other phytochemical preparations tested. Antimicrobial activity assays by broth microdilution technique confirmed the antibacterial potency of *T. vulgaris* EO (MIC = 1.43–22.99 mg/mL) and *O. vulgare* EO (MIC = 2.89–23.13 mg/mL). These results demonstrated the efficacy of *T. vulgaris* and

Recibido: 27/10/2025
Aceptado: 03/11/2025
Publicado: 23/12/2025

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Funding: This work was supported by grant from Secretaría de Ciencia y Técnica - Universidad Nacional de Río Cuarto (PPI 2024–2026, Disp. N° 009/2024)

*O. vulgare* EOs as natural antimicrobial agents, suggesting their potential application as sustainable alternatives to synthetic pesticides for the control of bacterial leaf blight in wheat crops.

**Keywords.** *Pseudomonas syringae*, *Origanum vulgare*, *Thymus vulgaris*, phytochemicals, antimicrobial activity

**Resumen.** El trigo (*Triticum aestivum* L.) es uno de los cereales más importantes a nivel mundial, pero su productividad se ve afectada por enfermedades bacterianas como el tizón foliar bacteriano, causado por *Pseudomonas syringae*. El uso excesivo de pesticidas sintéticos ha generado contaminación ambiental, resistencia microbiana y riesgos para la salud, destacando la necesidad de alternativas sostenibles. En este estudio se aislaron cepas fitopatógenas de *Pseudomonas* a partir de hojas de trigo sintomáticas recolectadas en Córdoba (Argentina). Los aislamientos fueron identificados fenotípicamente mediante pruebas bioquímicas y el esquema LOPAT, confirmando su pertenencia al Grupo I de *P. syringae*. Las pruebas de patogenicidad verificaron su capacidad para inducir síntomas típicos del tizón foliar y nueve aislados resultaron positivos para la producción de siringomicina, fitotoxina asociada a la necrosis del tejido huésped. Se evaluó la actividad antimicrobiana de distintas preparaciones fitoquímicas de *Thymus vulgaris* y *Origanum vulgare* (decocciones, extracto alcohólico, hexánico, clorofórmico y propanólico y aceites esenciales (AEs)) frente a cepas aisladas y de referencia de *P. syringae*. Los ensayos de difusión en disco mostraron que los AEs de *T. vulgaris* y *O. vulgare* presentaron los efectos inhibidores más fuertes. Las pruebas de microdilución en caldo confirmaron su alta potencia antibacteriana (CIM del AE de *T. vulgaris* = 1,43–22,99 mg/mL y CIM del AE de *O. vulgare* = 2,89–23,13 mg/mL). Los resultados demuestran la eficacia de los AEs de *T. vulgaris* y *O. vulgare* como agentes antimicrobianos naturales con potencial aplicación para el control sostenible del tizón foliar bacteriano en trigo.

**Palabras clave.** *Pseudomonas syringae*, orégano, tomillo, fitoquímicos, actividad antimicrobiana.

## INTRODUCTION

Common wheat (*Triticum aestivum* L.) is a crop of great value worldwide, constituting one of the main sources of human food (Wrigley, 2016). In Argentina, wheat is one of the most important crops, with an average sown area of 5.6 million hectares and an annual production of approximately 16 million tons (BCR, 2023).

During the wheat crop cycle, various biotic factors can alter the normal functions of plants. These include pathogens such as bacteria, fungi, viruses and nematodes, which are responsible for generating changes in the physiology or behavior of plants, leading to partial alteration or death of the plant or its organs (Duveiller *et al.*, 1997; Agrios, 2005). In Argentina, fungal and viral pathogens are the most studied, therefore, diseases caused by bacteria require more information and studies of their effects on crops (Pozzi *et al.*, 2023).

Among the most relevant bacterial species responsible for causing diseases in wheat are *Pseudomonas syringae* pv. *syringae*, the causal agent of bacterial leaf blight, *Xanthomonas translucens*, responsible for bacterial leaf streak, and *Clavibacter tessellarius*, which causes bacterial mosaic disease (Duveiller *et al.*, 1997). In the field, grain yield losses caused by bacterial leaf blight depend on several factors, including environmental conditions (cool temperatures, high relative humidity, and frost), disease incidence and severity, pathogen aggressiveness, crop resistance or susceptibility, and the phenological stage at which infection occurs (Valencia-Botín and Cisneros-López, 2012; Xin *et al.*, 2018). Populations of *P. syringae* pv. *syringae* are found epiphytically on the surface of wheat plants and other hosts; therefore, climatic conditions are more relevant to disease outbreaks than the presence of inoculum. The disease can affect the plant from the flag leaf stage until the end of the cycle, where small water-soaked spots are initially observed that expand and merge, forming dried, grayish-green areas. They then become necrotic and the lesions can progress to destroy more than 75% of the leaf blade (Duveiller *et al.*, 1997).

*P. syringae* produces different virulence factors that aid in the establishment and spread of the disease, and their presence depends on the bacterial strain. Among these virulence factors are phytotoxins and enzymes that decompose plant tissues (Xin *et al.*, 2018). One of the phytotoxins produced by *P. syringae* pv. *syringae* is syringomycin, a class of cyclic lipodepsinonapeptide that produces pores in the host plasma membrane, increasing permeability and causing electrolyte loss, resulting in tissue necrosis (Ichinose *et al.*, 2013).

The management of bacteria diseases has been based on the use of antibiotics, antibacterial chemicals, biocontrol agents, and resistant varieties. It is well known that chemical control measures can negatively affect human health, the environment, and biodiversity, as some pesticides are toxic and non-biodegradable (Santiago-Santiago *et al.*, 2024; Pantović *et al.*, 2025; Rai and Rai, 2025). Furthermore, the limited availability of bactericides, the ability of plant pathogenic bacteria to disperse even over long distances and persist in seeds, and the rapid acquisition of resistance to chemical agents create a considerable challenge in disease control (Bastas and Kannan, 2015). In this context, there is a marked trend toward reducing the use of agrochemicals in agriculture, generating the need to develop ecological and sustainable therapeutic alternatives (Santiago-Santiago *et al.* 2024; Rai and Rai, 2025). Research on phytopathogenic bacteria control using phytochemicals from aromatic plants has been increasing in the last years, and the results strongly suggest the potential of essential oils (EOs) for controlling these diseases (Kotan *et al.*, 2014; Ghalem, 2016; Santiago-Santiago *et al.*, 2024).

Among aromatic plant species, *Origanum vulgare* and *Thymus vulgaris* occupy a special position because their EOs and extracts are capable of inhibit bacteria and fungi (Rota *et al.*, 2008; Oliva *et al.*, 2015; Carezzano *et al.*, 2017; Bounar *et al.*, 2020; Kosakowska *et al.*, 2024). In addition, botanical compounds offer certain advantages, such as being degradable and more environmentally friendly than synthetic pesticides (Díaz and Aguilar, 2018).

The main objectives of this study were to isolate, characterize and evaluate the antimicrobial activity of EOs and extracts obtained from *O. vulgare* and *T. vulgaris* against phytopathogenic *Pseudomonas* isolated from wheat with bacterial leaf blight symptoms, with the aim of exploring new alternatives for the control of these bacterial diseases. Additionally, the isolates were tested for their ability to produce syringomycin, a phytotoxin produced by various *P. syringae* pathovars.

## MATERIALS AND METHODS

### Bacterial isolation from symptomatic wheat leaves

The wheat plants (*Triticum aestivum* L.) used in this study presented characteristic symptoms of bacterial leaf blight and were collected from an experimental station located in Marcos Juárez (Córdoba, Argentina) belonging to the Instituto Nacional de Tecnología Agropecuaria (INTA). For bacterial isolation, the leaves were aseptically cut and disinfected with ethanol (70% v/v) and then with a sodium hypochlorite solution (1% v/v). They were washed with sterile water and ground in a mortar with 1 mL of sterile distilled water. The remaining liquid was left steady for 5 min and sown on King B agar (KBA) (20 g/L peptone, 15 g/L magnesium sulfate-7-hydrate, 1.5 g/L dipotassium phosphate, 15 g/L agar-agar, 10 mL/L glycerol) with the addition of penicillin (50 IU/L). The plates were incubated for 24 h at 28°C or until colony development was observed. For long-term maintenance of the strains, King B broth (KBB) with the addition of glycerol (20% v/v) was used and stored at -20°C and -70°C (Oliva *et al.*, 2015).

### Phenotypic identification of the bacterial isolates

Conventional metabolic tests were performed on pure cultures to identify and classify the isolated bacteria. The following characteristics were determined: Gram stain, pigment production, cytochrome C oxidase, catalase, metabolism of glucose (oxidative/fermentative), aesculin hydrolysis, gelatine hydrolysis, nitrate reduction, indole formation, methyl red (MR), Voges-Proskauer (VP) and triple sugar iron agar (TSI). Subsequently, LOPAT tests were performed to differentiate plant pathogenic from saprophytic *Pseudomonas*: L: levan production, O: oxidase, P: potato rot, A: arginine dehydrolase, and T: tobacco hypersensitivity reaction (Lelliott *et al.*, 1966).

## Pathogenicity test

Wheat seeds were disinfected with alcohol (70% v/v) and sodium hypochlorite (3% v/v), washed three times with sterile distilled water and sown in pots containing a 2:1 soil/sand mixture. They were incubated in a greenhouse (28 °C, 14 h of light). When the plants reached the visible flag leaf stage (Zadoks scale Z3.9) (Rawson and Gómez Macpherson, 2000), they were infected with cultures of the different isolates ($10^6$ CFU/mL, equivalent to OD 620 nm = 0.04) by spraying onto the wheat leaves. The pots were then covered with nylon bags for two days, then removed and left in place until the first symptoms of the disease appeared (Duveiller, 1997). Three plants per isolate were inoculated, and three plants were inoculated with sterile water as controls. The assay was performed in duplicate.

## Phenotypic detection of syringomycin production

Syringomycin production was assayed using a general method for lipodepsipeptide detection according to Hwang *et al.* (2005) with modifications. *P. syringae* isolates were incubated in KBB for 18 h at 28°C. A 20 µl drop of each culture was then inoculated on glucose potato agar (GPA) plates and incubated for five days at 28°C. They were then sprayed with a *Geotrichum citrii auranti* spore solution (1/10) and left for 24 h at room temperature to allow fungal growth. The presence of lipodepsipeptide was considered positive by the development of a zone of *G. citrii auranti* growth inhibition around the *P. syringae* colonies.

## Plant material and phytochemical preparations

The dried plant material of *Origanum vulgare* (oregano) and *Thymus vulgaris* (thyme) was obtained from "Los Molles" agricultural establishment located in Merlo, San Luis province (Argentina). The phytochemical preparations (PP) included decoctions, alcoholic, propanolic, chloroformic and hexanic extracts

Decoctions were obtained from 10 g of dried plant material in 100 mL of distilled water, heated to boiling point for 15 min, filtered with a Whattman N° 2 paper and autoclaved. The decoctions obtained were stored at 4°C in a caramel-colored bottle until use.

The alcoholic extract was prepared by macerating 10 g of dried plant material in 100 mL of ethanol 96 % v/v and incubating it on a rotary shaker for 24 h at room temperatura (no more than 45°C) and then filtered through Whattman N° 2 paper (Carezzano *et al.*, 2023). The filtrate was stored in a caramel-colored flask and the plant material was extracted two more times. The solvent was evaporated using a rotary evaporator (IKA RV 10, rotation speed: 50 rpm, bath temperature: 70 °C and reduced pressure: 0.075 MPa), and the evaporation was completed by bubbling with nitrogen gas. The extracts were stored at -20 °C until use.

The propanolic, chloroformic and hexanic extracts were obtained from 10 g of powdered plant material that were placed inside a porous bag or "thimble" made of cellulose or resistant filter paper and then subjected to Soxhlet extraction (Abubakar *et al.*, 2020). The process continues until the extraction was completed, point at which the solvent flowing from the extraction chamber leaves no residue. Then the solvent in each extract was evaporated in a rotary evaporator (Rotavapor – IKA RV 10), and the evaporation was subsequently completed by bubbling with nitrogen gas. The extracts were stored at -20°C until use.

The yield of the evaporated dry extracts was calculated using the formula described by Falleh *et al.* (2008): Y (%) = 100 Me/Ms, where Y is the extraction yield in %, Me is the weight of the extract after solvent evaporation and Ms is the dry weight of the plant sample.

The plant extracts obtained with different solvents were resuspended with 1/8 Dimethyl sulfoxide (DMSO) at the time of evaluating the antimicrobial activity at a concentration of 100 mg/mL.

The EOs used in this study were obtained by Oliva et al. (2015), using a hydrodistillation technique in a Clevenger apparatus. The chemical composition of the EOs was analyzed using gas chromatography-mass spectrometry (GC-MS) (Oliva *et al.*, 2015). The EOs were stored at -20°C until use.

## Antimicrobial activity

The activity of the PP was evaluated on phytopathogenic strains isolated from wheat and three reference strains: *P. syringae* pv. *toma-*

*to* DC3000, *P. syringae* pv. *syringae* B728a and *P. savastanoi* pv. *glycinea* B076. For this, a screening was made using the disk diffusion technique and then the broth microdilution technique was performed to determine the minimun inhibitory concentration (MIC) and minimun bactericidal concentratio (MBC).

***Disk diffusion method.*** The antimicrobial assay of the PP was performed using the standard Kirby-Bauer disk diffusion method with some modifications (Bauer *et al.*, 1966). The bacterial strains were grown in 5 ml of KBB, incubated for 18 h at 28 °C, and turbidity was adjusted to 0.5 of the standard McFarland scale ($10^8$ CFU/mL). Then, 0.1 mL of inoculum was seeded on KBA plates and distributed over the surface using a Drigalsky spatula. Subsequently, 6 mm paper discs were impregnated with 10 ul of each plant extract and placed on the plates previously seeded with each strain. DMSO was used as a control for the solvent in which the plant extracts were prepared. The plates were incubated for 24 h at 28°C to determine antibacterial activity. The assay was performed in triplicate and repeated twice. Antimicrobial activity was determined by taking two perpendicular measurements of the growth inhibition zone diameter, which were averaged.

### *Determination of the Minimum Inhibitory Concentration (MIC).*

The antimicrobial activity was assessed using the broth microdilution method in 96-well microplates. This technique was used to determine the MIC of PP that showed a growth inhibition zone diameter equal to or greater than 10 mm in the disk diffusion assay.

Bacterial strains were first grown in KBB at 28 °C for 24 h. Serial ten-fold dilutions($10^{-1}$ to $10^{-5}$) of each inoculum were prepared and 170 µL of each dilution was transferred to individual wells of a 96-well microplate. Subsequently, 20 µL of a DMSO: water solution (1:8, v/v) and 10 µL of resazurin solution (0.01% w/v) were added. Microplates were incubated at 28 °C for 4 h, and the last dilution unable to reduce resazurin (remained blue) was selected for the next steps.

Serial two-fold dilutions of each PP were made in DMSO (1:8, v/v) and 20 µL of each were placed in wells containing 170 µL of the previously standardized microbial suspension. The plates were incubated at 28°C for 24 h, after which 10 µL of the resazurin indicator (0.01% w/v) was added to each well. A second incubation was carried out for 4 h at 28 °C. Color change was used as an indicator of microbial growth; a blue color indicated growth inhibition (oxidized resazurin), while a pink color indicated microbial growth (reduced resazurin). Therefore, the MIC was defined as the highest dilution (lowest concentration) of the PP that remained blue after incubation.

Positive controls consisted of 170 µL of inoculum with 20 µL of DMSO (pink, growth), and negative controls included 170 µL of KBB with 20 µL of PP and 170 µL of KBB with 20 µL of DMSO (both blue, no growth) (Oliva *et al.*, 2015).

### *Determination of the Minimum Bactericidal Concentration (MBC).*

From the microplates used to determine the MIC, 100 µL aliquots were taken from the MIC well and the five previous dilutions and sown on KBA plates, which were incubated at 28°C for 24 h.

The MBC was defined as the lowest PP concentration at which initial inoculum survival was less than 0.01%, corresponding to the absence of visible bacterial growth on the agar surface. All assays were performed in duplicate (Oliva *et al.*, 2015).

## RESULTS

### Phenotypic identification of the isolates

The samples showing characteristic symptoms of bacterial leaf blight; from them fifteen (15) isolates coinciding with the genus *Pseudomonas* were obtained. The colonies observed were creamy, rounded, with irregular edges, and some with a yellow-green fluorescent pigment. Gram-negative bacilli were observed under the microscope. These isolates were called Mtn, where "n" represents the sample number (from 1 to 15). Fourteen (14) of the 15 isolates presented the following characteristics: negative oxidase, oxidative glucose metabolism, alkaline/alkaline TSI, variable nitrate reduction, negative acid-mixed and butylene glycol fermentation, negative indole, positive esculin hydroly-

sis, negative gelatin and starch hydrolysis. Strain Mt15, unlike the rest, was positive for the mixed acid and butylene glycol fermentation tests, therefore it was discarded. Thirteen of the 14 selected isolates exhibited fluorescent pigments when exposed to ultraviolet light. LOPAT tests were then performed on the 14 isolates, which presented the following characteristics: all were positive for levan production, negative for oxidase, negative for potato rot, did not decarboxylate arginine and presented a positive hypersensitivity reaction to tobacco (Fig. 1). Based on the results, the 14 isolates were classified within LOPAT Group I, which includes the phytopathogenic *P. syringae* (Lelliott *et al.*, 1966).

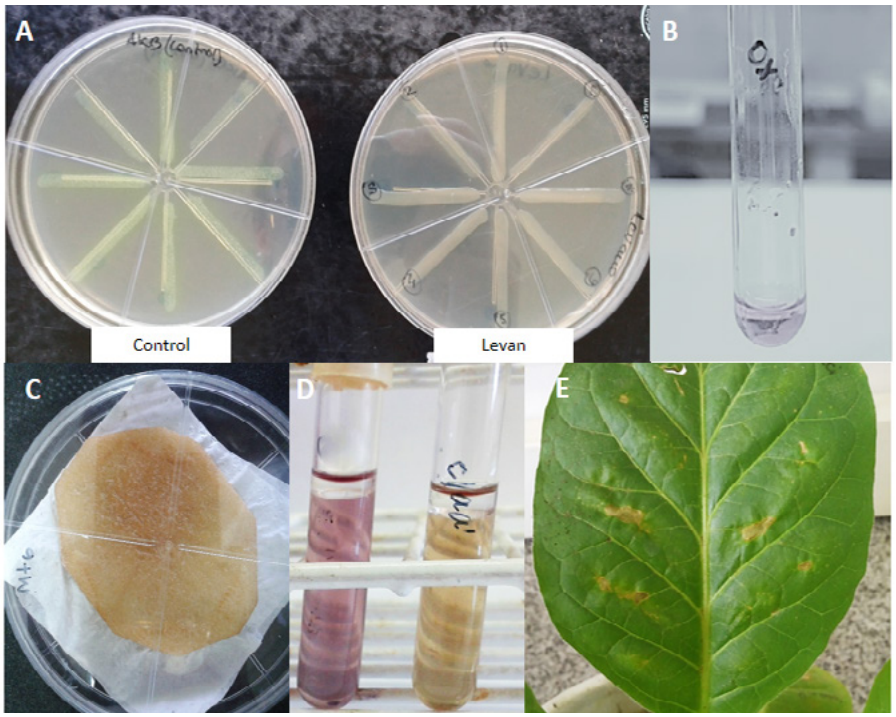


**Figure 1.** LOPAT tests. A. Positive Levan production. B. Negative oxidase. C. Negative potato rot. D. Negative arginine dehydrolase. E. Positive tobacco hypersensitivity reaction.

**Figure 2.** Pathogenicity test of the isolated phytopathogenic strains. Symptoms of leaf necrosis caused by *P. syringae* on wheat leaves.

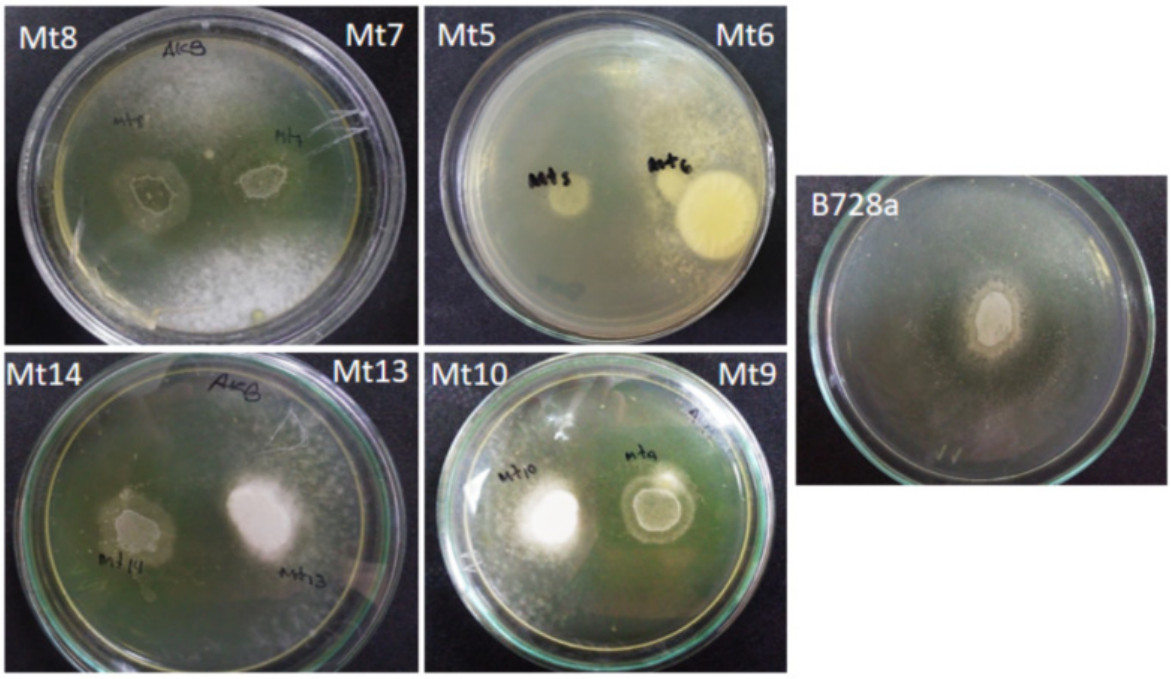


**Figure 3** Syringomycin production: Control (+): *P. syringae* pv. *syringae* B728a. Isolated phytopathogenic strains: Mt5, Mt6, Mt7, Mt8, Mt9, Mt10, Mt13, Mt14.

## Pathogenicity test

Pathogenicity test was performed on isolates identified as *P. syringae* using LOPAT tests. In accordance with Koch's postulates, healthy wheat plants were infected, and the first typical symptoms of bacterial leaf blight were observed 5 days post-infection: initially numerous small spots on the flag leaf and on the first and second lower leaves, then the lesions expand forming necrotic spots that merge and form grayish-green desiccated areas (Fig. 2). Inoculated bacteria were reisolated from the lesions and subjected to LOPAT tests, obtaining colonies with the same macro and microscopic characteristics as the original isolates.

## Phenotypic detection of syringomycin production

Phenotypic detection test for syringomycin production was performed on the 14 selected isolates. Production of this phytotoxin was observed in strains Mt1, Mt2, Mt4, Mt5, Mt7, Mt8, Mt9, Mt12 and Mt14, as evidenced by the growth inhibition of *G. citri aurantii*. *P. syringae* pv. *syringae* B728a was used as a positive control for toxin production in this assay. Figure 3 shows the growth inhibition zones of *G. citrii aurantii* produced by syringomycin.

## Phytochemical preparations

From dried plant material of oregano and thyme, highly aromatic decoctions of liquid consistency and dark green color were obtained. These decoctions were stored in dark flasks and later used to determine antimicrobial activity.

The plant extracts obtained with different solvents (alcohol, hexane, chloroform, and propanol) had a viscous consistency, a very dark green color, and highly aromatic. The alcoholic extract was obtained with a higher yield than the other extracts for two aromatic plants evaluated. The yields of the extracts obtained are shown in Table 1.

The EOs used in this study were obtained and analyzed by Oliva *et al.* (2015) by GC-MS, where 36 compounds were identified in *T. vulgaris* essential oil (TEO) and 24 in *O. vulgare* essential oil (OEO), representing more than 99% of their composition. Both presented high levels of carvacrol and low levels of thymol. The main components were *p*-cymene and γ-terpinene; the OEO also included hydrated *cis*-sabinene.

**Table 1.** Yield (%) of *O. vulgare* and *T. vulgaris* extracts obtained with different solvents

| Plant material | Yield (%) | | | |
|---|---|---|---|---|
| | AE | HE | CE | PE |
| *O. vulgare* | 11.90 | 0.44 | 0.57 | 3.20 |
| *T. vulgaris* | 12.60 | 3.95 | 0.43 | 0.97 |

References: AE: alcoholic extract; HE: hexanic extract; CH: chloroformic extract; PE: propanolic extract.

## Antimicrobial activity

In this study, the antimicrobial activity of decoctions, alcoholic extract (AE), hexanic extract (HE), chloroformic extract (CE), propanolic extract (PE), and EOs of *O. vulgare* and *T. vulgaris* were determined. The activity of the different phytochemical preparations was evaluated on 17 phytopathogenic *P. syringae* strains: 14 wheat isolates and three reference strains: *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *syringae* B728a and *P. savastanoi* pv. *glycinea* B076.

***Disk diffusion method.*** The evaluation of the antimicrobial activity of the decoctions using the disk diffusion technique determined that only the Mt13 strain was inhibited by the thyme decoction, with a growth inhibition zone size of 7.5 mm. The remaining strains evaluated were not inhibited.

The antimicrobial activity of the plant extracts obtained with different solvents was then evaluated and the following results were obtained. Thyme AE showed the best antimicrobial activity, being able to inhibit 15 strains (88.2%), with inhibition zone sizes between 8 and 13 mm, while oregano AE was active against six phytopathogenic strains (35.3%), with inhibition zone sizes less than 9 mm. Thyme HE inhibited three strains (17.6%) and oregano HE one strain (5.9%). Oregano PE inhibited one strain, while thyme PE and oregano and thyme CE did not show inhibitory activity against the strains studied (Table 2). Therefore, the extract with the highest antimicrobial activity was thyme EA, followed by oregano EA.

The evaluation of the antimicrobial activity of EOs from both aromatic plants determined that they exerted an inhibitory effect on the majority of the strains tested. OEO inhibited the growth of 16 strains, representing 94.1%, while TEO inhibited 15 strains, representing 88.2%. The growth inhibition zone sizes ranged from 7 to 29 mm in diameter. The most sensitive strain was *P. syringae* pv. *tomato* DC3000 for both OEO and TEO, with growth inhibition zone sizes of 29 and 26 mm, respectively, followed by the strain *P. syringae* pv. *syringae* B728a, while the wheat isolates that were inhibited presented growth inhibition halo sizes less than or equal to 10.5 mm for OEO and less than or equal to 9.5 mm for TEO (Table 2, Fig. 4).

**Table 2.** Antimicrobial activity of plant extracts and essential oils of *O. vulgare* and *T. vulgaris* on phytopathogenic *P. syringae* (average in mm).

| Phytopathogenic *P. syringae* | OAE | TAE | OHE | THE | OCE | TCE | OPE | TPE | OEO | TEO |
|---|---|---|---|---|---|---|---|---|---|---|
| **Wheat isolates** | | | | | | | | | | |
| Mt1 | NI | 10.5 | NI | NI | NI | NI | NI | NI | 7 | 11 |
| Mt2 | NI | 9 | NI | NI | NI | NI | NI | NI | 8.5 | 9.5 |
| Mt3 | NI | 12.5 | NI | NI | NI | NI | NI | NI | 7.5 | 8.5 |
| Mt4 | 8.5 | 9 | NI | NI | NI | NI | NI | NI | 7.5 | 8 |
| Mt5 | NI | NI | NI | NI | NI | NI | NI | NI | 7.5 | 8.5 |
| Mt6 | NI | NI | NI | NI | NI | NI | NI | NI | 7 | 10 |
| Mt7 | NI | 11.5 | NI | NI | NI | NI | NI | NI | 7.5 | 10 |
| Mt8 | 8 | 11 | 9 | NI | NI | NI | NI | NI | 9.5 | 10.5 |
| Mt9 | 7.5 | 10.5 | NI | NI | NI | NI | 7.5 | NI | 8.5 | 10.5 |
| Mt10 | 8 | 8.5 | NI | NI | NI | NI | NI | NI | NI | 8 |
| Mt11 | NI | 9 | NI | 7.5 | NI | NI | NI | NI | NI | NI |
| Mt12 | 8 | 10 | NI | 7 | NI | NI | NI | NI | 8.5 | 8.5 |
| Mt13 | 8.5 | 8.5 | NI | 7.5 | NI | NI | NI | NI | 7 | 7.5 |
| Mt14 | NI | 9.5 | NI | NI | NI | NI | NI | NI | 7 | 10 |
| **Reference strains** | | | | | | | | | | |
| *P. syringae* pv. *tomato* DC3000 | NI | 10.5 | NI | NI | NI | NI | NI | NI | 26 | 29 |
| *P. savastanoi* pv. *glycinea* B076 | NI | 13 | NI | NI | NI | NI | NI | NI | 10.5 | 9 |
| *P. syringae* pv. *syringae* B728A | NI | 8 | NI | NI | NI | NI | NI | NI | 13 | 16.5 |

References: OAE: oregano alcoholic extract, TAE: thyme alcoholic extract, OHE: oregano hexanic extract, THE: thyme hexanic extract, OCE: oregano chloroformic extract, TCE: thyme chloroformic extract, OPE: oregano propanolic extract, TPE: thyme propanolic extract, OEO: oregano essential oil, TEO: thyme essential oil, NI: not inhibited.

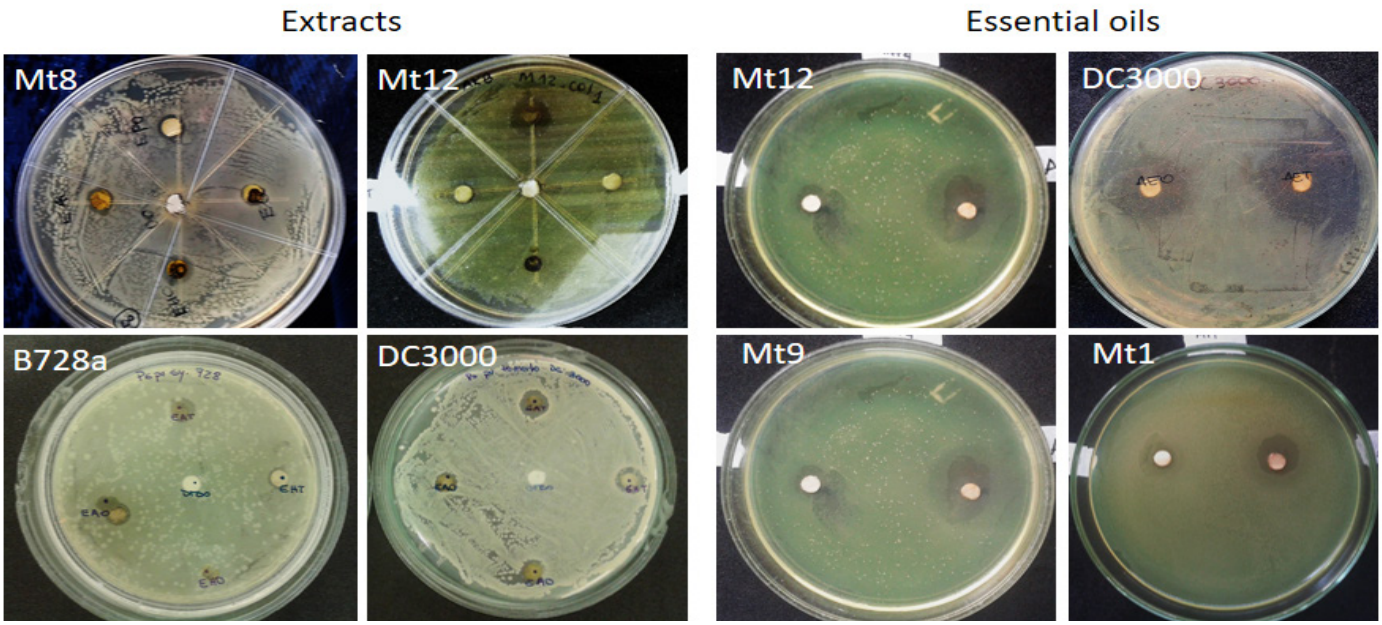


**Figure 4** Antimicrobial activity of different extracts and essential oils of *O. vulgare* and *T. vulgaris* using the disk diffusion technique on wheat isolates (Mt1, Mt8, Mt9, Mt12), and reference strains (*P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *syringae* B728a)

Therefore, based on the results obtained, it is possible to infer that phytochemical preparations from thyme demonstrated greater effectiveness than those obtained from oregano, since the percentages of inhibited strains were generally higher. The EOs of both aromatic plants showed the best inhibitory capacity (OEO: 88.2% and TEO: 94.1%), followed by the alcoholic extracts (TAE: 88.2% and OAE: 35.3%). TPE, OCE, and TCE did not present inhibitory activity against the strains studied.

***Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).*** In this work, the MIC and MBC of phytochemical preparations of oregano and thyme were determined for strains in which growth inhibition halos greater than or equal to 10 mm were observed by the disk diffusion technique. Thus, the following phytochemical preparations were evaluated: TAE, OEO and TEO.

The antimicrobial activity of TAE was assessed against wheat isolates: Mt1, Mt3, Mt7, Mt9, and Mt12, as well as the reference strains *Pseudomonas syringae* pv. *tomato* DC3000 and *P. savastanoi* pv. *glycinea* B076. The extract showed no detectable antimicrobial activity at the concentrations tested using the broth microdilution method.

The antimicrobial activity of OEO was evaluated against the reference strains: *P. syringae* pv. *tomato* DC3000, *P. savastanoi* pv. *glycinea* B076 y *P. syringae* pv. *syringae* B728a. OEO inhibited the growth of all three strains tested, with MIC values ranging from 2.89 to 23.13 mg/mL. Bactericidal activity was observed only against *P. syringae* pv. *syringae* B728a, with a MBC value of 5.78 mg/mL (Table 3).

The antimicrobial activity of TEO was evaluated against wheat isolates: Mt1, Mt6, Mt7, Mt8, Mt9, and Mt14, as well as the reference strains: *P. syringae* pv. *tomato* DC3000 y *P. syringae* pv. *syringae* B728a. TEO exhibited inhibitory activity against all tested strains except *P. syringae* pv. *syringae* B728a, with MIC values ranging from 1.43 to 22.99 mg/mL. No bactericidal activity of TEO was observed on any of the phytopathogenic strains analyzed (Table 3).

**Table 3.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alcoholic extract of thyme and EOs of oregano and thyme on phytopathogenic *P. syringae*.

| Phytopathogenic strains | TAE (0.048- 50 mg/ml) | | TEO (0.022-91.98 mg/mL) | | OEO (0.022-92.52 mg/mL) | |
|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC |
| **Wheat isolates** | | | | | | |
| Mt1 | - | ND | 5.74 | - | ND | ND |
| Mt3 | - | ND | ND | ND | ND | ND |
| Mt4 | ND | ND | ND | ND | ND | ND |
| Mt6 | ND | ND | 5.74 | - | ND | ND |
| Mt7 | - | ND | 5.74 | - | ND | ND |
| Mt8 | ND | ND | 2.87 | - | ND | ND |
| Mt9 | - | ND | 22.99 | - | ND | ND |
| Mt12 | - | ND | ND | ND | ND | ND |
| Mt14 | ND | ND | 22.99 | ND | ND | ND |
| **Reference strains** | | | | | | |
| *P. syringae* pv. *tomato* DC3000 | - | ND | 1.43 | - | 23.13 | - |
| *P. savastanoi* pv. *glycinea* B076 | - | ND | 5.74 | - | 2.89 | - |
| *P. syringae* pv. *syringae* B728a | ND | ND | - | ND | 5.78 | 1.44 |

References: (-): without MIC or MBC, ND: Not determined, TAE: thyme alcoholic extract, TEO: thyme essential oil, OEO: oregano essential oil.

## DISCUSSION

In this study, phytopathogenic bacteria of the genus *Pseudomonas* were detected as the cause of bacterial leaf blight in symptomatic wheat plants. The species of this genus are responsible for producing bacteriosis, resulting in significant yield reductions and consequent economic losses (Valencia-Botín and Cisneros-López, 2012). Bacterial diseases in wheat have been documented to reduce yield by up to 10% on average, with losses reaching 40% under favorable environmental conditions for pathogen development, such as high humidity and rainfall. However, the economic importance of these diseases can vary by region, country, and continent (Ocampo, 2013; Figueroa *et al.* 2018).

In Argentina, bacterial leaf blight was detected frequently during the 2001-2002 crop seasons, causing considerable damage due to environmental conditions conducive to its development, such as rain, frost, and wind (Simón and Fleitas, 2022). In recent years, an increase in the incidence of foliar bacterial diseases (spots and blights) in wheat crops has been recorded in our country, probably due to factors such as low resistance, climate change and seed contamination (Martino *et al.*, 2025). The INTA Marcos Juárez Agricultural Experimental Station conducted an evaluation of bacteriosis in bread wheat cultivars during the 2024 campaign, reporting that *P. syringae* pv. *syringae* was the most frequently isolated bacterium (Martino *et al.*, 2025).

One of the main virulence mechanisms of this pathogen is the production of phytotoxins, compounds produced during infection that facilitate the colonization of plant tissues and the development of symptoms, damaging plant cells (Bender *et al.*, 1999). It has been described that the level of toxin production and damage are correlated, so that non-toxigenic strains decrease or completely lose their virulence (Ichinose *et al.*, 2013). Phytotoxin detection contributes to the classification of members of the *P. syringae* complex into pathovars. In this study, syringomycin production was evaluated, and nine isolates were found to produce the phytotoxin. The pathovar *P. syringae* pv. *syringae* has been described as a producer of this phyto-

toxin (Bull *et al.*, 1998). This toxin participates in plant-pathogen interactions, contributes to the bacteria's virulence, and acts on the host cell membrane, causing pore formation, which increases its permeability and leads to electrolyte loss from plant cells. The H+−K+ exchange produces acidification of the cytoplasm, resulting in necrosis (Ichinose *et al.*, 2013). Therefore, studying the ability of strains to produce phytotoxins is essential not only to understand the pathogenic mechanisms of *P. syringae*, but also to develop control strategies.

Bacterial diseases in crops are of great importance due to the economic imbalances that imply the decrease or loss of agricultural production (Santiago-Santiago *et al.* 2024). Bacterial control of crop diseases represents a considerable challenge due to the limited availability of antimicrobial substances (Bastas and Kannan, 2015). In this context, there is an increasing number of studies dedicated to developing ecological and sustainable therapeutic alternatives. Research evaluating phytochemicals from aromatic plants strongly suggests the potential of EOs to control these diseases (Kotan *et al.*, 2014; Ghalem, 2016; Santiago-Santiago *et al.*, 2024).

EOs are secondary plant metabolites and are composed of a mixture of volatile compounds produced by aromatic plants. They are composed of low molecular weight compounds, terpenes, or phenylpropane derivatives, which give them various biological activities in humans, animals, and plants. Their main activities include antimicrobial, antioxidant, insecticidal, anti-inflammatory, analgesic, and antitumor properties, and they are widely used in food preservation (Adorjan and Buchbauer, 2010; Żukowska and Durczyńska, 2024).

In this research, the antimicrobial activity of oregano and thyme decoctions, extracts, and EOs against phytopathogenic *Pseudomonas* isolated from wheat and various reference strains of *P. syringae* was evaluated. Of the phytochemical preparations evaluated, the EOs were found to be the most effective in inhibiting the growth of the pathogens. These results could be explained by the differences in the quantity and quality of components present in the phytochemical preparations obtained and, consequently, their different terapeutic action (Dhami and Mishra, 2015). In addition, it has been described that some bacteria have mechanisms to resist or adapt to antimicrobial compounds and thus survive in adverse environments (Christaki *et al.*, 2020). Furthermore, members of the genus *Pseudomonas* are characterized by being highly resistant to chemical compounds, and in general, Gram-negative bacteria are less susceptible to EOs than Gram-positive bacteria (Kalemba and Kunicka, 2003).

The activity of EOs has been attributed to their constituent components, and their chemical structures determine the type of biological activity. Regarding the antibacterial mechanism, this is mainly due to the synergistic effects of their main components (Koroch *et al.*, 2007; Santiago-Santiago *et al.* 2024). The OEO and TEO used in this study contained thymol, carvacrol, and *p*-cymene, compounds with proven antibacterial activity. The OEO also contained *cis*-sabinene, which could be related to the differences in activity of the two EOs (Oliva *et al.*, 2015).

The EOs of *T. vulgaris* and *O. vulgare* have shown efficacy against different strains of phytopathogenic *P. syringae*, presenting better inhibitory activity than the antibiotic streptomycin, used as a treatment for bacteriosis in various plant productions (Oliva *et al.*, 2015). Kotan *et al.* (2009) evaluated the effects of *Thymus fallax* EO and its extracts against 25 phytopathogenic bacterial strains (*Clavibacter* spp., *Erwinia* spp., *Pantoea* spp., *Pseudomonas* spp. and *Xanthomonas* spp.). They found that thyme EO exhibited strong antibacterial activity against most of the strains tested, with MIC values ranging from 15.63 to 125 µL/mL. The hexanic extract showed moderate activity against several strains but with lower potency than the essential oil, with MIC values between 70 and 90 mg/mL, while in the chloroformic extract they reported lower antibacterial efficacy with activity against *Erwinia* spp. and *Xanthomonas* spp. with MIC ranges between 80 and 90 mg/mL. In a later study, Kotan *et al.* (2014) evaluated the antimicrobial activity of oregano (*Origanum onites*) EO and various extracts against 14 strains, including bacteria from the genera *Clavibacter*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. They determined that EO exhibited high antibacterial activity against all strains tested, with a MIC range of 7.81 to 31.25 µL/mL, while hexanic and chloroformic extracts inhibited most strains, except

for *Xanthomonas campestris* pv. *zinniae*, with a MIC range of 40 to 100 mg/mL. In the present study and based on the results obtained, it can be inferred that EOs were more effective than plant extracts in inhibiting the growth of phytopathogenic *Pseudomonas*, in accordance with the results reported in the aforementioned investigations.

Kosakowska *et al.* (2024) evaluated the antimicrobial activity of oregano and thyme EOs against *P. syringae*, *Xanthomonas hortorum* and *Erwinia carotovora*. In the study of thyme EO, MIC values were found in the range of 0.125 to 0.250 µL/mL, while for oregano EO the concentrations were higher, in the MIC range of 2 to 4 µL/mL. Therefore, thyme EO showed greater antibacterial potency than oregano. Carezzano *et al.* (2017) evaluated oregano and thyme EOs on different strains of *P. syringae*, demonstrating inhibitory activity with MIC values ranging from 1.43 to 11.5 mg/mL for thyme and 5.8 to 11.6 mg/mL for oregano. They also reported that both EOs were able to inhibit biofilm formation and syringomycin production in this phytopathogenic bacterium. Sotelo *et al.* (2023) isolated *P. syringae* strains that caused bacterial blight in soybean and found that OEO inhibited 100% of the strains tested, while TEO showed inhibitory effects in 78.6% of the strains. They reported MIC values between 11.56 mg/mL and 92.5 mg/mL for OEO, while the inhibitory values for TEO ranged from 5.74 mg/mL to 91.8 mg/mL. In agreement with the results reported by the aforementioned authors, the present study determined that the TEO presented lower MIC values than the concentrations reported for the OEO. However, both EOs represent a potential alternative for crop protection against bacterial diseases. Furthermore, EOs have been described to have a broad spectrum of activity against insects, mites, fungi and nematodes, constituting a promising option for integrated pest management (Isman, 2000).

## CONCLUSION

In the present study, phytopathogenic bacteria of the genus *Pseudomonas* were isolated and characterized from wheat crops grown in the province of Córdoba, Argentina. The isolates were phenotypically identified using the LOPAT scheme and pathogenicity assays, confirming their ability to cause disease in host plants. Among the phytochemical preparations evaluated, oregano and thyme EOs were shown to be effective inhibitors of the *in vitro* growth of the isolated phytopathogenic bacteria, as well as of *P. syringae* reference strains. These results highlight the antimicrobial potential of both EOs, that could be attributed to the presence of terpenic compounds, known for their antimicrobial activity when acting alone or synergistically. Therefore, considering the adverse effects associated with the use of synthetic pesticides and the growing need to find sustainable and safe alternatives for managing plant diseases and protecting the environment, oregano and thyme EOs are proposed as promising strategies for the control of phytopathogenic bacteria in wheat crops.

## ACKNOWLEDGMENTS

The authors gratefully appreciate the support from UNRC (Universidad Nacional de Río Cuarto) and CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas).

**Competing Interests:** No potential conflict of interest was reported by the authors.

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