Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Scientific Article

Wheat leaf blight-associated fungi and their biological control

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Section:
Special Number

Received:
November 18, 2024
Accepted:
June 10, 2025
Published:
June 26, 2025

Citation:
Sánchez-Alonso MG, Rivas-Valencia P, Robles-Yerena L, Rodríguez-Villareal RA *et al*., 2025. Wheat leaf blight-associated fungi and their biological control. Mexican Journal of Phytopathology 43(4): 74.
https://doi.org/10.18781/R.MEX.FIT.2024-26

ABSTRACT

Background/Objective. The objective was to determine the inhibitory effect of actinobacterial morphotypes on mycelial growth of fungi associated with wheat leaf blight**.**

Materials and methods. A total of 136 samples of wheat leaves were processed from the highlands of Mexico. They were sectioned, washed and placed in a humid chamber; fungi were isolated on potato dextrose agar. A morphological identification was carried out and the incidence of diseases was recorded by federal entity. Actinobacteria were isolated from soil samples using the dilution technique and microcolonies were isolated on nutrient agar. Preliminary *in vitro* bioassays were established at 27 °C with 144 actinobacteria and a dual confrotation with morphotypes presented a percentage of inhibition of mycelial growth (PIMG) greater than 40% and to identify those with potential for the control of fungi associated with leaf spots. The experimental design was completely randomized and an analysis of variance ($p \leq 0.05$) and mean test (Tukey $p \leq 0.05$) were performed.

Results. At least three actinobacteria inhibited mycelial growth of *Fusarium* spp. in a range of 42.5 - 66.9 %. The inhibitory efficacy of strain M2B M60 was highest with *Fusarium* isolate 1, that of M1B33 with *Fusarium* isolate 2 and that of M1B M4 was most effective with *Fusarium* isolate 3.

Conclusion. The use of actinobacteria represents an alternative for integrated management in the control of fungi associated with foliar blight in wheat, as they present an inhibitory effect on mycelial growth.

Key words: Actinobacteria, *Fusarium*, Specificity, Metabolites

INTRODUCTION

Agricultural production is under pressure to accelerate and increase its yields to meet the growing demand for food, as driven by population growth. In 2022, the global population reached 8 billion and is projected to rise to 8.5 billion by 2030 (UN, 2022). Cereals are a staple of the human diet (FAO, 2001); Sinaloa, Mexico, is the country's leading cereal producer, particularly of maize (SADER, 2022), and its production system is heavily reliant on agrochemicals (Cruz and Leos, 2018). Phytosanitary issues such as foliar spots impair plant function and result in yield losses ranging from 4% to as much as 38% in major production regions (*Mariscal-Amaro et al*., 2017). This highlights the need for efficient, sustainable, and cost-effective management strategies to achieve the yields expected by the growing population. The main fungi responsible for foliar spots or blights belong to the genera *Bipolaris*, *Fusarium*, and *Alternaria* (Prescott *et al.*, 1986); however, some causal agents remain unidentified (*Mariscal-Amaro et al.*, 2017). In Mexico, *F. graminearum*, *F. avenaceum*, *F. equisetii*, *F. proliferatum*, *F. subglutinans*, *F. oxysporum*, *F. thapsinum*, and *F. andiyazi* have been reported as species also associated with rot in various plant organs (Gilchrist-Saavedra *et al.*, 2005; Leyva-Mir *et al.*, 2017; Rangel-Castillo *et al.*, 2017).

It is worth noting that genetic improvement, fertilization rates, and both biological and chemical control are considered technologies for generating disease resistance, while training across the different segments of the production chain is viewed as a strategy to increase crop productivity (Cerutti, 2019; SENASICA, 2021; SADER, 2023). In the case of pesticides, their use poses a global health and environmental problem due to the presence of residues in food and contamination of soil and water (Rodríguez-Eugenio *et al.*, 2018; WHO, 2022), as pollution can result from bioaccumulation, transport, rainfall, evaporation, runoff, infiltration, and leaching (Jáquez-Matas *et al.*, 2022). The World Health Organization (WHO) is responsible for reviewing evidence and establishing internationally approved maximum residue limits. Nevertheless, developing countries need low-impact alternatives and the development of cost-effective technologies to either increase the use of or replace conventional, low-cost pesticides—many of which have been banned in developed nations but continue to be used in countries like Mexico (WHO, 2022).

A viable alternative is actinobacteria, a type of Gram-positive bacteria with varying oxygen requirements (Álvarez *et al.*, 2017), which possess antagonistic properties and the potential to inhibit the growth of agriculturally important phytopathogenic fungi. This is due to their secretion of secondary metabolites such as hydrolytic enzymes, chitinases, xylanases, and cellulases that degrade the cell walls of pathogens, as well as antibiotics (streptomycin and kamamycin), antifungal compounds, and pigments (Evangelista *et al.*, 2017; Parada *et al.*, 2017; Quiñones *et al.*, 2014; Cabrera *et al.*, 2020).

The most frequently identified genera of actinobacteria are *Streptomyces*, *Micronospora*, *Rhodococcus*, *Salinispora*, *Frankia*, *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Bifidobacterium* (Barka *et al.*, 2016). The inhibitory effect of actinobacteria isolated from soil samples has been observed in various contexts. In Nuevo Leon, an inhibitory effect was recorded against *Phytophthora capsici*, *Rhizoctonia solani*, and *Macrophomina phaseolina*, with inhibition ranging from 60% to 100% (Rodríguez-Villareal *et al.*, 2014). In Guanajuato, 82 strains were identified, three of which showed inhibitory effects against *R. solani*, *P. capsici*, and *F. oxysporum*, with an average radial inhibition rate of 81.8% (Sánchez-García *et al.*, 2019). In Hidalgo, the strain *Amycolatopsis* BX17 was identified as capable of completely inhibiting the mycelial growth of *F. graminearum* through the secretion of antifungal molecules (Cabrera *et al.*, 2020). Finally, in Jalisco, at least 49 strains were found to exhibit inhibition rates above 50% as antagonistic agents against *Fusarium* spp. under *in vitro* conditions (Quiñones *et al.*, 2014).

The antagonistic activity of actinobacteria is highly specific, and while certain species may act as antagonists in one plant crop, they can be pathogenic to others (Emmert and Handelsman, 1999; Glick, 1995). Therefore, the objective of this research was to identify actinobacterial morphotypes with inhibitory potential against fungi associated with wheat leaf blight, bearing in mind that *Fusarium* includes a wide range of species that cause root rot, leaf blight, and head blight symptoms, leading to yield losses of up to 50% (Ackermann and Pereyra, 2010).

MATERIALS AND METHODS

Sample collection

Commercial wheat fields at the grain-filling stage (from anthesis to physiological maturity) were visited in 136 locations across the High Valleys of Mexico. Each sample consisted of four leaves showing symptoms of blight and spotting. Of the above-mentioned localities, 25 locations were visited in Hidalgo, 45 in the State of Mexico, one in Morelos, five in Oaxaca, 15 in Puebla, and 45 in Tlaxcala. Samples were transported for processing to the Plant Pathology Laboratory at INIFAP-CEVAMEX, located in the municipality of Texcoco de Mora, State of Mexico.

Fungal Isolation

A total of 136 leaf samples were examined under a stereomicroscope (LEICA EZ4D, manufactured in Heerbrugg, Switzerland). Leaf segments (2 cm) showing symptoms were selected and surface-sterilized through three washes—1% sodium hypochlorite, 70% alcohol, and sterile water—for one minute each. The leaf segments were then placed in a humid chamber for 48 hours and inspected every 24 hours. Conidial masses that developed on the leaf surfaces were transferred to sterile 10 mL test tubes containing 1 mL of sterile water and vortexed to homogenize the suspension. A 20 µL aliquot was then dispensed onto potato dextrose agar (PDA) plates using a micropipette. After 24 hours, germinated conidia were examined using a compound microscope (LEICA EZ DM500, manufactured in Heerbrugg, Switzerland). Each germinated conidium was transferred to a new PDA Petri dish (modified technique from Morales-Rodríguez *et al.*, 2007).

The colonies obtained were identified using different culture media: PDA (for colony morphology and color), APZ (Potato Carrot Agar, to enhance colony growth), and SNA (Spezieller Nährstoffarmer Agar, used for the production of chlamydospores, microconidia, and macroconidia). Morphological identification was carried out using the keys of Leslie and Summerell (2006) and Zillinsky's (1984) guide to identifying common diseases of small-grain cereals. Morphometric analysis was conducted on 100 structures (conidia, phialides, macroconidia, conidiophores, etc.) to group the fungi by isolate.

Actinobacteria Isolation

Isolation was carried out using two composite soil samples from the municipality of Bustamante, Nuevo León: M1B (67 morphotypes), consisting of six subsamples, and M2B (77 morphotypes), consisting of five subsamples. Both were collected from the rhizosphere of avocado trees and obtained and purified at the General Teheran Experimental Station, Montemorelos, N.L. - National Institute of Forestry, Agricultural and Livestock Research (INIFAP). The isolation technique involved serial dilutions (10^{-2} and 10^{-3}) in sterile water, followed by dispersal of 50 µL onto nutrient agar medium. After 24 and 72 hours of incubation, the cultures were examined under a compound microscope, and microcolonies were transferred to new Petri dishes containing nutrient agar. Based on the morphological characteristics of the isolated colonies, the organisms were identified as belonging to the actinobacteria group.

Assays

Two preliminary bioassays were conducted to identify actinobacteria with the highest inhibition potential (>20%). The first included 144 morphotypes distributed across 36 Petri dishes plus one control dish. The second assay involved 16 morphotypes distributed across four Petri dishes plus one control. A subsequent dual confrontation test was performed between seven actinobacterial morphotypes and three previously selected fungi, distributed across 63 Petri dishes plus three controls. The variable used in all tests was the percentage inhibition of mycelial growth (PIMG), calculated using the formula from Salazar *et al.* (2001): PIMG = [(Mycelial growth in the control − Mycelial growth with treatment) / Mycelial growth in the control] * 100. Mycelial growth was measured in millimeters as the radial growth of the fungal colony between the fungal inoculum disk and the corresponding actinobacteria disk.

Preliminary bioassays. In 65 × 15 mm Petri dishes, a fungal inoculum disk (selected based on the most frequently reported fungus in the samples) was placed at the center, and four different disks of actinobacterial morphotypes were positioned at the outer edges of the same dish (no replicates). Additionally, a control dish was prepared with only the fungal inoculum disk placed at the center. The distance between the fungus and each actinobacteria disk was 1.5 cm. Mycelial growth data were recorded until the control fully covered the entire area of the Petri dish.

Dual culture assay. This assay was conducted only with actinobacteria that showed the highest inhibition percentages in the preliminary bioassay (>40%). Each actinobacteria strain was paired with three fungal isolates—two obtained from the wheat samples and a third, *Fusarium* isolate 3 (*Fusarium proliferatum*), which was donated from the personal collection of Dr. Leticia Robles. This particular species was included because of its agricultural relevance, as it is associated with premature yellowing or early maturity in wheat (Rangel-Castillo *et al.*, 2017). The actinobacteria and fungal disks were placed at opposite ends of the Petri dish, with a separation of 3 cm. Due to the rapid growth rate of the fungi during the assays, the transfer schedule was adjusted: the actinobacteria inoculum was placed first, followed by the fungal inoculum two days later, simulating a preventive treatment scenario.

Statistical analysis

A completely randomized experimental design was used for the dual culture assay, with three replicates. An analysis of variance ($p \leq 0.05$) was then performed, followed by a mean comparison test (Tukey, $p \leq 0.05$).

RESULTS

Identification of fungi associated with blight and foliar spots in wheat

Seven fungal species were identified, three of which are associated with wheat leaf blight (*Alternaria*, *Bipolaris,* and *Fusarium*), characterized by chlorotic halos that extend along the leaf blade. Two types of rusts were also identified (both from the genus *Puccinia*), though they were not included in the experiment due to being obligate parasites. Additionally, two fungi associated with foliar spots, also known as septoria leaf blotch (*Septoria* and *Zymoseptoria*), were identified.

***Alternaria* genus.** It was identified by the presence of conidia characteristic of the genus. Mata-Santoyo *et al.* (2018) report that *Alternaria* is associated with *Bipolaris sorokiniana* as a damage-enhancing agent or is identified as a saprophytic fungus in isolates obtained from wheat samples. However, Perelló *et al.* (2015) notes that *Alternaria* species have caused yield losses of up to 60% under favorable conditions, having been identified as pathogens responsible for foliar and grain diseases in wheat.

The conidia measured approximately 3–6 µm in thickness and 19–46.07 × 7.56–14.22 µm in length. *Alternaria* spp. colonies are dark green at maturity, while during active growth they display whitish to grayish tones. They are circular in shape, with a flat but cottony surface and filamentous edges (Figure 1).

***Bipolaris* Genus.** The observed symptoms included dark brown necrotic lesions along the leaves (Figure 2A). Under humid chamber conditions, conidiophore formation was detected on the leaf surface, along with dark, dense mycelium. The conidia and conidiophores allowed identification of the genus *Bipolaris* (formerly *Helminthosporium*) (Mata-Santoyo *et al.*, 2018). The observed conidia were oblong, olive-brown, septate, slightly curved, with smooth walls and a prominent basal scar (Figure 2B). Conidia measured approximately 34–63.12 × 10.06–18.5 µm, with three to nine septa per conidium. Conidiophores developed individually and measured on average 118.5–152 × 6.4–8.2 µm. Colonies were dark green, with a granular cottony texture, umbilicate elevation, and lobed margins (Figure 2C and D).

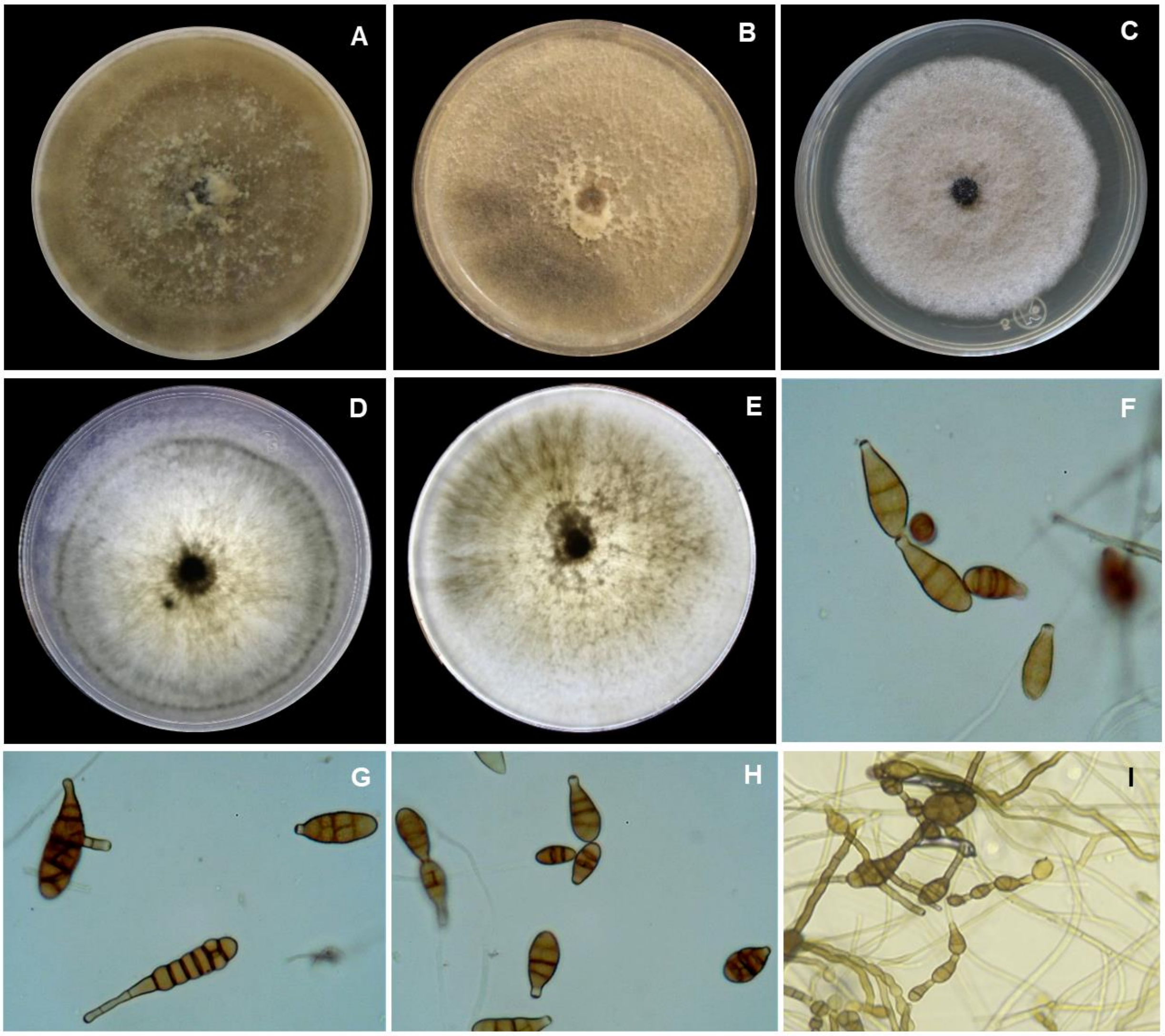

Figure 1. *Alternaria* spp. colonies. A) Colony on PDA culture medium. B) Colony on APZ culture medium. C) Early-stage mycelial growth on PDA culture medium. D) Colony on PDA medium showing one or two concentric rings observed under backlighting. E) Colony on PZA medium showing several concentric rings observed under backlighting. F-I) Conidia and conidial chains.

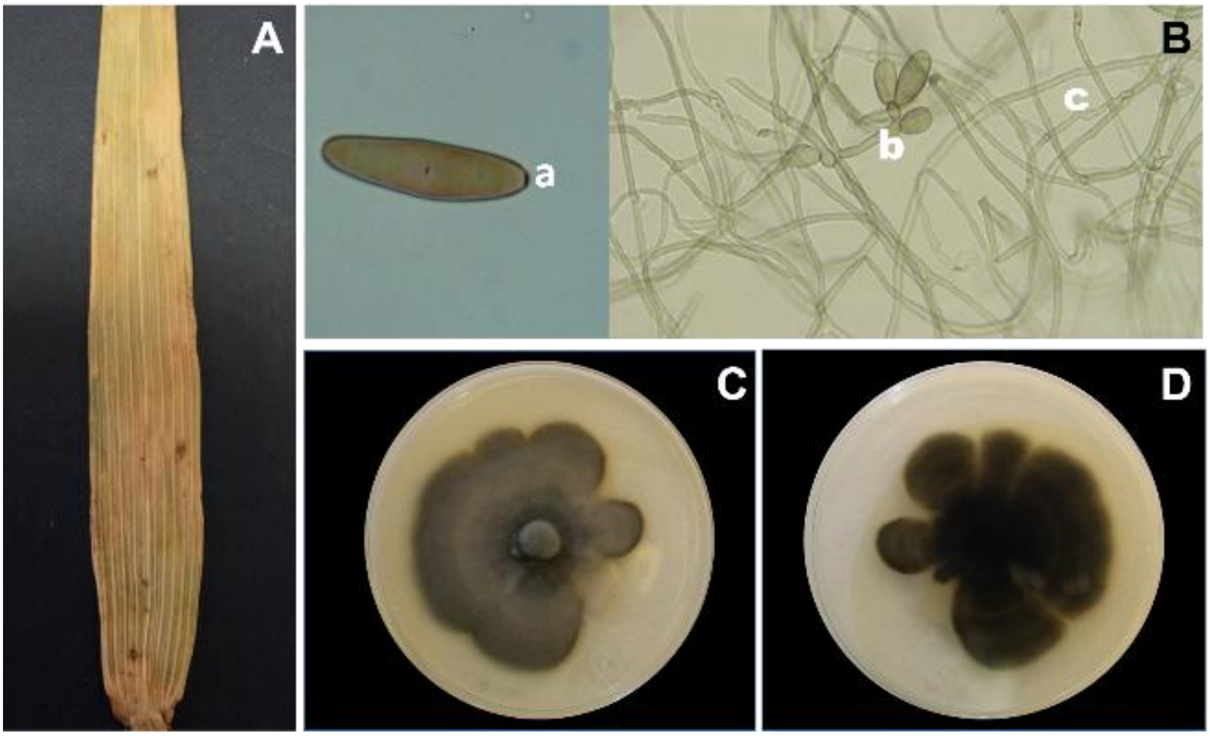

Figure 2. Symptoms and colonies of *Bipolaris* sp. A) Symptom of *Bipolaris* sp., of wheat leaf blight. B) Conidium with three slightly marked septa (a. basal scar), conidiophores (b), and mycelium (c). C) Colony on PDA culture medium. D) Reverse side of the Petri dish on PDA culture medium.

***Fusarium* Genus.** The observed symptom was a chlorotic spot with a whitish center (Figure 3); however, reports indicate that at early developmental stages the spots are grayish green, and at more advanced stages they become oval and grayish brown (Zillinsky, 1984). Nevertheless, the conidial mass used to isolate *Fusarium* was obtained from significantly dried leaves.

Based on the morphology of the structures, the genus was identified by the presence of macroconidia, microconidia, chlamydospores, and the type of phialides. The morphological characteristics suggested two types of *Fusarium* spp. isolates. On PDA culture medium, the first colony exhibited a yellow to reddish coloration, with circular growth, a flat but cottony surface, and filamentous edges—features consistent with descriptions of *F. graminearum* (Leyva-Mir *et al.*, 2017) (Figure 3); however, for future reference, it will be referred to as *Fusarium* isolate 1. Macroconidia recovered from SNA culture medium measured 21.01–63.14 × 3.08–4.85 µm, and chlamydospores measured 7.37–16.52 µm (Figure 3).

The second colony, grown on PDA culture medium, was whitish in color, with circular growth, slight ring formation, a cottony and flat surface (hereafter referred to as isolate 2) (Figure 3). Microconidia measured 5.55–11.2 × 1.73–3.79 µm, along with the presence of chlamydospores (Figure 3).

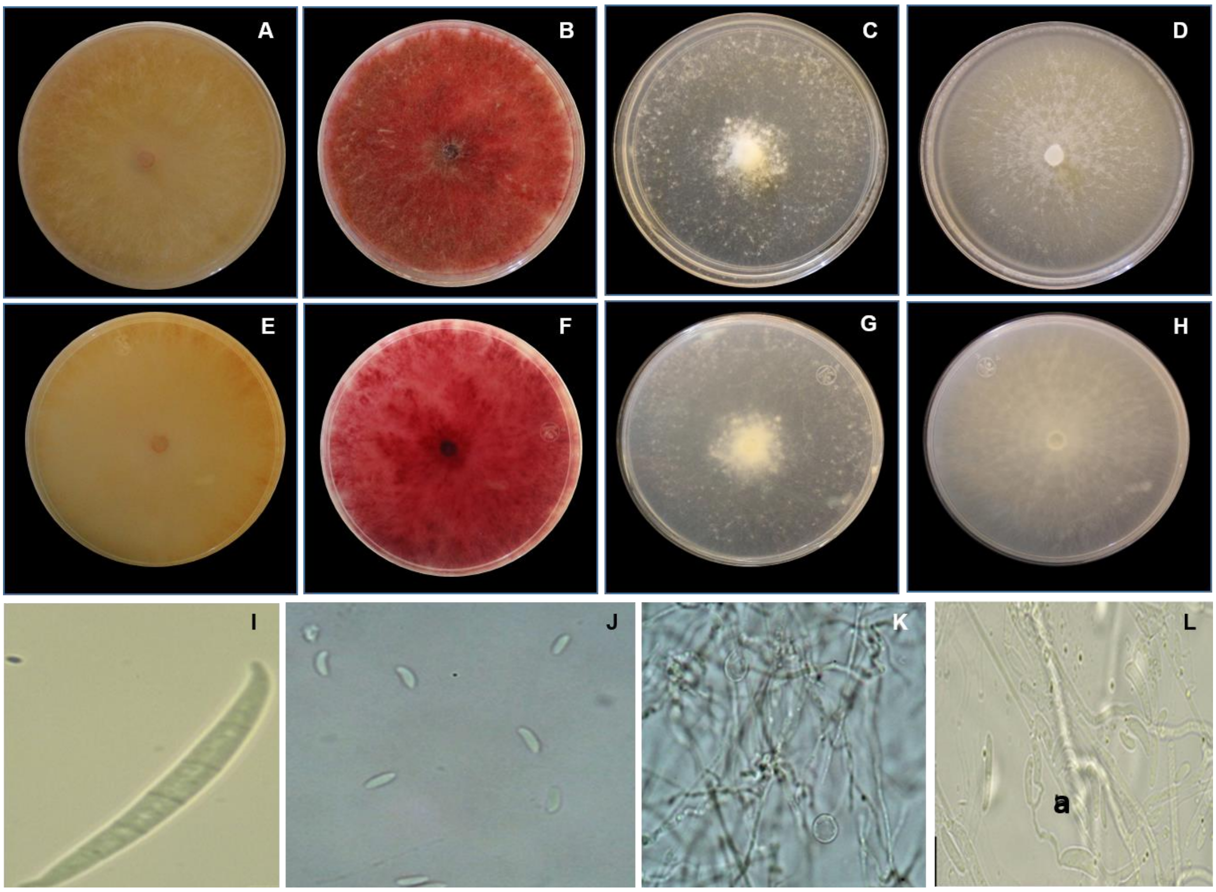

Figure 3. Characteristics of *Fusarium* spp. A and E) Front and reverse view of the colony on PDA culture medium of *Fusarium* isolate 1. B and F) Front and reverse view of the colony on SNA culture medium of *Fusarium* isolate 1. C and G) Front and reverse view of the colony on PDA culture medium of *Fusarium* isolate 2. D and H) Front and reverse view of the colony on SNA culture medium of *Fusarium* isolate 2. I) Macroconidium of *Fusarium* isolate 1. J) Microconidia of *Fusarium* isolate 2. K) Chlamydospores of *Fusarium* isolate 2. L) Microconidia and phialides (a) of isolate 2.

***Puccinia* genus.** Species of the genus *Puccinia* are known as the causal agents of rusts and are considered "the most diverse and economically significant groups of phytopathogenic microorganisms worldwide in agricultural and forestry production" (Zuluaga *et al.*, 2008). The characteristic symptom is the presence of pustules, which may contain two types of spores: urediniospores and teliospores. *Puccinia* 1 was observed with orange-red, spherical uredinia lacking ornamentation, measuring 22.53–28 µm in diameter. In contrast, *Puccinia* 2 showed uredinia distributed linearly along wheat leaves. These uredinia were yellow, slightly spherical, and measured 27.3–32.05 µm in diameter (Figure 4).

***Septoria* and *Zymoseptoria* genera.** During sampling, various degrees of septoria severity were observed. The symptoms included chlorotic halos containing pycnidia distributed throughout the halo (Figure 5).

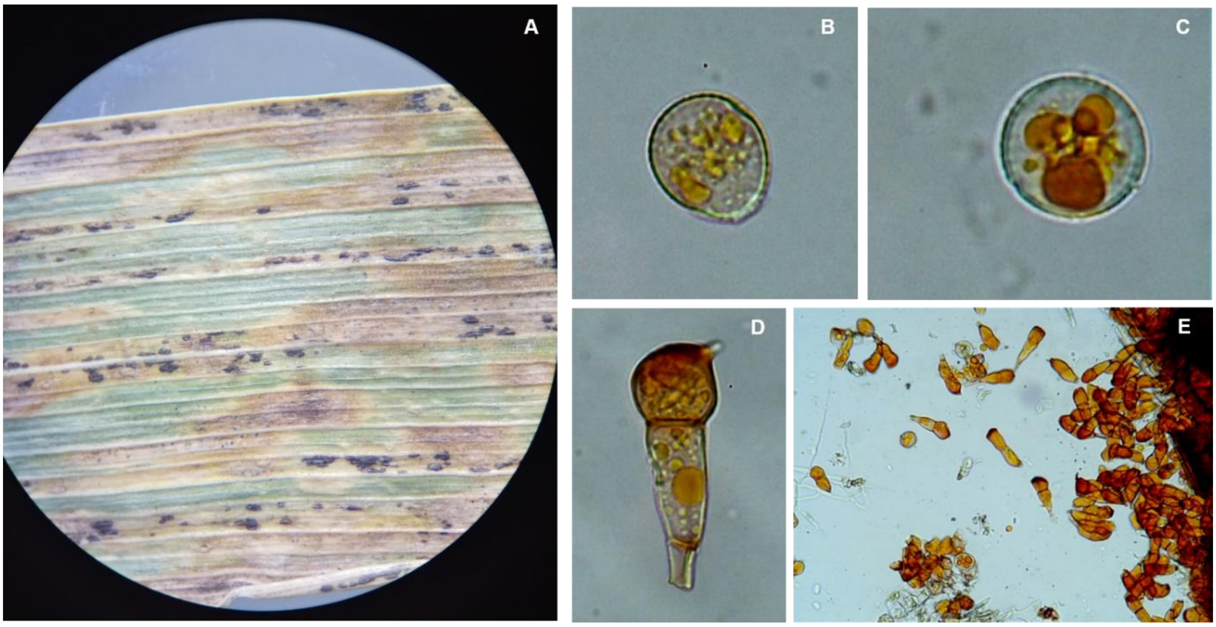

Figure 4. Rust pustules. A) Rust pustules on a wheat leaf. B) Urediniospore of *Puccinia* 1. C) Urediniospore of *Puccinia* 2. D) Teliospore of *Puccinia* 1. E) Teliospore of *Puccinia* 2.

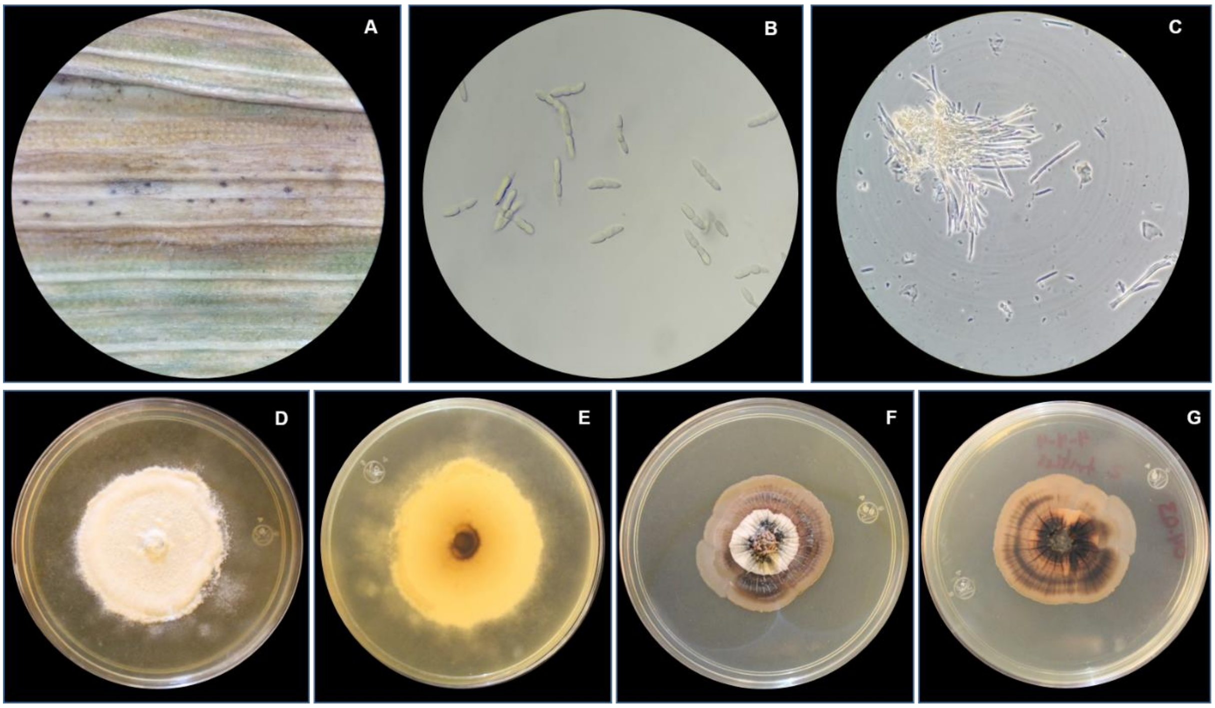

Figure 5. A) Chlorotic halos with pycnidia. B) Conidia of *Septoria* sp. C) Conidia of *Zymoseptoria* sp. observed under contrast phase. D) Front view of *Septoria* sp. colony on PDA. E) Reverse view of *Septoria* sp. colony on PDA. F) Front view of *Zymoseptoria* sp. colony on PDA. G) Reverse view of *Zymoseptoria* sp. colony.

The presence of *Septoria* and *Zymoseptoria* can sometimes be distinguished under a stereomicroscope by a slight difference in pycnidium color: *Zymoseptoria* tends to range from black to grayish, while *Septoria* ranges from black to dark brown.

The *Septoria* colony is slightly circular with some irregularities, a slightly convex surface, and slightly filamentous edges. The conidia are short, straight didymospores, unornamented, and pale yellow in color. Conidia measured approximately 14.6–20.2 × 2.86–3.48 µm.

In the case of *Zymoseptoria*, the colony was slightly circular with some irregularities, featuring a smooth surface and an umbilicate elevation (Figure 6). It is worth noting that *Zymoseptoria* colonies developed in two distinct forms: the first, at early stages, had a semi-liquid or viscous consistency with a pink coloration; the second, upon maturation, developed black mycelium with whitish segments. The conidia were filiform, unornamented, hyaline, and slightly curved, measuring 40–77.3 × 1.6–2.84 µm (Figure 5).

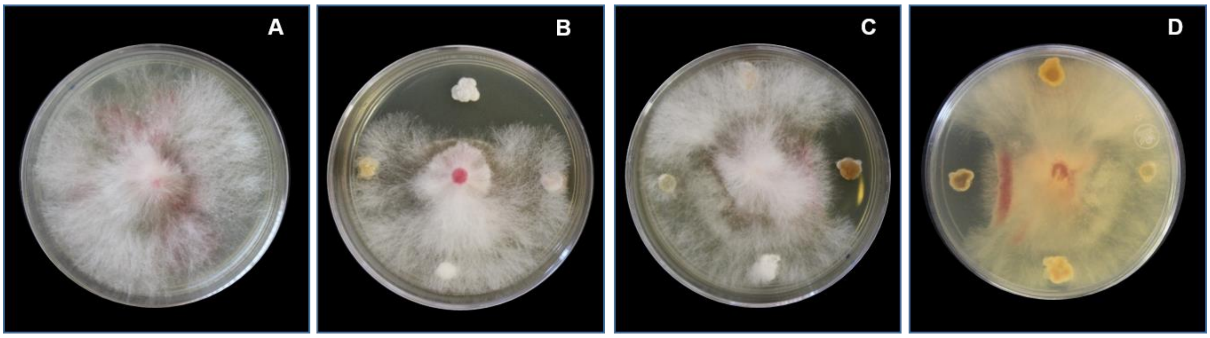

Figure 6. *Fusarium* isolate 1, nine days old. A) Control. B) and C) Interaction of the fungus with four actinobacteria, front view. D) Interaction of the fungus with four actinobacteria, back of the Petri dish.

Disease incidence

Once the main fungi present in the wheat samples were identified, disease incidence was determined based on the state in which each sample was collected (Table 1).

Table 1. Percentage of incidence of fungi associated with diseases in wheat crops sampled in the main producing states.

State	Incidence by genus (%)			
	***Alternaria* spp.**	***Bipolaris* spp.**	***Fusarium* spp.**	***Puccinia* spp.**
Hidalgo	20.0	4.0	52.0	92.0
Mexico	24.4	2.2	46.7	86.7
Morelos	0.0	0.0	0.0	100.0
Oaxaca	20.0	0.0	20.0	80.0
Puebla	13.3	0.0	60.0	80.0
Tlaxcala	8.9	0.0	35.6	88.9
Average	14.4	1.0	35.7	87.9

Considering the incidence of fungi in the evaluated samples, *Puccinia* and *Fusarium* were the most frequent, with 87.9% and 35.7%, respectively. Therefore, *Fusarium* was selected for the *in vitro* tests. The genera *Septoria* and *Zymoseptoria* could not be confirmed in all cases where symptoms were observed, and thus were not included in the incidence data.

Isolation of Actinobacteria and Preliminary Bioassays

A total of 144 actinobacteria were isolated from the soil samples M1B (67) and M2B (77) collected in the municipality of Bustamante, Nuevo Leon. The actinobacteria with the highest percentage of mycelial inhibition (42.9%) corresponded to 17 out of 144 morphotypes, representing only 11.1% of the evaluated population. The rest showed no inhibitory effect. The selected morphotypes, according to their group, were: from M1B—M1, M4, M12, M14, M16, M33, M41, M55, M57, and M60; and from M2B—M3, M15, M27, M45, M70, M73, and M74. In the second bioassay, the morphotypes showed variation in results compared to the first, with the second group showing a 25.1% decrease in PIMG (Table 2).

Table 2. Percentage inhibition of mycelial growth (PIMG) of preliminary bioassays of actinobacterial morphotypes against Fusarium isolate 1.

Group	First bioassay			Second bioassay		
	Morphotype	PIMG (%)	Average	Morphotype	PIMG (%)	Average
M1B	1	45.5	40.4	1	47.4	40.9
	4	40.9		4	63.2	
	12	40.9		12	52.6	
	14	40.9		14	36.8	
	16	36.4		16	47.4	
	33	40.9		33	57.9	
	41	50.0		41	57.9	
	55	36.4		55	21.1	
	57	31.8		57	-15.8	
M2B	3	50.0	45.4	3	-5.3	20.3
	15	50.0		15	5.3	
	27	31.8		27	-5.3	
	45	54.5		45	36.8	
	70	31.8		70	21.1	
	73	40.9		73	21.1	
	74	59.1		74	68.4	

The actinobacteria selected from the preliminary bioassays were MIB M4, MIB M33, MIB M41, M2B M60, and M2B M74, all of which showed a PIMG greater than 40%, confirming that some actinobacteria affect the mycelial growth of *Fusarium* morphotype 1 two days after inoculation (Figure 6).

Dual confrontation bioassay and growth rate

While the inhibitory capacity of some isolates is notable, a higher degree of specificity is also observed, given that the PIMG varies depending on the *Fusarium* isolate exposed. Three morphotypes—M1B M4, M1B M33, and M2B M60—stood out statistically with the highest inhibition percentages; however, each was more effective against a different isolate. M1B M4 was most effective against *Fusarium* isolate 3, M1B M33 against isolate 2, and M2B M60 against isolate 1 (Table 3 and Figure 7).

Table 3. Percentage inhibition of mycelial growth (PIMG) of three *Fusarium* isolates from wheat confronted with seven actinobacteria morphotypes.

Grupo	Morphotype	PIMG by *Fusarium* isolation					
		***Fusarium* isolation 1**		***Fusarium* isolation 2**		***Fusarium* isolation 3**	
M1B	M1	8.0	e	6.2	e	8.8	d
	M4	43.8	b c	42.5	a b	66.9	a
	M16	17.2	d	11.4	d	4.9	e
	M33	48.8	a b	47.1	a	47.9	b
	M41	38.3	c	31.3	c	43.4	b
M2B	M60	59.8	a	29.1	c	59.8	a
	M74	18.8	d	16.3	d	23.0	c

[z] Means with the same letter are not statistically different (Tukey, $p \leq 0.05$).

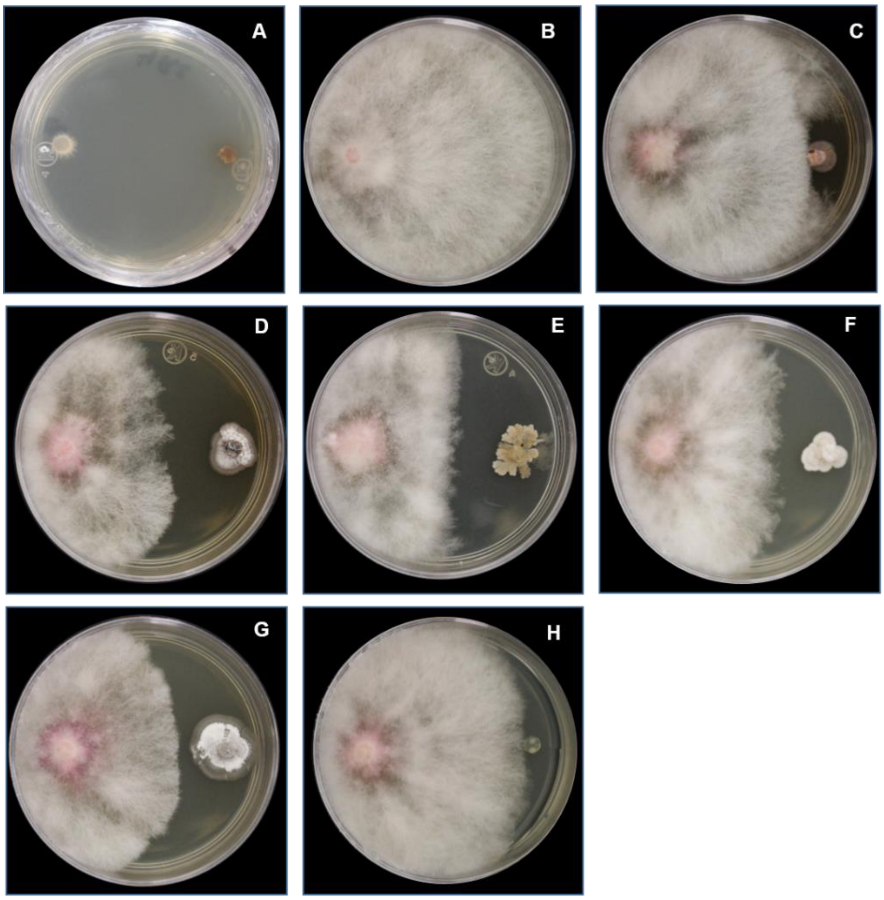

Figure 7. Dual confrontation with *Fusarium* isolate 1. A) Distribution of *Fusarium* discs. B) Growth of the control colony of *Fusarium* isolate 1. C) *Fusarium* isolate 1 and morphotype M1B M1. D) *Fusarium* isolate 1 and morphotype M1B M4. G) *Fusarium* isolate 1 and morphotype M2B M60. H) *Fusarium* isolate 1 and morphotype M2B M74. I) Mycelial growth of control *Fusarium* isolate 1.

In Table 4, it can be observed that *Fusarium* isolate 1 showed the highest growth rate starting on the third day after establishment, indicating that the difference in response among isolates of this genus depended on the growth rate of the fungus.

Table 4. Growth rate of *Fusarium* isolates from wheat leaves.

Fungus	Growth rate (cm day^{-1})								
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
Fusarium isolation 1	0.3	0.30	0.95	0.95	0.74	0.39	0.37	100	0.3
Fusarium isolation 2	0.51	0.52	0.53	0.44	0.39	0.33	0.28	0.29	0.28
Fusarium isolation 3	0.36	0.38	0.66	0.7	0.487	0.512	0.462	0.162	0.115

DISCUSSION

According to Savary *et al.* (2019), rust, along with *Fusarium* head blight, leaf spots, and powdery mildew, has caused wheat yield losses greater than 1% globally, which may reflect the evolution and resistance of pathogens to management strategies applied over time.

In this study, the presence of *Puccinia* spp. and *Fusarium* spp. was found to be predominant, while *Alternaria* spp., *Bipolaris* spp., *Septoria* spp., and *Zymoseptoria* spp. appeared with lower frequency in the High Valley of Mexico. This is generally consistent with the findings of Mariscal-Amaro *et al.* (2017), who reported *Bipolaris sorokiniana*, *Alternaria alternata*, *Septoria tritici*, *Fusarium equiseti*, *F. proliferatum*, *F. moniliforme*, *Curvularia* spp., and *Cladosporium* spp. as phytopathogenic fungi present on wheat leaves with high potential to cause root and stem rot symptoms, as well as to contaminate the grain and cause post-harvest rot.

In 2021, the National Service for Agrifood Health, Safety and Quality (SENASICA), through the (DGSV) General Directorate of Plant Health, promoted an operational strategy for the phytosanitary management of wheat. In the case of species from the genera *Puccinia* and *Fusarium*, the strategy refers only to chemical control and solely as a last resort (SENASICA, 2021). Previously, the use of *Trichoderma harzianum* was recommended as a preventive biological control applied to seed; however, integrated management requires treatments applicable not only to seed but also to seedlings or at critical development stages.

The use of actinobacteria for disease control has been explored at the in vitro level: the actinobacterium AAH5, isolated from soil samples from Saltillo, Coahuila, showed 49.4% inhibition against *Fusarium* spp. (*Dávila et al.*, 2013), which is consistent with the results of this research. However, the following findings were also highlighted: 1) there may be a synergistic effect among actinobacteria when they share the same space; 2) there is specificity between the actinobacteria morphotype and the fungal isolate.

Specificity, as well as the possible synergistic effect, may be due to the complex process of chemical signaling between organisms, as is the case with phytopathogenic bacteria and plants. According to Emmert and Hadelsman (*et al.*, 1999), actinobacteria exhibit a high degree of specificity regarding their ability to either cause disease or protect certain plants—that is, actinobacteria that are beneficial to some plants may cause disease in others. On the other hand, Gudesblat (2007) notes that bacteria, as part of an evolutionary process, developed various mechanisms to overcome the natural defenses of plants, as well as to detect the presence of related bacteria with which they may collaborate to successfully establish an infection. This may explain why the actinobacteria evaluated in the bioassays were able to detect the presence of other compatible actinobacteria (in the same Petri dish) and thereby enhanced the fungal growth inhibition effect. However, in the dual confrontation, the actinobacterium acted alone, resulting in a lower inhibitory effect.

This was observed in the case of morphotype M1B M1, which showed an inhibition effect of 45.5–47.4% in the bioassays, whereas in the dual confrontation (where only the actinobacterium and fungus interacted), it showed a mycelial growth inhibition range of only 6.1–8.8%.

Another possible explanation is the difference in the distance between the actinobacteria discs and the fungal discs. As noted by Rodríguez-Villareal *et al.* (2014), this may be related to the concentration of non-volatile compounds, since there is a direct relationship between the proximity of the actinomycete colony and the concentration of compounds. Thus, in the bioassays, the fungus—being closer to the actinobacteria—was exposed to a higher concentration of secondary metabolites (though not in contact with the actinobacteria colony itself), which was reflected in the higher inhibition percentage. It is worth noting that secondary metabolites produced by actinobacteria are of vital importance not only for the survival of the species but also for signaling and colonization of their habitat (González *et al.*, 2005). However, in addition to secondary metabolites, competition for space and nutrients is also a common mode of action in biocontrol agents against pathogens (Infante *et al.*, 2008).

CONCLUSIONS

Three actinobacteria morphotypes were identified with inhibitory activity against the mycelial growth of the genus *Fusarium*. The actinobacteria inhibited the three *Fusarium* isolates—associated with leaf blight—to varying degrees, indicating specificity, with mycelial growth inhibition ranging from 26.3% to 36.4%. Actinobacteria represent a potential alternative for the biological control of plant pathogenic fungi; however, extensive research is needed to characterize the chemical composition of the compounds produced by each morphotype and to determine their interactions with other actinobacteria.

Limitations

One of the limitations of this research is the production of actinobacteria. As with other living organisms, it is necessary to maintain their viability, concentration, and sufficient quantity to preserve their antagonistic effects.

Conflicts of Interest

None of the authors has any conflict of interest regarding this work.

Funding

This research was funded by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP).

Author Contributions

Project design and development: Dr. Rivas-Valencia, Dr. Robles-Yerena, and M.C. Sánchez-Alonso. Isolation of fungi associated with leaf spots: Dr. Robles-Yerena, Dr. Rivas-Valencia, M.C. Sánchez-Alonso, and Dr. Rodríguez-García. Isolation of actinobacteria: Dr. Rodríguez-Villareal and Dr. Rodríguez-Guerra. All authors have read and approved the published version of the manuscript.

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