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Search for fungi with potential mycoparasitic activity on causal agents of rust

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Abstract. *Luna-Escalona D, Alvarado-Rosales D, Equihua-Martínez A, Martínez-Trinidad T, Quezada-Salinas A, Vásquez-Murrieta S, Solís-Ramírez J, Jardines-Martínez A, King M, Noguez-Hernández R, Valadez-Moctezuma E. 2025. Search for fungi with potential mycoparasitic activity on causal agents of rust. Biodiversitas 26: 2706-2716.* Climate change has increased the incidence and severity of damage related to phytopathogens in agricultural and forestry production, resulting in a loss of 10-15% of the global production of major crops and adding up to hundreds of billions of dollars of additional costs across both industries. Phytopathogenic fungi are responsible for 70% to 80% of these adverse effects. To reduce these losses, producers have resorted to the use of chemical fungicides for disease control, which has, in turn, favored the development of chemoresistance to these compounds in phytopathogens. Therefore, searching for alternative management strategies, such as using mycoparasitic fungi, is a crucial endeavor in the global effort to protect crops from phytopathogens. In this context, our research team used white rust as the disease agent in *Chrysanthemum morifolium* plants at the maximum level on the severity scale, with the afflicted leaf area ranging from 56 to 88%. After three months of treatment with *Trichoderma asperelloides* TX19, a strain native to Mexico, demonstrated a reduction of 89% of the disease signs, highlighting the promising potential of mycoparasitic fungi in disease control.

Keywords: Biological control, mycoparasitic, reduction of disease, rust, *Trichoderma*

INTRODUCTION

Plant diseases cause an estimated global annual loss of 10% to 15% in the production of the world's major crops, with direct economic losses of up to hundreds of billions of dollars (Peng et al. 2021; Tubb and Seba 2021). Phytopathogenic fungi, a diverse and significant group, are of particular ecological and economic concern due to the substantial damage they cause globally (Jayawardena et al. 2021; Gomdola et al. 2022). Their increase as pathogenic agents and plant disease poses significant and growing risks to agricultural (Fones et al. 2020) and forestry production (Jean-François et al. 2023; Franić et al. 2023). This problem poses a risk to ecosystems (Jean-François et al. 2023), promotes uncertainty in food security (Singh et al. 2023), and disrupts the supply of plant inputs and their derivatives (Jez 2020). These examples highlight the global impact of phytopathogenic fungi and the urgent need for comprehensive management measures, such as the use of mycoparasitic fungi, to maintain balance with the environment (Ons et al. 2020).

The causative agents of rust, *Puccinia* spp., *Melampsora lini*, and *Phakopsora pachyrhizi*, are among the primary phytopathogenic fungi that affect crops worldwide (Dean et al. 2012). These types of phytopathogens are very diverse; they are classified as obligate biotrophs, which implies that they grow exclusively on their vascular plant hosts, including ferns, gymnosperms, and most members of the angiosperm families (Hovmøller et al. 2023). The spread of these fungi has intensified due to the transport of susceptible host plants and virulent rusts by unintentionally introducing diseased plants into hitherto disease-free host plant ranges, triggering major epiphytes. An example of the effects of an unintentional introduction of diseased organisms into new environments is the continuous spread of coffee rust (*Hemileia vastatrix*) in *Coffea* spp. plantations in all production areas (Rodrigues et al. 2022). This disease is one of the most destructive in the agricultural world, capable of causing losses of between 1,000 and 2,000 million dollars per year and one of the main limiting factors in coffee production worldwide (Tadesse et al. 2021). The harmful effects of rust are also apparent at the forest level, as seen in the United States, where 32.6 million hectares of whitebark pine are at risk due to infection by *Cronartium*

ribicola, the causative agent of white pine ampoule rust (Tomback et al. 2022).

Despite the development of alternative strategies, the main method of controlling these diseases at the agricultural and forestry levels remains the widespread use of chemical fungicides. However, the extensive use of chemical fungicides without biological bases has resulted in unintended consequences, including the emergence of chemo-resistant pathogens (Raymaekers et al. 2020), adverse effects on human health and environmental pollution, among other concerns (Ons et al. 2020; Raymaekers et al. 2020).

These trends have promoted the further development of environmentally friendly strategies, including biological control (Palmieri et al. 2022), which involves the reduction of pathogen and pest insect populations by natural enemies such as parasitoids, predators, pathogens, antagonists, or hyperparasites (Norris et al. 2020). Some of the first fungi associated with the biological control of rust are the species of hyphomycetes of the genus *Tuberculina*, considered hyperparasites of the spermogonial, aecial, and uredinial stages of rust (Wicker 1980). In 1890, Rostrup discovered the mycoparasitic properties of *Tuberculina maxima* after observing the fungi colonizing a sample of *Cronartium* fungus, the causal agent of pine rust in *Pinus monticola* (Wicker 1980). Since then, other species associated with the biological control of rust have been reported, including representatives of the genera *Verticillum* spp. and *Cladosporium* spp. (Sharma and Sankaran 2020). However, many other microorganisms could have potential uses as biocontrol agents for this type of disease. This research aimed to look for fungi with potential applications as biological control agents for rust.

MATERIALS AND METHODS

Collection of potential agents with mycoparasite activity

The search for diseased plants was carried out in the period from 2009 to 2023 in different locations in Mexico, such as the municipalities of Gustavo A. Madero, Miguel Hidalgo, Azcapotzalco, Xochimilco, Milpa Alta, Coyoacan in Mexico City, blueberry and apple production areas in Zacatlan de las Manzanas, State of Puebla; The Cedral Dam and Mineral del Chico in the state of Hidalgo; in addition to the forested area of the state of Colima. Plants found with rust symptoms were collected in paper bags and transported to the laboratory for analysis and searched for mycoparasite fungi.

First isolation of potential agents with mycoparasite activity

The samples were inspected visually, and then transverse cuts were made with a double-edged knife under stereoscopic microscope of plant tissue with signs of rust. The cuts were mounted on slides and observed under a compound microscope to identify the fungal structures of the probable mycoparasites. Fungi identified as mycoparasites of rusts were isolated on potato dextrose agar medium and incubated at 28°C for one week.

Identification of fungi

The identification of mycoparasitic fungi was complemented by molecular biology with the sequencing of the ITS region and the partial sequence of the elongation factor 1α (EF-1α) gene (O'Donnell et al. 2015; Raja et al. 2017). Once the fungal strains were corroborated, they were grown in dextrose potato broth at 100 rpm in a Shaker Innova 44 incubator for seven days until mycelial growth was observed (Triana-Vallejos et al. 2022). The biomass was collected by centrifugation at 8000 g at 4°C for 10 min (Covacevich and Fabiana 2014). For DNA isolation, CTAB protocol II was used (Hollingsworth 2006). DNA quality was verified in 1.2% agarose gel in TAE 1X buffer (Tris-base, glacial acetic acid, EDTA 0.5 M pH 8.0). High-molecular-weight DNA was visualized on a Universal Hood II model transilluminator (Bio-Rad), and its concentration was quantified in a NanoDrop spectrophotometer (Lambda Bio 12 Perkin Elmer) (Kirtane et al. 2021). For the identification of mycoparasitic fungi, the ITS region and a part of the gene encoding the elongation factor EF-1α were amplified by PCR. For the ITS region, the primers ITS 5HP (5′-GGA AGG AGA AGT CGT AAC AAG G-3′) and NL4 5′-GGT CCG TGT TTC AAG ACG G-3′ were used, and for the elongation factor 1α (EF-1α) with the primers, EF1 (5′-ATG GGT AAG GAG GAC AAG AC-3′) and EF2 (5′-GGAAGTACCAGTGATCATGTT-3) were used (O'Donnell et al. 2015), which amplify a region of 1200 bp and 650 bp respectively.

The PCR products were analyzed a 1.2% agarose gel. For band location purposes, 2 µL of the 100 bp molecular weight marker DNA (Promega) and 3 µL of the 1 kb marker O'GeneRulerTM (Fermentas) were used (Kirtane et al. 2021).

Evaluation of mycoparasitic activity

Evaluation of mycoparasitic activity in vitro

For the evaluation of this activity, water agar media enriched with teliospores of *Uromyces viciae-fabae* and *Ustilago maydis* + *Sporisorium reilianum* f. sp. *zeae* were prepared. For the preparation of the media, 1 g of teliospores, equivalent to 458,200,000 teliospores of each fungus mentioned above, were added to the water agar medium, sterilized, and emptied into Petri dishes. Then, 5 mL of a suspension of 1×10^{9} mL^{-1} of spores of the microorganism with mycoparasitic action potential were added, then incubated at 28°C under dark conditions for five days until the presence of mycelium was observed as part of the colonization process of the teliospores by the mycoparasite.

Evaluation of selective mycoparasite capacity

The capacity of selective mycoparasitic activity on *U. maydis*, the causal agent of charcoal in corn, was evaluated. This experiment used two treatments on maize fruits (corn) and *U. maydis* galls. In the first treatment, a small incision was made in the corn kernels, and then mycelial discs of 0.5 cm in diameter were deposited on them. The mycelial discs were placed directly on the whole corn kernels. The two procedures were repeated on galls of *U. maydis*

Evaluation of mycoparasite activity on rust-infected plants

In the first model, *Pelargonium inquinans* plants with *Uromyces geranii* were used to evaluate the mycoparasitic activity of the pre-selected fungi. The treatment plants were sprayed with a conidia suspension at a concentration of 1×10^7 CFU mL^{-1}, and the control plants were sprayed with sterile distilled water twice a week. The control was sprayed with sterile distilled water. Applications continued until mycoparasitic fungal growth was observed on the leaves.

In a second trial, *Chrysanthemum coronarium* plants infected with *Puccinia horiana* were used in a completely randomized design with five replicates for each treatment and control group (*C. coronarium* plants with rust), this was the second model employed. The plants of the treatment groups were sprayed twice a week with a suspension of VC18, TX19, and AC23 at a concentration of 1×10^7 CFU mL^{-1} each, and the groups were maintained under greenhouse conditions. The intensity of the disease was the parameter that was evaluated before and after the treatments; it was calculated as reported by Martanto et al. (2020). To determine the rate of affectation of the plants used in the in vivo mycoparasite experiments, the diagrammatic scale was used to assess the severity of bean rust caused by *Uromyces viciae-fabae* (Fragoso-Benhumea et al. 2022) (Figure 9).

Data analysis

The reduction in disease severity of the plants of each treatment was compared with the control. These results were analyzed by a one-way ANOVA test with p=0.05 and two Fisher's and Dunnett's mean tests.

RESULTS AND DISCUSSION

Collection and identification of fungi

Several distinctive mycoparasite rusts were found through the sampling process; however, not all mycoparasites could be isolated on Potato Dextrose Agar (PDA). One such case involved a mycoparasitic fungus interfering with the development of *Gymnosporangium clavipes* on *Crataegus mexicana* plants. Examination of the lesions revealed that the fungus was able to inhibit the emergence of *Gymnosporangium* aecial tubes, thereby preventing the dissemination of the fungus' eciospores by wind action and breaking the disease cycle, as shown in Figure 1. While this fungus may have great potential as a mycoparasite agent, it was, unfortunately, not possible to isolate it.

During the seven years of sampling, four fungi with potential parasitic mycotic activity were isolated in PDA: three from plants infected with rust and one from the soil found in a chinampa (Mesoamerican agricultural practice that relies on small, rectangular areas of fertile arable land to grow crops on the shallow lake beds in the Valley of Mexico). The collection data are shown in Table 1, and the fungi's identification is shown in Table 2.

The first mycoparasitic rust was found on *Tecoma stans* (Figure 2). The second was found by the Alcadía of Milpa Alta on fava bean plants (*Vicia faba*) (Figure 3). The mycoparasite fungus, AC23, was found on pustules of *Uromyces* spp., which itself was found infecting mistletoe plants of the genus *Struthanthus* spp., which in turn were parasitizing ash trees (Figure 4).

Table 1. Collection data of rust-diseased plants with the presence of a mycoparasite fungus

Year of collection	Locality	Coordinates of collection	Host or origin of the sample	Rust causal agent	ID of the fungus with potential mycoparasite activity
2018	Coyoacan, Town hall Ciudad de México, Mexico	19.32504, -99.17478	*Tecoma stans* (L.) Juss. ex Kunth	*Prospodium aculeatum*	VC18
2019	Xochimilco, Town hall Ciudad de México, Mexico	19.263791, -99.047496	Suelo	NA	TX19
2022	San Salvador Cuauhtenco, Milpa Alta, Ciudad de México, Mexico	9.19353, -99.10034	*Vicia faba* L.	*Uromyces viciae-fabae*	EM22
2023	Villa de Álvarez, Colima, Mexico	19.246678, -103.78177	*Struthanthus* spp.	*Uromyces* spp.	AC23

Note: NA: Not Applicable

Table 2. Molecular identification data of fungi with potential mycoparasitic activity

ID	Identification by BLASTn	ID percentage of identity	GenBank access number
EM22	*Eudarluca caricis*	99.03	SAMN42769536
AC23	*Acremonium sordidulum*	99.82	SAMN42769537
TX19	*Trichoderma asperelloides*	99.12	SAMN42769535
VC18	Unidentified		

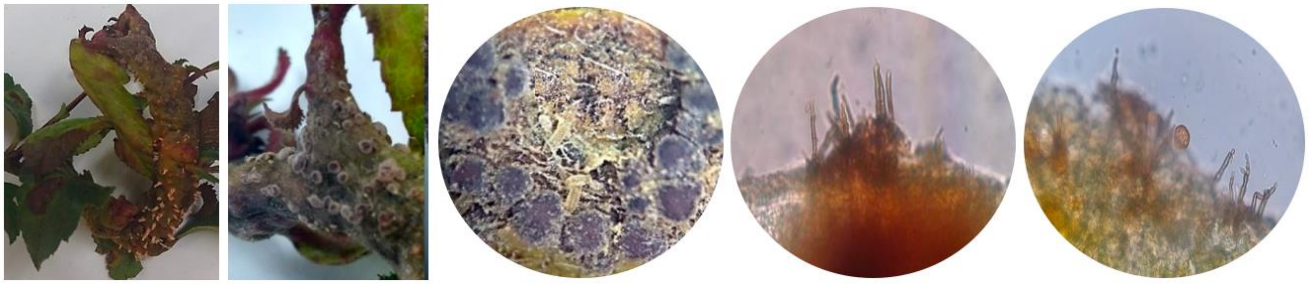


Figure 1. A-B. Outbreak of *Crateagus mexicana* with symptoms of hypertrophy and hyperplasia caused by colonization of *Gymnosporangium clavipes*. C. Approach of the velvety gray to brown tumors of 2 mm to 4 mm that developed on the aecial tubes of *G. clavipes*, preventing their development and release of their aeciospores. D-E. Cross-section of the tumors where melanic hyphae of the mycoparasitic are observed on some reminiscences of the at aecial tubes of *G. clavipes* and scarce presence of its aeciospores

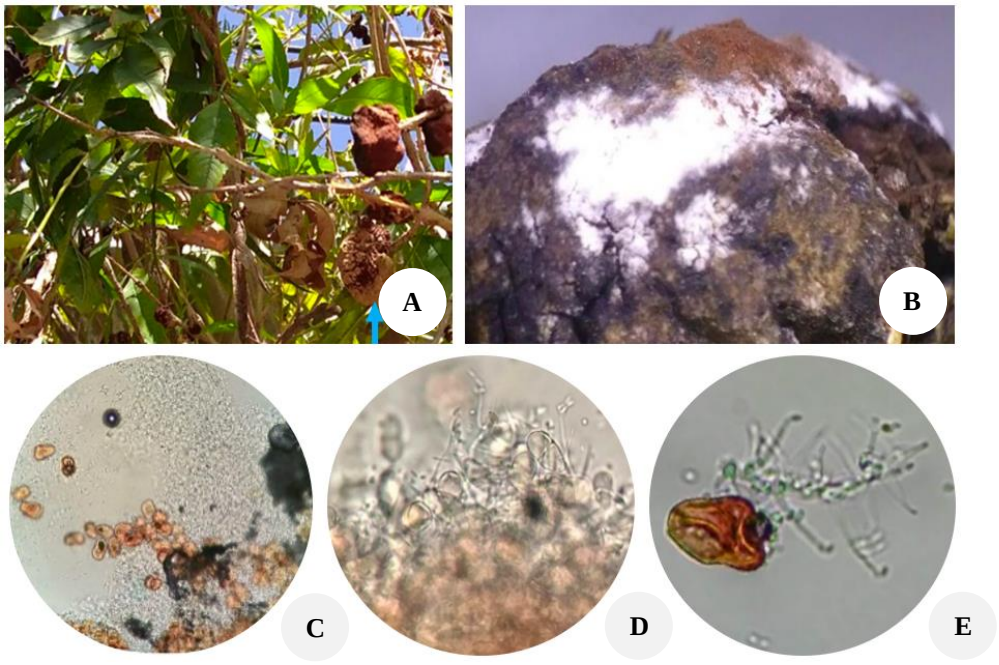


Figure 2. A. *Tecoma stans* plant infected with *Puccinia aculeatum*. B. Galls of *P. aculeatum* of powdery appearance, brown color. These fungi, present in the branches of *T. stans*, can reach diameters of 2 mm up to 15 cm. Some galls can be observed spots of velvety beige appearance that randomly covered part of the affected galls, corresponding to the growth of the parasitic fungus VC18. C-E. Observation at 40×, 400×, and 800× of the galls of *P. aculeatum* mycoparasitic (respectively) by a hyaline microsiphonate fungus and with the production of unicellular hyaline conidia

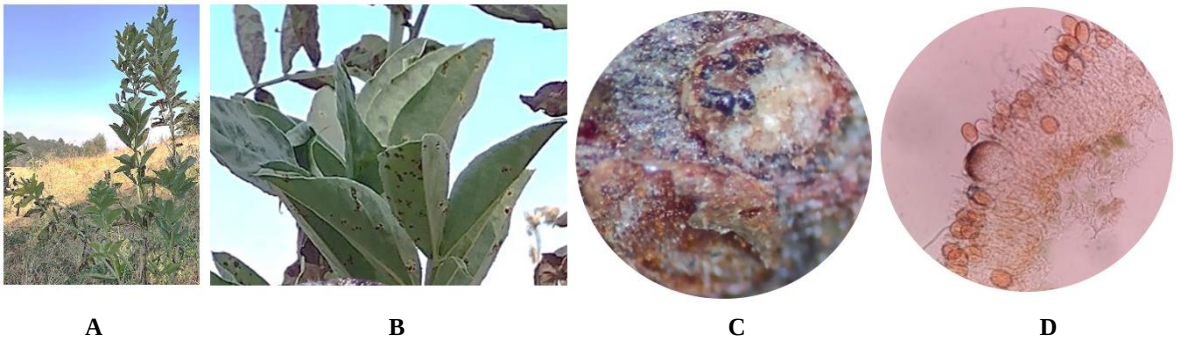


Figure 3. A-B. Fava bean plants (*Vicia faba)* were found to have rust, as brown pustules were observed on the underside of the leaf with a chlorotic halo with a diameter of 1-3 mm. C. Observation of the underside of the leaf under a stereoscopic microscope, showing a large production of uredospores of *Uromyces fabae* that broke the epidermis of the leaf and the presence of black pycnidia of the mycoparasite *Eudarluca caricis*. D. Cross-section of lesions and pycnidia of *E. caricis* growing on the uredospores

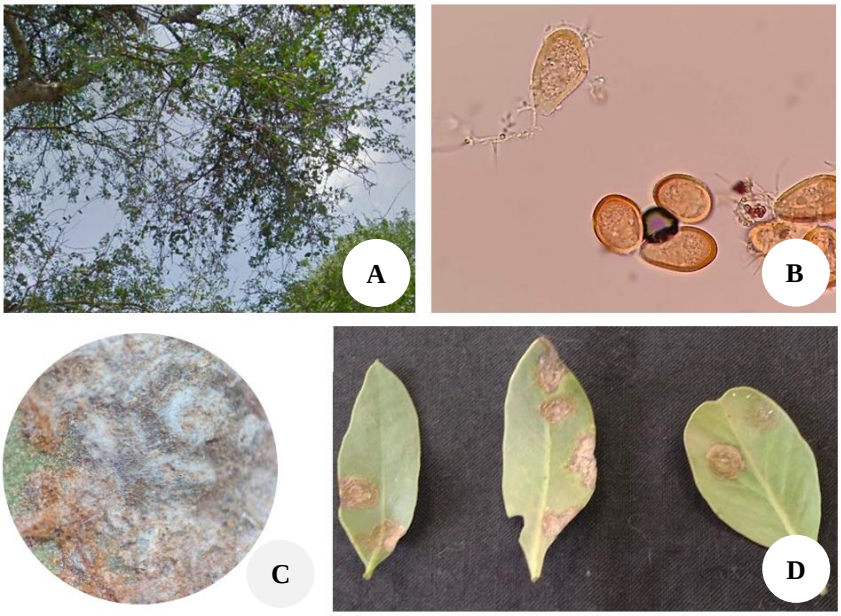


Figure 4. A. *Fraxinus uhdei* parasitized by *Struthanthus* spp. B. Uredospores of *Uromyces* spp. parasitized by *Acremonium sordidulum*; the mycelium of *A. sordidulum* surrounds uredospores with the production of unicellular conidiophores and hyaline conidia. C. Stereoscopic microscopic observation of pustular lesions of *Uromyces* spp. demonstrated by the presence of a mycelial growth with a whitish wooly appearance developing all over the pustule. D. Leaves of *Struthanthus* spp. with pustular lesions caused by *Uromyces* spp.; the two upper leaves also show the growth of the mycelial parasite *A. sordidulum*

Evaluation of mycoparasitic activity in vitro

The mycoparasitic above fungi were grown in a water agar medium supplemented with teliospores (as described above) without any other source of nutrients, such that only the fungi that could synthesize enzymes for the degradation and use of the spore components could develop. The specimens of VC18, EM22, and AC23 managed to grow to different degrees, as reported in Table 3 and Figure 5. Both VC18 and AC23 demonstrated their rapid capacity for biochemical adaptation by obtaining their energy and nutrients directly from the spores of *U. maydis* + *S. reilianum* and *U. vicie-fabae* without needing the addition of another carbon source (that would allow a gradual adaptation). The EM-22 fungus identified as *E. caricis* (despite being reported as a mycoparasite associated with 369 species of rusts (Gómez-Zapata et al. 2024), was able to penetrate directly into teliospores, ecios, and uredospores. This fungus has been used to produce the antibiotics Darlucine A and B, which are associated with the inhibition of rust development (Ashmitha Sri et al. 2020; Durgadevi et al. 2024). This fungus was eliminated from the rest of the trials due to its limited growth capacity in PDA medium, between 1.2-1.6 cm per week, and in the in vitro mycoparasite activity assay described in Figure 5. While AC23 managed to grow on 35-50% of the surface, VC18 covered more than 70% of the area of the culture medium with teliospores. The mycoparasitic activity and biochemical adaptability of the VC18 sample were demonstrated by its rapid growth after transitioning from a PDA medium with an easily assimilated carbon source to water agar media enriched with teliospores, a nutrient-restricted medium.

Evaluation of selective mycoparasite capacity

AC23 and VC18 were not able to grow on corn fruits, even though the corn fruits were injured to facilitate access to the plant's nutrients. While both samples were unable to develop on intact huitlacoche galls, they were able to start their development when the fungal spores were released by damaging the galls, showing that these fungi are not pathogenic for corn (Figure 6).

After the above-described series of tests that filtered the selection of potential mycoparasite candidates, it was found that two fungi isolated from the sampled rusts showed promising mycoparasite activity. To further enhance the potential of our research, a third fungus, the TX19 fungus, was incorporated into the trial. Despite being isolated from a sample of root rot in lettuce plants (Figure 7), this mycoparasite was chosen for its significant capacity to parasitize fungi such as *Fusarium* spp., *Monilinia* spp., and *Colletotrichum* spp., along with its impressive growth rate of 90 mm per week (Luna-Escalona 2025, unpubl. data).

Table 3. The mycoparasitic activity level of the VC18, WM22, and AC23 fungi on teliospores of *Ustilago maydis* and *Usitlago fabae*

ID of the fungus with potential mycoparasite activity	Growth in medium with spores of *U. maydis* + *S. reilianum*	Growth in medium with spores of *U. fabae*
VC18	+++	+++
EM22 (*Eudarluca caricis*)	+	+
AC23 (*Acremonium sordidulum*)	++	++

Note: *Growth of the parasitic fungus expressed in + (sparse growth, less than 30% of the plaque surface), ++ (approximately grew on 35-50% of the plaque surface), +++ (grew on more than 70% of the plaque surface)

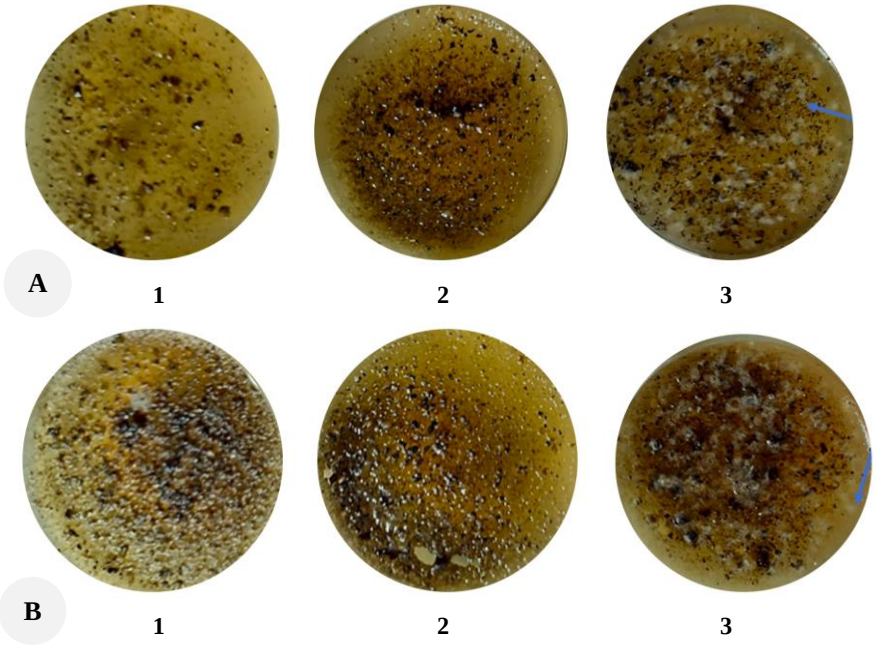


Figure 5. Results of the in vitro mycoparasitic test can be observed on: A. *Ustilago maydis* teliospores, B. *Ustilago fabae* teliospores. 1: EM22 fungi, 2: AC23 fungi, 3: VC18 fungi. The growth of these fungi appeared white and wooly on the surface of the plates

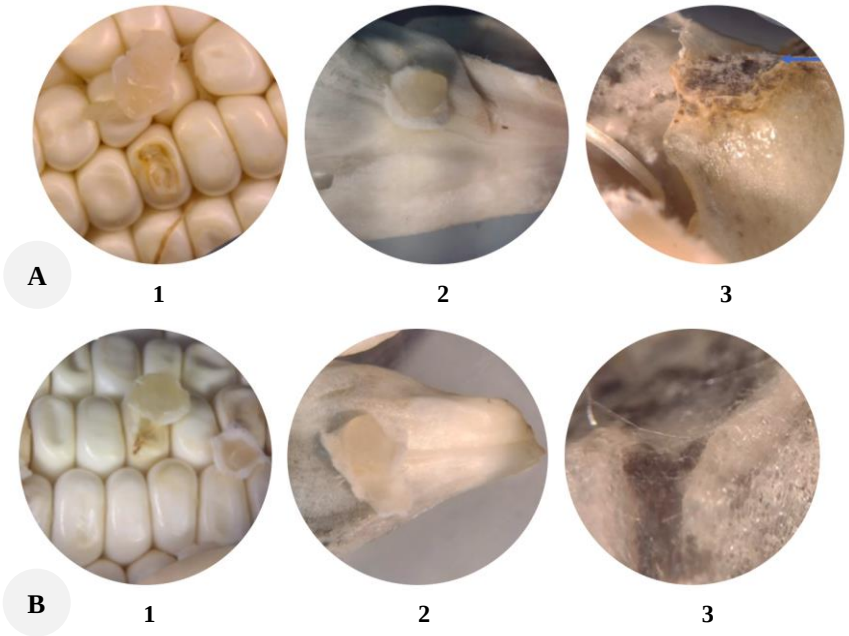


Figure 6. Specificity of mycoparasite activity on maize fruits, whole galls of huitlacoche, and in galls where *Ustilago maydis* teliospores were released. The premeditated lesion damaged corn fruits but was not damaged due to any colonization process of VC18 (A-1) and AC23 (B-1) fungi after inoculation. Intact undamaged galls with mycelial disc of VC18 (A-2) and AC23 (B-2) fungus after seven days of incubation on whole galls of huitlacoche. Growth of the VC18 (A-3) and AC23 (B-3) fungus on the galls of huitlacoche after injuring them and releasing the teliospores of *U. maydis*

A **B**

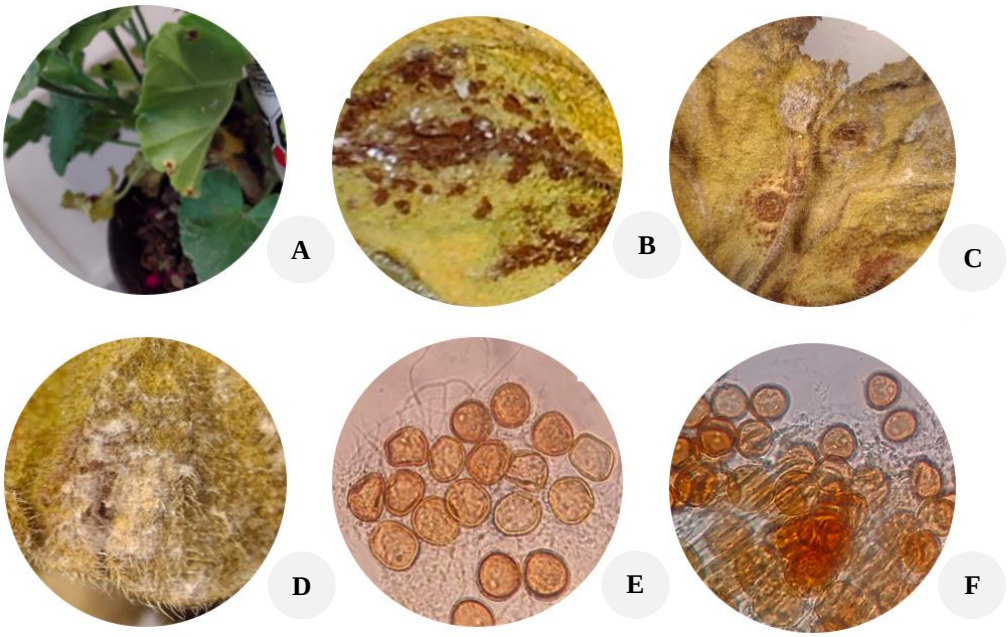
Figure 7. A. Purification of the TX19 fungus from the first isolation of the root rot sample. B. *Lactuca savita* sangria variety plant with the loss of turgor, a decrease in size, a change in coloration at the root level, a decrease in root hairs, and an observable area of necrosis

Evaluation of mycoparasite activity on rust-infected plants

In the mycoparasite test on rust-diseased *Pelargonium inquinans* plants (Figure 8), the plants began to selectively defoliate the rust-diseased leaves within one week after two applications of the TX19, AC23, and VC18. This rapid defoliation made it impossible to assess the mycoparasite process in the living host. However, the defoliated leaves of the plants from the TX19, AC23, and VC18 treatments were collected, and their inoculation was continued with the suspension of the fungi until their growth on the pustules of *U. geranii* was observed. As for the plants in the treatments, all of them were cured of rust after defoliating the diseased leaves after the second application of the treatments. This promising result underscores the need for further research in this area.

In the second model using white rust in *Chrysanthemum coronarium* plants, the mycoparasite activity was evaluated by a completely randomized experimental design. The plants used in the study presented damage classified as level 5 on the severity (Figure 10), corresponding to more than 56.1% of the leaf surface. These plants showed the presence of disease-specific pustules and an infection incidence of between 24% and 42% of the total population (Table 4).

The differences in disease intensity and severity of each treatment were meticulously considered at baseline and three months after the treatments were applied (Figure 10). This thorough approach was followed by an ANOVA test, which yielded a calculated F value of 38.82, surpassing the F tables value of 3.24 at a p-value of 0.05. These results allowed for the subsequent implementation of 2 mean analyses: Tukey and Dunnett. These tests, conducted with the same level of thoroughness, showed that the three treatments were successful in reducing the incidence and severity of white rust in *C. coronarium* plants to varying degrees compared to the control, as shown in Table 5. Notably, *T. asperelloides* emerged as the most effective treatment, reducing the disease intensity index by 89% and the severity scale from 5 to 1 in *C. coronarium* plants after 90 days of treatment.



A **B** **C**

D **E** **F**

Figure 8. A. Plant of *Pelargonium inquinans* diseased with rust. Defoliated leaves five days after inoculation with mycoparasite rust pustules with the fungi TX19 (B), AC23 (C) and VC18 (D) after 20 and 30 days of inoculation. Micoparasitation of uredospores of *P. inquinans* with the fungi VC18 (E) and AC23 (F) after 20 and 30 days of inoculation

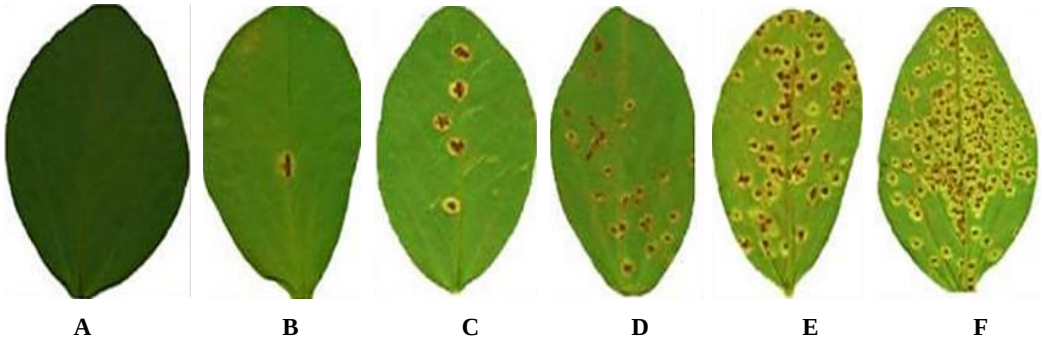


Figure 9. Diagrammatic scale used to assess the severity of bean rust caused by *Uromyces viciae-fabae*. A. Class 0 (0%). B. Class 1 (0.1 a 6.0%). C. Class 2 (6.1 a 12.0%). D. Class 3 (12.1 a 24%). E. Class 4 (24.1 a 56.0%). F. Class 5 (>56.1%). Taken from Fragoso-Benhumea et al. (2022)

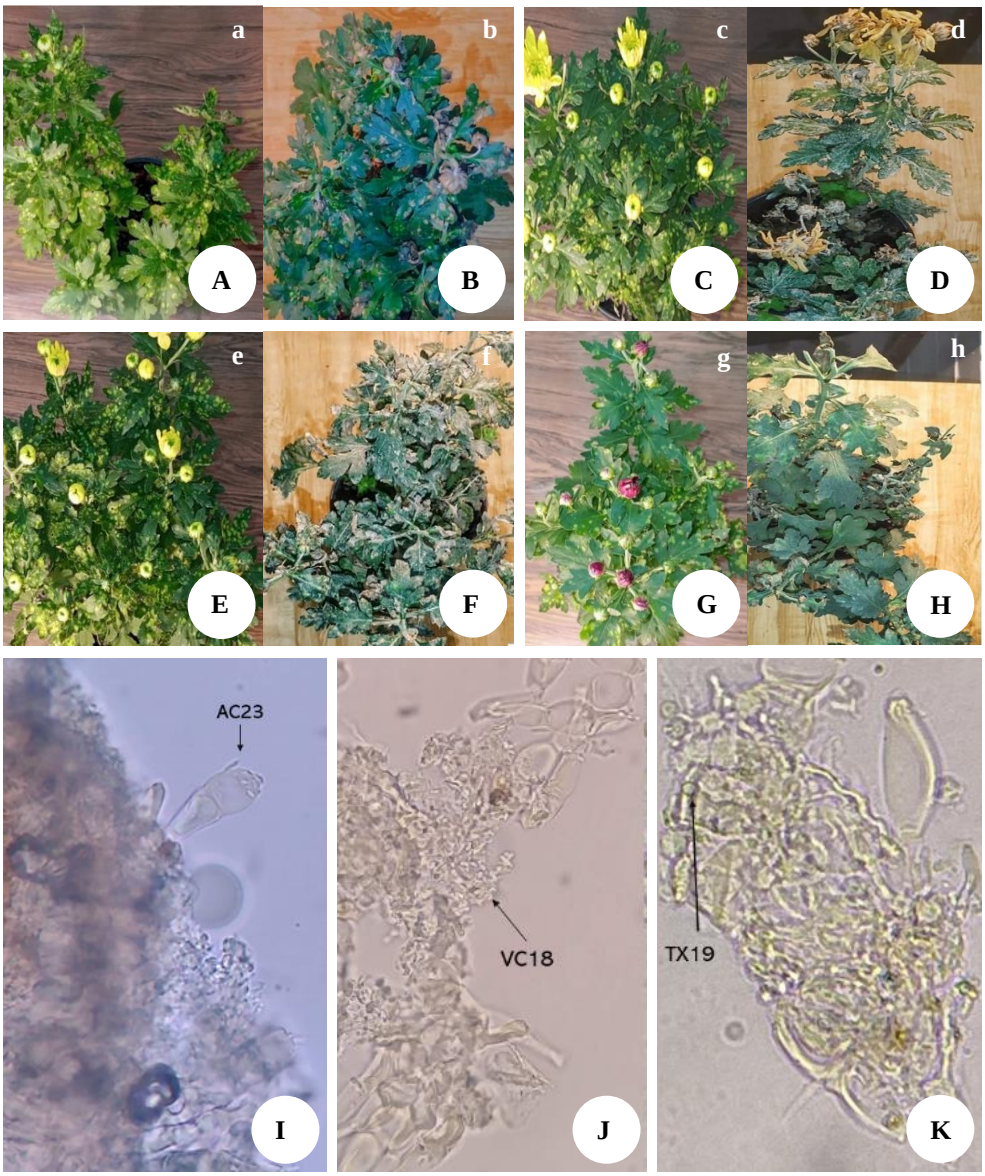


Figure 10. A-B. Disease control, *Chrysanthemum coronarium* plants infected with *Puccinia horiana*, C-H. Evaluation of the mycoparasitic activity of fungus TX19, AC23, and VC18 on *P. horiana* for three months, I-K. Microscopic observation of *P. horiana* lesions mycoparasitic by fungus TX19, AC23, and VC18 after 90 days of treatment. a. Severity scale Class 5, b. Severity scale Class 5, c. Severity scale Class 4, d. Severity scale Class 3, e. Severity scale Class 5, f. Severity scale Class 3, g. Severity scale Class 5, h. Severity scale Class 1

Table 4. Evaluation of the recovery process of *Chrysanthemum morifolium* plants because of treatments

Treatment	Mean percentages of involvement during the experiment (%)				Difference in mean percentages of involvement between day 0 to 90 of treatment
	Day 0	30 days	60 days	90 days	
Test	56.6	52.3	43.5	36.6	20.0
VC18	26.1	17.6	18.1	16.3	9.8
TX19	61.5	27.4	11.1	6.8	54.8
AC23	51.7	43.9	33.1	37.3	14.4

Table 5. Percentage of mycoparasitic on *Puccinia horiana* pustules, changes in the intensity and severity of white rust in *Chrysanthemum morifolium* plants because of treatments

Treatment	Change in severity scale after treatment	Infection intensity index. Day 1	Infection intensity index. Day 90	Percentage of mycoparasitism on *P. horiana* pustules
Test	5 a 5	41.4	36.6	NA
VC18 Unidentified *[c]	4 a 3	24.2	18.7	65
TX19s*[a]	5 a 1	41.9	4.7	94
AC23*[b]	5 a 3	38.9	29.6	70

Note: *The means of the treatments show a significant difference according to the Fischer method. a,b,c The means of the treatments show a significant difference compared to the control according to the Dunnett method

It is important to note that older studies of *T. asperelloides* have focused on its use as a plant growth-promoting microorganism (AlHadidi 2024). However, more recent efforts have started to study its use in the control of airborne phytopathogens. Boukaew and collaborators (2024a) evaluated in vitro the antagonistic effect of *T. asperelloides* on *Colletotrichum gloeosporioides* for postharvest control of anthracnose in *Capsicum* spp. plants. In addition, its antagonistic effects against fungi such as *Fusarium oxysporum* f. sp. *lentis* (Pooja 2023) and *Aspergillus* spp. have been explored alongside its aflatoxin-degrading capacity (Boukaew et al. 2024b). The results obtained in the research evaluating the in vivo mycoparasite activity of *T. asperelloides* on *P. horiana* pustules in *C. morifolium* plants at the greenhouse level help to demonstrate the potential of the TX19 strain as an aerial biological control agent. However, more tests are still needed to corroborate its efficiency in rust control and adaptability to environmental conditions at the field level.

It was observed that all *C. morifolium* plants used in the experiment after three months showed a difference in vigor, even though the intensity and severity of the disease were reduced in the treatments, as detailed above. The plants that presented the most significant deterioration were those that received the VC18 and AC23 treatments with a level of disease severity equal to 3 (Table 5). The plants that presented the second most deterioration were the control plants, with a level 5 of the severity of the disease caused by *P. horiana*, as observed in Table 5.

This deterioration of the parasitized control plants with *P. horiana* is due to its nature as an obligate biotrophic pathogen. *P. horiana* derives energy from the parasitized living plant cells, allowing it to survive and reproduce. This process causes a certain deterioration of the plant cell, but not its death, which is reflected in the detriment of the plant itself, as observed in Figure 10.B. However, when *P. horiana* is faced with external damage, such as the inefficient process of mycoparasitic activity carried out by the fungi used in the VC18 and AC23 treatments, the degree of deterioration of the plant changes. A possible justification for this phenomenon could be that the slow process of mycoparasitic activity allows *P. horiana* to trigger defense mechanisms against VC18 and AC23. This process would increase the energy requirement of *P. horiana*, thereby increasing the amount of nutrients extracted from the parasitized living cells of the plant, causing the premature death of these cells, which would present as a more significant deterioration of the plants that received these treatments compared to the control plants.

With these results we wish to provide evidence of the potential that microorganisms have for their use in the biological control of phytopathogenic diseases; Since the search and development of new proposals for biological control agents face challenges that must be overcome, such as the fear of the introduction of species foreign to pre-established ecosystems, to resolve this problem we must consider the fact that the microorganisms that are introduced In pre-established ecosystems, they generally tend to be displaced since the system tends to recover its homeostasis. In practice, this fact has been corroborated since to have efficient control of the disease, continuous applications of the biological control agent are necessary to maintain levels of populations that can confront the phytopathogen (Collinge et al. 2022). Or prioritize using endemic microorganisms as biological control agents depending on the geographic region where disease control is intended to be established (Vargas et al. 2023).

Another is the difference in costs compared to agrochemicals, if we compare them with traditional agrochemicals there is a big difference in terms of price (He et al. 2021) but in effectiveness these chemical products have lost efficiency due to the emergence of chemo-resistant strains which turns out to be a drawback in the management of diseases, for which the agrochemical industry

has had to develop new formulations to deal with diseases, increasing their prices (Shattuck 2021), equating them with the prices of biological control agents. However, we must remember the adverse effects of agrochemicals, such as environmental pollution, on human health, and that their inappropriate use during the control of phytopathogenic diseases favors the development of chemo-resistant strains (He et al. 2021).

Therefore, the search for new biocontrol agents is a viable alternative, although it represents new challenges. To achieve this objective, it is important to consider certain characteristics that these microorganisms must have to consider them as possible candidates for biological control agents. That is, they can displace or eliminate the phytopathogen using different strategies such as rapid growth, the ability to use it as a source of energy (mycoparasitization processes), production of secondary metabolites that could inhibit its growth, coupled with its ability to adapt to extreme environments and resilience to being displaced by pre-established communities in the ecosystems of the phyllosphere and/or rhizosphere (Pirttilä et al. 2021).

The evaluation of fungi with mycoparasitic activity involves several stages to select those with the best mycoparasitic potential. The first stage consists of laboratory tests, followed by greenhouse trials, and finally, field evaluations. The *Trichoderma* TX19 strain has successfully passed the first two testing stages; however, field tests must be conducted to confirm and assess its mycoparasitic activity. Regarding the variability in its mycoparasitic activity depending on the host plant, it is also advisable to carry out the corresponding tests since plants have different architectures that can influence the fungus's adherence and permanence, making it difficult for it to remain in the plant and preventing adequate protection of it.

In conclusion, the plants treated with the strain of *T. asperelloides* TX19 showed the highest vigor. This result suggests a dual beneficial effect on *C. coronarium* plants. The first is its efficient mycoparasitic action, and the second is its role as a growth promoter. These demonstrated benefits, combined with its tolerance to low percentages of humidity, UV radiation, abundant production of conidia, was isolated from alkaline soils and rapid growth, make *T. asperelloides* a fascinating candidate for use as a biocontrol agent in the management of phytopathogenic fungi, however, field tests must be conducted to confirm and assess its mycoparasitic activity.

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