

## Effect of Acibenzolar-S-methyl on the severity of *Pythium* sp. in tomato

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### Abstract

In tomatoes, the genus *Pythium* can cause economic losses of up to 50%. Acibenzolar-S-methyl is an inducer of plant defense, which has shown effectiveness against diseases caused by fungi, viruses and bacteria. The objectives of this study were to determine the *in vitro* effect of Acibenzolar-S-methyl on mycelial growth and on the severity of tomato seedling damping-off caused by *Pythium* sp. The *in vitro* effect was determined in V8 culture medium, whereas the severity of damping-off was determined in seedlings with two true leaves. Acibenzolar-S-methyl was applied via irrigation at concentrations of 10, 15, 20, 30 and 40 mg L<sup>-1</sup>, three days before inoculating the seedlings with the oomycete. *Pythium* severity was evaluated eight days after inoculating the pathogen. Acibenzolar-S-methyl did not affect the mycelial growth of the oomycete under *in vitro* conditions. In seedlings, the 15 mg L<sup>-1</sup> dose of the inducing product significantly reduced the damage caused by the oomycete, compared to the control treatment and the fungicide treatment. These results suggest that Acibenzolar-S-methyl could represent another option for managing tomato seedling damping-off caused by *Pythium* sp.

### Keywords:

*Solanum lycopersicum*, oomycete, root diseases.



## Introduction

In Mexico, the tomato (*Solanum lycopersicum*) is of great economic importance due to the value of its production (36 669 million pesos). In the 2023 agricultural year, 49 000 ha were cultivated, with a production of 3 636 927 t; the states of Sinaloa, San Luis Potosí, and Michoacán are the largest producers (SIAP, 2024). Nevertheless, tomato crops are affected by diseases caused by various types of pathogens, such as bacteria, nematodes, viruses, fungi and oomycetes, which cause significant losses to the crop.

Among these pathogens, *Pythium* stands out for its pathogenicity by causing seed rot in the field, seedling damping-off in pre-emergence and post-emergence, as well as root rot in adult plants. The genus *Pythium* is cosmopolitan and can cause economic losses of up to 50% in tomato (Quiroga-Madrigal *et al.*, 2007; Grijalba *et al.*, 2015), 70% in beans (Nzungize *et al.*, 2012), 12 to 54% in lettuce crops (Stanghellini and Kronland, 1986) and 10 to 100% in the production of chili seedlings (Jiménez-Pérez *et al.*, 2022), which can occur in both protected and open agriculture.

The management of diseases caused by *Pythium* has traditionally been done with chemical fungicides; however, the use of these products can lead to soil and water pollution, as well as negative health effects on agricultural workers (Nzungize *et al.*, 2012). There are currently several methods that individually or in combination can help reduce oomycete diseases in a significant way. For example, fungicide application on peach (*Prunus persica*) seedlings significantly reduced (62.5%) the severity of *Pythium ultimum*-induced damping-off, compared to control plants (Mannai and Boughalleb-M'Hamdi, 2021). Likewise, in chili (*Capsicum annuum*), the application of *Trichoderma harzianum* reduced the incidence and severity of chili wilt caused by *Phytophthora capsici* by 36 and 42%, respectively (Timila and Manandhar, 2020).

In this sense, the use of elicitor molecules that activate plant defense mechanisms has generated growing interest in recent years, as it promises to be an excellent alternative for managing phytopathogens (Cuellar-Espejel *et al.*, 2024). Acibenzolar-S-methyl (ASM) is a plant resistance inducer used to control a wide variety of diseases caused by fungi, viruses, and bacteria. ASM activates the acquired systemic resistance of plants, increasing the expression of genes related to defense and the accumulation of enzymes, such as  $\beta$ -1,3-glucanases, peroxidases, chitinases, and phenolic compounds (Nascimento *et al.*, 2016).

Nonetheless, in tomatoes, there is limited information on the use of ASM for managing seedling damping-off caused by *Pythium* sp. Traditionally, the use of fungicides, such as metalaxyl, fosetyl-Al, and propamocarb, has been the most common option for the management of oomycete diseases (Benavent-Celma *et al.*, 2022); however, the problems associated with the use of agrochemicals, such as environmental pollution, risks to human health, and the appearance of resistant strains, generate the need to look for more environmentally friendly alternatives.

Therefore, the purpose of this study was to evaluate the *in vitro* effect of ASM on the mycelial growth of *Pythium* sp., as well as its effect on the management of tomato seedling damping-off under controlled conditions.

## Materials and methods

### Location of the experimental site

The research work was conducted at the Phytopathology Laboratory of the Department of Agriculture and Livestock of the University of Sonora, located in the city of Hermosillo, Sonora, Mexico.

### *Pythium* sp. isolation

The oomycete was isolated from tomato seedlings with symptoms of wilting and basal rot of the stem. The collected roots and stems were taken to the laboratory and thoroughly rinsed under

running water, and segments approximately 1 cm in length with healthy and diseased tissue were cut. The segments were superficially disinfected with 1% sodium hypochlorite for one minute, rinsed three times with sterile distilled water, and placed in sterile gauze for drying. Tissue fragments were seeded in V8 medium (200 ml V8 juice, 3 g CaCO<sub>3</sub>, 16 g bacteriological agar) and incubated at 28 °C for three days.

An isolate of *Pythium* sp., pathogenic and purified from a hyphal tip, was randomly selected for the different assays of this study. The morphological identification, at the genus level, was carried out according to the characteristics described by Díaz-Celaya *et al.* (2011); to this end, mounts of tissue from 8-day-old cultures were prepared using a Carl Zeiss microscope.

### ***In vitro* effect of ASM on the mycelial growth of *Pythium* sp.**

The effect of Acibenzolar-S-methyl (Syngenta) on the mycelial growth of *Pythium* sp. was determined as described by Quiróz-López *et al.* (2021). For this assay, ASM was added to V8 medium before being emptied into Petri dishes. The evaluated doses of ASM in the V8 medium were 10, 15, 20, 30 and 40 mg L<sup>-1</sup>. Petri dishes with V8 medium without the inducing product were used as a control treatment. A 9 mm diameter V8 medium disc with *Pythium* sp. was placed at the center of each Petri dish, and the dishes were incubated at 28 °C. The diameter of colony growth was determined 48 h after incubation (hai); each treatment (ASM dose) consisted of four replications, and the entire experiment was performed twice under the same experimental conditions. The inhibition of mycelial growth of *Pythium* sp. was evaluated with the following formula:

$$\text{myceliuminhibition} = \frac{\text{controlcolonydiameter} - \text{treatmentcolonydiameter}}{\text{controlcolonydiameter} - 9\text{mm}} \times 100$$

### **Effect of ASM on the severity of seedling damping-off caused by *Pythium* sp.**

Saladette tomato seeds (Cazador F1, Böden Seed) were sown in pots (13 cm in diameter by 10 cm high) with soil autoclaved at 121 °C for 2 h. The pots with the tomato plants (one plant per pot) were maintained at a temperature of 29 ± 3 °C and a relative humidity of 70-80%, with frequent watering every third day as needed by the plants. The application of the defense inducer was carried out via irrigation with 40 ml per pot in tomato seedlings with two true leaves (17 days after sowing the seeds). The evaluated doses of ASM were 10, 15, 20, 30 and 40 mg L<sup>-1</sup>. Two control treatments were used: plants treated only with distilled water (without ASM and without inoculation) and inoculated plants (without ASM).

*Pythium* inoculation was performed three days after treating the tomato seedlings with ASM. For this, two wheat grains colonized with the oomycete were buried superficially at the base of the stem of each seedling (one on each side of the plant). There were 15 replications per treatment, arranged in a completely randomized design. The experiment to determine the effect of ASM on the severity of *Pythium* sp. in tomato seedlings was performed twice under the experimental conditions described above, and these were called experiment 1 and experiment 2.

The disease severity index was assessed at eight days after oomycete inoculation in plants, using the assessment scale proposed by Santini *et al.* (2006): 0= healthy root, healthy plant; 1= 10-25%, wilted plant; 2= 26-50%, wilted plant or chlorosis, with slight necrosis of the neck or stem; 3= 51-75%, wilted plant or chlorosis, with moderate necrosis of the neck or stem; 4= 76-100%, wilted plant or chlorosis, with extensive necrosis in the neck or stem and 5= dead plant. The severity of the disease was evaluated according to the following formula:

$$\text{Disease severity \%} = \frac{\sum n}{N \times 5} \times 100$$

Where:  $\sum n$ = the sum of the individual ranks; N= total number of plants measured; 5= highest value of the severity scale.

## Inoculum preparation

*Pythium* sp. was inoculated using the method of wheat grain colonized by the oomycete. For this method, healthy wheat grain, in good physical condition, was used, and 100 g was placed in a 250 ml flask, which was hydrated with 100 ml of distilled water for 24 h, and then sterilized (121 °C for 1 h) on two consecutive days. After sterilizing the grains, the pathogen (*Pythium* sp.) was inoculated. The inoculation of the grains was carried out with three pieces of V8 medium with mycelial growth of *Pythium* sp., which were then incubated at room temperature for eight days.

## Statistical analysis

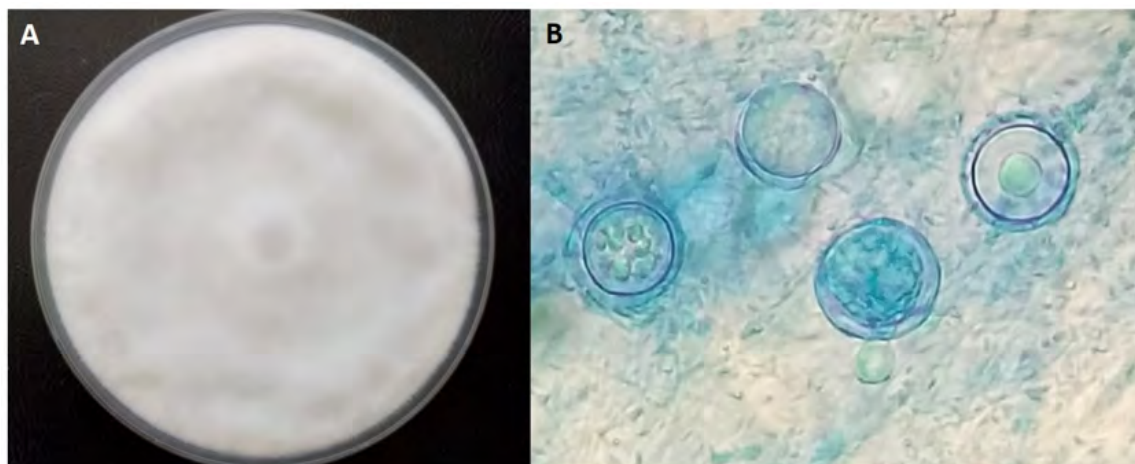
The *in vitro* effect of Acibenzolar-S-methyl on the mycelial growth of *Pythium* sp. and on the severity of tomato seedling damping-off caused by this oomycete was studied by an analysis of variance (Anova) and a Tukey comparison of means ( $p < 0.05$ ) using the R statistical package.

## Results and discussion

### Morphological identification of *Pythium* sp.

*Pythium* sp. presented coenocytic, abundant, and cottony white mycelium, with terminal spherical sporangia, spherical, terminal, intercalary oogonia, and monoclinal or diclinous antheridia present, and spherical and aplerotic oospores (Figure 1). These characteristics coincide with those reported by Díaz-Celaya *et al.* (2011) for species of the genus *Pythium* sp.

Figure 1. Morphological characteristics of *Pythium* sp.; A) mycelial growth in V8 medium and B) oospores.



### *In vitro* effect of ASM on the growth of *Pythium* sp.

In this essay, there was no effect of ASM doses on the mycelial growth of *Pythium* sp., as the V8 medium was completely covered with mycelium 48 h after in all treatments evaluated, suggesting that ASM has no toxic effect against *Pythium* sp (Table 1).



**Table 1. Effect of Acibenzolar-S-methyl (ASM) on the mycelial growth of *Pythium* sp., 48 h after inoculation in V8 medium.**

| Treatment                 | Diameter (mm)* | Inhibition (%) |
|---------------------------|----------------|----------------|
| Control (without ASM)     | 74.8 a         | -              |
| ASM 10 mg L <sup>-1</sup> | 75 a           | 0              |
| ASM 15 mg L <sup>-1</sup> | 74.8 a         | 0              |
| ASM 20 mg L <sup>-1</sup> | 74.5 a         | 0              |
| ASM 30 mg L <sup>-1</sup> | 74 a           | 0              |
| ASM 40 mg L <sup>-1</sup> | 75 a           | 0              |

\*= means within the same column with different letters indicate significant differences between treatments according to Tukey ( $p = 0.05$ ).

In this regard, Baysal *et al.* (2005) note that ASM is a potent activator of plant ASR and lacks antimicrobial properties on its own. According to our results, Benhamou and Bélanger (1998) indicate that ASM at a concentration of 3 mM did not affect the growth or development of *Pythium ultimum* mycelium compared to mycelial growth in control dishes. Similarly, Zhang *et al.* (2011) report that ASM at concentrations of 30 mg L<sup>-1</sup> had no effect on the mycelial growth of *Phytophthora capsici* or on the production of sporangia or the germination of zoospores. In contrast, other researchers have reported a direct toxic effect of ASM on the growth of fungi, such as *Verticillium dahliae*, *Rhizoctonia solani*, and *Curvularia eragrostidis* (Faessel *et al.*, 2008; Amini, 2015; Bussabong *et al.*, 2021).

### Severity of *Pythium* sp. in tomato seedlings treated with ASM

The first symptoms of damage caused by *Pythium* sp. in tomato seedlings were observed 48 h after inoculation, which manifested mainly as basal necrosis of the stem, which, as it progressed, covered the entire stem and caused the fall, wilting, and death of the seedlings. In this study, it was observed that ASM reduced the severity caused by *Pythium* sp. in treated tomato seedlings compared to seedlings inoculated only with the oomycete in the two experiments performed.

In the first experiment, it was observed that the application of ASM reduced the severity indexes of tomato seedling damping-off caused by *Pythium* sp. (Table 2).

**Table 2. Effect of the application of five doses of Acibenzolar-S-methyl (ASM, mg L<sup>-1</sup>) on the severity of seedling damping-off caused by *Pythium* sp., in tomatoes eight days after inoculation.**

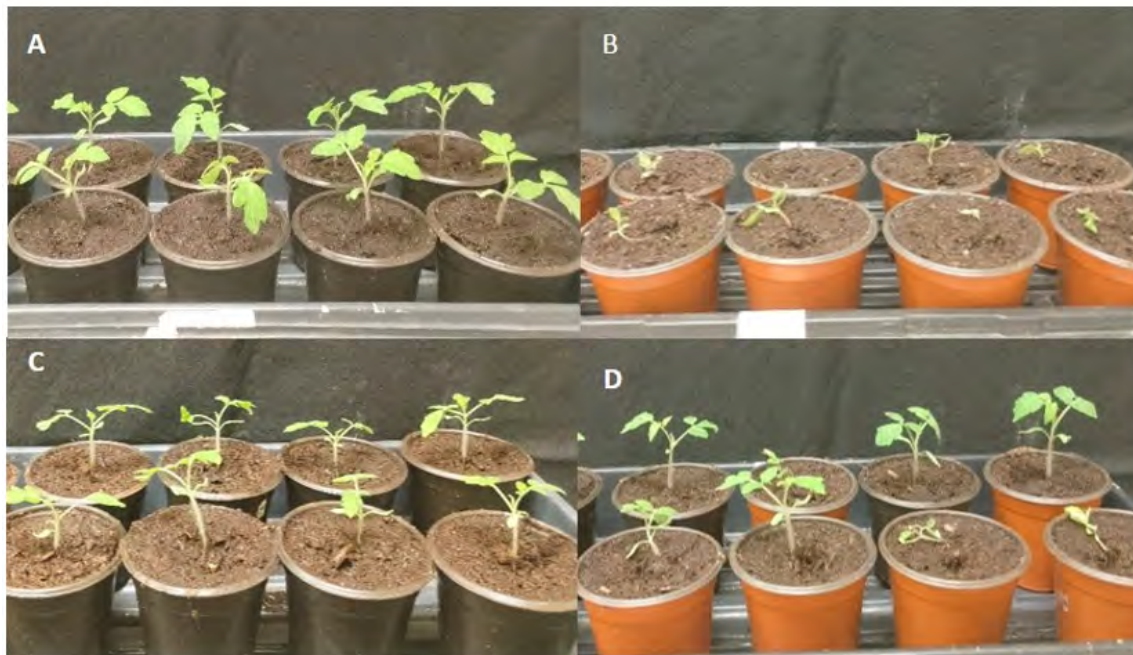
| Treatment (ASM mg L <sup>-1</sup> ) | Experiment 1   |              | Experiment 2   |              |
|-------------------------------------|----------------|--------------|----------------|--------------|
|                                     | Severity index | Severity (%) | Severity index | Severity (%) |
| 10                                  | 1.8 bc         | 37           | 1 cd           | 20           |
| 15                                  | 1 bc           | 22           | 1.8 bcd        | 36           |
| 20                                  | 1.5 bc         | 30           | 2.8 abc        | 56           |
| 30                                  | 1.7 bc         | 34           | 3 abc          | 60           |
| 40                                  | 2.5 ab         | 51           | 3.3 ab         | 66           |
| Fosetyl-Al                          | 2.6 ab         | 54           | 2 bc           | 40           |
| Control NO                          | 0 c            | -            | 0 d            | 0            |
| Control WO                          | 4.5 a          | 90           | 4.6 a          | 93           |

NO= control without oomycete; WO= control with oomycete, fosetyl-Al (3 g L<sup>-1</sup> of Aliette). Means within the same column with different letters indicate significant differences between treatments according to Tukey ( $p = 0.05$ ).

It was recorded that the dose of 15 mg L<sup>-1</sup> of ASM showed the lowest severity (22%) in tomato seedlings, compared to the control inoculated with *Pythium* sp., which showed a severity of 90%, and the treatment treated with the fungicide fosetyl-Al, with a severity of 54% (Figure 2).

**Figure 2. Effect of ASM on the severity of tomato seedling damping-off eight days after inoculation with *Pythium* sp.**

A) control (without oomycete, without ASM); B) control with *Pythium* sp. (without ASM); C) dose of 15 mg L<sup>-1</sup> of ASM and inoculated with *Pythium* sp. and D) dose of 3 g L<sup>-1</sup> Aliette (fosetyl-Al) and inoculated with *Pythium* sp.



Similarly, the other doses of ASM evaluated significantly reduced the severity of *Pythium* in tomato seedlings compared to the inoculated control (Table 2). The control seedlings without oomycete and without ASM showed no symptoms of damping-off, wilting, or basal necrosis of the stem during the development of the experiment.

In the second experiment, it was also observed that the application of ASM significantly reduced the severity indexes of seedling damping-off caused by *Pythium* sp., compared to seedlings inoculated with the oomycete alone (Table 2); the doses that showed the greatest efficacy in reducing seedling damping-off were 10 and 15 mg L<sup>-1</sup>, with severities of 20% and 36%, respectively, compared to the treatment inoculated and untreated with ASM, which showed 93% severity. In the treatments of the doses of 20, 30 and 40 mg L<sup>-1</sup> of ASM and the fungicide treatment, the severities of the disease were always lower than that assessed with the inoculated and untreated treatment.

The results of this study, both from experiments 1 and 2, indicate that ASM applied via irrigation reduces the damage or severity caused by *Pythium* sp. in tomato seedlings, even with better management efficacy than treatment with fungicide, which suggests that ASM could represent another alternative for the management of this disease in the seedling stage. Traditionally, the management of damping-off or rots by *Pythium* has been carried out through the use of fungicides, such as propamocarb, metalaxyl, captan, and fosetyl-Al, which provide efficient control of diseases caused by this pathogen in various crops. Nevertheless, the deficient and large-scale use of these agrochemicals can lead to environmental pollution (soil and water), health risks for farmers, as well as the development of resistant pathogens (Nzungize *et al.*, 2012; Adhikari *et al.*, 2024).

ASM is a salicylic acid analog compound that induces acquired systemic resistance in plants, which is effective against a wide range of pathogens, such as oomycetes, fungi, viruses and bacteria (Ito *et al.*, 2024). In this study, it was observed that the application of the ASM inducer in tomato seedlings significantly reduced the severity of the disease compared to plants treated only with the oomycete, and even showed better control efficacy than the fungicide treatment. This suggests that

ASM could represent a strategy for the integrated management of seedling damping-off caused by *Pythium*, thereby minimizing the negative effects of the use of chemical control.

Similar results to ours were reported by Radhakrishnan *et al.* (2011), who point out that the treatment of the rhizome of turmeric (*Curcuma longa* L.) with ASM suppresses the rot of the rhizome caused by *Pythium aphanidermatum* by increasing the activity of proteases, protease inhibitors, and peroxidases. Likewise, Benhamou and Bélanger (1998) point out that the foliar application of ASM in cucumber plants reduces the colonization of *Pythium ultimum* through a rapid and efficient massive accumulation of phenolic compounds in the sites where the pathogen tries to penetrate, which contrasts with the intense colonization and marked damage observed in plants not treated with ASM, suggesting that the application of the compound stimulates the defense response of plants.

The beneficial effect of applying ASM to reduce the incidence or severity of oomycetes has also been observed with *Phytophthora capsici*. For example, in squash (*Cucurbita pepo*), the application of ASM numerically reduced the final incidence of damage caused by *P. capsici* under field conditions, compared to the incidence observed in untreated plants, suggesting that ASM increases the resistance of squash plants to *P. capsici* (Ji *et al.*, 2011); on the other hand, in chili, the application of ASM significantly reduced the damage caused by *P. capsici* by 45% compared to untreated plants. In the latter study, increased resistance to *P. capsici* was associated with increased activity of phenylalanine ammonia-lyase enzymes and pathogenesis-related proteins (chitinases and  $\beta$ -1,3-glucanases), as well as an increase in phenolic compounds in plants treated with ASM.

On the other hand, it has been observed that the application of ASM also reduces the severity of diseases caused by *Pilgeriella anacardii*, the causative agent of black mold in crops of the cashew nut (*Anacardium occidentale*) (Viana *et al.*, 2012), of *Fusarium graminearum*, causative agent of fusarium spike in wheat crops (Shude *et al.*, 2022), of cucumber mosaic virus (CMV) and cucurbit yellowing and dwarfism virus (CYSDV) in melon crops (Kenney *et al.*, 2020) and of *Xanthomonas campestris* pv. *vitians*, causal agent of leaf spot in lettuce crops (Yigit, 2011).

## Conclusions

The results showed that the application of the ASM defense inducer in tomato seedlings, three days before inoculation with *Pythium* sp., significantly reduces the severity of damping-off caused by this pathogen. The defense inducer did not affect the mycelial growth of *Pythium* sp. at the doses tested under *in vitro* conditions. The dose of 15 mg L<sup>-1</sup> showed the lowest values of severity caused by oomycete, suggesting that ASM could represent another alternative for managing tomato seedling damping-off, thus minimizing the negative impact of pesticides.

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## Effect of Acibenzolar-S-methyl on the severity of *Pythium* sp. in tomato

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