



Scientific Article

# *In vitro* and *in planta* effect of silicon and phosphites in control of *Fusarium oxysporum* f. sp. *lycopersici* causing wilting of tomato

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## ABSTRACT

**Background/Objective.** Bioactive substances such as phosphites and silicon present a promising alternative for management of vascular wilt of tomato, due to their potential to inhibit pathogen growth and to induce plant defense mechanisms. The objective of this study was to evaluate the effect of three phosphite sources and a source of silicon on isolate *Fol59* of *Fusarium oxysporum* f. sp. *lycopersici* race 2 via *in vitro* and *in planta* assays.

**Materials and Methods.** In *in vitro* conditions, percentage of radial growth inhibition (PRGI) of isolate *Fol59* was determined in PDA medium supplemented with each bioactive substance in concentrations between 10 and 20 000 ppm following a completely randomized design (4 × 9) with four treatments and nine different concentrations; five technical replicates and three biological replicates. In *in planta* conditions, the area under the disease progress curve (AUDPC) and efficacy percentage were evaluated with a completely randomized design (4 × 2) with four treatments and two different concentrations under four experimental conditions: control, inoculated control, treatments without inoculation and inoculated treatments. 20 experimental units and three biological replicates were used per treatment.

**Results.** After seven days, the bioactive substances reduced radial growth of the fungus between 70 and 100% with significant differences among the concentrations. The largest inhibition was registered with silicon at 10 000 ppm (100%) followed by calcium phosphite at 2 000 ppm (99%). Fourteen days post inoculation, in *in planta* assays, the control showed 100% incidence and 98% severity. However, the potassium phosphite treatment (KPhi1) at 2 000 ppm reduced severity to 56%, which represents a 42% efficacy against the control.

**Conclusion.** Phosphites and silicon showed inhibitory effect on mycelial growth of the fungus under *in vitro* conditions. In *in planta* assays, potassium phosphite at 2 000 ppm was the most effective treatment, reducing the severity of vascular wilt of tomato caused by *Fol59*.

**Keywords.** Bioactive substances, Induced resistance, *In vitro* inhibition, Management, Soilborne pathogens



## INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most economically important crops worldwide. In Colombia, this crop holds considerable relevance within the horticultural sector, with an annual production of approximately 685 498 tons and an estimated cultivated area of 8 500 ha, concentrated primarily in departments of the Andean region such as Boyacá, Antioquia, Cundinamarca, and Santander (Agronet, 2024). However, the crop faces phytosanitary challenges associated with soilborne pathogens, among which vascular wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is particularly notable, being responsible for yield losses exceeding 70% and representing one of the principal constraints on tomato production worldwide (Vásquez-Ramírez and Castaño-Zapata, 2017; Carmona *et al.*, 2020).

This pathogen is characterized by its destructive potential and ability to persist in the soil through the formation of resistant structures known as chlamydospores, which can remain viable and dormant for prolonged periods (Kang *et al.*, 2014; McGovern, 2015). Management of Fol relies primarily on the application of synthetic chemical fungicides, which are largely applied indiscriminately, negatively affecting natural resources and beneficial microorganisms while increasing phytosanitary risk (Brauer *et al.*, 2019; Trejo-Téllez *et al.*, 2024). In this context, and from a crop sustainability perspective, it is necessary to develop integrated disease management strategies that are more environmentally friendly and contribute to reducing the impact of the pathogen (Achary *et al.*, 2017).

Among the most widely studied alternatives are resistance inducers such as inorganic salts, including monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ), phosphites ( $\text{H}_2\text{PO}_3$ ), and silicon. Phosphites (Phi), derived from phosphorous acid ( $\text{H}_3\text{PO}_3$ ), constitute the reduced form of phosphate (Pi) and are considered a structural analogue. These compounds can be combined with other elements such as calcium ( $\text{Ca}(\text{H}_2\text{PO}_3)_2$ ), potassium ( $\text{KH}_2\text{PO}_3$ ), manganese ( $\text{MnHPO}_3$ ), magnesium ( $\text{Mg}(\text{H}_2\text{PO}_3)_2$ ), or zinc ( $\text{Zn}(\text{H}_2\text{PO}_3)_2$ ), forming salts that are widely used as pesticides, fertilizers, and/or biostimulants in horticulture (Gómez-Merino and Trejo-Téllez, 2015). Beyond their role in plant nutrition, phosphites are studied for their potential in plant disease control, showing efficacy when applied preventively (Yáñez-Juárez *et al.*, 2018). Phi salts have demonstrated effectiveness in controlling pathogens, notably oomycetes, bacteria, and fungi (Puerari *et al.*, 2015), as well as in potentiating plant defense mechanisms (Cerqueira *et al.*, 2017).

Phosphites act through two mechanisms of action: the first corresponds to a direct effect, inhibiting mycelial growth and disrupting pathogen metabolism (Machinandiarena *et al.*, 2012; Felipini *et al.*, 2016; Rocha *et al.*, 2016; Mulugeta *et al.*, 2019). The second is an indirect effect, associated with the induction of plant defense mechanisms, primarily related to the synthesis and signaling of SA. The induced responses include the production of phytoalexins and phenylpropanoids (such as chlorogenic acid and caffeic acid), the generation of reactive oxygen species (ROS), the activation of pathogenesis-related (PR) proteins, and cell wall reinforcement (Rey-Burusco *et al.*, 2019; Feldman *et al.*, 2020; Huang *et al.*, 2020).

In the *Solanum tuberosum*–*Phytophthora infestans* pathosystem, the activation of defense pathways and gene regulation following phosphite application has been described (Rey-Burusco *et al.*, 2019; Feldman *et al.*, 2020). Likewise, transcriptomic analyses in other systems have revealed systemic responses induced by phosphites (Huang *et al.*, 2020). In tomato, plant defense has been reported to be activated through SA synthesis and

the expression of the *PR1* and *Bgl* genes; the latter is involved in the biosynthesis of a  $\beta$ -1,3-glucanase, which participates in the degradation or destabilization of the pathogen cell wall (Vinas *et al.*, 2020).

On the other hand, silicon is one of the most abundant elements in the Earth's crust (Paye *et al.*, 2018; Islam *et al.*, 2020) and it is found in the soil in three forms: crystalline, semi-crystalline, and amorphous, primarily as silicon dioxide ( $\text{SiO}_2$ ). Plants assimilate it through root uptake in the form of monosilicic acid ( $\text{H}_4\text{SiO}_4$ ) (Paye *et al.*, 2018). The benefits of Si are associated with increased resistance to insects, nematodes and diseases, as well as to abiotic stress conditions such as drought, salinity, and extreme temperatures (Khan *et al.*, 2020). Despite the various studies supporting the potential of phosphites and silicon in protecting plants against diverse diseases, knowledge gaps persist in the tomato–*Fol* pathosystem regarding the comparison among different commercial sources and the differentiation of their direct effects on the pathogen from their indirect effects on the plant, both under *in vitro* and *in planta* conditions. In this context, the objective of this study was to evaluate the direct and indirect effects of different phosphite and silicon sources on *Fusarium oxysporum* f. sp. *lycopersici*, through *in vitro* assays and in tomato plants, as a basis for their potential incorporation into integrated management strategies for vascular wilt of tomato.

## MATERIALS AND METHODS

**Microorganism.** The fungal isolate used in both *in vitro* and *in planta* assays corresponded to isolate *Fol59* of *Fusarium oxysporum* f. sp. *lycopersici* race 2, obtained from a tomato plant exhibiting characteristic symptoms of vascular wilt. The isolate was identified morphologically and through pathogenicity tests as race 2 (Carmona *et al.*, 2020).

**Biological material.** For the *in planta* assays, 30-day-old tomato plants of the variety Santa Cruz Kada (Impulsemillas®), susceptible to *Fol*, were used. Prior to sowing, seeds were surface-disinfested with a 2% sodium hypochlorite solution for 10 min, followed by 70% ethanol for 1 min and three rinses with sterile distilled water. Subsequently, the seeds were immersed for 1 h in the solutions corresponding to each treatment (Table 1) and germinated on sterile peat.

**Table 1.** Evaluated bioactive substances in *in vitro* and *in planta* assays in tomato (*Solanum lycopersicum*).

| Treatment                            | Composition / Sources                                                                       |
|--------------------------------------|---------------------------------------------------------------------------------------------|
| Potassium phosphite ( <b>KPhi1</b> ) | $\text{P}_2\text{O}_5$ 420 g L <sup>-1</sup> and $\text{K}_2\text{O}$ 280 g L <sup>-1</sup> |
| Potassium phosphite ( <b>KPhi2</b> ) | $\text{P}_2\text{O}_5$ 434 g L <sup>-1</sup> and $\text{K}_2\text{O}$ 403 g L <sup>-1</sup> |
| Calcium phosphite ( <b>CaPhi</b> )   | $\text{P}_2\text{O}_5$ 176 g L <sup>-1</sup> and $\text{CaO}$ 45.6 g L <sup>-1</sup>        |
| Silicon ( <b>Si</b> )                | $\text{SiO}_2$ 179.4 g L <sup>-1</sup>                                                      |

**Bioactive substances.** Four commercial sources of phosphites and silicon were selected for evaluation (Table 1). Potassium phosphites were selected based on commercial availability. Although both present similar concentrations of  $\text{P}_2\text{O}_5$ , KPhi2 contains a higher concentration of potassium. For each substance, a stock solution was prepared and sterilized by filtration through a 0.22  $\mu\text{m}$  membrane.

**Evaluation of bioactive substances on the growth of *Fol59* in vitro.** The effect of the selected bioactive substances (Table 1) on the growth of *Fol59* was evaluated, including a fungal control consisting of potato dextrose agar (PDA) medium without supplementation. PDA medium was supplemented with each bioactive substance at nine concentrations (Table 2), with pH adjusted to 5.6 in all treatments. Agar discs of 7 mm diameter containing *Fol59* mycelium (eight days of growth) were placed at the center of each Petri dish containing supplemented medium. Petri dishes were incubated at 25°C in the dark for seven days.

**Table 2.** Concentration of bioactive substances evaluated against *Fusarium oxysporum* f. sp. *lycopersici* (*Fol59*) in *in vitro* conditions.

| Treatments | Concentrations (ppm) |       |       |       |       |       |        |        |                 |
|------------|----------------------|-------|-------|-------|-------|-------|--------|--------|-----------------|
|            | C1                   | C2    | C3    | C4    | C5    | C6    | C7     | C8     | C9              |
| KPhi1      | 0                    | 100   | 500   | 1 000 | 2 000 | 5 000 | 10 000 | 20 000 | ND <sub>z</sub> |
| KPhi2      | 0                    | 100   | 500   | 1 000 | 2 000 | 5 000 | 10 000 | 20 000 | ND              |
| CaPhi      | 0                    | 10    | 50    | 100   | 250   | 500   | 750    | 1 000  | 2 000           |
| Si         | 0                    | 1 000 | 2 000 | 5 000 | 6 500 | 8 500 | 10 000 | 20 000 | ND <sub>z</sub> |

ND<sub>z</sub>: Non-defined.

**Growth measurement.** Upon completion of the incubation period, a photographic record of each experimental unit was made. Five technical replicates and three biological replicates were established for each treatment. The images obtained were processed using ImageJ software (version 1.52f), and the percentage of radial growth inhibition (PRGI) was calculated according to the formula described by Lee *et al.* (2014):

$$PRGI (\%) = \frac{(C-T)}{C} \times 100$$

Where: *C* corresponds to the colony area of the pathogen in the inoculated control and *T* to the colony area in the treatment.

**Evaluation of vascular wilt in tomato plants.** For the *in planta* bioassays, two concentrations of each bioactive substance were selected based on the results obtained *in vitro* and from a preliminary assay (data not shown). The concentrations corresponded to the recommended dose of the commercial product and to half of that dose (Table 3).

Four experimental conditions were established: 1) control, corresponding to non-inoculated and non-treated plants; 2) inoculated control (*Fol59*), corresponding to plants inoculated solely with isolate *Fol59* of *Fusarium oxysporum* f. sp. *lycopersici* race 2; 3) non-inoculated treatments, corresponding to plants treated with bioactive substances only; and 4) inoculated treatments, corresponding to plants treated with bioactive substances and inoculated with *Fol59*.

Two applications of bioactive substances were carried out at different times: the first by seed immersion for 1 h and the second on 30-day-old seedlings in the nursery. In both cases, 10 mL of the corresponding solution was applied per plant. At 24 h after the second application, seedlings were inoculated with isolate *Fol59* using the root immersion method (Carmona *et al.*, 2020), with a suspension of  $1 \times 10^6$  conidia mL<sup>-1</sup> for 15 min. Subsequently, seedlings were transplanted into 16 oz plastic containers with sterile soil. The assay was repeated three times (biological replicates), with 20 repetitions per treatment (one plant per repetition).

**Table 2.** Concentrations of bioactive substances evaluated in tomato plants under *Fusarium oxysporum* f. sp. *lycopersici* inoculated and non-inoculated conditions.

| Treatment                            | Concentrations (ppm) |        |
|--------------------------------------|----------------------|--------|
|                                      | C1                   | C2     |
| Potassium phosphite ( <b>KPhi1</b> ) | 1 000                | 2 000  |
| Potassium phosphite ( <b>KPhi2</b> ) | 5 000                | 10 000 |
| Calcium phosphite ( <b>CaPhi</b> )   | 1 000                | 2 000  |
| Silicon ( <b>Si</b> )                | 2 500                | 5 000  |

**Disease assessment.** Plants were assessed every two days, from the appearance of the first symptoms ( $\approx$  6 days after inoculation) until 14 days after inoculation (dai). The following variables were assessed: incidence and severity. Severity was estimated using a modified visual scale (Rongai *et al.*, 2017) ranging from 0 to 5, where 0 corresponds to a healthy plant and 5 to a dead plant.

Additionally, the Area Under the Disease Progress Curve (AUDPC) was calculated using the equation described by Pedroza and Samaniego (2009), where  $y$  corresponds to the disease percentage (incidence or severity) and  $t$  to the elapsed evaluation time in days.

$$AUDPC = \sum_i \frac{y_i + y_{i+1}}{2} * (t_{i+1} - t_i)$$

Likewise, based on the final severity values, treatment efficacy was calculated using the formula proposed by Abbott (1925), taking the inoculated control as reference.

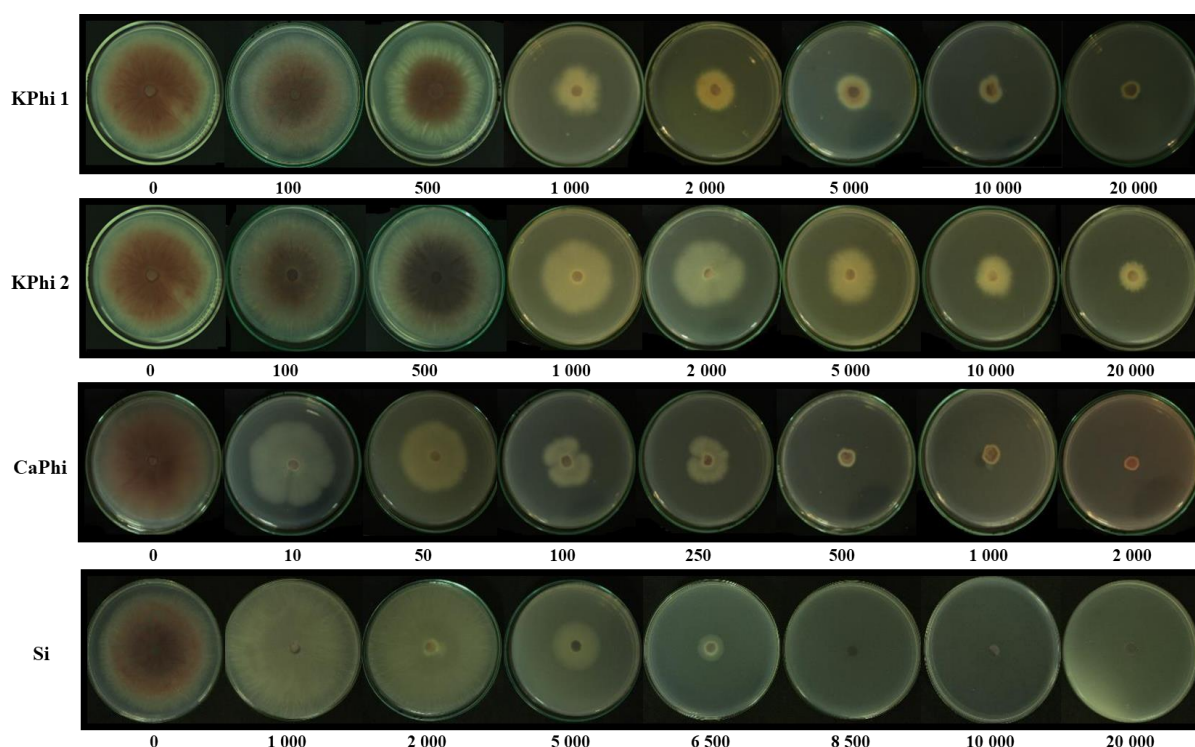
$$Efficacy (\%) = \frac{C - T}{C} \times 100$$

Where  $C$  corresponds to the value of severity in plants belonging to the inoculated control and  $T$  to the value obtained in the treated plants.

**Statistical analysis.** Data were analyzed using analysis of variance (ANOVA). For variables assessed over time (severity and incidence), a repeated measures model was used, whereas the Area Under the Disease Progress Curve (AUDPC) and the percentage of efficacy were analyzed by one-way ANOVA. When significant differences were detected ( $p \leq 0.05$ ), means were compared using Tukey's test. Statistical analyses were performed in SAS Enterprise Guide version 7 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

**Evaluation of bioactive substances on the growth of *Fol59* in vitro.** The evaluated bioactive substances showed a reduction in the radial growth of *Fol59*. Potassium phosphite KPhi1 exhibited a radial growth inhibition (PRGI) of 77.9% at a concentration of 1 000 ppm, reaching 97.4% inhibition at concentrations above 10 000 ppm. Potassium phosphite KPhi2, in turn, showed significant reduction from 1 000 ppm, with PRGI values of 73.4%, which were lower than those obtained with KPhi1, reaching 90.4% inhibition at concentrations above 10 000 ppm (Figure 1, Table 4). Overall, KPhi1 showed greater efficacy in reducing mycelial growth compared to KPhi2.



**Figure 1.** Effect of different concentrations of phosphites and silicon on mycelial growth of *Fol59* after seven days of incubation.

The results obtained under *in vitro* conditions showed that increasing concentrations of CaPhi significantly reduced the mycelial growth of *Fol59* (Figure 1), reaching up to 70% inhibition at a concentration of 50 ppm and 99% inhibition at 2 000 ppm (Table 4). On the other hand, treatment with silicon (Si) achieved 100% inhibition of *Fol59* mycelial growth at concentrations of 8 500 and 20 000 ppm (Figure 1). However, the lower Si concentrations (1 000 and 2 000 ppm) did not inhibit fungal growth, yielding negative inhibition values of -37.3% and -35.6%, respectively (Table 4, Figure 1).

**Reduction of vascular wilt in tomato plants.** Plants previously treated with bioactive substances (KPhi1-C1, KPhi1-C2, and KPhi2-C1) showed a significant reduction in AUDPC caused by *Fol59* compared to the inoculated control, maintaining this trend through the final day of assessment (14 dai). However, treatments with silicon and CaPhi did not show significant differences relative to the inoculated control. On the other hand, although some treatments showed greater efficacy under *in vitro* conditions, this response was lower in the *in planta* assays, possibly due to the influence of biotic and abiotic factors inherent to the *in vivo* system (Figure 2).

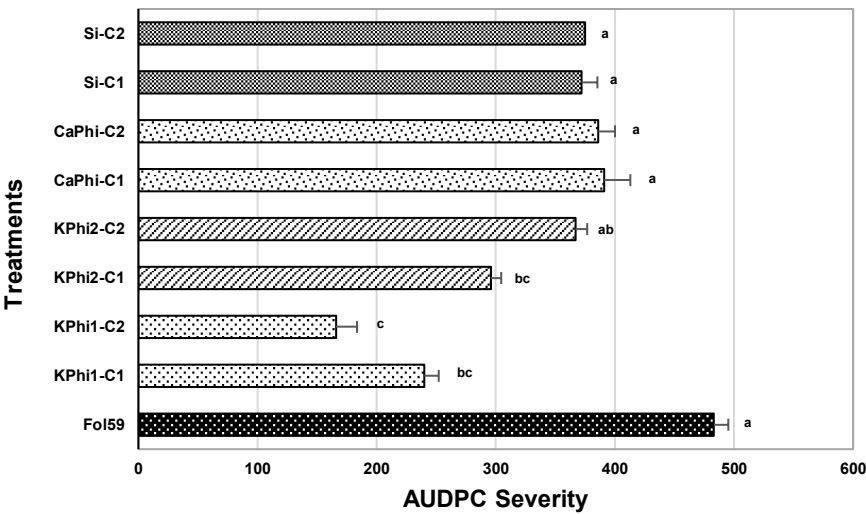
Among the products containing potassium phosphite as the active ingredient, only treatment KPhi1 showed notable effectiveness in reducing disease severity. Evaluated at both the manufacturer's recommended dose (C2) and half the dose (C1), KPhi1 reduced the severity of vascular wilt by 42 and 20%, respectively, in plants treated before and after inoculation with *Fol59*.

**Table 4.** Percentage of radial growth inhibition (PRGI) of *Fusarium oxysporum* f. sp. *lycopersici* using phosphites and silicon (KPhi1, KPhi2, CaPhi and Si) at different concentrations under *in vitro* conditions.

| Concentrations *(ppm) | <sup>z</sup> PRGI per treatment |                        |                        |                         |
|-----------------------|---------------------------------|------------------------|------------------------|-------------------------|
|                       | KPhi 1                          | KPhi 2                 | CaPhi                  | Si                      |
| 10                    | -                               | -                      | 45.5±3.6 <sup>ab</sup> | -                       |
| 50                    | -                               | -                      | 70.6±3.4 <sup>b</sup>  | -                       |
| 100                   | 15.8±5.4 <sup>b</sup>           | 7.6±4.9 <sup>b</sup>   | 80.6±4.3 <sup>a</sup>  | -                       |
| 250                   | -                               | -                      | 92.3±2.1 <sup>a</sup>  | -                       |
| 500                   | 24.5±5.5 <sup>ab</sup>          | 6.6±3.1 <sup>b</sup>   | 94.5±2.1 <sup>a</sup>  | -                       |
| 1 000                 | 77.9±6.9 <sup>a</sup>           | 73.4±6.2 <sup>ab</sup> | 96.9±2.2 <sup>a</sup>  | -37.3±10.3 <sup>b</sup> |
| 2 000                 | 95.4±1.7 <sup>a</sup>           | 74.5±5.4 <sup>ab</sup> | 99.4±0.2 <sup>a</sup>  | -35.6±7.5 <sup>b</sup>  |
| 5 000                 | 96.2±1.4 <sup>a</sup>           | 81.0±4.4 <sup>ab</sup> | -                      | 77.1±16.3 <sup>ab</sup> |
| 6 500                 | -                               | -                      | -                      | 97.7±2.0 <sup>a</sup>   |
| 8 500                 | -                               | -                      | -                      | 99.7±0.0 <sup>a</sup>   |
| 10 000                | 97.4±0.4 <sup>a</sup>           | 90.4±2.3 <sup>a</sup>  | -                      | 100±0.0 <sup>a</sup>    |
| 20 000                | 99.0±0.2 <sup>a</sup>           | 96.2±1.0 <sup>a</sup>  | -                      | 100±0.0 <sup>a</sup>    |

<sup>z</sup> Each value represents the average of three biological replicates and five technical replicates expressed in PRGI ± standard deviation (SD). Values with the same letter do not show significant differences between concentrations for each treatment. Tukey ( $p \leq 0.05$ ).

Silicon evaluated under *in vitro* conditions showed, at the recommended concentration, a growth inhibition of *Fol59* of 77%. However, the *in planta* experiments at both concentrations (C2 and C1) demonstrated lower efficacy in reducing vascular wilt caused by *Fol59*, with severity reductions of 22 and 20%, respectively (Table 5). In contrast, plants inoculated solely with *Fol59* showed progressive disease development, reaching a severity of 98% and an incidence of 100% at 14 dai (Table 5).



**Figure 2.** Area under disease progression curve (AUDPC) 14 days after inoculation, expressed as severity in tomato plants previously treated with bioactive substances (KPhi, CaPhi and Si) and inoculated with isolate *Fol59*. Bars with the same letter do not show significant differences according to the Tukey test ( $p \leq 0.05$ ).

Across the three biological replicates, the evaluated bioactive substances showed an effect in reducing the severity of vascular wilt in tomato plants. Treatment KPhi1-C2 exhibited the greatest disease reduction with a severity of 56% compared to the inoculated control, which reached a severity of 98% at 14 days after inoculation (Table 5). This treatment represented an efficacy of 42%, higher than that observed with CaPhi-C1 and CaPhi-C2, whose efficacy values were 10 and 13%, respectively.

Disease incidence exceeded 90% across all evaluated treatments and was similar to that recorded in the inoculated control, which presented an incidence of 100%. These results demonstrate the high aggressiveness of isolate *Fol59* in tomato plants under the assay conditions.

**Table 5.** Incidence and severity percentages caused for *Fusarium oxysporum* f. sp. *lycopersici* (*Fol59*) in tomato plants 14 days after inoculation (dai).

| Treatment                           | Incidence   |            | Severity   |            |
|-------------------------------------|-------------|------------|------------|------------|
|                                     | % Incidence | % Efficacy | % Severity | % Efficacy |
| Inoculated control ( <i>Fol59</i> ) | 100         |            | 98         |            |
| KPhi1-C1                            | 97          | 3          | 78         | 20         |
| KPhi1-C2                            | 92          | 8          | 56         | 42         |
| KPhi2-C1                            | 97          | 3          | 78         | 20         |
| KPhi2-C2                            | 97          | 3          | 80         | 27         |
| CaPhi-C1                            | 98          | 2          | 88         | 10         |
| CaPhi-C2                            | 100         | 0          | 85         | 13         |
| Si-C1                               | 98          | 2          | 78         | 20         |
| Si-C2                               | 98          | 2          | 76         | 22         |

During the *in planta* bioassays with silicon and potassium phosphite, tomato plants showed no signs of phytotoxicity. However, control plants treated with calcium phosphite exhibited symptoms of chlorosis on lower-stratum leaves, as well as leaf curling and reduced leaf size, at both evaluated concentrations (Figure 3). These symptoms were consistent across the three biological replicates, suggesting a possible phytotoxic effect associated with CaPhi treatment.

## DISCUSSION

Integrated disease management represents a key approach within sustainable agriculture, particularly in tomato cultivation, where agrochemical applications, especially fungicides, are frequent. One of the most promising strategies is based on resistance induction through the application of substances compatible with the environment and human health. In this context, various studies have described the protective effect of phosphites and silicon against pathogens and abiotic stress in different plant species by inducing a physiological state of alertness in the plant known as priming, which enhances the capacity to activate basal defense mechanisms, in addition to exerting a direct action on the pathogen, affecting its growth and sporulation (Trejo-Téllez and Gómez-Merino, 2018; Coskun *et al.*, 2019).



**Figure 3.** Symptoms of vascular wilting in tomato plants 14 days after inoculation (dai). A) Non-inoculated plants, including control and treatments with bioactive substances; B) *Fol59* inoculated plants, including inoculated control and treatments with bioactive substances.

Under *in vitro* conditions, potassium phosphite (KPhi) has demonstrated a significant inhibitory effect on various phytopathogens. Potassium phosphite formulations have shown a reduction of approximately 94% in mycelial growth of *Colletotrichum gloeosporioides* (Araújo *et al.*, 2010), while in *Fusarium circinatum* the reduction in mycelial growth can reach up to 53% depending on the applied dose (Cerqueira *et al.*, 2017). Similarly, in *Fusarium culmorum* and *F. graminearum*, mycelial development has been reduced by 60 to 80% even at low concentrations of 10 ppm KPhi (Hofgaard *et al.*, 2010).

Taken together, these findings suggest that the inhibitory effect of phosphite is not pathogen-specific, but rather reflects a direct mechanism of action that affects essential metabolic processes for fungal growth, such as phosphorylation and mycelial development (Machinandiarena *et al.*, 2012; Felipini *et al.*, 2016). This generalized antifungal action could explain the effects observed across different genera.

Although the direct mechanism of action of phosphites against microorganisms has not been fully elucidated, recent studies suggest that these compounds may interfere with phosphate-dependent cellular processes by acting as functional analogues thereof (Trejo-Téllez *et al.*, 2024). Furthermore, the antimicrobial activity of the phosphite ion may depend on the nature of the associated cation, the concentration of the phosphite anion and the acidification of the culture medium under *in vitro* conditions (Lobato *et al.*, 2010).

Similarly, silicon can contribute to pathogen management through the formation of physical and biochemical barriers that increase plant resistance to pests and diseases (Siddiq *et al.*, 2019). Bekker *et al.* (2009) reported that potassium silicate exerted a direct inhibitory effect on *F. oxysporum*, reaching up to 96.2% inhibition of mycelial growth at concentrations of 20 000 ppm and 100% at concentrations above 40 000 ppm. However, lower concentrations (5 000 and 10 000 ppm) showed negative inhibition values, indicating a possible stimulation of pathogen growth. This behavior is consistent with observations in the present study and can be explained by the phenomenon of hormesis, characterized by a biphasic response in which low doses stimulate organism growth and high doses exert an inhibitory effect (Dharma *et al.*, 2024).

In *Fol59*, the *in vitro* results demonstrated inhibition of mycelial growth following treatment with phosphites and silicon. Nevertheless, in the *in planta* assays, partial reduction of disease severity was obtained with potassium phosphite applications at the recommended dose (2 000 ppm). It has been reported that Phi treatment can activate the salicylic acid signaling pathway in response to pathogen infection, inducing the expression of defense-associated genes such as *PR1*, *PR2*, and antioxidant genes and enzymes involved in the generation of reactive oxygen species (Massoud *et al.*, 2012; Feldman *et al.*, 2020).

Phosphites can act efficiently as biopesticides against various soilborne phytopathogens (Puerari *et al.*, 2015; Mulugeta *et al.*, 2019; Morales-Morales *et al.*, 2022). In addition, they exhibit high systemic mobility, being translocated via the phloem from leaves to roots, which favors comprehensive plant protection (Yáñez-Juárez *et al.*, 2018). This transport capacity, together with their accumulation in certain storage tissues, contributes to their efficacy as phytosanitary management agents (Gómez-Merino and Trejo-Téllez, 2015; Jost *et al.*, 2015). On the other hand, plant response to phosphite may depend on its nutritional status with respect to inorganic phosphate (Pi). When plants have adequate Pi levels, Phi application can trigger defense mechanisms; however, under Pi

deficiency conditions, high Phi concentrations may accumulate and generate phytotoxic effects (Loera-Quezada *et al.*, 2015; Jost *et al.*, 2015).

The double application of KPhi1-C2 (on seed and onto the nursery substrate) suggests a protective effect in reducing the severity of wilt caused by *Fol59* by 42%. This reduction could be associated with the activation of plant defense mechanisms following KPhi treatment. In this regard, Feldman *et al.* (2020) determined, through molecular analysis using DNA microarrays, that 72 h after potassium phosphite application, potato (*Solanum tuberosum*) plants infected with *P. infestans* overexpressed genes involved in the activation of SA signaling pathways, as well as in the synthesis and modification of cell wall polymers, including suberins and gums.

Although both evaluated compounds contained the same active ingredient, KPhi2 did not show a comparable severity reduction to that obtained with KPhi1. This behavior suggests that the observed response does not depend solely on the phosphite anion, but also on characteristics inherent to the commercial formulation, the applied dose and the processes of absorption and translocation in the plant. The differences could be related to the presence of adjuvants such as solvents, surfactants or stabilizers capable of modifying the availability and biological performance of the active ingredient. It has been documented that these components can influence the stability, persistence, biological activity and potential phytotoxicity of agricultural inputs, particularly in complex systems such as soil (Knowles, 2008; Basilio *et al.*, 2024).

The results obtained with silicon showed an efficacy of 20 to 22% in reducing the severity of wilt caused by *Fol59*. Although these values did not show a comparable effect relative to the inoculated control, silicon could be considered as a complementary preventive management alternative in combination with potassium phosphites. Coskun *et al.* (2019) indicate that the effects of silicon are generally more consistent against biotrophic pathogens than against necrotrophic ones. In this regard, given that *F. oxysporum* exhibits hemibiotrophic behavior, the effectiveness of silicon could depend on the infection phase during which the pathogen interacts with plant tissues.

Silicon has been shown to act as a defense potentiator in various plant species. Castro-Rosalez (2017) reported that silicon application in tomato reduced the severity caused by *Fol* by 13.2%, in addition to inducing changes in root, stem and leaf micromorphology. This protection could be related to the accumulation of silicon in cell walls, forming mechanical barriers that hinder pathogen colonization (Siddiq *et al.*, 2019). Likewise, Wang *et al.* (2017) described that silicon applications in *Arabidopsis thaliana* promoted callose accumulation at penetration sites of the fungus *Golovinomyces cichoracearum* through activation of SA-dependent defense responses.

In the current agricultural context, characterized by the need to produce safe food with reduced environmental impact, the use of bioactive substances emerges as a complementary alternative within integrated management strategies and contributes to reducing dependence on agrochemicals (Kim *et al.*, 2016).

The results obtained in this study suggest that phosphite application can exert a combined effect of inhibiting *Fol59* growth and protecting tomato plants. A relevant aspect of this study was the difference observed between commercial formulations containing the same active ingredient, suggesting that phosphite efficacy does not depend solely on the phosphite anion, but also on components associated with the formulation. Likewise, the discrepancy between *in vitro* and *in planta* results highlights the complexity of the *Fol*-tomato pathosystem, particularly considering its vascular nature and its initial interaction

at the root level. These observations underscore the need for further studies evaluating the systemic accumulation of phosphite and its interaction with plant physiology under conditions closer to field settings. Nevertheless, to validate these findings under semi-commercial conditions, it will be necessary to develop studies that allow determination of the persistence of potassium phosphite in the plant and its protective capacity against *Fol*.

## CONCLUSIONS

Based on the results obtained, potassium phosphite at 2 000 ppm showed the greatest efficacy in reducing the severity of vascular wilt caused by isolate *Fol59* of *Fusarium oxysporum* f. sp. *lycopersici* race 2 in tomato plants. The evaluated phosphite and silicon sources demonstrated inhibitory capacity against pathogen growth under *in vitro* conditions, supporting their potential as complementary tools within integrated disease management strategies.

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