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A Potassium Phosphite Solution as a Dual-Action Strategy Against Bean Anthracnose: Antifungal Activity and Defense Gene Priming

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A Potassium Phosphite Solution as a Dual-Action Strategy Against Bean Anthracnose: Antifungal Activity and Defense Gene Priming

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Abstract: Anthracnose in bean is an important disease caused by *Colletotrichum lindemuthianum*, which affects crop productivity and infects the plant in all growth stages, affecting the quality of the pod and grains. The most viable strategy to control this disease is using bean cultivars; however, fungal variability is a limitation. Among the strategy proposed is using phosphite-based compounds, which can act as fungicides or priming stimulators. This study aimed to evaluate the antifungal activity of phosphite base solution (potassium phosphite (H_3PO_3), potassium hydroxide, and potassium citrate, in a formulation of phosphorus (P_2O_5) 28% and potassium (K_2O) 26%) on *C. lindemuthianum* under *in vitro* conditions. In addition, its effects as a defense inducer in Sutagao bean plants, assessing changes in disease severity and the expression of *PR1*, *PR3*, *PR4*, and *POD* defense-related genes in plants treated with the phosphite solution before infection with the fungus. The results showed the fungicidal effect of potassium phosphite solution on *C. lindemuthianum* by reducing 42% of the mycelial growth by 48% germination percentage with a dose of 5 mL L^{-1}. The effect of foliar of application of phosphite base solution on anthracnose severity showed a 17% reduction associated with the high expression of *PR1*, *PR3*, *PR4*, and *POD* defense genes, which increased in plants that were subsequently infected with the pathogen, demonstrating a priming effect. In conclusion, a potassium phosphite solution can be included in a management program to control bean anthracnose.

Keywords: anthracnose; defense-related genes; disease severity; potassium phosphite

1. Introduction

Colletotrichum lindemuthianum (Sacc. & Magnus) LambScrib, the anamorph of *Glomerella cingulata*, is the causal agent of anthracnose in the common bean (*Phaseolus vulgaris* L.), a devastating disease that leads the total crop loss under favorable conditions of high relative humidity (> 80%), conducting to severe economic losses [1,2]. *C. lindemuthianum* affects all plant organs and the disease symptoms include necrotic lesions on petioles, stems, branches and primary and secondary veins of leaves. Round sunken cankers appear on the pods, which can lead to pod malformation, low seed number and tissue death [3].

P. vulgaris is the most widely cultivated legume for direct consumption worldwide, as it is considered a source of dietary protein, particularly in developing countries [4]. The global demand for nutritious legumes, the adaptability of pathogens, and climate change make it necessary to control this disease worldwide [2].

Chemical fungicides were the first to control the plant diseases; however, *Colletotrichum* species respond differently to applying these products in the field [5]. Among the strategies proposed for disease control, priming is a novel technique in which the induction of a physiological state allows a plant to deploy a faster and more efficient defense response against stress compared to an unprepared plant [6].

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Phosphites derived from the neutralization of phosphorous acid (H_3PO_3) with a base, such as sodium hydroxide, potassium hydroxide, and ammonium hydroxide, have been used as alternative products for the management of plant diseases. They can be used to improve the performance of many crop species by inhibiting fungicide mycelial growth and germination of fungal spores [7,8]. They have also been used as fertilizers and/or biostimulants, which increase the absorption and assimilation of nutrients, and improve the quality of products and their tolerance to biotic and abiotic stresses [8,9]. The application of phosphites induces various plant defense mechanisms, even in the absence of pathogens [7,10,11], and increases systemic acquired resistance (SAR) signaling activities [8].

Therefore, studying the antifungal activity of phosphite-based products such as fungicides, and inductors of disease resistance in beans could contribute to management alternatives for anthracnose disease.

2. Materials and Methods

2.1. Isolate of C. lindemuthianum

The isolation of *C. lindemuthianum* was provided by the Alliance Bioversity International-CIAT entity and reactivated in Petri dishes containing PDA medium. A monosporic culture was performed to ensure the genetic uniformity of the fungus [12].

2.2. Effect of Potassium Phosphite Solution on C. lindemunthianum Under In Vitro Conditions

The direct effect of a phosphite base solution (potassium phosphite (H_3PO_3), potassium hydroxide, and potassium citrate, in a formulation of phosphorus (P_2O_5) 28% and potassium (K_2O) 26%) on the growth, morphology, and germination of *C. lindemuthianum* was conducted in in vitro tests. For this, a potato dextrose agar medium (PDA) was supplemented with the potassium phosphite solution at a dose of 5 mL L^{-1}. As a control, the fungus was grown on a PDA culture medium without potassium phosphite [9]. The mycelial growth of *C. lindemuthianum* was evaluated by transferring a 7 mm mycelial plug from a 7-day-old culture to a Petri dish containing PDA. The dishes were incubated at 25°C in a growth chamber. The final ratio of the colony was measured once the control mycelia fully colonized the medium. The mycelial growth rate was calculated by the equation

$$\text{MGR (cm/day)} = \frac{MG2-MG1}{t2-t1}, \quad (1)$$

where MG = mycelial growth and t = time in days.

The percentage of mycelial growth inhibition was calculated by the equation

$$\text{MGI (\%)} = \frac{MGc-MGt}{MGc} * 100, \quad (2)$$

where MGc = average diameter of the control *C. lindemuthianum* and MGt = average diameter of the treated *C. lindemuthianum*. A randomized design was employed with three replicates and the experiment was conducted three times.

The effect of potassium phosphite solution on *C. lindemuthianum* was determined after 18 days of growth by changes in colony morphology (color and appearance) and at the microscopic level by evaluating changes in mycelia and acervuli formation at 40X. A suspension of 5×10^4 conidia mL^{-1} was prepared for the conidia germination test. Twenty microliters of this suspension were sown and homogenized on the surface of the agar-water culture medium using a sterile rake and incubated for 24 h at 25°C [13]. Finally, 100 conidia were counted in an area of 2 cm^2, considering germinated conidia as those whose germ tube was twice the width of the conidia.

The data were analyzed using RStudio program (RSTUDIO-2023.09.1-494.EXE) and Microsoft Excel. The assumptions were validated through the Shapiro-Wilk normality test and Levene's test for homogeneity of variances. Subsequently, an analysis of variance (ANOVA) was performed, followed

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by a multiple comparison test using Tukey's method with a significance level of $p < 0.05$ to establish significant differences.

2.3. Inoculum Increase and Pathogenicity Test

To increase the inoculum of *C. lindemuthianum* for the pathogenicity tests, fragments of the monosporic fungi grown on PDA medium for over two weeks were placed into glass tumblers containing pre-sterilized beans (P. vulgaris) [14]. The tumblers were then incubated in darkness at 24 °C for 21 days, allowing the formation of acervuli and conidia of the pathogen. A conidial suspension was then prepared by maceration and filtration of the inoculated beans, counted in a Neubauer chamber, and adjusted to a concentration of 1×10^7 conidia mL^{-1}. This suspension was applied by brushing it onto both the upper and lower sides of the leaves of 14-day-old bean plants [15]. The plants were maintained at a relative humidity above 80% to promote infection.

2.4. Effect of Potassium Phosphite Solution on Anthracnosis Disease

To evaluate the potassium phosphite effect, 10-day-old plants of the Sutagao bean cultivar were sprayed with 1 mL of phosphite base solution at a concentration of 5 mL L^{-1}, as per the manufacturer's instructions. The application was done using an airbrush, targeting both the upper and lower sides of the leaves from a distance of 20 cm, during the afternoon hours. Five days after applying the potassium phosphite solution, the plants were inoculated with *C. lindemuthianum* as previously described. The plants were kept in a propagation greenhouse with an average temperature of 18-25 °C, relative humidity of 80%, and a natural photoperiod of 12 hours. The treatments included: (-PK/+Cl) plants without application of potassium phosphite and infected with *C. lindemuthianum*; (+PK/+Cl) plants treated with phosphite potassium and infected with *C. lindemuthianum*; (+PK/-Cl) plants treated with potassium phosphite and without *C. lindemuthianum* infection; (-PK/-Cl) plants without potassium phosphite application and not infected with *C. lindemuthianum*. The experiment was performed twice with 15 biological replicates for each treatment.

The effect of potassium phosphite solution on anthracnose in bean plants was determined by disease severity quantification. A descriptive ordinal scale was used for this purpose [16]. The evaluation was conducted every four days up to a maximum of 14 days post-inoculation (dpi). The area under the disease progress curve (AUDPC) was calculated from the severity data [17]. The RStudio program (RSTUDIO-2023.09.1-494.EXE) was used to analyze the AUDPC data. A repeated means analysis of variance (ANOVA) followed by Tukey's test was performed to determine significant statistical differences between treatments.

2.5. Effect of Potassium Phosphite Solution on Defense Genes Expression

To determine changes on defense gene expression by application of potassium phosphite in all treatments, the RNA was extracted from the leaves of bean seedlings at 0, 24, 48, 72, and 96 hours post-inoculation (hpi) using a CTAB-based protocol with LiCl [18]. Three leaves from each treatment were collected at each time point for RNA extraction. Subsequently, the RNA was treated with ThermoFisher® DNase I and its quality and concentration were measured using a Thermo Scientific™ NanoDrop™ Spectrophotometer. Elongation factor gene (EF1α) of P. vulgaris was amplified from each RNA to confirm the absence of DNA. The PCR reaction was conducted in a 20 µL reaction volume containing 1X Buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer (Foward 5′ CGGGTATGCTGGTGACTTTT 3′ and Reverse 5′ CACGCTTGAGATCCTTGACA 3′), 1 U Taq Polymerase, and 2.0 µL of DNase-treated RNA. The reaction conditions were as follows: 95 °C for 10 minutes, followed by 35 amplification cycles at 95 °C for 1 minute, 60 °C for 30 seconds, and 72 °C for 2 minutes. Finally, cDNA was synthesized from each RNA using ThermoFisher® M-MLV Reverse Transcriptase according to the manufacturer's protocol.

Before of gene expression assays, the amplification efficiency of each primer set (EF1-α, POD, PR-1, PR-3, and PR-4) [15] was made from ten-fold dilution series of P. vulgaris DNA (7.5 ng mL^{-1})

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by SYBR Green qPCR. Three replicates were prepared for each dilution and a standard curve was generated with the threshold cycle (Ct) values for each one. The efficiency was calculated by the equation

$$E = 10^{-1/slope} \quad (1)$$

The qPCR reactions were carried out in a final volume of 10 µL, containing 1 X BlasTaq™ 2 qPCR Master Mix, 0.2 µM of each primer, and 3 µL of cDNA. Amplification conditions were an initial incubation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 1 minute, 60°C for 30 seconds, and 72°C for 2 minutes. Dissociation curve (melting curve) analysis was performed with the thermal profile increasing from 60 °C to 95 °C in 15-second intervals [18] The reactions were run on a Jena Analytik qTOWER3 thermal cycler.

For the differential expression analysis of the defense genes, qPCR was performed using the relative quantification method with three cDNA replicates for each treatment at 24, 48, 72, and 96 hpi. In this analysis, the crossing point (CP) values of EF1-α (housekeeping gene) were compared to those of the defense-related genes. The qPCR reactions were conducted in a final volume of 10 µL, consisting of 5 µL BlasTaq™ 2X qPCR Master Mix, 0.2 µL of each primer at 10 µM and 3 µL cDNA. The qPCR amplification program was the same as the one used previously to describe the efficiency test and a dissociation curve (melting curve) was performed at the end with the thermal profile increasing from 60°C to 95°C in 15-second intervals. Relative expression for each gene was calculated using three repetitions of Ct values obtained for each treatment by the Pfaffl comparative method [19]. An ANOVA analysis of repeated means, followed by Tukey's test was performed to determine significant statistical differences between the treatments. The data were analyzed using the RStudio program (Rstudio-2023.09.1-494.EXE).

3. Results

3.1. Effect of Potassium Phosphite Solution on C. lindemuthianum

3.1.1. Effect on *C. lindemuthianum* Morphology

The macroscopic and microscopic characteristics of *C. lindemuthianum* differed between the cultures grown with and without potassium phosphite solution (Figure 1). The colony grown on PDA without supplementation exhibited a cottony growth pattern, black with a slightly gray center, 18 days after sowing (Figure 1a). Microscopically, this colony displayed hyaline septate mycelia with asexual fruiting bodies (acervuli), which are clusters of conidiophores from which conidia emerge (Figure 1b). The conidia observed were typical of the genus and were classified as hyaline with a cylindrical shape. In contrast, colonies grown on PDA supplemented with potassium phosphite (5 mL L^{-1}) had a black cotton-like appearance with a white center and a white edge (Figure 1c). Microscopically, the fungus showed deformations in the mycelium, characterized by overgrowth and widening of the hyphae (Figure 1d).

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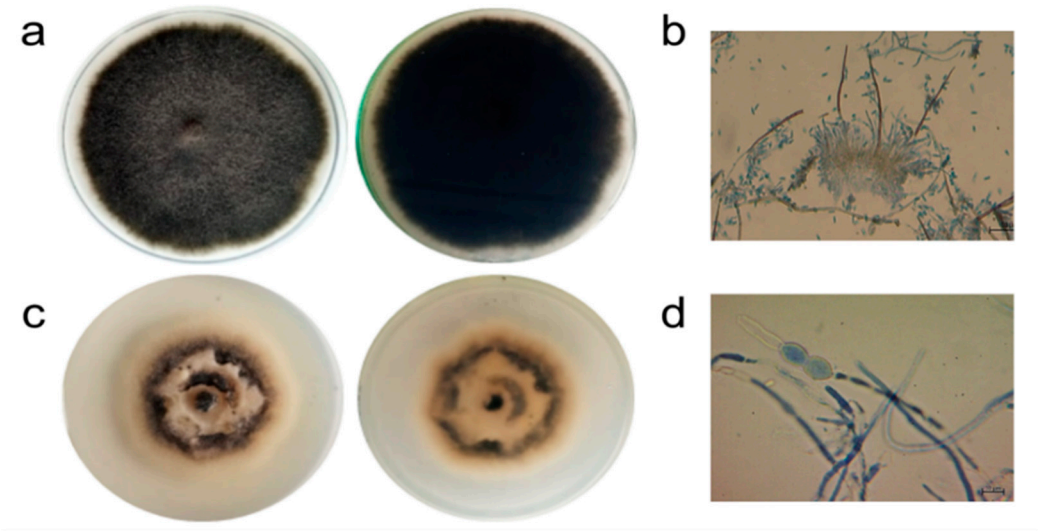

Figure 1. Macroscopic and microscopic characteristics of *C. lindemuthianum* grown on PDA with and without potassium phosphite solution (5 mL L^{-1}) 18 days after sowing. (a) *C. lindemuthianum* colony on PDA culture media without supplementation; the images on the left show the front of the colony, while those on the right show the back; (b) *C. lindemuthianum* colony on PDA culture media supplemented with potassium phosphite solution; the images on the left display the front of the colony, and those on the right display the back; (c) Acervuli and conidia of *C. lindemuthianum* observed at 40X magnification, grown on PDA without supplementation; (d) Deformations in *C. lindemuthianum* mycelia observed at 40X magnification, grown on PDA supplemented with potassium phosphite solution.

3.1.2. Effect on *C. lindemuthianum* Growth Rate and Conidia Germination

Colonies of *C. lindemuthianum* grown on PDA without supplementation potassium phosphite showed greater mycelial growth (Figure 2), as well as higher mycelial growth rate and germination percentage, compared to colonies grown on PDA supplemented with potassium phosphite (5 mL L^{-1}) (Table 1). The growth inhibition percentage in the PDA-supplemented culture media was 42 %, and the germination percentage decreased by 48 %, compared to the non-supplemented culture media (Table 1).

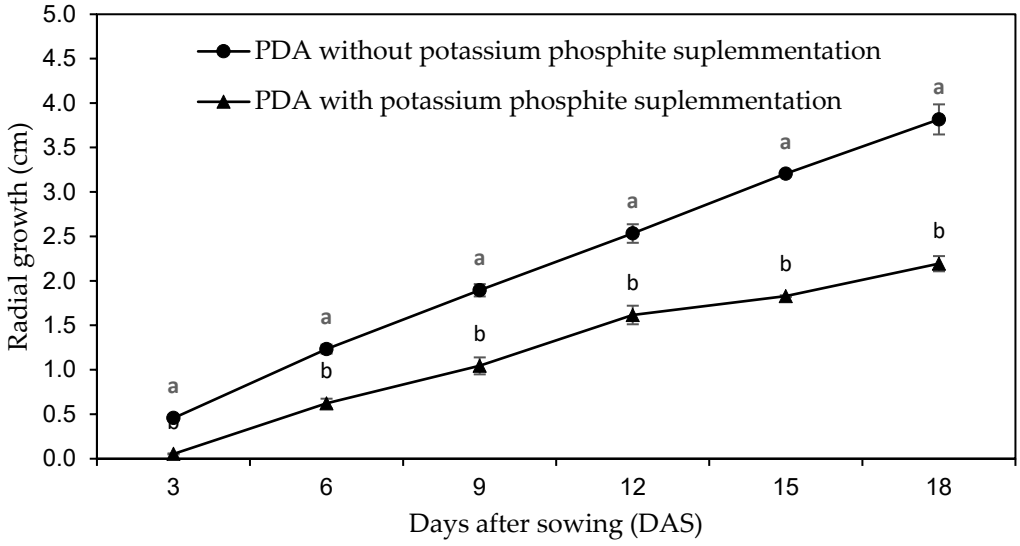

Figure 2. Radial growth curve of *C. lindemuthianum* in PDA culture media with and without potassium phosphite solution. Data are shown as the mean ±SD of three replicates. Different letters represent values with significant differences between treatments ($p<0.05$).

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Table 1. Mycelial growth rate, growth inhibition percentage, and conidial germination percentage of *C. lindemuthianum* in PDA culture media with and without potassium phosphite supplementation.

Treatment	Mycelial growth rate (cm/day)	Growth inhibition percentage (%)	Conidial germination percentage (%)
PDA without supplementation	0,22 a	-	49,44 a
PDA supplemented with potassium phosphite	0,14 b	42,39	25,78 b

Different letters represent values with significant differences between treatments ($p<0.05$).

3.2. Effect of Potassium Phosphite Solution on Bean Anthracnose

The effect of potassium phosphite on anthracnose was estimated in bean seedlings of the Sutagao cultivar infected with *C. lindemuthianum*. Leaves of plants previously treated with potassium phosphite before fungal infection (+PK/+Cl) showed smaller necrotic lesions in the secondary veins compared to those of infected plants that were not treated with potassium phosphite (-PK/+Cl) (Figure 3a). In addition, AUDPC data indicated a 17% reduction in disease severity in plants treated with the phosphite potassium before infection with *C. lindemuthianum* (+PK/+Cl), compared to untreated plants (-PK/+Cl). Significant differences between the two treatments were observed as early as 4 days post-inoculation (dpi) and persisted throughout the disease evaluation period (Figure 3b).

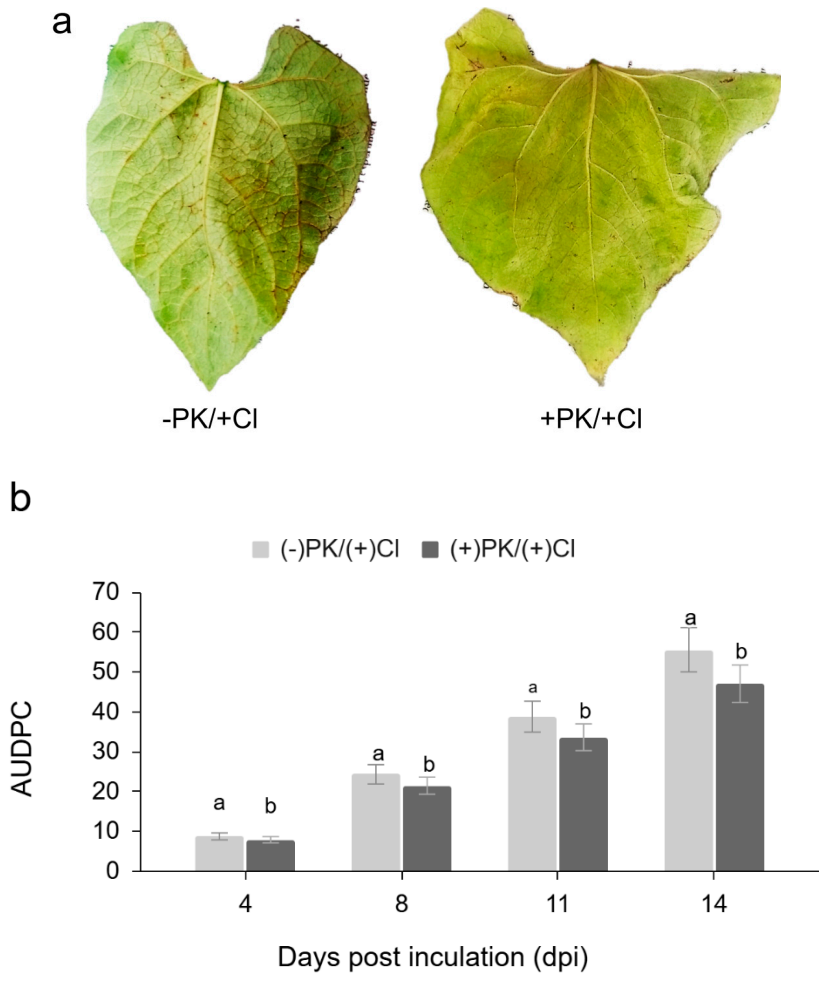

Figure 3. Effects of potassium phosphite solution on anthracnose disease in Sutagao bean plants. (a) Necrotic lesions caused by *C. lindemuthianum* in leaves at 14 dpi. The image on the left shows a leave infected with *C. lindemuthianum* but not pre-treated with potassium phosphite solution (-PK/+Cl); the image on the right shows

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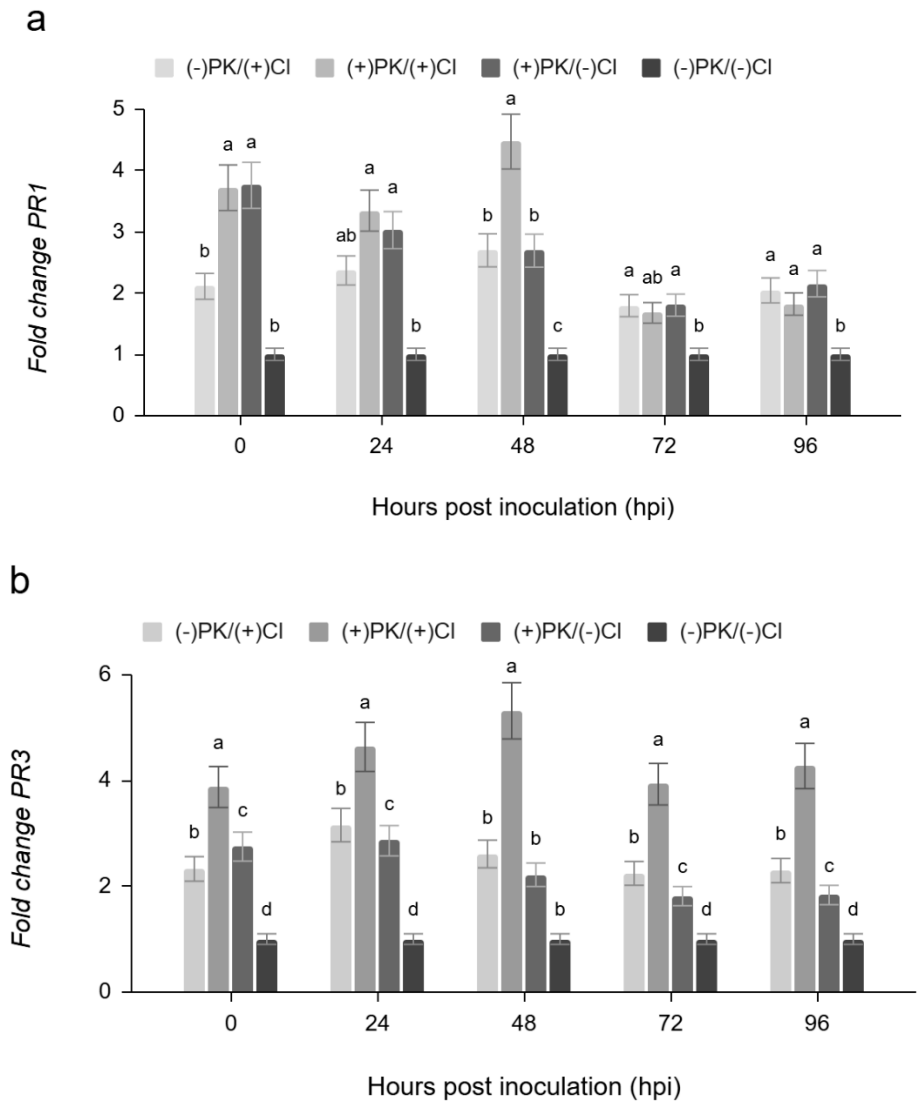
a leave infected and pre-treated with potassium phosphite solution (-PK/+Cl); (b) AUDPC values obtained after inoculation with *C. lindemuthianum* on 10-day-old plants of the Sutagao bean cultivar, previously treated with potassium phosphite solution at different time points. Different letters represent values with significant differences between treatments ($p<0.05$).

3.3. Defense Genes Expression on Bean Plants Treated with Potassium Phosphite Solution and Infected with C. lindemuthianum

The potassium phosphite solution significantly increased the expression of the plant defense genes *PR1*, *PR3*, *PR4*, and *POD*, in plants treated with this product without prior *C. lindemuthianum* infection (+PK/-Cl), demonstrating a defense-inducing effect (Figure 4). This gene induction was further enhanced when the plants were infected with the fungus (+PK/+Cl) (Figure 4). *PR1* and *PR3* exhibited a significant increase in expression at 48 hpi, although the expression of PR1 declined at 72 hpi, whereas *PR3*, remained elevated (Figure 4a, 4b). *PR4* and *POD* displayed early expression at 24 hpi, with *PR4* showing the highest expression level among all the genes evaluated (Figure 4c). These findings suggest a strong activation of the defense response, possibly linked to a priming effect (Figure 4).



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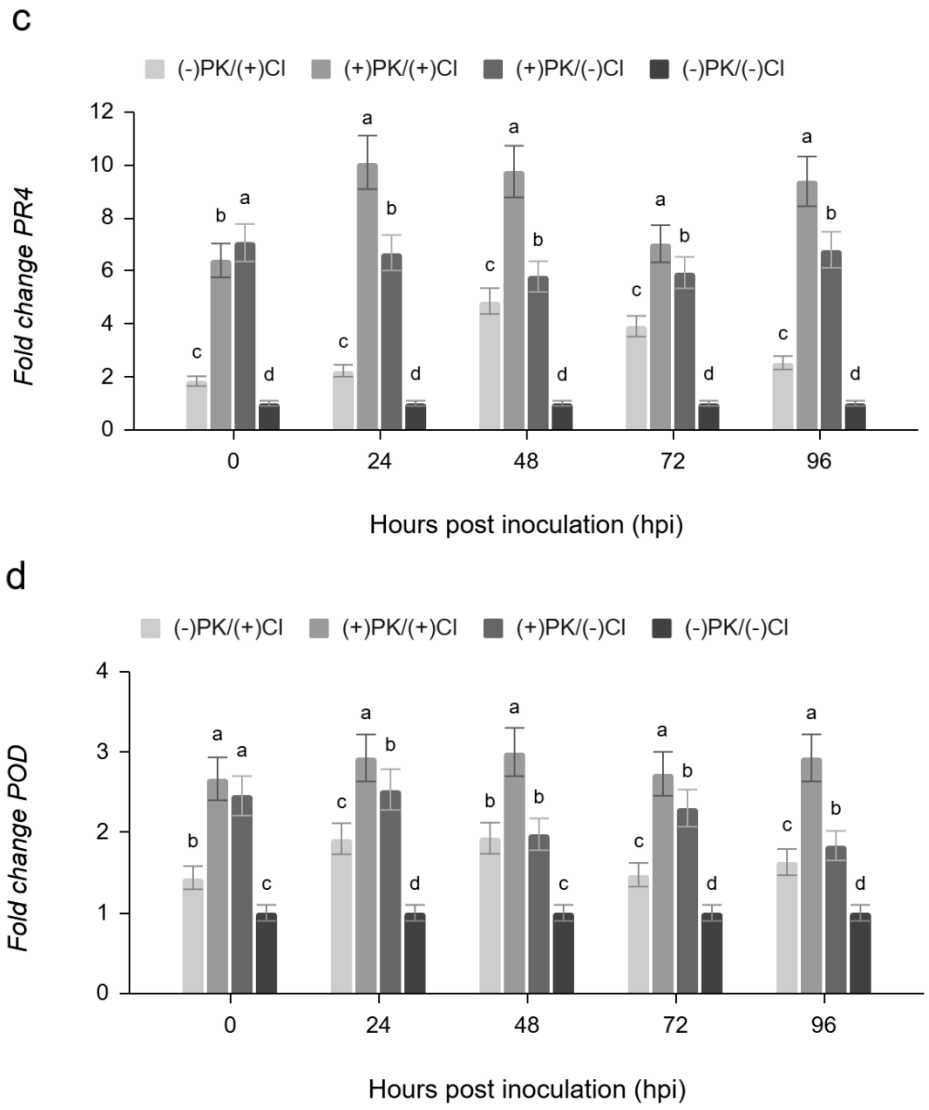

Figure 4. Differential expression of defense genes in Sutagao bean cultivar inoculated with *C. lindemuthianum* and treated with potassium phosphite solution at different time points. (a) Changes in *PR1* expression; (b) Changes in *PR3* expression; (c) Changes in *PR4* expression; (d) Changes in *POD* expression. Different letters indicate significant differences between treatments ($p<0.05$).

4. Discussion

Various studies have demonstrated the efficacy of phosphite base solutions similar to the product evaluated in this study (28% P_2O_5, and 26% K_2O) in controlling anthracnose caused by *Colletotrichum* species, as well as their fungicidal activity in *in vitro* evaluations [9]. In this work, *C. lindemuthianum* showed a significant reduction in growth (42%) and conidial germination percentage (48%) on PDA supplemented with 5 mL L^{-1} of potassium phosphite solution, similar to the decrease in mycelial growth (62.1%) and germ tube emission in *C. lindemuthianum* reported by others authors [20]. However, another study showed that a dose of 5 ml L^{-1} of phosphite A (26% P_2O_5 and 19% K_2O) and phosphite B solution (33.6% P_2O_5 and 29% K_2O) had a significant effect, inhibiting mycelial growth by 81.1% and 100%, respectively [9]. Evaluation of the dose of phosphite ion in a formulation of (0-40-20 of N-P-K) (pH 3) on *C. gloeosporioides*, the causal agent of anthracnose in apple, reduced the colony diameter by 94% [21]. In the same way, doses of 5.0 mL L^{-1} and 10.0 mL L^{-1} of potassium phosphite (35.10% P_2O_5, 25.65% K_2O) inhibited the conidia germination by around 51.1% and 63.1%, respectively, in *C. gloeosporioides* isolated from coffee anthracnose [22]. The effects of phosphite exposure on fungal cells have been associated with alterations in phosphorus metabolism and in

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compounds containing this element, including inhibition of key phosphorylation reactions in fungi [10,11]. Likewise, these compounds have been associated with changes in gene expression related to the synthesis of the cell wall and cytoskeleton proteins [23]. Other authors have reported that potassium phosphite produces increases in inorganic polyphosphates, alterations in nucleotide reserves, pentose phosphate metabolism, disruption of ionic balance, and changes in protein kinase activation, which regulate the formation of infective structures of the pathogen **[8,10,11,13,24]**.

However, the reduction of bean anthracnose severity by the application of the potassium phosphite solution was smaller (17%) than reported by other authors, ranging from 56-69% [9], 74-81% [25], and 71% [26]. The differences could be related to the bean material used and the concentration of the pathogen inoculum. The genetic and variety of bean plants used in this study (Sutagao cultivar, derived from the cross between Cabrera and G2333) differs from Perola [9], BRS Majestoso [25], and IPR Tangará [26], characteristics that determine resistance or susceptibility to the pathogen depending on its race. In addition, the inoculum concentration of *C. lindemuthianum* used to infect the plants in this study (1×10^7 conidia mL^{-1}) was higher than $7x10^5$ conidia mL^{-1} [9,25] and $1x10^6$ conidia mL^{-1} [26] used by other authors.

Furthermore, the quantity of phosphite and potassium in the product, as well as the dose and time of application, may have also influenced the results. Other studies [9,25,26] evaluated various phosphite formulations with different concentrations of P_2O_5 and K_2O than those used in this study. However, one of the phosphites evaluated by other authors [25] corresponded to the same one used in this work, but applied at three different times to plants with a more advanced stage of development, at stages V4 (three trifoliate leaves), R5 (pre-flowering), and R7 (pod formation).

Similarly, phosphites have been evaluated in seeds with reapplication 18 days after sowing at the moment of expansion of the second trifoliate leaf [26], which differs from the present study, in which only one application was performed. This suggest that to obtain better results in reducing anthracnose in bean caused by *C. lindemuthianum*, it is necessary to carry out several applications of this compound in seeds or at more advanced phenological stages, acting as a priming effect. This strategy improves the defense response of plants by triggering changes in the plant at physiological, transcriptional, metabolic, and epigenetic levels, preparing it with a better response capacity when exposed to a subsequent stimulus, such as attacks by a pathogen, activating immune mechanisms more quickly and strongly **[8,27]**.

The effect of phosphite as a strategy to reduce the severity of diseases in plants has been demonstrated mainly against oomycete pathogens, such as species of the genus *Phytophthora*, *Pythium*, and *Peronosclerospora sorghi.* However, it also acts against fungi such as *Alternaria alternata*, *Fusicladium effusum*, *Fusarium* spp., *F. oxysporum*, *Rhizoctonia solani*, *Verticillium* spp.; some protists such as *Plasmodiophora brassicae*; and bacteria such as *Pectobacterium carotovorum* and *Pseudomonas syringae* **[8,11,28]**. Nevertheless, changes in the expression of defense genes, in plants treated with these potassium phosphite products have not been thoroughly evaluated in plant-pathogen interactions that do not consider oomycetes.

This study demonstrated that the application of potassium phosphite solution to bean plants induced the expression of *PR1*, *PR3*, *PR4*, and *POD* defense genes. However, their expression increased when the plants were infected with *C. lindemuthianum*. Similar results have been described in potato plants that were previously foliar-sprayed with a potassium phosphite (KPhi)-based fungicide and infected with *Phytophthora infestans* [8]. In the present study, the expression of *PR3* was higher at 48 hpi, which was also reported to be the highest enzymatic activity for this protein (PR3) in potatoes treated with phosphite and infected with *P. infestans* [8]. Additionally, for *PR4*, another chitinase evaluated in this work, the expression level increased earlier than that for *PR3* (24 hpi) and was maintained until the last evaluation time (96 hpi) as well as for *PR3*. Similarly, high expression levels of genes associated with glucanases and chitinases in cucumber (*Cucumis sativus* L.) plants treated with potassium phosphite and in plants treated and inoculated with *Pseudoperonospora cubensis* [29], indicating a sensibilization of the plants that triggers a rapid defense response against pathogens. Therefore, chitinases are important in the early defense response, and their enhanced

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effect in plants previously stimulated with phosphite solution results, in increased sensitivity and activity against phytopathogens [30].

Concerning *PR1*, the potassium phosphite solution promoted its expression before infection with the pathogen. The highest expression of the gene was evident at 48 hpi, although it subsequently declined at 72 hpi, indicating that it did not maintain its expression. Increased expression of *PR1* in plants treated with phosphite before infection with a pathogen has been reported in plants principally infected with an oomycete. For example, pepper (*Capsicum annuum*) treated with potassium phosphite and inoculated with *Phytophthora capsici* and *Phytophthora cinnamomi* showed strong upregulation of soluble proteins and pathogenesis-related 1 (CaPR1) over time, which is associated with callose deposition and ROS production [31,32]. Although the detection of callose and H_2O_2 was not carried out in this study, previous work has compared the defense responses against *C. lindemuthianum* between the susceptible bean cultivar Sutagao, the same used in this study, and the resistant bean genotype G2333 [15]. These authors showed an increase in the expression of *PR1*, *PR3*, and *POD* genes at 1 dpi, coinciding with the time of H_2O_2 production in the resistant bean cultivar, in contrast to delayed expression of these genes in the susceptible bean cultivar. This indicates that *PR1*, *PR3*, and *POD* are markers associated with defense against the fungus and that the phosphite increased their expression in the Sutagao cultivar before and after infection.

It is important to highlight PR1 as a marker of systemic resistance, priming response, and transgenerational priming response against pathogen challenge [33]. It has been reported that an increase in some enzymatic activities, such as peroxidase and polyphenol oxidase, after treatment with phosphite and infection by a pathogen is related to the induction of a systemic defense response [28]. In this study, high POD expression was detected both in plants treated with phosphite and after inoculation with *C. lindemuthianum* from 24 hpi until the final evaluation time (96 hpi). This expression is related to the induction of ROS detoxification activity by the enzyme peroxidase (POD), which is generated as an antioxidant defense response to pathogen infection and further stimulated by phosphite [8,34]. In bean, an increase in POD enzyme activity has been reported in plants stimulated with phosphite, which is associated with detoxification **[8,35]**. In other plants stimulated with phosphite and infected before with the pathogen, an increase in POD activity has been reported, for example, cucumber plants with *Pythium ultimum* var [36]; apple (*Malus pumila*) with *Venturia inaequalis* [37]; potato plants (*S. tuberosum*) with *P. infestans* [8]

5. Conclusions

The potassium phosphite solution (P_2O_5 28% and K_2O 26%) has direct antifungal activity against *C. lindemuthianum*, the causal agent of anthracnose in common bean. It inhibits the growth, development, and conidial germination of the pathogen. The effect of this compound on Sutagao bean plants stimulated the expression of some defense-related genes, which significantly increased after pathogen infection, demonstrating the priming effect of this product.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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