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RESEARCH PAPER

Efficacy of phosphonic acid applied by spraying and seed treatment at various concentrations for controlling downy mildew disease in maize

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ABSTRACT

Downy mildew, caused by *Peronosclerospora* spp., is one of the major problems in maize cultivation. Several studies have reported the development of resistance in *Peronosclerospora* spp. to metalaxyl, which had previously been an effective control method for the disease. Other research suggests that the efficacy of phosphonic acid in controlling downy mildew in maize is inconsistent. The objective of this study was to evaluate the efficacy of seed treatment with phosphonic acid in controlling downy mildew in Bisi 18 maize. The treatments were arranged in a randomized block design with six replications. The observed variables included the incidence and severity of the disease, the area under the disease progress curve (AUDPC), maize yield, and the content of tannin and hydroquinone compounds in maize leaves. The results showed that both phosphonic acid applied by spraying and seed treatment significantly reduced the incidence and severity of downy mildew ($P < 0.05$). However, the concentrations of phosphonic acid used in seed treatment had no significant effect on the disease during 1–7 weeks after inoculation. Furthermore, all phosphonic acid treatments tended to increase the content of tannin and hydroquinone compounds in maize leaves, which should be confirm quantitatively.

Key words: maize downy mildew, phosphonic acid, seed treatment.

INTRODUCTION

Lampung Province is categorized as one of the largest maize-producing areas in Indonesia, where maize is considered one of the most important food crops because it provides products used for both food and feed. From 2020 to 2023, the maize planting area fluctuated between 2.33 to 2.76 million ha, while production remained relatively low, ranging from 5.53 to 5.99 tons per ha (BPS-Statistics Indonesia, 2023). The decline in maize production has been attributed to several factors, including plant diseases. One of the most significant diseases affecting maize is downy mildew, which is frequently reported as a severe threat that reduces maize yields in various regions of Indonesia, including Lampung Province (Ginting et al., 2023; Ginting et al., 2020; Muis et al., 2016; Widiantini et al., 2015; Hikmahwati et al., 2011).

Downy mildew disease in maize in Indonesia is reported to be caused by three *Peronosclerospora* species: *P. maydis*, *P. sorghi,* and *P. philippinensis* (Ginting et al., 2020; Muis et al., 2016; Hikmahwati et al., 2011). These pathogens have also been reported to infect maize plants in Lampung Province (Ginting et al., 2020; Muis et al., 2016; Muis et al., 2013). Infected maize plants exhibit two types of symptoms: systemic and local. Systemic symptoms occur when the pathogen reaches the plant's growing point. These symptoms appear as small chlorotic spots on the leaves of young infected plants, developing parallel to the leaf veins. As a result, the maize plants may become stunted. Local symptoms, on the other hand, manifest as chlorotic lines on the leaves when the pathogen does not reach the growing point. Additionally, the infection can produce visible signs of the disease in the form of conidia and conidiophores, which appear as a white, velvet-like layer on the lower surface of the infected leaves, especially in the morning.

Ginting et al. (2020) reported that downy mildew disease in maize often becomes epidemic in the field, even when farmers use superior seed varieties treated with metalaxyl. Although metalaxyl

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had been effective for more than two decades, it was reported to be ineffective in controlling the disease. This decline in efficacy was likely due to mutations in *Peronosclerospora*, the pathogen responsible for the disease (Isakeit & Jaster, 2005; Widiantini et al., 2015).

To overcome the problem, research needs to be conducted to identify alternative fungicides that can effectively suppress the pathogen. This study focuses on evaluating the efficacy of phosphonic acid as a potential alternative to metalaxyl. Phosphite, a salt derivative of phosphonic acid (H_3PO_3), is a fungicide that can move systemically within plants (Guo et al., 2021). In addition to its fungicidal activity, phospite has also been reported to act as a resistance inducer against pathogen infection (Guo et., 2021; Wu et al., 2019; Cerqueira et al., 2017). Wu et al. (2019) reported that phospite application increased various secondary metabolites associated with plant resistance to pathogens, including phenols. According to Jadesha et al. (2025), the resistance of maize inbred lines to downy mildew is also related to the presence of phenolic compounds, which are found in higher levels in resistant lines than in susceptible ones. Phenols are complex compounds that can be broken down into various derivatives such as tannins, hydroquinone, and others (Bhattacharya et al., 2010).

Although phosphonic acid has shown promise in controlling downy mildew in maize (e.g., Ginting et al., 2023), its efficacy has been inconsistent. This inconsistency may be due to delayed absorption of phosphonic acid during the vegetative stage, when plants are most susceptible to *Peronosclerospora* spp. Therefore, efforts are needed to enhance the early uptake of the active substance to maximize its effectiveness.

In this study, phosphonic acid was applied as a seed treatment to facilitate earlier absorption by the plants. The aims was to determine whether early application could enhance the resistance of maize plants to pathogens and improve its effectiveness in controlling downy mildew. For comparison, phosphonic acid was also applied via foliar spray. This comparison is crucial given the increasing resistance of the downy mildew pathogen to metalaxyl, which has long been the primary fungicide used for disease management in maize.

The objective of this study was to evaluate the efficacy of phosphonic acid applied as a seed treatment at various concentrations, as well as phosphonic acid applied through foliar spraying, in controlling downy mildew in the Bisi 18 maize variety.

MATERIALS AND METHODS

Research Site. The research was conducted from April to September 2024 at two locations: the Plant Disease Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Lampung, and a farmer's field in East Lampung Regency (5°11'10.9"S 105°45'22.4"E).

Experimental Design. The treatments were arranged in a randomized block design (RBD) with six replications. Each experimental unit consisted of a plot measuring 1.25 × 2 m, with seeds planted at a spacing of 25 × 75 cm, resulting in 14 plants per plot. The treatments were as follows: (1) Control (water/F0); (2) Phosphonic acid spray at a concentration of 6 mL/L (recommended concentration as control) applied at 1, 2, 3, 4, and 5 weeks after planting (WAP) (F1); (3) Seed treatment with phosphonic acid at a dose of 2.4 g/kg seed (F2); (4) Seed treatment with phosphonic acid at a dose of 4.8 g/kg seed (F3); (5) Seed treatment with phosphonic acid (PT Mitra Kreasidharma) at a dose of 9.6 g/kg seed (F4); (6) Seed treatment with phosphonic acid at a dose of 19.2 g/kg seed (F5).

Pathogen Identification. The *Peronosclerospora* species causing the disease in this experiment were identified by observing their conidia and conidiophores. The initial inoculum source was obtained from maize plants showing downy mildew symptoms, collected from farmers' fields in Natar District, South Lampung (5°20'20.6"S 105°14'09.6"E). The symptomatic plants, along with the soil around their roots, were placed in of 40 × 40 cm polybags and transported to a greenhouse at the Faculty of Agriculture, University of Lampung, for maintenance.

To prepare glass slides for observation, symptomatic maize plants were taken to the laboratory in the afternoon. The leaves were first gently washed under running tap water, wiped clean, and dried with tissue paper. The plants were then watered to remove any dirt or fungal debris. The symptomatic maize plants in polybags were placed on a water-filled tray to maintain high humidity, tightly covered with transparent plastic (60 × 100 cm), and incubated at 17 ºC for 8 hours (Ginting et al., 2020).

After incubation, the plastic cover was removed, and the lower surfaces of the leaves showing white, flour-like signs were sampled. Transparent tape was gently pressed onto the affected leaf surfaces to lift the conidia and conidiophores. The tape was then carefully removed and placed on a glass slide with a drop of 2%

methylene blue. The prepared slides were examined under a compound microscope to identify the conidia and conidiophores (Ginting et al., 2020).

Land Preparation, Maize Planting, and Inoculum Preparation. The land was prepared by clearing weeds, and hoeing the soil to a depth of approximately 20 cm to improve aeration. The area was divided into 36 experimental plots, each measuring 2 × 1.25 m². Planting holes were made to a depth of 3 cm with a spacing of 25 × 75 cm, and three Bisi 18 hybrid maize seeds were planted per hole. Before planting, seeds were washed to remove any fungicide coating. After germinated, thinning was performed to leave one healthy plant per hole, resulting in 14 plants per plot.

Inoculum Preparation and Inoculation. Inoculation was carried out using maize plants with downy mildew symptoms as the inoculum source. Maize seeds were planted in 25 × 25 cm polybags containing a mixture of soil, manure, and sand (2:1:1). Each polybag was planted with three Bisi 18 maize seeds. On the sixth day after planting (DAP), thinning was done to leave one plant per polybag. At, 7 DAP, plants were inoculated by dripping a *Peronosclerospora* conidial suspension directly onto the growing points.

The conidial suspension was prepared as follows: leaves from symptomatic plants (used for pathogen identification) were rinsed with sterile water. The plants in the polybags were placed on a tray and covered with transparent plastic (60 × 100 cm) at 17:00 WIB in a room maintained at 17 ºC. At 04:00 WIB the next morning, the plastic was removed, and the undersides of leaves with white fungal masses were brushed to collect conidia, which were suspended in 20 mL of sterile distilled water.

Inoculation of Source Plants and Test Plants. For source plants, maize seedlings at 7 DAP were cleared of dew or guttation water from their growing points using a pipette. Then, 0.1 mL (4 drops) of the conidial suspension was applied to each plant. Once these plants reached 4 weeks and showed symptoms, they were used as inoculum sources for inoculating test plants at 7 DAP.

Plant Maintenance and Harvesting. Plant maintenance included watering, fertilizing, and weeding. Fertilizers used were urea, triple superphosphate (TSP), and potassium chloride (KCL), applied in rows 5 cm from the plant stem. The recommended doses were urea (300 kg/ha), TSP (200 kg/ha), and KCL (50 kg/ha), equivalent to 75 g urea, 50 g TSP, and 12.5 g KCl per 2 × 1.25 m plot. Urea was applied in three splits: 35 g at 7 DAP (together with all TSP and KCl), 25 g at 30 DAP, and 15 g at 45 DAP. Weeding was done manually by hand and hoe.

Harvesting was conducted at 110 DAP when stems, leaves, and husks turned yellow and dry, and kernels became shiny and hard. The husks were peeled, and cobs were sun-dried.

Observation. Observed variables included disease incidence, disease severity, area under the disease progress curve (AUDPC), and maize yield.

Disease incidence (DI). DI was measured 1–7 weeks after inoculation (WAI) using (Ginting & Aeny, 2020):

$$DI\ (\%) = \frac{n}{N} \times 100$$

DI = Disease incidence (%);
n = Number of symptomatic plants;
N = Total plants observed.

Disease severity (DS). DS was also measured 1–7 WAI using (Ginting & Aeny, 2020):

$$DS\ (\%) = \frac{\sum (n \times V)}{N \times V} \times 100$$

DS = Disease severity (%);
n = Number of plants with a given score;
N = Total plants;
v = Disease score (Table 1);
V = Highest score.

Area Under Disease Progress Curve (AUDPC). AUDPC was calculated as (Ginting et al., 2020):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) \times \left(T_{i+1} - T_i \right)$$

AUDPC = Area under the disease progress curve,
X_i = Disease severit of i-th;
T_i = Observation time of i-th;
n = Number of observations.

For yield, harvested cobs were sun-dried, manually shelled, and weighed.

Qualitative Analysis of Phenolic Compounds (Tannin and Hydroquinone). Qualitative analysis was conducted at 28 days after inoculation (DAI) following

Soesanto et al. (2021). A 10 g of maize leaves were washed, ground with a mortar, mixed with 10 mL of 80% ethanol, and filtered with Whatman No. 1 paper.

***Tannin Test*:** To test for tannin content, 5 mL of the extract was placed into a test tube, and 3 drops of $FeCl_3$ solution were added. A color change in the extract to bluish-black or blue-green indicated the presence of tannins. To indicate the quality of tannin content, the color changes are grouped into 4 categories, i.e.,), - (no color change), + (slight change), ++ (slightly concentrated), +++ (concentrated), and ++++ (highly concentrated).

***Hydroquinone Test*:** To test for hydroquinone content, 5 mL of the extract was placed into a test tube, and 5 drops of 10% NaOH were added. A color change in the extract to red indicated the presence of hydroquinone. The method to indicate the quality of hydroquinone content was the same as that for tannin content.

Data Analysis. Data were tested for homogeneity (Bartlett's test) and additivity (Tukey's test). ANOVA was performed in MS Excel 2019, and means were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Symptoms of downy mildew disease in maize plants initially appeared on leaves near the growing point, close to the stem. These symptoms manifested as chlorotic areas developing parallel to the leaf veins and, in some cases, spreading across the entire leaf. Disease signs were often visible as a distinct whitish layer on the lower leaf surface. As the disease progressed, leaves and stems became stiff and brittle. In some plants, growth was stunted, and affected plants either failed to produce fruit or produced malformed cobs with poorly filled seeds. The symptoms and signs of maize downy mildew (MDM) observed in this study were consistent with those described by Ginting et al. (2020), Widiantini et al. (2015), and Hikmahwati et al. (2011).

Pathogen identification, conducted at the Plant Disease Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Lampung, revealed that the conidia were oval-shaped, measuring 14.9–15.8 µm by 17.0–17.3 µm. The conidiophores were 212 µm long and exhibited two levels of branching (Figure 1). Based on conidial and conidiophore morphology, the pathogen was identified as *Peronosclerospora sorghi* (CIMMYT, 2012). Ginting et al. (2020) reported that the causal agents of MDM in Lampung included three *Peronosclerospora* species*: P. sorghi, P. maydis,* and *P. philippinensis.*

The effects of phosphite acid treatment, applied either through spraying or seed treatment, on the incidence and severity of downy mildew, as well as the Area Under Disease Progress Curve (AUDPC), are presented in Figures 1, 2, and 3, respectively. Both application methods, spraying and seed treatment,

Tabel 1. Scores for downy mildew

Scores	Score description	Level of disease intensity
0	No symptoms	No symptoms
1	Symptoms occur in up to 10% of plants	Mild
2	Symptoms occur in more than 10 to 25% of plants	Somewhat severe
3	Symptoms occur in more than 25 to 50% of plants	Severe
4	Symptoms occur in more than 50% of plants	Very severe

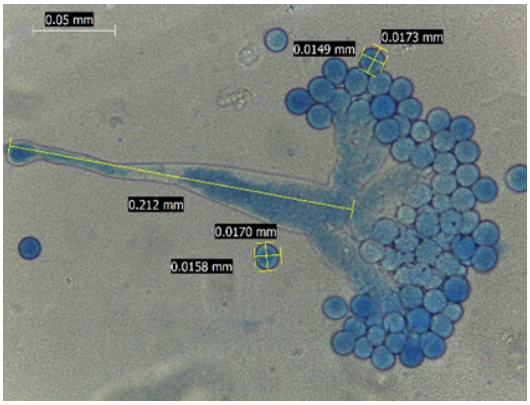

Figure 1. Conidiophore and conidia structure of *Peronosclerospora sorghi.*

significantly reduced downy mildew incidence ($P < 0.05$). However, the concentration of phosphite acid in the seed treatment did not significantly affect disease incidence during the 1–7 weeks after inoculation (WAI) period (Figure 2).

Similar results were observed for other variables. Phosphonic acid, whether applied through spraying or seed treatment, significantly reduced disease severity ($P < 0.05$). However, the concentration of phosphonic acid in seed treatment had no significant effect on disease severity between 1 and 7 (Figure 3). Both spraying and seed treatment with phosphonic acid also significantly reduced the AUDPC of downy mildew disease ($P < 0.05$), although the concentration of phosphonic acid in seed treatment had no significant effect on AUDPC (Figure 4).

In this experiment, analysis of the three variables, disease incidence, disease severity, and AUDPC, demonstrated the effectiveness of phosphonic acid in reducing downy mildew intensity in maize. These findings align with the report by Panicker & Gangadharan (1999), which indicated that phosphonic acid can reduce both incidence and severity of downy mildew caused by *P. sorghi* in maize. Additionally, Tias (2017) reported that phosphonic acid effectively controls maize downy mildew in the hybrid maize variety P-27. Phosphonic acid has also been reported to suppress conidial tube germination and growth of *P. maydis*, thereby reducing disease intensity (Sari, 2018). Beyond its direct inhibitory effects on pathogens, phosphite has also been reported to induce plant resistance to infection (Guo et., 2021; Wu et al., 2019; Cerqueira et al., 2017).

The results of the data analysis showed that the

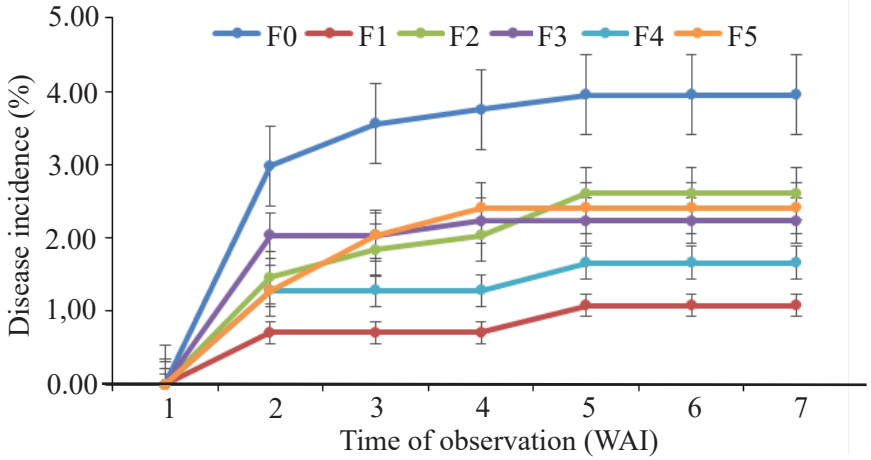

Figure 2. Effect of phosphitic acid fungicide concentration on the incidence of downy mildew in maize plants during 1–7 WAI (weeks after inoculation). F0 = control; F1 = spray 6 mL/L, applied 1–5 weeks after planting; F2 = seed treatment at 2.4 g/kg seed; F3 = seed treatment at 4.8 g/kg seed; F4 = seed treatment at 9.6 g/kg seed; F5 = seed treatment at 19.2 g/kg seed.

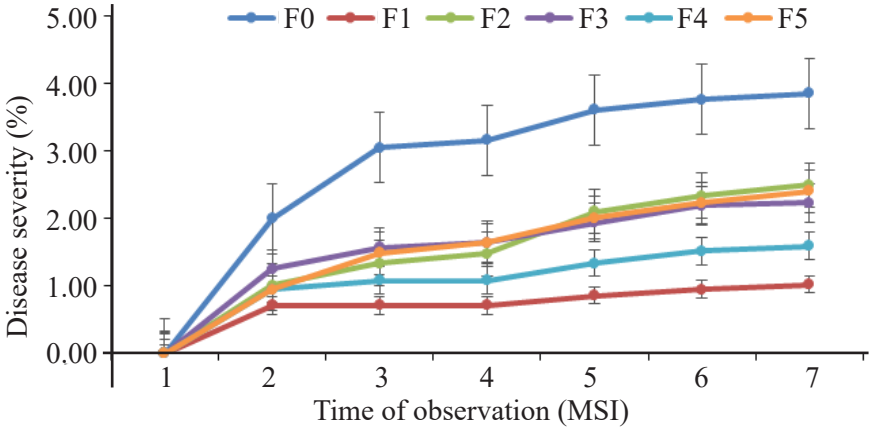

Figure 3. Effect of phosphitic acid fungicide concentration on the severity of downy mildew in maize plants during 1–7 WAI (weeks after inoculation). F0 = control; F1 = spray 6 mL/L, applied 1–5 weeks after planting; F2 = seed treatment at 2.4 g/kg seed; F3 = seed treatment at 4.8 g/kg seed; F4 = seed treatment at 9.6 g/kg seed; F5 = seed treatment at 19.2 g/kg seed.

application of 6 mL/L phosphonic acid at 1–5 weeks after planting (F1 treatment) significantly increased maize production. However, all other phosphonic acid seed treatments had no significant effect on yield (Figure 5). In other words, except for the F1 treatment, all phosphonic acid applications did not significantly increase maize production, despite reducing disease intensity. This suggests that while reducing downy mildew intensity helps lower disease pressure, it may not be sufficient to significantly enhance physiological processes in maize plants that drive yield improvement. Further research is needed to enhance phosphonic acid effectiveness, such as combining it with other chemical or biological agents, or exploring alternative control techniques.

Phytochemical analysis revealed that all phosphonic acid treatments increased tannin and hydroquinone content in maize plants, except for the F1U5 and F4U5 treatments, where tannin levels were similar to those in untreated plants (Table 2). At low concentrations, phosphonic acid can stimulate the plant's natural defenses, leading to increased activity of antimicrobial compounds and enzymes such as phytoalexins, peroxidases, and polyphenol oxidases (Havlin & Schlegel, 2021). A similar study should be conducted to confirm these phytochemical results, as the findings in this experiment were inconsisten.

For example, Guest & Bompeix (1990) found that phosphite treatment induces a strong and rapid defense response in the host plant, inhibiting pathogen proliferation. Although phosphite concentrations in planta only partially inhibit pathogen growth in vitro, it effectively controls *Phytophthora*-induced diseases in tobacco, capsicum, and cowpea. Additionally, foliar application of phosphonic acid has been shown to increase pectin levels in plant cell walls and inhibit

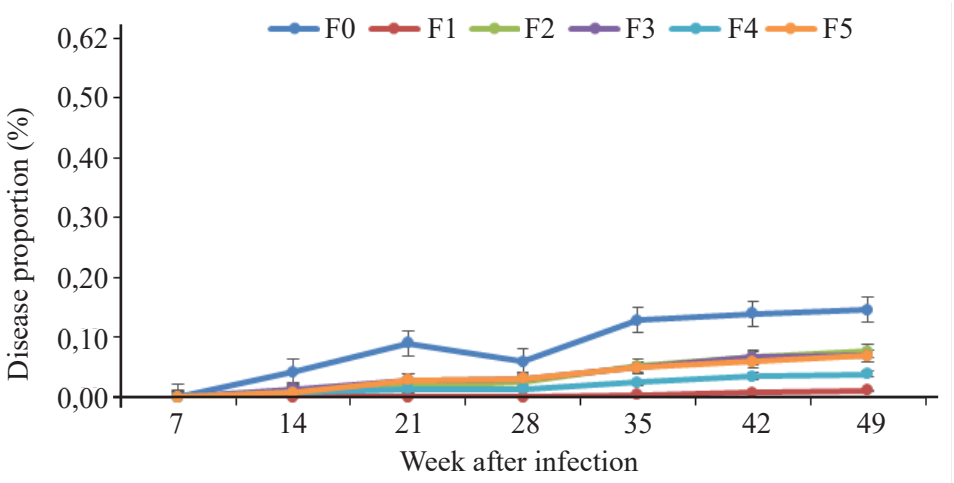

Figure 4. Effect of phosphitic acid fungicide concentration on the AUDPC of downy mildew in maize plants during 1-7 WAI (weeks after inoculation). F0 = control; F1 = spray at 6 mL/L, applied 1–5 weeks after planting; F2 = seed treatment at 2.4 g/kg seed; F3 = seed treatment at 4.8 g/kg seed; F4 = seed treatment at 9.6 g/kg seed; F5 = seed treatment at 19.2 g/kg seed.

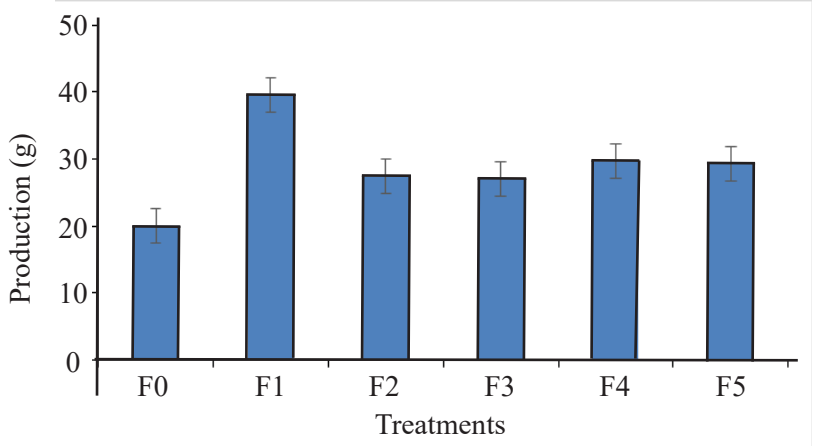

Figure 5. Maize crop production following the application of phosphitic acid at various doses. F0 = control; F1 = spray at 6 mL/L, applied 1–5 weeks after planting; F2 = seed treatment at 2.4 g/kg seed; F3 = seed treatment at 4.8 g/kg seed; F4 = seed treatment at 9.6 g/kg seed; F5 = seed treatment at 19.2 g/Kg seed.

polygalacturonase activity (Achary et al., 2017). Pectin, an important structural component of plant cell walls, serves as a barrier to pathogen entry, while polygalacturonase is an enzyme secreted by many pathogens, including oomycetes, to degrade pectin during infection (Götesson et al., 2002).

In this study, phosphonic acid application increased tannin and hydroquinone content in maize leaves. Tannins and hydroquinones are phenolic secondary metabolites. According to Kumar et al. (2020), various phenolic compounds can act as allelochemicals, phytoalexins, and phytoanticipins. Mohammadi et al. (2019, 2020) reported that phosphite application conferred resistance to *Phytophthora* infection in potato tubers, and this resistance was associated with the accumulation of specific phenolic compounds in phosphite-treated tubers. Han et al. (2021) observed that phosphite application increased phenolic content in potato leaves and enhanced expression of resistance genes. Wu et al. (2019) demonstrated that phosphite treatment elevated chlorogenic acid, caffeic acid, and salicylic acid levels in potato leaves. These compounds are recognized as key components of plant defense responses to both biotic and abiotic stresses.

CONCLUSION

The application of phosphonic acid, whether by spraying or seed treatment, significantly reduced both the occurrence and severity of downy mildew ($P < 0.05$). However, the concentration of phosphonic acid in seed treatment had no effect on the disease between 1 and 7 weeks after inoculation. All phosphonic acid treatments appeared to increase the content of tannin and hydroquinone compounds in maize plants, with a few exceptions as discussed above.

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AUTHORS' CONTRIBUTIONS

CG, TM, HaS, and HeS designed and planned the experiment. CG coordinated the experiment, including laboratory and field work, and contributed to writing the manuscript. TM, HaS, HeS, and HMA provided advice during the experiment and assisted in manuscript preparation. EAP participated in parts of the

Table 2. Phytochemical analysis of maize plants following the application of phosphitic acid at various concentrations

Treatments	Results	
	Tannin	Hydroquinone
F0U1	+	+
F0U4	+	+
F1U2	+++	++++
F1U5	+	+++
F2U3	+++	++++
F2U6	++	+++
F3U3	++	+++
F3U6	++++	+++
F4U2	++	+++
F4U5	+	+++
F5U1	+++	+++
F5U4	+++	+++

F0 = No fungicide; F1 = Phosphitic acid spray application, 6 mL/L, applied 1–5 WAP (weeks after planting); F2 = Phosphitic acid seed treatment, 2.4 g/kg seed; F3 = Phosphitic acid seed treatment, 4.8 g/kg seed; F4 = Phosphitic acid seed treatment, 9.6 g/kg seed; F5 = Phosphitic acid seed treatment, 19.2 g/kg seed; U = replicate; - = No color change; + = Slight change; ++ = Slightly concentrated; +++ = Concentrated; ++++ = Highly concentrated.

experiment, including laboratory and field work. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there are no competing interests, whether financial or non-financial, and no professional or personal relationships that could have influenced the work submitted for publication.

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