P-ISSN: 3009 – 7487
E-ISSN: 3009 – 7886

JOURNAL OF PLANT AND FOOD SCIENCES

June 2025

Dramatic Therapeutic Potential of Phosphite-Induced Resistance Against Fusarium Wilt in Pepper Plant

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Received: Received: 19th April 2025; in revised form: 19th April 2025/ Accepted: 11th June 2025/ Published: 13th June 2025
DOI: 10.21608/jpfs.2025.374287.1030

ABSTRACT

Wilt disease, caused by *Fusarium oxysporum*, is a major fungal disease responsible for considerable economic losses in a wide range of crops globally, including pepper (*Capsicum annuum* L.). Therapeutic nutrients play a crucial role not only in promoting plant growth but also in enhancing the plant's immune responses against pathogenic attacks. From enhancing defense responses to aiding cellular homeostasis, these nutrients have far-reaching impacts on plant health infection conditions. This study employed calcium phosphite (MAXIFOS Ca®) and copper phosphite (MAXIFOS Cu®) to stimulate growth-promoting strategies and activate resistance mechanisms in pepper plants (*Capsicum annuum* L.). Also, the antioxidant enzyme peroxidase (POD) and polyphenol oxidase (PPO) activities, as well as photosynthetic pigments, free proline, total phenol, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), were used to evaluate the promise of resistance of growing pepper plants. The results demonstrated that calcium phosphite was the most effective treatment, achieving a 35% reduction in the percent disease index (PDI) and enhancing plant protection by 57.7%. Furthermore, photosynthetic pigment content was substantially reduced in infected plants. The disease caused significant physiological and biochemical disruptions, including a 181% increase in MDA, 115 % increase in H_2O_2, a 32% increase in total phenol, and a 24 % increase in free proline. Treatment with tested inducers, especially calcium phosphite, significantly reversed these effects. MDA and H_2O_2 levels decreased by 63% and 34%, respectively. The results confirm the therapeutic role of of MAXIFOS Ca® and MAXIFOS CU® as a protection approach against *F. oxysporum* may be advised.

Keywords: *Fusarium oxysporum*, therapeutic nutrients, MAXIFOS Ca®, MAXIFOS Cu®, and immune responses.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is considered one of the most important and productive vegetable crops cultivated in subtropical regions worldwide (Datta *et al.*, 2021). However, its production is severely constrained by various biotic stresses, among which Fusarium wilt, caused by *Fusarium oxysporum*, represents a major threat.(Abdelaziz *et al.,* 2023). This soil-borne pathogen invades the plant's vascular system, leading to wilting, chlorosis, stunted growth, and often plant death (Srivastava *et al.,* 2016). Vegetable diseases that cause whole or part crop loss restrict plant defense (Shafique *et al.,* 2016). These fungi hinder the growth of peppers by more than 80%, and even infected plants that grow suffer from a lack of water and nutrients reaching the plant parts. This causes significant physiological and morphological changes that reduce or completely eliminate production loses (Abdelaziz *et al.,* 2022).

One of the most serious diseases that can affect pepper plants is leaf spot disease, which is brought on by *Fusarium oxysporum* (Bereika *et al.,* 2020; Manda *et al.,* 2020). *Fusarium oxysporum* can attack the host plant through roots, leaves, natural openings, and plant wounding. Fusarium infection has been reported to impair photosynthesis and reduce pigment content, leading to overall growth suppression and a significant decline in crop yield (Albalawi *et al.,* 2022; Hossain *et al.,* 2002). In recent years, increasing attention has been given to Therapeutic nutrition as sustainable alternatives to synthetic chemicals. Among them, phosphite-based compounds—particularly calcium phosphite and copper phosphite—have emerged as promising agents(Dervaric, 2023). These compounds not only exhibit direct antifungal activity, but also function as elicitors of plant immunity, triggering a cascade of physiological and biochemical responses that enhance resistance against various pathogens. Therapeutic nutrition refers to the strategic application of nutrients that activate and enhance key physiological processes in plants, thereby improving their ability to withstand biotic and abiotic stresses, mitigate disease symptoms, and reduce the adverse effects associated with plant pathogens (Attia *et al.*, 2016; El-Moneim *et al.*, 2021; Kumar & Verma, 2018; Sundström *et al.,* 2014). The novelty of our study was that Calcium phosphite (MAXIFOS Ca®) and copper phosphite (MAXIFOS CU®) for controlling *Fusarium oxysporum* as well as in enhancing pepper plant growth. Calcium is an essential mineral that promotes plant growth and development by regulating various physiological processes (Waraich *et al.*, 2012). Copper phosphite can improve the availability of this essential micronutrient. Copper is a cofactor for several enzymes involved in chlorophyll synthesis and photosynthetic electron transport (Batista *et al.,* 2020). Phosphite ions have been shown to induce systemic acquired resistance (SAR) in plants, making them more resistant to pathogens (Mohammadi *et al.,* 2021). Copper phosphite (MAXIFOS CU®) can help mitigate oxidative stress by enhancing the plant's antioxidant defenses, thereby protecting chlorophyll from degradation (Attia *et al.,* 2023).

Therefore, the present study aimed to evaluate and compare the protective effects of Calcium phosphite (MAXIFOS Ca®) and copper phosphite (MAXIFOS CU®) against *Fusarium oxysporum* in pepper plants. The investigation focused on assessing their roles in disease suppression, regulation of photosynthetic pigments, accumulation of phenols and proline, reduction of lipid peroxidation and H_2O_2 levels, and enhancement of antioxidant enzyme activity. The goal was to determine their potential as eco-friendly and effective tools for managing Fusarium wilt and improving plant health under pathogen stress.

MATERIALS AND METHODS

Fungal pathogen:

This fungal pathogen was obtained from Al-Azhar University's Regional Center for Mycology and its identity was verified through the use of established laboratory identification techniques (Hibar *et al.,* 2007).

Source of inducers:

Calcium phosphite (MAXIFOS Ca®) and copper phosphite (MAXIFOS Cu®), used as therapeutic nutrients in this study, were originally manufactured by MAFA-VEGETAL ECOBIOLOGY, Spain.

Trial Design:

For the purposes of this study, pepper seedlings (*Capsicum annuum* L., F1 hybrid) were transplanted into pots measuring 40 cm in diameter, each filled with 6.5 kg of a sand-to-clay mixture in a 1:3 (w/w) ratio. The pots were maintained under greenhouse conditions, with temperatures ranging from 22°C during the day to 18°C at night, and relative humidity levels between 70% and 85%. One week after inoculation, six treatment groups were arranged in a randomized order as follows:

- (T1) Healthy control
- (T2) Infected control
- (T3) Healthy plants treated with Calcium phosphite (MAXIFOS Ca®) 3ml/L
- (T4) Healthy plants treated with copper phosphite (MAXIFOS CU®) 3ml/L
- (T5) Infected plants treated with Calcium phosphite (MAXIFOS Ca®) 3ml/L
- (T6) Infected plants treated with copper phosphite (MAXIFOS CU®) 3ml/L

Each treatment was replicated three times.

Disease symptoms and disease index:

Disease symptoms, disease index (DI), and protection percentage were evaluated with slight modifications to a previously established method(Attia *et al.,* 2020).

Biochemical defense indicators:

Fresh pepper leaves were analyzed for photosynthetic pigments, including chlorophyll a, chlorophyll b , and carotenoids, following a standard procedure. (Attia *et al.,* 2020). . The proline content in fresh pepper plant leaves was measured following a method outlined by (Irigoyen *et al.,* 1992). The total dry phenol content was measured using the method described by (Dai *et al.,* 1993) .

Stress markers:

The method used by (Hu *et al.,* 2004) was applied to determine the amount of MDA in fresh pepper leaves. while hydrogen peroxide (H_2O_2) content was measured according to the protocol outlined by (Mukherjee & Choudhuri, 1983).

Assay of antioxidant enzyme activity:

For the assay of antioxidant enzyme activity, 2 g of pepper tissue was homogenized in 10 mL of phosphate buffer (pH 6.8, 0.1 M). The rate of change in absorbance due to pyrogallol oxidation was monitored using a UV spectrophotometer (Jenway) within 60 seconds at 470 nm and 25°C, following the procedure outlined by (Bergmeyer, 1965). The method employed by (Matta and Dimond, 1963) was utilized to calculate the activity of PPO.

Statistical analyses:

The data were analyzed using a one-way analysis of variance (ANOVA). Significant differences between treatments were determined using the least significant difference (LSD) test at $p < 0.05$, with the analysis conducted using CoStat software. The data ($n = 3$) are presented as means ± standard errors.

RESULTS AND DISCUSSION

Disease symptoms and disease index:

The reduction in disease index (DI) observed in treated plants compared to the infected control confirms the role of phosphite compounds in enhancing disease resistance(Attia et al., 2024). As shown in the results in Table 1, the incidence of infection caused by *Fusarium* reached 80.0%. However, calcium phosphite was the most effective treatment, reducing the percent disease index

(PDI) by 35% and increasing protection by 57.7%. Copper phosphite treatment followed, resulting in a PDI of 27.5% and a 66.6% increase in protection. Notably, copper phosphite achieved the greatest reduction in DI , suggesting a superior capacity to suppress pathogen development, possibly due to the combined antimicrobial properties of copper and the resistance-inducing nature of phosphite(Gómez-Merino *et al.,* 2022).

Table 1: Effect of Calcium phosphite and copper phosphite on disease index.

Treatments	Disease symptoms Classes					DI (disease index) (%)	Protection (%)
	0	1	2	3	4		
Infected control	0	0	2	3	5	82.5 [a]	0 [c]
Infected + Calcium phosphite	3	3	2	1	1	35 [b]	57.7 [b]
Infected + copper phosphite	3	4	2	1	0	27.5 [c]	66.6 [a]
L.S.D.						1.99	1.63

Photosynthetic pigments:

The results presented in Figure 1 show a significant reduction in the levels of chlorophyll a and chlorophyll b in Fusarium-infected plants. In contrast, carotenoid content was significantly increased by 30% in infected plants relative to healthy controls. Treatment with copper phosphite on healthy plants resulted in the most significant increases, with chlorophyll a level rising by 43% and chlorophyll b levels rising by 75%. Meanwhile, Ca phosphite treatment led to the most notable increase in carotenoid content, with a 62% rise compared to untreated healthy plants. Regarding the effect of the tested inducers on Fusarium-challenged plants, Ca phosphite appeared as the greatest effectual treatment, showing the largest significant increase in chlorophyll a content (98%). On the other hand, copper phosphite induced the greatest increase in chlorophyll b (90%) and carotenoid content (60%) compared to untreated infected plants. These findings suggest that the protective action of phosphites involves the stabilization of chloroplast structures and maintenance of photosynthetic machinery under pathogen stress(Li *et al.,* 2025). In addition to photosynthetic restoration, phosphite treatments activated key defense-related metabolic pathways, as evidenced by increased accumulation of total phenolic compounds(Mohammadi *et al.,* 2021). This disease mitigation was strongly associated with improvements in photosynthetic pigment content(Simkin *et al.,* 2022).This is due to its involvement in promoting improved nutrient intake and general plant health, which can lead to more strong chlorophyll production (Mohammadi *et al.,* 2020). Fusarium infection significantly reduced chlorophyll a and b, impairing photosynthetic performance, while carotenoid content increased—likely as a stress response(Abdelaziz *et al.,* 2022).

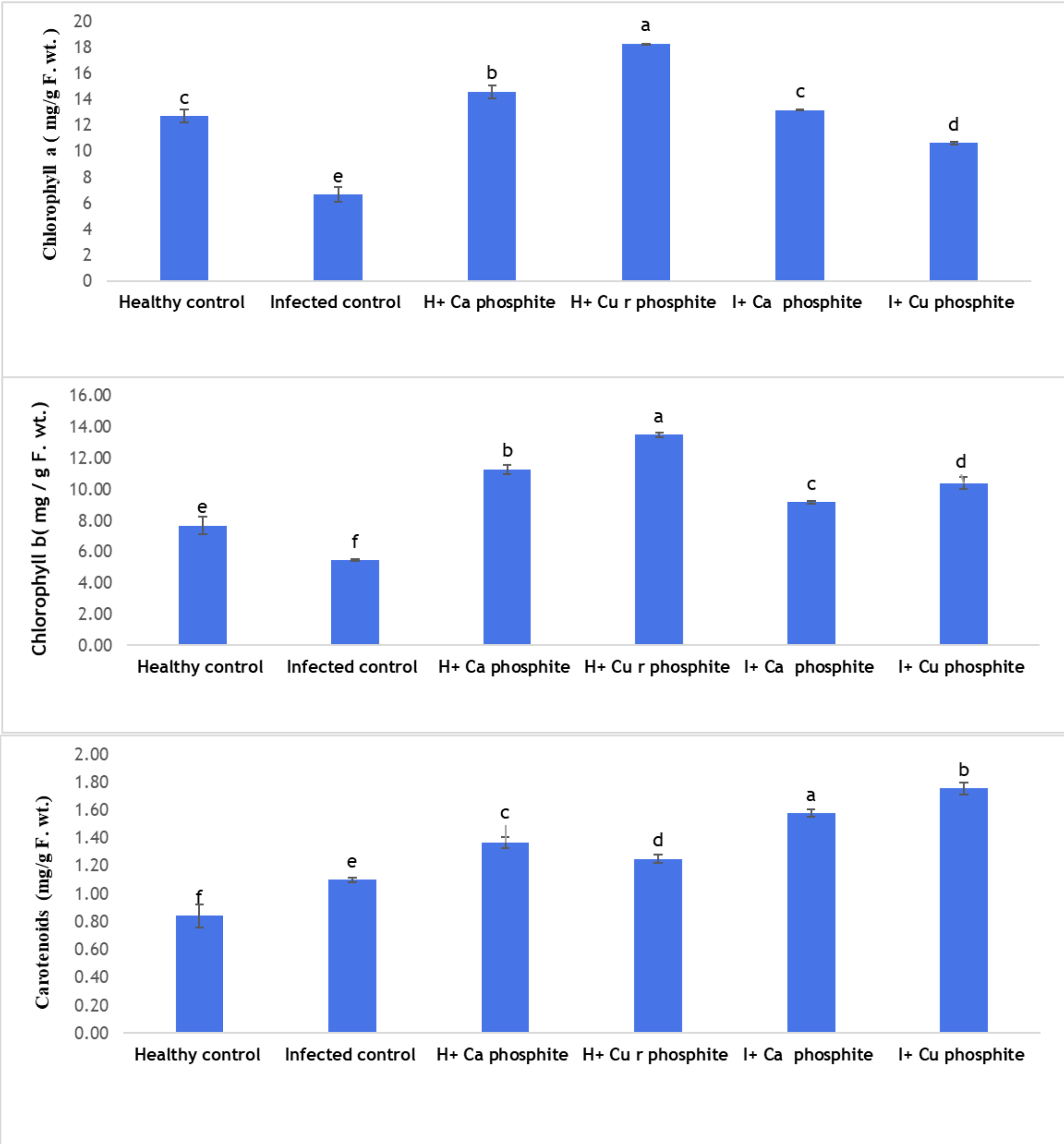

Figure 1 : Effect of Ca phosphite and CU phosphite on photosynthetic pigments of plants.

Free proline and Phenol content:

The data showed a significant increase in free proline levels in Fusarium-infected plants, with an enhancement of 27% compared to healthy controls (Figure 2). Regarding the effect of Ca phosphite and CU phosphite on healthy plants, it was noticed that the plant's proline increased by 94 % and 58 % in response to copper phosphite and calcium phosphite, respectively. Additionally, copper phosphite proved to be the most effective treatment, resulting in a significant increase in free proline content by 129%, followed by calcium phosphite, which increased proline levels by 93%, compared to untreated infected plants.

The data demonstrated that Fusarium infection led to a significant increase in the total phenol content of infected plants by 32% (Figure 3). Regarding the effect of Ca phosphite and CU phosphite on healthy plants, total phenol content increased by 68% and 40%, respectively. CU phosphite was found to be the most effective treatment, resulting in a substantial increase in total phenol content by 103%, while Ca phosphite increased it by 80.6%, compared to untreated infected plants (Figure 3). In this experiment, Fusarium infection resulted in increased levels of total phenols and free proline in the plants (Abdelhameed *et al.,* 2023;

Attia *et al.,* 2022). Furthermore, phenolic compounds may enhance the stability of cellular membranes by reducing their flexibility (Stanley & Parkin, 1991). Phenols are central to plant defense due to their roles as antioxidants and antimicrobial agents. The superior phenolic response induced by copper phosphite reinforces its role in promoting secondary metabolite biosynthesis as part of the plant's defense arsenal(Al-Khayri *et al.,* 2023). Furthermore, both treatments significantly enhanced proline accumulation, an important osmolyte and ROS scavenger. Copper phosphite, in particular, induced a 129% increase in proline in infected plants, surpassing the 93% increase observed with calcium phosphite. This suggests that phosphites not only confer structural protection but also regulate osmotic balance and stress-related metabolic adjustments. Phenolic compounds play a crucial role in preventing infection by either generating a wide range of metabolic byproducts involved in host defense systems or by reducing pathogen toxicity while boosting the plant's protective mechanisms (Shalaby & Horwitz, 2015). Several previous results corroborated that phosphite has been shown to induce systemic acquired resistance (SAR) in plants, making them more resistant to pathogens (Mohammadi *et al.,* 2021; Reverchon & Méndez-Bravo, 2021).

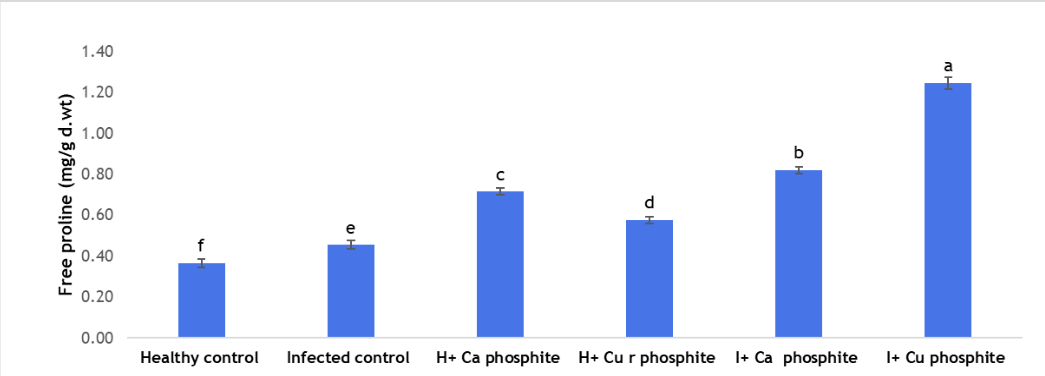

Figure 2: Effect of Ca phosphite and Cu phosphite on free proline of pepper plants.

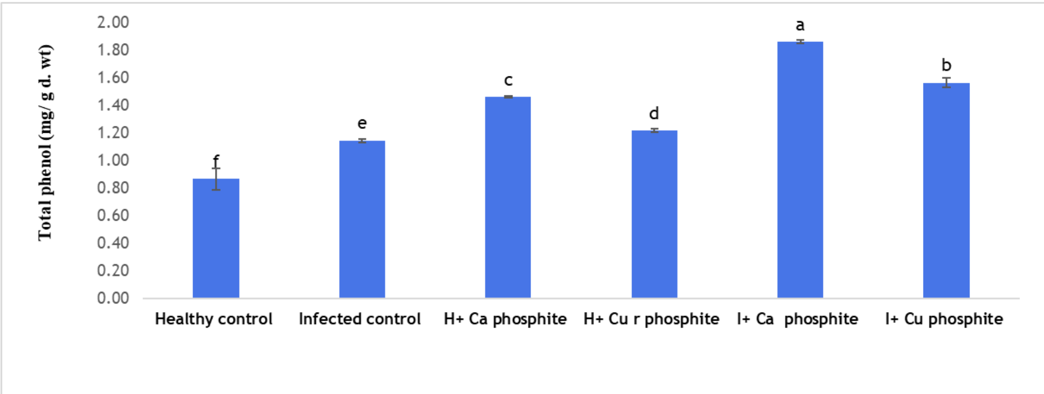

Figure 3: Effect of Ca phosphite and Cu phosphite on total phenol of pepper plants.

Stress markers (MDA and H_2O_2):

The data exhibited that Fusarium caused a notable and significant rise in (MDA and H_2O_2) of the infected plants (Table 2). Oxidative stress parameters further supported the role of phosphites in alleviating infection-induced damage (Liu *et al.,* 2016). In infected controls, levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were markedly elevated, indicating membrane lipid peroxidation and ROS accumulation(Akbar *et al.,* 2020). Also, a key oxidative stress marker, H_2O_2 levels were lower in phosphite-treated plants, with calcium phosphite showing the greatest reduction. This demonstrates the potential of calcium phosphite in enhancing antioxidant defenses and reducing cellular oxidative damage. Their protective effects are not limited to direct pathogen suppression but extend to the modulation of key physiological and biochemical pathways (Attia *et al.,* 2024). The notable superiority of copper phosphite in enhancing phenol, proline, and reducing MDA as well as H_2O_2 levels suggests its broader action spectrum and highlights its value in integrated disease management (Danish *et al.,* 2025).

Table 2: Effect of Ca phosphite and Cu phosphite on MDA and H_2O_2 of pepper plants. Data represent mean ± SD, n = 3, letters revered to significant in static analysis.

Treatments	MDA mg/g. f. wt.	H_2O_2 mg/g. f. wt.
Healthy control	2.39 ± 0.55 ^{cd}	0.44 ± 0.02 ^{d}
Infected control	6.72 ± 0.09 ^{a}	0.96 ± 0.04 ^{a}
H+ Ca phosphite	2.19 ± 0.36 ^{d}	0.28 ± 0.22 ^{f}
H+ Cu r phosphite	1.76 ± 0.13 ^{e}	0.35 ± 0.06 ^{e}
I+ Ca phosphite	3.76 ± 0.55 ^{b}	0.55± 0.48 ^{c}
I+ Cu phosphite	2.49 ± 0.48 ^{c}	0.63 ± 0.15 ^{b}
LSD at 5%	0.28901	0.0545

Antioxidant enzymes activity:

The data exhibited that Fusarium caused a notable and significant rise in (PPO and POD) activities of the infected plants (Figure 3 A and B). Regarding the effect of calcium phosphite and copper phosphite on infected plants, it was noticed that the PPO increased by (195 % and 122 %) in response to calcium phosphite and copper phosphite, respectively. Additionally, calcium phosphite was found to be the most effective treatment, causing a significant increase in PPO activity by 106%, followed by copper phosphite with a 64% increase, compared to untreated healthy plants (Figure 3A). Regarding the effect of calcium phosphite and copper phosphite on infected plants, the activity of POD increased by 123% and 113%, respectively, in response to calcium phosphite and copper phosphite. Additionally, copper phosphite was the most effective treatment, showing a significant increase in POD activity by 96%, followed by calcium phosphite with an 81% increase, compared to untreated healthy plants (Figure 3B). The enzymatic antioxidant defense system also responded positively to phosphite application. In infected plants, both polyphenol oxidase (PPO) and peroxidase (POD) activities were significantly elevated following treatment. Calcium phosphite induced the strongest increase in PPO (195%), while copper phosphite stimulated the highest POD activity (96%). This differential activation suggests that each phosphite compound may influence distinct branches of the oxidative defense network, thereby contributing synergistically to stress mitigation. Overall, the observed improvements across disease severity, pigment content, phenolic metabolism, osmolyte accumulation, oxidative stress markers, and enzymatic antioxidant activity clearly indicate that calcium and copper phosphites act as multifunctional elicitors of plant defense. In contrast, calcium phosphite's greater effect on chlorophyll a, PPO activity, and H_2O_2 scavenging suggests a stronger link to photosynthetic protection and ROS regulation (Chaves *et al.,* 2021). Copper is involved in the formation of peroxidase, an enzyme that protects cells from oxidative damage (Klotz *et al.,* 2003). By enhancing the plant's antioxidant system, copper phosphite helps maintain higher levels of chlorophyll and other pigments under stress conditions (Chaves *et al.,* 2021; Mohammadi *et al.,* 2021).

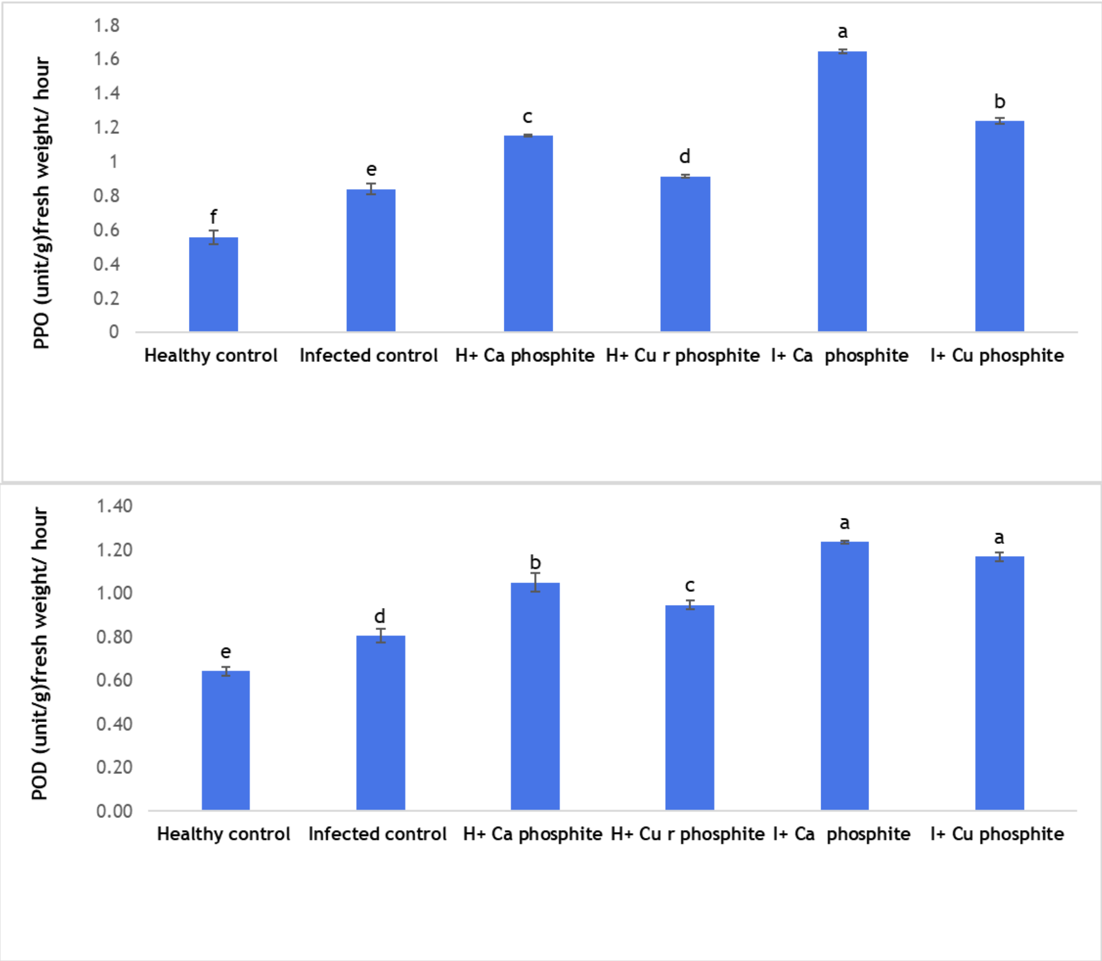

Figure 4: Effect of MAXIFOS Ca® and MAXIFOS CU® on (PPO and POD) activities of plants. H = healthy, I = infected. Data represents SD, n = 3), letters revered to significant in statically analysis).

Conclusion

The present study demonstrated that both calcium phosphite (MAXIFOS Ca®) and copper phosphite (MAXIFOS CU®) significantly mitigated the harmful effects of *Fusarium oxysporum* infection in pepper plants. The protective effects were evident through reduced disease severity, restoration of photosynthetic pigments, enhanced increase of phenolic compounds and proline, lowered oxidative stress markers (MDA and H_2O_2), and increased activity of antioxidant enzymes. Among the treatments, MAXIFOS CU® exhibited a more pronounced effect in terms of disease suppression, phenol and proline accumulation, POD activity, and membrane protection, indicating a strong induction of systemic resistance and stress adaptation. MAXIFOS Ca®, on the other hand, was more effective in enhancing chlorophyll a content, PPO activity, and H_2O_2 scavenging, suggesting its role in preserving photosynthetic function and reducing oxidative burden. Collectively, these findings support the conclusion that phosphite-based inducers can serve as effective components in sustainable disease management strategies, offering a dual benefit of pathogen control and physiological enhancement of plant health.

Ethical approval

There are no experiments on people or animals in this study.

Conflict of interest

All authors proclaim that there is no conflict of interest.

Authors' contributions

Conceptualization; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A.; Methodology; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A; Data Analysis; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A.; Figures and tables preparation; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A; Writing original draft preparation; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A., Writing review and editing M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A.; Resources; M.A.N, S.M.E, K.K.E, M.M.A, M.M.E and M.S.A.; All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Eng. Mahmud M. Elsayed for his valuable assistance throughout the study. Additionally, the authors are grateful to the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, for their support in promoting this research.

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