

Determination of the efficacies of different phosphites in the management of tomato bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato*

Pseudomonas syringae pv. *tomato*'nun neden olduğu domates bakteriyel benek hastalığının yönetiminde farklı fosfitlerin etkinliklerinin belirlenmesi

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ARTICLE INFO	ABSTRACT
<p>Article history: Recieved / Geliş: 27.06.2022 Accepted / Kabul: 12.09.2022</p> <p>Keywords: Tomato Phosphite <i>Pseudomonas syringae</i> pv. <i>tomato</i> Control</p> <p>Anahtar Kelimeler: Domates Fosfit <i>Pseudomonas syringae</i> pv. <i>tomato</i> Mücadele</p> <p>✉Corresponding author/Sorumlu yazar: Saad Mohamed HAJI NOUR sacdhaji45@gmail.com</p> <p>Makale Uluslararası Creative Commons Attribution-Non Commercial 4.0 Lisansı kapsamında yayınlanmaktadır. Bu, orijinal makaleye uygun şekilde atıf yapılması şartıyla, eserin herhangi bir ortam veya formatta kopyalanmasını ve dağıtılmasını sağlar. Ancak, eserler ticari amaçlar için kullanılamaz.</p> <p>© Copyright 2022 by Mustafa Kemal University. Available on-line at https://dergipark.org.tr/pub/mkutbd</p> <p>This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.</p> 	<p>This study tested the efficacy of five different phosphites (calcium, copper, magnesium, potassium and zinc/manganese phosphites) and a fungicide Fosetyl-Aluminum to inhibit bacterial speck disease severity caused by <i>Pseudomonas syringae</i> pv. <i>tomato</i> (<i>Pst</i>) on tomato leaves. The phosphites were applied at the recommended doses to the <i>Pst</i> inoculated plants in pots by foliar spraying at one-week intervals for a total of 4 weeks. The plants were kept in a controlled greenhouse under relative humidity (%75-90) and temperature (22-24 °C) until disease symptoms appeared in the control plants. Phosphites and Fosetyl-Aluminum inhibited the <i>Pst</i> symptoms on tomato leaves by 42.1-75.0% in the first and 22.8-90.3% in the second experiments. This study demonstrated the direct influence of phosphites on tomato bacterial speck. The study suggested that phosphites can be an effective alternative for the chemical control of tomato bacterial disease. The bacterial agent, <i>Pst</i>, causes bacterial speck disease in tomatoes. The initial symptoms of the disease are water-soaked, small dark brown spots surrounded by a yellow halo on tomato leaves. Since the pathogen is seed-borne, control of the disease is difficult.</p> <p>ÖZET</p> <p>Bu çalışmada, beş farklı fosfit (kalsiyum, bakır, magnezyum, potasyum ve çinko/mangan fosfit) ve fungusit Fosetyl-Alüminyum'un <i>Pseudomonas syringae</i> pv. <i>tomato</i> (<i>Pst</i>)'nun neden olduğu bakteriyel benek hastalığının domates yapraklarındaki gelişimini baskılama durumları test edilmiştir. Fosfitler saksıda bulunan <i>Pst</i> inokule edilmiş domates bitkilerine önerilen dozda 4 hafta boyunca haftada bir kez yapraktan püskürtme şeklinde uygulanmıştır. Bitkiler yüksek nem (%75-90) ve sıcaklıkta (22-24 °C) kontrollü serada kontrol bitkilerinde hastalık belirtileri gözleninceye kadar bekletilmiştir. Fosfitler ve Fosetyl-Alüminyum domates yapraklarında <i>Pst</i> belirtilerini birinci denemede %42.1-75.0, ikinci denemede ise %22.8-90.3 oranında azaltmıştır. Bu çalışma fosfitlerin domates bakteriyel benek hastalığının engellenmesi üzerine olan direk etkiyi ortaya koymuştur. Çalışma sonucunda fosfitlerin hastalığın mücadelesinde kimyasallara alternatif uygulama olarak kullanılabilceği vurgulanmıştır. Bakteriyel etmen, <i>Pst</i>, domateslerde bakteriyel benek hastalığına yol açar. Hastalığın yaprak belirtileri önceleri su emmiş, daha sonra etrafı sarı bir hale ile çevrili küçük koyu kahverengi lekeler şeklindedir. Hastalık etmeninin tohum kaynaklı olmasından dolayı hastalıkla mücadele oldukça zordur.</p>
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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) originated on the other side of the planet from where we are now. It is believed to be indigenous to the Western South American continent (modern-day Peru). The fruit was known as a tomato by the native Aztecs, who ate it raw and cooked it with other ingredients. Even though no one knows when humans first began cultivating them, they have been domesticated for more than 2.500 years, according to current estimates (Anonymous, 2019).

Tomatoes contribute to about a quarter of all vegetable production. Several formulations are available, and their beneficial impacts on human health are primarily derived from their containing high levels of antioxidants such as lycopene, folic acid, ascorbic acid, flavonoids, alpha-tocopherol, potassium, and phenolic compounds (Erba et al., 2013).

Countries like China, India, the United States of America (USA), Turkey, Egypt, Iran, Italy, Spain, Brazil and Mexico are the world's top tomato-growing countries. There are approximately 5.02 million hectares under tomato cultivation, with a total production of 170.75 million tons and a productivity of 33.99 tons ha⁻¹. According to FAO data for 2020, China contributes a significant portion of the entire tomato production amount of 186.821,216 tons, or 34.72%, of the total tomato production amount (FAOSTAT, 2021).

The yield of tomato plants is affected by a variety of bacterial, fungal, and viral diseases as well as pests. The prevalence of bacterial diseases that affect tomato plants is a significant problem, particularly in tomato fields worldwide. The most common bacterial diseases faced, particularly in greenhouse tomatoes, are pith necrosis and stem rot (*Pseudomonas corrugata*, *Pseudomonas cichorii*, *Pseudomonas viridiflava*, *Pseudomonas mediterranea*, *Pectobacterium carotovorum* subsp. *carotovorum*), bacterial canker and wilt (*Clavibacter michiganensis* subsp. *michiganensis*), bacterial speck (*Pseudomonas syringae* pv. *tomato*) and bacterial spot (*Xanthomonas* spp.) (Jones et al., 2014).

Pseudomonas syringae pv. *tomato* (Okabe) Young, Dye and Wilkie is the most common bacterial agent that causes bacterial speck disease. The initial symptoms of bacterial speck disease are water-soaked, like small spots that appear on the leaves of the tomato plant. These spots turn into black-brown in the center, surrounded by a yellow ring. The affected area of the leaf is enclosed in a black-brown chlorotic halo. It causes stunted growth and decreases the yield of tomato plants. The temperature range between 13 °C to 28 °C with high humidity plays a vital role in the transmission and progress of *Pst*. The existence of a disease can be harmful to tomatoes grown in a greenhouse or an open field (Preston, 2000).

This bacterial infection is a seed-borne disease that can survive in infected plant residues, soils, and seeds. The bacterium also prefers high humidity and blowout gliding or splashing water for a better infection. Genetic resistance is one of the initial steps in disease control. Some wild tomato varieties have been tested for resistance; however, no commercially available tomato cultivars were found to be tolerant of bacterial speck disease (Stamova, 2009; Janssen et al., 2018). The best way to control the disease is to use high-quality, pathogen-free seeds, immersion of seeds at 50 °C for 25-30 minutes, soil solarization, crop rotation, and preferring drip irrigation. Among other measures, immediately removing and destroying all infected plants is an initial method to control the disease. There are some copper compounds available to control bacterial diseases that provide safety and protection in nurseries, fields, and greenhouses in the early stages, but they are insufficient for the upgoing stages (Jones et al., 2014; Anonymous, 2017; Horuz et al., 2018; Anonymous, 2021).

Aside from all of these control methods, it is essential to provide appropriate plant nutrition and fertilization, as shown in cultural measures, to ensure that plants survive (DonHuber et al., 2012). On the other hand, plants acquire the majority of the nutrients they require from the soil through their roots. However, if the soil is inadequate morphologically, the required nutrients will not be available to the plants. As a result, the plants will not develop or grow properly. Recently, Karnez et al. (2021) aimed to determine the suppression level of the disease caused by

P. syringae pv. *tomato* by using vermicompost fertilization and found that contents of macro and micro nutrients were increased by vermicompost on tomato and the disease was highly suppressed by vermicomposts. The effect of five plant activators (Crop-Set, ISR2000, KingBo, Sergomil L60, Turf-Set) and a biopreparat Serenade® SC (1.34% *Bacillus subtilis* QST 713) has been investigated against bacterial speck disease in the plastic greenhouse (Aktepe, 2022). According to the results, it was determined that the treatments reduced the speck on the leaves by 16-50% and by 25-50% on the stems. Although all applications were found to be successful in suppressing the speck disease symptoms on the leaves and stem, Turf-Set, ISR-2000 and Sergomil applications were determined to be the most successful applications. In many studies, the antimicrobial activities of essential oils and nanoparticles have been also investigated against many plant pathogenic bacteria and it has been reported that they have high efficacies (Bozkurt et al., 2020; Şahin et al., 2021; Soylu et al., 2022; Şahin et al., 2022).

Reduced phosphorus (P) compounds containing phosphite (Phi) have been investigated since the 1930s as potential sources to meet P requirements (MacIntire et. al., 1950; Jackman et. al., 1970). Interest in the use of reduced P compounds in agriculture increased in the 1970s when it was shown that Phi compounds exhibited antifungal properties, particularly with Oomycetes fungi (Guest & Grant, 1991). Over the last several decades, because of significantly less complex and costly approval processes required for fertilizers compared to fungicides, Phi-based fungicide products were widely integrated into agricultural plant disease management programs. They are often labelled as biostimulants or fertilizers in the market (Lovatt & Mikkelsen, 2006). It has been demonstrated that using a foliar spray of potassium phosphite in conjunction with a non-systemic fungicide is effective in controlling *Phytophthora infestans* in potatoes. In a recent study, researchers observed a reduction in disease severity when they tested combinations of potassium phosphite and potassium phosphite+*Trichoderma* spp. against *Colletotrichum lindemuthianum*, which causes anthracnose in widely used beans. The study was performed under greenhouse conditions (Liljeroth et al., 2016).

In a recent study conducted by Costa et al. (2020), it was determined that manganese and zinc phosphites have the potential to control *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (*Xapf*) the causal agent of common bacterial blight. When comparing the Mn and Zn phosphite treatments to the control treatment, the area under the common bacterial blight progress curve was reduced by 34% and 59%, respectively. Mn and Zn phosphites were found to be effective in priming common bean plants to increase their resistance to *Xapf* infection.

The purpose of this study was to examine the efficacy of five different phosphites (calcium, copper, magnesium, potassium and zinc/manganese phosphites) and a fungicide (Fosetyl-Al) to control the tomato bacterial speck disease in a greenhouse with a heat-controlled system as two repeated pot experiments.

MATERIALS and METHODS

Bacterial pathogen

In the experiment, a *Pseudomonas syringe* pv. *tomato* strain DG 1-2 1R, isolated and identified, was used as the pathogen for foliar spraying. The isolate is kept at -20 °C in the Erciyes University, Faculty of Agriculture, Plant Protection Department, Bacteriology culture collection. The media King B (KB) was used for the growth of bacteria in the laboratory conditions and Yeast Dextrose Calcium Carbonate Agar (YDCA) was used for short-term storage at 4 °C in the fridge (Lelliott & Stead, 1987).

Research area

Between August and December 2021, this study was conducted in greenhouses with automatic heated systems at Erciyes University Faculty of Agriculture.

Tomato seedlings

In the pot trials, the Veyron F1 tomato variety was used in the first experiment, and the Figen F1 tomato variety was used in the second experiment. The seedlings were obtained from Istanbul Fide, Antalya. In the first experiment, 63 tomato seedlings were used, and another 84 tomato seedlings were used in the second experiment.

Plant growth

For the pot trials, seedling bags of 22x40 cm in size in the first trial and 17x30 cm in the second trial were used, and the soil mixture was prepared as 2200 g of sand + clay + peat (30% peat, 70% sand + clay) was distributed for each bag.

Phosphites and fungicide applications

Phosphite-containing fertilizers and the plant activator used in the study, their nutrient contents and applied doses are listed below (Table 1). Fertilizers and fungicide were sprayed onto the tomato plants four times once a week at the doses recommended by the manufacturer for use on tomatoes.

Table 1. Phosphite fertilizers used in the study, their contents and doses

Çizelge 1. Çalışmada kullanılan fosfitli gübreler, içerikleri ve dozları

Fertilizer Type	Nutritional Content	Recommended Dose (L)	Applied (ml/ml)	dose
Copper Phosphite	5% Nitrogen (N), 5% Copper (Cu), 13% Phosphorus Penta Oxide (P ₂ O ₅)	250 – 300 ml / 100	1.5 / 500	
Zinc/Manganese Phosphite	5% Nitrogen (N), 5% Zinc (Zn), 5% Manganese (Mn), 20% Phosphorus Penta Oxide (P ₂ O ₅)	250 – 300 ml / 100	1.5 / 500	
Calcium Phosphite	5% Nitrogen (N), 6% Calcium Oxide (CaO), 20% Phosphorus Penta Oxide (P ₂ O ₅)	250 – 300 ml / 100	1.5 / 500	
Magnesium Phosphite	5% Nitrogen (N), 5% Magnesium Oxide (MgO), 30% Phosphorus Penta Oxide (P ₂ O ₅)	200 – 250 ml / 100	1.25 / 500	
Potassium Phosphite	20% Potassium Oxide (P ₂ O ₅), 30% Phosphorus Penta Oxide (P ₂ O ₅)	250 – 300 ml / 100	1.5 / 500	
Fungicide, Fosetyl Al	80% Fosetyl Al	250 g / 100	1.25 g/500	

Bacterial culture refreshment

The stock of bacterial isolate kept in the same ratio of glycerol (40%) and nutrient broth in the refrigerator at -20 °C was streaked onto KB medium using the three-line method and incubated at 25 °C for 48-72 hours. Then, *Pseudomonas syringae* pv. *tomato* (Pst) colonies growing in white rod-shaped creamy colour were purified and used in the pathogenicity test.

Pathogenicity test of Pst

To increase the virulence of Pst, standard tomato seedlings at 3–5 true leaves were sprayed with the isolate DG 1-2. Five tomato plants were used for this test. Approximately one full loop of the freshly grown bacterial isolate was taken and suspended in 9 mL of sterile distilled water. Then, using a spectrophotometer, the prepared suspensions were adjusted to an absorbance value of 0.2 at a 600 nm wavelength, and the bacteria in the suspension were adjusted to a density of 10⁷ cells ml⁻¹. Finally, this inoculum was sprayed onto the plant leaves until runoff. The sprayed plants were kept in the climate cabinet (16 hours of the light period, 8 hours of dark environment, 27°C temperature, 70-80% humidity) until disease symptoms were observed.

Re-Isolation of pathogenic bacterium from diseased plants

Initially starting from yellow to dark brown, black lesions were seen on the leaves of the diseased plants, and the infected leaves were removed with the help of clean pruning shears. After the surface disinfection of the taken parts was made in 70% alcohol, they were taken into a sterile mortar and thoroughly crushed, and a homogeneous suspension was obtained with 3-4 ml of sterile distilled water. A loopful suspension was streaked onto KB in 100 mm diameter Petri dishes using the three-line method. The petri dishes were incubated at 25°C for 3-5 days to allow the growth of bacteria. The white and creamy-coloured bacterial re-isolate that developed was purified and inoculated onto freshly prepared YDC agar media and stored at +4°C and in glycerol at -20°C in the refrigerator until use.

Application of phosphites and fungicide to tomato seedlings

The phosphite-containing fertilizers used in the study were applied to the plants by foliar spraying for a total of 4 weeks at one-week intervals at the recommended doses for each one. Fertilizers were applied to the plants 7, 14, 21 and 28 days after the seedlings were transplanted. Control plants were only sprayed with distilled water.

Inoculum preparation

In this study, highly virulent and freshly grown in KB medium DG 1-2 1R isolate was used. The suspension was prepared from that isolate in sterile distilled water. This suspension was adjusted to an absorbance value of 0.2 at a 600 nm wavelength in a spectrophotometer. Then, the bacterial density in this suspension was determined as 3×10^8 cells ml^{-1} using the petri count method. After that, the suspension was diluted one time. This diluted suspension (3×10^7 cells ml^{-1}) was sprayed to the control, and the phosphites or fungicide applied tomato leaves until runoff. The pathogenic bacterium was treated to the plants on September 23, 2021 and October 7, 2021, in the fourth week after seedling transplantation and just before the third dose of phosphites applications in the first and second trials, respectively.

The effect of phosphites on tomato bacterial speck disease

To examine the effect of phosphite-containing commercial fertilizers on tomato bacterial speck, a pot trial was set up in the heated greenhouse of Erciyes University Faculty of Agriculture. A total of 6 applications (Zinc/Manganese phosphite, Copper phosphite, Magnesium phosphite, Potassium phosphite, Calcium phosphite, and Fosetyl-Aluminium) for the bacterial isolate were used to determine the effect of phosphite-containing fertilizers on diseases in the trial, including positive and negative control applications. The experiment was established with eight applications. For each application, nine tomato seedlings were used in the first trial and 12 tomato seedlings in the second trial, respectively. In the first experiment, the seedlings were transplanted on August 20, 2021 and the efficacy of the treatments was evaluated on 19.10.2021, respectively. In addition, the plants for the second experiment were transplanted on 11.09.2021 and all the plants were evaluated for disease development on November, 8, 2021.

Experimental design and evaluation of the experiments

The experiment was established in a randomized block design with 3 replications in the first trial and 4 replications in the second trial, with 3 plants evaluated in each replication. The positive control group, which was only sprayed with bacteria and no fertilizer was applied, was observed daily and the development of symptoms of the disease was followed. The observations were continued until the symptoms were seen. When symptoms such as dark brown lesions surrounded with a yellow halo on the leaves of the control plants were observed. A modified 0 to 5 scale (0: no symptom; 1: 1-10% of the leaves infected; 2: 11-25% of the leaves infected; 3: 26-50% of the leaves infected; 4: 51-75% of the leaves infected; 5: 76-100% of the leaves infected) was used for disease severity (DS)

(Kirli, 2016). DS was calculated using the Tawsend-Heuberger formula. The efficacies of the treatments were also calculated according to the Abbott formula. The formulas used were as follows:

Disease severity (%) = $[(\sum \text{number of diseased leaves in each grade} \times \text{grade}) / (\text{total number of leaves investigated} \times \text{the highest disease index})] \times 100$ **Eq.(1)**

Efficacy (%) = $[(\text{incidence rate in the control} - \text{incidence rate in the treated group}) / \text{incidence rate in the control}] \times 100$ **Eq.(2)**

Twelve leaves were controlled in each plant for symptom development. The experiment was repeated twice under greenhouse conditions. The statistical differences between the applications were calculated with the Tukey HSD multiple comparison test ($p \leq 0.05$ significance level) in the SPSS statistical program and one-way ANOVA analysis of variance. According to the results obtained, all different groups were lettered, and the results were interpreted.

RESULTS and DISCUSSIONS

Pathogenicity test

The bacterial isolate DG 1-2 that was sprayed on tomato plants induced dark brown lesions surrounded with a yellow halo two weeks after inoculation. Some symptomatic leaves were collected and transferred to the laboratory for re-isolations. To fulfill KOCH's postulates, eight cream-colored colonies were purified and identified as *Pst* according to classical tests like colony morphology and fluorescence on KB, hypersensitive reaction on tobacco leaves.

The effect of phosphites on tomato bacterial speck disease

The first experiment was carried out in the controlled greenhouses of the Faculty of Agriculture between September, 15, 2021 and October, 19, 2021. According to the results, since the disease severity in the positive control was 37.5%, disease severity in five different phosphites and Fosetyl-Al applications ranged from 9.4% to 21.7%. All applications inhibited the disease development between 42.1% and 75.0%. Among treatments, zinc/manganese phosphite (75.0%), magnesium phosphite (66.4%), and Fosetyl-Al (56.6%) showed the highest disease reduction (Figure 1).

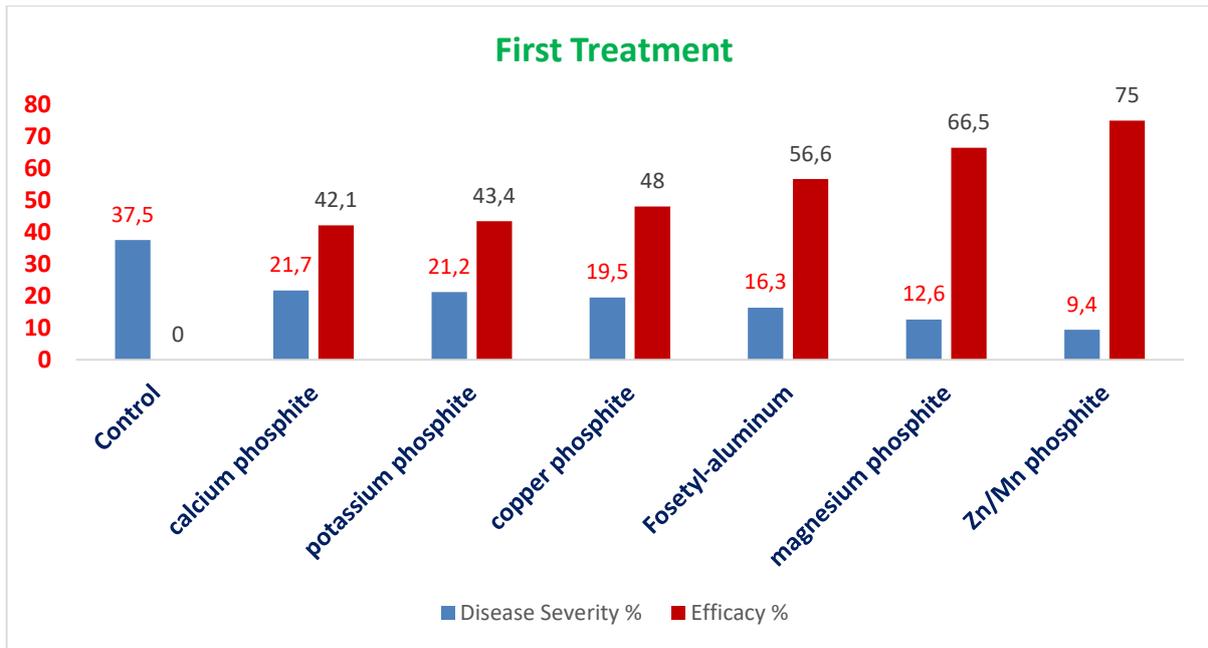


Figure 1. Efficacy of treatments on *Pst* development in the first experiment

Şekil 1. Birinci denemede *Pst* gelişimi üzerine uygulamaların etkinliği

In the second experiment, the phosphites and Fosetyl-Al treatments prevented the development of bacterial speck disease on tomatoes from 22.8% to 90.3% (Figure 2). While the disease severity rate in the positive control was 43.0%, the disease severities in treated plants were 33.19% in copper phosphite, 30.9% in calcium phosphite, 15.4% in potassium phosphite, 10.4% in zinc/manganese phosphite, 9.3% in magnesium phosphite, and 4.2% in Fosetyl-Al applications. Compared to all phosphites, Fosetyl-Al showed the most significant reduction in disease development with a rate of 90.3% efficacy. In addition, magnesium phosphite reduced the disease severity at 78.35%, zinc/manganese phosphite at 75.8% and potassium phosphite at 63.2% rates.

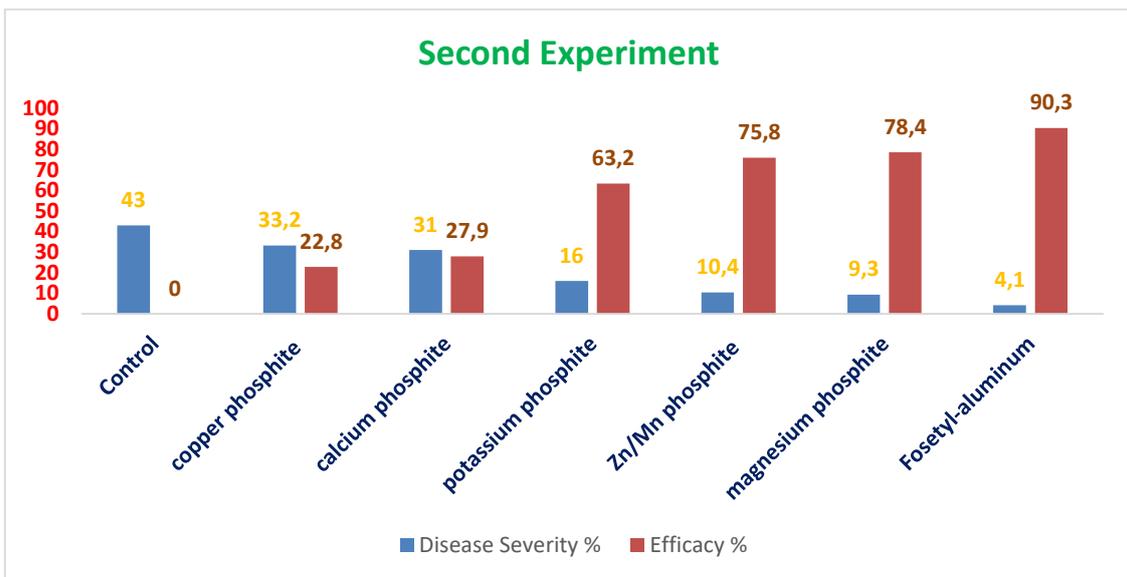


Figure 2. Efficacy of treatments on *Pst* development in second experiment

Şekil 2. İkinci denemede *Pst* gelişimi üzerine uygulamaların etkinliği

Initially, the first and second treatments were statistically analyzed separately, but both treatments yielded similar results. Thus, data from two experiments were combined and the average of experiments was statistically evaluated. According to both experiments, potassium phosphite (53.3%), magnesium phosphite (72.4%), Fosetyl-Al (73.4%), and Zn/Mn phosphite (75.3%) reduced the *Pst* development over 50%. Additionally, it was determined that all applications were statistically different from the positive control group and successfully prevented tomato plants from the disease (Table 2).

Table 2. Efficacy of treatments on *Pst* development (First and second experiments)

Çizelge 2. Pst gelişimi üzerine uygulamaların etkinliği (Birinci ve ikinci denemelerde)

Treatments	First experiment		Second experiment		Mean of Two experiments	
	Disease severity (%)	Efficacy %	Disease severity (%)	Efficacy %	Disease severity (%)	Efficacy %
Control	37.5 ^{a*}	-	43.0 ^{a*}	-	40.3 ^a	-
Calcium Phosphite	21.7 ^b	42.1	31.0 ^b	28.0	26.3 ^b	35.0
Potassium Phosphite	21.2 ^b	43.4	15.9 ^c	63.2	18.5 ^c	53.3
Copper Phosphite	19.5 ^{bc}	48.0	33.2 ^b	22.8	26.4 ^b	35.4
Fosetyl-Al	16.3 ^{bcd}	56.6	4.2 ^d	90.3	10.2 ^{cd}	73.4
Magnesium Phosphite	12.5 ^{cd}	66.5	9.3 ^c	78.4	11.0 ^{cd}	72.4
Zn/Mn Phosphite	9.4 ^d	75.0	10.4 ^c	75.8	9.9 ^d	75.4

* The mean disease severity given in each column followed by the different letters are significantly different according to Tukey HSD multiple comparison test ($p \leq 0.05$).

In this study, the effects of five different phosphites and fungicide, Fosetyl-Al on the development of tomato bacterial speck disease were investigated by pot experiments repeated twice under heated and controlled greenhouse conditions. In both trials, all treatments suppressed disease development between 42% and 75% in the first trial and 22% to 90% in the second trial, respectively. All applications were found to be successful in the control of the disease with an effect of more than 50%.

Tomato is one of the most produced vegetables in the world. Nowadays, tomato production can be done in greenhouses for 12 months with the development of modern greenhouse technologies. Due to the increase in tomato production and the prolongation of the production season in the greenhouse, the importance and necessity of cultural practices have risen. The biotic disease agents can attack tomatoes due to the favorable conditions in greenhouses. It is reported that nearly 200 plant pathogens, including bacteria, cause diseases in tomatoes (Jones et al., 2014). The pathogens belonging to *Pseudomonas* and *Xanthomonas* genera cause the most economic losses in tomatoes (Popović & Ivanović, 2015; Horuz et al., 2018; Mensi et al., 2018).

The bacterium *Pseudomonas syringae* pv. *tomato* is the causal agent of bacterial speck disease on tomatoes. It is a widespread disease of tomatoes that can be found mostly where tomatoes are grown. When the disease severely affects leaves early in the growing season, it can significantly reduce yield. When symptoms appear on tomato fruit, the disease could have a much greater effect on quality. The best way to control this disease is by using high-quality, pathogen-free seeds, soaking of seeds at 50 °C for 25-30 minutes, soil solarization, crop rotation, and preferring drip irrigation.

Among other measures, immediately removing and destroying all infected plants is an initial method to control the disease. There are some copper compounds available to control bacterial diseases that provide safety and protection in nurseries, fields, and greenhouses in the early stages, but they are insufficient for the later stages. A number of chemical and physical treatments have been applied directly to the plant, soil, or seed to limit the introduction in infected areas with *Pst* (Shenge et al., 2008; Li et al., 2017; Horuz et al., 2018; Elsharkawy, et al.,

2020). Since the use of antibiotics is prohibited to control bacterial diseases and chemical control is insufficient, cultural measures have importance in bacterial speck control. It is feasible to increase plant disease resistance by applying adequate fertilizers. Nutrients are vital for the growth of plants and beneficial microorganisms and control of plant diseases indirectly. They affect the resistance and susceptibility of the host plant to diseases by causing various changes in plant morphology. Phosphorus (P) is one of 17 essential elements required for plant growth (Vance et al., 2003).

The P concentration in plants ranges from 0.05 to 0.50% dry weight. This element plays a role in some processes, such as energy generation, nucleic acid synthesis, photosynthesis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N) fixation. Phosphites stand out among the fertilizers available in the agricultural market. The phosphite is a generic name used for inorganic salts of phosphorous acid (H_3PO_3). These salts show high solubility, rapid plant absorption, significant selectivity and systemic translocation (Guest and Grant, 1991; Dalio et al., 2014). Phosphites are obtained by the reaction of phosphorous acid with K^+ , Ca^{2+} , Mn^{2+} , Zn^{2+} , and other micronutrients. In agriculture, phosphites are used as fertilizers and systemic fungicides (Achary et al., 2017; Najdabbasi et al., 2022). Indeed, phosphites are extensively available either as a superior source of plant phosphorus nutrition (P) or can trigger plant host defense as plant defense activators that are translocated in both xylem and phloem to prevent pathogen invasion over a wide range of hosts (Hardy et al., 2001; McDonald et al., 2001).

The efficacy of phosphites has already been verified for many plant fungal diseases including soybean anthracnose and downy mildew (Silva et al., 2011; da Silva Junior et al., 2021), apple scab and Moldy-Core Decay (Reuveni et al., 2003; Felipini et al., 2016), common bean anthracnose (Figueira et al., 2020), Phytophthora crown rot on zucchini (Gilardi et al., 2020), coffee leaf rust (Junior et al., 2021) and potato late blight (Liljeroth et al., 2016; Najdabbasi et al., 2022). However, few studies conducted on the effect of several phosphites for plant bacterial diseases including potato soft rot (Lobato et al., 2011), fire blight (Bahadou et al., 2017) and common bacterial blight (Costa et al., 2020).

According to the findings, Zn/Mn phosphites showed the highest disease suppression (75%) among all sprays in both experiments. Similarly, Fagundes-Nacarath et al. (2018) revealed that Zn phosphite was much more fungistatic than Cu phosphite in white mold severity in common beans. The efficiency in the severity of soybean anthracnose was 85% and 27% in Mn and Zn phosphite treated plants, respectively (da Silva Junior et al., 2021). When the common bean plants were sprayed with the solutions (7.5 ml L^{-1}) of Mn and Zn phosphites, the area under the common bacterial blight progress curve (AUCBBPC) was significantly lower by 34% and 59% for Mn and Zn phosphite treatments (Costa et al., 2020). In common bean (*Phaseolus vulgaris*), the anthracnose severity was highly reduced with the application of the K phosphite, Zn phosphite, Mn phosphite (74-90%) when compared to the control treatment (Gadaga et al., 2017).

Disease suppression results received from the K phosphite treated plants ranged from 30% to 80% in previously conducted several studies (Lobato et al., 2011; Felipini et al., 2016; Martinez et al., 2016; Gadaga et al., 2017; Mehta et al., 2022). The efficacy of K phosphite was 43% and 63% in both trials, thus, partial similarities were obtained with the latest works. Calcium phosphite and magnesium phosphite reduced bacterial speck disease severity by 35.04% and by 72.40%, respectively.

In this study, the effect of these two phosphites on tomato bacterial speck disease was demonstrated for the first time. Our study suggested that phosphites had the potential in inhibiting *Pst* development on tomato leaves, thus, could be included into integrated disease management programs.

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STATEMENT OF CONFLICT OF INTEREST

The author(s) declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

REFERENCES

- Achary, V.M.M., Ram, B., Manna, M., Datta, D., Bhatt, A., Reddy, M.K., & Agrawal P.K. (2017). Phosphite: A novel P fertilizer for weed management and pathogen control. *Plant Biotechnology Journal*, 15, 1493-1508. <https://doi.org/10.1111/pbi.12803>
- Aktepe, B.P. (2021). The effect of different plant activators and biological preparete on the biological control of bacterial speck disease in tomato. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 26 (2), 355-364. <https://doi.org/10.37908/mkutbd.908921>
- Anonymous (2017). Small brown/black spots on a green tomato characteristic of bacterial speck. Photo courtesy of bacterial speck of tomato. Available: <https://pddc.wisc.edu> (Accessed: March 31, 2022).
- Anonymous (2019). A Cultural History of Tomatoes. <https://www.babbel.com/en/magazine/tomato-history> (Accessed: March, 10, 2022).
- Anonymous (2021). Bacterial Speck / Tomato / Agriculture: Pest Management Guidelines / UC Statewide IPM Program (UC IPM). <https://www2.ipm.ucanr.edu/agriculture/tomato/Bacterial-Speck/> (Accessed: March, 31, 2022).
- Bahadou, S.A., Oujija, A., Boukhari, M.A., & Tahiri A. (2017). Development of field strategies for fire blight control integrating biocontrol agents and plant defense activators in Morocco. *Journal of General Plant Pathology*, 99, 51-58. <https://doi.org/10.4454/jpp.v99i0.3909>
- Bozkurt, I.A., Soyulu, S., Kara, M., & Soyulu, E.M. (2020). Chemical composition and antibacterial activity of essential oils isolated from medicinal plants against gall forming plant pathogenic bacterial disease agents. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 23, 1474-1482. <https://doi.org/10.18016/ksutarimdog.vi.723544>
- Costa, L.C., Debona, D., Silveira, P.R., Cacique, I.S., Aucique-Pérez, C.E., Resende, R.S., Oliveira, J.R., & Rodrigues, F. Á. (2020). Phosphites of manganese and zinc potentiate the resistance of common bean against infection by *Xanthomonas axonopodis* pv. *phaseoli*. *Journal of Phytopathology*, 168 (11-12), 641-651. <https://doi.org/10.1111/jph.12944>
- da Silva Junior, M.B., de Resende, M.L.V., Pozza, E.A., Resende, A.R., Vasconcelos, V.A.M, Monteiro, A.C.A., Silveira, G.C.D., & dos Santos, B.D.M. (2021). Phosphites for the management of *anthracnose* in soybean pods. *Journal of Plant Pathology*, 103 (2), 611-617. <https://doi.org/10.1007/s42161-021-00747-y>
- Dalio, R.J.D., Fleischmann, F., Humez, M., & Osswald, W. (2014). Phosphite protects *Fagus sylvatica* seedlings towards *Phytophthora plurivora* via local toxicity, priming and facilitation of pathogen recognition. *PLoS One*, 9 (1), Article: e87860. <https://doi.org/10.1371/journal.pone.0087860>
- Elsharkawy, M., Derbalah, A., Hamza, A., & El-Shaer, A. (2020). Zinc oxide nanostructures as a control strategy of bacterial speck of tomato caused by *Pseudomonas syringae* in Egypt. *Environmental Science and Pollution Research*, 27, 19049-19057. <https://doi.org/10.1007/s11356-018-3806-0>

- Erba, D., Casiraghi, M.C., Ribas, A. A, Cáceres, R., Marfà, O., & Castellari, M. (2013). Nutritional value of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniques. *Journal of Food Composition and Analysis*, 31 (2), 245-251. <https://doi.org/10.1016/j.jfca.2013.05.014>
- Fagundes-Nacarath, I.R.F., Debona D., Brás, V.V., Silveira, P.R., & Rodrigues, F.A. (2018). Phosphites attenuate *Sclerotinia sclerotiorum* induced physiological impairments in common bean. *Acta Physiologia Plantarum*, 40, 198. <https://doi.org/10.1007/s11738-018-2776-7>
- FAOSTAT (2021). Available at: <https://www.fao.org/faostat/en/#home> (Accessed date: June, 8, 2022).
- Felipini, R., Boneti, J.I., Cezar, A., Neto, R., & Veleirinho, B. (2016). Apple scab control and activation of plant defence responses using potassium phosphite and chitosan. *European Journal of Plant Pathology*, 145, 929-939. <https://doi.org/10.1007/s10658-016-0881-2>
- Figueira, E.P.P., Felipini, R., Boneti, J.I., Cezar, A., Neto, R., & Veleirinho, B. (2020). Histochemical changes induced by *Trichoderma* spp. and potassium phosphite in common bean (*Phaseolus vulgaris*) in response to the attack by *Colletotrichum lindemuthianum*. *Semina: Ciências Agrárias*, 41 (3), 811-828. <https://doi.org/10.5433/1679-0359.2020v41n3p811>
- Gadaga, S.J.C., Abreu, M.S., Resende, M.L.V., & Ribeiro, J.P.M. (2017). Phosphites for control of anthracnose in common bean. *Fitopatologia: Pesquisa Agropecuária Brasileira*, 52 (1), 36-44. <https://doi.org/10.1590/s0100-204x2017000100005>
- Gilardi, G., Koike, S., Lanini, T., Mitchell, J., & Smith, R. (2020). Effect of biocontrol agents and potassium phosphite against *Phytophthora* crown rot, caused by *Phytophthora capsici*, on zucchini in a closed soilless system. *Scientia Horticulturae*, 265, 109207. <https://doi.org/10.1016/j.scienta.2020.109207>
- Guest, D.I., & Grant, B.R. (1991). The complex action of phosphonates as antifungal agents. *Biological Reviews*, 66, 159-187. <https://doi.org/10.1111/j.1469-185X.1991.tb01139.x>
- Hardy, G.S.J., Barrett, S., & Shearer, B. (2001). The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology*, 30, 133-139. <https://doi.org/10.1071/AP01012>
- Horuz, S., Ocal, A., & Aysan, Y. (2018). Efficacy of hot water and chemical seed treatment on bacterial speck of tomato in Turkey. *Fresenius Environmental Bulletin*, 27, 3185-3190.
- Huber, D., Römheld, V., & Weinmann, M. (2012). *Relationship between Nutrition, Plant Diseases and Pests. Marschner's Mineral Nutrition of High. Plants (Third edition)*. pp. 483-643.
- Jackman, R., Lambert, J., & Rothbaum, H. (1970). Red phosphorus as a fertiliser for grass-clover pasture. *New Zealand Journal of Agricultural Research*, 13, 232-241. <https://doi.org/10.1080/00288233.1970.10425396>
- Janssen, D., García, C., Ruiz, L., De Cara-García, M., Simon, A., & Martínez, A. (2018). Disease resistance in tomato crops produced in Spain. *Acta Horticulturae*, 1207, 63-68. <https://doi.org/10.17660/ActaHortic.2018.1207.8>
- Jones, J., Sally, A.M., Thomas, A.Z., & Timur, M.M. (2014). *Bacterial speck, Compendium of tomato diseases and pests*. 2nd edn. APS press, pp. 54-55.
- Júnior, J.H., Debona, D., Zambolim, L., & Rodrigues, F.Á. (2021). Factors influencing the performance of phosphites on the control of coffee leaf rust. *Bragantia*, 80, 1-7. <https://doi.org/10.1590/1678-4499.20200176>
- Karnez, E., Güldoğan, Ö., Ercan, N., Korkmaz, K., & Aysan, Y. (2021). Domateste bakteriyel benek hastalığının mücadelesinde vermikompost uygulamasının etkisi. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 26 (3), 726-735. <https://doi.org/10.37908/mkutbd.986521>
- Kirli, M.M. (2016). Farklı plastik örtülerin domates bakteriyel kara leke hastalığına etkisi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, 45 s.
- Lelliott, R.A., & Stead, D.E. (1987). *Methods for the diagnosis of bacterial disease of plants*. In: *Methods in Plant Pathology*, (Preece TF, Ed.), Blackwell Scientific Publications, Oxford. pp. 176-177.

- Li, Y., Yang, D., & Cui, J. (2017). Graphene oxide loaded with copper oxide nanoparticles as an antibacterial agent against, *Pseudomonas syringae* pv. *tomato*. *RSC Advances*, 7 (62), 38853-38860. <https://doi.org/10.1039/C7RA05520J>
- Liljeroth, E., Lankinen, Å., Wiik, L., Burra, D.D., Alexandersson, E., & Andreasson, E. (2016). Potassium phosphite combined with reduced doses of fungicides provides efficient protection against potato late blight in large-scale field trials. *Crop Protection*, 86, 42-55. <https://doi.org/10.1016/j.cropro.2016.04.003>
- Lobato, M., Machinandiarena, M., Tambascio, C., Dosio, G., Caldiz, D., Daleo, G., Andreu, A., & Olivieri, F. (2011). Effect of foliar applications of phosphite on post-harvest potato tubers. *European Journal of Plant Pathology*, 130, 155-163. <https://doi.org/10.1007/s10658-011-9741-2>
- Lovatt, C., & Mikkelsen, R. (2006). Phosphite fertilizers: What are they? Can you use them? What can they do? *Better Crops*, 90, 11-13.
- MacIntire, W., Winterberg, S., Hardin, L., Sterges, A., & Clements, L. (1950). Fertilizer evaluation of certain phosphorus, phosphorous, and phosphoric materials by means of pot cultures. *Agronomy Journal*, 42, 543-549. <https://doi.org/10.2134/agronj1950.00021962004200110004x>
- Martínez, S. (2016). Effects of combined application of potassium phosphite and fungicide on stem and sheath disease control, yield, and quality of rice. *Crop Protection*, 89, 259-264. <https://doi.org/10.1016/j.cropro.2016.08.002>
- McDonald, A.E., Grant B.R., & Plaxton, W.C. (2001). Phosphite (phosphorous acid): Its relevance in the environment and agriculture and influence on plant phosphate starvation response. *Journal of Plant Nutrition*, 24, 1505-1519. <https://doi.org/10.1081/PLN-100106017>
- Mehta, S., Kumar, A., Achary, V.M.M., Ganesan, P., Patel, A., Singh, A., Rathi, N., Das, T.K., Lal, S.K., & Reddy, M.K. (2022). Antifungal and defense elicitor activity of potassium phosphite against fungal blast disease on *ptxD-OE* transgenic indica rice and its acceptor parent. *Pesticide Biochemistry and Physiology*, 182, Article 105026. <https://doi.org/10.1016/j.pestbp.2021.105026>
- Mensi, I., Jabnoun-Khiareddine, H., Zarrougui, N.E., Ben Zahra, H., Cesbron, S., Jacques, M.A., & Daami-Remadi, M. (2018). First report of tomato bacterial speck caused by *Pseudomonas syringae* pv. *tomato* in Tunisia. *New Disease Reports*, 38, 21. <https://doi.org/10.5197/j.2044-0588.2018.038.021>
- Najdabbasi, N., Mirmajlessi, S.M., Dewitte, K., Mänd, M., Landschoot, S., & Haesaert, G. (2022). Combination of potassium phosphite and reduced doses of fungicides encourages protection against *Phytophthora infestans* in potatoes. *Agriculture*, 12 (2), 189. <https://doi.org/10.3390/agriculture12020189>
- Popović, T., Ivanović, Ž., & Ignjatov, M. (2015). First report of *Pseudomonas viridiflava* causing pith necrosis of tomato (*Solanum lycopersicum*) in Serbia. *Plant Disease*, 99 (7), 1033-1033. <https://doi.org/10.1094/PDIS-01-15-0052-PDN>
- Preston, G.M. (2000). *Pseudomonas syringae* pv. *tomato*: the right pathogen, of the right plant, at the right time. *Molecular Plant Pathology*, 1 (5), 263-275. <https://doi.org/10.1046/j.1364-3703.2000.00036.x>
- Reuveni, M., Sheglov, D., & Cohen, Y. (2003). Control of moldy-core decay in apple fruits by β -aminobutyric acids and potassium phosphites. *Plant Disease*, 87 (8), 933-936. <https://doi.org/10.1094/PDIS.2003.87.8.933>
- Shenge, S., Mabagala, D., Mortensen, R.C.N., & Wydra, K. (2008). Molecular characterization of *Pseudomonas syringae* pv. *tomato* isolates from Tanzania. *Phytoparasitica*, 36 (4), 338-351. <https://doi.org/10.1007/BF02980813>
- Silva, O.C., Santos, H.A.A., Dalla Pria, M., & May-De Mio, L.L. (2011). Potassium phosphite for control of downy mildew of soybean. *Crop Protection*, 30 (6), 598-604. <https://doi.org/10.1016/j.cropro.2011.02.015>

- Soylu, S., Kara, M., Türkmen, M., & Şahin, B. (2022). Synergistic effect of *Foeniculum vulgare* essential oil on the antibacterial activities of Ag- and Cu-substituted ZnO nanorods (ZnO-NRs) against food, human and plant pathogenic bacterial disease agents. *Inorganic Chemistry Communications*, 146, Article 110103. <https://doi.org/10.1016/j.inoche.2022.110103>
- Stamova, L. (2009). Resistance to *Pseudomonas syringae* pv. *tomato* race 1. *Acta Horticulturae*, 808, 219-222. <https://doi.org/10.17660/ActaHortic.2009.808.33>
- Şahin, B., Aydın, R., Soylu, S., Türkmen, M., Kara, M., Akkaya, A., Çetin, H., & Ayyıldız, E. (2022). The effect of *Thymus syriacus* plant extract on the main physical and antibacterial activities of ZnO nanoparticles synthesized by SILAR Method. *Inorganic Chemistry Communications*, 135, Article 109088. <https://doi.org/10.1016/j.inoche.2021.109088>
- Şahin, B., Soylu, S., Kara, M., Türkmen, M., Aydın, R., & Çetin, H. (2021). Superior antibacterial activity against seed-borne plant bacterial disease agents and enhanced physical properties of novel green synthesized nanostructured ZnO using *Thymbra spicata* plant extract. *Ceramics International*, 47, 341-350. <https://doi.org/10.1016/j.ceramint.2020.08.139>
- Vance, C.P., Uhde-Stone, C., & Allan, D.L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157 (3), 423-447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>