T.R.
ERCİYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

EFFICACY OF PHOSPHIDE BASED FERTILIZERS ON BACTERIAL SPECK DISEASE OF TOMATO

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Master's Thesis

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KAYSERİ

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COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained in accordance with academic and ethical rules. I also state that, as required by these rules and behaviors, I have fully cited and referenced all materials and results that are not inherent in this study.

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The Master's thesis entitled **“Effıcacy Of Phosphıde Based Fertılızers On Bacterıal Speck Dısease Of Tomato”** was prepared in accordance with Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

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FOSFİT BAZLI GÜBRELERİN DOMATES BAKTERİYEL BENEK HASTALIĞINA ETKİSİ

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ÖZET

Bakteriyel etmen, *Pseudomonas syringae* pv. *tomato* (*Pst*), domateslerde Bakteriyel Benek Hastalığına neden olur. Hastalığın ilk belirtileri, domates yapraklarında sarı bir hale ile çevrili, suyla ıslanmış, küçük koyu kahverengi lekelerdir. Patojen tohum kaynaklıdır, bu nedenle hastalığın kontrolü zordur. Bu çalışmada, beş farklı fosfitin (kalsiyum, bakır, magnezyum, potasyum ve çinko/manganez fosfit) ve bir fungisit olan Fosetyl-Alüminyum'un yapraklarda *Pst*'nin hastalık şiddetini önlemedeki etkinliği test edilmiştir. Fosfitler, saksılarda *Pst* bulaştırılmış bitkilere, önerilen dozlarda birer hafta arayla toplam 4 hafta boyunca yapraktan püskürtme ile uygulanmıştır. Bitkiler, kontrol bitkilerinde hastalık belirtileri görülene kadar yüksek nem (%75-90) ve optimum sıcaklık (22-24 °C) koşulları altında kontrollü bir serada tutulmuştur. Sonuç olarak, uygulamalar istatistiki olarak farklılık göstermiştir. Uygulamaların etkinliği ilk denemede %42- %75, ikinci denemede ise %22- %90 arasında bulunmuştur. Çalışma, fosfitlerin domates yapraklarında *Pst* gelişimini azaltma potansiyeline sahip olduğunu bulmuş ve entegre hastalık yönetiminde fosfitlerin de kullanılabileceği sonucuna varmıştır.

Anahtar Kelimeler: Domates, Fosfit, *Pseudomonas syringae pv. tomato*, Kontrol

EFFICACY OF PHOSPHIDE BASED FERTILIZERS ON BACTERIAL SPECK DISEASE OF TOMATO

Sa ad Mohamed HAJI NOUR

Erciyes University, Graduate School of Natural and Applied Sciences
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ABSTRACT

The bacterial agent, *Pseudomonas syringae pv. tomato* (*Pst*), 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. The pathogen is seed-borne, that's why, control of the disease is a challenge. In this study, we tested the efficacy of five different phosphites (calcium, copper, magnesium, potassium and zinc/manganese phosphites) and a plant activator, Fosetyl-Al to inhibit the disease severity of *Pst* on leaves. The phosphites were applied to the *Pst* inoculated plants in pots by foliar spraying for a total of 4 weeks at one-week intervals at the recommended doses. 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. As a result, the treatments were statistically significant. The efficacies were between 42% to 75% in the first trial and 22% to 90% in the second trial, respectively. The study concluded that the phosphites have potential to reduce *Pst* development in tomato leaves and could be used in integrated disease management.

Keywords: Tomato, Phosphite, *Pseudomonas syringae pv. tomato*, Control

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ABBREVIATIONS

PST	: *Pseudomonas syringae pv. tomato*
PH	: Potential of hydrogen
YDCA	: Yeast Dextrose Calcium Carbonate Agar
g	: Gram
L	: Litre
KB	: King's medium B
ml	: Mililitre
nm	: Nanometer
PCR	: Polymerase chain reaction
cm	: Centimeter
Ppm	: Parts per million
µL	: Microliter
FAO	: Food and Agricultural Organization
SPSS	: Statistical Package for the Social Sciences
ºC	: Celsius degree
%	: Percent
K	: Potassium
DNA	: Deoxyribonucleic acid
RNA	: Ribonucleic acid
P	: Phosphite

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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).

After being introduced to Europe from the Andean region in the 16th century, the tomato has spread worldwide and is now the most important vegetable crop in the world (Bergougnoux, 2014). By the late 18th century, the tomato had spread throughout Europe, but it was not until the mid-1800s that it began to spread into Africa and Asia. It was introduced to Syria for the first time by British consul John Barker, who oversaw its expansion throughout the region. Quickly after, it spread to other regions, including Iran, and even made its way to Asian countries such as China and Japan. Over time, it became a key component of Middle Eastern cuisine (Anonymous, 2019).

Along with the significance of tomatoes are some of the most important vegetables on the planet, accounting for the second most widely grown and consumed vegetable in Western countries (Willcox *et. al.*, 2003).

Tomatoes contribute to about a quarter of all vegetable production. Several elements 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).

According to Weisburger (2002), tomato consumption is associated with a lower risk of chronic diseases such as cardiovascular disease and cancer, among other things. A key ingredient in the traditional national cuisine, the tomato is one of the most important crops

in the world's agri-food industry and one of the most important crops in most countries' agro-food sectors. Italy was the world's third-largest producer of processed tomatoes in 2019, with an annual production of approximately 5 million tons of raw material, trailing only California, which produced 13 million tons, and China's 5.6 million tons (Bonzi, 2019). When it comes to tomato processing, Turkey holds a significant position worldwide. About 25–30% of the tomatoes produced in Turkey are processed. The majority of the remaining tomatoes are eaten fresh. Tomato paste accounts for 80% of the total amount of tomatoes processed, with canned food accounting for the remaining 15%. The remaining tomatoes are ketchup and tomatoes. They are used to produce tomato-based products such as tomato juice and sauce (Ertürk and Çirka, 2015).

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. 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. China is followed by India, Turkey, the USA, Egypt, Iran, Italy, Spain, Brazil and Mexico (*FAOSTAT*, 2021).

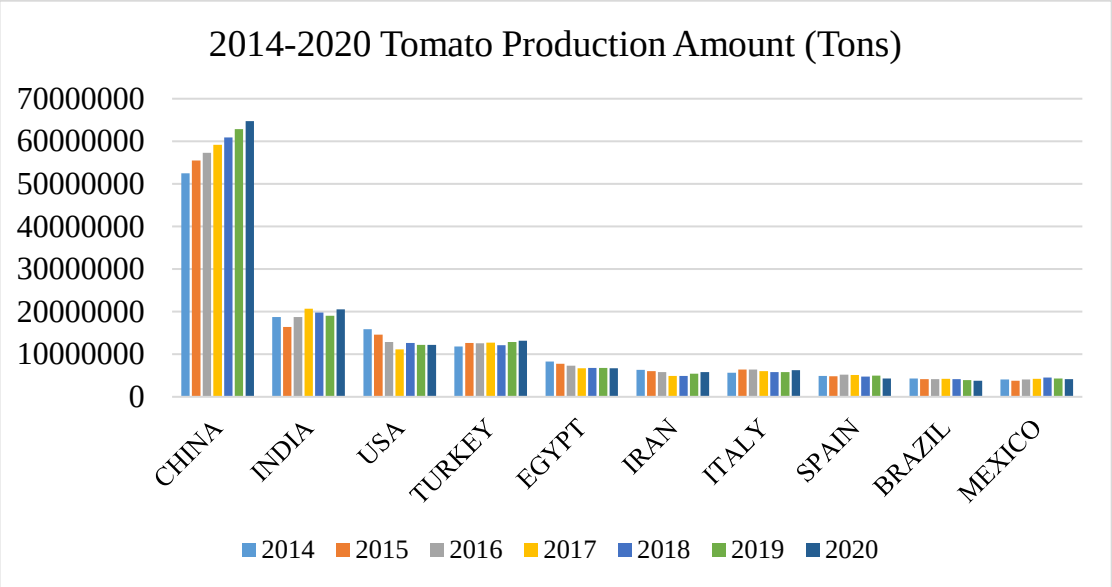


*Figure 1.*World tomato production 2014-2020

In most nations, except of China, India, and Turkey, their tomato yields had fallen as shown in Figure 1. For example, between 2014 and 2020, tomato production in Brazil and Egypt declined.

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 cause problems in tomato plants in most countries globally is a significant problem in tomato fields in particular. 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 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 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).

The number of reports of bacterial speck in tomatoes, caused by *Pseudomonas syringae pv. tomato,* has increased in various parts of the world (Mıller and Jones,2014). A novel pathogenic race (race 1) of *Pst* was discovered in processing tomato farms in Southwestern Ontario in four survey years (1982–1985). Race 1 was identified in 8 and 14 percent of the fields surveyed in 1982 and 1983, respectively, based on a single mass sample collected from each field. The presence of race 1 in 65–100 percent of the areas studied in 1983, 1984, and 1985 was confirmed when 5 to 50 lesions from each field were examined (Lawton and Macneill, 1986). In 1987, symptoms of bacterial speck disease of tomato *(Pst*) were reported and observed in many different tomato-cultivated areas in Chile (Valenzuela *et al.*, 2021)

A similar problem was reported for the first time in Tanzania in 2007, when the prevalence of bacterial speck disease in tomatoes was described in part in the tomato cultivation regions of Tanzania. A total of 56 isolates were taken, with 23 of them being identified as *Pseudomonas syringae pv. tomato* (Shenge *et al.*, 2008). During the spring seasons of 2002 and 2003, the southwestern region of Turkey, particularly Antalya, was heavily impacted by a severe outbreak of disease that affected tomato seedlings and commercial companies. A water-soaked, dark a brown to black spot on new tomato seedling leaves was the primary symptom of the disease. After re-isolating and running all the required steps of Koch's postulates, it was discovered that the prevalence and occurrence of tomato bacterial speck disease (*Pst*) (Yilmaz *et al.*, 2007).

The bacterium *Pseudomonas syringae pv. tomato* causes bacterial speck disease in tomatoes. As a result, it is among the most destructive bacterial diseases globally (Chen *et al.*, 2020). While it is one of the most dangerous diseases that may infect tomato plants, bacterial speck disease, which infects the plant at its seedling stage, can cause it to lose up to 75% of its quality and yield, causing it to be eliminated (Li et al., 2017)

The bacterial speck disease of tomatoes caused by *Pst* was once considered as minor importance in terms of the economy (Anonymous,1986). However, it is now recognized as having a significant impact on financial losses in the tomato commercial and industrial sectors (Yunis *et al.*, 1980; Anonymous, 2001; Bashan and De-Bashan, 2002). 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).

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. 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). Most living organisms, especially animals and humans, have relied on plants for nutrients for a long time. 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. Thus, fertilizers are essential and required for the plant. The use of animal or/and plant residues as fertilizers is traced back to 5900–2400 B.C. In addition, it is shown that Europeans and Asians have used animal waste and water management to improve their crops for centuries (Bogaard *et al.*, 2013).

Human beings have been highly focused throughout history, from prehistoric to ancient times, on improving the production of their crops and the addition of different organic and mineral elements. For the last three centuries, researchers have been attentive to the fact that various livestock wastes, plant residues, crushed bones, and minerals applied directly to the soil can enhance plant growth and development. It was discovered by chance, while scientists were attempting to get as close to the element as possible (Russel and Williams, 1977).

The world population began to overgrow in the 18th century, resulting in a shortage of food production. As a result, the first chemical fertilizer, superphosphate, was discovered by treating bones with sulphuric acid in the early nineteenth century. Phosphate with coprolite rock quickly replaced bones as the primary source of phosphate (P). In early 1861, the first potassium (K) fertilizer industry was built in Germany. During World War I, a potassium fertilizer industry was established in North America, and calcium nitrate was the first nitrate fertilizer produced. Following the discovery of ammonia in 1913, several nitrate fertilizers were developed. However, their efficacy was low. In addition to developing efficiency, the Tennessee Valley Authority (TVA) established procedures that produce more than 75% of the fertilizers such as ammonium nitrate, diammonium phosphate, nitric phosphate, ammonium polyphosphate, and urea ammonium phosphate (Hignett, 1985).

The significance of fertilizer and fertilization can be understood through this historical procedure. A properly applied and good fertilization program provides the nutrients required by the plant and provides the plant with resistance to a wide range of diseases and pests.

For plant growth, phosphate is a fundamental macronutrient that interacts with all the metabolic and botanical components within each section of the plant cell. It also regulates all metabolic actions in the plant, such as photosynthesis, gaseous exchange, and glycolysis (Versaw and Garcia, 2017).

There are a variety of phosphite group fertilizers that are used in agricultural applications for plant disease and weed control. Several different types of phosphorus can be found, including potassium phosphite, copper phosphite, magnesium phosphite, manganese phosphite, zinc phosphite, aluminium phosphite, and calcium phosphite (MacIntire, Winterberg and Journal, 1950). Phosphites, also known as phosphonic acid derivatives, were first used as *aluminium-tris (O-ethyl phosphonate*) and Fosetyl aluminium fungicides in the early 1900s, taking advantage of the fungicidal properties of phosphites. Phi compounds have been used as agricultural fungicides for several decades, and Phi residues are more soluble in soils than Pi (Havlin and Schlegel 2021). Phosphites have taken the place of phosphate fertilizers in agricultural areas. Griffith et al. (1993) suggested that plants absorb Phi and Pi by the same active transport system, competing for entry into the cell, although some suggest that plant cells may absorb Phi more rapidly than Pi observed that phosphite and phosphate have the same carrying system, but plant cells absorb the phosphite form more rapidly than the phosphate form. Interestingly, McDonald et al. (2001) argued that Phi may strengthen the effects of Pi deficiency by tricking Pi deficient cells into sensing they are Pi sufficient. Phi may also be recognized or sensed in plants as Pi for sustaining plant growth and function under low soil P. thus, phosphites play an essential role in promoting plant growth and development in soils with phosphorus deficiency. Although some consider Phi effects on plant diseases an "indirect" effect, Phi can enhance plant health directly through the control of selected fungi on plants. Several works completed on the use of Phi to control or reduce the severity of plant diseases. 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 (Liljeroth *et al.*, 2016). Due to phosphite fertilizer and phosphorus-containing fertilizers, many plants have increased yield and quality, such as broccoli, tomatoes, onions, potatoes, peppers, cherries, peaches, oranges, and rice (Gaskell *et al.*, 2000).

Lovatt, C. (2013) evaluated if timing Phi foliar applied fertilizers can increase the efficacy, fruit set, and size of avocado and citrus trees. Eleven-year-old avocado and six-year-old from budding mandarin trees were used in the study by supplying potassium phosphite and low-biuret urea fertilizers directly to the foliage of the trees. According to the findings of this study, applying foliar fertilizer at the optimal period on an alternate-yielding tree was critical to effectively increasing the yield of the fruit at a size that is economically desirable for both mandarin oranges and avocados. In a single winter pre-bloom, the mandarin foliar treatment of low-biuret urea boosted fruit output with crosswise diameters of around 2.3 and 2.4 inches by 13,200 fruits (2860 kg) per 200 trees for every acre for two years. In avocados, just a single foliar treatment for potassium phosphite enhanced fruit output from 6.3 to 11.5 ounces by 8140 fruit (4268 kg) per 110 trees per acre for three years. These findings support the notion that specialized nutrients given to the foliage, including flowers, can efficiently fulfill crop nutrient needs while stimulating specific metabolic and physiological mechanisms, leading to higher fruit formation, size, quality, and producer revenue.

Manna *et al*., (2016) inspected to measure the development of a phosphite-mediated fertilization and weed control system for rice. The rice plants were assembled using a codon-optimized *phosphite oxidoreductase gene* (ptxD) from *Pseudomonas stutze*; since this gene, the general phenotypic, root development, and physiology were all significantly enhanced. In contrast to the typical production in phosphite-treated transgenic plants, phosphite performs as a trans locative, non-selective, pre-and post-thread herbicide while maintaining plant yield. As a result, Phi has the potential to be an outlay agricultural strategy that would allow us to grow crops in soils with low orthophosphate availability and deal with the problems of P depletion and herbicide resistance.

In a study done by Costa *et al.*, (2020) it was determined that the common bean has the potential to be treated with manganese and zinc phosphites for the control of *Xanthomonas axonopodis pv. phaseoli var. fuscans.* the causal agent of common bacterial

blight was inoculated into the plants 48 hours after being sprayed with the solution of (7.5 ml/L) Mn and Zn phosphites, while control plants were sprayed only with water. 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.A study concluded by Quaglia *et al.*, (2021) investigated whether zinc phosphate treatments of tomato plants can reduce the severity of bacterial speck disease by reducing the effect of *Pseudomonas syringae pv. tomato* on morphological and biochemical plant defense responses in tomato plants. A zinc phosphate was applied at a concentration of 10 ppm (25.90 mM) before spraying the tomato plant was inoculated with strain DAPP-PG 215, race 0 of *Pst*. The percentage of chlorosis and necrosis on each leaf and the number of necrotic spots were used to measure disease symptoms. As a result, soil treatments with zinc phosphate could protect tomato plants against *Pst* attacks by inducing morphological and biochemical plant defense responses and direct antibacterial activity.

The purpose of this study was to examine the efficacy of five different phosphites and a plant fungicide, Fosetyl-Aluminium to control the tomato bacterial speck disease in a greenhouse with a heat-controlled system as two repeated pot experiments.

CHAPTER 1

GENERAL INFORMATION

In the last ten decades, there has been a severe global increase in the excess growth of human beings, which has resulted in fears of not having enough food and other resources. Sustainable yield growth is one of the most important approaches to solving this problem. Tomatoes are one of the most important crops for human consumption, and it is essential to increase the yield and safety of the tomato plant. Unfortunately, numerous bacterial, fungal, and viral diseases and pests are detrimental to tomato production and reduce yields.

Currently, pesticides are extensively used, resulting in pollution of the soil, people, and the environment as a result of this. Therefore, various research projects are attempted to find alternative control strategies for plant diseases through research to reduce pollution. The purpose of this study, which was carried out following the same objectives and strategies as the previous one, was to slow down the progression of the disease by activating disease resistance mechanisms in the plant and to supplement the nutrient source that the plant requires by using phosphite (phosphonate)-sourced fertilizers to supplement the nutrient source that plant required. This study demonstrates an important contribution to the long-term viability of agriculture, the protection of human health, and the prevention of environmental pollution threats. A correlation exists between plant nutrition and the presence and progression of the disease. However, the effects of phosphite-containing fertilizers on the suppression or prevention of various diseases in plants and the activation of plant resistance mechanisms have only been studied in a few studies.

The present literature views summarize the findings of numerous studies conducted to determine the efficacy of phosphite-based fertilizers on several plant diseases.

1.1. The Importance of Phosphate in Agriculture

According to research concluded by Rickard (2000), a series of experiments were carried out on an open farm and in a greenhouse to investigate if the phosphites affect the yield of vegetables, including onions, potatoes, celery, and sweet peppers. After applying phosphites to the foliage of the vegetables, it was found that phosphites significantly increased the yield of vegetables.

The roots' function is to absorb minerals and water from the soil, making them one of the most important parts of plant physiology.To promote root development, phosphate has a vital role to play. This includes the adjustment of root structure and thickness, the regulation of primary and lateral root development, which stimulates the investigation of the presence of phosphorus on the topsoil, and the improvement of the density of root hair with growth (Vance *et al.*, 2003).

Schroetter *et al.*, (2006) tested the efficiency of potassium phosphite as a P source for maize plants in a long-term field trial and a pot experiment. In the study field, two distinct P fertilization procedures were carried out. 45 kg ha^{-1} P treatment as triple superphosphate in combination with an organic fertilizer in the type of farmyard manure (4.8 t ha^{-1} DM) and others without phosphite fertilizer. The pot trial was performed, cleaned gravel sand was applied as the substrate, and deionized water was served for watering. In the beginning, there were just three separate groups of soil P fertilization developed, and each set included a total of 12 repetitions. The first group got P fertilization (1,008 mg P per pot) from dissolved potassium dihydrogen phosphate (KH_2PO_4). In contrast, the second treatment was given an equal quantity of P in the form of mixed potassium dihydrogen phosphate (KH_2PO_3). Finally, another batch of containers was made without the addition of P. For the field-based treatment, the maize plants were harvested two weeks following foliar fertilization; for the pot-based treatment, the harvest took place ninety days after the seeds were sown. The results of the study showed that plant roots are good at absorbing this P compound. After foliar phosphite application, this chemical was visible in all maize plant sections, proving its phloem and xylem mobility. The formation of Phosphite was very significant in developing corncobs. Only small amounts of phosphate are oxidized during plant metabolism. The stunted growth found in plants that had previously been treated with Phosphite was most noticeable in situations when there was

a lack of Phosphite due to the inhibition of the organic processes that enable plants to react generally to P deficiencies.Growth and development of the plant after nitrogen fertilizers, phosphorus is one of the most critical elements in plant growth and development, accounting for up to 0.2 percent of the dry weight of the plant. Phosphorus is the only fertilizer that limits the number of nutrients usable for crop development and growth after nitrogen fertilizers (Azziz G., *et al.*, 2012).

Phosphorus is a critical component of the *nucleic acids DNA* and *RNA*, as well as the *phospholipids* in cell membranes and the energy nucleoside ATP. It is therefore considered essential to all forms of life on Earth, including both lower and higher plants, because of its role in genetic heredity, cell wall structure, signal transduction pathways, and metabolism (Butusov and Jernelöv, 2013).

Estrada-Ortiz *et al*., (2013) investigated the impact of phosphite on the production of strawberries and the quality of their fruit. Throughout the research, the experiments were carried out in a tunnel-type greenhouse, utilizing drip irrigation and volcanic rock as a substrate. The strawberry plants were applied to five different treatments with various percentages of Phi (0, 20, 30, 40, and 50%). Steiner's approach was assessed. The initial sample took place 30 days after treatments and the next 131 days later, according to the blooming and fruit development periods, respectively. The Steiner's nutritional solution was retained at 50%, and starting with the fruiting phase, it was maintained at 75%. As a result, no significant differences were observed when compared to the control. In addition to 20% Phi slightly improving yield and fruit size, plants given 30 percent or more phosphorus had the highest values for pH, electrical conductivity, anthocyanin concentration, yield, and fruit size when compared to both the control group and the other treatments.

Phosphorus (P) is a fundamental and essential nutrient required in large amounts for the development process and reproduction. In addition, it is an essential fertilizer that is undoubtedly necessary to maintain a high level of agricultural productivity. However, applying phosphate fertilizer to soil causes the remaining microbial activity and fixations to be disabled immediately (López-Arredondo *et al.*, 2014).

1.2. Effect of Phosphites on Plant Diseases

Zahid et al., (1999) investigated the influence of potassium phosphonate on *Phytophthora cinnamomi*, the causal agent of root rot of *Pinus radiata*. *P. radiata* seedlings transplanted into washed river sand/peat potting mix inoculated with *P. cinnamomi* displayed severe wilt and root rot signs within seven weeks and died within 12 weeks. In contrast to direct inoculation, stem wound inoculation with pathogen mycelium resulted in only restricted lesions. Applications of three different doses of potassium phosphate (0.01, 1.0, and 5.0%) to the foliage or the roots of transplanted plants into pots with pathogen-infested sand or sand/peat mixture, root rot symptoms have been diminished significantly. When applied to a sand/peat potting mix, phosphonate treatments were more efficacious than when applied to washed river sand. According to the findings, the pathogen's recovery from infected plant roots and the potting mix was lower in pots saturated with phosphonate than in pots treated with foliar sprays.

Reuveni et al., (2003) assessed the effects of potassium phosphite and DL-b-aminobutyric acids (BABA) on the control of moldy-core (*Alternaria alternate*) in apples The experiment was performed both in the laboratory and in the field. Inoculation of wounded mature fruits in the laboratory trial indicated that 50 µg/ml of potassium phosphite inhibited decay by *A. alternata* by 40 to 58%, and 500 µg/ml completely inhibited decay by the pathogen. According to the findings of *in vitro* study, BABA compounds did not affect conidial germination or mycelial growth, potassium phosphite had a significant inhibitory effect on both developmental stages of the fungus. Fieldwork research performed in a commercial apple orchard revealed that three foliar applications of BABA or potassium phosphite, applied from the start of flowering until petal fall, reduced the number of fruits contaminated with the moldy core by 40 and 60%, respectively. Based on observations, potassium phosphite and BABA may effectively control moldy-core disease in apple trees.

Silva *et al.*, (2011) investigated if the potassium phosphite can control *Peronospora manshurica*, the causal agent of downy mildew of soybean. Field experiments were carried out in the southern Brazilian state of Parana during the growing seasons of 2006/2007 and 2007/2008. At two growth stages, V6 (fifth trifoliolate leaf) and R2 (full flowering), four rates of potassium phosphite (0, 375, 750, and 1500 g P_2O_5 + K_2O ha^{-1}) were applied. With an increase in the rate of phosphite applied, there was a linear decrease in the severity of downy mildew and a substantial improvement in the leaf area index. A

considerable increase in yield was recorded during the 2006/2007 growing season, which was attributed to the use of the highest rate of phosphite.

Felipini *et al.*, (2016) determined whether two elicitors (potassium phosphite and chitosan) had any influence on apple scab (*Venturia inaequalis*) prevention and even if they had any capacity to stimulate plant defenses. Seven days before fungus inoculation, potassium phosphite and chitosan were treated on apple seedlings, and disease severity was assessed 14 days after inoculations. The metabolic profile of leaves treated with phosphite or chitosan that were shown to be susceptible and moderately susceptible to infection was subsequently analyzed using attenuated total radiance transform infrared spectroscopy. The effect of product treatments on the germination of *V. inaequalis* was investigated. Despite product application, moderately susceptible leaves had higher peroxidase activity. Chitosan diminished spore germination by 45% but did not affect disease severity. While potassium phosphite (2 μL mL−1) reduced scab severity by as much as 62%, it also promoted the accumulation of salicylic acid, protocatechuic acid, and epicatechin in sensitive leaves. The scientists concluded that the resistance induced by potassium phosphite may play an important role in scab management.

Abbasi and Lazarovits (2016) were inspected to determine if the seed treatment with phosphonate (AG3) can suppress the *Pythium damping-off* of cucumber seedlings. In monitored environments and the field, a phosphonate (AG3) formulation was applied as a seed treatment to prevent *Pythium damping-off* of cucumber (*Cucumis sativus L.)* plants. The cucumber seed was handled by immersing it in a phosphonate solution for ten minutes. The seedlings were planted in a peat-based mix or sandy loam soil mixed with *Pythium aphanidermatum or P. ultimum* inoculum or in muck soil normally inoculated with irregular, *P. ultimum,* and other *Pythium spp.* More than 80% of the damping-off in all infected substrates was reduced by phosphonate seed treatment in a controlled environment. During testing conducted on field plots, cucumber stands increased by 63 percent in the six weeks after sowing treated seed in *pythium*-infested muck soil. In comparison, the control group's stand percentage was just 18 percent. In contrast, phosphonate seed treatment is an efficient and cost-effective method of preventing cucumber plants from the detrimental effects of *Pythium damping-off.*

Ramezani *et al.*, (2017) investigated the impact of potassium phosphite (KPhi) at molecular and biochemical levels in response to *Pseudoperonospora cubensis* infection in cucumber plants. The experiment was carried out on four weeks old cucumber seedlings. Initially, the plants were separated into five groups, each consisting of six pots, each containing one plant. Various treatments were applied to each group. The first group served as a control, and distilled water was used. The leaves in the second group were treated with only 2 g L-1 KPhi three times a day for one day. The leaves were picked at time intervals ranging from 24, 48, 72, and 96 hours, four days after KPhi treatment. On the third treatment, the leaves were treated with 2 g L^{-1} KPhi three times daily for one day. *P. cubensis* inoculation was performed four days after KPhi application, and leaves were gathered at intervals of 24, 48, 72, and 96 hours eight days after inoculation (Pre-inoculation). Fourth treatment Leaves were treated with 2 g L^{-1} KPhi three times a day for one day, eight days after *P. cubensis* inoculation. After four days, the harvesting of leaves occurred at 24, 48, 72, and 96 hours (Post-inoculation). In the final treatment, only *P. cubensis* was used to inoculate the leaves, harvested after eight days at intervals of 24, 48, 72, and 96 hours (*P. cubensis*). Levels in cucumber plants in response to *P. cubensis* infection Real-time PCR was used to investigate the differential expression of *P. cubensis* defense genes. At 96, 72, and 48 hours, the highest levels of *Thaumatin-like protein* (TLP), *Ribosome-inactivating protein* (RIP), and *defensin* gene expression were found in pre-inoculation plants. These findings demonstrated that these genes are involved in cucumber leaves' defense response following KPhi treatment and pathogen inoculation. When looking at the biochemical level, it was discovered that pre-inoculation plants had higher levels of some phenylpropanoid pathway end-products such as phytoalexins, phenolic components, flavonoids, and anthocyanins, as well as higher levels of phenylalanine ammonia-lyase (PAL) enzymatic activity, when compared to post-inoculation plants at all time points.

Figueira et al., (2020) tested the efficacy of potassium phosphite (Fertilis®), *Trichoderma harzianum* (isolate TOD1) and *Trichoderma virens* (isolate TM4) against *Colletotrichum lindemuthianum*, which causes anthracnose in common bean varieties.The efficacy of treatments on the histochemical changes, such as the hypersensitivity reaction and the severity of the disease was investigated. The application of potassium phosphite alone, in combination with *T. harzianum* and *T. virens*

significantly reduced disease severity by 68%, 84%, and 71%, respectively. It has been observed that the combination of these two compounds causes a rapid change in the histochemical properties of the plant which are responsible for resistance.

Gilardi *et al.*, (2020) investigated the five biocontrol agents and potassium phosphite for their capacity to control *Phytophthora capsici* on hydroponically grown zucchini plants at varied concentrations and application rates. Five to seven days before planting the zucchini seedlings, a pathogen-infected peat substrate was treated with an experimental biocontrol agent (*Trichoderma gamsii + T. asperellum*) containing a commercial formulation of *Trichoderma gamsii + T. asperellum*, followed by five days of follow-up treatment intervals. The experimental biocontrol agents included *Trichoderma* sp. TW2, a combination of *Pseudomonas* FC7B, FC8B, FC9B, and *Pseudomonas* sp. PB26, *Fusarium solani* FUS25. The potassium phosphite was applied directly to the growing medium or via a nutrient solution every six days, beginning at pathogen infestation and ending 5–7 days before planting. When applied to zucchini, potassium phosphite significantly reduced Phytophthora crown rot disease severity by 62–94 %, resulting in a more consistent disease severity reduction than that achieved with experimental BCAs, either alone or in combination, or with the *Trichoderma gamsii + T. apserellum* formulated mixture (which reduced disease severity by 29–47 %). Scientists concluded that growers would have an excellent opportunity to control *P. capsici* on soilless zucchini due to this study.

da Silva Junior *et al.*, (2021) researched the influence of phosphites in the control of anthracnose in soybean pods, as well as their role in pathogen transmission and the activities of plant defense enzymes. The soybean plants were treated with potassium, manganese, zinc, and copper phosphites, as well as the fungicide epoxiconazole with piraclostrobin at different growth stages of soybean. The severity of anthracnose, the incidence of *C. truncatum* in soybean seeds, the activity of the peroxidase, catalase, and superoxide dismutase enzymes, as well as the concentrations of soluble lignin and total soluble phenols, were all analyzed. It is concluded that copper, manganese, and potassium phosphites could diminish the severity of soybean anthracnose and the transmission of the *C. truncatum* in soybean seeds, increased lignin content and enzymatic activity.

Júnior *et al.*, (2021) investigated the influence of several phosphites on the control of the fungus *Hemileia vastatrix*, the causal agent of coffee leaf rust (CLR), and is believed to be the most severe pathogen influencing coffee yield worldwide. Two dosages of phosphate solutions standard (from 1.5 to 3 $mL \cdot L^{-1}$) and double (from 3 to 6 $mL \cdot L^{-1}$) were applied to three-month-old coffee plants in the following doses: K (40% $P_2 O_5$; 1.5 and 3 $mL \cdot L^{-1}$), K (30% $P_2 O_5$; 3 and 6 $mL \cdot L^{-1}$), Cu (2.5 and 5 $mL \cdot L^{-1}$), Mn/Zn (3 and 6 $mL \cdot L^{-1}$) and Na (3 and 6 $mL \cdot L^{-1}$) 24 hours before inoculations with the pathogen. After being sprayed with phosphite solutions, the leaves were either not washed or washed with deionized water. The control treatment consisted of spraying plants with deionized water. Then, inoculation was conducted on the abaxial side of the first pair of enlarged leaves (1 mg per leaf) using a camel hairbrush and a suspension of urediniospores. Regardless of the washing procedure, all phosphites applied at regular concentrations (1.5 to 3 $mL\ L^{-1}$) reduced the total number of pustules per leaf by 28-69%. The Cu phosphite showed superior performance on the CLR control (60 and 69%), regardless of the dose utilized or the washing conditions utilized in the experiment. Only Cu and Na phosphites decreased CLR severity at the standard doses (53-61%) without leaf washing. The CLR severity was decreased using K (30% and K 40%) and Cu phosphites when applied at the standard doses with leaf washing (41-59%). The efficiency of the phosphites for CLR control varied according to the accompanying cation, dose and washing conditions. The Cu phosphite resulted in the best CLR control regardless of the dose used and the washing conditions.

Mehta *et al.*, (2022) explored the anti-fungal and protection elicitor activity of potassium phosphite (Phi) against the pathogen *Mycoplasma oryzae* on the rice cultivar, Samba Mahsuri, in India, as well as its *Agrobacterium rhizogenes* variant (ptxD-OE) that pushes the limits of the phosphite dehydrogenase enzyme. In the presence of potassium phosphite, it elevated the levels of photosynthetic pigments, which was accompanied by increased activity of protective enzymes. Furthermore, the application of Phi to the rice leaf resulted in the activation of defensive-associated genes (OsCEBiP and OsPDF2.2) in the plant. Moreover, phosphite treatment also decreased the production of reactive *lipid peroxidation*, which helped diminish the cell damage caused by *Magnaporthe oryzae* in rice.

Najdabbasi *et al.*, (2022) studied the effectiveness of potassium phosphite (KPhi) in combination with recommended and reduced doses of different fungicides (mancozeb (MCB), cyazofamid (CFD), mandipropamid (MPD), azoxystrobin (AZ), and benthiavalicarb-isopropyl (ISO)) in the control of Phytophthora late blight infection caused by *Phytophthora infestans* on potatoes. The protective impacts of various combinations were first evaluated *in vivo*, then, compared with a greenhouse screening. When used at recommended and reduced doses, the fungicides CFD and MCB were less efficient in decreasing *P. infestans* infections than when combined with KPhi. CFD, MPD, and MCB were the most effective treatments when combined with KPhi. However, the combination of KPhi with AZ, ISO/MCB, and CFD at reduced doses exhibited strongly protective activity compared to other similar combinations. It reduced the severity of infection by *P. infestans* by up to 89%. Greenhouse experiments also revealed that a combination of KPhi and CFD at both doses resulted in the most significant reduction in disease severity (up to 90%) within 35 days of infection. They concluded that the synergy between phosphite and fungicides may significantly protect against Phytophthora infection in potatoes.

Su *et al.*, (2022a) showed whether potassium phosphate could boost the antagonistic capacity of *Bacillus amyloliquefaciens* in the management of tomato bacterial wilt. A combination of potassium phosphite (K-Phite) and biocontrol agent *Bacillus amyloliquefaciens QPF8* was used in the study. According to the experiment, the first and second K-Phite could significantly inhibit the growth of *R. solanacearum* and enhance the antagonistic capability of *B. amyloliquefaciens F8.* Meanwhile, greenhouse soil experiments revealed that the combined treatment had a significantly higher control efficiency for tomato bacterial wilt than either *B. amyloliquefaciens F8* or K-Phite alone. Ultimately, the findings illustrate a new approach for the application-based control of tomato bacterial wilt disease and a new integrated pattern based on enhancing the antagonistic capability of biocontrol microbes by K-Phite.

Su *et al.*, (2022b) showed if Potassium phosphite (KPhi) can increase the capability of the soil microbiome to control the tomato bacterial wilt disease caused by *Ralstonia solanacearum*. Two numerous different soils, Luvisol soil from a grape plantation in Hebei Province, were collected from the upper soil layer (5–30 cm) of an open field covered with grass (HN) and planted with grapes for two years (HG). A 6.5 pH KPhi

suspension was prepared with potassium hydroxide. In both soil samples, the effects of KPhi on the number of culturable *Actinobacteria* were determined. The same approaches were used to assess KPhi's influence on the Nanjing soil microbial species; KPhi treatment boosted antagonistic soil microorganisms like *Paenibacillus* and *Streptomyces*. It reduced the number of dominant genera of gram-negative bacteria like *Pseudomonas*, *Massilia*, and *Flavobacterium.* In addition, the KP-modified soil microbiome was observed to suppress the growth of *R. solanacearum* in soil. According to the findings, the addition of potassium phosphite to the soil microbiome regulated the soil microbiome's antagonistic ability to suppress *R. solanacearum*.

CHAPTER 2

MATERIAL AND METHODS

2.1. Material

2.1.1. Bacterial Pathogen and the Media

In the experiment, a *Pseudomonas syringe pv. tomato* strain DG 1-2 1R, isolated and identified by Dr. Sümer Horuz 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) (20 g proteose peptone, 10 ml glycerin, 1.5 g K_2HPO_4, 1.5 g $MgSO_4.7H_2O$, 15 g agar, 1000 ml distilled water, pH: 7.2) was used for the growth of bacteria in the laboratory conditions and Yeast Dextrose Calcium Carbonate Agar (YDCA) (10 g yeast extract, 20 g dextrose, 20 g $CaCO_3$, 18 g agar, 1000 ml distilled water) was used for short-term storage at 4 °C in the fridge (Lelliott and Stead, 1987).

The KB medium was sterilized in an autoclave at 1.2-atmosphere pressure at 121 °C for 20 minutes, and after cooling to 50 °C, it was poured into Petri dishes and stored in the refrigerator at +4 °C until use. Another medium, YDCA, was divided into glass tubes (16 cm long, 16 cm in diameter) of 10 ml each. Their mouths were closed with cotton and then sterilized in an autoclave. After the sterilization process, the tubes, which were cooled to 50 °C, were mixed homogeneously in the tube mixer, then left to freeze for 24 hours on horizontally 2 cm high wooden plates, and then stored at +4 °C in the refrigerator until use.

2.1.2. Research area

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

2.1.3. 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.

2.1.4. 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 prepared as 2200 g of sand + clay + peat (30% peat, 70% sand + clay) was distributed for each bag.

2.1.5. Phosphites and Plant Activator 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 plant activator were sprayed on 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.

Fertilizer Type	**Nutritional Content**	**Recommended Dose**	**Applied dose**
Copper Phosphite	5% Nitrogen (N), 5% Copper (Cu), 13% Phosphorus Penta Oxide (P_2O_5)	250 – 300 ml / 100 L	1.5 ml/ 500 ml
Zinc/Manganese Phosphite	5% Nitrogen (N), 5% Zinc (Zn), 5% Manganese (Mn), 20% Phosphorus Penta Oxide (P_2O_5)	250 – 300 ml /100 L	1.5 ml/500 ml
Calcium Phosphite	5% Nitrogen (N), 6% Calcium Oxide (CaO), 20% Phosphorus Penta Oxide (P_2O_5)	250 – 300 ml / 100 L	1.5 ml/500 ml
Magnesium Phosphite	5% Nitrogen (N), 5% Magnesium Oxide (MgO), 30% Phosphorus Penta Oxide (P_2O_5)	200 – 250 ml / 100 L	1.25 ml/500 ml
Potassium Phosphite	20% Potassium Oxide (P_2O_5), 30% Phosphorus Penta Oxide (P_2O_5)	250 – 300 ml / 100 L	1.5 ml/500 ml
Plant Activator, Fosetyl Al	80% Fosetyl Al	250 g / 100 L	1.25 g/500ml

2.2. Method

2.2.1. 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, *Pst* colonies growing in white rod-shaped creamy colour were purified and used in the pathogenicity test.

2.2.2. 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 10^7 cells/ml. Finally, this inoculum was sprayed onto the plant leaves until runoff. The sprayed plants were kept in the climate cabinet (16 hours of light period, 8 hours of dark environment, 27°C temperature, 70-80% humidity) until disease symptoms were observed.

2. 2. 3. 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 a clean scissor. 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.

2.2.4. Application of Phosphites and Plant Activator 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.

2.2.5. Inoculum Preparation

Highly virulent and freshly grown in KB medium DG 1-2 1R isolate was used. 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 $3x10^8$ cells/ml using the Petri count method. After that, the suspension diluted one time. This diluted suspension ($3x10^7$ cells/ml) was sprayed on the control, and the phosphites or plant activator was applied to 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 phosphite applications in the first and second trials, respectively.

2.2.6. 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.

2.2.7. 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)(Figure 2). DS was calculated using the Tawsend-Heuberger formula. Efficacies of the treatments were also calculated according to Abbott formula. The formulas used were as follows:

Disease severity (%) = [(∑ number of diseased leaves in each grade × grade)/(total number of leaves investigated × the highest disease index)] × 100

Efficacy (%) = [(incidence rate in the control – incidence rate in the treated group)/incidence rate in the control] × 100

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.

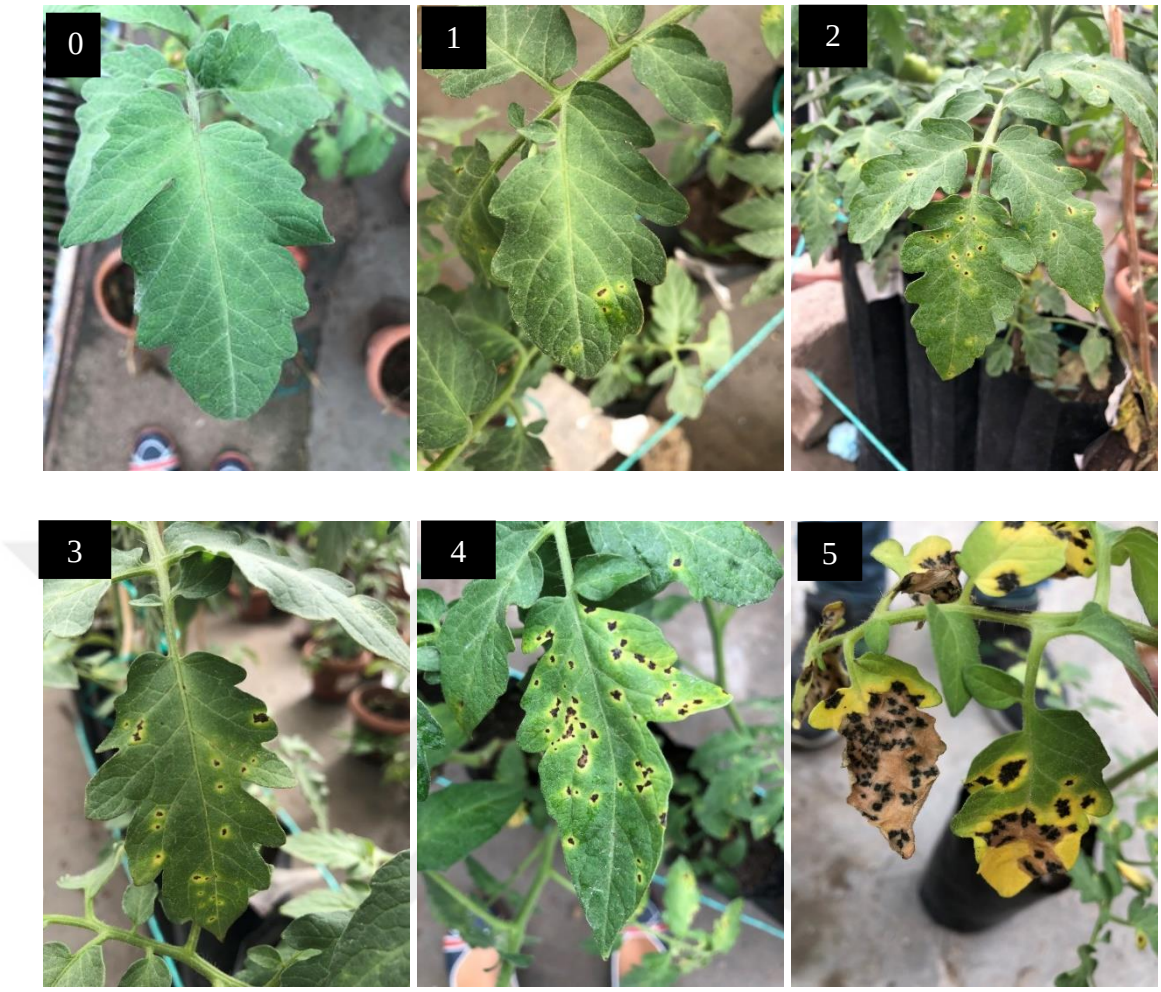


Figure 2. Modified **0** to **5** scale **0**: no symptom; **1**: 1-10% of the leaves infected, **2**: 11-25% of the leaves infected,**3**: 26-50% of the leaves infected,**4**: 51-75% of the leaves infected, **5**: 76-100% of the leaves infected.

CHAPTER 3

RESULTS

3.1. Pathogenicity Test

The bacterial isolate DG 1-2 that was sprayed to on tomato plants induced dark brown lesions surrounded with 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.

3.2. 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 37.53%, disease severity in five different phosphites and Fosetyl-Al applications ranged from 9.38% to 21.73%. All applications inhibited the disease development between 42.10% and 75.01%. Among treatments, zinc/manganese phosphite (75.01%), magnesium phosphite (66.45%), and Fosetyl-Al (56.57%) showed the highest disease reduction (Figure 3.1).

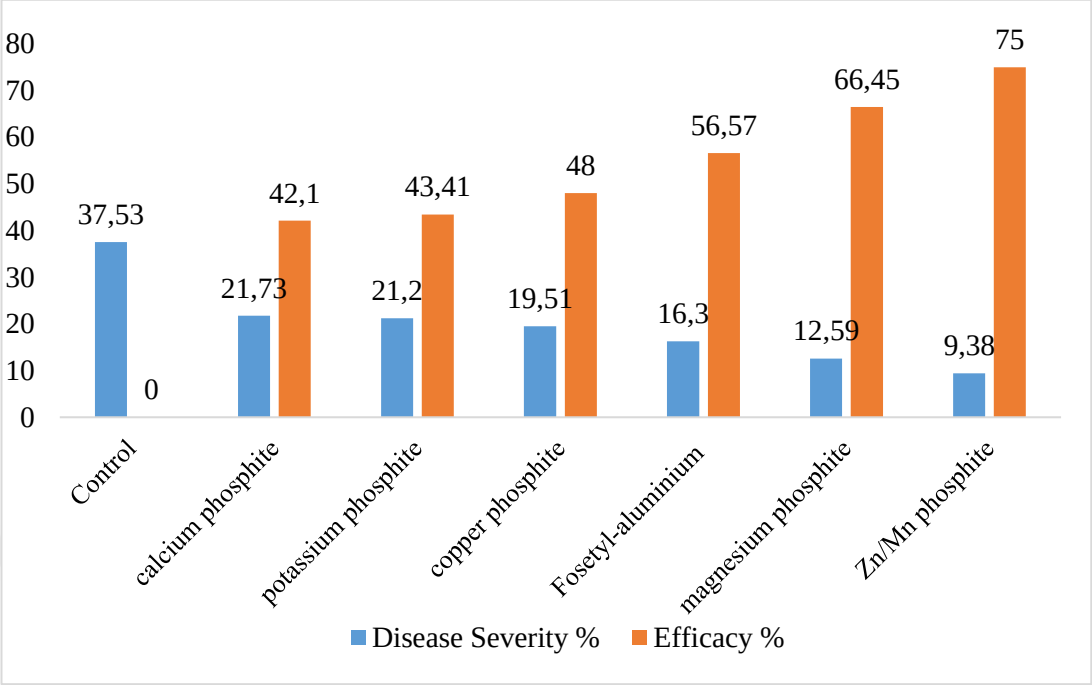


Figure 3.1. Efficacy of treatments on Pst development (First experiment)

In the second experiment, the phosphites and Fosetyl-Al treatments prevented the development of bacterial speck disease on tomatoes from 22.81% to 90.30%. While the disease severity rate in the positive control was 43.00%, the disease severities in treated plants were 33.19% in copper phosphite, 30.94% in calcium phosphite, 15.38% in potassium phosphite, 10.42% in zinc/manganese phosphite, 9.31% in magnesium phosphite, and 4.17% in Fosetyl-Al applications. Compared to all phosphites, Fosetyl-Al showed the most significant reduction in disease progression with a rate of 90.30% efficacy. In addition, magnesium phosphite reduced the disease severity at 78.35%, zinc/manganese phosphite at 75.77% and potassium phosphite at 63.19% rates (Figure 3.2).

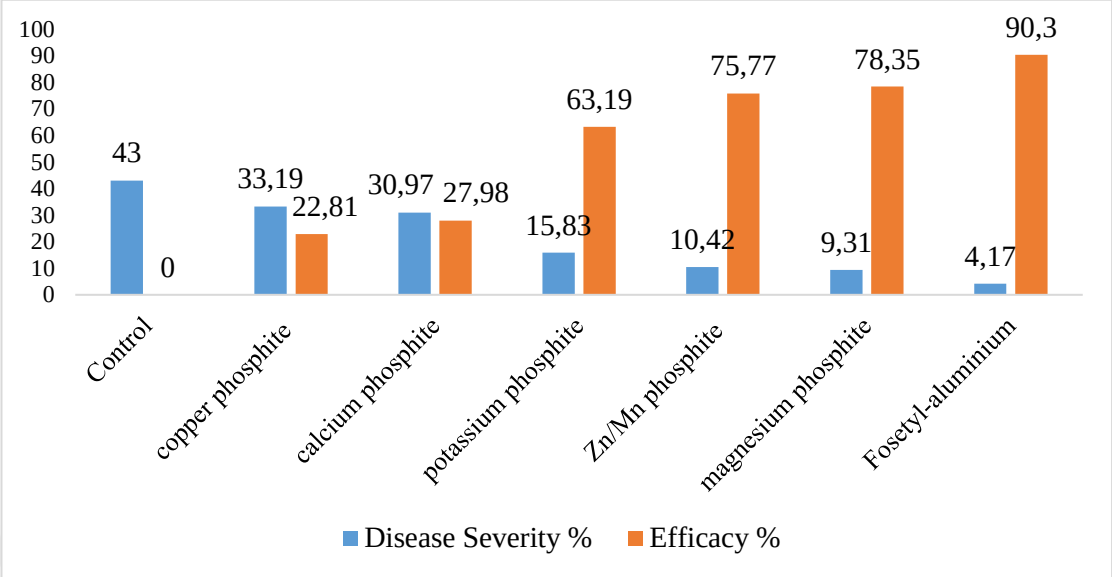


Figure 3.2. Efficacy of treatments on Pst development (Second experiment)

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 (53.31%) (Figure 3.3a), Magnesium phosphite (72.40%) (Figure 3.3b), Fosetyl- Al (73.43%) (Figure 3.4a), and Zn/Mn Phosphite (75.39%) (Figure 3.4b) reduced the *Pst* development over 50%. Additionaly, it was determined that all applications were statistically different from the positive control group and successfully prevented tomato plants from the disease (Table 2).

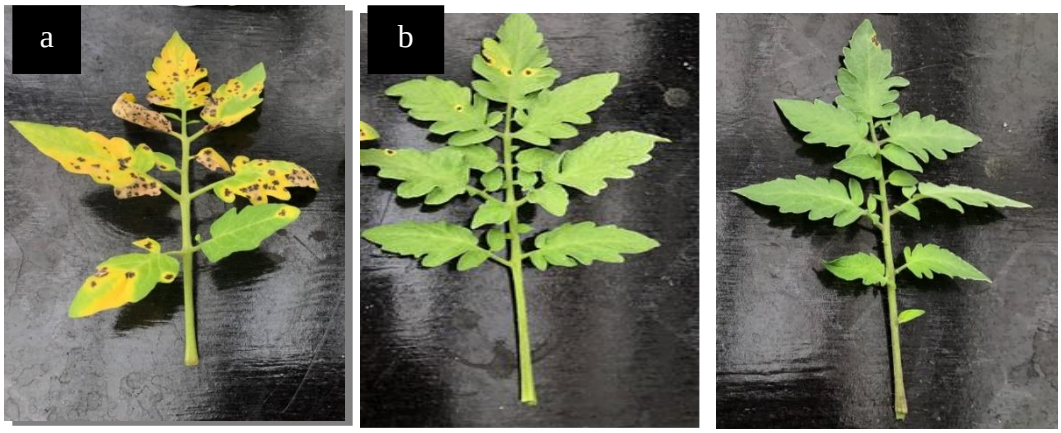


Figure 3.3. Disease development on control (left) and K Phosphite (a), Mg Phosphite (b) applied tomato plants

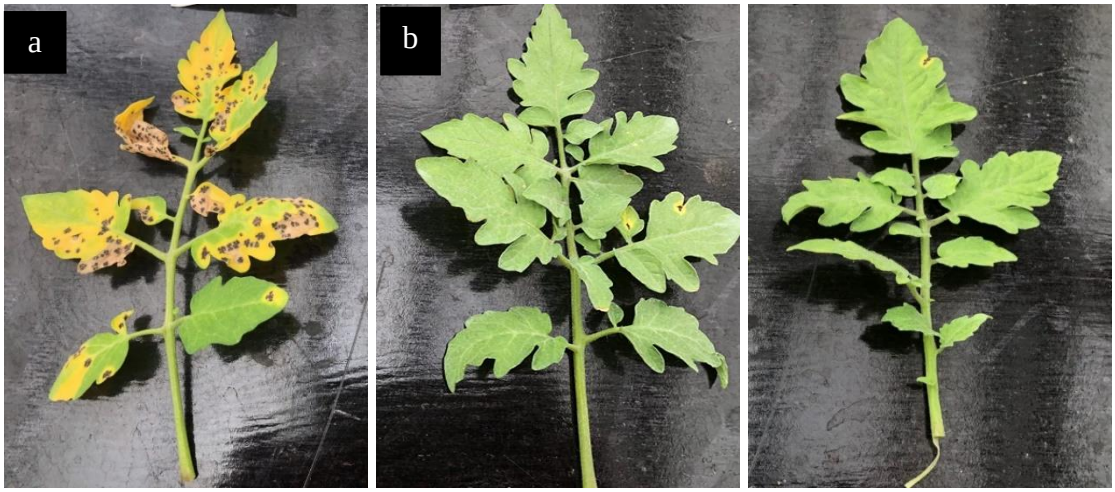


Figure 3.4. Disease development on control (left) and Fosetyl-Al (a) ,Zn/Mn Phosphite (b) applied tomato plant



Figure 3.5. Disease development on control on a stem(left) , calcium phosphite (a) and copper phosphite (b) applied to the tomato plant.

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

Treatment groups	First experiment		Second experiment		Mean of Two experiments	
Treatments	Disease severity %	Efficacy %	Disease severity %	Efficacy %	Disease severity %	Efficacy %
Control	37.53 [a*]		43.00 [a*]		40.26[a]	
Calcium Phosphite	21.73 [b]	42.10	30.97 [b]	27.98	26.35[b]	35.04
Potassium Phosphite	21.23 [b]	43.43	15.83 [c]	63.19	18.53[c]	53.31
Copper Phosphite	19.51 [bc]	48.01	33.19 [b]	22.81	26.35[b]	35.41
Fosetyl-Al	16.30 [bcd]	56.57	4.17 [d]	90.30	10.23[cd]	73.43
Magnesium Phosphite	12.59 [cd]	66.45	9.31 [c]	78.35	10.95[cd]	72.40
Zn/Mn Phosphite	9.38 [d]	75.01	10.42 [c]	75.77	9.90[d]	75.39

CHAPTER 4

DISCUSSION-CONCLUSION AND RECOMMENDATIONS

4.1. Discussion

In this study, the effects of five different phosphites and plant activator 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 favourable 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 tomato (Popović ve 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 harshly impacts 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. Using 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. 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 gained importance in bacterial speck control. It is feasible to increase plant disease resistance by applying adequate fertilizers. Nutrients are vital for 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. 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 defence 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 on 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 mould severity in common bean. 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 sprayed with the solutions (7.5 ml/L) of Mn and Zn phosphites, the area under 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 control treatment (Gadaga et. al., 2017).

Disease suppression results received from the K phosphite-treated plants were ranged from 30% to 80% in several pieces of researches (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 our 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 potential in inhibiting *Pst* development on tomato leaves, thus, could be included in integrated disease management programmes.

4.2 Conclusion and Recommendations

To our knowledge, this is the first study to demonstrate the direct influence of phosphites on tomato bacterial speck. Our data suggested that phosphites can be an effective alternative for the control of tomato bacterial diseases. Studies on the effects of phosphites

under field conditions, on the genes responsible for plant resistance and on other bacterial diseases are planned for the future.

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English- Professional working proficient;

Turkish- Limited working proficient,

Arabic- Limited working proficient,

Afan Oromo-Beginner