CUKUROVA UNIVERSITY

INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Disease Management of Two Important Bacterial Diseases on Tomato By Using Nanoparticles, Some Chemicals and Plant Activators

Omar Hatif ABDULRAZZAQ

Plant Protection Department

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This DoctorateThesis was evaluated by the following Jury Members on 20/10/2023 and was approved by unanimity/majority of votes.

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Omar Hatif ABDULRAZZAQ

Advisor: Prof. Dr Yeşim AYSAN

Plant Protection Department

ABSTRACT

Clavibacter michiganensis subsp. michiganensis, causal agent of Bacterial Canker and Wilt Disease and *Xanthomonas perforans* causal agent of Bacterial Spot Disease are the main pathogens on/in tomatoes. In this study, the effects of two nanomaterials (nano silver AgNps and nano copper CuNps), a plant activator (ISR2000), a commercial fungicide called KOCIDE, and two new chemical compounds named sodium pentaborate pentahydrate (SPP) and disodium octaborate tetrahydrate (DOT) were investigated on Bacterial Canker and Wilt diseases caused by *Clavibacter michiganensis subsp. michiganensis* and Bacterial Spot disease caused by *Xanthomonas perforans* on/in tomatoes. While nanomaterials (AgNps and CuNps) and chemicals (SPP and DOT) showed promising results *in vitro* trials, they could not achieve the same effect *in vivo* trials. In particular, SPP and DOT exhibited phytotoxic effects on tomato leaves. Therefore, further research should be conducted on combining these chemicals as active ingredients in pesticides. In greenhouse and climate chamber trials, ISR2000 significantly reduced disease severity, emerging as the most successful application. This application has suppressed Bacterial Wilt Disease by 77.91%, Bacterial Spot Disease by 54.84% in tomato plants, and Seedborne Bacterial Spot Disease by 84.64%. KOCIDE, a copper-based preparation, was another successful application by effectively suppressing disease severity. This application has suppressed Bacterial Wilt Disease by 46.25%, Bacterial Spot Disease by 22.79% in tomato plants, and Seedborne Bacterial Spot Disease by 80,41%. These results demonstrate that plant resilience-enhancing preparations are the most successful treatments in suppressing infection, especially against chemical-resistant pathogens. These applications are of great importance as alternative environmentally friendly approaches, particularly against chemical-resistant pathogens.

Keywords: Nanoparticular, bacterial spot, bacterial wilt, SPP, DOT

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DOKTORA TEZİ

Nanopartiküller, Bazı Kimyasallar ve Bitki Aktivatörü ile Domateste İki Önemli Bakteriyel Hastalığın Hastalık Yönetimi

Omar Hatif ABDULRAZZAQ

Danışman: Prof. Dr Yeşim AYSAN

Bitki Koruma Anabilim Dalı

ÖZ

Bakteriyel Kanser ve Solgunluk Hastalığına neden olan *Clavibacter michiganensis subsp. michiganensis* ve Domateste Bakteriyel Leke Hastalığına neden olan *Xanthomonas perforans* domatesleri etkileyen iki ana patojendir. Bu çalışmada iki nanomateryal (nano gümüş AgNps ve nano bakır CuNps), bir bitki aktivatörü (ISR2000), ticari bir fungisit olan KOCIDE ve sodyum pentaborate pentahidrat (SPP) ve disodyum octaborate tetrahidrat (DOT) adı verilen iki yeni kimyasal bileşiğin etkisi araştırılmıştır. *In vitro* denemelerde nanomateryaller (AgNps ve CuNps) ve kimyasallar (SPP ve DOT) umut verici sonuçlar verirken, *in vivo* denemelerde aynı etkiyi gösterememiştir. Özellikle SPP ve DOT domates yapraklarında fitotoksik etki göstermiştir. Bu nedenle bu kimyasalların pestisitlerde aktif madde olarak kombinasyon şeklinde kullanımıyla ilgili daha fazla araştırma yapılmalıdır. Hem Sera hem de iklim odasındaki deneme sonuçlarında ise ISR2000 hastalık şiddetini büyük ölçüde azaltarak en başarılı uygulama olarak belirlenmiştir. Bu uygulama domates bitkisinde Bakteriyel Solgunluk Hastalığını %77.91 oranında, Bakteriyel Leke Hastalığını %54.84 oranında ve tohum kaynaklı Bakteriyel Leke Hastalığını ise %84.64oranında baskılamıştır. Bakırlı preparat olan KOCIDE hastalık şiddetini iyi oranda baskılayan bir diğer başarılı uygulama olmuştur. Bu uygulama domates bitkisinde Bakteriyel Solgunluk Hastalığını %46.25 oranında, Bakteriyel Leke Hastalığını %22.79 oranında ve tohum kaynaklı Bakteriyel Leke Hastalığını ise %80.41 oranında baskılamıştır. Bu sonuçlar bitki dayanıklılığını arttırıcı preparatların enfeksiyonu baskılamada en başarılı uygulama olduğunu göstermektedir. Bu uygulamalar özellikle kimyasallara direnç gösteren patojenlere karşı alternatif çevreye dost uygulamalar olarak büyük önem taşımaktadır.

Anahtar Kelimeler: Nanoparticül, Bacterial Leke, Bacterial Solgunluk, SPP, DOT

EXTENDED ABSTRACT

Tomato, Lycopersicon esculentum Mill, is a crop from the Solanaceae family with a high nutritional value due to essential elements and vitamins such as vitamin C. It contains many levels of body-building minerals, such as iron, phosphorus, calcium, and phosphorus. Tomato is a globally produced crop. The tomato is a member of the family Solanaceae and grows as an annual shrub. Tomato are short-lived herbaceous perennials in areas free of pests and diseases. It is believed to have originated in the narrow, dry, and tropical coastal areas of Ecuador, Peru, Bolivia, and parts of Northern Chile. It is native to tropical America. It was brought to Mexico and Central America, where it was utilized by the Aztec and Toltec people and native people living in Vera Cruz and Puebla in Mexico, who were probably responsible for domesticating the animal. The seeds of the tomato plant were brought back to Italy by returning explorers as early as the year 1554, and the plant gained popularity very fast in the warm climate of the Mediterranean. Tomatoes eventually made their way to Northern Europe from that region. The term "tomato" comes from the Spanish word "tomati," which is derived from the South American words "xitomate" and "zitotomate.", the tomato was produced in the countries of Europe as an aesthetic plant known as the "love apple." However, it was not commonly acknowledged as a food at the time. It was thought to be poisonous, maybe because it is a member of the same family as deadly nightshade. Tomatine is a potentially lethal alkaloid found in tomato leaves and immature fruits. After it was determined that the tomato did not contain toxins, it was swiftly adopted as a crop for human use, and its cultivation rapidly expanded globally. After being unknown in the Old World until the sixteenth century and being sporadically used in the nineteenth century, the tomato did not gain widespread popularity as a star vegetable in commercial agriculture and backyard gardens until the twentieth century. The Lycopersicon esculentum species and other related species are used as the foundation or topping for various raw and cooked dishes, and the freshness of these dishes is frequently praised. Tomato Producers face different disease and pest problems in tomato production areas. Several bacterial, fungal, and viral diseases are responsible for significant losses in quality and yield. The tomato (Lycopersicon esculentum) is becoming increasingly significant globally for research into the basic principles of plant growth and development and consumption as a fresh crop and a key ingredient in many prepared cuisines. Members of the Lycopersicon genus may survive in various environmental and dietary situations. A handful of the species have been crossed to produce numerous kinds intended to provide a field crop for a single harvest or, particularly when protected, a succession of fruits for the fresh market over an extended time. Through the combined efforts of plant breeders and agricultural technologists, field tomatoes are susceptible to mechanized harvesting, enabling the cost-effective production of enormous tonnages. Many different canned, frozen, preserved, or dried foods contain the products. According to data collected from FAO, global tomato production for processing and fresh consumption in 2021 amounted to just over 189.1 million metric tons, up 2% from the

184.8 million mT produced in 2020 and 4% from the average of the previous three years (2018-2020) (182.7 million tons). The annual tomato production in many countries has reached or surpassed one million tons. Türkiye's production of tomatoes reached over (13 million tons), double that of Italy but only a fifth of China's (67 million Tons), accounting for nearly 36% of the global harvest.

Plant pathogens represent a danger to the productivity of agriculture because they are the source of diseases that significantly damage the economy and environment. Bacterial Spot Disease caused by Xanthomonas perforans and Bacterial Canker and Wilt Disease caused by Clavibacter michiganensis subsp. michiganensis are the main pathogens on/in tomatoes. vesicatoria). The pathogens induce various disease symptoms, such as wilt, necrosis, gummosis, and vascular or parenchymatous diseases, on diverse plant families' leaves, fruits, and stems, including numerous economically significant crops like tomatoes and peppers.

The distribution of Xanthomonas species is spread worldwide. Xanthomonas euvesicatoria and Xanthomonas vesicatoria on tomato and pepper; Xanthomonas perforans and Xanthomonas gardneri are becoming increasingly isolated from the USA, Canada, South America, Africa, and Europe. Bacterial spot disease is caused by the pathogen Xanthomonas axonopodis pv. vesicatoria (Xanthomonas axonopodis pv. vesicatoria) is a devastating pepper (Capsicum annuum) disease in Türkiye. Bacterial spot disease, caused by Xanthomonas, is a severe pepper disease in the eastern Mediterranean provinces of Adana and Osmaniye in Türkiye. The disease affects 52 to 100 %of pepper fields in these provinces. Bacterial spot disease can infect all portions of a tomato plant above ground, including the leaves, stems, and fruit. Bacterial spots Symptoms appear on leaves as small (less than 3.18 mm) circular spots that are sometimes water-soaked. Spots may appear yellow green initially, then deepen to brownish-red as they age. Extensive leaf yellowing and leaf loss can occur when the disease is severe. Spots on green fruit are often tiny, elevated, and blister-like, with a yellowish halo. As the fruit matures, the spots increase (to a maximum size of 6.14 mm) and become dark, scabby, and scratchy. Mature patches might be elevated or depressed with elevated margins. The disease cycle found that Bacterial spot pathogen inoculum can be found in seeds, tomato volunteers, and plant detritus from diseased plants. All this is considered a source for the pathogen and a method to survive environmental factors. Temperatures between 24°C and 30°C and high humidity favor disease development

Tomato Bacterial Spot was first observed in South Africa in 1914. The disease is one of the most destructive tomato diseases globally and significantly impacts the yield of tomato plants cultivated in warm, humid regions. Typically, infection results in leaf and fruit lesions, causing defoliation and fruit yield loss. Xanthomonas is a widespread genus of plant-pathogenic bacteria found in numerous climate zones; the disease is caused by four species of Xanthomonas (Xanthomonas euvesicatoria, Xanthomonas. gardneri, Xanthomonas. perforans, and Xanthomonas.

One of the most damaging tomato diseases is bacterial canker and wilt disease caused by the pathogen Clavibacter michiganensis subsp. michiganensis. Disease was described for the first time in North America in 1909. It is the source of a vascular bacterial blight feared by producers in many production areas. Easily transmitted by seeds and seedlings, it occurs in the field and under protection. The pathogen causes disease symptoms on all above-ground green parts and fruits, decreasing yield. The pathogen is a non-motile that can be seed-borne and a gram-positive bacterium. This vascular pathogen typically invades and multiplies in the xylem through natural openings or lesions, resulting in symptoms of wilt and canker Bacterial infections have a global impact on tomato plants, particularly in regions characterized by high humidity and sub-humid conditions. Both are the two main pathogens that impact tomatoes. In this study, the effect of two nanomaterials (nano silver AgNps and nano copper CuNps), a plant activator (ISR2000), a commercial fungicide KOCIDE, and two novel chemical compounds called sodium pentaborate pentahydrate (SPP) and disodium octaborate tetrahydrate (DOT) against two serious diseases were examined. This study started by determining the minimal inhibitory concentration for all treatments against both pathogens using the speared plate method and by determining the inhibition zone for each treatment using the disc diffusion method.

Furthermore, minimum pathogen concentrations were determined for both pathogens. However, this study aims to investigate the effect of the treatments on disease severity and the area under the disease progress curve (AUDPC) caused by the pathogen Clavibacter michiganensis subsp. michiganensis under greenhouse conditions. Moreover, the effects of the treatments against Xanthomonas perforans on the plant leaves under a climate chamber and the effect of treatments on the seed germination and disease severity of Xanthomonas perforans as a seed-borne pathogen. The result of the minimum inhibitory concentration test shows the (AgNps) against both pathogens is (0.02) ppm (CuNps) against both pathogens (0.0035) ppm and (DOT) against both pathogens (0.0025) ppm and (SPP) against both pathogens (0.00375) ppm. Furthermore, the result from greenhouse trials on Clavibacter michiganensis subsp. michiganensis shows that the ISR2000 was the best in reducing the disease severity by (13.25%) with efficacy reaching (77.91%) followed by the KOCIDE (32.25%), with efficacy reaching (46.25%) compared to the positive control (60.00%). The result from the climate chamber showed that ISR2000 was superior in reducing Xanthomonas perforans severity on the tomato leaves by (23.20%) with efficacy reaching (54.84%) and the KOCIDE was able to reduce the severity by (39.66%) with efficacy reached (22.79%) and the nanomaterial treatment CuNps (42.23%) with efficacy reached (17.80%) compared to the positive control treatment (51.37%). The result from the seed trials showed that ISR2000 was able to reduce the severity of the disease on cotyledons by (10.19%) efficacy (84.64%) and KOCIDE (12.99%) efficacy (80.41%) and AgNps(43.40%) efficacy (34.56%) and CuNps(46.84%) efficacy (29.37%). Compared to the positive control treatment severity (66,32).

This study provides a summary indicating that the nanomaterials AgNps and CuNps, as well as the novel chemical materials SPP and DOT, have efficacy in vitro for pathogen prevention. However, their effectiveness in vivo is not comparable. The efficacy of the plant activator ISR2000 and the fungicide KOCIDE has been demonstrated in several in vivo trials, including greenhouse and climate chamber experiments and seed treatments, for controlling both pathogens.

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SYMBOLS AND ABBREVIATIONS

AgNps : Silver nanoparticles
ASM : Acibenzolar-S-methyl
AUDPC : Area under the disease progress curve
AuNPs : Gold Nanoparticle
BCA : Biological Control Agent
BST : Bacterial Spot Disease
Cfu : Colony formation unit
Cu : Copper
CuNps : Copper nanoparticles
DLA : Diseased leaf area
DNA : Deoxyribonucleic acid
FAO : The Food and Agriculture Organization
FeS2NPs : Iron nanoparticles
FQ-Cu : Fixed quaternary ammonium copper
HP : Hydrogen peroxide
Hrp : Harpin Protein
IPM : Integrated pest management
IR : Induced systemic resistance
Mid : Middle
MV-Cu : Multivalent copper
Nm : Nanometre
NPs : Nanoparticles
PDA : Potato Dextrose Agar
RNA : Ribonucleic acid
SAR : Systemic acquired resistance
SPP : Sodium pentaborate pentahydrate
TiO2NP : Titanium Oxide Nanoparticle
TSA : Tryptic soy agar
TÜİK : Türkiye İstatistik Kurumu
Xoo : *Xanthomonas oryzae* pv. *Oryzae*
ZnNP : Zinc Nanoparticle
ZnONP : Zinc Oxide Nanoparticle

1. INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill, is a crop from the *Solanaceae* family with a high nutritional value due to essential elements and vitamins such as vitamin C. It contains many levels of body-building minerals, such as iron, phosphorus, calcium, and phosphorus. Tomato is a globally produced crop. The tomato is a member of the family *Solanaceae* and grows as an annual shrub.(Zwain and Altabo, 2018) Tomato are short-lived herbaceous perennials in areas free of pests and diseases. It is believed to have originated in the narrow, dry, and tropical coastal areas of Ecuador, Peru, Bolivia, and parts of Northern Chile. It is native to tropical America. It was brought to Mexico and Central America, where it was utilized by the Aztec and Toltec people and native people living in Vera Cruz and Puebla in Mexico, who were probably responsible for domesticating the animal. The seeds of the tomato plant were brought back to Italy by returning explorers as early as the year 1554, and the plant gained popularity very fast in the warm climate of the Mediterranean. Tomatoes eventually made their way to Northern Europe from that region. The term "tomato" comes from the Spanish word "tomati," which is derived from the South American words "xitomate" and "zitotomate.", the tomato was produced in the countries of Europe as an aesthetic plant known as the "love apple." However, it was not commonly acknowledged as a food at the time. It was thought to be poisonous, maybe because it is a member of the same family as deadly nightshade. Tomatine is a potentially lethal alkaloid found in tomato leaves and immature fruits. After it was determined that the tomato did not contain toxins, it was swiftly adopted as a crop for human use, and its cultivation rapidly expanded globally (Blancard et al., 1992). After being unknown in the Old World until the sixteenth century and being sporadically used in the nineteenth century, the tomato did not gain widespread popularity as a star vegetable in commercial agriculture and backyard gardens until the twentieth century. The *Lycopersicon esculentum* species and other related species are used as the foundation or topping for various raw and cooked dishes, and the freshness of these dishes is frequently praised. Tomato Producers face different disease and pest problems in tomato production areas. Several bacterial, fungal, and viral diseases are responsible for significant losses in quality and yield (Arshad et al., 2014). The tomato (*Lycopersicon esculentum*) is becoming increasingly significant globally for research into the basic principles of plant growth and development and consumption as a fresh crop and a key ingredient in many prepared cuisines. Members of the *Lycopersicon* genus may survive in various environmental and dietary situations. A handful of the species have been crossed to produce numerous kinds intended to provide a field crop for a single harvest or, particularly when protected, a succession of fruits for the fresh market over an extended time. Through the combined efforts of plant breeders and agricultural technologists, field tomatoes are susceptible to mechanized harvesting, enabling the cost-effective production of enormous tonnages. Many different canned, frozen, preserved, or dried foods contain the products. Over the past ten years, tomato fruits have been the focus of considerable molecular research because of their

economic significance and because they can be grown using genetic engineering and molecular biology techniques. (Hobson and Grierson,1993). Tomatoes significantly contribute to strengthening the immune system due to their content of carbohydrates, organic acids, amino acids, vitamins, pigments, numerous mineral substances, phenolic compounds, and high antioxidant activity (Oğuz et al., 2014) According to data collected and updated in December 2022 by the FAO, global tomato production for processing and fresh consumption in 2021 amounted to just over 189.1 million metric tons, up 2% from the 184.8 million mT produced in 2020 and 4% from the average of the previous three years (2018-2020) (182.7 million tons). According to the statistics in the table.1.1, the annual tomato production in many countries has reached or surpassed one million tons. Türkiye's production of tomatoes reached over (13 million tons), double that of Italy but only a fifth of China's (67 million Tons), accounting for nearly 36% of the global harvest. (FAO, 2022).

Table 1.1. The major tomato-producing countries in the world (thousand tons) (FAO, 2022)

Country	2016	2017	2018	2019	2020	2021
China	57.31	59.18	60.92	62.87	64.77	67.54
India	18.73	20.71	19.76	19.01	20.57	21.18
Türkiye	12.60	12.75	12.15	12.84	13.20	13.10
USA	12.88	11.14	12.61	12.16	12.23	10.48
Egypt	7.32	6.73	6.78	6.81	6.73	6.25
Italy	6.44	6.02	5.80	5.78	6.25	6.64
Iran	5.83	4.90	4.93	5.46	5.79	3.39
Spain	5.23	5.16	4.77	5.00	4.31	4.75
Mexico	4.05	4.24	4.56	4.27	4.14	4.15
Others	46.99	47.20	47.96	48.81	48.83	51.66
World	177.38	178.02	180.23	183.02	186.82	189.13

According to TUIK data, in 2021, Türkiye will produce approximately 32 million kilograms of vegetables. In 2021, the tomato, the most produced vegetable in Türkiye, will account for 41.2% (13.1 million tons) of total vegetable production. In Türkiye, tomatoes are grown both in open fields and undercover. Türkiye produced 4.4 million kilos of tomatoes under cover in 2021. Tomatoes can be cultivated in any soil type, from alluvial to clay. Tomatoes cultivated in sandy-loam soils yield early harvests. In addition, tomatoes are resistant to moderate sodium conditions (Maltaş and Kaplan 2015). Considering the tomato cultivation areas in Türkiye, it is seen that the cultivation areas have decreased by 5.2% in 2021 compared to 2020. Türkiye's total tomato production in 2021 decreased by 0.8% compared to the previous year and became 13.1 million tons. Yield increased by 4.6% compared to the previous year and reached 79.4 tons/ha in 2021, as shown in Table 1.2.

Table 1.2. Türkiye's tomato cultivation area, production, and yield (TÜİK, 2022)

Year	Cultivation area (thousand ha)	Production (thousand tons)	Yield (ton/ha)
2017	177	12.750	72.0
2018	169	12.150	71.9
2019	173	12.842	74.2
2020	174	13.204	75.9
2021	165	13.095	79.4

Antalya ranks first on Türkiye's province-by-province list of 2021 tomato cultivation areas, with approximately 19 thousand hectares. In 2021, Antalya will be followed by Bursa with roughly 16 thousand ha and Manisa with roughly 13 thousand ha. In 2021, tomato production was mostly realized in the Mediterranean at 32.7%, the Aegean at 24.4%, the East Marmara at 12.5%, and the West Marmara at 8% (TÜİK, 2022). In 2021, production in Antalya, the Türkiye leader in tomato production, increased by 8.9% compared to the previous year, as shown in Table 1.4.

Table 1.3. Distribution of Türkiye's tomato production by provinces (thousand tons) (TÜİK, 2022)

Province	2017	2018	2019	2020	2021	2022
Antalya	2.53	2.51	2.53	2.57	2.80	2.55
Bursa	1.45	1.36	1.49	1.34	1.35	5.12
Manisa	975.00	856.00	922.00	1.12	1.10	849.00
İzmir	966.00	947.00	1.01	1.09	1.02	855.00
Mersin	1.04	924.00	1.04	930.00	886.00	854.00
Muğla	649.00	676.00	701.00	660.00	628.00	552.00
Çanakkale	608.00	583.00	605.00	627.00	581.00	611.00
Konya	249.00	249.00	303.00	552.00	542.00	849.00
Balıkesir	519.00	420.00	445.00	486.00	417.00	511.00
Others	3.31	3.25	3.41	3.46	3.49	7.92
Türkiye	12.75	12.15	12.84	13.20	13.10	13.00

Plant pathogens represent a danger to the productivity of agriculture because they are the source of diseases that significantly damage the economy and environment. Bacterial Spot Disease caused by *Xanthomonas perforans* and Bacterial Canker and Wilt Disease caused by *Clavibacter michiganensis* subsp. *michiganensis* are the main pathogens on/in tomatoes.

Tomato Bacterial Spot was first observed in South Africa in 1914. The disease is one of the most destructive tomato diseases globally and significantly impacts the yield of tomato plants cultivated in warm, humid regions. Typically, infection results in leaf and fruit lesions, causing defoliation and fruit yield loss. *Xanthomonas* is a widespread genus of plant-pathogenic bacteria found in numerous climate zones; the disease is caused by four species of *Xanthomonas (*Xanthomonas *euvesicatoria, Xanthomonas. gardneri, Xanthomonas. perforans, and Xanthomonas vesicatoria*).(Jones et al. 2004;Timilsina et al. 2020). The pathogens induce various disease

symptoms, such as wilt, necrosis, gummosis, and vascular or parenchymatous diseases, on diverse plant families' leaves, fruits, and stems, including numerous economically significant crops like tomatoes and peppers (Balogh et al. 2003). The distribution of *Xanthomonas* species is worldwide spread. *Xanthomonas euvesicatoria and Xanthomonas vesicatoria* on tomato and pepper; *Xanthomonas perforans* and *Xanthomonas gardneri* are becoming increasingly isolated from the USA, Canada, South America, Africa, and Europe (Sharma et al. 2018;Almashhadany 2019). Mirik et al. mentioned that Bacterial spot disease is caused by the pathogen *Xanthomonas axonopodis pv. vesicatoria* (*Xanthomonas axonopodis* pv. *vesicatoria*) is a devastating pepper (*Capsicum annuum*) disease in Türkiye. Bacterial spot disease, caused by *Xanthomonas,* is a severe pepper disease in the eastern Mediterranean provinces of Adana and Osmaniye in Türkiye. The disease affects 52 to 100 %of pepper fields in these provinces. Bacterial spot disease can infect all portions of a tomato plant above ground, including the leaves, stems, and fruit. Bacterial spots Symptoms appear on leaves as small (less than 3.18 mm) circular spots that are sometimes water-soaked. Spots may appear yellow green initially, then deepen to brownish-red as they age. Extensive leaf yellowing and leaf loss can occur when the disease is severe. Spots on green fruit are often tiny, elevated, and blister-like, with a yellowish halo. As the fruit matures, the spots increase (to a maximum size of 6.14 mm) and become dark, scabby, and scratchy. (Sahin and Aysan ,2005), Mature patches might be elevated or depressed with elevated margins. The disease cycle found that Bacterial spot pathogen inoculum can be found in seeds, tomato volunteers, and plant detritus from diseased plants. All this is considered a source for the pathogen and a method to survive the environmental factors. Temperatures between 24°C and 30°C and high humidity favor disease development. (Jones et al.,1986).

The pathogen spreads through irrigation drops and aerosols carried by the wind and through some cultural practices. The pathogen's spread will be aided by handling plants with damp foliage (Pohronezny ,1990). The pathogens enter via stomata, hydathodes, and wounds caused by wind-driven sand, insect bites, or mechanical means. Up to 4 m away from nearby symptomatic plants, the bacteria can be recovered from asymptomatic plants in as little as one week. Because overhead watering systems are used in greenhouses, bacterial spot disease can spread quickly throughout the commercial seedling production process, reaching a distance of up to 2 m in just three weeks after the initial plant infection. Under ideal circumstances, symptoms appear on tomato seedlings five days after the initial infection (Jones et al. 1991).

One of the most damaging tomato diseases is bacterial canker and wilt disease caused by the pathogen Clavibacter *michiganensis* (Sen et al. 2018). Disease was described for the first time in North America in 1909. It is the source of a vascular bacterial blight feared by producers in many production areas. Easily transmitted by seeds and seedlings, it occurs in the field and under protection. The pathogen causes disease symptoms on all above-ground green parts and fruits, decreasing yield. The pathogen is a non-motile that can be seed-borne and a gram-positive bacterium.

This vascular pathogen typically invades and multiplies in the xylem through natural openings or lesions, resulting in symptoms of wilt and canker (Tipu et al. 2018;Blank et al. 2016).

Depending on the age of the host plant, cultivar susceptibility, and virulence of *Clavibacter michiganensis*, as well as certain environmental conditions, such as temperature and humidity, plants infected with *Clavibacter michiganensis* show various symptoms. Early infections of plants (as seeds or young seedlings) cause them to develop systemic infections (also known as primary infections), which reduce fruit quality and yield and frequently result in plant mortality. As opposed, older plants typically have secondary infections known as foliar infections, which result in chlorosis of the leaves but may or may not have an impact on the quality and yield of the current crop. During a systemic infection, plants may not show any symptoms at first. Symptoms will appear after 6–8 weeks when the pathogen has multiplied to a high level (109–1010 per gram of plant tissue homogenate).(de León et al. 2011). The internal vasculature turns brown due to *Clavibacter michiganensis* invasion and multiplication in the xylem vessel, which also causes the vascular tissues to degrade over time. In the initial stages of the disease, this prevents water from moving up and causes wilting. When a tomato plant is infected in its late developing stages, it may not gradually show symptoms or wilt.

They can endure infection and possibly even yield commercial fruit but can also effectively spread infection over the following growing season.(Eichenlaub and Gartemann 2011)(Sharabani et al. 2013). *Clavibacter michiganensis* is the only member of the Gram-positive, plant-pathogenic actinomycetes (family Microbacteriaceae) genus Clavibacter. According to their respective host ranges, this species is divided into five subspecies. *C. michiganensis subsp. sepedonicus* causes potato ring rot (*Solanum tuberosum*;), *C. michiganensis subsp. tesselarius* causes wheat leaf freckles and leaf spots (*Triticumaestivum*;), and *C. michiganensis subsp. insidiosus* causes lucerne wilting and stunting (*Medicago sativa*;). *Clavibacter michiganensis* can grow at temperatures between 20°C and 30 °C, can persist at 50 °C, and grows optimally at 25°C. The bacterium can grow on synthetic media, but it takes between 3 and 7 days for colonies to appear on selective agar plates. The optimal pH range for this bacterium's growth is between 7 and 8, but *Clavibacter michiganensis* can still grow in plant xylem at a pH of 5.(Ialacci et al. 2016;Sen et al. 2018)

Clavibacter michiganensis typically induces systemic infections in tomato plants. Due to a local infection, the pathogen can also cause spots on leaves, petioles, peduncles, and fruits, typically under overhead irrigation. A wide variety of symptoms differ according to the production environment (greenhouse or field), plant age at the time of infection, cultural practices, cultivar, etc. Symptoms can be divided into two categories based on whether the infection is systemic or superficial within the vascular tissue. In systemic infections, the disease is frequently identifiable at an early stage by dull-green, viscous areas between the leaf veins that quickly dry out. Next, areas of pale brown necrosis that are frequently marginal appear, giving the plant a scorching look. Small necrotic zones can merge to form larger necrotic zones. As the systemic infection progresses, one or a few of the lower leaves turn downward, and often, the leaflets along one side of a leaf become

flaccid, at least during periods of increased evapotranspiration. Under favorable conditions for symptom development (25–30°C) and evapotranspiration stress, entire leaves wilt and shrink in days. Eventually, the entire plant wilts and dries up. When bacteria multiply on the plant surface or within surface wounds and stomata, superficial infections occur; the leaves, stems, and calyces may appear to have been sprinkled with coarse flour.

Close inspection reveals lesions typically white to pale orange and raised or depressed. Spots on stems are typically less noticeable than those on foliage. A common leaf symptom is a dark brown stain surrounded by a yellow-to-orange area; this is caused by an infection of a water-excreting gland (hydathode). The earliest infected leaflets then curl, and their margins turn yellow and necrotic. Plants grow inadequately and eventually die from dehydration. Sometimes, yellow streaks will form along the stem, split open at the nodes to produce cankers. Sometimes, blister lesions on stems are observed. On affected fruits, symptoms may manifest as small, slightly elevated lesions with a white margin or halo. These lesions may grow to a few millimeters in diameter and develop brown, roughened centers known as 'bird's-eye' patches. These areas may develop in greenhouses with overhead irrigation. Several lesions may form close to the calyx in clusters of fruits. Under the calyx scar, the vascular tissues and those leading to the seeds may be dark yellow to brown. Canker-like symptoms are uncommon; the most prevalent name for the disease, bacterial canker, is a misnomer.(Tipu et al. ,2018;Oepp et al. 2017;Ahmad et al. 2015;Baysal et al. 2011).

The primary inoculum sources for disease epidemics are infected seed and plant debris.(Quesada-Ocampo et al. 2012). The main vector throughout massive distances is the seed. Pathogen transmission rates from seed to seedling can range from 0.25 to 85%, and populations of as little as five bacteria per seed can cause seedling infection. Under ideal circumstances, even a transmission rate of 0.01% can start a major epidemic.(Fatmi and Bolkan 2017) (Lelis et al. 2014). Within an established crop, the subsequent interplant spread of *Clavibacter* spp (secondary infection) is primarily caused by cultural practices like pruning, clipping, contact between sick and healthy plants, rain, splashing, irrigation techniques, and the application of nutrient solutions during fertigation (Oepp et al. 2017; Werner et al. 2002; Sen et al. 2018). Stomata, hydathodes, roots, and wounded tissues, such as damaged trichomes, can all lead to secondary infections (Chang 1992). After establishing an infection, *Clavibacter* spp invades xylem vessels and spreads systemically throughout a host. When infections occur on mature plants, they can be asymptomatic: yield loss was not significant for plants infected after the 18-19 leaf stage (83). Before beginning a new growing season, producers should take stringent precautions against numerous potential pathogen sources, as asymptomatic plants can be a source of infection.(Sharabani et al. 2018)

Plant pathogens represent a danger to the productivity of agriculture because they are the source of diseases that significantly damage the economy and environment. Their impact has worsened recently due to economic globalization and climate change, which encourage the emergence and quick spread of new infections (Chakraborty et al., 2022). The latest developments

in crop protection have been directed toward an overall reduction in dependency on traditional pesticides and the obligatory adoption of an integrated pest management (IPM) program that is addressed in the rules of several countries. As a result, there is a growing interest in practical and sustainable alternatives to traditional pesticides. Many microbial biocontrol agents (BCA) have recently been developed to manage bacterial and fungal infections. Biological control is a potential option (Bonaterra et al., 2022). Plant pathologists and others in the agricultural sector confront enormous challenges. Nanotechnology is a new weapon against these developing issues with disease management and plant health. The utilization of nanotechnology in plant disease management, diagnosis, and genetic transformations remains in its earliest stages and has just begun to be examined in plant pathology literature (Elmer and White, 2018). Nanoparticles are any material with one or more dimensions between 1 and 100 nm. This definition has enabled a wide range of both synthetic and natural composites/materials as nanoparticles. Nanoparticles occur naturally in many forms, including volcanic dust and oceanic salt sprays (Frewer et al., 2011). Because of nanoparticles' small size and large ratio of surface space to volume, they can efficiently bind, integrate, and absorb substances such as small-molecule drugs, DNA, RNA, proteins, and probes. In addition to surface space, nanoparticles differ from their bulk counterparts in other properties. (Albanese et al.,2012).

This study provides a summary indicating that the nanomaterials AgNps and CuNps, as well as the novel chemical materials SPP and DOT, have efficacy *in vitro* for pathogen prevention. However, their effectiveness *in vivo* is not comparable. The efficacy of the plant activator ISR2000 and the fungicide KOCIDE has been demonstrated in several *in vivo* trials, including greenhouse and climate chamber experiments and seed treatments, for controlling both pathogens.

2. PRELIMINARY WORK

2.1. Management of Plant Bacterial Disease

Hirano and Upper (1983), mentioned that controlling bacterial diseases is one of the most challenging aspects of growing crops. It consists of numerous integrated activities to eradicate disease sources, protect plants from infection, and reduce susceptibility. Only copper compounds and antibiotics are included in the assortment of chemical preparations. However, the use of the mentioned group is prohibited in the European Union. Therefore, other techniques, such as those found on beneficial bacteria and yeasts, are more prevalent. Several fundamental concepts underlie strategies for managing bacterial plant diseases. These include eradication, exclusion, treatment, protection (prophylaxis, avoidance), and legal applications (laws and regulations).

According to Agrios (2005), Most guidelines do not have zero-tolerance policies for certain diseases as their end goal. They are designed to make disease incidence and severity more moderate and tolerable levels.

Sobiczwski (2008) stated that the method selection against a particular disease should be based on an in-depth knowledge of the causal agent's characteristics, particularly its pathogenic capabilities and chances of survival on plant surfaces, in plant tissues, and outside host plants. The focus of protective activities should be the localization and eradication of the primary inoculum source. If possible, eradication (the destruction or removal of the pathogen or the elimination of the plant hosting the pathogen) should be implemented. Seeds and nursery stock production should be concentrated in regions free of quarantine diseases (monitoring, inspections, and seed and nursery stock indexing are required). Disease-resistant or disease-tolerant cultivars should be planted where economically significant diseases are endemic. Chemical preparations (other than those based on copper; in some nations, antibiotics are permitted under extremely stringent regulations) and biopreparations should be used to protect plants. Appropriate soil and cultivation practices (plant rotation, fertilization, irrigation, etc.) must be implemented to reduce plant susceptibility.

2.1.1. Chemical Controlling Methods

As reported by Zaumeyer (1958), The most important reason is probably that antibiotics are the best antibacterial substances we have, and almost all antibiotics have been made in the past for use in clinical treatment, not plant farming. İntial research into the chemical treatment of bacterial diseases focused on a "kitchen sink" method, which meant testing a wide range of compounds against various diseases..For this kind of study, many essential chemical elements have been studied.

Shoda (2000), stated that the idea of using chemicals for managing bacterial diseases has been largely influenced by elements like the availability of effective modes of action, the ability to reach the pathogen on plant surfaces, the pathogen's susceptibility to the specific chemical, the economic value of the crop at risk, and the market potential of using the chemical in an industrial

setting. Compared to fungicides, for example, there are not as many chemicals on the market that kill germs that cause plant diseases.

Effect Copper (CU) compounds

Shoemaker and others (1981) mentioned that the study shows how this new multi-site Cu/Zn hybrid nanoparticle could manage bacterial spots. Every 7 to 10 days, copper compounds, either alone or combined with the protective fungicide mancozeb, are typically applied as bactericides. The results of a study conducted in Florida during the mid-1960s indicated that the combination of copper and Mancozeb was more effective than copper alone for controlling bacterial spots of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*. These results have served as the basis for control recommendations for other bacterial diseases.

Cooksey (1990) reported that It was considered nothing short of amazing that antibiotics could treat patients with deadly and incapacitating illnesses. Antibiotics' promise to treat plant diseases, particularly those brought on by bacteria, was soon realized by plant pathologists. To prevent plant diseases in the 1950s, some 40 antibiotics with bacterial or fungal origins were tested.

Hausbeck and others (2000), found that A result shows that, Except for Mancozeb, chemical applications reduced population sizes and dissemination of *Clavibacter michiganensis* among tomato seedlings in the greenhouse and influenced subsequent plant growth and yield in the field. While applications of copper hydroxide, copper hydroxide/mancozeb, copper hydroxide/mancozeb (premixed 12 h prior to spraying), streptomycin, and streptomycin/copper hydroxide to seedlings in the greenhouse did not significantly differ from the inoculated control, these treatments tended to increase the survival of inoculated transplants in the field relative to the inoculated control. In the field, the yields of inoculated controls were 63% (1995) and 51% (1996) of those of uninoculated controls. Except for Mancozeb in 1995, all interventions produced yields comparable to those obtained with the uninoculated control in both years. Field survival and yield were severely compromised when transplants contained a pathogen population of 31 108 CFU/g of tissue. Except for Mancozeb, all interventions reduced *Clavibacter michiganensis* populations to $<5.0 \times 105$. None of the interventions reduced the incidence of fruit spotting significantly compared to the inoculated control.

McManus and others (2002), Since the late 19th century, when Alexis Millardet unintentionally found the effectiveness of neutralized copper sulfate in preventing grapevine downy mildew, copper fungicides have been used for managing plant diseases. The discovery of the first practical technique for controlling diverse phytopathogens transformed agricultural productivity. Although several fungicidal active ingredients have since been discovered, copper-based plant protection solutions are still commonly utilized in both conventional and organic agriculture. Copper compounds and streptomycin (introduced in the 1950s) proved to be the most effective bactericide spray treatments for bacterial disease management on plants, primarily targeting *Pseudomonas* spp.,

Xanthomonas spp., *Erwinya amylovora* and *Escherichia coli*. Nearly 60 years ago, the development of antibiotics for treating bacterial diseases transformed human medicine.

According to Perry and Wright (2013), there is a link between the selection of resistance in pathogen populations, the intensive use of copper and antibiotic sprays over several years, and numerous treatments during a single season. Furthermore, resistance has often arisen from the acquisition of genes encoding resistance determinants, involving non-target microbiota in the horizontal transfer of resistance determinants within agricultural ecosystems. We now understand that the contemporary human population's antibiotic resistance dilemma is caused by horizontal gene transfer, a process by which bacteria can acquire resistance determinants even from phylogenetically distant species. Nevertheless, copper octanoate alone or in a tank mixed with other chemicals only worked for making seedlings. Using different compounds should make tomatoes healthier, reduce the spread of diseases into fields, and lower the amount of copper left in the environment.

Bastas (2014), shows result in his published paper shows the effectiveness of some chemicals, a plant growth regulator (Prohexadione-Ca (PC)), two plant activators (hydrogen peroxide (HP)) and harpin protein (Hrp), fungicides, maneb+copper (MC), copper compounds (copper sulphate pentahydrate (CSP) copper hydroxide (CH) and copper oxychloride (CO)) and an antibiotic, streptomycin sulphate on the pathogen were assessed *in vitro* and *in vivo* on two tomato cultivars, Newton and Orient. In plants treated with Hrp and HP, a reduction in disease severity (approximately 45%) was correlated with the suppression of bacterial growth over the duration of infection. The activities of POX, GPX, and PAL enzymes were measured on the fifth day as resistance markers, and applications of Hrp, PC, and HP caused a progressive and statistically significant increase of both enzymes in locally treated tissues. Only PC interventions have reduced plant heights, and PC is up to 40% effective at reducing plant heights. CSP and CO, in combination with Maneb, were the most efficacious copper compounds on disease severity (about 50%). In addition, the combination of CSP with Hrp, HP, and PC substantially decreased the pathogen population in vitroand had a greater impact on disease symptoms than CSP alone. We conclude that Hrp, HP, and PC may be beneficial in controlling the external symptoms of this disease in greenhouses while reducing the amount of copper applied to crops.

Horváth and others (2015), and others like Liu et al. (2012), reported that since the mid-1900s, BST management has frequently utilized antibiotics and copper-based substances. However, due to the regular usage of these substances, *Xanthomonas* bacteria quickly developed resistance to copper and antibiotics. According to reports from Horvath and others, nearly all the *Xanthomonas perforans* strains discovered in Florida commercial tomato crops are copper-resistant.

Sundin and others (2016), mentioned that antibiotics differed from the metal-based bactericides that farmers had access to in the 1950s and 1960s due to their potency at low doses and no toxicity against plants. Antibiotics' main targets were bacterial vegetable diseases and apple and pear fire blight. Unfortunately, developing germs resistant to antibiotics has reduced the effectiveness

of antibiotics against plant bacterial disease. The usage of copper and streptomycin has been hampered by the evolution of resistance in populations of plant pathogens, even though these bactericides have generally been successful disease management agents.

Another study by Strayer-Scherer and others (2018), investigated the antibacterial effects of the new copper composites core-shell copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu) as possible alternatives to commercially available micron-sized copper bactericides for controlling copper-tolerant *Xanthomonas perforans*. In a test tube, 100 µg /ml of solid copper from CS-Cu and FQ-Cu inhabitate the copper-tolerant *Xanthomonas perforans* strain for 1 hour. None of the micron-sized copper rates (100–1,000 µg /ml) from KOCIDE 3000, on the other hand, decreased the number of bacteria by a large amount of copper-tolerant Xanthomonas perforans populations were found to be higher after 48 hours of interaction compared to the control water (P 0.05). Copper-sensitive *Xanthomonas perforans* were killed in 1 hour by all copper-based therapy. Greenhouse studies showed that all copper composites lowered the severity of bacterial spot disease significantly (P 0.05) more than copper-mancozeb and water controls. Even though there was no significant effect on yield, copper composites reduced disease severity much more than water controls. In field tests, copper composites used 80% less metallic copper than copper-mancozeb (P 0.05). This study shows that it is possible to control copper-tolerant *Xanthomonas perforans* and tomato bacterial spot with copper composites.

Carvalho and others (2019) found that many studies have searched for new ways to use Cu to break the resistance of the bacterial pathogen. One of these studies is by Renato Carvalho and his team, adding another compound with Cu. This study examined how antibacterial a copper-zinc (Cu/Zn) hybrid nanoparticle was on strains that could handle copper and strains that could not. Compared to the non-treated and micron-size industrial copper controls, the hybrid nanoparticle greatly cut bacterial growth in the lab. Compared to the control group, the level of disease in tomato transplants treated with the hybrid nanoparticle was much less severe when treated with the hybrid nanoparticle, and no phytotoxicity was seen in the plants. We also looked at the effect of hybrid nanoparticles on the bacterial pigment *xanthomonadin* by using Near-InfraRed Raman spectroscopy to tell if bacteria were breaking down. Compared to samples treated with micron-size copper or left untreated, the hybrid nanoparticle greatly affected *Xanthomonas perforans'* ability to make *xanthomonadin*.

In a study published by Abrahamian and others (2019), they used 19 different chemical agents, biological control agents, plant activators, and novel products were tested to see if they might inhibit bacterial spots on tomato seedlings and in the field. The effectiveness of single cycles or mixes of several compounds on seedlings was compared to a control group that was not treated and a copper standard. Stand-alone applications of acibenzolar-S-methyl (ASM), copper octanoate, quinoxyfen, oxysilver nitrate, and pentasilver hexaoxoiodate lowered disease by a large amount compared to controls that had been inoculated. Compared to the control, the disease was much less common in

seedling programs that used ASM or copper octanoate. Oxytetracycline and streptomycin did not work in seedling studies to reduce the severity of diseases when there was a lot of disease pressure. Even though there were big differences in how bad the disease was between treatments, there was no big difference in the general yield between treatments in field trials. Overall, ASM was very effective at preventing diseases in the greenhouse and the field, whether used alone or as part of a program with other chemicals.

2.1.2. Plant Activator

According to Noutoshi and others (2012), Induced resistance (IR) causes numerous forms of systemic resistance throughout a plant's tissues. IR is based on two fundamental mechanisms: direct activation of defense responses in systemic tissue after local stimulation and Priming entails activating systemic responses, but only when the pathogen reaches these sites and Priming of systemic responses.

According to Aranega-Bou and others (2014). plant activators are natural or synthetic chemical compounds that protect plants against pathogens by initiating defense priming. Unlike traditional fungicides or pesticides, plant activators have long-lasting effects on plant resistance because they do not directly harm pathogens. The use of plant activators to combat newly emerging plant diseases is on the rise; however, most of the molecular mechanisms underlying immune induction by these activators remain unknown. Almost all plant activators induce defensive responses, imitating systemic acquired resistance (SAR) or induced systemic resistance(IR).

Mauch-Mani and others. (2017), mentioned that Induced resistance allows plants to activate the appropriate defenses set in each situation to avoid misallocating resources and minimize trade-offs between defenses against distinct enemies. However, due to the time required to implement the response, plants may sustain substantial damage before the defense response takes effect.

Li and others (2020) Gozzo and Faoro (2013) and others like found that Priming likely evolved to compensate for this vulnerability, as well as to allow plants to detect environmental cues and to foster a state of readiness that enables a rapid, robust response to pathogen attack.

Effect of ISR 2000

ISR 2000TM is a commercial product designed to improve growth, yield responses, and disease resistance in a wide range of crops, including vegetables and fruit. The active ingredient is *Lactobacillus acidophilus*, plant extract, yeast extract, and benzoic acid. Lactobacillus acidophilus (Moro, 1900) Hansen & Mocquot, 1970 (*Lactobacillales: Lactobacillaceae*) is a probiotic species of bacteria that has an important potential for suppression of unwanted microflora in humans and animals.

Soykan, (2010), in her master's degree thesis, approved that ISR 2000 could reduce the disease severity by 31.17% and disease percentage index by 62.09% in root application.

Yildiz (2016), proved that ISR 2000 gave a good amount of protection by Affecting the development of tomato bacterial wilt disease in pots, which was 23.7 % compared to the positive control at 0.0% and also proved the effect of ISR 2000 on the development of tomato bacterial wilt disease in field experiment 22.9 % compare to 0.0% on positive control.

Another study by Karut, Horuz, and Aysan (2019) approved the effect of the ISR 2000 on seeds infected by *Clavibacter michiganensis* ; the ISR 2000 was effective by 89.0 % on the pathogen population.

Aktepe (2021), found that ISR 2000 could suppress the percentage of the bacterial speck disease in tomato caused by the pathogen *Pseudomonas syringae pv. tomato* 43.33% in three trials, respectively.

Kaymak (2022) studied some commercial products on root and crown rot caused by *Phytophthora cactorum* in apple cultivation, showing that ISR2000 could reduce the Disease severity by 12.5 %, and the Efficacy was 62.5 %.

2.1.3. Nanotechnology

Elmer and White (2018), stated that Nanotechnology is a new weapon in our arsenal against these escalating plant and disease management challenges. Nanotechnology can be used for plant disease management, diagnosis, and genetic modification. Engineered nanoparticles consist of materials between 1 and 100 nm, including metalloids, metallic oxides, nonmetals, carbon nanomaterials, functionalized dendrimers, liposomes, and quantum dots. Their small dimension, large surface area, and high reactivity allow them to be utilized as bactericides/fungicides and nano fertilizers. Nanoparticles can be designed as biosensors for plant disease diagnostics and as carriers for genetic material, probes, and agrochemicals. In the past decade, phytopathology-related nanotechnology reports have multiplicatively increased. Nanomaterials have been incorporated into disease management strategies and diagnostics and used as molecular tools. Shukla and others (2019), stated that nanocoatings are of great interest to the scientific community because using suitable and protective packaging materials can significantly extend shelf life. Many metal-based NPs (nanoparticles), including FeNPs, AgNPs, TiO2NPs, AuNPs, CuNPs, FeS2NPs, ZnNPs, ZnONPs, and carbon-based NPs, including carbon nanotubes and fullerene are used as seed priming agents to promote seed growth, germination, and stress tolerance in plants.

According to Vijayakumar and others (2019), The majority of the reports reviewed in this article are focused on pathogen inhibition using metalloid/metallic oxide nanoparticles as bactericides/fungicides and as nano fertilizers to improve human health. The use of nanoparticles as plant disease diagnostic biosensors is also examined. As the global demand for food production increases with a changing climate, nanotechnology could sustainably mitigate many challenges in disease management by reducing chemical inputs and encouraging rapid pathogen detection.

Nanotechnology has also contributed to developing polymers or nanocomposites with effective gas and water barrier properties that are smart, durable, and cost-effective.

Effect of Nanosilver (AgNps)

In their research study, Kim and others (2012), focused on the fungicidal properties of nano-sized silver colloidal solution employed as an antifungal agent for treating diverse plant pathogens. They used 10, 25, 50, and 100 ppm of WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R silver nanoparticles (AgNPs). On potato dextrose agar (PDA), malt extract agar, and maize meal agar plates, eighteen distinct plant-pathogenic fungi were treated with these AgNPs. He computed fungal inhibition to evaluate the antifungal Efficacy of silver nanoparticles against pathogens. According to the results, AgNPs possess antifungal properties against these plant pathogens on multiple levels. Treatment with WA-CV-WB13R AgNPs inhibited the majority of fungi to their maximal extent. According to the results, the greatest inhibition of plant pathogenic fungi was observed with PDA and 100 ppm AgNPs.

Yin et al. (2012) mentioned that Among nanoparticles, silver nanoparticles (AgNPs) are intensively used in many materials owing to their antibacterial effects. In most fields, including natural ecosystems, silver nanoparticles (AgNPs) are the most extensively used particles. In one of the experiments, the effect of AgNPs on seed germination was determined using AgNPs synthesized by various methods. The germination of common wetland plants was evaluated using two AgNPs: polyvinylpyrrolidone-coated silver nanoparticles of 20 nm (PVP-AgNPs) and gum arabic-coated silver nanoparticles of 6 nm (GA-AgNPs). It was discovered that direct exposure to GA-AgNPs reduced the growth of the greatest number of plants. In a sediment exposure experiment, however, the effect was less pronounced. Except for one species, PVP-AgNPs and AgNO3 solution exhibited no significant effect. Thus, effects were observed to differ between species.

Noshad and others (2019), referred to another study on the targeted species *Clavibacter michiganensis subsp michiganensis*, the causative pathogen of Tomato canker disease. They hypothesized that the synthesized AgNPs might be beneficial for controlling this pathogen. Antibiotic activity was detected at a low concentration of 1 mM, whereas the zone of inhibition increased positively at 2.5 mM. Our results demonstrate that the current process is an outstanding candidate for the industrial production of AgNPs and can potentially control the bacterial pathogen *Clavibacter michiganensis.*

Another study by Noshad and others (2019), examined the influence of biogenic AgNPs on the germination and growth of Solanum lycopersicum seeds. Compared to seeds not treated with silver nanoparticles (AgNPs), the germination rate and seedling growth of seeds treated with AgNPs were significantly higher. In addition, its bactericidal effect against *Clavibacter michiganensis (Clavibacter michiganensis)* infection in Solanum lycopersicum was determined.

Mishra et al. (2020) highlighted the potential function of biosynthesized AgNPs against *Xanthomonas oryzae pv. oryzae*; the pathogen responsible for the devastating sheath blight disease of rice. At 20, 30, and 50 g/mL, the potent antibacterial activity of biosynthesized AgNPs (size 12 nm) against Xoo was observed. Even at the reduced 5 g/mL concentration, AgNPs significantly inhibited biofilm formation by *Xoo* ($p = 0.001$). Maximum biofilm inhibition ($p = 0.000$) was observed at 50 g/mL AgNPs concentration relative to the control. In addition, the anti-disease properties of biosynthesized AgNPs were validated under greenhouse conditions. Xoo-inoculated rice plants exhibited 33.91 % DLA (% Diseased leaf area) compared to 9.25% DLA (% Diseased leaf area) in plants sprayed with AgNPs. Our data suggest that biosynthesized AgNPs-based nanoformulations can effectively manage rice blight. In addition, AgNPs' antibiofilm strategies can be utilized against a broad spectrum of bacterial phytopathogens. In light of the rapid emergence of antibiotic-resistant microbial strains, this study provides an alternative, effective platform for applying nanoformulation to enhance agricultural sustainability.

Noshad and others (2020), show in one of their studies that they investigated the bactericidal potential of AgNPs (silver nanoparticles) against the bacterial canker of tomato (BCT) using a green chemistry technique. Using a mycelial aqueous extract of the agriculturally beneficial fungus Pythium oligandrum, AgNPs were produced. UV–Vis spectroscopy for the absorbance pattern confirmed the formation of AgNPs, while transmission electron microscopy (TEM) investigated their morphology. The X-ray diffractogram of biogenic AgNPs revealed a crystalline structure with an average particle size of 12 nm. The seeds treated with AgNPs demonstrated a normal germination rate and seedling growth. An in-vitro study revealed that the prepared AgNPs inhibited the bacterial pathogen to the greatest extent. The introduction of AgNPs significantly prevents and inhibits the bacterial pathogen *Clavibacter michiganensis* on tomato plants within a greenhouse. These results indicate that this method is a viable contender for the industrial production of AgNPs.These particles function as an inhibitor and broad-spectrum antibacterial agent against *Clavibacter michiganensis,* providing a novel and environmentally friendly alternative for BCT management.

Atiq and others (2022), reported that silver and copper nanoparticles were evaluated to manage citrus canker under laboratory conditions. The silver + copper nanoparticles produced the largest inhibition zone (21.06 mm), followed by silver (18.26 mm) and copper nanoparticles (15.04 mm), respectively. In the field, copper nanoparticles exhibited the highest disease incidence (41.24%) and silver and copper nanoparticles exhibited the lowest disease incidence (35.23%) compared to the control. Under field conditions, the lowest disease severity (23.23%) was observed when silver and copper nanoparticles were administered in combination, followed by silver nanoparticles (28.31%) and copper nanoparticles (35.23%). Complete Randomised Design (CRD) was utilized for the laboratory and greenhouse experiments, whereas Randomised Complete Block Design (RCBD) was employed for the field experiment.

A study conducted by Méndez-Andrade and others (2022), shows the bactericidal effect of AgNPs against *Clavibacter michiganensis* infection in tomato plants was determined. Forty-two days after inoculation with *Clavibacter michiganensis* , the disease incidence in plants treated with AgNPs at 50 mg L1 did not exceed 20%, and disease severity was reduced by 36%, which correlates with the inhibition of bacterial growth in the tissue (up to 95%). The physiological analysis also demonstrated the growth-promoting effect of AgNPs on diseased plants, with shoot length (22,1%) and root dry weight (25,73%) increasing compared to the pathogenic control. AgNPs increased the leaf concentrations of CAT, APX, POD, and total phenol and flavonoid enzyme activity. This bactericidal activity and activating an antioxidative defense system may account for the slowed onset of symptoms and decreased bacterial growth in AgNPs-treated plants.

Méndez-Andrade and others (2022), in a study of *Ralstonia solanacearum* exposed to AgNPs (MIC/MBC 200/400 lg ml-1) exhibited cellular envelope injury, cell bulging, and pit formation. The nucleic acid discharge increased gradually from 8% to 34% (r2 = 0.97). At 400 lgAgNPs ml-1, both the metabolic activity and surface adhesion ability of *R. solanacearum* were eliminated. Results indicated that the AgNPs synthesized in this study possessed sufficient anti-pathogenic potential and could be used as a frugal and safe alternative to agrochemicals. Moreover, the results of this study are likely to serve as an essential indicator for the development of effective nano-control agents, which would assist in the management of some lethal phytopathogens capable of causing substantial losses to agricultural production systems.

Dilbar and others (2023), referred to The spherical morphological shape of the nanoparticle with a size below 100 nm was confirmed by SEM and TEM, while TGA determined its stability. The in-vitro and in-plant antibacterial activities of the particles against *Xanthomonas campestris* demonstrated maximum inhibition by uncentrifuged silver nanoparticles recombination with S.parviflora plant extracts (SpAgNPs+PE), followed by centrifuged nanoparticles (SpAgNPs) and plants extract alone (PE). These results suggest that biosynthesized-AgNPs can be used to control *X.campestris* effectively.

Effect of Nanocopper (CuNps)

Praburaman and others (2016), reported that CuO NPs of 50–100 nm size biosynthesized with Piper beetle leaf extract were used against the bacterial phytopathogens *Ralstonia solanacearum and Xanthomonas axonopoda pv. alfalfa*. At 25 µg/mL, each strain's reported inhibition zone values were 9 and 7 mm. .

Rezaie, and others (2017), intensively investigated the CuNPs and CuONPs' antibacterial activity against environmental bacterial strains (plant, urinary tract, and other human bacterial pathogens).

Cumplido-Nájera and others (2019), research to determine the effect of copper nanoparticles and potassium silicate on tolerance to *C. michiganensis* in tomato. CuNPs and potassium silicate

effectively reduced the severity of *C. michiganensis*, the reduction in yield loss due to microorganisms was 16.1%. Biochemical analyses revealed that applying copper nanoparticles and potassium silicate enhanced the activity of the enzymes SOD, PAL, GPX, and APX, as well as the concentration of reduced glutathione and total phenols in the leaves. The activity of the PAL and GPX enzymes and the levels of lycopene and -carotene were increased in the fruits. Copper nanoparticles and potassium silicate increased tomato plants' tolerance to *Clavibacter michiganensis* by altering the levels of enzymatic and non-enzymatic compounds crucial for plant defense.

Chikte and others (2019), mentioned that Cu NPs and CuO NPs of 75 and 163 nm were used to control *Xanthomonas* axonopodis pv, respectively. *punicae* (*xap*), the pathogen responsible for bacterial blight disease in pomegranate. Minimum inhibitory concentration (MIC) values for Cu NPs and CuO NPs against *xap* were 2.50 and 1,560 μg/mL, respectively. Minimum bactericidal concentration (MBC) values were 5 and 3150 μg/mL, respectively. Under greenhouse conditions, the foliar application of Cu NPs (400 μg/mL) reduced disease incidence by 90% and 15% on 6-month-old plants in the early and established phases of infection, respectively. In a field experiment with severely infected 7-year-old plants, the postinfection foliar application of CuNPs reduced disease incidence by 20%.

Varympopi and others (2020), test two concentrations of nano copper against various plant pathogenic bacetria. CuNP1 and CuNP2 in two respective concentrations (1500 ppm or 300 ppm). Dynamic Light Scattering, Transmission Electron Microscopy, Attenuated Total Reflection measurements, X-ray Photoelectron Spectroscopy, X-ray Diffraction and Scattering, and Laser Doppler Electrophoresis were used to characterize both products. The gram-negative species *Agrobacterium tumefaciens, Dickeya dadantii*, *Erwinia amylovora*, *Pectobacterium carotovorum*, *Pseudomonas corrugata, Pseudomonas savastanoi* pv. *savastanoi*, and *Xanthomonas campestris* pv. The evaluation was based on comparisons with two commercial bactericides, KOCIDE (copper hydroxide) and Nordox (copper oxide). CuNP1 inhibited the development of five species and slowed the development of *P. corrugata but* did not affect *X. c. pv campestris.* The MICs were considerably lower than those of commercially available formulations. CuNP2 hindered the development of *E. amylovora* and *P. s.* pv. *savastanoi*. Again, its aggregate activity was greater than that of commercially available formulations. The first report of an extensive *in vitro* evaluation of CuNPs with greater potential than their conventional counterpart suggests that synthesizing stable CuNPs can lead to developing low-cost, sustainable commercial products.

El-Batal and others (2020), mentioned in a study to determine the antibacterial and antibiofilm capabilities of the mono-dispersed copper oxide nanoparticles (CuO NPs)-streptomycin nano-drug synthesized by a cost-effective and environmentally favorable gamma irradiation technique. UV–Vis, XRD, FTIR, HRTEM, DLS, HRSEM, and EDX elemental analyses comprehensively characterized the incorporation of CuO NPs-streptomycin. The antibacterial and antibiofilm activities of CuO NPs-streptomycin against pathogenic bacteria causing brown, ring, soft

rot, and black limb diseases in potato plants were evaluated in vitro. It was determined The proposed reaction mechanism regarding the synergistic potential of CuO NPs and streptomycin. The incorporated CuO NPs-streptomycin exhibited a Surface Plasmon Resonance-specific absorption peak at 585.0 nm. The HRTEM, HRSEM, and XRD results confirmed the monodispersed crystalline nature of the synthesized CuO NPs-streptomycin with a particle size of 20.20 nm. CuONPs. streptomycin displayed promising antibacterial activity (35.50 mm ZOI) against *Clavibacter michiganensis subsp. sepedonicus*. In addition, the biofilm inhibition percentage of CuO NPs-streptomycin against C. *michiganensis* subsp. *sepedonicus, Ralstonia solanacearum,* and *Dickeya solani* were approximately 90.99%, 84.23 %, and 83.42%, respectively. Consequently, based on the prominent characteristics, this research could provide insights for identifying dangerous agricultural challenges, potato packaging and processing, and a novel nano-drug formulation for invading potato pathogenic bacteria during cultivation.

Ngoc and others (2021), investigated The effect of varying concentrations of copper ions on the particle size and morphology of nano Cu2O-Cu/alginate. Transmission electron microscopy (TEM) and X-ray diffraction (XRD) measurements revealed that the synthesized nano Cu2O-Cu/alginate was predominantly composed of Cu2O-Cu in the form of a Cu2O@Cucore–shell structure with particle sizes of 4.2, 5.3, and 8.4 nm for 60, 80, and 100 mM of copper ion initial concentrations, respectively against *Xanthomonas oryzae pv. oryzae*, the nano Cu2O-Cu/alginate, exhibited potent antibacterial activity. The results demonstrated that nano Cu2O-Cu/alginate containing 30 mg.l1 of copper ions completely inhibited the growth of Xanthomonas OO bacteria, which cause rice leaf blight. Nano Cu2O-Cu/alginate can be used as a fungicide to replace toxic agrochemicals and could be applied to the development of sustainable agricultural production.

Abd-Elsalam and others (2022), Copper nanoparticles are among the most important nanomaterials due to their diverse properties, applications, and manufacturing economics. Copper nanoparticles give fertilizers and insecticides a larger specific surface area. Nanomaterials also make providing nutrients with enhanced crop protection simpler in a site-specific and regulated manner than using multiple agrochemical suppliers.

2.1.4 New Chemical Material

According to Elmer and White (2018), Plants are perpetually vulnerable to biotic and abiotic stresses, resulting in yearly crop losses. The present scenario of affairs represents a grave threat to global food security and safety. Professional plant pathologists must have theoretical and practical knowledge of plant diseases and the factors involved and the ability to identify effective control measures. That is why the scientific community is searching for new ways and materials to accomplish the food security goal.

Effect of disodium octaborate tetrahydrat (DOT)

Pratt and Quill (1996), Found that By applying 2% and 4% solutions of disodium octaborate tetrahydrate (DOT) as soon as the Sitka spruce, Picea sitchensis stumps were felled and inoculated with basidiospores of *Heterobasidion annosum*, the degree of heartwood infection was dramatically decreased. Sapwood infection was so uncommon across all interventions, including controls, that it was impossible to assess the Efficacy of DOT in this tissue. According to the trial's findings, DOT may be hazardous to *H. annosum* at lower amounts of borate loading than was previously thought to be conceivable. The borate loading in the stumps seemed to reach equilibrium throughout the experiment's ten months, independent of the initial concentration used. These treatments did not affect Melanotus proteus' stump-surface fruiting, a potential fungus rival of *H. annosum.* Gentz and Grace (2006), mentioned that Boron, particularly in its complex forms as disodium octaborate tetrahydrat DOT, is utilized as a wood preservative to defend against decay fungi and certain insects, such as termites and wood-boring beetles. Boron is also used in insecticides to combat urban pests such as cockroaches and fleas.

Sayin, and others (2016), conducted a study to demonstrate the Antibacterial and Antibiofilm Effects of Boron on Different Bacteria In this study, boric acid (BA) and disodium octaborate tetrahydrate (DOT) were assessed for their antibacterial effects and antibiofilm capacities on some strains of clinical and type cultures that are of concern for veterinarians (*Staphylococcus aureus* ATCC 25923, *Aeromonas hydrophil*a ATCC 19570, *Pseudomonas aeruginosa* ATCC 27853, *Brucella melitens*, Scanner electron microscopy was used to track the suppression of biofilm. By employing the broth technique and microdilution, the lowest MIC values for BA and DOT were determined from *Pseudomonas aeruginosa* ATCC 27853 and were 0.385 and 0.644 mg/ml, respectively.The most resilient to BA and DOT was *Staphylococcus aureus*. Using the microplate method, we found that a clinical isolate of *Lactococcus garviea* and *Pseudomonas aeruginosa* ATCC 27853 showed the strongest positivities for biofilm development.

Erper, and others (2019), Refer to their study on the Antifungal Effect of Boron Compounds Against Three *Rhizoctonia solani* the Causing Root and Crown Rot. The boron compound that proved the most harmful was Disodium tetraborate. The four boron compounds' MIC values ranged between 0.25 and 0.5%. According to soil bioassays, the four boron compounds and tolclophos methyl suppressed the mycelial growth of the AG-4 HG-II and -III isolates when used at 1% or less doses. Winter squash seeds were shown to be phytotoxic to boron compounds at concentrations of 0.125–0.25% in terms of root elongation. However, no concentration of the investigated compounds was found to be phytotoxic in seed germination. According to the findings, boron compounds may be used instead of synthetic fungicides to prevent winter squash's Rhizoctonia root and crown rot.

A study by Gür, and others (2021), showed The efficiency of 14 different boron compounds against *Pseudomonas syringae pv.tomato*. The study was tested at five doses (1, 5, 10, 20 and 40 mM). The effectiveness of tomato was tested in vitroat a concentration of 108 CFU ml, and the top

4 Boron compounds (ammonium tetrafluoroborate, sodium tetrafluoroborate, zinc borate, and disodium octaborate tetrahydrate) were coated with 5 mM doses of the infected cv. To assess bacterial populations and seed emergence rates in the seeds, H2274 tomato seeds were used. The most effective active ingredient in suppressing P. s., with a 92% ratio, was Disodium octaborate tetrahydrate, one of the 14 distinct Boron compounds employed in the studies. Pv. Sodium tetrafluoroborate treatments had a 39% success rate in increasing tomato populations in tomato seedlings. According to the study, some boron compounds may be a practical, efficient, and environmentally friendly chemical for lowering P. s. pv. Within the bounds of appropriate agricultural techniques, tomato in tomato seeds.

Erkan and Aşci (2022), Refer to (DOT) dosages of 0 (control), 15, 30, 45, and 60 mg L-1, were tested to evaluate how they affected the growth and germination of rocket and cress seeds in pot trials. In particular, the 45 mg L-1 application DOT benefited rocket and cress seeds' germination and dry matter content. It was observed that there were statistically significant growth differences between the doses utilized.

Effect of sodium pentaborate pentahydrate (SPP)

While there are not many studies on the activity of sodium pentaborate pentahydrate on phytobacteriology, Doğan and others (2014) , mentioned in a study conducted in 2014 that of the fundamental refined boron compounds, sodium pentaborate pentahydrate SPP (NaB5O8.5H2O), contains 5 moles of water. Furthermore, the study shows the antimicrobial activity of the SPP on *Streptococcus*.

This study has been begun due to the little research available on the effects of nanomaterial and plant activators, particularly SPP and DOT.

3. MATERIALS AND METHODS

3.1 Material

Pathogens isolation: *Clavibacter michiganensis* and *Xanthomonas perforans* were obtained from the Phyto bacteriology laboratory at Cukorova University (Prof. Dr. Yesim Aysan). Grown on the Tryptic Soy Agar (TSA) media and kept in the incubator for 48 h with a temperature of 25 ±3 °C

Experimental Material: Petri Plate (9 cm in diameter), Auto Clave, Tryptic Soy Agar (TSA) (Merck 1.05458.0500; 40 gr/lt),), gas burner, alcohol 70%, scalpel blades, scalpel blade handle, isolation hood, incubator, parafilm, markers pens, lapels, clear test tubes, purified water, salt solution (NaCl), inoculation loop, glass Spreader, sprayer, syringe. For *in vivo* trials, plastic pots (14 cm in diameter), mixtures of soil (peat/clay/sand), tomato plants, tobacco plants, tomato seeds, Cheesecloth, Glass Laboratory Bottles, shakers, vortex,

The nanoparticles, some chemicals and plant activators: All the nanoparticles and the chemicals and plant activators used in our trials were provided Okhygiene Medikal ve Kimyasal Ürünler Kozmetik San.Tic.Ltd, as mentioned in Table 3.1

Table 3.1. The nanoparticles, some chemicals and plant activators used in trails

Material	Status	Source
Silver nanoparticles (AgNps)	Liquid	Okhygiene Medikal ve Kimyasal Ürünler Kozmetik San.Tic.Ltd
Copper nanoparticles (CuNps)	Liquid	
Disodium octaborate tetrahydrate (DOT)	Powder	
Sodium pentaborate pentahydrate (SPP)	Granule	
ISR2000 (855.81 g/l *Lactobacillus acidophilus* fermentation product, Yucca plant extract, yeast extract, riboflavin, benzoic acid, nicotinamide and thiamine)	Liquid	Improcrop USA
KOCIDE-2000 (Copper hydroxide)	Granule	Corteva agriscience

3.2. Method

3.2.1. Pathogenicity test for *Clavibacter michiganensis* and *Xanthomonas perforans* on Tomato Plants

Two young Tutkun F1 cultivar tomato seedlings were cultivated for the pathogenicity test, which was conducted to confirm the isolates. The *Xanthomonas perforans* pathogenicity test was performed with a spraying technique. A plate containing recently cultivated colonies of *Xanthomonas perforans* was subjected to the addition of purified water to harvest all the colonies. The pathogen solution prepared at a measurement level of 0.2 at 600 nanometers in a spectrophotometer was sprayed on tomato leaves by using a sterile hand under controlled room conditions (at 28 °C, 75% humidity, 8/16 hours light/dark). Symptoms were observed around 12-14 days following the

application. After brown spot symptoms with or without a yellow halo were observed on the leaves of pepper plants, pathogenic bacteria were isolated from a single spot according to bacteriological techniques and the stages of Koch's postulate were completed (Lelliott and Stead, 1987). Different colonies of the re-isolated isolate were stored in liquid medium containing 0.85% NaCl in sterile tubes at room temperature in the dark.

To confirm the *Clavibacter michiganensis* isolates, a pathogenicity test was performed on two young Tutkun F1 cultivar tomato seedlings. Different procedures were utilized for the *Clavibacter michiganensis* test, including syringe inoculation of the fresh tomato seedling's stem and room-temperature testing conditions (at 28 ℃, 75% humidity, 8/16 hours light/dark). The signs and symptoms appear 5 to 10 days after applying. After brown symptoms were observed in the vascular tissue of tomato plants, pathogenic bacteria were isolated from this infected tissue according to bacteriological techniques and the stages of Koch's postulate were completed (Lelliott and Stead, 1987). Different colonies of the re-isolated isolate were stored in liquid medium containing 0.85% NaCl in sterile tubes at room temperature in the dark.

The pathogens were reisolated from the plant tissues. An infected leaf was used for isolation for the *Xanthomonas perforans* pathogen, while for the *Clavibacter michiganensis* pathogen, an infected portion of the stem was used for isolation. The isolation process involved the purification of all samples using the single colony method.

3.2.2 The Preparation of The Nanoparticles, Some Chemicals and Plant Activators

In this trial, we utilized four distinct chemical substances, each showing excellent results in maintaining the proliferation of pathogens at different concentrations. Obtained material from Prof. Dr. Yesim AYSAN and Prof. Dr. Mustafa MİRİK, as shown in the table below. According to the instructions of Prof. Dr. Mustafa Mirik, the material was prepared by adding the specified quantity of the material to 50 ml of the liquid (agar\ distilled water) and mixing it completely before adding it to the total amount of liquid (agar\purified water) (Figure 3.1A)

3.2.3. Minimum Inhibitory Concentration (MIC)

Following the preparations of the chemical material and pathogen isolations, a trial was performed to determine the minimum inhibitory concentration that would affect the pathogens. To achieve this, many spread plate method experiments were conducted. Three concentrations and three plates per treatment are used for each of the four chemicals and pathogens. The pathogen concentration was 10^7 CFU and 100µl for each plate. After application, the plates were evaluated in every four days The incubation temperature for each application was 25±2 °C (Figure 3.1B). This trial was performed to determine the inhibitory zones of the MIC for the chemical materials by the disc diffusion method (Nosh et al., 2019). This method was conducted to observe the sensitivity of the pathogens to the chemicals. A pathogen suspension was made for both pathogens with a

concentration of 10^7 CFU and grown on a petri dish with a spread plate method. The chemical was added to the plate as a 6mm filter paper containing 10µl for each paper, and three plates conducted the trial for each and with three different periods, 1,3,24 h respectively and the measurement was taken by vernier caliper.

Figure 3.1. A: shows the preparation of the nanoparticles and some chemicals. B: shows the spread plate method

3.2.4. Determine The Specific Concentrations of The Pathogens for *In Vivo* Trials.

A preliminary investigation was conducted on newly cultivated tomato plants to ascertain the pathogen concentrations for the experimental trials. These trials were conducted under controlled laboratory and greenhouse conditions, encompassing *Clavibacter michiganensis* and *Xanthomonas perforans* pathogens.

Both bacterial pathogen agents were utilized in a pathogenicity assessment conducted on recently grown plants. The method used for the inoculation of *Xanthomonas perforans* was the application of a pathogen suspension spray over the leaves. The symptoms generally develop after around three to four weeks. Leaves showing symptoms of *Xanthomonas perforans* infection were gathered from the affected plant, and afterward, the pathogens were isolated and purified (Figure 3.2A). The inoculation procedure for *Clavibacter michiganensis* required the utilization of a syringe

to inject the pathogen into the lower part of the Stem. Afterward, around three weeks were given for the pathogen to develop within the plant before symptoms became apparent. The Stem of the infected plant had been cut in half, and the pathogen was reisolated and purified in our laboratory (Figure 3.2B).

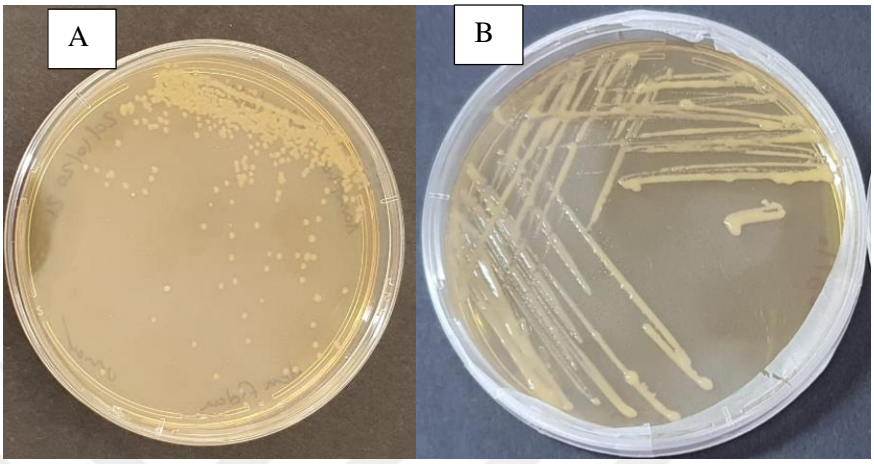

Figure 3.2. (A) *Xanthomonas perforans* (B) *Clavibacter michiganensis* colonies

The experiment was conducted under two different conditions, and initial experiments were conducted within the controlled laboratory environment of the Phyto Bacteriology Lab at Cukorova University's Plant Protection Department. The second experiment was conducted within the greenhouse facilities of Cukorova University. Pathogen suspensions were prepared for both pathogens, and these suspensions were afterward diluted using the serial dilution method. The dilutions ranged from 10^1 to 10^6 in terms of concentration. Every concertation was tested on two fresh tomato plants using spraying *Xanthomonas perforans* and injection for *Clavibacter michiganensis.* The symptoms develop within 3-5 weeks following the first sign of *Xanthomonas perforans* infection. The first sign of symptoms associated with *Clavibacter michiganensis* frequently appears within approximately two to three weeks.

3.2.5. Effect of Nanomaterials, Chemicals and Plant Activator on Bacterial Cancer and Wilt Disease Caused by *Clavibacter michiganensis.*

The trial was conducted at the greenhouses of the Plant Protection Department at Cukorova University. The greenhouse evaluation started by obtaining young Tutkun F1 cultivar tomato seedlings from the Atlas Fide Corporation plant ages three to four weeks. After the plants arrived at the Cukorova Phytobacteriology Laboratory, they were transferred to larger pots containing soil and a commercially available peat mixture. These potted plants are then carried to the greenhouse within the plant protection department, where they undergo irrigation. The bacterial pathogen *Clavibacter michiganensis* was used for inoculations in this experiment. Bacterial cells were harvested from freshly grown plates on Tryptic soy agar (TSA) at 25°C for 72h to prepare pathogen inoculum

suspension. The inoculum was suspended in 9 ml sterile saline buffer (NaCI 0.85%), and the concentrations were adjusted 1x10^6 CFU/ml) with a measurement level of 0.2 at 600 nanometers in a spectrophotometer (Shimadzu UV-120-01, North America). All chemical treatments have been prepared using the referred concentration based on MIC study.The disease severity of all treatments was evaluated based on a 0-4 scale. Plants with no symptoms received a score of 0; those with ¼, ½, or ¾ of the plant withered (from the bottom up) received scores of 1, 2, or 3, respectively. A score of 4 indicated withered and dead plants (Klement et al., 1990).

On November 14, 2022, 42 plants, each having 4-5 true leaves, were tested by inoculation with *Clavibacter michiganensis* inoculum using the stem inoculation technique. Each plant stem tissue was inoculated with 100 µl suspension of *Clavibacter michiganensis* using an insulin syringe. The plants were kept under a controlled condition (27±3 °C and 60% humidity), primarily a glasshouse (Figure 3.3A). For nine readings, with an interval of five days between each reading. All treatments were applied using the spraying technique except for the ISR2000 treatment, which was immersed in a solution for 15 minutes before being transplanted into the plastic pots (figure3.3B) (Table 3.2).

Table 3.2. Method of applying treatments in the greenhouse

Date	Treatment	Method
08-11-2022	ISR2000	immersion for 15 minutes
09-11-2022	ISR2000	Spraying
11-11-2022	All the chemical treatment	Spraying
14-11-2022	The pathogen *Clavibacter michiganensis*	Inject
15-11-2022	ISR2000 - All the chemical treatment	Spraying
22-11-2022	All the chemical treatment	Spraying

Figure 3.3. A show the plant in greenhouse B shows the ISR2000 treatment immersed in a solution for 15 minutes before being transplanted into the plastic pots and the plant in the greenhouse trial

As we mentioned, the evaluation was maintained during 9 weeks, and the first evaluation was done after 21 days of chemical treatment. After that, using evaluation continued every five days

as the next table shows that Stem inoculated plants were examined for bacterial wilt symptoms. The disease symptoms were evaluated 3., 4., 5., 6., 7., 8 and 9 weeks after the plant inoculations using the 0-4 scale described below (Table 3.3). Furthermore, the area under the disease progress curve (AUDPC) was recorded for nine weeks. Data on disease severity and AUDPC was analyzed using analysis of variance (ANOVA) in CoStat Statistics Software (CoHort Software, Pacific Grove, CA, U.S.A. Version 6.4). The area under the disease progress curve (AUDPC) was calculated with the formula below.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where "t" is the time of each reading, "y" is the percentage of affected foliage at each reading and "n" is the number of readings. The variable "t" can represent Julian days, days after planting or days after emergence.

Table 3.3. Shows the scale for Bacterial Cancer and Wilt Disease caused by *Clavibacter michiganensis*

Scale value	Symptoms
0	No symptom
1	Wilting plant of % 1-25
2	Wilting plant of % 26-50
3	Wilting plant of % 51-75
4	Wilting plant of % 76-100 and dead plant

3.2.6. Effect of Nanomaterials, Chemicals and Plant Activator on Bacterial Leaf Spot Disease Caused by *Xanthomonas perforans*

This trial was conducted in the climate chamber of the phytobaceriology lab. İn Plant Protection Department at Cukorova University. The evaluation started by obtaining tomato plants from the climate chamber trial conducted at the greenhouses of the Plant Protection Department at Cukorova University. The greenhouse evaluation started by obtaining tomato plants from the Hishtil Fidecilik A.Ş plant ages three to four weeks. After the plants arrived at the Cukorova phytobacteriology laboratory, they were transferred to larger pots containing soil and a commercially available peat mixture.

These potted plants are then carried to the climate chamber in the plant protection department, where they undergo irrigation. The bacterial pathogen *Xanthomonas perforans* was used for inoculations in this experiment. Bacterial cells were harvested from freshly grown plates on Tryptic soy agar (TSA) at 25°C for 48h to prepare pathogen inoculum suspension. The inoculum was

suspended in 9 ml sterile saline buffer (NaCI 0.85%), and the concentrations were adjusted $1x10^6$ cfu/ml) with a spectrophotometer (Shimadzu UV-120-01, North America) and add to distilled water to be ready to use. All chemical treatments have been prepared in the Phytobacteriology laboratory with the concentration mentioned in (Table 3.4).

On July 24, 2023 (Table 3.4), 48 plants, each with 4-5 true leaves, were tested by spraying with *Xanthomonas perforans* inoculum using the leave leaves technique (Figure 3.4). Each plant leaf was sprayed with a suspension of *Xanthomonas perforans* using a small spray bottle. The plants were preserved within a controlled environment, primarily a climate chamber. All treatments were applied using the spraying technique except for the ISR2000 treatment, which was immersed in a solution for 15 minutes before being transplanted into the plastic pots.

Table 3.4. Method of applying treatment in the climate chamber

Date	Treatment	Method
24-07-2023	ISR2000	immersion for 15 minutes
29-11-2023	ISR2000	Spraying
3-08-2023	All the chemical treatment	Spraying
05-08-2023	The pathogen *Xanthomonas*	Spraying
06-11-2022	ISR2000 - All the chemical treatment	Spraying

The first reading was after the last chemical spraying in 21 days. The plant was examined for bacterial spot symptoms. The disease symptoms were evaluated 3 using the adjusted scale from 1-6, 1 = symptomless, 2 = a few necrotic spots on a few leaflets, 3 = a few necrotic spots on many leaflets, 4 = many spots with coalescence on few leaflets, 5 = many spots with coalescence on many leaflets, 6 = severe disease and leaf defoliation. (Abbasi et al. 2002).

A disease severity (DS) was calculated using the following formula:

$$DS = \frac{\sum(\text{class x no.of leaves in class})}{\text{Total no.of leaves x (no.of classes} - 1)} \text{x } 100$$

Data on disease severity was analyzed using analysis of variance (ANOVA) in CoStat Statistics Software (CoHort Software, PacificGrove, CA, U.S.A. Version 6.4).

Figure 3.4. Spraying the seedling with the pathogen suspension

Effect of Nanomaterials, Chemicals and Plant Activator on Seed-Borne Bacterial Spot Disease Caused by *Xanthomonas perforans*

The tomato seeds used in this study were acquired from Adana markets and belonged to a susceptible cultivar. All the chemical substances were prepared. There are two additional materials, ISR 2000 and KOCIDE, which come with recommended dosages provided by the manufacturer. The study was conducted in a climate-controlled room in the Phytobacteriology Laboratory of the Plant Protection Department at Cukorova University. For each treatment, three replicates of tomato seeds (50 seeds per treatment) from the susceptible cultivar seeds were immersed in a suspension of *Xanthomonas* pathogen at a concentration of 10^6 CFU for 30 minutes (Figure 3.5A). The suspension was then filtered using a double-layered cheesecloth. Afterward, the seeds were immersed in a chemical suspension consisting of six different chemical solution treatments and one treatment of a plant activator solution for 30 minutes. Each seed was randomly distributed into plastic containers containing peat soil and provided with everyday watering using distilled water (Figure 3.5B). The containers were then placed under observation in the climate-controlled room.

The temperature range observed is between 25-28 °C. Each cotyledon was inspected for spot symptoms between 14 to 21 days after planting. The assessment involved recording the transmission of bacterial spots in seedlings, which was calculated by dividing the number of seedlings displaying characteristic signs of bacterial spots by the total number of seedlings that germinated and then multiplying the result by 100. The assessment of disease severity was conducted through visual examination and assigned scores based on a modified scale ranging from 0 to 3 (table 3.5). A score of 0 indicates a plant that seems healthy, while a score of 1 represents the presence of 1-2 spots on the cotyledon. A score of 2 indicates the presence of 3-4 spots, and a score of 3 indicates the presence of more than 5 spots. Mirik, (2005) has previously described this scoring system.

$$DS = \frac{\sum(P \times Q)}{(M \times N)} x100$$

Disease severity formula P = severity score, Q = number of infected plant cotyledons having the same score; M = Total number of cotyledons observed, N = Maximum rating scale number.

Table 3.5. Cotyledon disease severity index

scale	describe
0	shows a healthy-looking plant (no symptoms)
1	1-2 spot on cotyledon
2	3-4 spots
3	More than 5 spots

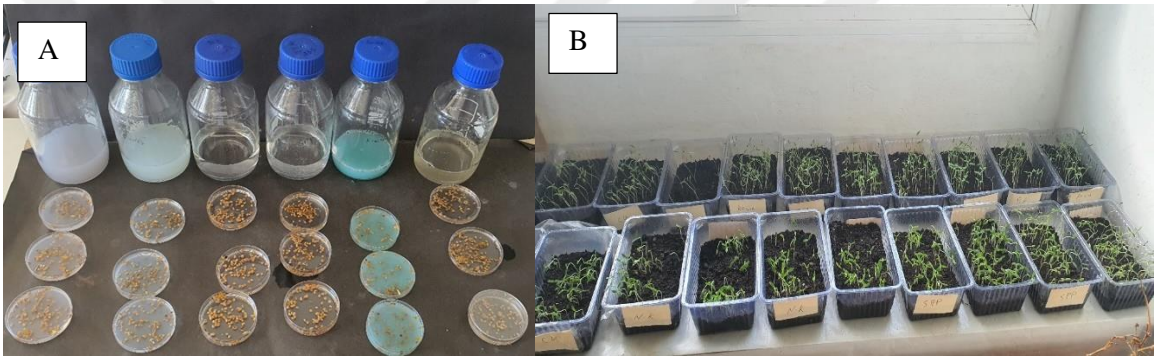

Figure 3.5. A: shows the seeds immersed in chemical suspensions of nanomaterials and chemical solutions and plant activator, B: shows the seed trail containers.

4. RESULTS AND DISCUSSIONS

4.1. Pathogenicity test for *Xanthomonas perforans* and *Clavibacter michiganensis*

Both pathogens showed a positive result in the pathogenicity test conducted on the tomato plant (Table 4.1). the symptoms of *Xanthomonas perforans* become apparent after 12 days (Figure 4.1A), whereas the symptoms of *Clavibacter michiganensis* develop after 10 days (Fıgure 4.1B); this indicates that both pathogens can already infect the plant and elicit the development of the disease on tomato plants.

Table 4.1. Shows the result of the Pathogenicity test for *Clavibacter michiganensis* and *Xanthomonas perforans*

Pathogen	Method	Positive\ Negative	Code
Clavibacter michiganensis	injection	+	Ya-1080
Xanthomonas perforans	spraying	+	Ya-873

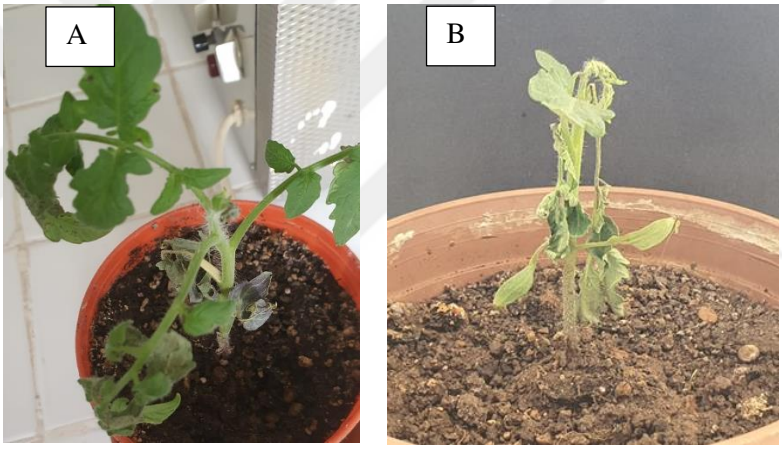

Figure 4.1. A shows the result of *Clavibacter michiganensis* pathogenicity test B shows the result of *Xanthomonas perforans* pathogenicity test.

4.2 The Result of Determining Treatments' Minimum Inhibitory Concentration (MIC) Against *Xanthomonas perforans* and *Clavibacter michiganensis*

According to the result of MIC of AgNps, Neither *Xanthomonas perforans* (Figure 4.2) A nor *Clavibacter michiganensis* (Figure 4.2 B) showed any colony growth on the petri dish containing silver nanoparticles (AgNps) at a concentration of 0.02 ppm. Therefore, this concentration can be considered the minimum inhibitory concentration (MIC) for this treatment (Table 4.2).

According to the result of CuNps for MIC: Neither *Xanthomonas perforans* (Figure 4.3 A) nor *Clavibacter michiganensis* (Figure 4.3 B) showed any colony growth on the petri dish containing copper nanoparticles (CuNps) at a concentration of 0**.**0035 ppm. Therefore, this concentration can be considered the minimum inhibitory concentration (MIC) for this treatment. (Table 4.3). MIC of the DOT shows that *Neither Xanthomonas perforans* (Figure 4.4 A) nor *Clavibacter michiganensis*

(Figure4.4B) showed any colony growth on the petri dish containing Disodium octaborate tetrahydrate (DOT) at a concentration of 0.0025 ppm. Therefore, this concentration can be considered the minimum inhibitory concentration for this treatment (Table 4.4). According to result of MIC of of the SPP, Neither *Xanthomonas perforans* (Figure 4.5 A) nor *Clavibacter michiganensis* (Figure 4.5 B) *showed* any colony growth on the petri dish containing Sodium pentaborate pentahydrate (SPP) at a concentration of 0.00375 ppm. Therefore, this concentration can be considered the minimum inhibitory concentration for this treatment (Table 4.5). So, in the end, minimum inhibitory concentration of all treatments against *Xanthomonas perforans* and *Clavibacter michiganensis* was determined (Table 4.6).

Table 4.2. Shows the dosage concentrations of AgNps against *Xanthomonas perforans and Clavibacter michiganensis to* find the MIC

	Xanthomonas perforans			*Clavibacter michiganensis*		
Treatments (PPM)	R1	R2	R3	R1	R2	R3
AgNps (0.0125)	+	+	+	-	-	-
AgNps (0.015)	+	+	+	+	+	+
AgNps (0.0175)	+	+	+	+	+	+
AgNps (0.02)	-	-	-	-	-	-
AgNps (0.025)	-	-	-	-	-	-
AgNps (0.05)	-	-	-	-	-	-
Control	+	+	+	+	+	+

Table 4.3. Shows the dosage concentrations of CuNps against *Xanthomonas perforans and Clavibacter michiganensis to* find the MIC

	Xanthomonas perforans			*Clavibacter michiganensis*		
Treatments (PPM)	R1	R2	R3	R1	R2	R3
CuNps (0.00125)	+	+	+	+	+	+
CuNps (0.0025)	+	+	+	+	+	+
CuNps (0.00375)	-	-	-	-	-	-
Control	+	+	+	+	+	+

Table 4.4. Shows the dosage concentrations of DOT against *Xanthomonas perforans and Clavibacter michiganensis* to find the MIC

	Xanthomonas perforans			*Clavibacter michiganensis*		
Treatments (PPM)	R1	R2	R3	R1	R2	R3
DOT (0.00125)	+	+	+	+	+	+
DOT (0.0025)	-	-	-	-	-	-
DOT (0.00375)	-	-	-	-	-	-
DOT (0.005)	-	-	-	-	-	-
DOT (0.01)	-	-	-	-	-	-
DOT (0.015)	-	-	-	-	-	-
DOT (0.025)	-	-	-	-	-	-
DOT (0.05)	-	-	-	-	-	-
DOT (0.075)	-	-	-	-	-	-
Control	+	+	+	+	+	+

Table 4.5. Shows the dosage concentrations of SPP against *Xanthomonas perforans* and *Clavibacter michiganensis* to find the MIC.

	Xanthomonas perforans			*Clavibacter michiganens*is		
Treatments (PPM)	R1	R2	R3	R1	R2	R3
SPP (0.0125)	+	+	+	+	+	+
SPP (0.0025)	+	-	+	+	+	-
SPP (0.00375)	-	-	-	-	-	-
Control	+	+	+	+	+	+

Table 4.6. Shows the minimum inhibitory concentration (MIC) of all treatments against *Xanthomonas perforans* and *Clavibacter michiganensis* .

Material	Status	(MIC) ppm
Silver nanoparticles (AgNps)	liquid	0.02
Copper nanoparticles (CuNps)	liquid	0.0035
Disodium octaborate tetrahydrate (DOT)	powder	0.0025
Sodium pentaborate pentahydrate (SPP)	granule	0.00375

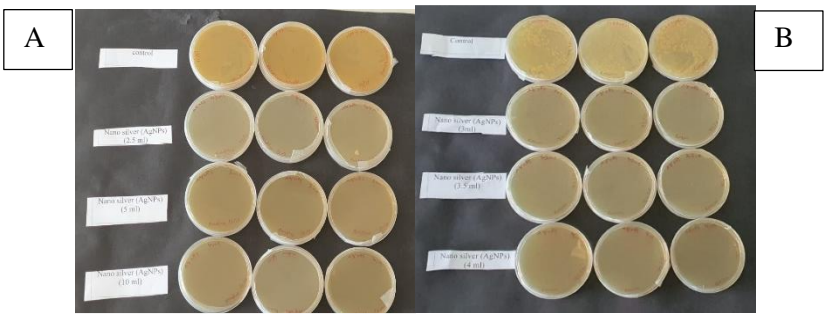

Figure 4.2. Shows the dosage concentrations of AgNps against (A) *Xanthomonas perforans and* (B) *Clavibacter michiganensis to* find the MIC

Figure 4.3. Shows the dosage concentrations of CuNps against (A) *Xanthomonas perforans* (B) *Clavibacter michiganensis* to find the MIC

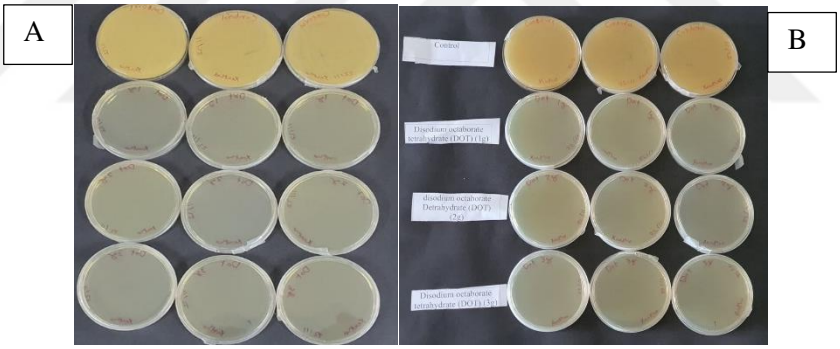

Figure 4.4. Shows the dosage concentrations of CuNps against (A) *Xanthomonas perforans* (B) *Clavibacter michiganensis* to find the MIC

Figure 4.5. Shows the dosage concentrations of SPP against (A) *Xanthomonas perforans* (B) *Clavibacter michiganensis to* find the MIC

4.3. Result Inhibition zones of minimum inhibitory concentration for *Xanthomonas perforans* and *Clavibacter michiganensis*

All the treatments were successfully inhibited the growing of *X. perforans* on the petri dish (Figure 4.6). There was no significant differences between time of the treatment, and the highest radius range of inhibition was by the treatment of CuNps at (1.13) mm AgNps at (0.7) mm.DOT, SPP (0.63)(0.34)mm, respectively (Table 4.7). Noshad et al. (2019) showed the same result with slightly different concentrations against *Xanthomonas campestris.*

Table 4.7. Shows Inhibition zones of minimum inhibitory concentration against *Xanthomonas perforans*

Treatments	Inhibition zones (mm)		
Time	1Hour	3Hour	24Hour
AgNps + *Xanthomonas perforans*	0.7	0.7	0.7
CuNPS + *Xanthomonas perforans*	1.13	1.13	1.13
DOT + *Xanthomonas perforans*	0.63	0.63	0.63
SPP + *Xanthomonas perforans*	0.34	0.34	0.34

The treatments of CuNps gave a good result by inhibiting the pathogen *Clavibacter michiganensis* on a petri dish (Figure 4.7) with the highest radius range of inhibition (0.66)mm followed by the treatment of AgNps at (0.36) , DOT, and SPP (0.166) (0.66)mm, respectively was, with no significant differences in time of the treatments (Table 4.8). Noshad et al. (2019), demonstrated the same result using the AgNps against *Clavibacter michiganensis* and other plant pathogenic bacteria.

Table 4.8. Shows Inhibition zones of minimum inhibitory concentration against *Clavibacter michiganensis*

Treatments	Inhibition zones (mm)		
Time	1Hour	3Hour	24Hour
AgNps + *Clavibacter michiganensis*	0.36	0.36	0.36
CuNPS + *Clavibacter michiganensis*	0.66	0.66	0.66
DOT+ *Clavibacter michiganensis*	0.166	0.166	0.166
SPP + *Clavibacter michiganensis*	0.066	0.066	0.066

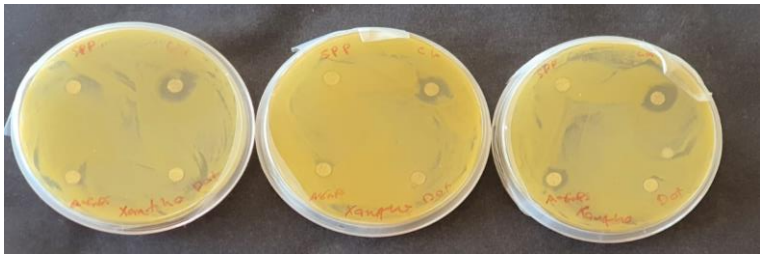

Figure 4.6. Shows Inhibition zones of minimum inhibitory concentration of treatments against *Xanthomonas perforans*

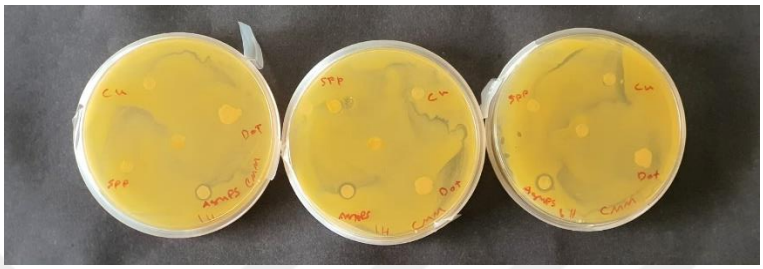

Figure 4.7. Shows Inhibition zones of minimum inhibitory concentration against *Clavibacter michiganensis*

4.4. The Result of Determining The Specific Concentrations of The Pathogens for *In Vivo* trials

Spot symptoms were observed on tomato plants in both greenhouse and climatic room conditions across all treatment concentrations of *Xanthomonas perforans* in all weeks of the test (Table 4.9 and 4.10). A minimal concentration of 10^6 CFU was determined to be sufficient for inducing disease symptoms (Figure 4.8), and thus, this concentration was selected for all subsequent trials. Wilting symptoms were observed on tomato plants in the greenhouse and climatic room conditions across all treatment concentrations of *Clavibacter michiganensis* all week of the test. A minimal concentration of 10^6 colony-forming units (CFU) was determined to be sufficient for inducing disease symptoms (Figure 4.9), and thus, this concentration was selected for all subsequent trials (Table 4.11 and 4.12).

Table 4.9. Shows the number of spots on tomato leaves of different concentrations of *Xanthomonas perforans* in greenhouse conditions.

concentration	1w	2w	3w	4w	5w	6w
10^1	11	18	28	33	36	42
10^2	4	11	32	36	38	40
10^3	6	8	22	45	51	51
10^4	0	6	7	14	21	21
10^5	1	7	21	25	30	32
10^6	0	5	16	24	32	38

Table 4.10. Shows the number of spots on tomato leaves of different concentrations of *Xanthomonas perforans* in climate chamber conditions

concentration	1w	2w	3w	4w	5w	6w
10^1	4	16	22	25	25	25
10^2	1	4	9	11	13	15
10^3	1	4	8	24	24	25
10^4	1	7	7	9	13	15
10^5	2	3	9	12	17	18
10^6	1	8	11	16	22	23

Table 4. 11. Shows disease severity of different concentrations of *Clavibacter michiganensis* in greenhouse conditions.

Concentration (cfu)	Plant Height (cm)	Disease symptoms (cm)
10^1	34	30
10^2	37	32
10^3	72	44
10^4	65	31
10^5	60	27
10^6	78	11

Table 4.12. Shows disease severity of different concentrations of *Clavibacter michiganensis in* climate chamber conditions.

Concentration(cfu)	Plant Height(cm)	Wilting symptoms (cm)
10^1	40	38
10^2	36	33
10^3	66	37
10^4	65	26
10^5	72	33
10^6	71	11

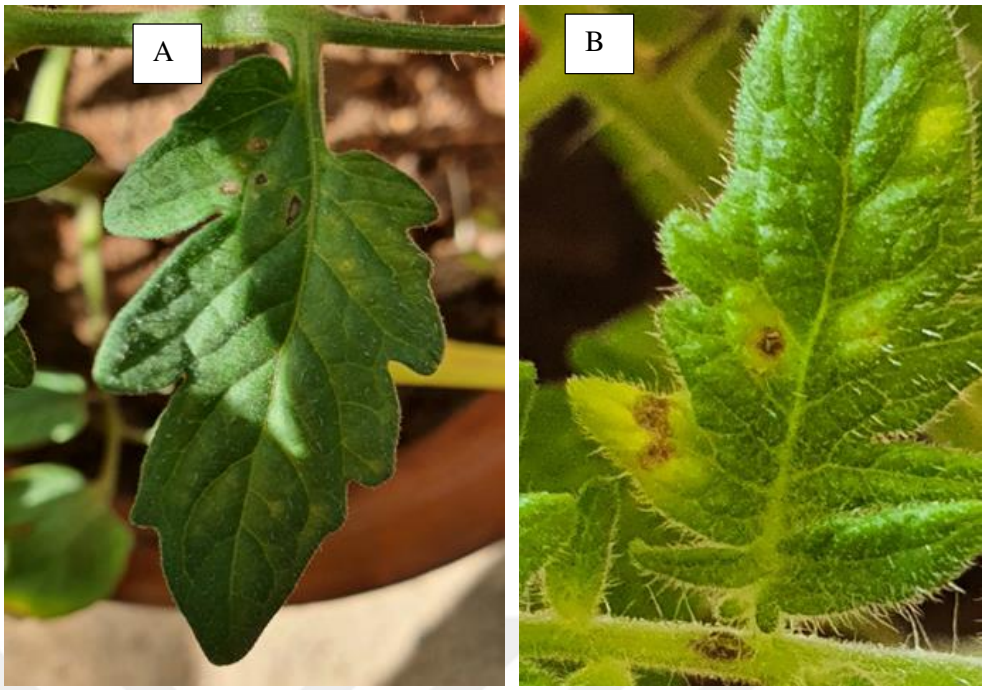

Figure 4.8. (A) shows the symptoms of a lower concentration of *Xanthomonas perforans* in greenhouse conditions (B) shows the symptoms of lower concentration of *Xanthomonas perforans* in climate chamber conditions.

Figure 4.9. (A) shows the symptoms of lower concentration of *Clavibacter michiganensis* in greenhouse conditions (B) shows the symptoms of lower concentration of *Clavibacter michiganensis* in climate chamber conditions.

4.5. The Result of The Effect of Nanomaterials, Chemicals and Plant Activators on Bacterial Cancer and Wilt Disease Caused by *Clavibacter michiganensis*

Based on the data shown in Table 4.13, it can be observed that ISR2000 shows a higher level of effectiveness compared to the other treatments. The KOCIDE treatment shows a great result compared to the positive control treatment (Figure 4.11). The ISR2000 shows a notable decrease in disease severity, reducing to 13.25%. Additionally, the treatment showed a high level of efficacy, with a percentage of 77.91%. The KOCIDE treatment shows a disease severity of 32.25% and an efficacy of 46.25%, placing it second. However, nanochemical treatments did not successfully decrease the severity of the disease and were not entirely effective in controlling the infection. The two chemical materials, DOT and SPP, show encouraging outcomes in disease management, with disease severity scores of 40.5% and 43.5%, respectively, and efficacy for DOT, 32.5 and 27.5 for SPP. Nanomaterial treatments did not decrease the severity of the disease AgNps, CuNps, 60.00% and 66.62%, respectively, with no efficacy impact compared to the positive control (Figure 4.10).

At the same time, the result of the area under the disease progress curve (AUDPC) for nine weeks in the figure below shows that the ISR2000 treatment has a stable rhythm by reducing the (AUDPC) to 182.0 from the first week till the last week of reading. This was followed by the treatment of KOCIDE 441.0, DOT (556.5) and SPP (598.5) compared to the positive control treatment 812.0, while the nanomaterial treatments AgNps, CuNps did not impact the (AUDPC) (808.5-927.5) respectively compared to the positive control treatment (Figure 4.12).

Table 4.13. Shows the result data of the Effect of nanomaterials, chemicals and plant activators on Bacterial Cancer and Wilt Disease caused by *Clavibacter michiganensis*

Treatment	Mean	AUDPC	Disease severity (%)	Efficacy%
ISR2000	0.53 a	182.00 a	13.25	77.91
KOCIDE	1.29 a	441.00 ab	32.25	46.25
DOT	1.62 b	556.50 bc	40.50	32.50
SPP	1.74 b	598.50 bc	43.50	27.50
AgNps	2.40 cd	808.50 cd	60.00	00
Positive control	2.40 cd	812.00 cd	60.00	-
CuNps	2.66 d	927.50 d	66.62	-11.04

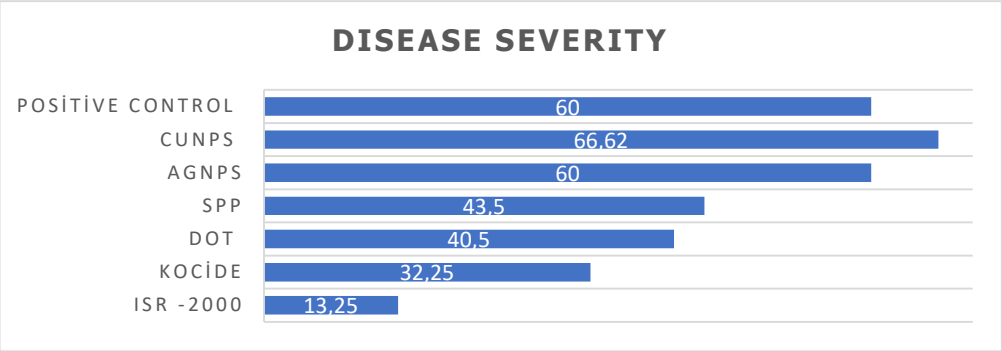

Figure 4.10. Shows the Effect of nanomaterials, chemicals and plant activators on Bacterial Cancer and Wilt Disease severity caused by *Clavibacter michiganensis* under greenhouse conditions

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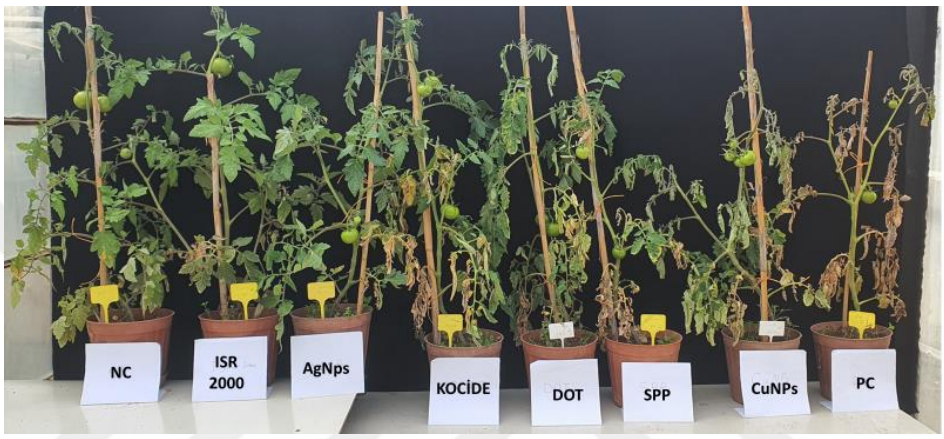
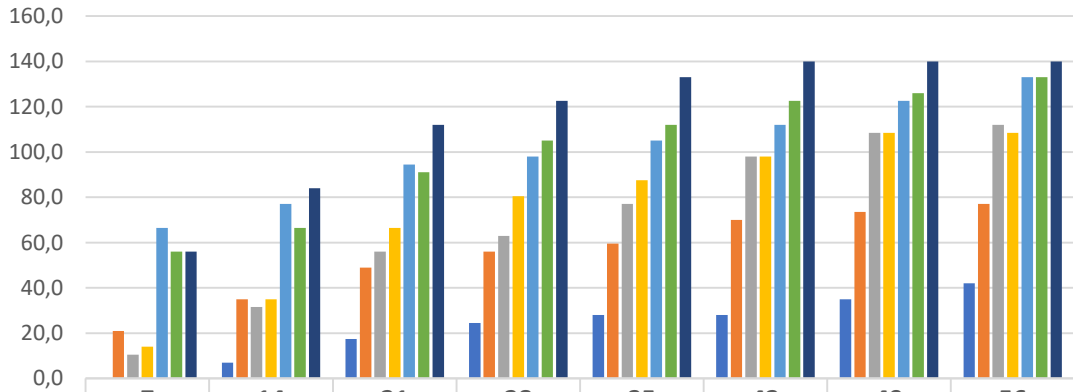


Figure 4.11. Shows the Effect of nanomaterials, chemicals and plant activators on Bacterial Cancer and Wilt Disease caused by *Clavibacter michiganensis* under greenhouse conditions



	7	14	21	28	35	42	49	56
Isr	0,0	7,0	17,5	24,5	28,0	28,0	35,0	42,0
Kocide	21,0	35,0	49,0	56,0	59,5	70,0	73,5	77,0
Dot	10,5	31,5	56,0	63,0	77,0	98,0	108,5	112,0
Spp	14,0	35,0	66,5	80,5	87,5	98,0	108,5	108,5
Agnps	66,5	77,0	94,5	98,0	105,0	112,0	122,5	133,0
P.C	56,0	66,5	91,0	105,0	112,0	122,5	126,0	133,0
Cunps	56,0	84,0	112,0	122,5	133,0	140,0	140,0	140,0

Figure 4.12. Shows the AUDPC of all treatments.

4.6. Effect of Nanomaterials, Chemicals and Plant Activator on Bacterial Leaf Spot Disease Caused by *Xanthomonas perforans* in Climate Chamber Conditions.

The data was collected 21 days after applying chemical treatments with a scale for disease severity from 0-5 (Figure 4.14). The data Table 4.14 below shows that the treatments of ISR2000 and KOCIDE provided the highest results in disease severity reduction, with respective values of 23.20 % and 39.66%. Additionally, the efficacy of these treatments was found to be 54.84% and 22.79%, respectively. The application of nanomaterials in the treatment of CuNps showed good results, as shown by a reduction in disease severity to 42.23% and an efficacy of 17.80%. This result was compared to the positive control treatment, which showed a disease severity of 51.37%. The SPP and DOT treatments showed promising results in reducing disease severity, with 44.83% and 50.71% reductions, respectively. However, it is noticeable that the SPP treatment shows a higher efficacy level than the DOT treatment, with reductions of 12.72% and 1.29%, respectively, for both treatments. The treatment of AgNps did not show a good result, reducing the severity to 52.96 % and the same for efficacy to 3.09% with no significant difference from the positive control treatment (Figure 4.13).

Table 4.14. Shows the Effect of the nanomaterials, chemicals and plant activator on disease severity of *Xanthomonas perforans* in climate chamber conditions.

Treatment	Disease severity %	Efficacy %
ISR2000	23.20 a	54.84
KOCIDE	39.66 b	22.79
CuNps	42.23 bc	17.80
SPP	44.83 c	12.72
DOT	50.71 d	1.29
AgNps	52.96 d	3.09
Positive Control	51.37 d	-

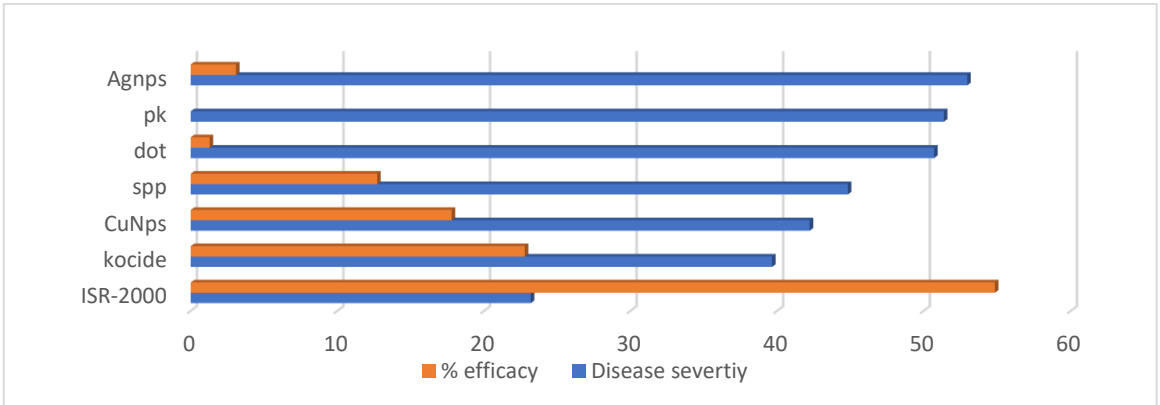


Figure 4.13. Shows the Effect of the nanomaterials, chemicals and plant activator on the disease severity of *Xanthomonas perforans* and the efficacy of the treatments.

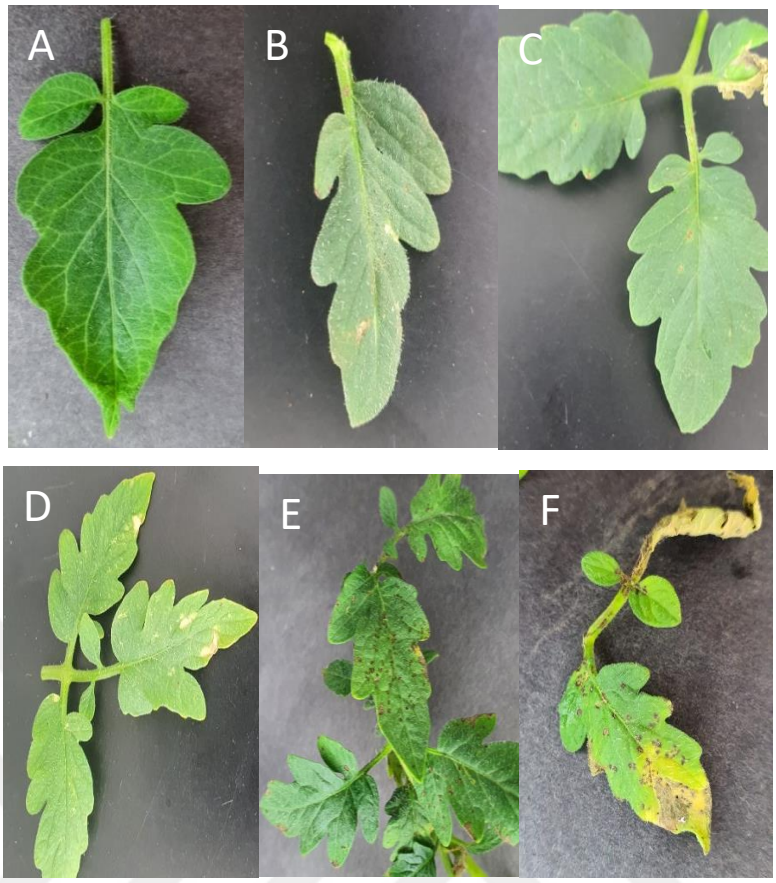


Figure 4.14. shows the scale for the disease severity for *xanthomonas perforans* (A) 0 = symptomless (B) 1 = a few necrotic spots on a few leaflets (C) 2 = a few necrotic spots on many leaflets (D) 3 = many spots with coalescence on few leaflets (E) 4 = many spots with coalescence on many (F) 5 = severe disease and leaf defoliation

Twenty-one days after the seed was sowed in plastic pots and transferred to the climate chamber, each cotyledon was examined for spot symptoms (Figure 4.16). Disease severity was evaluated visually and scored using a modified scale range of 0 to 3, as previously described. Furthermore, the result data was collected and analyzed statically in the table 4.15 below. The result showed that the ISR2000 had the best impact in decreasing the data severity by 10,19%, and it was the best efficacy treatment by 84,64%; in second place, the treatment of KOCIDE gave a very significant result by decreasing the severity to 12.99% with efficacy reached 80.41%. The nanomaterial treatment also gave a promising result by decreasing the severity by 43.40% for the AgNps treatment and 34.56% by the efficacy, and the CuNps decreased the severity to 46.84 % with 29.37% in terms of efficacy. The new chemical treatment of DOT and SPP did not give significant results in disease severity at 64.20% and 68.88%, respectively, and efficacy reached 3.20% and 3.86%, respectively (Figure 4.15)

Table 4.15. Shows the Effect of nanomaterials, chemicals and plant activators on seed-borne Bacterial Spot Disease caused by *Xanthomonas perforans* in climate chamber conditions.

Treatment	Disease severity %	Efficacy %
ISR2000	10,19 a	84,64
KOCIDE	12,99 a	80,41
AgNps	43,40 b	34,56
CuNps	46,84 b	29,37
DOT	64,20 c	3,20
SPP	68,88 c	3,86
positive control	66,32 c	-

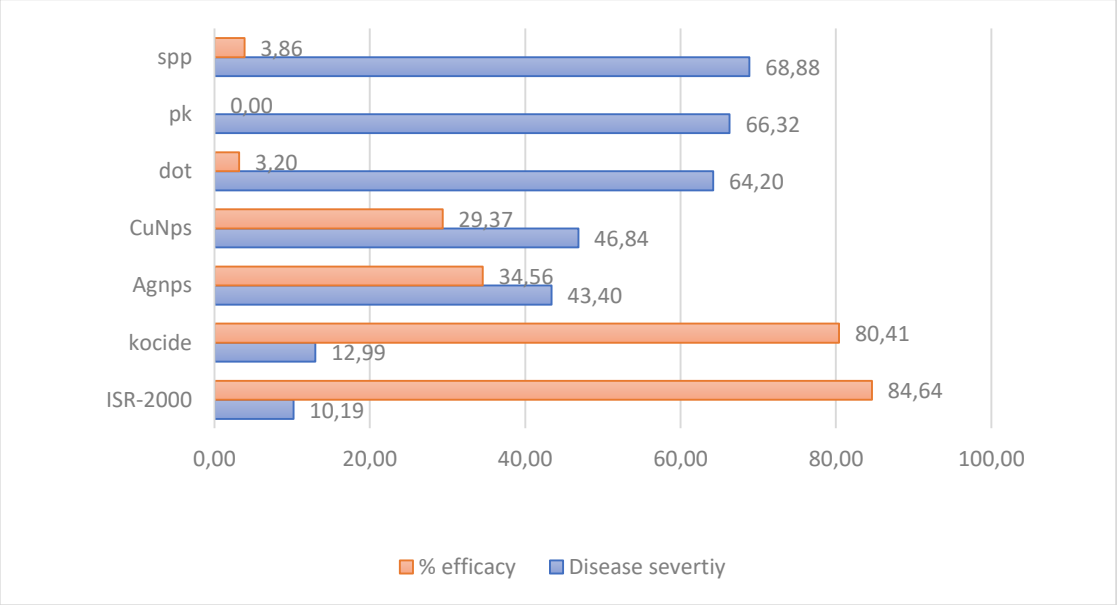


Figure 4.15. shows the Effect of nanomaterials, chemicals and plant activators on seed-borne Bacterial Spot Disease caused by Xanthomonas perforans in climate chamber conditions.

Figure 4.16. Shows the difference between a healthy cotyledon and an infected cotyledon by *Xanthomonas perforans*

After 21 days, the seed was planted in plastic containers and transferred to the climate chamber; seed germination data was collected to demonstrate the treatment's Effect on seed germination, as shown in the table 4.16 below. The result demonstrates that the treatments affected the seed germination negatively. The treatments of the SPP and DOT decreased the seed germination by 33.11% and 26.35%, respectively, and it was lower than the positive control, which decreased the seed germination by 17.57%. The treatments of KOCIDE, ISR2000, CuNps, and AgNps had a very good impact on seed germination: 0.68%, 2.7% and 2.7% and 4.05%, respectively, with no significant differences compared to the negative control (Figure 4.17).

Table 4.16. Shows the Effect of nanomaterials, chemicals and plant activators on seed germination

Treatment	Seed germination (%)	Decrease in germination (%)
Negative Control	98,67 a	-
KOCIDE	98,00 a	0,68
CuNps	96,00 a	2,7
ISR2000	96,00 a	2,7
AgNps	94,67 a	4,05
Positive Control	81,33 b	17,57
DOT	72,67 c	26,35
SPP	66,00 c	33,11

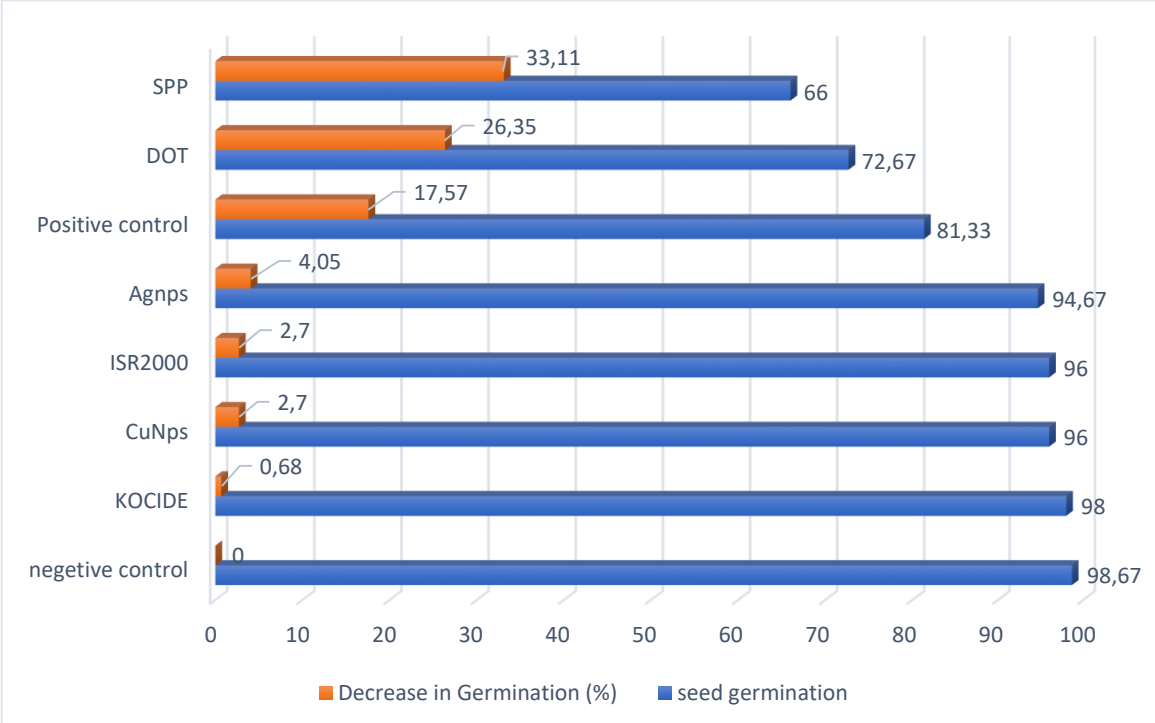


Figure 4.17. Shows the Effect of nanomaterials, chemicals and plant activators on seed germination.

5. DISCUSSION

Nanotechnology represents a novel tool in our efforts to address the growing challenges of plant and disease management. Nanotechnology can be utilized for plant disease management, diagnosis, and genetic modification (Elmer and White, 2018). Copper (Cu) and silver (Ag) compounds have been widely employed as inorganic agrochemicals for crop protection and as fertilizers because of their antibacterial capabilities and because they are important micronutrients for plants. However, excessive Cu application may harm crops and cause environmental pollution. The antibacterial capabilities of copper compounds in the form of nanoparticles (NPs) may be improved, which could result in lower doses. Recent studies have demonstrated the effectiveness of Cu-based NPs against various plant pathogens (Printz et al. 2016). Because they are less expensive than Ag and TiO2, Cu-based NPs have an advantage over traditional insecticides and other inorganic-based nanomaterials.

Nevertheless, the chemical makeup and oxidation state of the Cu-based NPs, rather than the size, shape, or type of bacterium, determine their antibacterial properties: (i) copper (Cu); (ii) nanocomposites based on copper (Cu); and (iii) various nanostructures containing copper (Cu), such as copper oxides (CuO and Cu2O); sulfates, sulfides, phosphates, etc. Additionally, the antibacterial activity of Cu-based NPs is determined by the synthesis parameters, including solvents, precursors, temperature, etc. Cu-based NPs can be used in agriculture, however, the synthesis process must be eco-friendly and scalable. Chemical approaches, such as hydrothermal and polyol procedures, are said to be the most prevalent among the synthesis techniques to create Cu-based NPs (Hermida-Montero et al. 2019).

The minimum inhibitory concentrations of AgNps and CuNps were determined *in vitro* poison agar assays by applying the spread palate method and disc diffusion method to evaluate the efficacy of Agnps and Cunps against two phytopathogenic bacteria, *Clavibacter michiganensis* and *Xanthomonas perforans*. Overall, AgNps and CuNps were hazardous to bacterial pathogens at concentrations of 0.02 ppm and 0.0035 ppm, respectively. The current study demonstrated that all treatments could act as a bacteriocide on bacterial diseases with the smallest possible interferences. According to Noshad et al., (2020), the AgNps and Cunps performed similarly against the *Clavibacter michiganensis* and other plant pathogenic bacteria, but with distinct considerations.

The result of the greenhouse trials shows that plant activator ISR2000 and fungicide KOCIDE were Superior in 9 weeks of control of the disease, and they kept the AUDPC of the disease in stable ranges. They were effective on Bacterial Cancer and Wilt Disease caused by *Clavibacter michiganensis in* disease severity and efficacy 13.25% and 32.25%, respectively.

Soykan (2010), reported a similar result in a study on bacterial wilt in tomatoes caused by *Clavibacter michiganensis that* ISR2000 reduced the disease severity to 31.17%, with efficacy reaching 62.09%.

The results collected from climate chamber trials showed the advantage of ISR2000 and KOCIDE over other treatments. Both treatments reduced the severity of *Xanthomonas perforans* on the tomato leaves in climate conditions room by 23,20% and 39,66%, respectively. The only difference is that in these trials, the CuNps treatment showed a promising result by suppression of the severity 42,23%. Aktepe (2021), mentioned in her study on speck disease in tomato caused by *Pseudomonas syringae pv. tomato* similar result, the study demonstrated that the plant activators are very successful in controlling the plant bacterial disease, and the study shows that the ISR2000 reduced the disease severity by 43.33% with efficacy reaching 35.00% compared to the positive control. Yildiz (2016), proved that ISR 2000 gave a good amount of protection by affecting the development of tomato bacterial wilt disease in pots, which was 23.7 % compared to the positive control and also proved the effect of ISR2000 on the development of tomato bacterial wilt disease in field experiment 22.9 % compared to the positive control.

Plant activator ISR2000 and fungicide KOCIDE and the nanomaterial treatments AgNps, CuNps showed an excellent result against the *Xanthomonas perforans* on seeds. By reducing the severity and increase the germination rate of the seeds. Except the SPP and DOT treatment. Noshad et al., (2019). Demonstrated similar results about AgNps and how it affects the seed germination of tomato plants infected with *Clavibacter michiganensis.* The SPP and DOT had a phytotoxic effect on plant leaves. Türkiye preserves 70% of the boron element, a usable source of new formulation. Therefore, more research should be conducted on the use of these chemicals. Perhaps their use in combination form as an active pesticide ingredient could be more suitable.

ISR2000, with its unique components that enhance the natural defense system of plants, providing natural resilience against disease agents and stress conditions in plant production, has proven to be the most successful application in this thesis study. Such products can assist plants in becoming more resilient against disease agents and stress conditions by activating their natural defense mechanisms. ISR2000 or similar plant activators can be used in the agricultural sector to increase crop yields and reduce the use of chemical pesticides in disease management. It can also be added to integrated management when chemical pesticide use is not feasible throughout the plant's growth stages.

6. CONCLUSION

This study has demonstrated the reducing effect of nanomaterials (nano silver AgNps and nano copper CuNps), a plant activator (ISR2000), a commercial fungicide called KOCIDE, and two new chemical compounds named sodium pentaborate pentahydrate (SPP) and disodium octaborate tetrahydrate (DOT) on Bacterial Spot disease caused by *Xanthomonas perforans* and Bacterial Canker and Wilt disease caused by *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes.

This study aims to investigate the effect of the treatments on disease severity and the area under the disease progress curve (AUDPC) caused by the pathogen *Clavibacter michiganensis* under greenhouse conditions. Moreover, the effects of the treatments against *Xanthomonas perforans* on the plant leaves under a climate chamber and the effect of treatments on the seed germination and disease severity of *Xanthomonas perforans* as a seed-borne pathogen.

It started with determining the minimum inhibitor concentration against both pathogens and as a result, it was determined as AgNps (0.02) ppm, CuNps (0.0035) ppm, DOT (0.0025) ppm and SPP (0.00375) ppm.

The result from greenhouse trials on *Clavibacter michiganensis* shows that the ISR2000 was the best in reducing the disease severity by (13.25%) with efficacy reaching (77.91%) followed by the KOCIDE (32.25%), with efficacy reaching (46.25%) compared to the positive control (60.00%). The ISR2000 was the best treatment as a curative and protective against Bacterial Cancer and Wilt Disease. Besides, the other treatments gave the plant many curatives except the CuNps, which did not have any significant differences from the positive control treatment. KOCIDE could be used successfully in the area without copper product resistance.

The result from the climate chamber showed that ISR2000 was superior in reducing *Xanthomonas perforans* severity on the tomato leaves by (23.20%) with efficacy reached (54.84%) and the KOCIDE was able to reduce the severity by (39.66%) with efficacy reached (22.79%) and the nanomaterial treatment CuNps (42.23%) with efficacy reached (17.80%) compared to the positive control treatment (51.37%). ISR2000 was also determined as the most effective treatment for Bacterial Leaf Spot Disease caused by *Xanthomonas perforans.* KOCIDE, CuNps and SPP were determined as the other effective treatments against Bacterial Leaf Spot Diseases.

The result from the seed trials showed that ISR2000 was able to reduce the severity of the disease on cotyledons by (10.19%) efficacy (84.64%) and KOCIDE (12.99%) efficacy (80.41%) and AgNps (43.40%) efficacy (34.56%) and CuNps (46.84%) efficacy (29.37%). Compared to the positive control treatment severity (66,32). ISR2000, AgNps, KOCIDE, CuNps and SPP showed no negative impact on seed germination as a negative control on seed-borne Bacterial Spot Disease.

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