EVALUATING THE EFFICACY OF ENDOPHYTIC *PSEUDOMONAS* SPP. THROUGH ROOT COATING TO PREVENT SOME IMPORTANT BACTERIAL PATHOGENS OF TOMATO (*Solanum lycopersicum*)

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ABSTRACT

MSc Thesis

EVALUATING the EFFICACY of ENDOPHYTIC *Pseudomonas* spp. THROUGH ROOT COATING to PREVENT SOME IMPORTANT BACTERIAL PATHOGENS of TOMATO (*Solanum lycopersicum*)

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Tomato production faces significant challenges from bacterial wilt and canker (*Clavibacter michiganensis* subsp. *michiganensis*, *Cmm*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*, *Pst*), which are highly virulent pathogens causing substantial yield losses. Current control strategies rely heavily on copper-based antimicrobial compounds, which pose environmental and resistance-related concerns. This study investigated the potential of *Pseudomonas fluorescens* strains as sustainable biocontrol agents against these pathogens. Four fluorescent Pseudomonas strains (46/a1, 27/3, M17, M11) were selected for *in vivo* evaluation based on their *in vitro* antagonistic activity, as determined by inhibition zones. These strains were introduced into tomato plants via root inoculation, and their efficacy against *Cmm* and *Pst* was assessed. Statistical analysis revealed that strain M17 showed no significant impact on disease severity compared to control treatments, indicating limited or no biocontrol efficacy. In contrast, strains 27/3, 46/a1, and M11 significantly reduced disease severity. Strain 27/3 reduced disease severity by 44% against *Cmm* and 48% against *Pst*, while strain 46/a1 achieved reductions of 40% for both pathogens. Strain M11, however, was the most effective strain, reducing disease severity by 28% against *Cmm* and 32% against *Pst*.

December 2024, 49 pages

Key Words: *Clavibacter michiganensis* subsp. *michignanensis*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas fluorescens*, tomato, disease management

ÖZET

Yüksek Lisans Tezi

DOMATESİN (*Solanum lycopersicum*) BAZI ÖNEMLİ BAKTERİYEL PATOJENLERİNİN ÖNLENMESİNDE ENDOFİTİK *Pseudomonas* spp.'nin KÖK KAPLAMA YOLUYLA ETKİNLİĞİNİN DEĞERLENDİRİLMESİ

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Danışman: Doç. Dr. Didem CANİK OREL

Domates üretimi, önemli verim kayıplarına neden olan yüksek derecede virülent patojenler olan bakteriyel solgunluk ve kanser (*Clavibacter michiganensis* subsp. *michiganensis, Cmm*) ile bakteriyel benek (*Pseudomonas syringae* pv. *tomato, Pst*) nedeniyle önemli zorluklarla karşı karşıyadır. Mevcut kontrol stratejileri büyük ölçüde çevresel ve dirençle ilgili endişeler bakır bazlı antimikrobiyal bileşiklere dayanmaktadır. Bu çalışma, bu patojenlere karşı sürdürülebilir biyokontrol ajanları olarak *Pseudomonas fluorescens* strainlerinin potansiyelini araştırmıştır. *İn vitro* antagonistik aktiviteleri temel alınarak dört floresan Pseudomonas straini (46/a1, 27/3, M17, M11) *in vivo* değerlendirme için seçilmiştir. Bu strainler, domates bitkilerine kök inokülasyonu yoluyla uygulanmış ve *Cmm* ile *Pst*'ye karşı etkinlikleri değerlendirilmiştir. İstatistiksel analiz, M17 straininin kontrol uygulamaları ile karşılaştırıldığında hastalık şiddeti üzerinde anlamlı bir etkisinin olmadığını ve dolayısıyla sınırlı veya hiç biyokontrol etkinliği göstermediğini ortaya koymuştur. Buna karşılık, 27/3, 46/a1 ve M11 strainleri hastalık şiddetini önemli ölçüde azaltmıştır. 27/3 straini, hastalık şiddetini *Cmm*'ye karşı %44 ve *Pst*'ye karşı %48 oranında azaltırken, 46/a1 straini her iki patojene karşı %40 oranında azalma sağlamıştır. M11 straini ise en etkili strain olarak belirlenmiş ve *Cmm*'ye karşı %28, *Pst*'ye karşı ise %32 oranında hastalık şiddetini azaltmıştır.

Aralık 2024, 49 sayfa

Anahtar Kelimeler: *Clavibacter michiganensis* subsp. *michignanensis, Pseudomonas syringae* pv. *tomato, Pseudomonas fluorescens,* domates, hastalık kontrolü

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Ahmednur Yusuf IDAN
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THESIS APPROVAL

LIST of SYMBOLS

°C	Celsius
Cm	Centimeter
g	Gram
l	Litre
Mm	Millimolar
mm	Millimeter
mL	Millilitre
Na	Sodium
Ni	Nickel
Pb	Lead
Zn	Zinc

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
BCA	Biological Control Agents
EB	Endophytic Bacteria
FAO	Food and Agriculture Organization
IAA	Indole-3-acetic acid
PGPR	Plant Growth-Promoting Rhizobacteria
Ros	Reactive Oxygen Species
TÜİK	Türkiye İstatistik Kurumu (Turkish Statistical Institute)
USDA	United States Department of Agriculture

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1. INTRODUCTION

Taxonomically, tomatoes (*Solanum lycopersicum*) belong to the Solanaceae (nightshades) family. This economically highly important family contains some of the most grown crops such as potatoes, pepper, tobacco, eggplants, etc. The tomato originated in western South America, specifically in regions that are now Ecuador, Chile, and Peru (McCue, 1952). It was introduced to Europe through Spain in the 16th century and was later introduced to European colonies (Bergougnoux, 2014). Tomato grows for 90 to 150 days and thrives from 18 to 26 °C, with nighttime temperatures between 10 and 20 °C. Dry climates are preferable because tomatoes are less likely to experience diseases, pests, and fruit rot. Tomato has a low tolerance for cold weather and growing conditions below -2 °C can lead to severe yield losses. Apart from needing a pH of 5 to 7, and well-drained soil, tomatoes don't have specific requirements for soil type and can thrive in a variety of soil conditions. Tomatoes are predominantly self-pollinating plants, each plant containing female and male parts on their flowers. However, cross-pollination can occasionally occur through the action of wind or insects.

The tomato is a nutritious plant, rich in vitamins, minerals, phenolic compounds, and carotenoids, including significant levels of Lycopene and β-carotene. Lycopene has antioxidant, anti-inflammatory, and anticancer properties and gives the ripened tomato its red color (Jacob et al., 2008a; Kheyrodin & Kheyrodin, 2017; Edinger & Koff, 2006;). Frequent use of tomato-based products has been linked to a lower risk of thrombosis, inflammation, cardiovascular disease, and prostate cancer (Giovannucci, 2002; Jacob et al., 2008a, 2008b). Tomatoes have a high water content, with 100 grams of tomatoes containing over 94 grams of water, according to the USDA. This is higher than the water content of watermelon, which is 91 grams per 100 grams.

According to the FAO's 2022 report, global tomato production has reached 186 million metric tonnes, making tomatoes the most widely produced vegetable in the world (FAO 2022). Economically, it is among the most significant crops cultivated globally, with China being the largest producer, followed by India, Türkiye, the United States, Egypt, Italy, Mexico, Brazil, Nigeria, and Spain (FAO 2022). Asia was the leading region in tomato production in 2022, accounting for 63.9% of global output (Figure 1.1). Tomatoes can be consumed raw or processed into various products, including soup, and paste, thereby broadening its market appeal.

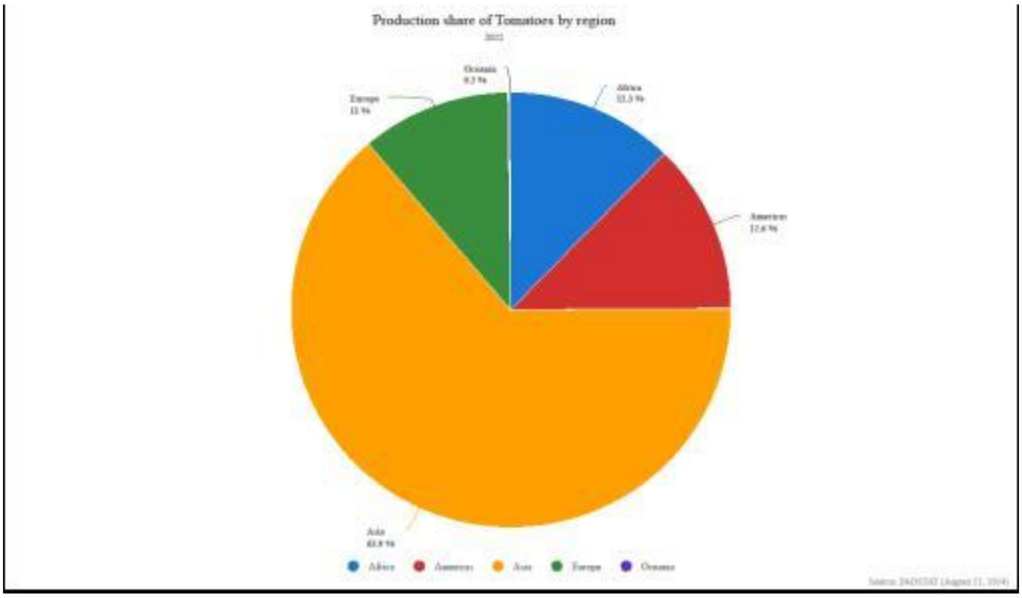

Figure 1.1 Tomato production around the world by region (FAO, 2022)

The highest importer of tomatoes is the United States followed by Germany and France. While largest exporter was reported to be Mexico, followed by the Netherlands, and Spain, while Türkiye is the 6th largest exporter of tomatoes (FAO, 2022).

Türkiye is among the world's top tomato producers (FAO, 2022). Tomato exports contributed USD 450 million to the economy of Türkiye in 2023 (Anonymous, 2024). The leading provinces in tomato production are Antalya followed by Bursa, Manisa, İzmir, and Mersin (Figure 1.2 and Figure 1.3).

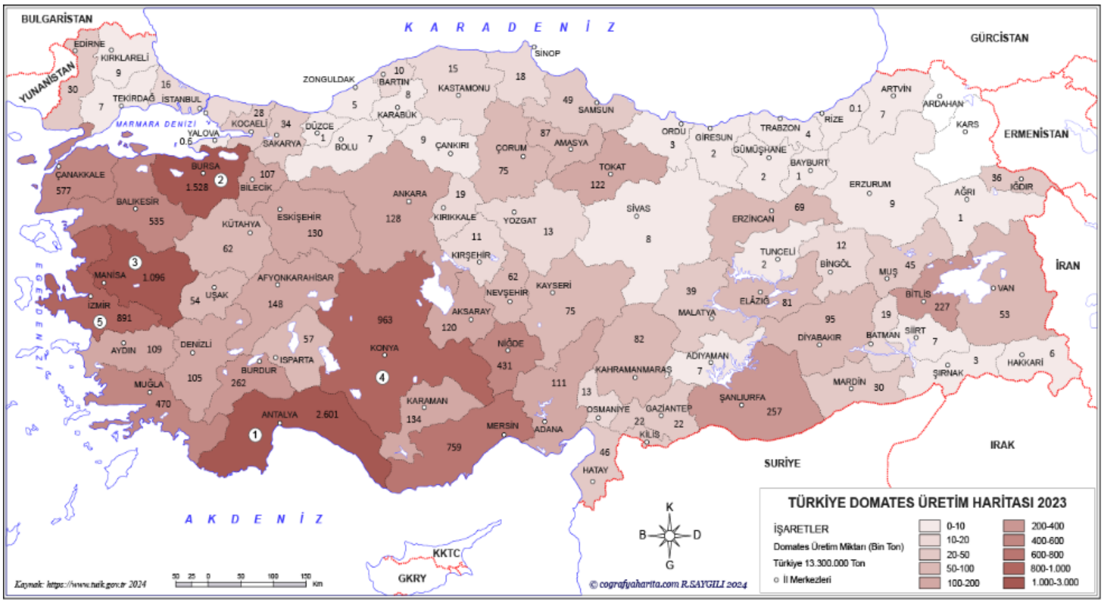

Figure 1.2 Tomato production map in Türkiye (Anonymous, 2023)

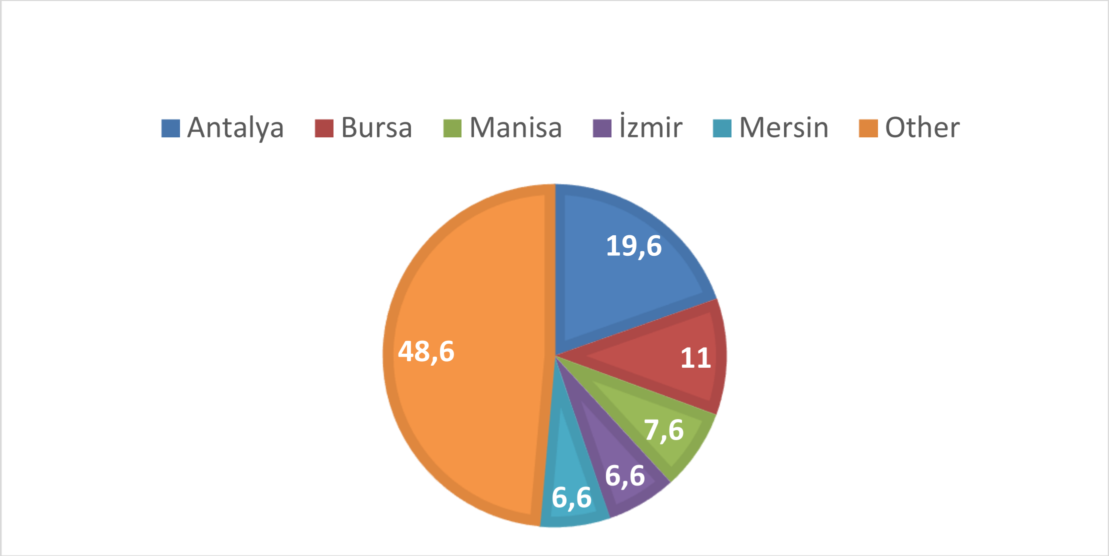

Figure 1.3 Türkiye's tomato production distribution by provinces (Anonymous, 2024)

Tomato production is significantly impacted by various challenges, including pests as well as bacterial and fungal pathogens. Among the most critical bacterial pathogens affecting tomatoes are *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas* spp., and *Rhizoctonia solani*. This study evaluated the efficacy of endophytic *Pseudomonas fluorescens* strains as a sustainable alternative control strategy against *Cmm* and *Pst*, aiming to mitigate crop losses and reduce dependency on conventional, unsustainable management practices.

1.1 Overview of Bacterial Wilt and Canker (*Clavibacter michiganensis* subsp. *michiganensis - Cmm*)

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is a subspecies in the *Clavibacter michiganensis* species that comprises five subspecies, all of which are host-specific pathogens that reside within the vascular tissues of plants (Eichenlaub & Gartemann, 2011; Peritore-Galve et al., 2021). It colonizes and multiplies in xylem vessels. *Cmm* is a non-motile, seed-borne, phytopathogenic, Gram-positive actinomycete bacterium (Eichenlaub & Gartemann, 2011). Some members of the Solanaceae family serve as its host, with tomatoes being the most important. It appeared in the records in Michigan, United States, in 1909 (Gartemann et al., 2003). Since its first documentation in the USA, it has spread to almost all tomato-growing countries where it caused outbreaks in many of them. It is a highly important pathogen worldwide and is a quarantine organism in the European Union, Africa, and many other nations.

Disease symptoms can be seen in various parts of the plant including vascular tissues, leaves, and fruits. The severity of the symptoms depends on environmental factors, the stage of infection, and the age of the plant (Sharabani et al., 2013). Early stages of infection are characterized by unilateral wilting, where only the side of the leaflets and leaves wilt while the rest appear healthy (Rivera-Sosa et al., 2021). Cutting open the stem of an infected plant may reveal a brown discoloration of the vascular system. Leave symptoms first appear as small, water-soaked lesions. Yellow to dark brown necrotic margins can develop on the edges of the leaves that move inward and merge which is often referred to as “firing” (Nandi et al., 2018a). Leaves may also curl towards the later stages of the infection (Sharabani et al., 2013).

Canker symptoms which primarily affect the stems are the reason this pathogen got its name. Canker symptoms initially appear as small, water-soaked lesions on the stem. Over time these symptoms enlarge and darken (Peritore-Galve et al., 2021). Eventually, the infected areas become sunken. The cankers can spread along the stem, sometimes encircling it. When a canker girdles the stem, it disrupts the flow of water and nutrients, leading to wilting and potentially killing the plant above the infection site (Peritore-Galve et al., 2021). In more severe infections the stems split open. In response to the infection, the plant may produce a sticky, amber-colored gum that exudes from the cankers. The gum is most found oozing from the edges of the cankered areas or from cracks in the stem where the cankers have caused splitting. The fruit symptom is the presence of small, white lesions that resemble a bird's eyes and have a brown core.

Plants that are infected usually exhibit reduced growth, and overall reduced fitness. Disruption of nutrition intake and water transportation by the pathogen is the cause of this stunting. As the infection progresses, the plant may turn entirely yellow and lose its leaves prematurely. Fruit production usually drops along with it. 46 to 93% of plants may die from an epidemic of *Cmm* and yield losses reaching up to 80% (Peritore-Galve et al., 2021). During widespread epidemics, fruit weight can decrease by more than 50% compared to the average (Peritore-Galve et al., 2021).

Cmm can be transmitted through various mechanisms, contaminated seeds being the main means of transmission (Frenkel et al., 2015; Nandi et al., 2018b). The bacteria can be found on the surface of seeds or within, making seed-borne transmission a serious risk. Even just a small number of contaminated seeds can spread disease over a field or greenhouse. When infected seeds germinate, the bacteria can infect the emerging seedlings (Frenkel et al., 2015). Infected seedlings often serve as an initial source of inoculum, leading to further spread within a crop.

Cmm can be transferred from plant to plant via mechanical means. Common operations like pruning, trellising, and harvesting can leave small wounds on plants, allowing bacteria to enter and infect healthy plants. Tools, hands, and equipment that come into contact with infected plants can easily transmit bacteria to uninfected plants. *Cmm* can be spread by water, particularly during rainy weather or overhead irrigation. Water splashing from infected plants can carry the bacteria to nearby healthy plants. This form of transmission is especially problematic in densely planted fields or greenhouses. Although *Cmm* is not a soil-borne pathogen, it can survive in the soil for short periods, particularly in plant debris (Peritore-Galve et al., 2021). The bacteria can be spread through contaminated soil, especially when planting in the same location year after year without proper crop rotation.

Moving infected transplants from one location to another can introduce the bacteria into previously uninfected areas. Even asymptomatic plants can carry the bacteria, making it difficult to identify and remove infected transplants before they spread the disease. Residual plant material left in the field or greenhouse after harvest can harbor *Cmm*. This material can serve as a source of inoculum for the next crop, particularly if proper sanitation practices are not followed.

Cmm chromosome comprises 3.298 Mb and exhibits a high guanine + cytosine content of 72.6% (Gartemann et al., 2008). The pathogen harbors two native plasmids, pCM1 (24.7 kbp) and pCM2 (70 kbp), that encode pathogenicity factors (Eichenlaub & Gartemann, 2011; Gartemann et al., 2008; Meletzus et al., 1993; Metzler et al., 2006). Studies have shown that in the absence of the two native plasmids, the pathogen fails to induce disease symptoms, albeit successful endophytic colonization of the host (Nandi et al., 2018b). The absence of either of the plasmids has been reported to cause reduced virulence and delayed onset of the disease symptoms. In addition to the two plasmids, *Cmm* has a 129 kb chromosomal pathogenicity island that plays a role in its pathogenicity (Nandi et al., 2018b).

The Pat-1 protein is thought to play a role in the degradation of host plant tissues. It contains a conserved GDSGG motif which is likely part of the enzyme's catalytic triad, essential for its proteolytic activity (Burger et al., 2005). Studies suggest that Pat-1 and related proteases not only contribute to the pathogenicity of *Cmm* but also potentially trigger immune responses in host plants, functioning as both virulence factors and immunity elicitors (Hwang et al., 2022).

Interestingly, not all strains carrying the *pat-1* gene display the same pathogenic behavior, highlighting the complexity of interactions between the pathogen and its host (Nandi et al., 2018b). The variability in plasmid content among different strains further contributes to differences in virulence levels.

Proteases are thought to contribute to the *Cmm* pathogenicity by promoting the destruction of plant tissues, which allows the pathogen to penetrate and spread within the host. *tomA*, a gene within the PAI, generates a protein that detoxifies α-tomatine, a poisonous chemical produced by tomatoes as a defensive strategy (Gartemann et al., 2008).

1.2 Overview of Bacterial Speck of Tomato (*Pseudomonas syringae* pv. *tomato* - *Pst*)

Pseudomonas syringae pv. *tomato* (*Pst*) is a motile, aerobic, rod-shaped, Gram-negative phytopathogen, and the causal agent of the bacterial speck of tomato. It is a member of the phytopathogenic species complex *Pseudomonas syringae*. This pathogen can live epiphytically on leaves. Outbreaks caused by this pathogen can occur in the presence of favorable conditions. It can also be seed- or soil-borne (Gullino et al., 2009). *Pst* is found in all tomato-grown areas. Apart from its importance as a pathogen, it is also scientifically important and used as a model organism to study and understand phytopathogens (Xin & He, 2013).

Symptoms of the pathogen can be seen on the leaves, fruits, stems, and flowers. The pathogen is favored by cool, moist conditions in which the symptoms become more recognizable (Preston, 2000). Early seedling infection can cause stunted growth, including smaller, deformed leaves. Small, dark brown to black spots appear on the leaves, surrounded by a yellow halo (Preston, 2000). These lesions are the most characteristic symptom of bacterial speck and can lead to leaf curling or deformation. In severe infections, large areas of the leaf tissue can become necrotic, resulting in leaf death and defoliation (Preston, 2000).

Fruit symptoms, on the other hand, are small, superficial black spots that can become sunken as the fruit matures, and form on the fruit irrespective of the maturity stage (Preston, 2000). These specks can lead to a significant economic loss of the fruit.

Symptoms are not typically observed on the stems but in severe cases may develop elongated, dark streaks. Overall, *Pst* significantly impacts tomato yield and quality, particularly in cool, wet climates.

Pst spreads through several mechanisms that favor its transmission across tomato plants. Similar to *Cmm*, the pathogen can survive on or within tomato seeds, making seed transmission a significant route for the disease (Dutta et al., 2014). Infected seeds can carry the pathogen, even if they do not show visible signs of contamination. Once the seeds are planted, the pathogen infects the seedlings, leading to early symptoms that can spread to the entire crop.

This pathogen uses a range of sophisticated strategies to establish infection and inhibit the immune response of the host. One of its most important components is the Type III secretion system, which delivers effector proteins directly to plant cells (O'Malley & Anderson, 2021; Preston, 2000). These effectors affect plant cellular processes, weaken host immune responses, and stimulate bacterial development, ultimately causing illness (O'Malley & Anderson, 2021).

AvrPtoB and Hop proteins are critical effectors that disrupt the plant immune system. AvrPtoB lowers plant defenses while stimulating immune responses in specific conditions (Wang et al., 2019). These effectors can disrupt hormone signaling pathways, such as salicylic acid and jasmonic acid, which are critical for plant defense (Chen et al., 2017). Furthermore, *Pst* uses coronatine, a phytotoxin that mimics plant hormones, specifically jasmonic acid, to further depress the plant immune system (Worley et al., 2013). Coronatine stimulates stomatal reopening after the plant attempts to close them to resist pathogen entry, which facilitates bacterial invasion (Toum et al., 2016; Worley et al., 2013).

Pst uses these strategies to alter host processes and overcome plant resistance, resulting in the formation of specks on leaves and a reduction in fruit quality and yield.

1.3 Overview of *Pseudomonas fluorescens*

Pseudomonas fluorescens is a Gram-negative, rod-shaped bacterium, extensively studied for its beneficial traits in various fields, including agriculture, biotechnology, and ecology (Muriel Rhodes, 1959). It belongs to the Pseudomonadaceae family and is commonly found in soil, water, and plant surfaces, where it plays a critical role in promoting plant health and controlling plant diseases (Novik et al., 2015). *Pf* is significant for its potential to produce secondary metabolites, such as antibiotics and siderophores, which can hinder the growth of pathogenic microbes (Novik et al., 2015; Wang et al., 2024). Studies have shown that *Pf* produces pyoverdine, a fluorescent siderophore that binds iron with high affinity, thereby depriving competing organisms of this essential nutrient (Trapet et al., 2016). This characteristic makes *Pf* an effective agent in suppressing the growth of various soil-borne pathogens (Novik et al., 2015). Furthermore, its genome has been extensively studied to understand the genetic basis of its interaction with plants and pathogens.

Additionally, *Pf* is a model organism in studying biofilm formation and bacterial motility (Baum et al., 2009). It is also important in bioremediation research due to its ability to degrade pollutants, such as hydrocarbons and pesticides, in contaminated environments (Gutiérrez et al., 2020). In agriculture, *Pf* is recognized as a PGPR, meaning it enhances plant growth either directly by facilitating nutrient uptake or indirectly by suppressing plant diseases. *Pf* has been shown to produce various growth-promoting substances, including IAA, which stimulates root elongation, and ACC deaminase, which helps plants cope with stress by lowering ethylene levels (Chen et al., 2017; Kochar et al., 2011; Nascimento et al., 2019).

Pf is used as a BCA against a variety of plant pathogens, both bacterial and fungal (Couillerot et al., 2009; Farahat et al., 2016). *Pf* suppresses these pathogens through multiple mechanisms, including competition for nutrients and space, production of antifungal, antibacterial compounds such as phenazine, pyrrolnitrin, pyoluteorin, and 2,4-diacetylphloroglucinol, and induction of systemic resistance in plants (Hu et al., 2005; Shanmugaiah et al., 2010; Souza & Raaijmakers, 2006; Velusamy et al., 2006).

Ecologically, *Pf* plays a critical role in nutrient cycling and maintaining soil health. Its ability to degrade organic matter contributes to the mineralization of nutrients, which are then available for plant uptake (Novik et al., 2015). *Pf* also participates in the nitrogen cycle by promoting nitrogen fixation in plants through its association with legume roots (Wu et al., 2024).

Pf also contributes to the remediation of polluted environments by degrading toxic compounds such as polycyclic aromatic hydrocarbons, chlorinated solvents, and heavy metals (Alfarras et al., 2022; Bugg et al., 2000; Vandenbergh & Kunka, 1988). Its versatile metabolism allows it to adapt to various ecological niches, making it a key player in maintaining environmental homeostasis and biodiversity.

1.4 Management of Bacterial Phytopathogens with *Pf* strains

Phytopathogens can severely damage crops, leading to massive yield losses if left untreated. Traditionally, chemical pesticides and synthetic treatments have been used to control these pathogens. However, their overuse has raised environmental concerns, including soil and water contamination, and the development of resistant pathogen strains. In recent years, BCAs have emerged as a promising alternative to chemical pesticides. *Pf* strains have attracted a lot of attention among these BCAs because of their capacity to inhibit a variety of bacterial and fungal phytopathogens.

BCAs refer to natural organisms that are used to suppress pest populations or diseases. In the context of phytopathology, beneficial microbes, including bacteria and fungi, are used to control plant pathogens. Its primary mode of action includes competition for space and nutrients, production of antimicrobial compounds, and induction of systemic resistance in plants. Many *Pf* strains produce antibiotics such as phenazine, pyrrolnitrin, and 2,4-Diacetylphloroglucinol (DAPG), which directly inhibit the growth of bacterial and fungal pathogens. *Pf* strains also secrete siderophores, iron-chelating compounds that sequester iron from the environment, making it less available to pathogens. This can effectively starve the pathogen of a critical nutrient. By colonizing the rhizosphere aggressively, *Pf* strains outcompete pathogens for essential resources. This competitive exclusion is particularly effective in preventing pathogen establishment and proliferation. By stimulating the plant's immune system, Pf strains can also increase plants' resistance to various infections.

Compared to chemical treatments, biological control offers a more sustainable approach, as it mitigates environmental risks and does not contribute to the growing problem of chemical resistance in pathogens. *Pf* strains have shown promising results in controlling bacterial, fungal, and even nematode phytopathogens (Dubey & Vishwavidyalaya, 2014; Siddiqui et al., 2006; Suresh et al., 2022; Vanitha et al., 2009).

Pf A506 strain is an effective BCA in managing bacterial fruit blotch in watermelon. This disease, caused by *Acidovorax avenae* subsp. *citrulli* is a significant threat to watermelon crops (Fessehaie & Walcott, 2005). A study evaluating the efficacy of *Pf* A506 found that it significantly reduced the growth of the pathogen and decreased seed infestation, which is a critical factor in the spread of bacterial fruit blotch (Fessehaie & Walcott, 2005). The success of *Pf* A506 demonstrates the potential of specific *Pf* strains to suppress bacterial phytopathogens by competing for resources and producing metabolites that inhibit pathogen growth. Another example of the biocontrol potential of *Pf* is seen in the management of halo blight, a bacterial disease caused by *Pseudomonas syringae* pv. *phaseolicola* (Alström, 1991). This disease can cause severe lesions on common bean plants, reducing yield and quality. *Pf* strain S 97 was reported to significantly reduce the incidence of halo blight lesions by inducing systemic resistance in the bean plants (Alström, 1991).

Pf strains can influence the microbial composition of the soil, promoting the growth of beneficial microbes and creating a suppressive soil environment that inhibits pathogen activity. In a study involving *Pf* strain P142 and *Bacillus velezensis* (*B. velezensis*) B63, both strains significantly reduced wilt symptoms in greenhouse-grown tomatoes caused by bacterial pathogens (Elsayed et al., 2020). The study also observed notable shifts in the composition of prokaryotic communities within the rhizosphere, suggesting that inoculation with *Pf* strains not only suppresses pathogens but also promotes a healthier microbial balance in the soil (Elsayed et al., 2020). This microbial shift can further enhance plant growth and resistance to diseases. As soil health is a critical component of sustainable agriculture, the ability of *Pf* strains to contribute to suppressive soils makes them an ideal tool in integrated pest management strategies.

Rice is the primary food for more than half of the world's population, and diseases like bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae*, pose a serious threat to global rice production (Umesha, 2014). In a study involving 10 *Pf* strains isolated from the rice rhizosphere, seed treatments with these isolates resulted in improved seedling germination and a significant reduction in bacterial leaf blight symptoms (Umesha, 2014). This is a critical finding, as it shows that *Pf* strains can not only protect rice plants from devastating bacterial diseases but also promote healthier, more vigorous plant growth from the seedling stage.

Moving forward, more research is needed to explore the full potential of *Pf* strains in field conditions, optimize their application methods, and understand their interactions with other beneficial microbes. Additionally, integrating *Pf* into broader integrated pest management programs will be crucial for maximizing their benefits and minimizing the reliance on harmful chemical treatments.

2. SUMMARY of the LITERATURE

2.1 Studies on the Promotion of Plant Growth by Endophytes

Hall et al. (1996) studied PGPR *Pseudomonas putida* GR12-2 and found that it promotes root elongation in canola, lettuce, and tomato.

Ait Barka et al. (2006) studied *Burkholderia phytofirmans* PsJN and investigated their ability to enhance the chilling tolerance of grapevine plantlets. Grapevine plantlets inoculated with PsJN showed improved growth both at cold and normal temperatures. PsJN-inoculated plantlets have also shown increased photosynthetic activity and starch accumulation.

Gravel et al. (2007) inoculated greenhouse-grown tomato plants with with *P. putida* and *Trichoderma atroviride* and investigated their impact on plant growth and fruit yield. The researchers have reported that both microorganisms significantly improved overall plant health and marketable fruit yield under hydroponic conditions.

Figueiredo et al. (2008) studied the effects of co-inoculating *Paenibacillus polymyxa* and *Rhizobium tropici* on drought stress reduction in common bean plants. The findings showed that co-inoculation with both bacterial strains increased plant growth, nitrogen content, and nodulation, especially under severe drought circumstances.

Kıdoğlu et al (2009) studied the effects of plant growth-promoting rhizobacteria on tomato yield in a soilless perlite system. Among native bacterial strains tested, *Bacillus* spp. strain 66/3 increased marketable yield by 37% in fall and 18% in spring compared to controls, outperforming the commercial strain *B. amyloliquefaciens* FZB24. Other strains, *Pseudomonas putida* 18/1K and *Pseudomonas fluorescens* 70, also showed promising results.

Shi et al. (2009) studied three endophytic bacteria from sugar beet root and investigated their phytohormone IAA, Absic acid, Giberellic acid, and Zeatin riboside producing ability. The three bacterial isolates, *Bacillus pumilus*, *Chryseobacterium indologenes*, and *Acinetobacter johnsonii*, produced considerable amounts of IAA and enhanced root and shoot growth in beet seedlings under both sterile and glasshouse conditions. Inoculation with these bacteria resulted in enhanced plant height, fresh and dry weight, and leaf count.

Karthikeyan et al. (2012) studied salt-tolerant, ACC deaminase-encoding, diazotrophic endophytic bacteria isolated from *Catharanthus roseus* and their role in salt stress reduction. *Achromobacter xylosoxidans* AUM54 was selected among 27 isolates due to its superior nitrogen fixation, IAA generation, and phosphate solubilization. Inoculation with AUM54 reduced ethylene levels by 11-23%, increased antioxidative enzyme activity, and dramatically improved plant growth metrics under salt stress, including germination rate, root dry weight, and alkaloid content.

Montañez et al. (2012) identified cultivable endophytic bacteria from maize cultivars that promote plant growth by nitrogen fixation, IAA generation, phosphate solubilization, and siderophore synthesis. The bacteria belonged to the genera Rhanella, Pantoea, Rhizobium, Pseudomonas, and Burkholderia. Inoculation tests revealed that several strains drastically increased maize shoot biomass, with *Herbaspirillum frisingense* performing particularly well across multiple cultivars.

Faria et al. (2013) studied twelve endophytic bacteria from orchid meristems, identifying eight strains as *Paenibacillus* spp. These strains were evaluated for IAA production and plant growth stimulation. While none solubilized phosphate, all produced measurable IAA levels, and the *Paenibacillus lentimorbus* and *P. macerans* strains significantly increased the root biomass and survival rates of *Cattleya loddigesii* seedlings during acclimatization.

Abd El-Daim et al. (2014) studied the effects of bacterial seed treatments on heat stress tolerance in wheat seedlings. Wheat seedlings pretreated with *B. amyloliquefaciens* (*B. amyloliquefaciens*) UCMB5113 and *Azospirillum brasilense* NO40 saw increased heat tolerance, improved growth and survival under heat stress. NO40 and UCMB5113 inoculated seedlings were reported to have maintained better water content, fresh weight, and dry weight compared to the untreated controls.

Szilagyi-Zecchin et al. (2014) studied and identified six endophytic bacterial strains of *Bacillus* sp. and as *Enterobacter* sp. isolated from corn roots in which four of them have shown nitrogen fixation ability, and production of high levels of IAA, siderophores, and lytic enzymes.

Güneş et al (2015) studied the biochemical mechanisms of ten plant growth-promoting rhizobacteria (PGPR) strains. *Bacillus megaterium* M3 showed the highest production of amino acids, organic acids (malonic, oxalic), and plant hormones (IAA, GA, SA) while enhancing enzyme activities. *B. megaterium* Tv-17C was notable for cysteine and valine production, while *Pantoea agglomerans* RK-92 excelled in urease and dehydrogenase activities.

Soares et al. (2016) studied the endophytic bacterium *B. amyloliquefaciens* C6c, isolated from the invasive plant English ivy. The bacterium produced IAA, solubilized phosphate, and secreted hydrolytic enzymes, all of which promoted plant development. It also generated antifungal lipopeptides, which suppressed fungal infections. Inoculation with C6c drastically increased English ivy growth, improved nitrogen uptake, and protected against fungal infections like *Alternaria tenuissima*.

Sarkar et al. (2018) studied ACC deaminasemel-producing *Burkholderia* sp. strain P50 isolated from rice fields and found that it significantly increases rice seedling growth and salt tolerance. They also found that the P50 improves antioxidant enzyme activity, increases proline synthesis, and improves the general health of rice seedlings by enhancing nutrient uptake and lowering the negative effects of ROS produced by salt stress.

Abdelshafy Mohamad et al. (2020) studied primarily Bacillus genus belonging to beneficial endophytic bacterial population isolated from *Thymus vulgaris*. They exhibited several plant growth-promoting traits, including nitrogen fixation, siderophore production, phosphate solubilization, auxin synthesis, and secretion of lytic enzymes. Three of the strains (EGY05, EGY21, EGY25) were able to promote tomato growth under salt stress and reduced activity of antioxidant enzymes. They also effectively controlled *Fusarium oxysporum* under salt stress and reduced disease symptoms significantly.

Jhuma et al. (2021) studied and isolated salt-tolerant, plant growth-promoting rhizobacteria from *Oryza sativa* roots in saline conditions. Four bacterial groups were identified from 75 isolates: *Enterobacter*, *Achromobacter*, *Bacillus*, and *Stenotrophomonas*. These bacteria showed remarkable plant growth-promoting characteristics, such as IAA production, phosphate solubilization, nitrogen fixation, and EPS production, particularly in high salt circumstances. *Stenotrophomonas pavanii* and *B. aryabhattai* were very successful in increasing biofilm development, which improved plant tolerance to salinity stress.

Mei et al. (2021) studied phosphate-solubilizing bacterial endophytes isolated from plants, specifically their potential to enhance plant development *in vitro* and greenhouse conditions. Five bacterial strains, *Pantoea vagans*, *Pseudomonas psychrotolerans*, *B. subtilis*, *B. safensis,* and *Pantoea agglomerans*, showed substantial phosphate solubilization. These bacteria also produced auxin, ACC deaminase activity, nitrogen fixation, and siderophores, all of which promote plant growth. *P. agglomerans* IALR1325 demonstrated the greatest plant growth promotion in both tall fescue and greenhouse trials with tomatoes and peppers.

Singh et al. (2021) studied *Pseudomonas aeruginosa* B18 strain isolated from sugarcane roots. The study found that this strain promoted plant growth and bicontrol activity against the sugarcane smut pathogen *Sporiosorium scitamineum*. Whole-genome sequencing of strain B18 showed genes involved in nitrogen fixation, phosphate solubilization, ACC deaminase activity, siderophore formation, and plant hormone synthesis, including IAA.

Qin et al. (2022) studied the effects of inoculating sugarcane with the nitrogen-fixing endophytic bacteria *Klebsiella variicola* DX120E. The findings revealed considerable improvements in sugarcane growth, including higher plant height, stem diameter, biomass, and Brix levels. DX120E increased critical enzymes associated with nitrogen and carbon metabolism, such as nitrate reductase and sucrose phosphate synthase, whereas a metabolomic study revealed altered carbohydrate and amino acid metabolism in both leaves and stems.

Zahra et al. (2023) studied the ability of salinity-tolerant endophytic bacteria to enhance mungbean development under salt stress. Six bacterial strains, including *Pseudomonas extremorientalis, Bradyrhizobium japonicum*, and *Serratia quinivorans,* showed great salt tolerance and plant growth-promoting characteristics such as phosphate solubilization, IAA synthesis, nitrogen fixation, and zinc solubilization. Innoculation with these strains considerably increased mungbean development, boosting root and shoot length, fresh and dry weight, proline, glycine betaine, and total soluble protein levels, demonstrating their potential as biofertilizers for salinity-affected soils.

2.2 Studies on Biological Control of Endophytes

Özaktan et al (1999) studied *Erwinia herbicola* strains for biocontrol of fire blight in pears. Among 167 epiphytic bacterial isolates, 70 showed antagonistic activity against *Erwinia amylovora*. Strains 1B and 24 demonstrated high efficacy, reducing blossom blight by up to 98% and completely suppressing fruit infections in talc-based formulations. These formulations extended bacterial viability up to 180 days at 10°C and showed better efficacy than lyophilized or whey-based options.

Abo-Elyousr and El-Hendawy (2008) studied the use of *P. fluorescens* and acibenzolar-S-methyl (BTH) to treat bacterial spot disease in tomatoes caused by *Xanthomonas axonopodis* pv. *vesicatoria*. Both treatments improved seed germination, reduced disease severity, and raised tomato yield in greenhouse and outdoor settings. The most effective treatment was foliar application of *P. fluorescens*, which reduced disease while increasing seedling strength and biomass. The combined treatment of *P. fluorescens* with BTH afforded better control.

Karagöz & Kotan (2010) studied 40 plant growth-promoting rhizobacteria and endophytic bacteria isolates for managing bacterial leaf spot in lettuce caused by *Xanthomonas axonopodis pv. vitians*. Identified isolates included *Pantoea agglomerans* (33), *Bacillus* spp. (6), and *Paenibacillus polymyxa* (1). All isolates demonstrated nitrogen fixation and phosphate solubilization. In greenhouse tests, *Bacillus megaterium* KBA-10, *P. agglomerans* KBA-8, and *P. agglomerans* RK-198 reduced disease severity by 77.7%, 89%, and 100%, respectively, while also promoting lettuce growth. These results highlight promising biocontrol potential and growth enhancement properties.

Lanna Filho et al. (2013) studied the biocontrol ability of *B. pumilus* and *B. amyloliquefaciens* against *Pst*. Using a GFP-tagged strain of the pathogen, the study found that both *Bacillus* species greatly reduced the pathogen population on the tomato phylloplane, reducing disease severity by up to 97%.

Hong et al. (2015) studied four novel leaf-inhabiting endophytic bacteria from *Arabidopsis thaliana* (*A. thaliana*)and assessed their antipathogen activity. Two *Rhodococcus* isolates were discovered as the genus' first known leaf-inhabiting endophytes. The bacteria had various degrees of antagonism against *F. oxysporum* and *P. syringae*. Notably, *B. cereus* KB1 substantially improved disease resistance in tomato plants against *B. cinerea* and *P. syringae*.

Hong et al. (2016) studied the biocontrol potential of *Paenibacillus polymyxa* AC-1 against *Pst*, the bacterial pathogen that causes bacterial speck disease in *A. thaliana* and tomato. AC-1 reduced the growth of *Pst in vitro* and showed high antagonistic activity. Furthermore, AC-1 was able to colonize *Arabidopsis* roots and leaves, spreading systemically to aerial tissues. The study also emphasized the significance of ABA and jasmonic acid signaling pathways in AC-1 colonization, indicating its potential as a BCA in agriculture.

Akköprü & Özaktan (2018) studied the biocontrol and growth-promoting effects of *Pseudomonas putida* AA11/1 and acibenzolar-S-methyl (ASM) on cucumber cultivars susceptible and tolerant to angular leaf spot (ALS) disease caused by *Pseudomonas syringae* pv. *lachrymans*. ASM reduced disease severity by up to 92% but caused phytotoxicity, while AA11/1 reduced severity by 34% in the susceptible cultivar and improved yields in both cultivars (68% in tolerant and 33% in susceptible) under disease-free conditions. AA11/1 enhances plant tolerance and yield without reducing pathogen populations, supporting its use in integrated management strategies.

Abo-Elyousr et al. (2019) studied the biological management of *Cmm* with formulated plant growth-promoting bacteria. Four bacterial species were tested: *B. subtilis*, *B. amyloliquefaciens*, *Pf*, and *P. aeruginosa*. Under greenhouse conditions, formulated *B. amyloliquefaciens* (74.4%) and *P. aeruginosa* (66.7%) demonstrated the greatest reduction in disease severity. Bacterial treatments also increased seed germination, seedling vigor, and plant growth.

Canik Orel (2020) stuied the preventive effects of commercial *Bacillus subtilis* QST 713 and endophytic bacteria (*Pseudomonas gessardii* Lt13 and *Bacillus mojavensis* Lt24) against *Pseudomonas cichorii* (*Pc*) and *Pseudomonas viridiflava* (*Pv*) infections in lettuce. QST 713 was effective against both pathogens at all application times. Lt13 was most effective against Pc at 24 hours prior application, while Lt24 showed significant prevention of *Pv* and *Pc* infections at 24- and 48-hours prior applications. Early application (0–24h) provided the highest efficacy, highlighting the timing's critical role in disease management.

Akköprü et al (2021) studied the effects of endophytic bacteria *Ochrobactrum* spp. strain CB36/1 and *Pantoea agglomerans* strain CC37/2 on cucumber growth, fruit quality, yield, and resistance to ALS disease in a soilless system. Both strains persisted in plant tissues, increasing total yield by 22% and 21%, respectively. CC37/2 significantly reduced ALS severity by 41% and improved yield under disease stress. CB36/1 improved fruit fresh weight and yield under disease conditions. The findings highlight the potential of these endophytes in reducing chemical inputs and enhancing sustainable agriculture.

Yildirim et al. (2021) studied the effects of seed-coating *Capsicum annuum* seeds with endophytic *Pseudomonas* and *Bacillus* strains on seed quality, specifically germination and growth of seedlings in immature and mature seeds. Seed coating, particularly with *Bacillus subtilis* Bs1 and *Pseudomonas gessardii* L13, resulted in significantly higher germination rates, seedling emergence, and shorter germination times than control seeds.

Başaran (2023) investigated the potential of *Pseudomonas* spp. and *Bacillus* spp. isolates to control *Erwinia persicina,* which causes soft rot in lettuce. Among the tested strains, *Bacillus mojavensis* Bmj1, *Pseudomonas putida* L2B, and *Bacillus* strain Ba7 significantly reduced symptoms when applied to lettuce leaves 24–72 hours before pathogen inoculation. The 24-hour application of Bmj1 and L2B was particularly effective. In contrast, root applications did not yield significant disease suppression.

3. MATERIALS and METHODS

The endophytic bacteria used in this study were obtained from healthy tomato seedlings variety “Redguard” which were obtained from Kaş/Antalya region. However, *Cmm* and *Pst* were readily available in the bacterial collection of the Bacteriology Laboratory at Ankara University, Department of Plant Protection.

Table 3.1 Taxonomy of the beneficial endophytic and pathogenic strains used in this study

Strain	Taxonomy	Host
27/3	*Pseudmonas fluorescens*	Tomato
M11	*Psedumonas fluorescens*	Tomato
M17	*Psedumonas fluorescens*	Tomato
46/a1	*Psedumonas fluorescens*	Tomato
Pst K11	*Pseudomonas syringae* pv. *tomato*	Tomato
Cmm S49	*Clavibacter michiganensis* subsp. *michiganensis*	Tomato

3.1 Isolation of Endophytic Bacteria

3.1.1 Surface sterilizing tomato seedlings

Approximately 8 weeks old, healthy tomato seedlings (variety Redguard) were selected as potential hosts for endophytic fluorescent *Pseudomonas* spp. to ensure that only endophytic bacteria were isolated, a rigorous surface sterilization protocol was followed. Seedlings were removed from the pots and were immersed in 0.5% NaOCl solution for 3 minutes, and then rinsed in sterile distilled water for 3 minutes. This process was repeated three times to effectively eliminate any epiphytic bacteria. Following sterilization, seedlings were dried on sterile blotting papers.

Once dry, the seedlings were sectioned into approximately 1 cm segments from various parts of the plant using a sterile scalpel blade. These segments were transferred to BIOREBA extraction bags containing 0.9% NaCl. The plant tissues were then crushed within the bags to release and extract endophytic bacteria from the internal tissues. Serial dilutions 10^2 to 10^4 were prepared from the resulting suspension.

For isolation, 100 µL from each dilution was spread onto King's B medium plates using a Drigalski spatula. The final rinsing water from the sterilization process was also plated on the King's B medium to verify the effectiveness of surface sterilization and confirm the endophytic origin of isolated bacteria.

3.1.2 Determination of fluorescent Pseudomonads

Comprehensive biochemical analyses were performed to identify *Pseudomonas fluorescens* colonies. Colonies exhibiting diffusible pigmentation on King's B medium plates were initially screened, as fluorescent *Pseudomonas* spp. were the target. These colonies were then purified through single-colony isolation. Additionally, colonies were examined under UV light at 360 nm (Figure 3.1). Colonies displaying a bluish fluorescence under UV illumination were selected as candidates and further purified by single-colony isolation. To assess their pathogenic potential, colonies were incubated in Nutrient Broth Yeast Extract Agar (NBYA) at 37 °C. The absence of growth at this temperature indicated that the isolates were unlikely to be human pathogens.

LOPAT tests were conducted to confirm the species identity of the isolates as *Pseudomonas fluorescens* as given by Braun-Kiewnick and Sands (2000). These tests were:

Levan production: Isolates were cultured on sucrose-rich media to detect the synthesis of levan, a fructan polysaccharide. The absence of mucoid colonies indicated a negative result.

Oxidase activity: The presence of cytochrome c oxidase was evaluated using an oxidase reagent. A color change to dark purple or blue signified a positive reaction.

Pectolytic activity: Isolates were inoculated onto pectin-containing agar to assess their ability to degrade pectin. The lack of clear zones around bacterial growth denoted a negative result.

Arginine dihydrolase activity: The enzymatic hydrolysis of arginine to ornithine was tested using Thornley’s medium (1960) given in **Hata! Başvuru kaynağı bulunamadı.**, with an alkaline pH shift indicating a positive outcome.

Tobacco hypersensitivity: The potential to elicit a hypersensitive response in tobacco plants was examined by inoculating tobacco leaves and observing for tissue necrosis at the inoculation site within 24–48 hours. The absence of necrosis indicated a negative result.

Table 3.2 Composition of Thornley's medium for detecting arginine dihydrolase activity in Pseudomonas species

Component	Per L
Peptone	1.0 g
NaCl	5.0 g
Dipotassium Hydrogen Phosphate (K_2HPO_4)	0.3 g
Agar	3.0
Phenol red	1.0 mg
Arginine HCl	10.0 g

Isolates exhibiting the characteristic LOPAT profile for *Pseudomonas fluorescens* were confirmed as *Pseudomonas fluorescens*.

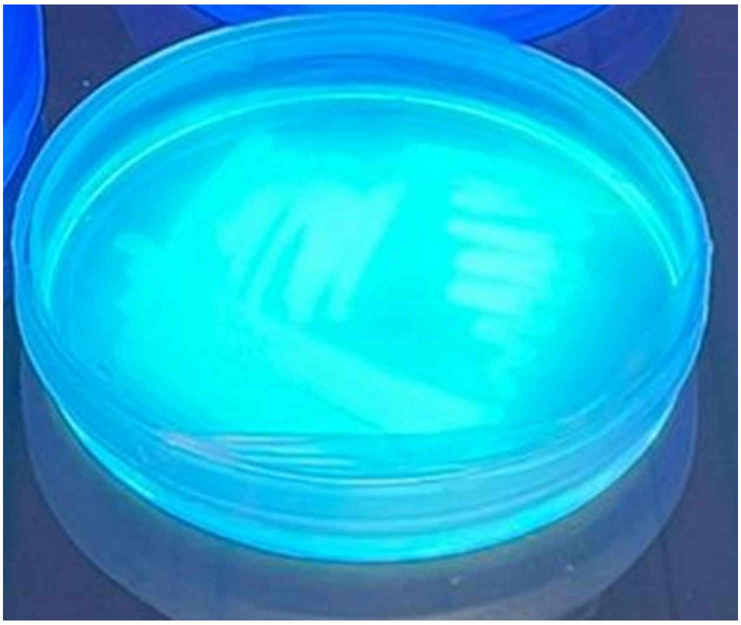

Figure 3.1 The fluorescent image of the endophyte strains under U.V. light at 360nm

3.2 *In vitro* Experiments

The Kirby-Bauer (1966) disc diffusion method was used for the *in vitro* experiments. The fluorescent strains, whose antagonistic effect would be tested, were grown in NBYA medium. Colonies were taken with a sterilized loop and were suspended in sterilized 0.9% NaCl. The concentration of each strain was then adjusted with a spectrophotometer at OD 600= 1×10^8 CFU/mL. The pathogenic *Cmm* strain S49 and *Pst* strain K11 were grown in Nutrient Agar (NA) medium at 28 ºC and 24h culture of each pathogens were used for both *in vitro* and *in vivo* experiments. The concentration of the pathogenic strains was adjusted to 1×10^4 CFU/mL with a spectrophotometer at OD 600.

First, the pathogenic bacterium was streaked onto Muller-Hinton included agar plate as 100 µL by a Drigalski spatula to cover the whole agar surface. Then the petri dish was incubated for 30 min at room temperature to let the pathogenic bacteria dry on the surface. Then, 20 µL suspension of each endophytic *Pseudomonas* strain was infiltrated into a sterile empty cellulose disc (Oxoid, USA) individually for disc diffusion test (Figure 3.2). Infiltrated discs were put onto agar surfaces equal distances from each other, which were already spread with 100 µL of pathogenic bacterium. the same procedure was done with both pathogenic *Cmm* and *Pst* and all the candidate *Pseudomonas* strains were examined 3 as 3 replicates. Then the petri dishes were incubated for 48 hours at 28 °C. The inhibition zones formed around the bacteria-containing disks were measured as mm. The largest zone-making strains were selected as the antagonists and used for root coating.

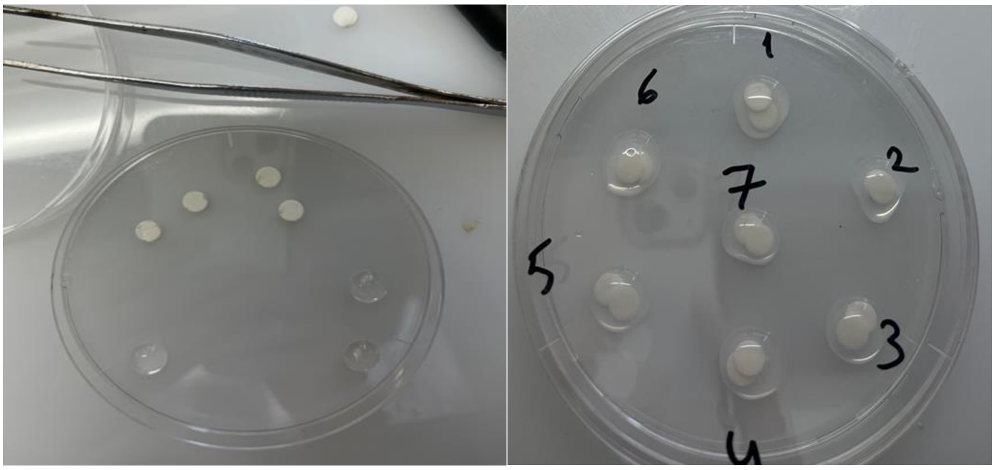

Figure 3.2 Application of the Kirby-Bauer disc diffusion method by using one of the *Pf* strains on *Cmm*

3.3 *In vivo* Experiments

Endophyte strains that formed inhibition zones were selected for the *in vivo* experiment. 8 weeks old, healthy Tomato seedlings were used in the experiment. Seedlings were removed from their pots, and the roots were wounded by shaving with a sterile scalpel blade to let the bacteria into the plants. Wounded seedlings were then later immersed in 1 x 10^8 CFU/mL endophyte-containing suspension. One hour later, each plant was returned to its original pot. Pathogens were introduced into the plants 24 hours later. For *Cmm* inoculation the endophyte-inoculated seedlings were immersed in 10^4 CFU/mL pathogenic *Cmm*-containing suspension. The seedlings were treated with *Cmm* or with dH_2O for 1 h. After inoculation, the seedlings were re-potted and were removed to a controlled climate room at 25 ^{0}C and with 16h light, and 8h dark conditions. For *Pst* inoculation, the bacterial suspension was uniformly sprayed onto the leaf surfaces using a hand spray bottle, ensuring thorough and comprehensive coverage of leaf surfaces.

Special attention was given to the abaxial (underside) surfaces of the leaves, targeting the stomatal openings to facilitate bacterial entry. To minimize excess moisture on the leaf surfaces, watering was withheld immediately prior to the procedure. Each endophytic *Pf* was applied to each pathogenic bacteria as 5 repetitions. The experiment included control groups of *Cmm* only as the positive control for *Cmm*-infected seedlings, *Pst* only as the positive control for *Pst*-infected seedlings, and dH_2O immersed/sprayed seedlings as the negative control. Symptoms were observed in 14 and 28 days.

Figure 3.3 Left: Seedling immersed in a pathogen-containing suspension. Right: Seedlings post-inoculation, housed in the climate-controlled room

3.4 Evaluation of the Disease Severity Index

The assessment of disease symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* was conducted 28 days post-inoculation using a 0-4 scale, as described by Akat and Özaktan (2011), with the following criteria:

0: No disease symptom was observed,

1: 1/4 of the plant from bottom to top is withered or dried,

2: 1/2 of the plant from bottom to top is withered or dried, 3: 3/4 of the plant from bottom to top is withered or dried,

4: The entire plant is withered or dried.

The assessment of disease symptoms caused by *Pseudomonas syringae* pv. *tomato* was conducted 28 days post-inoculation using a 0-5 scale, as described by Akat and Özaktan (2011), with the following criteria:

0: no disease symptom,

1: 1-10% of the leaves infected,

2: 11-25% of the leaves infected,

3: 26-50% of the leaves infected,

4: 51-75% of the leaves infected,

5: 76-100% of the leaves infected.

The Disease Severity Index (DSI) was determined using the following formula: **DSI =** [(∑ number of diseased leaves in each grade × grade) / (total number of leaves investigated × the highest disease index)] × 100.

The results obtained were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test at P<0.05 using the statistical software JMP Pro® 16 (SAS Institute, Cary, NC).

Abbot’s formula was used to understand the percentage of effectiveness of a treatments (Abbot, 1925).

Efficacy (%) = $\frac{X-Y}{X} \times 100$

X = Disease severity in the positive control.
Y = Disease severity in the treated group.

4. RESULTS

During the isolation of the endophytic bacteria, those that produced fluorescent pigment were screened. Four fluorescent colonies were subsequently purified. These colonies morphologically had smooth edges and smooth surface in King's B medium. All strains produced diffuse pigment on King's B medium and were observed as bluish under UV light at 360nm (Figure 4.1). The LOPAT tests results for isolates are give in Table 4.1.

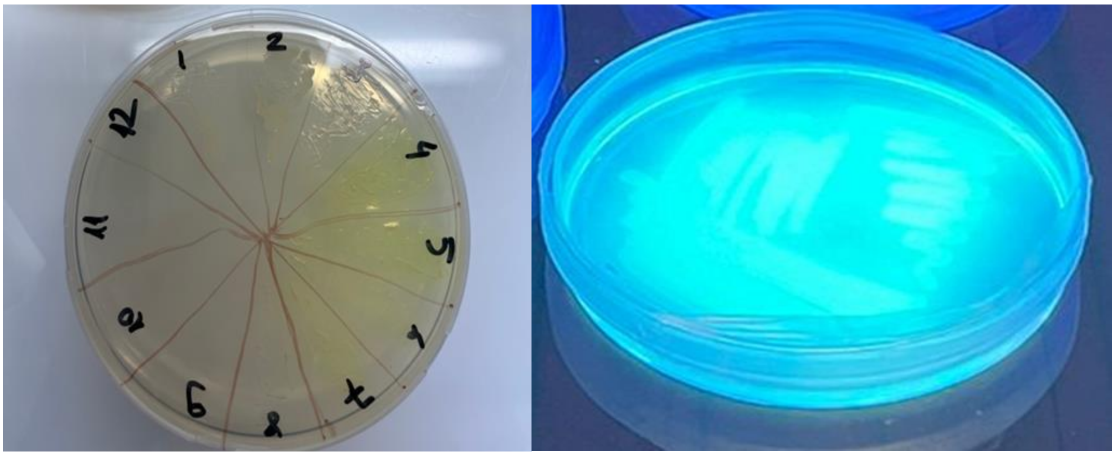

Figure 4.1 The *Pf* colonies which produced diffuse pigment on King's B medium (left) and bluish reflection under UV light at 360nm (right)

4.1 *In vitro* Experiment Results

M11 and M17 both created overlapping inhibition zones in the 10^8 concentrations against *Cmm* and *Pst*. M11 showed the highest antagonistic activity against both pathogens, followed by M17. For *Cmm*, 27/3 demonstrated notable inhibitory activity, while for *Pst*, both 27/3 and 46/a1 displayed similar inhibition zones, indicating comparable efficacy. Detailed inhibition zone measurements for each endophytic bacterial strain are provided in Table 4.2 and Table 4.3.

Table 4.1 LOPAT test results and growth at 37 °C for *Pseudomonas fluorescens*

Strain	**Taxonomy**	**Host**	Growth at 37C	Levan	Oxidase	Pectolytic activity	Arginin hydrolase	HR on Tobacco	Growth at 37 °C
27/3	*Pseudmonas fluorescens*	Tomato	-	+	+	-	+	-	-
M11	*Psedumonas fluorescens*	Tomato	-	+	+	-	+	-	-
M17	*Psedumonas fluorescens*	Tomato	-	+	+	-	+	-	-
46/a1	*Psedumonas fluorescens*	Tomato	-	+	+	-	+	-	-

Table 4.2 Inhibition zones made by endophytic bacteria against *Cmm* in the 10^8 concentration

Cmm EB	Mean (mm)
M11	9
M17	8
27/3	4
46/a1	3

Table 4.3 Inhibition zones made by endophytic bacteria against *Pst* in the 10^8 concentration

Pst EB	Mean (mm)
M11	8
M17	7
27/3	3
46/a1	3

4.2 *In vivo* Experiment Results

Fourteen days post-inoculation, plants were monitored for initial symptoms of *Cmm* and *Pst* infections to assess early disease progression. At this early stage, plants in the *Cmm*$^+$ *and Pst*$^+$ control groups began to exhibit characteristic disease symptoms, including unilateral wilting, chlorosis, and necrotic lesions. In contrast, plants treated with endophytic bacteria showed considerably reduced symptom development, suggesting a protective effect from the endophytic bacterial strains.

Given the preliminary nature of the 14-day observations, it was determined that an additional 14 days would be needed to comprehensively evaluate disease progression and the long-term impact of EB treatments. By the 28-day mark, significant differences in symptom severity were observed across treatments. Plants in the untreated *Cmm*$^+$ and *Pst*$^+$ control groups exhibited full symptomology for their respective pathogens. For *Cmm*-infected plants, symptoms included wilting, chlorosis, and firing of the leaves while *Pst*-infected plants displayed leaf spot development, and chlorosis (Figure 4.1). These observations align with established symptom profiles for *Cmm* and *Pst*, indicating effective pathogen establishment in the control groups.

In the EB-treated groups, symptom severity varied between strains:

Strain M11: Plants inoculated with strain M11 showed minimal symptom development compared to the control groups, with symptoms either absent or present only in a mild form. By the 28-day mark, M11-treated plants maintained significantly better overall health than untreated controls.

Strain M17: Unlike M11, M17-treated plants displayed symptoms similar to those of the control groups, particularly by the 28-day mark. This indicates limited protective efficacy against both *Cmm* and *Pst* under *in vivo* conditions.

Strain 27/3: Plants treated with strain 27/3 showed significant symptom suppression, with fewer and less severe symptoms than the controls, though more than those treated with M11.

Strain 46/a1: Similar to 27/3, strain 46/a1 provided significant symptom reduction. Plants inoculated with 46/a1 showed significantly less severe symptoms compared to the control groups, with symptom levels comparable to those of 27/3-treated plants.

The symptoms were assessed and graded using a modified scale system: a 0–4 scale for *Cmm* and a 0–5 scale for *Pst* (Akat & Özkan, 2011), following the system initially developed by Thirthaallappa Lohithaswa (2000) and with the modification of Canik Orel (2020). This scoring system provided a quantitative measure of symptom severity, enabling a more objective comparison across treatments. Control groups consistently scored at the highest levels of symptom severity, reflecting the full impact of pathogen inoculation without biocontrol intervention.

Figure 4.2 From top left to bottom right, *Cmm*+ control, *Pst*+ control, *Cmm*+ M11, *Pst*+ 27/3

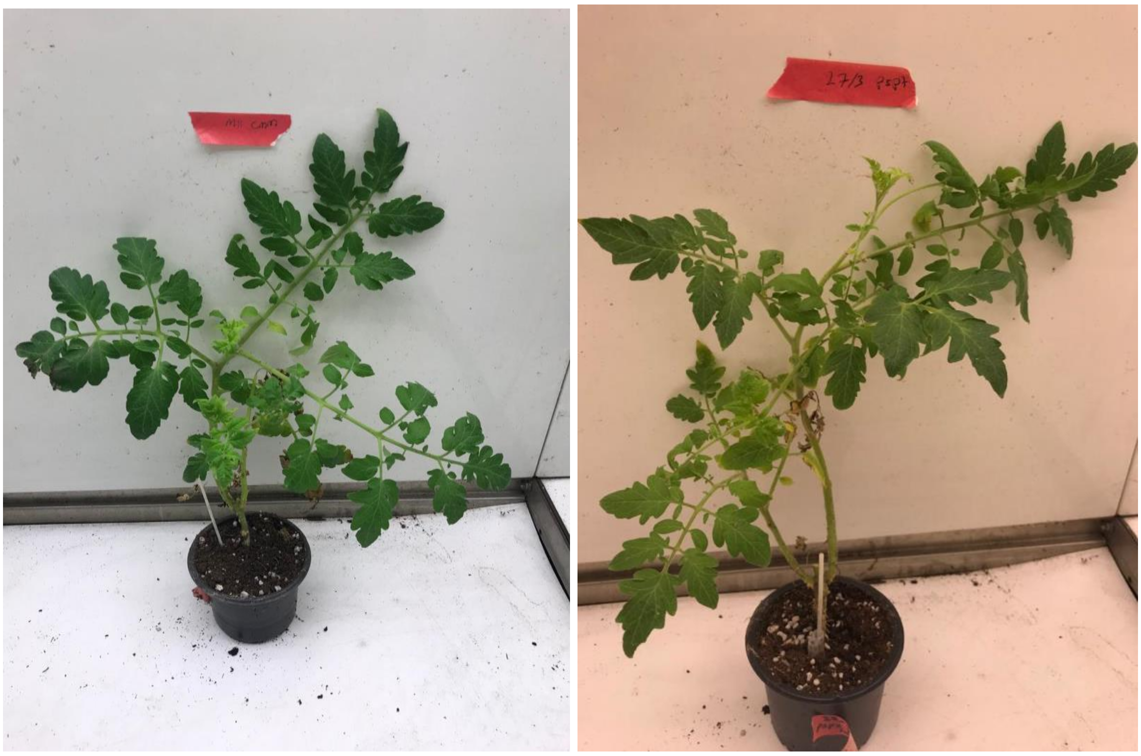

Figure 4.2 From top left to bottom right, *Cmm*+ control, *Pst*+ control, *Cmm*+ M11, *Pst*+ 27/3 (continue)

4.3 Disease Severity Index Statistical Analysis

Table 4.4 presents the statistical analysis results for *Cmm* (left) and *Pst* (right)

Table 4.4 The statistical analysis of the in vivo effects of endophytic bacterial strains on disease severity reduction in *Cmm* (left) and *Pst* (right)

***Cmm* EB**	**Scale Rate**	**DSI (%)**
Cmm	3,8 A	76 A
M17	3,6 A	72 A
27/3	2,2 B	44 B
46/a1	2 B	40 B
M11	1,4 B	28 B
dH2O	0 C	0 C
LSD	1,09	21,78
CV	25,28	25,28
F	18,5802	18,5802

***Pst* EB**	**Scale Rate**	**DSI (%)**
Pst	3,60 A	80 A
M17	3,20 AB	64 AB
27/3	2,40 BC	48 BC
46/a1	2,00 C	40 C
M11	1,60 C	32 C
dH2O	0 D	0 D
LSD	1,08	21,9
CV	25,53	25,03
F	15,5556	17,851

Table 4.5 DSI and treatment efficacy for *Pseudomonas fluorescens* strain treatments against *Cmm*

Treatment	DSI (%)	Efficacy (%)
Cmm	76	0
M17	72	5.2
27/3	44	42.1
46/a1	40	47.3
M11	28	63.1

Table 4.6 DSI and treatment efficacy for *Pseudomonas fluorescens* strain treatments against *Pst*

Treatment	DSI (%)	Efficacy (%)
Pst	80	0
M17	64	20
27/3	48	40
46/a1	40	50
M11	32	60

The results indicate that strain M17 demonstrates a low reduction in disease severity, with efficacy of 20% for *Pst* and 5.26% for *Cmm* ***Hata! Başvuru kaynağı bulunamadı.*** and **Hata! Başvuru kaynağı bulunamadı.**. This reduction is not statistically significant compared to the controls (DSIs of 80% for *Pst* and 76% for *Cmm*), suggesting that M17 does not provide substantial protective effects against either pathogen under *in vivo* conditions.

In contrast, strain 27/3 exhibits a statistically significant reduction in disease severity, achieving disease suppression efficacy of 40% for *Pst* and 42.11% for *Cmm*. These results represent a considerable improvement over the controls, indicating that 27/3 possesses moderate biocontrol efficacy against both pathogens *in vivo*.

Strain 46/a1 also shows a significant decrease in disease severity, with efficacy of 50% for *Pst* and 47.37% for *Cmm*. This level of disease suppression is notably effective compared to the controls, suggesting that 46/a1 has a consistently significant antagonistic effect on both pathogens *in vivo*.

Strain M11 demonstrates the most effective disease suppression, with efficacy of 60% for *Pst* and 63.16% for *Cmm*. These results indicate a statistically significant reduction in disease severity relative to the controls, positioning M11 as the most promising candidate among the tested strains for biocontrol of both pathogens. The performance of M11 highlights its potential as a robust biocontrol agent capable of substantially mitigating disease symptoms in tomato plants.

5. DISCUSSION

The study aimed to evaluate the potential of endophytic fluorescent Pseudomonas strains as biocontrol agents for managing *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato* in tomato seedlings. This approach aims to reduce reliance on traditional chemical controls, which carry environmental and health risks. *In vitro* and *in vivo* experiments assessed the efficacy of four strains (M11, M17, 27/3, and 46/a1) in suppressing pathogen growth. While some strains showed significant promise, their efficacy varied between controlled in vitro assays and complex in vivo conditions.

Control of bacterial pathogens in tomatoes remains a significant agricultural challenge, given the limited effectiveness and environmental concerns associated with traditional chemical treatments (Lalancette & McFarland, 2007; Lamichhane et al., 2018). Chemical treatments, such as copper-based bactericides and antibiotics, are limited by environmental toxicity, pathogen resistance, and potential harm to beneficial microorganisms (Batuman et al., 2024; Rai et al., 2018). Besides, antibiotics are not approved for use as pesticides in many countries. There is currently no effective chemical treatment for *Cmm* globally, making eradication essential upon detection. Alternative treatments have been used to prevent contamination of seeds by the pathogen, such as acid extraction, fermentation of fruit after harvest to eliminate the pathogen from the seeds and heat treatment, but limitations persist. In this context, endophytic bacteria like *Pseudomonas fluorescens* offer a promising biocontrol alternative due to their antagonistic properties and plant growth-promoting effects (Ganeshan & Kumar, 2005; Jan et al., 2011).

In vitro experiments offer an initial assessment of the antagonistic ability of promising bacterial strains to inhibit the growth of other microorganisms directly. The DSI and the efficacy values from *in vivo* experiments offer a robust measure of the efficacy of each strain in reducing disease symptoms in natural conditions.

In this study, strain M11 exhibited the strongest inhibition zones (9 mm for *Cmm*, 8 mm for *Pst*), suggesting high antimicrobial activity, possibly through siderophore or antibiotic production, which inhibit pathogen growth *in vitro*.

Strain M17 exhibited inhibition zones of 8 mm (*Cmm*) and 7 mm (*Pst*), indicating robust antagonistic effects. This result implies a robust production of inhibitory metabolites or competitive mechanisms against both pathogens.

Strains 27/3 and 46/a1 displayed moderate inhibition (4 mm and 3 mm for *Cmm*, respectively), with similarly low inhibition zones (3 mm) against *Pst*. These results suggest that 27/3 and 46/a1 may rely more on indirect suppression mechanisms than direct inhibition.

In vivo experiments measured disease severity indices and the efficacy values to assess practical biocontrol potential of these strains. The values revealed the potential of each strain as a biocontrol agent under natural conditions, where plant-microbe interactions and environmental factors influence efficacy.

Strain M11 achieved the lowest DSIs (28% for *Cmm*, 32% for *Pst*) with efficacies of 63.16% (*Cmm*) and 60% (*Pst*). These results indicate that M11 is highly effective in reducing disease severity in plants., combining direct inhibition with systemic resistance induction and effective colonization (Tshikhudo et al., 2023).

Strain M17 showed high DSIs (64% for *Cmm*, 72% for *Pst*) with efficacies of 5.2% (*Cmm*) and 20% (*Pst*). Despite strong *in vitro* inhibition, M17’s low *in vivo* performance suggests limitations in metabolite production or competition with plant microbiota under *in vivo* conditions.

Strain 27/3 reduced DSIs to 44% (*Cmm*) and 48% (*Pst*), corresponding to efficacies of 42.1% (*Cmm*) and 40% (*Pst*). Its moderate efficacy indicates the potential for indirect mechanisms like niche competition or host immunity enhancement (Santoyo et al., 2016).

Strain 46/a1 achieved DSIs of 40% for both pathogens, with efficacies of 47.3% (*Cmm*) and 50% (*Pst*). Despite low *in vitro* inhibition, 46/a1 demonstrated effective *in vivo* suppression, likely through mechanisms beyond direct antimicrobial activity, such as enhancing host immune responses.

The significant differences between *in vitro* and *in vivo* efficacy, particularly for M17, underscore the complexities of biocontrol under natural conditions. Factors such as nutrient availability, plant-microbe interactions, and competition within the plant microbiome influence success. While *in vitro* assays are valuable for initial screening, they cannot fully replicate these interactions, emphasizing the need for rigorous in vivo testing.

Strains like M11, with consistent performance across *in vitro* and *in vivo* conditions, represent robust candidates for biocontrol applications. Meanwhile, 27/3 and 46/a1, which demonstrated lower *in vitro* inhibition but significant *in vivo* efficacy, highlight the importance of indirect mechanisms that operate under natural conditions. Future research should investigate the specific biocontrol strategies employed by these strains, including metabolite production, systemic resistance induction, and competitive interactions.

Environmental factors, such as soil composition and moisture, should also be assessed to optimize the biocontrol potential of these strains. Combining strains or developing formulations to enhance bacterial persistence and colonization may further improve efficacy. The promising results for *Pseudomonas fluorescens* strains in this study support the broader use of beneficial microbes in sustainable agriculture, offering eco-friendly alternatives to conventional chemical treatments.

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