COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

SCHOOL OF POST GRADUATE STUDIES

DEPARTMENT OF BIOTECHNOLOGY

MSc. THESIS ON

BIOCONTROL EFFECT EVALUATION OF *PSEUDOMONAS FLUORESCENS* AGAINST *ALTERNARIA SOLANI* CAUSING EARLY BLIGHT DISEASE ON TOMATO (*SOLANUM LYCOPERSICUM*) PLANTS GROWN IN SOME DISTRICTS OF NORTH WOLLO, ETHIOPIA

Prepared By: **ID No**

Birhan Berihun **GSR 15053/15**

Advisor: Abebe Girma (Associate Professor)

Master's Thesis Submitted to the Department of Biotechnology, College of Natural and Computational Science for the partial fulfillment of the requirement of Masters of Science in Biotechnology

June 2025

Woldia, Ethiopia

APPROVAL SHEET

This is to certify that the thesis entitled “bio control effect evaluation of *Pseudomonas fluorescens* against *Alternaria solani* causing early blight disease on tomato (*Solanum lycopersicum*) plants grown in some districts of North Wollo, Ethiopia” submitted in partial fulfillment of the requirements for the degree of Master's with General Biotechnology, and has been carried out by Birhan Berihun under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to Biotechnology Department.

Abebe Girma (Associate Professor) _____ _____

Advisor Signature Date

As member of the Board of Examiners of the Master of Sciences (MSc.) thesis open defense examination, we have read and evaluated this thesis prepared by Mr. Birhan Berihun Abebe entitled with: “ bio control effect evaluation of *Pseudomonas fluorescens* against *Alternaria solani* causing early blight disease on tomato (*Solanum lycopersicum*) plants grown in some districts of North Wollo, Ethiopia”. We hereby certify that; the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (MSc.) in General Biotechnology.

Board of Examiners

_____ _____ _____

Name of External Examiner Signature Date

_____ _____ _____

Name of Internal Examiner Signature Date

_____ _____ _____

Name of Chairman Signature Date

DECLARATION

I declare that this thesis is a result of my genuine work and all sources of information used for writing it have been duly acknowledged. This thesis has been submitted to Woldia University in partial fulfillment of the requirements for the degree of Master of Science in General Biotechnology, and is deposited at the library of the University to be made to borrowers under the rules and regulations of the library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowable without special permission if an accurate acknowledgement of source is made. Requests for permission for extended quotations from or reproduction of this manuscript in whole or in part may be granted by the Head of the Department of Biotechnology or the Dean of the School of Graduate Studies, when in his or her judgment the proposed use of the material is for a scholarly interests. In all instances, however, permission must be obtained from the author.

Name: Birhan Berihun

Place: Woldia University

Date of submission: June, 2025

Signature: _____

BIOGRAPICAL SKETCH

The author, Birhan Berihun, was born on 01 may 1987, at Woldia Wereda, North Wollo Zone to his father Berihun Abebe and his mother Abebu Amede. He attended Elementary education (grade one to eight) at Jenetober Primary School from 1997 -2004 and secondary education (grade nine to twelve) at Sirinka Secondary School from 2005-2008. After completing his elementary and secondary education, he joined to Woldia University and studied biotechnology for four years and he has been graduated in the year 2013. After graduation, he was employed by Woldia Univesity on January 2013 as graduate assistant (GAII) in biotechnology department. The University offers him free scholarship for Master of Science (MSc.) study in General Biotechnology and attended his MSc, Program.

ACKNOWLEDGEMENTS

I am deeply grateful to God, whose grace, mercy, and blessings have sustained me throughout my academic journey and made the completion of this MSc thesis possible. My sincere appreciation goes to my advisor, Abebe Girma (Associate Professor), for his exceptional guidance, patience, and support. His insightful feedback and constant encouragement have been invaluable to both my academic and personal growth. I would also like to thank the faculty members for their dedication, mentorship, and contributions to my learning, as well as my thesis committee for their thoughtful feedback and critical insights. My appreciation extends to the department and university staff for their administrative and technical support, and to the University for the Financial Assistance provided during my studies. Finally, heartfelt thanks to my family and friends for their love, encouragement, and unwavering belief in me. Your support has been a source of strength throughout this journey. May God bless you all, Amen!

ABBREVIATIONS

BCAs	Biological Control Agents
FAO	Food and Agricultural Organization
HCDA	Horticulture Crop Development Authority
MR-VP	Methyl Red-Voges- Proskauer
NA	Nutrient Agar
PBS	Phosphate Buffered Saline
PDA	Potato Dextrose Agar
PIA	Pseudomonas Isolation Agar
TSB	Tryptic Soy Broth
CRD	Complete Randomized Design

ABSTRACT

Tomatoes (Solanum lycopersicum), a common and widely utilized vegetable in Ethiopia, encounter various production challenges, including diseases such as late blight, early blight, and bacterial wilt. This study focuses on isolating and screening Pseudomonas fluorescens as a bio control agent against Alternaria solani, which causes early blight in tomato plants. The objectives of the study includes isolation and characterization of P. fluorescens from soil and healthy tomato plant rhizospheres, isolating Alternaria solani from diseased tomato leaves, evaluating its pathogenicity in vivo, assessing the antagonistic activity of P. fluorescens against it, and determining the in vivo effectiveness of selected P. fluorescens isolate. The study was conducted at Woldia University's Biotechnology Laboratory, focusing on three kebeles in North Wollo, Ethiopia. Samples of rhizosphere soil and infected tomato leaves were collected from farmers during the dry season. P. fluorescens was isolated using the serial dilution up to biochemical test method cultured on Pseudomonas Isolation Agar (PIA). Alternaria solani was isolated from infected leaves using surface sterilization and cultured on Potato Dextrose Agar (PDA). Pathogenicity tests for Alternaria solani were conducted on tomato seedlings in a controlled greenhouse environment. In vitro antagonism of P. fluorescens labeled PFS12, PFK13, and PFSA31, along with a standard chemical of mancozeb and a control group of P. fluorescens against A. solani was assessed using the dual culture method, while in vivo efficacy was evaluated through greenhouse experiments. The research followed a Completely Randomized Design with three replications. Data were taken on the growth of the pathogen. The growth diameter of A. solani colonies was measured in centimeter, and the percent growth inhibition was calculated. Two way (ANOVA) was used to determine significance difference among treatments, means were compared using Fisher's protected least significant difference (LSD) test at a 5% significance level and, Correlation analysis test for the link between the pathogen and Pseudomonas fluorescens isolates .Isolates PFS12 and PFK13 showed moderate effectiveness against the radial growth of A. solani, achieving percent growth inhibitions of 56.04% and 55.04%, respectively. The standard chemical treatment (mancozeb) followed closely with a growth inhibition of 54.84%. The control group (Pseudomonas fluorescens) also demonstrated a moderate growth inhibition of 57.65% against A. solani. Data were gathered regarding disease parameters. The day after transplanting, the percent disease index was significantly lower in all treated groups compared to the control (water). The isolate PFSA31 achieved the lowest percent disease index of 24.733% which was comparable to the standard chemical treatment at 28.467%, with both treatments significantly different from the control (water) at 60.333%. The findings showed the bio control potential of selected P. fluorescens isolates as effective and environmentally sustainable alternatives to synthetic fungicides for the management of early blight disease in tomato cultivation, emphasizing the importance of utilizing indigenous strains for optimal performance.

Keywords: *Alternaria solani*, Biological control, Growth, *Pseudomonas fluorescens*, Tomato blight, Yield

TABLE OF CONTENTS

Contents Pages

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

1. INTRODUCTION

1.1. Background and Justification

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated and consumed horticultural crops globally. It plays a significant role in both household nutrition and income generation for farmers due to its versatility in fresh consumption and processing into products such as sauce, paste, ketchup, and juice. Tomatoes are rich in vitamins A, B, and C, and essential minerals including potassium, phosphorus, and iron (Akram *et al.*, 2020). They thrive across various agro-ecological zones, typically at altitudes ranging between 700 and 2000 meters, under optimal temperatures of 21–24°C (Singh & Surender, 2018). In East Africa, and particularly in Ethiopia, tomatoes are an important vegetable crop cultivated by smallholders, commercial farms, and national agricultural enterprises. The Rift Valley region, especially the Awash River Valley and lake areas, is the center of intensive tomato production using supplemental irrigation, mainly during dry seasons (Gemechu & Beyene, 2019). Despite the expansion in tomato cultivation and its growing economic significance, national average yields in Ethiopia remain below the global average and lower than those in neighboring countries (Yigezu Wendimu, 2021). Several biotic and abiotic factors contribute to this yield gap, including poor agronomic practices, post-harvest handling issues, and the prevalence of destructive diseases. Among these, early blight, caused by the fungal pathogen *Alternaria solani*, is one of the most damaging. Most studies have reported yield losses of up to 80% under severe infestation, leading to serious economic consequences for farmers (Panno *et al.*, 2021). To manage such diseases, many farmers rely heavily on chemical fungicides, which can offer rapid and effective control. However, their application poses several drawbacks: high costs, increased pest resistance, potential contamination of soil and water, and adverse health effects on humans and non-target organisms (Gatahi, 2020). Additionally, the reduced availability of certain fungicides due to regulatory restrictions further limits farmers' options, especially in low-income regions. In light of these challenges, interest has grown in biological alternatives, particularly the use of plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens, Bacillus*, *Streptomyces*, and *Trichoderma* species (Boro *et al*., 2022). PGPR are known not only for enhancing plant growth but also for their ability to suppress phytopathogens through multiple mechanisms. These include the production of antibiotics, siderophores, lytic enzymes, and the induction of systemic resistance in host plants (Lahlali *et al.*, 2022). Unlike chemical treatments, PGPR-based bio control is environmentally friendly, reduces chemical residue in food, and contributes to sustainable

agricultural practices (Basit *et al.*, 2021). Among PGPR, *Pseudomonas fluorescens* has gained considerable attention due to its broad-spectrum antifungal activity. It colonizes the rhizosphere effectively and suppresses pathogens through the production of secondary metabolites such as phenazines, hydrogen cyanide (HCN), and 2, 4-diacetylphloroglucinol (DAPG), as well as through competition for nutrients and space. Several studies have confirmed the antagonistic potential of *P. fluorescens* against *A. solani,* reporting growth inhibition rates ranging from 50% to 60% in vitro (Maurya *et al.*, 2014). Despite the promising potential demonstrated in earlier studies, there remains a critical gap in localized research. Most previous investigations were conducted outside Ethiopia, under different agro-climatic conditions and using non-native microbial strains. Very few studies have focused on identifying and evaluating indigenous *P. fluorescens* strains for their bio control efficacy against *A. solani* under Ethiopian conditions, particularly in North Wollo, where tomato farming is widespread but suffers from high disease pressure and poor disease management practices. This region faces unique challenges, including inadequate disease control strategies, limited access to biological products, and insufficient extension services, all of which contribute to yield losses. Given these gaps, the current research aims to isolate and screen indigenous *P. fluorescens* strains from tomato-growing areas in North Wollo, Ethiopia, and assess their in vitro antagonistic potential against *Alternaria solani*. By identifying effective local isolates, this study seeks to offer sustainable, eco-friendly alternatives to chemical fungicides. Ultimately, the findings will support the development of integrated disease management strategies, contributing to improved tomato yields, enhanced farmer livelihoods, and greater food security in the region.

1.2. Statement of the Problem

Tomato (*Solanum lycopersicum*) is a vital crop for food security and income generation in Ethiopia, including the North Wollo district. However, its productivity is severely threatened by early blight, primarily caused by the fungal pathogen *Alternaria solani*. Under favorable environmental conditions, this disease can cause yield losses of up to 80%, leading to significant economic hardship for smallholder farmers (Mengesha & Hussien, 2017). Despite its importance, tomato production in North Wollo faces numerous challenges, including poor post-harvest handling practices and limited access to effective disease management strategies (Muhie, 2023). Currently, farmers predominantly rely on synthetic fungicides such as tower and mancozeb to control early blight. While these chemicals may provide short-term control, their excessive and repeated use has led to increased pathogen resistance,

reducing their effectiveness. Moreover, the overuse of chemical fungicides raises serious environmental concerns, including soil and water contamination, and poses health risks to farmers and consumers. The high costs associated with chemical inputs further strain farmers' resources, making sustainable disease management difficult for small-scale growers. The extensive dependence on chemical fungicides also neglects the ecological impacts and contributes to the emergence of fungicide-resistant pathogen strains, complicating control efforts further. Additionally, there is a notable lack of locally adapted, environmentally friendly alternatives that could serve as sustainable disease control measures (Ahmed *et al*., 2024; Lahlali *et al*., 2022). Despite the potential benefits of biological control agent, such as *Pseudomonas fluorescens*, their application, especially within the Ethiopian context, remains underexplored. Existing studies are mostly confined to other regions and focus on different pathogens, with limited in vivo assessments relevant to local farming conditions in North Wollo. This knowledge gap hampers the development of safe, cost-effective, and environmentally sustainable strategies for tomato disease management in Ethiopia. Therefore, there is an urgent need to explore the isolation and application of native *Pseudomonas fluorescens* strains as biological control agents against *A. solani*. Such efforts could reduce reliance on chemical fungicides, mitigate environmental and health risks, and improve tomato yields sustainably. While the bio control potential of *P.fluorescens* against *A.solani* has been demonstrated in various studies, its effectiveness can vary significantly depending on environmental conditions, crop variety, soil characteristics, and the specific strains of both the pathogen and the bio control agent. In Ethiopia, particularly in the North Wollo region, there is limited local data on the native strains of *P. fluorescens* and their antagonistic activity against *A. solani* under local agro-ecological conditions.

1.3. Objectives

1.3.1. General objective

- ✓ The main objective this study was to determine the bio control effect evaluation of *Pseudomonas fluorescens* against *Alternaria solani* causing early blight disease on tomato (*Solanum lycopersicum*) plants grown in some districts of North Wollo, Ethiopia.

1.3.2. The Specific objectives

- ✓ To isolate and purify *Alternaria solani* from diseased tomato leaves.
- ✓ To conduct morphological, and microscopic tests for identifying *Alternaria solani.*
- ✓ To determine the pathogenicity of *Alternaria solani* under greenhouse conditions.
- ✓ To isolate and purify *Pseudomonas fluorescens* from the rhizosphere of healthy tomato plants.
- ✓ To evaluate the in vitro antagonistic activity of *Pseudomonas fluorescens* against *Alternaria solani.*
- ✓ To assess the in vivo efficacy testing of selected *Pseudomonas fluorescens* in controlling early blight disease in tomato plants.

1.4. Significance of the Study

There are important agricultural and financial ramifications to researching the isolation and screening of *Pseudomonas fluorescens* as a bio control agent against *Alternaria solani,* the pathogen that causes tomato fruit blight. While utilizing *Pseudomonas fluorescens* as a biological control agent offers the potential for farmers in North Wollo to reduce their reliance on chemical agents, thus minimizing production costs and fostering sustainable agricultural practices, it is essential to emphasize the need for thorough validation of its efficacy. Additionally, there is a significant health and environmental advantage to using *Pseudomonas fluorescens,* which reduces chemical runoff and possible toxicity to non-target organisms, improving ecosystem health and protecting human health. Furthermore, as food security is a major global concern, the study's societal advantages and global relevance cannot be understated. This research promotes agricultural sustainability and productivity by reducing tomato blight losses, which benefits communities in the world and Ethiopia.

2. LITERATURE REVIEW

2.1. Biology of Tomato

The wild tomato, a vine-like herb from the nightshade family, is thought to be the ancestor of today's cultivated tomato varieties (Wakil *et al.,* 2018). The tomato belongs to the family *Solanaceae,* the genus *Solanum,* which is part of *Lycopersicon,* which includes 12 wild relatives of the tomato native to Western South America. Tomato is widely grown in the Central, East and West Africa particularly, in Ghana and Nigeria. It was then distributed as a wild plant in the wider range of tropics and subtropics. The genus *Solanum* also includes 4 species of which *Juglandifolia* and *Lycopersicoides* are considered wild relatives of the tomato (Matumwabirhi & Kulimushi, 2020). Carolus Linnaeus first named the tomato *Solanum lycopersicum* in 1753. However, Philip Miller placed the cultivated tomato in the new genus *Lycopersicon,* separated from *Solanum* based on anther morphology (Kulus & Dariusz, 2022). Recently, tomato was transferred to the original genus *Solanum* L. based on morphological, biochemical and molecular (Bergougnoux & Véronique, 2014). The genus *Solanum* is classified in the *Solanaceae* family, which is called the *Solanaceae* family. The *Solanaceae* family includes many species, as well as food plants (tomato, potato, pepper, and eggplant), medicinal plants (lethal nightshade, henbane, and datura), and ornamental plants (petunias), among many (Motti & Riccardo, 2021). In terms of nutritional content, a tomato is categorized as a vegetable even though it is botanically a subset of a berry or fruit. Although they are planted as annuals, tomatoes are actually perennial dicots with a short lifespan. There are two types of stem growth habits: upright and prostrate. It reaches a height of two to four meters. The leaves measure 15–50 cm in length and 10–30 cm in width, grouped in a spiral pattern. Six to twelve flowers are produced by the clustered inflorescence. The plant is partially cross-pollinated but primarily self-pollinated. The fruit is a meaty berry that is 2–15 cm in diameter and globular to oblate in shape. The fruit is green and hairy when it is immature. Red, orange, and yellow fruits are considered ripe. The tomato contains a large number of kidney- or pear-shaped seeds. They are light brown and hairy.

2.2. Varieties of Tomato

Based on factors including fruit color and shape, plant height, days to maturity, disease resistance, and other growth traits that are often determinate or indeterminate, different tomato varieties that are marketed worldwide have been categorized (Kenneth & Oduor, 2016).Some foreign tomato varieties include Big Boy, Beef master, and Goliath, all originating from Canada. Tomato F1 No. 7 and Tomato F1 Tyking 5 are from

Vietnam. Tomato Red Cherry, Tomato Floradade, Tomato Jam Roma, and Royal Sluis are from the USA. Starke Moneymaker and Starke Heinz 1370 originate from South Africa (Wakil *et al*., 2018). Melka Shola and Marglobe, along with other released and tested varieties such as Fetan, Bishola, Eshete, and Metadel, are also cultivated in Ethiopia (Beyene, 2019).

2.3. Cultivation of Tomato

Globally, tomatoes occupy a total of about 5 million hectares of land, yielding about 180.48 million tons (Kumar *et al.,* 2020).China is the main tomato producer (50 million tons), followed by the United States and India (Guan *et al.,* 2018).Nigeria, Egypt, and Morocco are the largest tomato producers in Africa. Tomato is the most popular and widely cultivated vegetable in Ethiopia, the annual tomato cultivation in Ethiopia is around 1.8 million tones (Knapp & Peralta, 2016).Tomatoes respond well to abroad range of temperatures. The temperature range for seed germination is 10-35°C, and the optimum temperature range is 17-20°C. Optimum growth rate is achieved at 22 °C, while decline above 30 °C and below 12 °C. Fruit ripening is inhibited above 30 °C and below 16 °C (Silva *et al.,* 2017).Rough fruits are caused by growing temperatures below 16 °C root growth does not occur below 16 °C .Tomatoes require adequate, uniform moisture, especially during flowering and fruit formation. Tomatoes grow best in well-drained soil with plenty of organic matter. Protection from wind is important, especially at the beginning of production. Plastics such as mulch, row covers, and tunnel cores are also used to help this crop. According to Osman and Towhid (2018) estimated that weather has a significant effect on the marketable yield of ripe tomatoes, which is approximately 10,000 to 30,000 kg/ha.

2.4. Uses and Nutritional Values of Tomato

Tomato fruits are said to contain sufficient amounts of lycopene and vitamins A, B, and C. In addition, tomato fruits contain moderate amounts of potassium, iron, and phosphorus (Kaboré *et al.*, 2022).It was reported that consumption of lycopene, the substance that gives tomatoes their reddish color, minimizes the incidence of prostate, lung, and gastrointestinal cancers. Moreover, tomatoes are known to support heart health by lowering cholesterol levels and reducing blood pressure, thanks to their high content of potassium and antioxidants. They also contribute to skin health, as the vitamins and antioxidants in tomatoes can help protect the skin from sun damage and improve overall complexion (Erba *et al*., 2013). Furthermore, the dietary fiber found in tomatoes aids in digestion and promotes gut health, making them a valuable addition to a balanced diet.

According to Giovannucci *et al*. (2002), estimated that 14 carotenoids found in human serum, tomato and tomato products contribute to nine and are the predominant source of about one-half, including lycopene.

2.5. Climatic Requirements for Tomato Growth

Tomato plant growth depends critically on environmental conditions can significantly affect the growth of tomato production. Light, carbon dioxide, suitable temperature, and availability of water and nutrients are the main requirements for the growth of tomato plants (Kumar *et al.,* 2012).Tomato plants grow better in bright conditions, but day length does not affect their growth, but they grow quickly under short or long day conditions and complete their life cycle between 90 and 150 days (Naseer *et al.,* 2022).On the other hand, growing tomatoes requires stable and suitable narrow temperatures. Thus, large temperature fluctuations can reduce the quality of the fruit and reduce the yield. Optimum daily average temperature of 18-26 °C and night average temperature of 18-21 °C favor the growth of tomato plants (Hendricks, 2012). Tomato cultivation in greenhouses requires the use of a drip irrigation system and a fumigation system. Adequate nutrient supply has been shown to enhance tomato yield, improve the nutritional quality, and enrich the flavor of the fruit. Additionally, the proper application of nutrients contributes to better post-harvest storage of tomatoes. Conversely, excessive use of fertilizers can negatively affect tomato production, reduce nutrient use efficiency, and significantly contribute to environmental pollution (Hasnain *et al.,* 2020).

2.6. Soil Requirements for Tomato Growth

Tomatoes can be grown in a variety of soil types, as long as the soil is warm, well-aerated, has a high water-holding capacity, and is free from salinity (Schwarz *et al*., 2014). Tomato plants can grow in almost all soil type; however, they perform best in well-drained, light clay soils with a pH range of 5 to 7. High soil salinity can negatively impact their growth (Kaae & Helen, 2023).In general, sandy loam soils that are well-prepared and deeply friable are essential for the development of a strong root system (Tracy *et al.*, 2013).

2.7. Constraints to Tomato Production

Tomato production in the country is hampered by several problems such as physiological disorders, pests and diseases, and postharvest diseases (Gemechis *et al.,* 2012). The most common pests are African flies, aphids, leaf miners, spiders, thrips, white flies, and weeds (Bora & Mayurakshi, 2023). Whiteflies and red spider mites mainly affect tomato plants during the dry season by sucking the plant sap, which reduces growth rate and productivity (Kihoro & Esther, 2014).Weeds, such as nightshades and black hairs, compete with tomato

plants for light, space, and nutrients, negatively impacting fruit color, taste, and texture, while physiological disorders from water and nutrient stress, like low calcium causing floral abscission and black spots, low potassium leading to uneven ripening, and excessive nitrogen hindering fruit production, also affect tomato yield (Gemechis *et al.,* 2012).The most important obstacles to tomato production are diseases, which cause economic losses of 15–95% (Geoffrey *et al.,* 2014). According to Blancard and Dominique (2012) mention that several disease-causing pathogens have been reported to inhibit tomato production in Sanaa and East Africa, especially Ethiopia, the most destructive being late wilt, early wilt, bacterial wilt, bacterial canker, bacterial spot, Fusarium wilt, yellow leaf curl virus, and tomato spot virus, and early blight can be major constraints to tomato fruit production in the world.

2.7.1. Physiological disorders

Physiological disarranges are primarily caused by water and supplement stresses (Mishra & Archana, 2021). For occasion, low levels of calcium in the soil result in bloom conclusion spoil, causing dark spots at natural product bottoms; levels of potassium in the soil result in uneven maturing of tomato natural products; over the top levels of nitrogen block the generation of tomato natural products. Over the top nitrogen makes tomato plants develop greater and more grounded with bigger branches than ordinary but meddling with natural product generation (Phanindra *et al.,* 2024). In expansion, over the top nitrogen is considered as an imperative figure in the advancement of bloom conclusion decay.

2.7.2. Pests and weeds

The common pests such as African bollworms, aphids, leaf diggers, insect bugs, thrips, whiteflies, and weeds, including nightshade and black jack, compete for resources with tomato plants, with whiteflies and red spider mites notably affecting tomato plants during the dry season by reducing growth rates and productivity, while weeds interfere with tomato production by impacting color, flavor, and texture of the produce (Jones *et al.,* 2014).

2.7.3. Diseases

Diseases affecting tomato plants result in yield losses due to the premature death of foliage, affected stems, and diseased fruits. These issues are more pronounced in humid regions with high rainfall compared to drier areas (Vallad *et al.,* 2018). Diseases play a significant role in the yield gap, creating a need for effective management of tomato crops to maximize yield. A variety of tomato diseases pose serious challenges in

Ethiopia and other tomato-producing countries. These include bacterial diseases such as bacterial wilt and bacterial spot, fungal diseases like early blight, late blight, and fusarium wilt, as well as viral diseases such as leaf curl and spotted wilt (Brahimi *et al.,* 2017).Early scourge is prompted by the organism *Alternaria solani* and is checked by light brown, getting to be darker as they develop (Haider & Ehsan, 2020).

2.8. Management of Tomato Fungal Diseases and Pathogens

Plant diseases constitute a major constraint to crop production (Begna & Temesgen, 2020). This often leads to significant crop losses, ranging from mild damage to as much as 80%. Tomato plants are vulnerable to various diseases wherever they are cultivated. Fungal pathogens such as *Alternaria solani* (Ellis and Martin) Jones and Grout, which causes early blight, and *Fusarium oxysporum* f. sp. *lycopersici,* responsible for wilt disease, are among the primary factors contributing to yield reduction in tomato crops (Soni & Neelam, 2020). Early blight is a three-phase disease that causes leaf spots, stem cankers, and fruit rot. Fungal diseases like this are responsible for substantial economic losses faced by tomato growers each year in many regions where tomatoes are cultivated (Gupta & Thind, 2018). Fungal infections are typically caused by fungal spores that land on the leaves, germinate, and then penetrate the plant tissue through the stomata, wounds, or occasionally directly through the plants surface. The filaments grow rapidly in the affected plant tissue, from which they absorb nutrients and may also release substances that are toxic to the plant (Pankaj *et al*., 2020). The affected plant tissue typically dies. While the harmful effects of the fungus are usually confined to the affected area, certain fungi (such as *Fusarium* and *Verticillium* spp.) can invade the plant's vascular tissues (xylem) and spread throughout the plant (Pankaj *et al.*, 2020). The most noticeable symptom of fungal diseases is the appearance of leaf spots. These spots are typically round or oval but can also be polygonal or spindle-shaped with pointed ends. In the early stages of infection, moist areas may appear on the leaves, which will later die off. As the infection progresses, the leaf spots develop a dead brown center, surrounded by a light or dark-colored halo. Concentric rings in varying shades of brown or gray may form around the center.

2.8.1. Incidence and severity of tomato plant disease

Plant disease is assessed by evaluating its incidence, which refers to the number or proportion of plant parts such as leaves, stems, or fruits that exhibit disease symptoms compared to the total number of units observed (Opoku & Asante, 2014).However, disease severity refers to the extent of leaf area or amount of plant tissue affected by the disease, as well as the resulting yield loss it causes (Durairaj *et al*., 2016). Measuring disease incidence is a relatively quick method in epidemiological studies to track the spread of a disease across a field,

region, or country (Soni & Neelam, 2020).The economic threshold of a disease is the level of damage at which the cost of control measures is equal to the additional returns gained from protecting the crop (Safi *et al.*, 2020).This threshold varies based on the crop's tolerance level (damage threshold), which is influenced by factors such as the crop's growth stage at the time of infection, management practices, environmental conditions, changes in pathogen virulence, and the introduction of new control methods (Bhagat & Pratibha, 2018).According to Opoku and Asante (2014), a disease is considered epidemic when its incidence and severity increase rapidly from a low to a high level. In contrast, an endemic disease maintains a relatively stable level of occurrence over time. The progression of disease severity depends on the degree of interaction among the host, the pathogen, and the surrounding environment (Melomey *et al*., 2019).Diseases like cereal smuts, neck blast of rice, brown rot of stone fruits, and vascular wilts in annual crops show a direct correlation between disease incidence, severity, and yield loss, as each affected plant or fruit typically results in a complete loss. In contrast, for diseases such as most leaf spots, root lesions, and rusts where a plant is classified as diseased regardless of whether it has a single lesion or numerous ones incidence may not accurately reflect disease severity or the extent of yield loss. Yield loss is defined as the difference between the potential (attainable) yield and the actual yield obtained (Pankaj *et al*., 2020).

2.8. 2. Impact of *Alternaria solani* on tomato growth

Tomato (*Solanum lycopersicum*) is highly susceptible to early blight, a major foliar disease caused by *Alternaria solani*, which leads to significant economic losses globally and in tropical regions like Ethiopia. Yield losses ranging between 35% and 78% in different tomato varieties under field conditions without fungicidal protection (Naik, 2020). Adhikari *et al*. (2020) observed up to 80% reduction in marketable fruit yield during high disease pressure seasons. (Panno *et al*., 2021) documented 50–75% yield loss due to early blight in open-field tomato cultivation, especially where integrated pest management was not adopted.

In the Ethiopian context, Emana *et al.* (2017) noted that early blight caused 30–60% yield reduction, particularly affecting smallholder farmers who rely on open-pollinated varieties and traditional disease management approaches. These studies confirm that *A. solani* is not only ergonomically damaging but also poses a direct threat to food security and farmers' livelihoods due to the scale of yield reduction. .This pathogen lives both in soil and air, and can cause serious losses due to premature fruit drop. *Alternaria solani* has been documented to infect tomato plants under both dry and wet conditions and can grow in a wide temperature range from 4 °C to 36 °C air (Soni & Neelam, 2020).The hyphae of *Alternaria solani* are septate, branched, and light brown, becoming darker as they mature. Grown alone or in small groups, *Alternaria*

solani conidiophores are septate, straight or flexible, dark, and 50–90 μm in size (Lodhi & Singh, 2021).According to Marak *et al*. (2014) reported that a number of strains of *Alternaria solani* have been identified that show great morphological and physiological variation in addition to their genetic structure and pathogenicity.

2.8.2.1. The pathogen classification and morphology

Alternaria solani belongs to the kingdom of fungi, phylum Eukarya, Hyphomycetes, Hyphales, order Porosporae (Dhaval *et al.,* 2021). The shape of the colonies can vary widely, but they are often effusive, gray and brown to black, with a cocoon or velvety texture (Marak *et al*., 2014).Growth is rapid in many growing media, but sporulation requires special conditions. Orange to dark red pigments are formed, which color the substrate (Khadidja & Kadari, 2020). Conidiophores can reach lengths of up to 110 mm and diameters of 6-10 mm, being thick-walled, straight or flexible, septate, and occurring singly or in small clusters. Conidia are typically 75-350 μm long and 20-30 μm wide, light olive-brown, oblong, double-walled, and formed singly or in short chains, with beaks 5-9 mm in diameter, filiform, and pale brown (Alhussaen & Khalaf, 2012). Spore size varies, so that spores overlap in size with other large spored *Alternaria* species. Foliar symptoms, host region, and cultural characteristics aid in identification. According to Jambhulkar *et al.* (2016) estimated that biochemical or molecular techniques best confirm the identity of the pathogen, and sporulation requires special conditions.

2.8.2.2. Signs and symptoms of the disease

Tomato blights such as early blight and late blight can impact leaves throughout all growth (Xie *et al.,* 2015).Initially appearing as small spots, these lesions exhibit a bull's-eye pattern with concentric rings when reaching a quarter inch or larger in diameter. The affected areas expand, with yellowing of surrounding tissue often occurring, leading to significant leaf loss under extreme temperature and humidity conditions. Stem lesions, akin to leaf lesions, have the potential to encircle the plant if they develop close to the soil. Fungal entry into fruits typically transpires at the calyx or stem attachment point, resulting in infection. Fruits affected by these blights, as noted by exhibit concentric rings and lesions that can grow extensively, often covering nearly the entire fruit (Attia *et al.,* 2020).

2.8.2.3. Host susceptibility

Tomato plants are susceptible to *Alternaria solani* throughout their growth stages, with vulnerability escalating as the plants mature. Notably, plants with reduced leaf greenness and high yields, or those maturing early, are more predisposed to infection compared to those with ample foliage and lower yields or delayed maturation (Ravichandra, 2021). The susceptibility to *Alternaria solani* varies across different tomato cultivars and hybrids, indicating a consistent trend among various types (Adhikari *et al.,* 2021). Furthermore, the host's age significantly impacts susceptibility; although optimal weather conditions might not trigger outbreaks in young, resilient tomato plants, the overall susceptibility of the host is influenced by biological factors like the initial inoculum level and the host's physiological age.

2.8.2.4. Pathogen lifecycle and source of inoculum

Early blight is widespread in most regions where tomatoes or potatoes are grown but is particularly common in tropical and temperate regions (Adhikari *et al.,* 2017).Because several episodes of infection can occur during the season. *Alternaria solani* is a polycyclic pathogen. It is an imperfect fungus because sexual reproduction is currently unknown (Dhaval *et al.,* 2021). Primary infections in new tomato or potato plantings are caused by overwintering. The pathogen overwinters in plant debris as mycelium or conidia. In addition, chlamydial spores have been identified as a source of early blight overwintering inoculum that allows the disease to persist in or on the soil surface under cold conditions (Rodrigues *et al.,* 2010).The inoculum can survive in uncultivated soil debris for five to eight months. The resistance of hyphae to decomposition is increased by their dark color. Spores are mostly stored in infected debris and seeds (Cheruiyot & Jepto, 2023). Survival in debris and seeds is largely determined by meteorological, edaphic, and biotic factors. The fungus is best preserved in dry voids and between crops in plant litter and seeds (Wang & Dongwei, 2020). It can also survive on volunteer tomato plants (in warmer climates) and other cultivated and wild nightshade plants such as potato, eggplant, nettle, and black nightshade (Dourado *et al.,* 2014).Warm and humid conditions favor spore germination, where the optimum temperature is 8-32˚C (Animashaun & Mufutau, 2015). Germination involves the growth of germ tubes that penetrate directly into the host or through a stoma or damage to the plant. The primary inoculum produces conidia in the spring, which are then spread by spray or wind to the lower leaves of the plant, where they germinate and infect (Boyno *et al.,* 2022). Wind, rain, and insects are the main methods of spreading spores. Rapid spread only begins when the infection has reached the stage where healthy leaves wither and plants begin to die. The wind speed curve resembles the spread of spores (Dongre *et al.,* 2023*).* Free humidity favors spore germination during infection, but relative humidity close to saturation

can also cause it. At 20°C, spores can germinate after only two hours of humidity. Germ tube elongation requires a longer irrigation period. Germ tubes form an aspersorium and penetrate the epidermis directly or through wounds or stomata. Depending on the age and sensitivity of the plants, the incubation time varies significantly (Saha & Das, 2013).Initial infections become necrotic and chlorotic halos form. Conidia produced by the mycelium of necrotic areas infect healthy plants and cause secondary infections (Sippel, 2016). Spawning takes place between 5 and 30 degrees Celsius, and 20 degrees is ideal (Kushwaha, 2020). According to Yadav *et al.* (2015) reported that the strongest sporulation occurs after heavy rain or dew. A large number of spores are produced during alternating wet and dry periods. Spore formation begins during daylight, but spores accumulate within 7–14 days and then disperse during the day.

2.8.2.5. Epidemics

Moisture plays an important role in the development of early blight (Saha & Poly, 2013). Free water is essential for the development of the disease, and according to studies, the wet length of the leaves can explain up to 90% of the variation in the onset and severity of the disease. Early development is also promoted by increased leaf maturity, high fruit load, overcrowded plants, higher than average rainfall or dew, and shade (Sarkar *et al.,* 2022). Epidemics usually only occur late in the season when plants are more susceptible.

2.8.2.6. Factors favoring early blight of tomato

The production of *Alternaria solani* spores is induced by day light (Roy, Chapol K, et al., 2019). Prolonged periods of wetness are often needed for the production of *Alternaria solani* spores. However, sporulation of the fungus can occur when moist and dry conditions alternate. Thus, conidiophores formed in moist night can bear conidia in the following moist night after a period of dry day. Varying levels of minimum, optimum, and maximum temperatures for germination of *Alternaria solani* spores (Yadav *et al.,* 2015). According to Hussain *et al.* (2019) estimated that an optimum temperature of 20^{o}C is required for germination of spores of *A. solani* in a two-hour time period. The minimum temperature of 10^{0}C and a maximum temperature of 35^{0}C are required for the germination of *A. solani* spores. *Alternaria solani* thrives in warm temperatures (20-25^{0}C) and prolonged periods of leaf wetness resulting from high humidity. These conditions are conducive for spore germination and dissemination of spores from diseased plants. Tomato early blight has often been associated with plants under stress from nitrogen deficiency. Although symptoms of tomato early blight may appear in the early stages of the cropping season, susceptibility to the disease increases with the age of plant tissues, especially after fruit and tuber initiation (Salomon *et al.,* 2009).Early blight is also favored by cultural

practices including overhead irrigation, which increases wetness; poor sanitation, which maintains the pathogen availability, use of infected seeds and non-rotation of crops, among other practices (Matumwabirhi & Kulimushi, 2020).

2.8.3. Management of tomato early blight

Tomato early blight is a localized infection, it is said to be difficult to control. This is often related to the fact that *Alternaria solani* has a wide host range, many infectious strains, and a long active phase. Tomato yield reductions of up to 80% have been reported, with disease severity ranging up to 72%. A one percent increase in disease severity reduces tomato yield by up to 1.4 percent (Raza *et al.,* 2016).Tomato early blight can be controlled by one or a combination of three approaches: cultivation methods, chemical application, and use of resistant cultivars (Adhikari *et al.,* 2017). Some tomato cultivars have been reported to be resistant to early blight. However, early blight tolerant cultivars did not fare well in terms of yield. Furthermore, transferring resistance to most tomato varieties is not easy. Thus, cultural management strategies, in addition to the regular use of synthetic bacterial and fungal fungicides are the most common measures to control tomato early blight. The most common chemical compounds used worldwide to control tomato early blight are mancozeb, zineb, ridomil MZ-72, Saaf, and copper oxychloride; propiconazole, methylthiophanate, propamocarb, azoxystrobin, cymoxanil, propineb,and chlorthalonil. These chemicals must be applied every seven days to effectively control the pathogen (Yang *et al.,* 2017).The most common cultural practices associated with early blight control in tomatoes are sanitation, rotation of tomatoes with non-host crops for two to three years, use of pathogen-free seeds and plantings, removal of infected plant material from the garden, and voluntary control of crops and weeds (such as potatoes and horseradish). Appropriate irrigation strategies, such as early morning watering to reduce the duration of leaf wetness, furrow, and drip irrigation rather than overhead irrigation, are useful for early blight control in tomatoes (Adhikari *et al.,* 2017). In addition, maintaining plant vigor by adding adequate nitrogen and phosphorus, especially at the fruit stage, has been reported to have a significant effect on early blight control in tomatoes (Yang *et al.,* 2017). Water treatment of infected seeds at 50 ^{o}C for 25 minutes before sowing is useful to prevent seed dispersal (Müller *et al.,* 2016).Early blight control can be achieved with plant extracts and antagonistic cells and/or their secondary metabolites (Matumwabirhi & Kulimushi, 2020). Most of the bioagents reported for early blight control are *Pseudomonas aeruginosa, P. putida, P. cepacia, P. gladioli, P. fluorescens, Trichodermaviride, T. harzianum,*and *Bacillus subtilis.* The mechanisms of action of most antagonistic microorganisms are not well understood. However, they have been associated with one or a combination of the following strategies: direct parasitism, which often results in the

death of plant pathogens; competition for nutrients and space, which limits pathogen growth, and direct production of antibiotics, which prevents their development on plant pathogens (Matumwabirhi & Kulimushi, 2020) reported that *Pseudomonas* gladioli initiate systemic resistance in host plants by accumulating higher amounts of phenol and increasing peroxidase activity. Various plant extracts, including *Cinnamomum zeylanicum, Ferula foetida, Glycyrrhizaglabra, Hemidesmusindicus, and Syzygiumaromaticum*, have been reported to exhibit antifungal activity against the tomato early blight pathogen.

2.8.4. Management of early blight using microbial antagonists

Microbial adversaries are inviting to the environment and have negligible impacts on non-target life forms, counting people, creatures, and plants (Shoaib *et al.,* 2019). Microbial opponents have been detailed to have shifted exercises that ruin the development and improvement of numerous plant pathogens. The instruments of activity through which microbial enemies secure plants from pathogen assault are various and contrast from one adversary to another.

Microbial adversaries have been related to one or a combination of the following techniques: coordinate parasitism, which frequently leads to the passing of plant pathogens; supplement and space competition, compelling pathogen development and coordinate generation of anti-microbial compounds ruining the advancement of plant pathogens (Attia *et al.,* 2020). Most detailed microbial adversaries utilized in overseeing early curse incorporate *Bacillus subtilis, Pseudomonas aeruginosa, P. putida, P. cepacia, P. gladioli, P. fluorescens, Trichoder maviride,*and*T. harzianum*. Tall changeability of the capability of opposing microorganisms in controlling one or another plant pathogen is the fundamental confinement in consolidating microbial based pesticides in the administration of plant infections (Maji & Chakrabartty, 2014). The adequacy of microbial enemies against plant pathogens is affected by changed components, including temperature, relative stickiness, the nature of the host plant, and the nature of the plant pathogen, among other variables (Maji & Chakrabartty, 2014).

2.8.5. Use of resistant cultivars and its limitations

Host resistance is an effective and efficient component of integrated plant disease management, and some tomato cultivars offer moderate resistance to diseases such as bacterial wilt, early blight, and late blight (Adhikari *et al.,* 2017).The use of resistant cultivars has been reported to be the most effective and practical way to manage disease. Although the species can be found worldwide, the distribution of individual strains is

not uniform, resulting in the breakdown of resistance in a highly tolerant variety from a different geographic region (Onduso, 2014).

2.9. Biological Control of Diseases

Biological control is increasingly considered as an alternative to chemical control of plant diseases in sustainable agriculture (Abdalla *et al.,* 2014). Its measures are based on the use of organisms antagonistic to the target pathogens. Mycoparasitism, which can result from physical inter hyphal disruption or the formation of volatile and non-volatile metabolites, is one way antagonistic organisms can interact (Imran & Muhammad, 2022). Several microorganisms, including the obligate *Pseudomonas fluorescens* Joseph *et al.* (2017) have been reported to be effective biological control agents (BCAs) against *Alternaria solani.* Biological control of soil-borne plant pathogens has increased research worldwide due to the lack of resistant crop varieties, the harmful effects of applying large amounts of fungicides to the soil, and the emergence of resistance to soil-borne plant pathogens (Yazici *et al.*, 2011). The use of biological control agents to control soil-borne pathogens has become more popular in recent years due to environmental concerns associated with chemical control. Biological control methods are widely accepted and recommended as essential practices in sustainable agriculture, and the greatest potential for biological control is in microorganisms, arbuscular mycorrhizal fungi (AMF),and some naturally occurring antagonistic rhizobacteria such as *Bacillus* sp., *Pseudomonas* sp (Yendyo *et al.,* 2018). According to Dorjey *et al.* (2017) reported that the application of AMF to agricultural crops can provide protection against soil-borne pathogens by reducing root diseases caused by several soil-borne pathogens. Pathogen control and suppression by mycorrhizal fungi involves multiple mechanisms, including pathogen exclusion, altered nutrition, cell wall lignification, and secretion of small molecular compounds (Dorjey *et al*., 2017). Other biological agents that have been used to control the disease include *Fluorescent pseudomonads,* such as *Pseudomonas fluorescens,* which are antagonistic to soil-borne pathogens by producing antimicrobial agents, compete for space, and nutrients, and indirectly induce systemic resistance (Zhang *et al.,* 2018). Bacteriophages capable of attacking *Ralstonia solanacearum* have been used as a bio control agent (Tan *et al.,* 2013).Plant growth promoting bacterial strains (PGPR) have been described as promising bio control agents for the control of *R. solanacearum*.

2.9.1. *Bacillus subtilis* as a biological control agent

Bacteria in the *Bacillus subtilis* group are Gram-positive, possess flagella arranged peritrichously, and produce spores that serve as reproductive units (Ramyabharathi & Raguchander, 2014).These species possess several

characteristics that make them ideal for the study of chromosomal replication. *Bacillus* belongs to the family *Bacillaceae,* order *Bacillales,* class *Bacilli,* and phylum *Firmicutes*. A distinguishing feature of the *Bacillaceae* family is the production of circular, oval, or tubular endospores. Species within the *Bacillus* genus are differentiated from other members of the *Bacillaceae* family by their aerobic nature (either strict or facultative), their ability to produce catalase, and their rod-shaped morphology (Walia *et al.,* 2014). *B. subtilis*strains employ several modes of action, either individually or synergistically, to inhibit the development of phyto pathogens. These include competition for available space and nutrients, antibiosis, and the induction of host plant defense mechanisms (Wang *et al*., 2018). Antibiosis occurs due to the release of secondary metabolites, including lipopeptides, enzymes, and various low-molecular-weight volatile compounds. Lipopeptides include surfactin, fengycin, and iturin Padró *et al.* (2021), bacitracin Matumwabirhi and Kulimushi (2020). Enzymes include chitinase Wang *et al*. (2018) and chitosanase (Gayathri *et al*., 2025). Volatile compounds produced by *B. subtilis* strains include 2-nonanone, 2-methylpyrazine, and β-benzen eethanamine, which inhibit the development of various fungal pathogens by preventing mycelial growth and spore germination (Almoneafy *et al*., 2014).Certain volatile compounds secreted by *B. subtilis* are also known to stimulate activities that promote plant growth (Ramyabharathi & Raguchander, 2014). According to Abdelmoteleb *et al.* (2022)evaluated that the *B. subtilis* strain "ALICA" was evaluated against various plant pathogenic fungi, including *A. alternata*, and was found to produce antifungal lip peptides such as subtilosin, subtilisin, and two hydrolytic enzymes—β-1,3-glucanase and protease. These enzymes are believed to degrade components of fungal cell walls, including β-1, 4-glucan and glucosidic bonds. According to Ramyabharathi and Raguchander (2014) estimated that the *B. subtilis*strain EPCO16 was tested against *Fusarium oxysporum*f.sp. *lycopersici* and was found to produce antifungal metabolites, including bacillomycin, fengycin, iturin, and bacilysin, along with volatile compounds exhibiting antifungal activity, such as hexadecanoic acid methyl ester, dodecanoic acid, pentadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, 1,2-benzenedicarboxylic acid, and dibutyl ester. The study also recorded a significant reduction in disease incidence, along with enhanced plant growth and increased fruit yield in plants treated with the antagonist, compared to control plants.

2.9.2. *Streptomyces species* as a biological control agent

Streptomyces species are filamentous, Gram-positive, aerobic bacteria commonly found in soil and water. These species form a mycelium that produces chains of spores upon maturation (Hasani *et al.*, 2014). Several *Streptomyces* species play a role in the decomposition of soil organic matter, thereby enhancing soil fertility

(Golińska, 2020). They also produce secondary metabolites that function as plant growth promoters, including gibberellins, indole-3-acetic acid, and siderophores, among others (Goudjal *et al*., 2016). *Streptomyces* species are known to produce several antibiotics that inhibit the establishment and growth of other microorganisms, including plant pathogens. These antibiotics include oligomycinA , kanosamine, zwittermicin A, and xanthobactin, among others (Aigle *et al.*, 2014).*Streptomyces* species have also been shown to stimulate the host plant's defense system against plant pathogens (Suroto *et al*., 2017).However, the use of Streptomyces species as antagonistic microbes in agricultural production is challenging due to their low sporulation capacity, moderate antagonistic activity, and the short shelf life of their formulations (Vurukonda *et al*., 2018).

2.9.3. *Trichoderma* species as a biological control agent

Trichoderma species are known to exhibit a range of activities that prevent the establishment and development of plant pathogens. These include mycoparasitism, the production of antibiotics, competition for resources, and the induction of systemic resistance in host plants. Interestingly, these modes of action are often employed synergistically, making *Trichoderma* species more effective against plant pathogens (Asad & Ahmad, 2022). *Trichoderma* species are known to produce compounds that promote the growth of host plants and enhance productivity. These include ethylene, terpenoids, and phytoalexins, among others (Sarma *et al.*, 2014).The process of mycoparasitism begins with the recognition of the pathogen, followed by the coiling of *Trichoderma*hyphae around the hyphae of the plant pathogen. Subsequently, *Trichoderma* species penetrate the plant pathogen's cell wall through the secretion and release of cell wall-degrading enzymes, with mutanase and α-1, 3-glucanase being among the most well-known (Oloo & Judith, 2013).Antibiosis occurs due to the production of microbial compounds that are harmful to phytopathogens. *Trichoderma* species have been linked to the production of a variety of antifungal secondary metabolites. These include pyrones (e.g., 6-pentyl-α-pyrone), koninginins, viridins, azaphilones, isocyanide metabolites, and peptaibols, among others.

2.9.4. *Pseudomonas fluorescens* as a biological control agent

Pseudomonas fluorescens is a gram staining ,In dole and starch hydrolysis negative, catalase, gelatin liquefaction, oxidase, Triple sugar iron , citrate utilization ,and fluorescent pigmentation positive bacterium (Alelign, 2021). The bacterium is rod-shaped and moves by means of flagella (Panpatte *et al.,* 2016). *Pseudomonas* genus belongs to the family *Pseudomonadaceae*, order *Pseudomonadales*, class Gammaproteo bacteria, and phylum Proteobacteria. *Pseudomonas fluorescens* is a saprophytic bacterium mostly found in soil, water, and aquatic environments , in the rhizosphere of various crop plants (Hol *et al.*,

2013).*Pseudomonas* members are primarily aerobes. Optimal growth temperatures for *Pseudomonas fluorescens* range between 25-35^{0}C (Matumwabirhi & Kulimushi, 2020).*Pseudomonas fluorescens* is known for activities that impede the establishment and development of plant pathogens. In addition to competing for available nutrients and space, *Pseudomonas fluorescens* is known for the production of secondary metabolites, including antibiotics, along with; 2, 4-diacetyl phloroglucinol, phenazine, pyoluteorin, andbiosurfactant antibiotics; iron chelating siderophores such assalicylic acid, pyochelin,andpyoverdine (Neidig *et al.*, 2011).Hydrogen cyanide; lytic enzymes, including chitinaseand β-1, 3-glucanase, among others. Production of siderophores as an antagonistic strategy is used by *Pseudomonas fluorescens* mainly in environments where competition for available iron is required (Albert *et al.*, 2016). Lytic enzymes are responsible for the digestion of chitin, β-1, 3-glucan, and proteins composing cell walls of phytopathogenic fungi. *Pseudomonas* species have also been associated with activities favoring plant growth along with the secretion of plant hormones such as auxins, cytokinins, and gibberellins, and the solubilization of essential minerals such as nitrogen, phosphorous, and iron evaluated *Pseudomonas fluorescens* against tomato pathogens, including *Alternaria solani,* plant growth promoting activities and inhibition of the percent disease index for both infections resulting from the production of siderophores, hydrogen cyanide, ammonia,andindole-3-acetic acid by both antagonistic bacteria (Mahatma & Mahatma, 2015). According to Pawaskar and Mahesh (2023) estimated that, *Pseudomonas fluorescens* against *Alternaria solani in vitro* and on chili seeds and reported concurrent production of siderophore, indole-3-acetic acid, hydrogen cyanide, phosphate solublization, among others, as mechanisms used by the antagonist to hinder growth of plant pathogens and promote plant growth.

2.9.4.1. In vitro evidence

C. Roy *et al.* (2019) demonstrated that different *P. fluorescens* strains inhibited radial mycelia growth of *A. solani* by 52% to 61% using the dual culture method. Biswas *et al*. (2020) found that the Pf-5 strain of *P. fluorescens* suppressed *A. solani* growth by 58% in dual plate assays. Singh *et al.* (2018) reported that *P. fluorescens* isolates produced antifungal metabolites that reduced pathogen growth by 55–63% in vitro, with visible mycelia degradation.

2.9.4.2. In vivo evidence

Matumwabirhi and Kulimushi (2020) applied *P. fluorescens* as a foliar spray and recorded a 48% reduction in disease severity and 25% increase in tomato yield compared to untreated controls. Toma *et al*. (2024) also observed that *P. fluorescens* reduced early blight disease index by 42% in greenhouse trials and enhanced total

fruit yield by 28%. Shahid *et al*. (2022) demonstrated that tomato plants treated with *P. fluorescens* exhibited delayed symptom expression and improved plant vigor under field conditions, contributing to significantly higher yield performance (20–30% increase).

2.9.4.3. Gaps identified in literature

Despite these promising results, most bio control studies have been conducted in Asia or under greenhouse conditions using non-native strains, with limited applicability to Ethiopian agro-ecologies. There is a lack of data on indigenous strains of *P. fluorescens* and their adaptation to North Wollo soils and environmental conditions. Few studies have directly compared local isolates to standard strains or synthetic fungicides like mancozeb. The existing literature seldom addresses the practical field-level integration of *P. fluorescens* into tomato farming systems in Ethiopia.

2.9.4.4. Importance and objectives of the present study

Given the above gaps, this study was initiated to, isolate and screen native strains of *Pseudomonas fluorescens* from tomato rhizospheres in North Wollo. Evaluate their antagonistic potential against *Alternaria solani* under in vitro conditions. Compare the performance of these isolates with standard chemical controls (mancozeb) and known standard *P. fluorescens* strains. The ultimate goal is to identify effective, eco-friendly alternatives to synthetic fungicides, contributing to sustainable tomato production and improving farmer resilience in Ethiopia’s smallholder systems.

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted at the Biotechnology Laboratory of Woldia University, located in Woldia town, the administrative capital of the North Wollo Zone in the Amhara Regional State, Ethiopia. Woldia is situated at a latitude of 11°49′ N and a longitude of 39°36′ E, at an elevation of approximately 2,112 meters above sea level. The area falls within the Weyna Dega (midland) agro ecological zone and is characterized by moderate temperatures and seasonal rainfall patterns. The climate in Woldia is typically subtropical highland, with average annual temperatures ranging from 10°C to 28°C and annual rainfall between 700 mm and 1,200 mm, mainly occurring from June to September with the total population more than 83,000 residents. The predominant soil types in the area are vertisols and cambisols, which support diverse agricultural production under both rains, fed and irrigated conditions. Agriculture is the primary livelihood activity in Woldia and its surrounding areas, employing the majority of the population. The region is known for growing a variety of crops, including cereals (e.g., teff, sorghum, and maize), pulses (e.g., lentils and chickpeas), and horticultural crops, particularly tomatoes, onions, and peppers. Livestock farming is also common, with cattle, goats, sheep, and poultry being the main animals reared. Despite its agricultural potential, the region is frequently challenged by biotic and abiotic stressors, including plant diseases such as early blight, caused by *Alternaria solani,* which significantly affects tomato production. Yield losses of up to 80% have been reported under severe disease conditions, particularly in the absence of effective management strategies. Although farmers frequently use chemical fungicides such as mancozeb and chlorothalonil, their effectiveness has declined over time due to fungicide resistance and increasing concerns about their environmental and health impacts. For this study, soil and rhizosphere samples were collected from three tomato-producing Kebeles in North Wollo, Sirinka (1,891 meters elevation), a mid-altitude area with temperatures ranging from 12°C to 27°C and annual rainfall between 680 mm and 1,200 mm. Sanka (1,680 meters elevation), slightly lower altitude area with temperatures ranging from 12°C to 21°C and annual rainfall between 900 mm and 1,400 mm. Kobo (1,468 meters elevation, warmer and drier lowland area with temperatures ranging from 18°C to 27°C and rainfall between 410 mm and 820 mm. These Kebeles were chosen due to their suitability for tomato cultivation and their high incidence of early blight disease, making them appropriate sites for sampling and microbial isolation in the context of this bio control study (Hussain & Shazia, 2024).

3.2. Study Design and Period

The experimental research design employed a complete randomized design (CRD) to ensure systematic and unbiased results. The study took place from September 2024 to April 2025 at the Woldia University Microbial Biotechnology Laboratory, focusing on selected tomato cultivation sites in the North Wollo Zone. The research integrated both in vitro (laboratory) and in vivo (greenhouse) experiments to rigorously assess the efficacy of *Pseudomonas fluorescens* against *Alternaria solani*.

3.3. Sample Size and Collection

Rhizosphere soil and infected tomato leaf samples were collected during the dry season in September 2024 from three tomato-producing Kebeles, Sirinka, Sanka, and Kobo, located in the North Wollo Zone of the Amhara Regional State, Ethiopia. These sites were selected based on a preliminary field survey assessing the prevalence and severity of early blight disease caused by *Alternaria solani*. Within each Kebele, three representative tomato farmlands showing high disease incidence were purposively selected as sampling locations. From each selected farmland, rhizosphere soil samples were collected at a depth of approximately 5 to 15 cm using sterile spatulas, and three infected tomato leaves were excised using sterilized blades. All samples were collected while wearing sterile gloves to avoid cross-contamination. In total, nine soil samples and nine leaf samples were collected, three of each from every Kebele. These eighteen primary samples were then triplicated to ensure statistical validity and experimental reproducibility, resulting in fifty four final samples (six samples per Kebele × three Kebeles × three replications). The samples were immediately placed in sterile, labeled paper bags indicating the date and location of collection. After collection, all samples were aseptically transported in cooled containers to the Biotechnology Laboratory of Woldia University. Upon arrival, the samples were stored at 4°C until further microbiological and pathological analysis.

Figure 3. 1: Sample collection from different Kebeles

A: Infected tomato leaf samples, B: Rhizophere soil samples

3.4. Treatments and Experimental Set Up

The experiment was consisted from nine treatments and three replications. The total combination was 9*3 = 27 trials.

Table 3.1: *Pseudomonas fluorescens* treatments from study areas

Treatments	Substrate code	Sampling sites
Trt 1	PFS12	*Pseudomonas fluorescens* From Sirinka 1
Trt 2	PFS21	*Pseudomonas fluorescens* From Sirinka 2
Trt 3	PFS22	*Pseudomonas fluorescens* From Sirinka 3
Trt 4	PFK13	*Pseudomonas fluorescens* From Kobo 1
Trt 5	PFK21	*Pseudomonas fluorescens* From Kobo 2
Trt 6	PFK22	*Pseudomonas fluorescens* From Kobo 3
Trt 7	PFSA31	*Pseudomonas fluorescens* From Sanka 1
Trt 8	PFSA32	*Pseudomonas fluorescens* From Sanka 2
Trt 9	PFSA33	*Pseudomonas fluorescens* From Sanka 3

3.5. Experimental Procedure and Preparation

3.5.1. Isolation, purification and characterization of *Alternaria solani*

Tomato leaves exhibiting characteristic symptoms of early blight were collected from infected plants in the study area. Using sterile blades, the infected portions of the leaves were cut into small segments (approximately 0.5–1 cm^2). These segments were surface-sterilized by immersion in 1% sodium hypochlorite solution for one minute, followed by four successive rinses with sterile distilled water to remove any residual disinfectant. The sterilized leaf tissues were then dried on sterile filter paper under aseptic conditions and placed on Petri dishes containing sterilized Potato Dextrose Agar (PDA) medium. The plates were incubated at 28°C for seven-ten days to promote the growth and sporulation of the fungal pathogen. Fungal colonies emerging from the plated tissues were carefully sub-cultured onto fresh PDA plates using a sterile inoculating needle. Single spore isolation was performed to obtain pure cultures. These pure isolates were maintained on

PDA and periodically sub-cultured to preserve their viability and purity. Preliminary identification of the pathogen was based on colony morphology and growth characteristics, including texture and growth form. Further microscopic examination of the pure cultures was conducted using a light microscope at 40× magnification. Morphological characterization focused on the hyphae and conidia, particularly their shape, size, color, septation, and arrangement in chains (Alhussaen & Khalaf, 2012).

3.5.2. Preparation of *A.solani* inoculum

Ten ml of sterile distilled water were poured on14-day old single spore PDA cultures of *Alternaria solani*. Colonies were scraped using sterile glass slide. To remove debris, the resulting conidial suspension was sieved through a sterile muslin cloth. Using a haemocytometer, the concentration of the suspension in conidia was calculated and adjusted to $3x10^6$ spores/ml (Roy, Chapol, *et al.*, 2019).

3.5.3. Assessment of pathogenicity of *A.solani*

A greenhouse experiment was conducted at Woldia University in a structure measuring 3 × 3 × 2 meters. The greenhouse was designed with fine mesh netting on the sides and covered with plastic on the top to regulate temperature, pH, pest entry, and rainwater exposure. Tomato seedlings were grown in 22 cm diameter plastic pots. Each pot was filled with a sterilized potting mixture composed of sand and sandy loam in a 2:1 ratio. The soil mixture was autoclaved at 121°C for one hour and allowed to cool for seven days before use. Five tomato seeds were sown per pot and thinned to three plants once seedlings reached approximately 10 cm in height. Pathogenicity of *Alternaria solani* was assessed following Koch's postulates. At 40 days after sowing, healthy tomato seedlings were sprayed with a conidial suspension (3×10^6 spores/mL) using a hand sprayer. Control plants were treated with sterile distilled water. To ensure optimal humidity for disease development, all inoculated and control plants were covered with transparent plastic bags for 48 hours. Environmental conditions such as temperature and humidity were closely monitored throughout the experiment. Symptoms began to appear 5 to 7 days post-inoculation on the lower leaves of inoculated plants. These symptoms included small, dark brown spots that gradually expanded into concentric rings characteristic of early blight. As the disease progressed, necrotic lesions enlarged, often coalescing and causing leaf chlorosis and defoliation. To confirm infection, symptomatic leaves were collected, and *A. solani* was re-isolated using standard isolation techniques. The re-isolated pathogen was cultured on PDA and compared morphologically and culturally with the original strain used for inoculation. The consistency in colony morphology and microscopic features validated the fulfillment of Koch's postulates. Data recorded during the experiment

included the number of days until symptom onset, severity of symptom expression, lesion diameter, percentage of infected leaves, plant height, and leaf chlorosis. These observations confirmed the pathogenicity of *Alternaria solani* under controlled greenhouse conditions (Stammler *et al*., 2014).

3.5.4. Isolation and purification of *Pseudomonas fluorescens*

Pseudomonas fluorescens was isolated from rhizosphere soil samples using the serial dilution technique. One gram of each soil sample was suspended in 9 ml of sterile distilled water and vortexes thoroughly to create a uniform suspension. Serial dilutions were prepared up to 10^{-7}. From each dilution, 100 µL aliquots were spread on Pseudomonas Isolation Agar (PIA) plates using the spread plate method. The plates were incubated at 28°C for 24-48 hours to allow bacterial growth. Following incubation, distinct bacterial colonies were observed on the agar surface. Colonies with morphological characteristics typical of *Pseudomonas fluorescens*, notably their ability to fluoresce under UV light, were selected. These colonies were aseptically transferred to fresh PIA plates using a sterile inoculating needle for purification. Sub-culturing was repeated to obtain pure cultures (Hammami *et al.*, 2013).

3.5.5. Cultural and morphological characterization of *Pseudomonas fluorescens*

The purified isolates were characterized based on their cultural and morphological features, including colony shape, size, pigmentation, texture, and fluorescence under ultraviolet light. Colonies that exhibited the typical greenish pigmentation and fluorescent glow were tentatively identified as *Pseudomonas fluorescens* (Kipgen *et al*., 2021).

3.5.6. Biochemical characterization of *Pseudomonas fluorescens*

To confirm the identity of the isolates, standard biochemical tests were performed, including Gram staining, oxidase, catalase, methyl red test, gelatin hydrolysis test, starch hydrolysis test, indole test, triple sugar iron test, motility test, *fluorescens* pigmentation test and citrate utilization tests. Confirmed isolates were then maintained in nutrient broth supplemented with glycerol (15–20%) and stored at 20°C for further use (Suresh *et al.*, 2021).

3 .5.6.1.Motility test

The motility test for *Pseudomonas fluorescens* was conducted using sulfide indole motility (SIM) medium to assess bacterial motility, hydrogen sulfide production, and indole formation. A sterile inoculating needle was

used to stab the SIM medium to a depth of 2-3 cm, introducing the *Pseudomonas fluorescens* isolate. The inoculated tubes were incubated at 37°C for 24 hours. After incubation, the medium was examined for turbidity away from the stab line, indicating motility; if growth spread throughout the medium, it confirmed the bacterium's motility. The medium was also checked for a black precipitate, indicating hydrogen sulfide production, and for a red ring after adding Kovac's reagent, indicating indole production. The expected result for *Pseudomonas fluorescens* was positive motility, with growth diffusing from the stab line, confirming its motile nature (Bagul *et al.,* 2023).

3.5.6.2. Catalase test

Pure cultures of *Pseudomonad fluorescens* isolates were taken on a clean glass and one or two drops of three percent H_2O_2 was added. Formation of bubble was considered as positive for catalase test (Mohammed *et al.,* 2020).

3.5.6.3. Oxidase test

A filter paper was dipped in 1 per cent oxidase reagent or tetramethyl-p-phenylenediamine reagentand let to dry. Fresh pure cultures of *Pseudomonas fluorescens* isolates were rubbed onto treated filter paper and checked for variations in color. When the color changes to dark purple within 5 to 10 seconds the test isolates were considered oxidase positive (Nepali *et al*., 2018).

3.5.6..4. Starch hydrolysis test

Overnight grown cultures of *Pseudomonas fluorescens*in broth (10 µl) were spotted on to sterile starch agar plates and incubated at 28±2 ^{o}C for 24 to 48 hours. Later, the plates were flooded with gram's iodine solution and the appearance of transparent zone around the colony was considered as positive, whereas no clear zone around the bacterial growth indicates negative reaction for the test (Nepali *et al.,* 2018).

3.5.6.5. Triple sugar iron (TSI) slant/deep test

To identify the results of the TSI (Triple Sugar Iron) test for *Pseudomonas fluorescens* after incubation, examine the medium for color changes, fermentation, gas production, and hydrogen sulfide (H_2S) formation. TSI agar contains three sugars dextrose, lactose, and sucrose phenol red for detecting carbohydrate fermentation, and ferrous ammonium sulfate to indicate H_2S production through blackening in the butt of the tube. After inoculating the agar by stabbing the center and streaking the surface, and incubating at 37°C for 24

hours, observe the slant and butt. A yellow color indicates fermentation of sugars, while a red color suggests no fermentation. Gas production is indicated by bubbles or cracks in the agar. Black precipitate in the butt signifies H_2S production, indicating the organism's ability to reduce sulfur compounds. Analyzing these observations will provide insights into the biochemical characteristics of the *Pseudomonas fluorescens* isolate (Nepali *et al.,* 2018).

3.5.6.6. Methyl red test

Test tubes containing autoclaved glucose phosphate broth were inoculated with the *Pseudomonas fluorescens* cultures and incubated at 28±2 ^{0}C for 48 hours. Later, 5-6 drops of methyl red indicator was added and appearance of red color production was considered positive, whereas yellow color production was considered as negative for the test (Nepali *et al.,* 2018).

3.5.6.7. Gelatin hydrolysis test

The overnight pure cultures of the *Pseudomonas fluorescens* isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated at 28±2 ^{o}C for 24 hours. Later, they were refrigerated for 30 minutes at 4 ^{o}C. After refrigeration, the isolates that showed liquefaction of gelatin were considered positive for the test and the ones that solidified gelatin were negative for the test (Soesanto *et al.,* 2011).

3.5.6.8. Citrate utilization test

Simon's citrate agar slants were inoculated with overnight cultures and incubated at 28±2 ^{0}C for 24 hours. Color change from green to blue were taken as positive for the test, whereas those that did not record any change in color will be negative for citrate utilization (Güler & Küçük, 2010).

3.5.6.9. Indole test

Sulfide indole motility agar slants were inoculated with the overnight grown cultures of the *Pseudomonas fluorescens* isolates and incubated at 28±2 ^{o}C for 24-48 hours. Later, ten drops of Kovac's indole reagent were added to each tube and observed for red color. Appearance of red color was considered as positive and no color changes considered as negative for indole test (Onchwari, 2016).

3.5.6.10. Fluorescent pigmentation test

The Petridis containing sterilized *Pseudomonas* isolation agar medium were inoculated with the isolate of *Pseudomonas sp*. incubated for five days and observed. Yellowish green fluorescent pigment observed under UV light (365 nm) indicated positive results (Anitha & Kumudini, 2014).

3.5.7. In vitro evaluation of *Pseudomonas fluorescens* against *Alternaria solani*

The antagonistic potential of *Pseudomonas fluorescens* isolates against *Alternaria solani* was evaluated in vitro using the dual culture technique, following a Completely Randomized Design (CRD) with three replicates per treatment, which is suitable for uniform laboratory settings with randomly assigned treatments. Seven-day-old cultures of *A. solani* were prepared by cutting 5 mm agar plugs using a sterile cork borer and placing them near the edge of sterile Potato Dextrose Agar (PDA) plates. Due to the slow growth of the fungus, plugs were incubated three days prior to inoculation of the bacterial antagonists. Isolates of *P. fluorescens*, including both newly isolated and reference strains, were spot-inoculated on the opposite side of the same plate, approximately 2 cm from the edge and equidistant from the fungal plug. Control plates with only *A. solani* served as a baseline. Prior to greenhouse evaluation, all bacterial isolates were screened under laboratory conditions for key bio control traits, including antibiotic production (via agar diffusion methods), lytic enzyme activity (e.g., chitinase and protease), and bioactive compound production (e.g., siderophores, hydrogen cyanide, and volatile organic compounds) to assess their potential antifungal efficacy. The plates were incubated at 28°C for five- seven days, and the radial growth of *A. solani* was measured daily from day 3 to day 13. Zones of inhibition between the bacterial colonies and fungal mycelium were recorded, and percent growth inhibition (PGI) was calculated using the formula PGI (%) = ((C − T) / C) × 100, C- mycelia growth of pathogen in control T- mycelia growth of pathogen in dual culture plate. Based on PGI values, antagonistic activity was categorized following the scale, very low (0–25%), low (26–50%), moderate (51–75%), and high (76–100%). This in vitro screening was an essential step to identify the most promising *P.fluorescens* strains for subsequent greenhouse evaluation (Lalhruaitluangi C *et al.*, 2022).

3.5.8. Design and set up of the pot experiments

Microbial antagonists selected from the in vitro screening were further evaluated for their efficacy in managing tomato early blight under in vivo conditions using pot experiments conducted in the greenhouse at Woldia University between October 2024 and February 2025. Certified tomato seeds of the susceptible variety

Roman BF, obtained from the Sirinka Agricultural Center near Woldia University's botanical garden, were sown in sterilized nursery beds. Thirty days after sowing, healthy seedlings were transplanted into plastic pots measuring 30 cm in diameter and 30 cm in height, each filled with a sterilized potting mix composed of sand and sandy loam at a 2:1 ratio, previously autoclaved at 121°C for one hour. Each pot contained three rows with five plants per row, maintaining inter-row and intra-row spacing of 0.5 meters, and pots were arranged with 0.5-meter spacing between them. The experiment was arranged in a Completely Randomized Design (CRD) with three replicates per treatment. Treatments included, one tomato plants inoculated with *Alternaria solani* and treated with selected *Pseudomonas fluorescens* isolates (experimental treatments), two plants inoculated with *A. solani* but untreated (negative control), and three plants neither inoculated nor treated (positive control). The antagonists were applied as soil drench and foliar spray at specified intervals following pathogen inoculation to assess their bio control efficacy. The greenhouse experiment lasted for 60 days post-transplanting. Data recorded included disease incidence, severity and index assessed from 10^{th} to 60^{th} days. Final data collection occurred at the end of the 60-day period, when comprehensive assessments of disease suppression and plant growth promotion were conducted to evaluate the effectiveness of the microbial antagonists in managing early blight in tomato under controlled conditions (Ahmad *et al.*, 2024).

3.5.9. Preparation of the antagonists

3.5.9.1. Preparation of standard *Pseudomonas fluorescens*

Standard *Pseudomonas fluorescens* was acquired from Ethiopian Public Health Institute (EPHI) referral and reference laboratory and was used as a source of the bacterium and for comparison. Using a sterile micropipette, one ml of the formulation was measured and diluted as recommended for the in vitro evaluation of the bacterium against *A.solani* (Zegeye *et al.*, 2011).

3.5.9.2. Preparation of cultures filtrates from *Pseudomonas fluorescens* isolates

Culture filtrates of *Pseudomonas fluorescens* isolates were prepared using nutrient broth composed of 5 g/L peptone, 3 g/L beef or yeast extract, and 0.5 g/L sodium chloride in 1,000 mL distilled water (pH 7.0 at 25°C). Approximately 8.5 g of the medium was dissolved in 500 mL distilled water, sterilized by autoclaving at 121°C and 1 bar pressure for 15–20 minutes, and then cooled. Seven-day-old *P. fluorescens* cultures grown on Pseudomonas Isolation Agar were suspended in 10 mL sterile distilled water by flooding and scraping with sterile glass slides. This suspension was aseptically transferred into 500 mL of sterile nutrient broth in conical

flasks, which were sealed with cotton wool and aluminum foil to prevent contamination. Flasks were incubated at room temperature (24 ± 2°C) on laboratory benches for 9 days. The fermentation broth was then filtered through sterile muslin cloth, and the resulting culture filtrate was collected in sterile 1,000 mL conical flasks for further use (Suresh *et al.,* 2021).

3.5.10. In vivo efficacy testing of *Pseudomonas fluorescens*

To assess the in vivo efficacy of *Pseudomonas fluorescens* in controlling early blight disease caused by *Alternaria solani* in tomato plants, several carefully controlled steps should be followed. First, select a tomato variety known to be susceptible to early blight, and grow the plants in pots or a greenhouse under consistent, controlled conditions to ensure all plants were at similar growth stages and healthy at the start of the experiment. *P. fluorescens* suspension was prepared in section above 3.11.2 by culturing it in nutrient broth. Treatments were applied on a 10 day interval commencing 50 days after sowing in the greenhouse and 20 days after transplanting in the pot. One thousand ml of culture filtrates from isolates of selected antagonists were prepared and were thoroughly mixed with one ml acquawet to allow it to stick on the leaf surface. The treatments were sprayed on the leaves of tomato plants using hand sprayers. Since cases of phytotoxicity were recorded on tomato leaves for the normal strength, the culture filtrates were diluted to half strength by adding an equal volume of sterile water. One standard *Pseudomonas fluorescens*, Mancozeb or antifungal chemicals, one negative control or water and three isolated *Pseudomonas fluorescens* were applied according to guidelines. A total of 6 sprays were done for the whole experiment (Meena & Marimuthu, 2012). Monitor the plants over 2–4 weeks, recording signs of early blight, including leaf spots and wilting, and measure disease incidence as a percentage of plants affected. Assess disease severity on a scale (e.g., 0–5, where 0 indicates no symptoms and 5 indicate severe symptoms (González-Concha *et al.*, 2023).

3.6. Data Collection

To collect data for this study, the research aimed to screen *Pseudomonas fluorescens* as a bio control agent against *Alternaria solani*, the causal agent of early blight in tomato plants. The study involved the isolation and characterization of *P.fluorescens* and *A. solani* from rhizosphere soil and infected tomato leaves, respectively. Sampling procedures included documentation of sampling locations, environmental conditions, and characteristics of collected specimens. The physical and cultural characteristics of the isolated microbial colonies were recorded, including colony color, shape, size, and texture. Microscopic features were also

examined for identification. In addition, biochemical tests were performed on *P. fluorescens* isolates to confirm their identity. To evaluate the antagonistic potential of *P. fluorescens,* radial growth of *A. solani* was measured daily from day 3 to day 13 under both treatment and control conditions in a laboratory dual culture assay. Growth inhibition was calculated using the formula: Growth Inhibition (%) = ((C − T) / C) × 100, where C is the colony diameter in the control and T is the colony diameter in the treatment. In greenhouse trials, disease progression on tomato plants was monitored from the tenth to the sixteenth day after inoculation. Disease parameters such as disease incidence, severity, and disease index were recorded for each treatment group in pots, following standard procedures (Matumwabirhi & Kulimushi, 2020; Wspanialy & Moussa, 2020).

$$\text{Percent Disease Incidence} = \frac{\text{(Number of infected leaf/plant per pot)} \times 100}{\text{(Total number of leaf/plant observed per pot)}}$$

$$\text{Percent Disease Severity} = \frac{\sum \text{(Disease rating} \times \text{number of plants scored for each rating)} \times 100}{\text{(Total number of leaf observed} \times \text{maximum rating scale)}}$$

$$\text{Percent Disease Index} = \frac{\sum \text{(Percent disease incidence + Percent disease severity)} \times 100}{\text{(Total number of leaves per pot observed} \times \text{maximum rating scale)}}$$

Rating scale	Disease severity percent	Disease reaction
0	0	No symptom
1	1-10	Mild symptom
2	11-25	Moderate symptom
3	26-50	Symptom
4	51-75	Very Symptom
5	≥76	Totally severe

3.7. Data Analysis

The recorded data was analyzed and interpreted using SPSS (Version 27.1) and Statistix 10. Two way ANOVA was used to determine statistical significance among treatments and fishers protected least significance difference (LSD) test at 5% significance level, for mean comparison .Finally correlation analysis was used under in vivo conditions to link the relationship between disease parameters (Incidence , severity and Index).

4. RESULTS AND DISCUSION

4.1. Results

4.1.1. Isolation and morphological characterization of isolated *Alternaria solani*

Pure colonies of *Alternaria solani* that were grown on Potato Dextrose Agar (PDA) exhibited distinct characteristics for identification. The morphological and microscopic characterization of *Alternaria solani* isolates revealed distinct variations across several features. Colony coloration ranged from white (Ass11, Assa12, Ask12, Ask13) to dark brown (Ass12, Ass13, Assa13, Ask11), grey (Ass13), and blackish hues (Assa11). Colony textures varied from cottony (Ass11, Assa12, Ask11, Ask12, and Ask13) to fluffy (Ass12, Ass13, Assa11, and Assa13). Growth patterns were predominantly fast and smooth, with isolates showing either regular (Ass11, Ass13, Assa12, Ask11) or irregular growth (Ass12, Assa11, Ask12). The reverse sides of the colonies exhibited primarily dark brown (Ass11, Ass13, Assa13, Ask12) or white pigmentation (Ass12, Assa12, Ask11, Ask13), with one isolate (Assa11) displaying a yellow reverse. Colony shapes were mainly circular and raised (Ass13, Assa11, Assa12, Assa13, Ask11, Ask12), with some exhibiting hairy margins (Ass11, Ass12, Assa13, Ask13). Colony sizes varied significantly, ranging from 36.9 µm (Ass13) to 93.2 µm (Ask13). Microscopic analysis showed that the hyphae were mostly branched filaments (Ass12, Ass13, Assa13, Ask11, Ask12, Ask13), with some isolates forming septate hyphae with chains (Ass11) or bearing conidia with beaks (Assa11, Assa12). Septation patterns included horizontal septation ranging from 2 to 7 and vertical septation from 1 to 4, providing a comprehensive profile of the morphological and microscopic diversity among the examined *A. solani* isolates. A detailed morphological analysis had been presented in Figure 4.2 and Table 4.2.

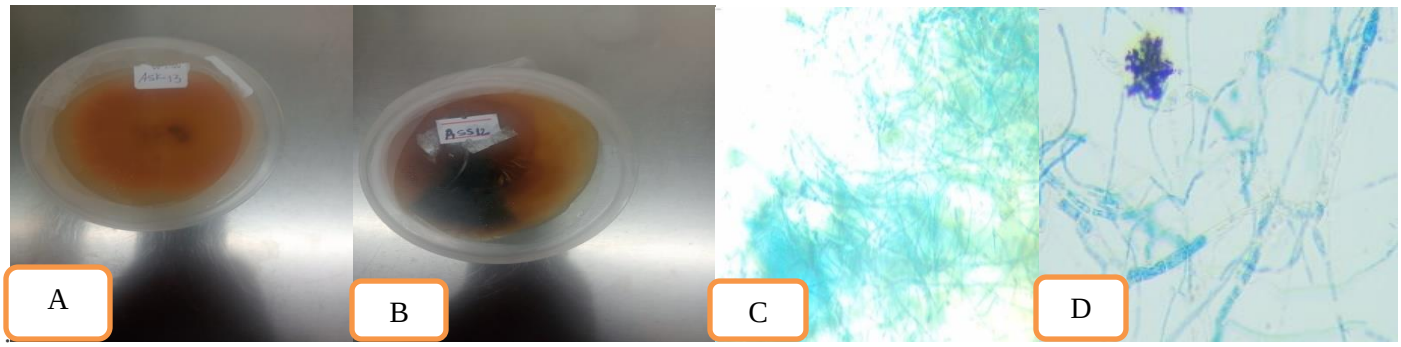


Figure 4.2: Cultural and morphological characteristics of isolated *Alternaria solani*

A: *Alternaria solani* seven- day old colony (obverse), B: *Alternaria solani* ten- day old colony (reverse), C: *Alternaria solani* hyphae (x40), D: *Alternaria solani* conidia (x40).

Table 4.2: Cultural, morphological and microscopic characteristics of isolated *Alternaria* solani

Isolate Code	Color	Texture	Growth Form	Reverse Side	Shape	Size (um)	Hyphae	Septation	
								Horizontal	Vertical
Ass11	White	Cottony	Fast , Smooth, regular	Dark brown	hairy margin	78.4	Septate hyphae, septate chain forming	3-5	1-3
Ass12	Dark brown	Fluffy	Weak, Smooth, irregular	White	hairy margin	42.5	branched filament	3-6	2-4
Ass13	Grey	Fluffy	Fast, Smooth, regular	Dark brown	Circular, raised colonies	36.9	branched filament	3-5	1-2
Assa11	Blackish	Fluffy	Fast, Smooth, irregular	Yellow	Circular, raised colonies	38.7	Septate, Beaks – like structure	2-6	1-3
Assa12	White	Cottony	Fast, Smooth, regular	White	Circular, raised colonies	91.5	Septate, Beaks on conidia	3-7	1-3
Assa13	Dark brown	Fluffy	Fast, Flat growth circular	Dark brown	hairy margin	75.4	branched filament	4-7	1-2
Ask11	Dark brown	Cottony	Medium, Smooth, regular	White	Circular, raised colonies	70.3	branched filament	4-7	1-2
Ask12	White	Cottony	Fast, Smooth, irregular	Dark brown	Circular, raised colonies	92.3	branched filament	2-5	1-2
Ask13	White	Cottony	Fast	White	hairy margin	93.2	branched filament	2-6	1-3

4.1.2. Pathogenicity of *Alternaria solani*

Tomato seedlings (20 days old) inoculated with a conidial suspension of *Alternaria solani* developed characteristic early blight symptoms 15 days post-inoculation. Affected foliage displayed dark brown, oval to angular lesions measuring 2–7 mm in diameter, exhibiting distinct concentric rings typical of *A. solani* infection. Lesions gradually expanded, leading to complete blighting of the infected leaves. To satisfy Koch's postulates, the pathogen was re-isolated from symptomatic leaf tissues using standard tissue isolation techniques on Potato Dextrose Agar (PDA). The resulting single-spore colonies exhibited cultural and morphological characteristics identical to those of the original isolate. Microscopic examination confirmed

that the hyphal and conidial features of the re-isolated fungus were consistent with *A. solani*, thereby confirming the identity of the pathogen. So confirmed the re -isolated pathogen was causal agent.

Figure 4.3: Early blight symptoms on infected tomato leaf and healthy tomato leaf under greenhouse

A: healthy tomato leaf from greenhouse pot, B: early blight symptoms on infected tomato leaf from greenhouse pot.

4.1.3. Isolation and morphological characterization of isolated *Pseudomonas fluorescens*

The bacteria *Pseudomonas fluorescens* was isolated from rhizosphere soil using pseudomonas isolation agar, one gram of rhizosphere soil was collected and serially diluted up to 10^{-7}. From this dilution, 27 distinct bacterial colonies were isolated. Among 27 *Pseudomonas fluorescens* isolates, only nine were morphologically characterized based on features such as colony shape, cell shape, color, elevation, and surface texture, pigmentation, and Gram reaction. In terms of surface coloration, most isolates displayed a greenish tint (Pfs12, Pfsa31, Pfsa32, Pfsa33, and Pfk13), while others showed a blue-green tint (Pfs21, Pfs22, Pfk21, Pfk22). The reverse side of the colonies was predominantly yellowish-green in Pfs12, Pfs21, Pfs22, Pfsa31, Pfsa32, and Pfsa33; whereas Pfk13, Pfk21, and Pfk22 exhibited off-white reverse. Colony textures ranged from smooth (Pfs12, Pfsa31, Pfsa32, Pfsa33, Pfk21, Pfk22) to glossy (Pfs21, Pfs22, Pfk13). The colony margins were generally rounded, except for Pfk13, which displayed an irregular margin. All isolates were rod-shaped, with colony sizes ranging from 1 to 3 mm. In terms of cellular arrangement, most isolates formed small clusters (Pfs12, Pfs21, Pfs22, Pfk21, Pfk22), while Pfsa31, Pfsa32, Pfsa33, and Pfk13 appeared as single cells. Colony elevation was predominantly convex, except in Pfk21 and Pfk22, where a flat elevation was observed. These findings provide a comprehensive morphological profile of the isolated *P. fluorescens* strains, as detailed in Figure 4.4 and Table 4.3.

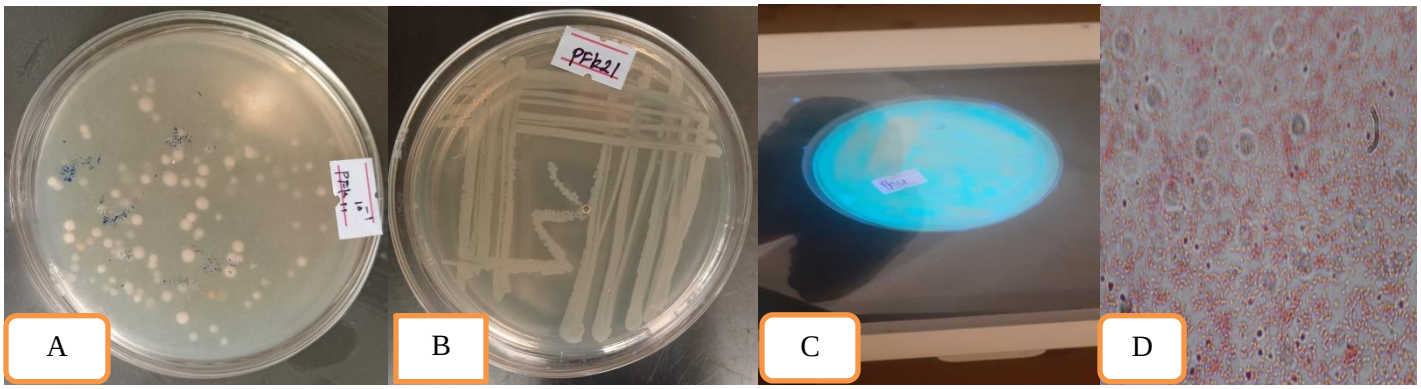


Figure 4.4: Cultural and morphological characteristics of isolated *Pseudomonas fluorescens*

A: *Pseudomonas fluorescens* colonies, B: *Pseudomonas fluorescens* sub culturing, C: Isolated *Pseudomonas fluorescens* under ultraviolet light, D: Microscopic observation of *Pseudomonas fluorescens*.

Table 4.3: Cultural and morphological characterization of isolated *Pseudomonas fluorescens*

Sampling Code	Surface Color	Reverse color	Texture	Margin	Shape	Size	Arrangement	Elevation
Negative Control								
P.fluorescens	Greenish	Yellowish green	Smooth	Round	Rod	1-3 mm	small cluster	Convex
Pfs12	Greenish	Yellowish green	Smooth	Round	Rod	1-3 mm	small cluster	Convex
Pfs21	Blue Green	Yellowish green	Glossy	Round	Rod	1-3 mm	small cluster	Convex
Pfs22	Blue Green	Yellowish green	Glossy	Round	Rod	1-3 mm	small cluster	Convex
Pfsa31	Greenish	Yellowish green	Smooth	Round	Rod	1-3 mm	Single cell	Convex
Pfsa32	Greenish	Yellowish green	Smooth	Round	Rod	1-3 mm	Single cell	Convex
Pfsa33	Greenish	Yellowish green	Smooth	Round	Rod	1-3 mm	Single cell	Convex
Pfk13	Greenish	Off white	Glossy	Irregular	Rod	1-3 mm	Single cell	Convex
Pfk21	Blue Green	Off white	Smooth	Round	Rod	1-3 mm	small cluster	Flat
Pfk22	Blue Green	Off white	Smooth	Round	Rod	1-3 mm	small cluster	Flat

4.1.4. Biochemical characterization of isolated *Pseudomonas fluorescens*

Out of the 27 *Pseudomonas fluorescens* isolates obtained, nine representative isolates were selected for detailed biochemical characterization. All nine isolates tested positive for citrate utilization, catalase activity, oxidase activity, motility, triple sugar iron (TSI) reactions, fluorescent pigment production, and gelatin liquefaction- traits that are characteristic of *P. fluorescens* and support their species-level identification. In contrast, all isolates were negative for starch hydrolysis and indole production and exhibited Gram-negative staining, confirming their Gram-negative cell wall structure characterized by a thin peptidoglycan layer and an outer membrane, and indicating specific metabolic limitations. Catalase and oxidase positivity confirmed the ability of the isolates to break down hydrogen peroxide and the presence of cytochrome c oxidase, respectively-both hallmark features of *Pseudomonas* species. Motility tests revealed that all isolates were motile and possessed flagella, suggesting enhanced adaptability to diverse environments. The starch hydrolysis and indole tests confirmed the absence of amylase and the inability to convert tryptophan to indole, consistent with the known biochemical profile of *P. fluorescens*. TSI agar tests showed that most isolates fermented glucose without producing hydrogen sulfide (H_2S) and did not ferment lactose or sucrose. However, isolates Pfsa31, Pfsa32, Pfk21, and Pfk22 did not ferment glucose, suggesting possible strain-specific variations or experimental inconsistencies. All isolates were gelatinase-positive, and citrate utilization confirmed their ability to use citrate as a sole carbon source. Additionally, the methyl red test was positive in all isolates, indicating stable acid production during glucose metabolism. The production of fluorescent pigments under UV light further confirmed the identity of the isolates as *P. fluorescens*. A detailed summary of these results is presented in Figure 4.5 and Table 4.4.

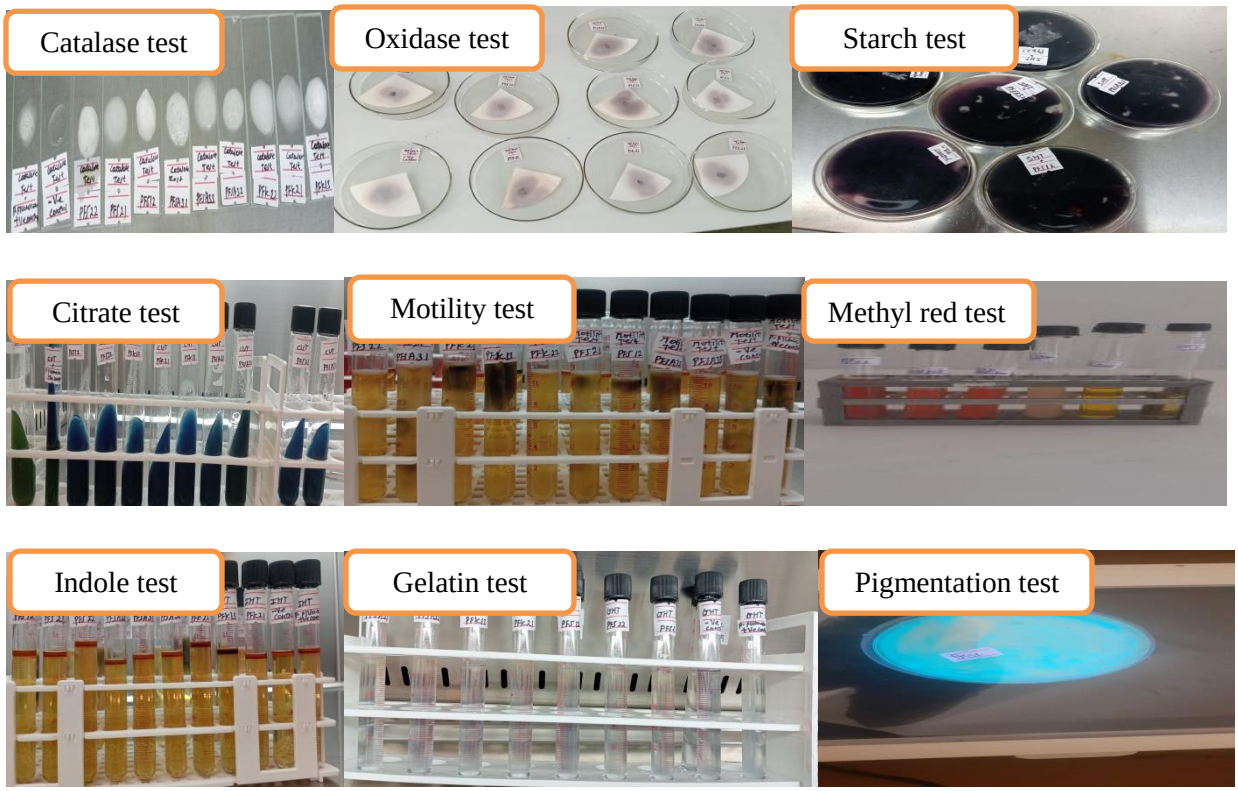


Figure 4.5: Different biochemical test of isolated *Pseudomonas fluorescens*

Table 4.4: Biochemical characteristics of isolated *Pseudomonas fluorescens*

Isolate Code	Gram Staining	Catalase test	Oxidase Test	Motility Test	Starch hydrolysis test	Indole test	Gelatin liquefaction Test	Methyl red test	Citrate Utilization Test	*fluorescens* Pigmentation test
Negative Contro										
P.fluorescens	-	+	+	+	-	-	+	+	+	+
Pfs12	-	+	+	+	-	-	+	+	+	+
Pfs21	-	+	+	+	-	-	+	+	+	+
Pfs22	-	+	+	+	-	-	+	+	+	+
Pfsa31	-	+	+	+	-	-	+	+	+	+
Pfsa32	-	+	+	+	-	-	+	+	+	+
Pfsa33	-	+	+	+	-	-	+	+	+	+
Pfk13	-	+	+	+	-	-	+	+	+	+
Pfk21	-	+	+	+	-	-	+	+	+	+
Pfk22	-	+	+	+	-	-	+	+	+	+

+: indicates positive, -: indicates negative

4.1.5. In vitro evaluation of isolated *Pseudomonas fluorescen* against *Alternaria solani*

Pseudomonas fluorescens isolates coded PFS12, PFS21, PFS22, PFK13, PFK21, PFK22, PFSA31, PFSA32 and PFSA33 were selected based on preliminary experiments, along with a standard *P.fluorescens* strain obtained from EPHI, and anti-fungi chemicals (mancozeb) were evaluated for their in vitro antagonism activity against *A. solani*. Each treatment was replicated three times. The experimental design was a Complete Randomized Design (CRD) in triplicate. Data were taken on the growth of the pathogen. The radial growth of the pathogen's colonies (measured in centimeters) was recorded daily, beginning on the third day after the bio control agents (BCAs) were introduced, until the thirteenth day, when no further growth was observed in the control plates. The selected *P.fluorescens* isolates, anti-fungi chemicals (mancozeb) and the standard strain significantly inhibited the radial growth of *A.solani* from the third day onward, with suppression continuing until no additional colony expansion was detected in the control plates. A total of nine *P.fluorescens* isolates, standard check (mancozeb), and control (*Pseudomonas*) strain were screened for their inhibitory effect on the radial growth of *A. solani* using the dual culture technique. All isolates demonstrated moderate growth inhibition against *A.solani*. The pathogen's growth in dual culture plates continued until it reached the leading edge of the antagonists. In the in vitro antagonism activity of the radial growth of pathogen, radial growth of treatment and percent growth inhibition did not significantly (at $p>0.05$) differ among treatments on the day after transplanting. Significant ($p\leq0.05$) increases were recorded for the radial growth of pathogen, radial growth of treatments and percent growth inhibition in all the treatments with time. Comparison of means was done using Fischers' protected LSD test (at $p\leq0.05$) using SPSS version 27.1 and mean interaction effect was performed using Statstix 10 (Appendix 6.11). Among the different isolates of *P.fluorescens* and anti- fungi chemicals (mancozeb),the standard strain exhibited the highest suppression, recording the least pathogen colony diameter (2.40 cm) , followed by PFS12 (2.49 cm), PFK13 (2.57 cm) and PFSA31 (2.58) respectively. The standard *P.fluorescens* strain exhibited the highest percent growth inhibition (57.65%), followed by PFS12 (56.04%), PFK13 (55.04%), and PFK21 showed the lowest antagonistic effect (52.91%). Isolates like PFS12 and PFK13 displayed moderate inhibitory effects, while the standard *P.fluorescens* strain demonstrated a stronger antagonistic potential. In this study, no zones of growth inhibition were observed between the colonies of *P.fluorescens* and those of *A.solani*. The detailed in vitro antagonism activity of *P.fluorescens* isolates, control and standard check was summarized in Figure 4.6 and 4.7, Table 4.5 and 4.6.

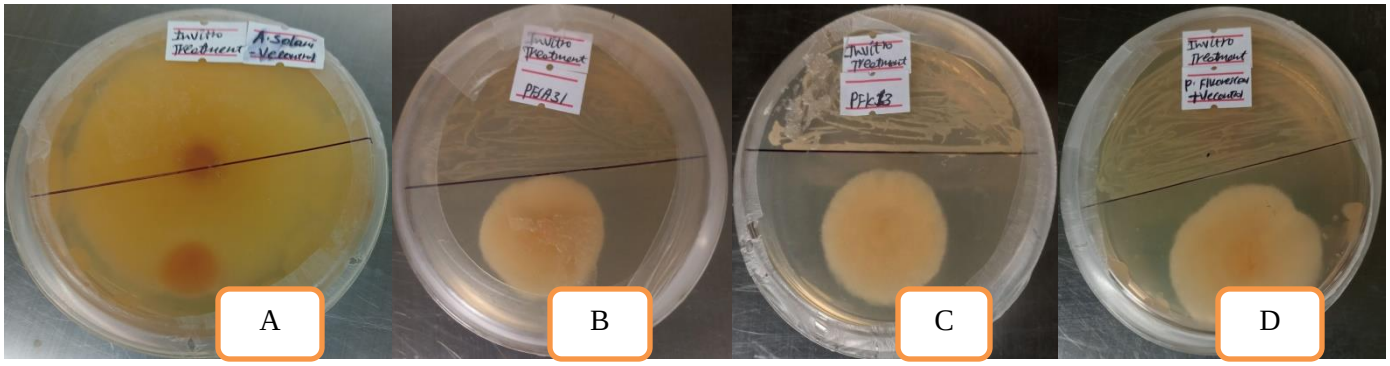


Figure 4.6: Effects of bacterial antagonists on the radial growth of isolated *Alternaria solani*

A: *Alternaria solani* (control), B: PFSA31 against *Alternaria solani, C:* PFK13 against *Alternaria solani*, and D: standard *Pseudomonas fluorescens* against *Alternaria solani.*

Table 4.5: Antagonistic activity of isolated *Pseudomonas fluorescens*, standard check (Mancozeb) and control (standard *P.fluorescens*) against *Alternaria solani* with treatments

Experimental site	Treatments	Radial growth of pathogens(cm)	Radial growth of treatments(cm)	Inhibition (%)
	A. solani	5.81a	.00e	.00g
	PFS12	2.49e	3.32ab	56.04b
	PFS21	2.63c	3.18cd	53.98e
	PFS22	2.68b	3.12c	53.07f
	PFK13	2.57d	3.24bc	55.04c
	PFK21	2.71b	3.10d	52.91f
	PFK22	2.60cd	3.21a	54.23de
In vitro	PFSA31	2.58d	3.23c	54.65cde
	PFSA32	2.70b	3.11d	53.05f
	PFSA33	2.60cd	3.21c	54.61cde
	Mancozen	2.58d	3.23c	54.84cd
	St.P.fluorescens	2.40f	3.41a	57.65a
	Grand Mean	2.8607	2.9465	50.006
	C.V (%)	1.74	4.46	2.31
	LSD ($p \leqslant 0.05$)	0.0327	0.0866	0.7619

Means followed by the same letter (s) in the same column did not significantly different (at 5%). *Pseudomonas fluorescens* isolates: PFS12 (*Pseudomonas fluorecsens* from Sirinka), PFS21(*Pseudomonas fluorecsens* from Sirinka), PFS22 (*Pseudomonas fluorecsens* from Sirinka), PFK13 (*Pseudomonas fluorecsens* from Kobo), PFK21 (*Pseudomonas fluorecsens* from Kobo), PFK22 (*Pseudomonas fluorecsens* from Kobo), PFSA31 (*Pseudomonas fluorecsens* from Sanka), PFSA32 (*Pseudomonas fluorecsens* from Sanka) and PFSA33 (*Pseudomonas fluorecsens* from Sanka), St.*P.fluorescens*: Positive control, mancozeb: standard check, *A.solani*: Negative control, mean: grand mean, C.V (%): coefficient of variation, LSD: least significant difference.

Table 4.6: Antagonistic activity of isolated *Pseudomonas fluorescens*, standard check (Mancozeb) and control (standard *P.fluorescens*) against *Alternaria solani* with days

Experimental Site	Day	Mean of RGP (cm)	Mean of RGT (cm)	Mean of PGI (%)
	13	3.9619a	4.3781a	52.487a
	11	3.8475b	4.1425b	51.840b
	9	3.7039c	3.8294c	50.787c
	7	2.5292d	2.4708d	49.354d
In vitro (In laboratory)	5	1.6858e	1.5608e	48.038e
	3	1.4358f	1.2975f	47.530e
	Grand Mean	2.8607	2.9465	50.006
	C.V (%)	1.74	4.46	2.31
	LSD ($p \leqslant 0.05$)	0.0231	0.0612	0.5388

Means of RGP and RGT were significantly different from one another (at 5%) but means of PGI did not significantly different from one another (at 5%). Day: day after plating bacterial antagonists, RGP: radial growth of pathogen, RGT: radial growth of treatment, PGI: percent growth inhibition, Mean: grand mean, C.V (%): coefficient of variation, LSD: least significance difference.

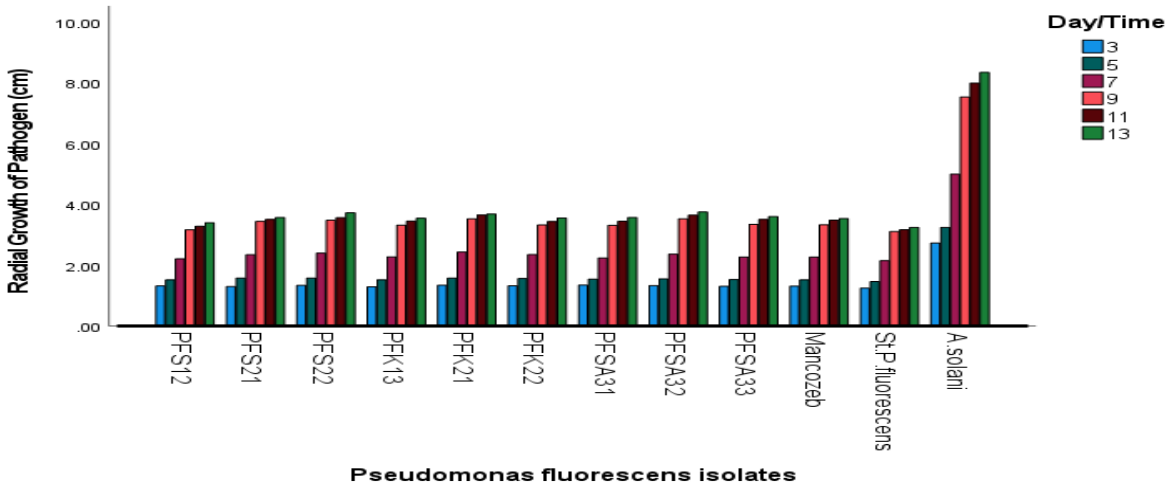


Figure 4.7: Mean diameter of radial growth of pathogen in the presence of isolated *Pseudomonas fluorescens*

4.1.6. In vivo efficacy testing of isolated *Pseudomonas fluorescens* in the managing of tomato early blight

In this study, six promising BCAs with notable inhibitory effects on the in vitro growth of *Alternaria solani* were selected for evaluation. From these, four treatments were chosen for greenhouse trials to assess their effectiveness in controlling early blight in tomatoes. The BCAs included three isolates of *Pseudomonas fluorescens* (PFS12, PFK13, and PFSA31) and one strain of *Pseudomonas fluorescens*. Water served as the control treatment, while mancozeb was used as the standard chemical check. Each treatment was replicated three times. The experiment followed by Complete Randomized Design with three replications. Mean comparisons were performed using Fisher's protected LSD test (at $p \leq 0.05$) with SPSS version 27.1 and mean interaction effect was performed using Statstix 10. Results showed that percent disease incidence was significantly lower in all BCA treatments compared to the control (water). The disease incidence observed with mancozeb was comparable to that of BCAs. Similarly, percent disease severity and the percent disease index were significantly lower in all treatments relative to the control (water). Disease incidence ranged from 6.26% to 7.79% across all treatments, while the control treatment (water) had a significantly higher incidence of 15.94%. Disease severity ranged from 5.67% to 6.75% for all treatments, whereas the control treatment recorded a significantly higher severity of 14.22%. The percent disease index ranged from 24.29% to 28.47% for all treatments, while the control treatment had a significantly higher disease index of 60.33%. The findings suggest that BCAs are effective in managing early blight in tomatoes under greenhouse conditions, reducing growth with the disease.

4.1.6.1. Percent disease incidence for early blight in tomato plants treated with the various antagonists

In the greenhouse experiment, the percent disease incidence for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting. Over time, a significant ($p\leq0.05$) increase in disease incidence was observed in all treatments. However, all treatments significantly ($p\leq0.05$) reduced the percent disease incidence compared to the control treatment (water) throughout the experiment (Appendix 6.11). Specifically, on the 10^{th}, 40^{th}, 50^{th}, and 60^{th} days after transplanting, the standard check (mancozeb) and *P.fluorescens* isolates coded PFS12, PFK13, and PFSA31 were significantly ($p\leq0.05$) lowered the percent disease incidence compared to the control (water). The percent disease incidence ranged from 6.255% to 7.7889% for all treatments, while the control (water) treatment recorded a significantly moderate disease incidence of 15.944%. Significant differences ($p\leq0.05$) in percent disease incidence were observed between all treatments and the control (water) from the 10^{th} to the 60^{th} day after transplanting (Table 4.6). This study demonstrated the efficacy of selected microbial antagonists in reducing early blight on tomato plants under greenhouse conditions. The application of these antagonists significantly protected tomato leaves from *A.solani* infection and prevented the pathogen from spreading across leaf surfaces. By mitigating the impact of *A. solani,* the antagonists helped maintain the photosynthesis process, thereby supporting overall plant health and productivity. Detailed data on the percent disease incidence for *P. fluorescens* isolates, the control, and the standard check are summarized in Table 4.7 and Figure 4.8.

Table 4.7: Percent disease incidence for early blight in tomato plants treated with the various antagonists with treatments and days

Experimental site	Treatments	Mean of PDI	Day	Mean of PDI
	PFS12	6.7167cd	60	7.106d
	PFK13	6.8833c	50	7.583d
	PFSA31	6.2556d	40	8.511c
In vivo (Greenhouse)	St.*P.fluorescens*	6.4778cd	30	10.322b
	Mancozeb	7.7889b	20	10.961a
	Water	15.944a	10	5.583e
	Grand Mean	8.3444		8.3444
	C.V (%)	10.41		10.41
	LSD ($p\leqslant0.05$)	0.5777		0.5777

Means followed by the same letter (s) in the same column and experimental site did not significantly different (at 5%) for both treatment and day. LSD: Least significant difference, % CV: Percent of coefficient of variation, *Pseudomonas fluorescens* isolates: PFS12, PFK13 and PFSA31, St.*P.fluorescens:* Standard *Pseudomonas fluorescens,* Mancozeb: Synthetic fungicide, water: control, PDI: percent disease index.

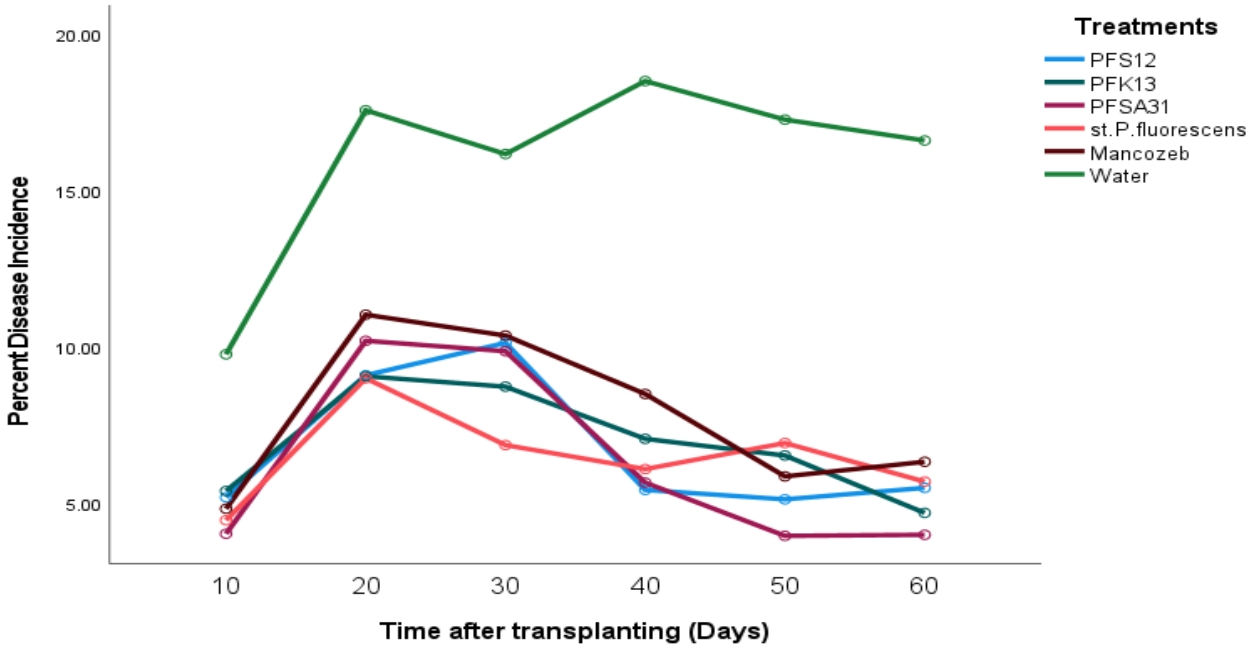


Figure 4.8: Effect of bio-control agents' on the percent disease incidence for tomato early blight disease progress curve of marginal mean at greenhouse

4.1.6.2. Percent disease severity for early blight in tomato plants treated with the various antagonists

In the greenhouse, the percent disease severity for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting. Over time, a significant ($p\leq0.05$) increase in disease severity was observed in all treatments. However, all treatments significantly ($p\leq0.05$) reduced the percent disease severity compared to the control (water) treatment as the experiment progressed (Appendix 6.13). The percent disease severity ranged between 5.667% and 6.750% for all treatments; while the control (water) treatment recorded a significantly moderate disease severity of 14.222%.The percent disease severity was somewhat lower in pots treated with mancozeb compared to those treated with the various microbial antagonists, though the differences were not substantial. Detailed data on the percent disease severity for *P.fluorescens* isolates, the control, and the standard check were summarized in Table 4.8 and Figure 4.9. This study highlights the effectiveness of the tested treatments in reducing disease severity and protecting plants under greenhouse conditions.

Table 4.8: Percent disease severity for early blight in tomato plants treated with the various antagonists with treatments and days

Experimental site	Treatments	Mean of PDS	Day	Means of PDS
	PFS12	6.750b	60	6.139e
	PFK13	6.222bc	50	6.861d
	PFSA31	6.111cd	40	7.889c
In vivo (Greenhouse)	St.*P.fluorescens*	5.667d	30	9.611b
	Mancozeb	6.389bc	20	10.333a
	Water	14.222a	10	4.528f
	Grand Mean	7.5602		7.5602
	C.V (%)	11.05		11.05
	LSD ($p \leqslant 0.05$)	0.5552		0.5552

Means followed by the same letter (s) in the same column and experimental site did not significantly different (at 5%) for treatment but means were significantly different from one another (at 5%) for day. LSD: Least significant difference, % CV: Percent of coefficient of variation, *Pseudomonas fluorescens* isolates: PFS12, PFK13 and PFSA31, St.*P.fluorescens*: Standard *Pseudomonas fluorescens*, Mancozeb: Synthetic fungicide, water: control, PDS: percent disease severity.

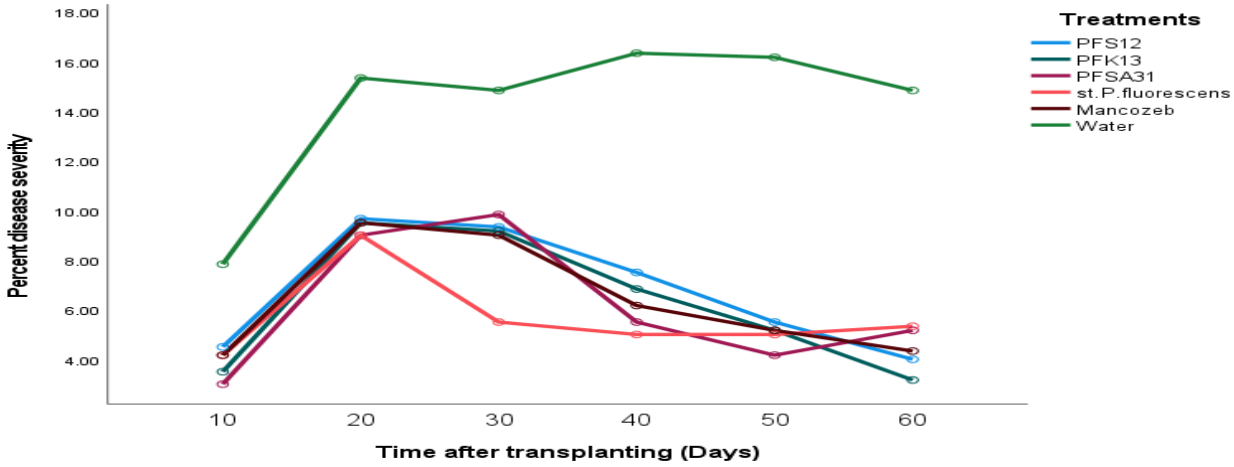


Figure 4.9: Effect of bio control agents' on percent disease severity for tomato early blight disease progress curve of marginal mean at greenhouse

4.1.6.3. Percent disease index for early blight in tomato plants treated with the various antagonists

In the greenhouse, the percent disease index for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting. Over time, significant ($p\leq0.05$) increases in the percent disease index were observed in all treatments. However, all treatments significantly ($p\leq0.05$) reduced the percent disease index compared to the control (water) treatment as the experiment progressed (Appendix 6.15). The percent disease index ranged between 24.289% and 28.467% for all treatments, while the control treatment recorded a significantly higher disease index of 60.333%. The effects of mancozeb in reducing the percent disease index were significantly ($p\leq0.05$) different from those of the various microbial antagonists, demonstrating its superior efficacy. Detailed data on the percent disease index for the treatments, including *P.fluorescens* isolates, the control, and the standard check, were summarized in Table 4.9 and Figure 4.10. These findings underscore the effectiveness of the tested treatments in minimizing disease index under greenhouse conditions.

Table 4.9: Percent disease index severity for early blight in tomato plants treated with the various antagonists with treatments and days

Experimental site	Treatment	PDIx	Day	Mean of PDIx
	PFS12	26.933c	60	26.489e
	PFK13	26.211cd	50	28.889d
	PFSA31	24.733 de	40	32.800c
In vivo (Greenhouse)	St.*P.fluorescens*	24.289e	30	39.867b
	Mancozeb	28.467b	20	42.700a
	Water	60.333a	10	20.222f
	Grand Mean	31.828		31.828
	C.V (%)	7.08		7.08
	LSD ($p\leqslant0.05$)	1.4974		1.4974

Means followed by the same letter (s) in the same column and experimental site did not significantly different (at 5%) for treatments but means were significantly different from one another (at 5%) for day. LSD: Least significant difference, % CV: Percent of coefficient of variation, PFS12, PFK13 and PFSA31, St.*P.fluorescens*: Standard *Pseudomonas fluorescens*, Mancozeb: Synthetic fungicide, water: control, PDIx: percent disease index.

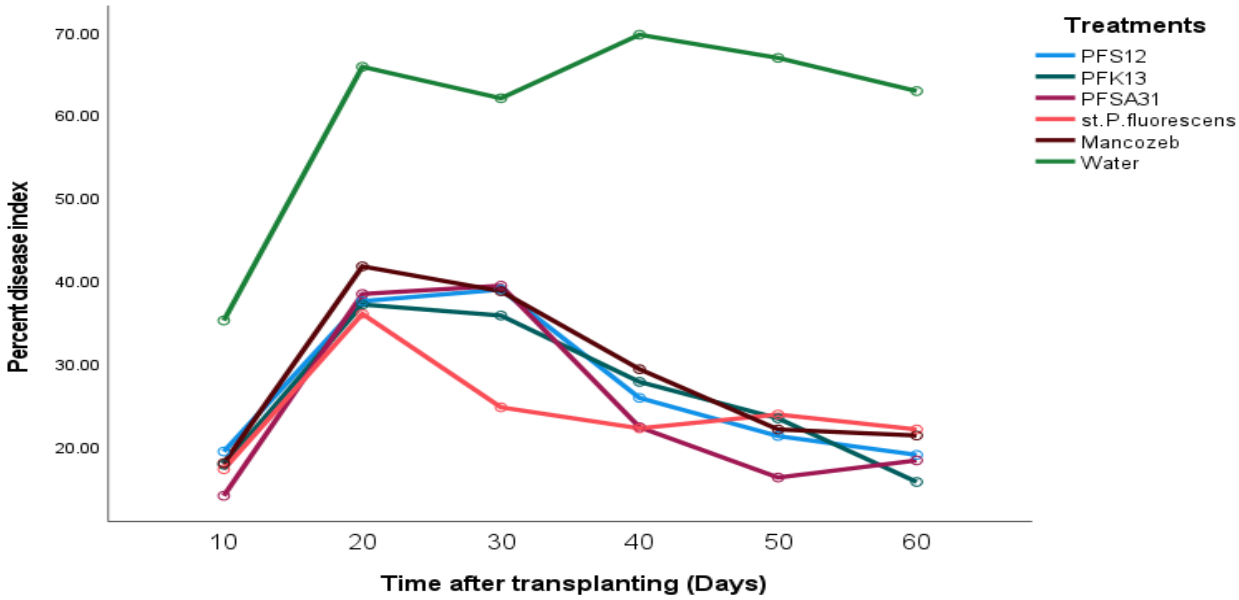


Figure 4.10: Effects of bio-control agents' on percent disease index for tomato early blight disease progress curve of marginal mean at greenhouse

4.1.7. Correlations between tomato early blight disease growth parameters

The in vitro assays demonstrated that *Pseudomonas fluorescens* isolates, particularly PFS12, PFK13, and PFSA31, produced notable zones of inhibition against *A. solani*, indicating strong antagonistic potential. These results translated effectively into greenhouse trials, where the same isolates significantly reduced disease incidence, severity, and index in tomato plants. This correlation suggests that the antimicrobial compounds produced by these isolates in vitro likely play a vital role in suppressing pathogen growth in tomato. In the greenhouse, the percent disease incidence, percent disease severity and percent disease index for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting correlations were recorded between different disease parameters. After a time at greenhouse, the percent disease incidence, percent disease severity and percent disease index was significantly varied (at $p\leq0.05$) and (at $p\leq0.01$).

Table 4.10: Correlations between tomato early blight disease growth parameters

	Percent Disease Incidence	Percent Disease Severity	Percent Disease Index
Percent Disease Incidence	1	.935**	.985**
		.000	.000
	108	108	108
Percent Disease Severity	.935**	1	.982**
	.000		.000
	108	108	108
Percent Disease Index	.985**	.982**	1
	.000	.000	
	108	108	108

The table indicates correlation between tomato early blight disease parameters, **: highly Significant (at $p \leq 0.01$) and *: Significant (at $p \leq 0.05$).

4.2. Discussion

The successful isolation of *Alternaria solani* from infected tomato leaves provided critical morphological evidence consistent with established descriptions of this pathogen. The observed colonies exhibited characteristic pigmentation, with dark coloration on the reverse side and greyish on the front, indicative of typical *A. solani* morphology. Microscopic examination revealed septate, branched hyphae that darkened over time, aligning with previous reports on the pathogen's structural traits. The conidiophores were short, septate, and brownish, while the conidia were notably large, brownish, and occurred singly or in pairs. The septation patterns, with horizontal septa ranging from 2 to 7 and vertical septa from 1 to 4, further corroborated the identity of *A. solani.* These morphological features not only confirm successful pathogen identification but also reflect the characteristic structural variations within *A. solani* strains, which can influence their pathogenicity and response to control measures. The morphological consistency with prior studies highlights the robustness of this identification approach and underscores the importance of detailed morphological characterization in confirming pathogen identity in psychopathological research. These characteristics corroborate findings of (Alhussaen & Khalaf, 2012; Gund, 2015).

A pathogenicity test was performed in the greenhouse to verify the disease-causing ability of the *A. solani* isolates. After fifteen days post-inoculation, early blight symptoms appeared on the tomato plants. The infected leaves showed dark brown, oval to angular spots ranging from 2 to 7 mm in diameter, often exhibiting concentric rings that gradually expanded. These lesions enlarged over time, ultimately causing

complete blighting of the affected leaves. The pathogen *A. solani* was successfully re-isolated from the symptomatic leaves using the standard tissue isolation technique, and the colonies obtained from single spores displayed morphological characteristics that matched those of the original pathogen.The observed symptoms align with those previously reported for *Alternaria solani* infections on tomato leaves in other studies (Kumar *et al*., 2020; Stammler *et al*., 2014).

Pseudomonas fluorescens was isolated from soil samples by utilizing pseudomonas isolation agar. A gram of rhizosphere soil was collected and subjected to serial dilution up to 10^-7. From this dilution, a total of 27 distinct bacterial colonies were obtained. However, only nine of these isolates were selected for morphological characterization, which was based on features such as colony shape, cell shape, color, surface texture, pigmentation, elevation, and Gram reaction .These characteristics were consistent with those previously reported for *P.fluorescens* species (Anitha & Kumudini, 2014; Kumar et al., 2023).

The biochemical characterization of the nine *Pseudomonas fluorescens* isolates confirmed their identity based on several key traits. All isolates tested positive for citrate utilization, catalase activity, oxidase activity, motility, triple sugar iron (TSI) reactions, fluorescent pigmentation, and gelatin liquefaction. Conversely, they were negative for starch hydrolysis, Gram staining, and indole production. These findings support the classification of these isolates as *Pseudomonas fluorescens*, as they display the characteristic positive metabolic traits associated with this species. Additionally, the results highlight the isolates' specific metabolic capabilities, such as citrate utilization and enzyme production, while also reflecting certain limitations, notably in starch degradation and indole synthesis. This biochemical profile underpins their potential role as biocontrol agents in agricultural settings.These characteristics were consistent with those reported for (Belkar & Gade, 2012; Ningaraju, 2020).

In vitro efficacy testing of isolated *Pseudomonas fluorescens* against *Alternaria solani*, under laboratory conditions was performed. Radial growth measurements of *A. solani* colonies were taken daily from the third to the thirteenth day after introducing the bio control agents (BCAs). The selected *P. fluorescens* isolates, along with the antifungal chemical (mancozeb) and a standard strain, significantly suppressed *A. solani* growth from the third day onward, with no further colony expansion in control plates observed by the study's end. Nine *P. fluorescens* isolates were tested using the dual culture technique, along with a positive control, negative control and standard check. Among the antagonists tested, *Pseudomonas fluorescens* isolates PFS12, PFK13, and PFSA31 exhibited the strongest inhibition against the radial growth of *Alternaria solani*. In vitro, the radial growth of pathogens, radial growth of treatments and percent growth inhibition for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting. Over

time, significant ($p \leq 0.05$) increases in the radial growth of pathogens, radial growth of treatments and percent growth inhibition were observed in all treatments. However, all treatments significantly ($p \leq 0.05$) inhibit the radial growth of pathogens compared to the control (*A.solani*) treatment as the experiment progressed The positive control (standard *P. fluorescens*) achieved the most significant suppression, with the smallest pathogen colony diameter of 2.40 cm, followed closely by PFS12 (2.49 cm), PFK13 (2.57 cm), and PFSA31 (2.58 cm). In terms of percent growth inhibition, the control (standard *P. fluorescens*) recorded the highest rate at 57.65%, followed by PFS12 at 56.04%, PFK13 at 55.04% and PFSA31 at 54.65%. Conversely, PFK21 demonstrated the lowest antagonistic effect, yielding a percent growth inhibition of 52.90%. These results underscore the variability in the effectiveness of different *P. fluorescens* isolates in suppressing *A. solani* growth, with certain isolates performing comparably to control (standard *P.fluorescens*) and, the standard chemical (mancozeb). Our findings were consistent with those of Dugassa *et al*. (2021) who reported percent growth inhibition ranging between 47–60% for effective *P. fluorescens* strains against *A. solani* in vitro. This alignment not only validates the efficacy of the selected local isolates but also emphasizes their potential as sustainable alternatives to chemical fungicides in tomato disease management. These results were consistent with earlier studies, affirming that the antagonists evaluated in this research showed differing degrees of effectiveness in inhibiting the in vitro growth of *Alternaria solani*. These results were consistent with earlier studies, affirming that the antagonists evaluated in this research showed differing degrees of effectiveness in inhibiting the in vitro growth of *Alternaria solani*. These findings align with studies by Lalhruaitluangi C *et al*. (2022) and Matumwabirhi and Kulimushi (2020),whose reported that effectiveness of bio-control agents in the management of early blight of tomatoes outperformed bacterial antagonists like *B. subtilis* and *P. fluorescens* in inhibiting fungal pathogens. However, contrasting results were reported by Matumwabirhi and Kulimushi (2020), who found *Pseudomonas fluorescens* isolates to be somewhat similar effective with that of control (standard *P.fluorescens*) against *A. solani*, suggesting that the efficacy of microbial antagonists may vary depending on the pathogen. This study identified growth inhibition zones between *Psudomonas* spp. Colonies and *Alternaria solani,* indicating the production of secondary metabolites that inhibit pathogen growth. This observation aligns with previous research that has been documented various secondary metabolites from *Pseudomonas fluorescens* effective against *Alternaria solani.* Furthermore, the *Pseudomonas fluorescens* isolates demonstrated the ability to mycoparasitize *A. solani* by expanding over its colonies, a finding consistent with earlier studies. In contrast, only faint inhibition zones were observed between *Pseudomonas fluorescens* and *A. solani*, highlighting the variability in antagonist effectiveness based

on the specific pathogen. These finding align with studies by (Neidig *et al.,* 2011),who reported that secondary metabolites of *Pseudomonas fluorescens*.

In vivo efficacy testing of isolated *Pseudomonas fluorescens*, positive control (*Pseudomonas fluorescens*) and the standard fungicide cehmical (mancozeb) in managing early blight (*Alternaria solani)* in tomato plants under greenhouse conditions. In the greenhouse, the percent disease incidence, percent disease severity and percent disease index for tomato early blight did not significantly varied (at $p>0.05$) among treatments starting from the day after transplanting Significantly different (at $p\leq0.05$) in percent disease incidence, severity, and index among treatments from the first 10^{th}day up to last 60^{th}day after transplanting microbial treatments provided early protection against the pathogen. Although disease levels gradually decreased over time in all treatments, the microbial antagonists and mancozeb consistently maintained significantly lower disease growth parameters compared to the untreated control (water), emphasizing their protective role in disease suppression. Regarding disease incidence, microbial antagonists (PFS12, PFK13, and PFSA31), mancozeb and standard *Pseudomonas fluorescens* significantly ($p\leq0.05$) reduced the percentage of disease incidence throughout the experiment. The day after transplanting, disease incidence in treated plants ranged from 6.255% to 7.7889%, whereas the untreated control (water) exhibited a much higher incidence of 15.944%. This indicated that microbial treatments played a crucial role in preventing *A. solani* infection and limiting its spread across leaf surfaces. These result aligned with the finding of Matumwabirhi and Kulimushi (2020) :Naglaa *et al.* (2016) reported that effectiveness of *Trichoderma* Spp., *Bacillus* Spp. and *Pseudomonas fluorescens* in the management of early blight of tomatoes. Similarly, disease severity remained significantly lower in treated plants compared to the control (water), with severity values ranging from 5.667% to 6.75% in treated pots, while the control (water) recorded a severity of 14.222%. Although disease severity was slightly lower in pots treated with *Pseudomonas fluorescens* isolates than in those treated with standard fungicides (mancozeb) and positive control (*P.fluorescens*), the differences were not substantial. This suggested that *P. fluorescens* isolates provided disease control comparable to chemical fungicides and positive control (*P.fluorescens*), supporting their potential use in sustainable disease management. These result collaborate with the finding of Lalhruaitluangi *et al.* (2022); Kulimushi *et al*. (2021) reported that in-vitro evaluation of antagonistic activity of *Trichoderma* spp. and *Pseudomonas fluorescens* isolates against *Alternaria solani* causes early blight of tomato. The percent disease index followed a similar trend, with all treatments showing significant reductions ($p\leq0.05$) compared to the control (water). The disease index ranged from 24.289% to 28.467% in treated tomato plants, while the control (water) recorded a significantly higher disease index of 60.333%. *Pseudomonas fluorescens* isolates exhibited a significantly difference ($p\leq0.05$) in reducing disease

index compared to standard chemicals and *P.fluorescens* strains, confirming its superior efficacy in disease suppression. This result aligned with the finding of Kulimushi *et al.* (2021) who evaluated that potential of *Trichoderma* spp., *Bacillus* subtilis and *Pseudomonas fluorescens* in the management of early blight in tomato. However, the microbial treatments still provided substantial protection by reducing disease development and helping to maintain plant health. The reduction in disease incidence, severity, and index across treated plants suggested that microbial antagonists acted through multiple mechanisms, including competition for nutrients, production of antimicrobial compounds, and induction of systemic resistance, which effectively suppressed *A. solani.* Overall, these findings underscored the potential of microbial antagonists as an environmentally friendly alternative to chemical fungicides. Overall, the results highlight the potential of microbial antagonists, particularly *Pseudomonas fluorescens* isolates, as effective and environmentally friendly alternatives to chemical fungicides. Among the treatments, *P. fluorescens* isolates achieved an average disease severity reduction of up to 72%, compared to 85% for chemical fungicides. Although chemical treatments showed slightly higher efficacy, *P. fluorescens* offered substantial control and could be considered for integration into sustainable disease management strategies. The application of both treatments significantly reduced early blight severity and incidence, preserving leaf integrity and potentially enhancing photosynthetic efficiency and yield. These findings suggest that microbial bio control agents can either supplement or partially replace chemical fungicides, reducing dependency on synthetic chemicals in tomato production.

Generally, the in vitro assays revealed that *Pseudomonas fluorescens* isolates, particularly PFS12, PFK13, and PFSA31, exhibited strong antagonistic activity against *Alternaria solani*, as indicated by the formation of prominent inhibition zones. Notable reductions in disease incidence, severity, and disease index were observed, supporting a positive correlation between in vitro antagonistic potential and in vivo bio control efficacy. This alignment suggests that antimicrobial metabolites produced by these isolates may play a crucial role in inhibiting pathogen growth within the host environment. Initially, no statistically significant differences ($p > 0.05$) were observed among treatments for disease parameters; however, as the disease progressed, significant variations emerged ($p \leq 0.05$ and $p \leq 0.01$), indicating time-dependent effects of the bio control agents. Correlation analysis among disease incidence, severity, and index revealed strong interrelationships, suggesting that these parameters are closely linked and can be used collectively to monitor disease progression and evaluate treatment efficacy. These findings not only reinforce the potential of selected *P. fluorescens* isolates as effective bio control agents against early blight but also highlight the importance of longitudinal assessment in accurately capturing treatment impacts over time.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Tomato (*Solanum lycopersicum*) is a key crop in Ethiopia, providing both nutritional value and economic benefits to farmers, especially in regions like North Wollo. However, tomato production is severely threatened by early blight disease, primarily caused by the fungus *Alternaria solani*. This pathogen can lead to yield losses as high as 80%, resulting in significant economic hardship for smallholder farmers. Traditionally, the management of early blight has relied heavily on chemical fungicides such as mancozeb and chlorothalonil. While these chemicals can be effective, their overuse has led to increased resistance in the pathogen population, as well as concerns about environmental pollution and health risks for both farmers and consumers. Given these challenges, there is a growing need for sustainable and environmentally friendly alternatives. Biological control agents (BCAs), specifically beneficial bacteria like *Pseudomonas fluorescens*, offer a promising solution. These microorganisms are known to suppress plant pathogens through various mechanisms, including competition, antibiosis, and induction of plant resistance. Despite their potential, there has been limited research on the effectiveness of *P. fluorescens* as a bio control agent against early blight in Ethiopia, particularly under local conditions in North Wollo. This study addressed this gap by isolating *P. fluorescens* from the rhizosphere of healthy tomato plants and *A. solani* from infected leaves collected from North Wollo. Laboratory (in vitro) and greenhouse (in vivo) experiments were conducted to evaluate the antagonistic activity of several *P. fluorescens* isolates against *A. solani*. The results showed that isolates PFS12 and PFK13 achieved moderate inhibition of the pathogen's radial growth, with percent growth inhibition values of 56.04% and 55.04%, respectively. These results were comparable to the standard chemical fungicide mancozeb, which showed 54.84% inhibition. In greenhouse trials, the isolate PFSA31 resulted in the lowest disease index (24.73%), which was similar to the chemical control (28.47%) and significantly better than the untreated control (60.33%). In summary, the findings demonstrate that selected *P. fluorescens* isolates can effectively reduce the incidence and severity of early blight in tomatoes, both in laboratory and greenhouse conditions. This suggests that bio control using *P. fluorescens* could serve as a viable, safer alternative to chemical fungicides for managing early blight in tomatoes in North Wollo. The adoption of such biological control methods would not only help protect tomato yields and farmer incomes but also contribute to safer food production and a healthier environment.

5.2. Recommendations

Based on the findings of this study, several steps can be taken to advance the application of the potential *Pseudomonas fluorescens* isolates (PFS12, PFK13, and PFSA31) as effective bio control agents against *Alternaria solani*. These isolates can be developed into commercial bio formulations following optimization of carrier materials and storage conditions to ensure field viability. Extensive field trials across different tomato-growing regions and climatic conditions will be essential to validate their long-term effectiveness and consistency in disease suppression. Further research should explore the molecular mechanisms underlying their antagonistic activity to better understand their mode of action, including gene expression and secondary metabolite production. Additionally, combining these isolates with other biological control agents may enhance their efficacy through synergistic interactions, contributing to integrated disease management strategies. Evaluating the influence of environmental factors such as soil type and moisture on their performance will help tailor application strategies for different agro ecosystems. A comparative cost-benefit analysis with conventional fungicides will be also necessary to assess economic viability, while studies on the reduction of chemical residues in treated crops can highlight the public health and environmental benefits of adopting these biological control solutions in sustainable agriculture

6. REFERENCES

Abdalla, Saifeldeen A, Algam, Soad AA, Ibrahim, Elshiekh A, . . . M, A. (2014). In vitro screening of *Bacillus* isolates for biological control of early blight disease of tomato in shambat soil. *World Journal of Agricultural Research, 2*(2), 47-50.

Abdelmoteleb, A., Moreno-Ramírez, L., Valdez-Salas, B., Seleiman, M. F., El-Hendawy, S., Aldhuwaib, K. J., . . . González-Mendoza, D. (2022). New *Bacillus subtilis* strains isolated from Prosopis glandulosa rhizosphere for suppressing Fusarium spp. and enhancing growth of Gossypium hirsutum L. *Biology, 12*(1), 73.

Adhikari, Pragya, Adhikari, Tika, Louws, Frank, . . . Dilip. (2020). Advances and challenges in bacterial spot resistance breeding in tomato (*Solanum lycopersicum L*.). *International Journal of Molecular Sciences, 21*(5), 1734.

Adhikari, Pragya, Oh, Yeonyee, Panthee, & R, D. (2017). Current status of early blight resistance in tomato: an update. *International journal of molecular sciences, 18*(10), 2019.

Adhikari, Tika B, Muzhinji, Norman, Halterman, Dennis, . . . J, F. (2021). Genetic diversity and population structure of *Alternaria* species from tomato and potato in North Carolina and Wisconsin. *Scientific reports, 11*(1), 17024.

Ahmad, T., Nie, C., Cao, C., Xiao, Y., Yu, X., & Liu, Y. (2024). First record of *Alternaria tenuissima* causing Aloe barbadensis leaf blight and leaf spot disease in Beijing, China. *Crop Protection, 175*, 106447.

Ahmed, R., Afzal, T., Ikram, M. T., Anwer, M., ur Rehman, S., Faisal, M., . . . Amin, M. A. (2024). Vitro and In-Vivo Testing of Early Blight of Tomato (*Alternaria solani*). *Plant and Environment, 5*(02), 12-21.

Aigle, Bertrand, Lautru, Sylvie, Spiteller, Dieter, . . . Jean-Luc. (2014). Genome mining of *Streptomyces* ambofaciens. *Journal of Industrial Microbiology and Biotechnology, 41*(2), 251-263.

Akram, M., Munir, N., Daniyal, M., Egbuna, C., Găman, M.-A., Onyekere, P. F., & Olatunde, A. (2020). Vitamins and Minerals: Types, sources and their functions. *Functional foods and nutraceuticals: bioactive components, formulations and innovations*, 149-172.

Albert, E., Segura, V., Gricourt, J., Bonnefoi, J., Derivot, L., & Causse, M. (2016). Association mapping reveals the genetic architecture of tomato response to water deficit: focus on major fruit quality traits. *Journal of Experimental Botany, 67*(22), 6413-6430.

Alelign, S. (2021). Evaluation of the efficacy of *Trichoderma* and *Pseudomonas* species against bacterial wilt *Ralstonia* isolates of tomato (*Lycopersicum* species). *African Journal of Microbiology Research, 15*(5), 262-271.

Alhussaen, & Khalaf. (2012). Morphological and physiological characterization of *Alternaria solani* isolated from tomato in Jordan Valley. *Research Journal of Biological Sciences, 7*(8), 316-319.

Almoneafy, Abdulwareth A, Kakar, Kaleem Ullah, Nawaz, Zarqa, . . . Guan-Lin. (2014). Tomato plant growth promotion and antibacterial related-mechanisms of four rhizobacterial *Bacillus* strains against *Ralstonia solanacearum*. *Symbiosis, 63*, 59-70.

Animashaun, & Mufutau. (2015). *The use of hot water treatment by small holders for the control of alternaria alternata, the cause of black mould disease of tomato.* University of Essex,

Anitha, & Kumudini. (2014). Isolation and characterization of *fluorescent pseudomonads* and their effect on plant growth promotion. *Journal of Environmental biology, 35*(4), 627.

Asad, & Ahmad, S. (2022). Mechanisms of action and biocontrol potential of *Trichoderma* against fungal plant diseases-A review. *Ecological Complexity, 49*, 100978.

Attia, Mohamed S, El-Sayyad, Gharieb S, Abd Elkodous M, El-Batal, & I, A. (2020). The effective antagonistic potential of plant growth-promoting rhizobacteria against *Alternaria solani*-causing early blight disease in tomato plant. *Scientia Horticulturae, 266*, 109289.

Bagul, Vishakha, Magar, Sunita, Markad, Harshavardhan, . . . Pradnya. (2023). Isolation and Characterization of *Pseudomonas fluorescens* Isolates from Different Rhizospheric Soils of Latur District of Maharashtra.

Basit, Abdul, Shah, Syed Tanveer, Muntha, Sidra Tul, . . . I, H. (2021). Plant growth-promoting Rhizobacteria (PGPR) as biocontrol agents for viral protection. *Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management*, 187-225.

Begna, & Temesgen. (2020). Major challenging constraints to crop production farming system and possible breeding to overcome the constraints. *International Journal of Research Studies in Agricultural Sciences (IJRSAS), 6*(7), 27-46.

Belkar, & Gade. (2012). Biochemical characterization and growth promotion activities of *Pseudomonas fluorescens*. *J. Pl. Dis. Sci*, *7*(2), 170-174.

Bergougnoux, & Véronique. (2014). The history of tomato: from domestication to biopharming. *Biotechnology advances*, *32*(1), 170-189.

Beyene, G. E. G. T. M. (2019). Evaluation of Tomato (*Solanum lycopersicum L*. mill) Varieties for Yield and Fruit Quality in Ethiopia. A Review. *Evaluation*, *9*(17).

Bhagat, & Pratibha. (2018). *Epidemiology and Management of Major Diseases of Tomato.* JNKVV, Jabalpur,

Biswas, Asib, Islam, Ashraful, Ahmed, Mosaddek, . . . Abdul. (2020). Different sources nitrogen based fertilizers on growth, yield and nutritional quality of tomato (*Lycopersicon esculentum Mill*.). *Acta Sci. Malays.(ASM)*, *4*, 39-44.

Blancard, & Dominique. (2012). *Tomato diseases*: Academic Press San Diego, CA, USA:.

Bora, & Mayurakshi. (2023). *Study On Major Insect Pests Of Tomato And Their Eco-Friendly Management.* Assam Agricultural University,

Boro, Manswama, Sannyasi, Shuvankar, Chettri, Dixita, . . . Kumar, A. (2022). Microorganisms in biological control strategies to manage microbial plant pathogens: a review. *Archives of microbiology*, *204*(11), 666.

Boyno, Gökhan, Demir, Semra, Danesh, & Rezaee, Y. (2022). Effects of some biological agents on the growth and biochemical parameters of tomato plants infected with Alternaria solani (Ellis & Martin) Sorauer. *European Journal of Plant Pathology*, *162*(1), 19-29.

Brahimi, Mohammed Hedija, Boukhalfa, Kamel, Moussaoui, & Abdelouahab. (2017). Deep learning for tomato diseases: classification and symptoms visualization. *Applied Artificial Intelligence*, *31*(4), 299-315.

Cheruiyot, & Jepto, E. (2023). *Biocontrol Potential Of Trichoderma Harzianum Rifai,(1969) And Beauveria Bassiana (Bals.-Criv.) Vuill.(1912) Against Phytopthora Infestans And Alternaria Solani Causing Blight In Tomato.* University Of Eldoret,

Dhaval, Pansuriya, Shete, Poonam P, Faraaz, Mohammed Hedija, . . . Dipen. (2021). Early blight (*Alternaria solani*) etiology, morphology, epidemiology and management of tomato. *Pharma Innovation*, *10*, 1423-1428.

Dongre, Mayur Arun, Ashraf, Mohd Shaikhul, Koshariya, Ashok Kumar, & P, R. (2023). *Plant Pathology and Plant Diseases*: AG publishing house (AGPH Books).

Dorjey, STANZIN, Gupta, VISHAL, Razdan, & VK. (2017). Evaluation of *Pseudomonas fluorescens* isolates for the management of Fusarium oxysporum f. sp. lycopersici and Rhizoctonia solani causing wilt complex in tomato. *Indian Phytopathol, 70*, 127-130.

Dourado, M. N., Souza, L. A., Martins, P. F., Peters, L. P., Piotto, F. A., & Azevedo, R. A. (2014). *Burkholderia* sp. SCMS54 triggers a global stress defense in tomato enhancing cadmium tolerance. *Water, Air, & Soil Pollution, 225*, 1-16.

Dugassa, Alemayehu, Alemu, Tesfaye, Woldehawariat, & Yitbarek. (2021). In-vitro compatibility assay of indigenous *Trichoderma* and *Pseudomonas* species and their antagonistic activities against black root rot disease *(Fusarium solani*) of faba bean (Vicia faba L.). *BMC microbiology, 21*, 1-11.

Durairaj, Chinnasamy, Sambathkumar, Subramaniam, Mohankumar, & Subbarayalu. (2016). Integrated pest management of cruciferous vegetables. *Integrated Pest Management of Tropical Vegetable Crops*, 149-165.

Emana, B., Afari-Sefa, V., Nenguwo, N., Ayana, A., Kebede, D., & Mohammed, H. (2017). Characterization of pre-and postharvest losses of tomato supply chain in Ethiopia. *Agriculture & Food Security, 6*, 1-11.

Erba, D., Casiraghi, M. C., Ribas-Agustí, A., Cáceres, R., Marfà, O., & Castellari, M. (2013). Nutritional value of tomatoes *(Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniques. *Journal of Food Composition and Analysis, 31*(2), 245-251.

Gatahi, D. M. (2020). Challenges and opportunities in tomato production chain and sustainable standards. *International Journal of Horticultural Science and Technology, 7*(3), 235-262.

Gayathri, M., Sharanya, R., Renukadevi, P., Malathi, V. G., Ghosh, A., Nallusamy, S., . . . Alkahtani, S. (2025). Genomic configuration of *Bacillus subtilis (*NMB01) unveils its antiviral activity against Orthotospovirus arachinecrosis infecting tomato. *Frontiers in Plant Science, 16*, 1517157.

Gemechis, Ambecha O, Struik, Paul C, Emana, & Bezabih. (2012). Tomato production in Ethiopia: constraints and opportunities. *Tropentag 2012, International Research on Food Security, Natural Resource Management and Rural Development. Resilience of Agricultural Systems against Crises: Book of Abstracts, 373*.

Gemechu, G. E., & Beyene, T. M. (2019). Evaluation of tomato (*Solanum lycopersicum L*. mill) varieties for yield and fruit quality in Ethiopia. A review. *Evaluation, 89*(10.7176).

Geoffrey, Sigei K, Hillary, Ngeno K, Kibe, M Antony, . . . C, M. (2014). Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. *International Journal of Business and Management, 9*(9), 205.

Giovannucci, Edward, Rimm, Eric, Liu, Yan, . . . Walter. (2002). A Prospective Study of Tomato Products, Lycopene, and. *Journal of the National Cancer Institute, 94*(5), 391.

Golińska, P. (2020). Synthesis of nanoparticles by actinomycetes: mechanism and applications. In *Microbial Nanotechnology* (pp. 1-19): CRC Press.

González-Concha, L. F., Ramírez-Gil, J. G., Mora-Romero, G. A., García-Estrada, R. S., Carrillo-Fasio, J. A., & Tovar-Pedraza, J. M. (2023). Development of a scale for assessment of disease severity and impact of tomato brown rugose fruit virus on tomato yield. *European Journal of Plant Pathology, 165*(3), 579-592.

Goudjal, Yacine, Zamoum, Miyada, Sabaou, Nasserdine, . . . Abdelghani. (2016). Potential of endophytic *Streptomyces* spp. for biocontrol of Fusarium root rot disease and growth promotion of tomato seedlings. *Biocontrol Science and Technology, 26*(12), 1691-1705.

Guan, Zhengfei, Biswas, Trina, Wu, & Feng. (2018). The US tomato industry: An overview of production and trade: FE1027, 9/2017. *EDIS, 2018*(2).

Güler, İ., & Küçük, Ç. (2010). Isolation and Characterization of *Pseudomonas* Isolates for Antagositic Activities. *Journal of Applied Biological Sciences*(3), 25-30.

Gund, P. (2015). Studies on variability in *Alternaria solani* causing early blight of tomato. *M. Sc.(Agri.) Thesis, MPKV Rahuri, Maharashtra. India*.

Gupta, S. K., & Thind, T. (2018). *Disease problems in vegetable production*: Scientific Publishers.

Haider, & Ehsan. (2020). *Assessment Of Phytoextracts Against Leaf Spot Of Brinjal Caused By Alternaria Alternata (Fr.) Keissler.* University Of Agriculture, Faisalabad,

Hammami, Inés, Hsouna, Anis Ben, Hamdi, Naceur, . . . Ali, M. (2013). Isolation and characterization of rhizosphere bacteria for the biocontrol of the damping-off disease of tomatoes in Tunisia. *Comptes Rendus. Biologies, 336*(11-12), 557-564.

Hasani, Amin, Muhammad Ahmar, Kariminik, Ashraf, Issazadeh, & Khosrow. (2014). *Streptomycetes*: characteristics and their antimicrobial activities.

Hasnain, Muhammad, Chen, Jiawei, Ahmed, Nazeer, . . . Ping. (2020). The effects of fertilizer type and application time on soil properties, plant traits, yield and quality of tomato. *Sustainability, 12*(21), 9065.

Hendricks, P. (2012). *Life cycle assessment of greenhouse tomato (Solanum lycopersicum L.) production in Southwestern Ontario.* University of Guelph,

Hol, WH Gera, Bezemer, T Martijn, Biere, & Arjen. (2013). Getting the ecology into interactions between plants and the plant growth-promoting bacterium *Pseudomonas fluorescens*. *Frontiers in plant science, 4*, 81.

Hussain, Azhar, Ali, Shaukat, Abbas, Haider, . . . Wali, S. (2019). Spatial distribution of early blight disease on tomato, climatic factors and bioefficacy of plant extracts against *Alternaria solani*. *Acta Scientiarum Polonorum. Hortorum Cultus, 18*(6), 29-38.

Hussain, & Shazia. (2024). Advancing Plant Health Management: Challenges, Strategies, and Implications for Global Agriculture. *International Journal of Agriculture and Sustainable Development, 6*(2), 73-89.

Imran, & Muhammad. (2022). *Biological Control Of Tomato Early Blight Disease Caused By Alternaria Solani*. King Abdulaziz University,

Jambhulkar, Prashant Prakash, Jambhulkar, Nitiprasad, Meghwal, Madanlal, . . . Shankar, G. (2016). Altering conidial dispersal of *Alternaria solani* by modifying microclimate in tomato crop canopy. *The Plant Pathology Journal, 32*(6), 508.

Jones, Jeffrey Bryant, Zitter, Thomas Andrew, Momol, Timur M, . . . A, S. (2014). Compendium of tomato diseases and pests.

Joseph, Abiodun, Igbinosa, Osaretin Best, Alori, Elizabeth T, . . . Patrick, A. (2017). Effectiveness of *Pseudomonas* species in the management of tomato early blight pathogen *Alternaria solani*. *African journal of microbiology research, 11*(23), 972-976.

Kaae, & Helen, G. (2023). *Growth, Yield, Physiological And Fruit Quality Response Of Two Tomato (Solanum Lycoperscion L.) Hybrids To Different Irrigation Regimes Under Field Conditions.*

Kaboré, Kabakdé, Konaté, Kiéssoun, Sanou, Abdoudramane, . . . Hama, M. (2022). Tomato by-products, a source of nutrients for the prevention and reduction of malnutrition. *Nutrients, 14*(14), 2871.

Kenneth, & Oduor. (2016). *Agro-morphological and nutritional characterization of tomato landraces (Lycopersicon species) in Africa.* University of Nairobi,

Khadidja, & Kadari. (2020). Isolation, Characterization of Phytopathogenic Fungi *Alternaria* sp., and Physico-Chemical Study. *Faculty of Natural and Life Science Departement of Biologie*.

Kihoro, & Esther. (2014). *Use of Mulches to Manage Spider Mites and White Flies in Open Field and Greenhouse Tomatoes.* University of Nairobi,

Kipgen, Thalhun Lhingkhanthem, Bora, Lohit Chandra, Goswami, Gunajit, . . . Chandra, K. (2021). Isolation and characterization of *fluorescent Pseudomonas* with bio-control potential against Ralstonia solanacearum. *Indian Phytopathology, 74*, 1055-1064.

Knapp, S., & Peralta, I. E. (2016). The tomato (*Solanum lycopersicum L*., Solanaceae) and its botanical relatives. *The tomato genome*, 7-21.

Kulimushi, Séraphin M, Muiru, William M, Mutitu, & W, E. (2021). Potential of *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* in the management of early blight in tomato. *Biocontrol Science and Technology, 31*(9), 912-923.

Kulus, & Dariusz. (2022). Genetic diversity for breeding tomato. *Cash Crops: Genetic Diversity, Erosion, Conservation and Utilization*, 505-521.

Kumar, Amarjeet, Kumar, Varun, Gull, Amir, . . . Ahmad, G. (2020). Tomato (*Solanum Lycopersicon*). *Antioxidants in vegetables and nuts-Properties and health benefits*, 191-207.

Kumar, KP Sampath, Paswan, Shravan, Srivastava, & Shweta. (2012). Tomato-a natural medicine and its health benefits. *Journal of Pharmacognosy and Phytochemistry, 1*(1), 33-43.

Kumar, Rajath, Santhosh, GP, Ganesha, & BH. (2023). Forest ecosystem soil attributes influence density of *Pseudomonas fluorescens. Indian Journal of Ecology, 50*(3), 778-784.

Kushwaha, D. a. (2020). *FUNGI (Viruses, Bacteria and Mycoplasma)* (Vol. 1): Dr. AK KUSHWAHA.

Lahlali, Ezrari, Radouane, Kenfaoui, Esmaeel, El Hamss, . . . Barka. (2022). Biological control of plant pathogens: A global perspective. Microorganisms 2022, 10, 596. In: s Note: MDPI stays neu-tral with regard to jurisdictional claims in ….

Lalhruaitluangi C, Tiameren AoN, Daiho L, Banik, Susanta, Ao, . . . SP, K. (2022). In-vitro Evaluation of Antagonistic Activity of Native *Trichoderma* spp. and *Pseudomonas fluorescens* Isolates against *Alternaria solani* Causing Early Blight of Tomato. *International Journal of Plant & Soil Science, 34*(13), 120-127.

Lalhruaitluangi, C., Ao, N. T., Daiho, L., Banik, S., Ao, M. A., & Kanaujia, S. (2022). In-vitro Evaluation of Antagonistic Activity of Native *Trichoderma* spp. and *Pseudomonas fluorescens* Isolates against *Alternaria solani* Causing Early Blight of Tomato. *International Journal of Plant & Soil Science, 34*(13), 120-127.

Lodhi, & Singh, D. (2021). Studies on *Alternaria* blight of Niger and its management.

Mahatma, & Mahatma. (2015). Soil suppressive microorganisms and their impact on fungal wilt pathogens. In *Organic Amendments and Soil Suppressiveness in Plant Disease Management* (pp. 249-274): Springer.

Maji, S., & Chakrabartty, P. (2014). Biocontrol of bacterial wilt of tomato caused by'Ralstonia solanacearum'by isolates of plant growth promoting rhizobacteria. *Australian Journal of Crop Science, 8*(2), 208-214.

Marak, Tanya R, Ambesh, Bharat Singh, Das, & Srikanta. (2014). Cultural, Morphological And Biochemical Variations Of *Alternaria* Solani Causing Diseases On Solanaceous Crops. *The Bioscan, 9*(3), 1295-1300.

Matumwabirhi, & Kulimushi. (2020). *Effectiveness of trichoderma Spp., bacillus Spp. and pseudomonas fluorescens in the management of early blight of tomatoes.* University of Nairobi,

Maurya, Manoj Kumar, Singh, Ramji, Tomer, & Ajay. (2014). In vitro evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen. *Journal of Biopesticides, 7*(1), 43.

Meena, & Marimuthu. (2012). Effect of application methods of *Pseudomonas fluorescens* for the late leaf spot of groundnut management. *Journal of Biopesticides, 5*(1), 14.

Melomey, Leander D, Danquah, Agyemang, Offei, Samuel K, . . . Michael. (2019). Review on tomato *(Solanum lycopersicum, L.*) improvement programmes in Ghana. *Recent advances in tomato breeding and production, 49*.

Mengesha, G., & Hussien, T. (2017). *Management Of Tomato (Solanum Lycopersicum L.) Late Blight [Phytophthora Infestans (Mont.) De Bary] Through Host Plant Resistance And Fungicide Application In Gamo Gofa Zone, Southern Ethiopia.* Haramaya University,

Mishra, & Archana. (2021). Physiological disorders of tomato-an overview. *International Journal of Farm Sciences, 11*(4), 46-50.

Mohammed, Ayomide F, Oloyede, Adejare R, Odeseye, & O, A. (2020). Biological control of bacterial wilt of tomato caused by Ralstonia solanacearum using *Pseudomonas* species isolated from the rhizosphere of tomato plants. *Archives of Phytopathology and Plant Protection, 53*(1-2), 1-16.

Motti, & Riccardo. (2021). The *Solanaceae* family: Botanical features and diversity. *The wild solanums genomes*, 1-9.

Muhie, S. H. (2023). Plant biostimulants in organic horticulture: a review. *Journal of Plant Growth Regulation, 42*(5), 2698-2710.

Müller, N. A., Wijnen, C. L., Srinivasan, A., Ryngajllo, M., Ofner, I., Lin, T., . . . Sinha, N. R. (2016). Domestication selected for deceleration of the circadian clock in cultivated tomato. *Nature genetics, 48*(1), 89-93.

Naglaa, AS Muhanna, Essa, TA, Manal, AH El-Gamal, . . . SM. (2016). Efficacy of free and formulated arbuscular mycorrhiza, *Trichoderma* viride and *Pseudomonas fluorescens* on controlling tomato root rot diseases. *Egyptian Journal of Biological Pest Control, 26*(3), 477.

Naik, K. (2020). Effects of selected biological control agents and plant extracts on early blight disease management and yield of cherry tomato. *Journal of Pharmacognosy and Phytochemistry, 9*(5), 587-592.

Naseer, Muhammad, Persson, Tomas, Hjelkrem, Anne-Grete, . . . Michel. (2022). Life cycle assessment of tomato production for different production strategies in Norway. *Journal of Cleaner Production, 372*, 133659.

Neidig, Nina, Paul, Rüdiger J, Scheu, Stefan, . . . Alexandre. (2011). Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. *Microbial ecology, 61*, 853-859.

Nepali, Bikram, Bhattarai, Sabin, Shrestha, & Jiban. (2018). Identification of *Pseudomonas fluorescens* using different biochemical tests. *International Journal of Applied Biology, 2*(2), 27-32.

Ningaraju, T. (2020). Collection, isolation and characterization of the *Pseudomonas fluorescence*, from Rhizosphere of Different crops (Ragi, Pigeonpea and groundnut). *IJCS, 8*(4), 2429-2433.

Oloo, & Judith. (2013). *Evaluation of local Trichoderma isolates for their efficiency in biological control of Fusarium oxysporum f. sp phaseoli in common bean.* University of Nairobi,

Onchwari, R. G. (2016). *Isolation and characterization of rhizosphere bacteria with potential to improve the plant growth of banana plants in Juja, Kenya.* COHES, JKUAT,

Onduso, J. N. (2014). *Management of bacterial wilt of tomato by use of resistant rootstock.* University of Nairobi,

Opoku, & Asante, B. (2014). *Incidence and severity of major fungal diseases of tomato (solanum lycopersicum l.) in three districts within forest and forest-savannah agro-ecological zones of Ghana.*

Osman, & Towhid, K. (2018). Sandy soils. *Management of soil problems*, 37-65.

Padró, Maria D Arias, Caboni, Emilia, Morin, Karla Azucena Salazar, . . . Víctor. (2021). Effect of *Bacillus subtilis* on antioxidant enzyme activities in tomato grafting. *PeerJ, 9*, e10984.

Pankaj, Rautela, Gupta, Supriya, Azad, CS, . . . RP. (2020). Diseases of Tomato Crops and Their Management. In *Diseases of Fruits and Vegetable Crops* (pp. 181-209): Apple Academic Press.

Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., . . . Matić, S. (2021). A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the mediterranean basin. *Agronomy, 11*(11), 2188.

Panpatte, Deepak, Jhala, Yogeshvari, Shelat, Harsha, . . . Rajababu. (2016). *Pseudomonas fluorescens*: a promising biocontrol agent and PGPR for sustainable agriculture. *Microbial inoculants in sustainable agricultural productivity: Vol. 1: Research perspectives*, 257-270.

Pawaskar, & Mahesh, M. (2023). *Evaluating the Potential of Hypersaline Bacteria as a Biocontrol Agents Against Microbial Pathogens Affecting Chilli (Capsicum Annuum L.) Plant.* Goa University,

Phanindra, Parvathaneni, Vinay, Muchumarri, Paul, Siripurapu Sumanth, & Kotiyal, A. (2024). Regulations of Flowering in Fruit Crop for Higher Yield and Quality Production. *A Monthly Peer Reviewed Magazine for Agriculture and Allied Sciences*, 91.

Ramyabharathi, S., & Raguchander, T. (2014). Mode of action of *Bacillus subtilis* EPCO16 against tomato Fusarium wilt. *Biochem. Cell. Arch, 14*(1), 47-50.

Ravichandra. (2021). *Postharvest Plant Pathology*: CRC Press.

Raza, Waqas, Ghazanfar, Muhammad Usman, Iftikhar, Yasir, . . . Hamid, M. (2016). Management of early blight of tomato through the use of plant extracts. *Management, 1*(5), 1123-1133.

Rodrigues, Tatiana TMS, Maffia, Luiz A, Dhingra, Onkar D, . . . SG, E. (2010). In vitro production of conidia of *Alternaria solani. Tropical Plant Pathology, 35*, 203-212.

Roy, Chapol, Akter, Nafiza, Sarkar, Mohammad, . . . Miskat. (2019). Control of early blight of tomato caused by *Alternaria solani* and screening of tomato varieties against the pathogen. *The Open Microbiology Journal, 13*(1).

Roy, Chapol K, Akter, Nafiza, Sarkar, Mohammad KI, . . . AA, M. (2019). Control of early blight of tomato caused by and screening of tomato varieties against the pathogen. *The Open Microbiology Journal, 13*(1).

Roy, C., Akter, N., Sarkar, M., Pk, M. U., Begum, N., Zenat, E., & Jahan, M. (2019). Control of early blight of tomato caused by Alternaria solani and screening of tomato varieties against the pathogen. *The Open Microbiology Journal, 13*(1).

Safi, Habibullah, Hussain, Shaukat, Shahid, Muhammad, . . . Muhammad. (2020). Incidence and severity of early blight of tomato in Peshawar, Mardan and Malakand divisions and variability amongst the isolates of *Alternaria Solani* Jones and Mart. *International Journal of Agriculture, Environment and Biotechnology, 13*(2), 175-183.

Saha, & Das. (2013). Assessment of losses due to early blight (*alternaria solani* ell. And mart.) and influence of weather factors on disease development in tomato. *Journal of Agrometeorology, 15*(1), 82-85.

Saha, & Poly. (2013). Assessment of losses due to early blight (*alternaria solani* ell. And mart.) and influence of weather factors on disease development in tomato. *Journal of Agrometeorology, 15*(1), 82-85.

Salomon, D., Bonshtien, A., Mayrose, M., Zhang, C., Shokat, K. M., & Sessa, G. (2009). Bypassing kinase activity of the tomato Pto resistance protein with small molecule ligands. *Journal of Biological Chemistry, 284*(22), 15289-15298.

Sarkar, Mangaldeep, Upreti, Rashmi, Kumar, & Sunil. (2022). Current Status of Tomato (*Solanum Lycopersicum* L.) Diseases and Their Management. In *Diseases of Horticultural Crops: Diagnosis and Management* (pp. 465-521): Apple Academic Press.

Sarma, Birinchi K, Yadav, Sudheer K, Patel, Jai S, . . . B, H. (2014). Molecular mechanisms of interactions of *Trichoderma* with other fungal species. *Open Mycol J, 8*(1), 140-147.

Schwarz, Dietmar, Thompson, Andrew, Kläring, & Hans-Peter. (2014). Guidelines to use tomato in experiments with a controlled environment. *Frontiers in Plant Science, 5*, 625.

Shahid, M., Singh, U. B., Ilyas, T., Malviya, D., Vishwakarma, S. K., Shafi, Z., . . . Singh, H. V. (2022). Bacterial inoculants for control of fungal diseases in *Solanum lycopersicum* L.(tomatoes): a comprehensive overview. *Rhizosphere microbes: biotic stress management*, 311-339.

Shoaib, Amna, Awan, Zoia , Khan, & Kashif. (2019). Intervention of antagonistic bacteria as a potential inducer of disease resistance in tomato to mitigate early blight. *Scientia Horticulturae, 252*, 20-28.

Silva, Kumar, Lalit, Shabani, Farzin, Picanço, & MC. (2017). Assessing the impact of global warming on worldwide open field tomato cultivation through CSIRO-Mk3· 0 global climate model. *The Journal of Agricultural Science, 155*(3), 407-420.

Singh, & Surender. (2018). Agrometeorological requirements for sustainable vegetable crops production. *J. Food Prot, 2*(3), 1-22.

Singh, Vipin Kumar, Singh, Amit Kishore, Singh, Prem Pratap, . . . Ajay. (2018). Interaction of plant growth promoting bacteria with tomato under abiotic stress: a review. *Agriculture, Ecosystems & Environment, 267*, 129-140.

Sippel, S. R. (2016). Contested red gold: The tomato in the context of European–Moroccan relations. In *Migration and Agriculture* (pp. 147-167): Routledge.

Soesanto, Loekas, Mugiastuti, Endang, Rahayuniati, & Feti, R. (2011). Biochemical characteristic of *Pseudomonas fluorescens* P60. *Journal of Biotechnology and Biodiversity, 2*, 19-26.

Soni, & Neelam. (2020). *Status and factors affecting development of early blight of tomato incited by Alternaria solani (Ellis Martin) Jones & Grout.* Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur,

Stammler, Gerd, Böhme, Franziska, Philippi, Jasmin, . . . Vanessa. (2014). Pathogenicity Of Alternaria-Species On Potatoes And Tomatoes.

Suresh, Perumal, Vellasamy, Shanmugaiah, Almaary, Khalid S, . . . B, Y. (2021). *Fluorescent pseudomonads* (FPs) as a potential biocontrol and plant growth promoting agent associated with tomato rhizosphere. *Journal of King Saud University-Science, 33*(4), 101423.

Suroto, Dian Anggraini, Kitani, Shigeru, Miyamoto, Kiyoko T, . . . Takuya. (2017). Activation of cryptic phthoxazolin A production in Streptomyces avermitilis by the disruption of autoregulator-receptor homologue AvaR3. *Journal of bioscience and bioengineering, 124*(6), 611-617.

Tan, S., Jiang, Y., Song, S., Huang, J., Ling, N., Xu, Y., & Shen, Q. (2013). Two *Bacillus* amyloliquefaciens strains isolated using the competitive tomato root enrichment method and their effects on suppressing Ralstonia solanacearum and promoting tomato plant growth. *Crop Protection, 43*, 134-140.

Toma, Anika Islam, Ali, Md Hosen, Al Aff, Tawseef, . . . Rahman, M. A. (2024). Potentiality of Formulated *Pseudomonas Fluorescens* and *Bacillus Subtilis* for the Management of Sheath Blight Disease of Rice Under Field Condition. *Journal of the Bangladesh Agricultural University, 22*(3), 342-351.

Tracy, Saoirse R, Black, Colin R, Roberts, Jeremy A, . . . J, S. (2013). Exploring the interacting effect of soil texture and bulk density on root system development in tomato (*Solanum lycopersicum L.*). *Environmental and Experimental Botany, 91*, 38-47.

Vallad, Gary E, Messelink, Gerben, Smith, & A, H. (2018). Crop protection: Pest and disease management. *Tomatoes, 2nd ed.; Euvelink, E., Ed.; CAB International: Boston, MA, USA*, 207-257.

Vurukonda, Sai Shiva Krishna Prasad, Giovanardi, Davide, Stefani, & Emilio. (2018). Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *International Journal of Molecular Sciences, 19*(4), 952.

Wakil, Waqas, Brust, Gerald, Perring, & Thomas. (2018). Tomato and management of associated arthropod pests: Past, present, and future. *Sustainable management of arthropod pests of tomato*, 3-12.

Walia, Abhishek, Mehta, Preeti, Chauhan, Anjali, . . . CK. (2014). Effect of *Bacillus subtilis* strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84*, 145-155.

Wang, & Dongwei. (2020). *Trade-offs in soil filamentous fungi*: Freie Universitaet Berlin (Germany).

Wang, Hui, Shi, Yuying, Wang, Doudou, . . . Aoxue. (2018). A biocontrol strain of *Bacillus subtilis* WXCDD105 used to control tomato Botrytis cinerea and Cladosporium fulvum Cooke and promote the growth of seedlings. *International Journal of Molecular Sciences, 19*(5), 1371.

Wspanialy, P., & Moussa, M. (2020). A detection and severity estimation system for generic diseases of tomato greenhouse plants. *Computers and Electronics in Agriculture, 178*, 105701.

Xie, Chuanqi, Shao, Yongni, Li, Xiaoli, . . . Yong. (2015). Detection of early blight and late blight diseases on tomato leaves using hyperspectral imaging. *Scientific reports, 5*(1), 1-11.

Yadav, Sm, Singh, Vineeta, Chand, & Ramesh. (2015). Mass Sporulation Of *Alternaria Solani* Causing Early Blight Of Tomato. *Indian Phytopathol, 68*(1), 83-86.

Yang, Y., Zhu, G., Li, R., Yan, S., Fu, D., Zhu, B., . . . Zhu, H. (2017). The Rna Editing Factor Slorrm4 Is Required For Normal Fruit Ripening In Tomato. *Plant Physiology, 175*(4), 1690-1702.

Yazici, Sabriye, Yanar, Yusuf, Karaman, & Isa. (2011). Evaluation of bacteria for biological control of early blightdisease of tomato. *African Journal of Biotechnology, 10*(9), 1573-1577.

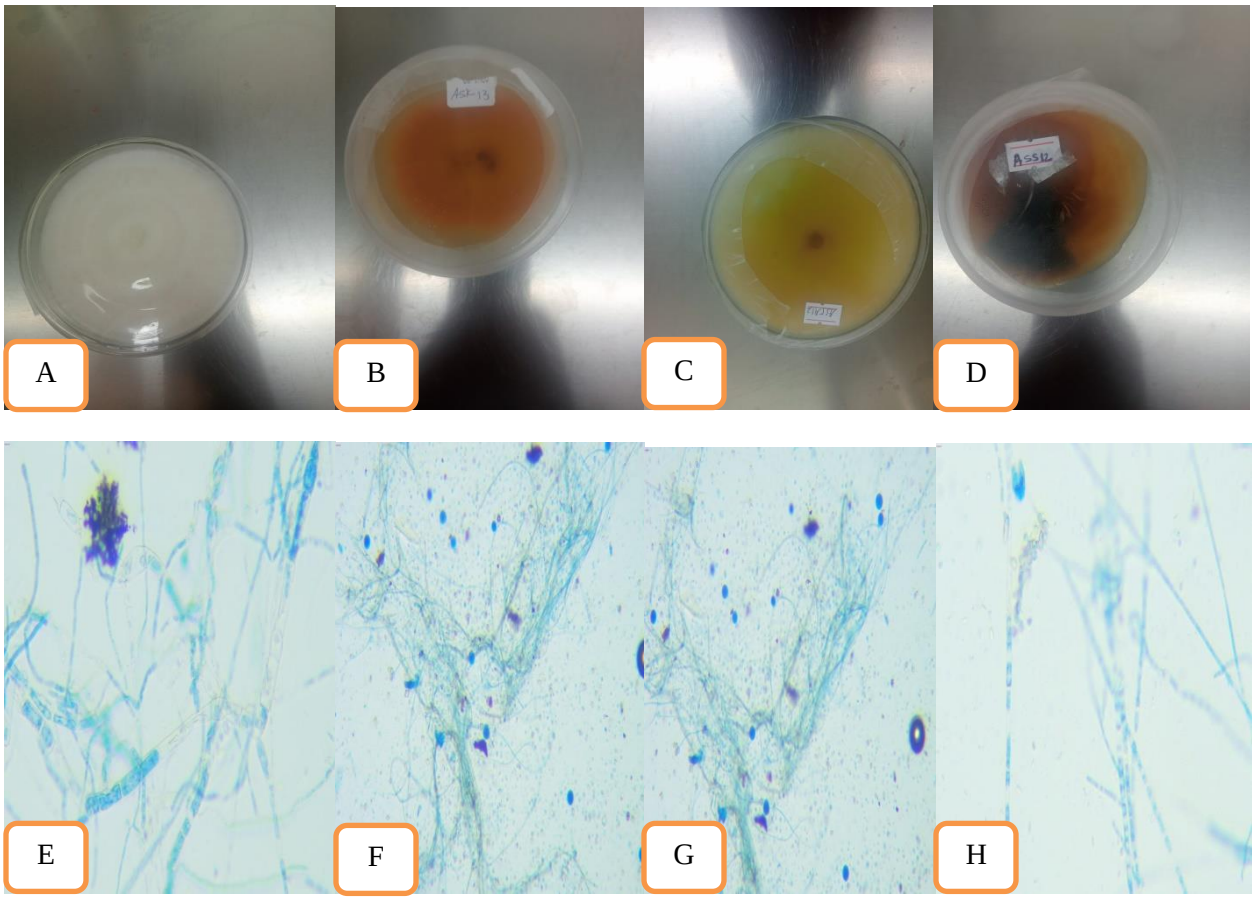
Yendyo, Shiva, Ramesh , GC, Pandey, & Binayak. (2018). Evaluation of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* for biological control of Ralstonia wilt of tomato. *F1000Research, 6*, 2028.

Yigezu Wendimu, G. (2021). The challenges and prospects of Ethiopian agriculture. *Cogent Food & Agriculture*, *7*(1), 1923619.

Zegeye, Ephrem Debebe, Santhanam, Amutha, Gorfu, Dereje, . . . Bekele. (2011). Biocontrol activity of *Trichoderma* viride and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions. *Journal of Agricultural Technology*, *7*(6), 1589-1602.

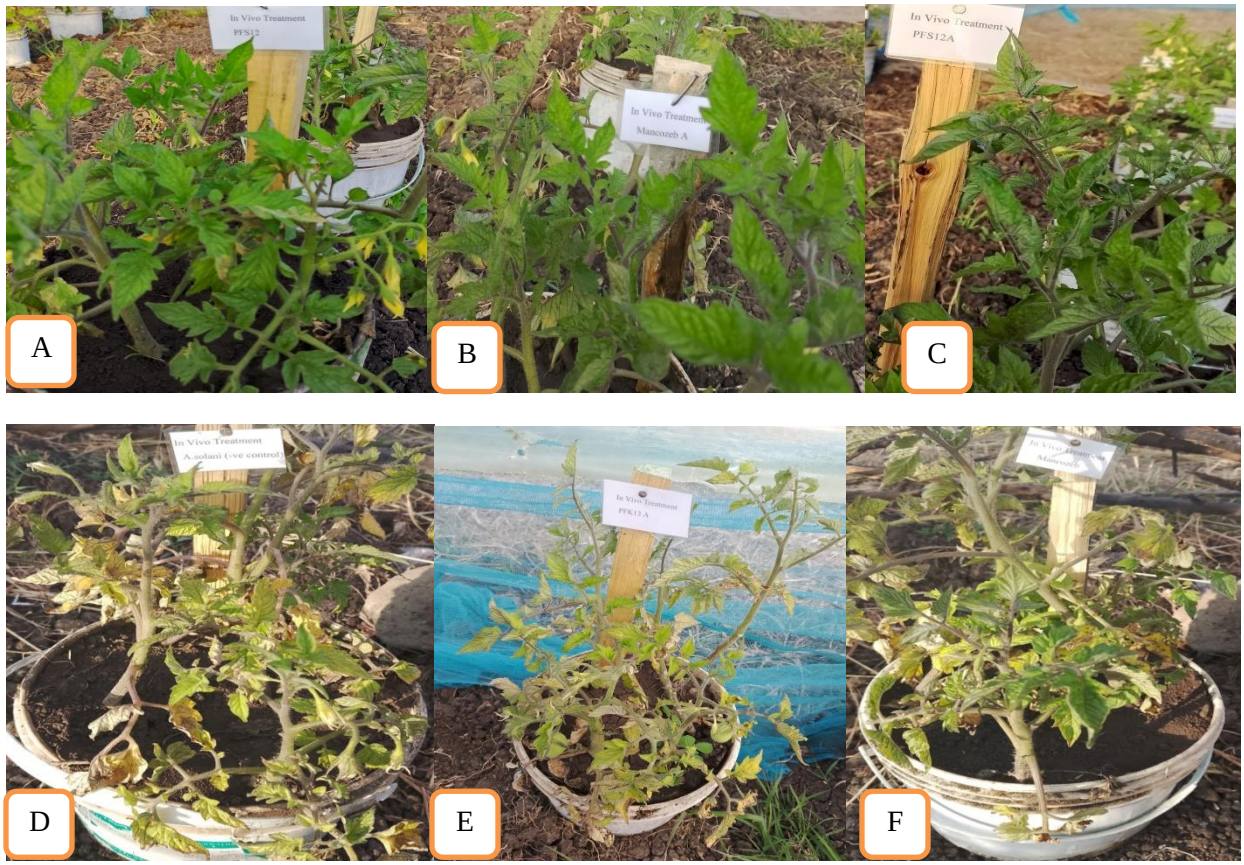
Zhang, S., Wang, L., Zhao, R., Yu, W., Li, R., Li, Y., . . . Shen, L. (2018). Knockout of SlMAPK3 reduced disease resistance to Botrytis cinerea in tomato plants. *Journal of agricultural and food chemistry*, *66*(34), 8949-8956.

7. APPENDICES



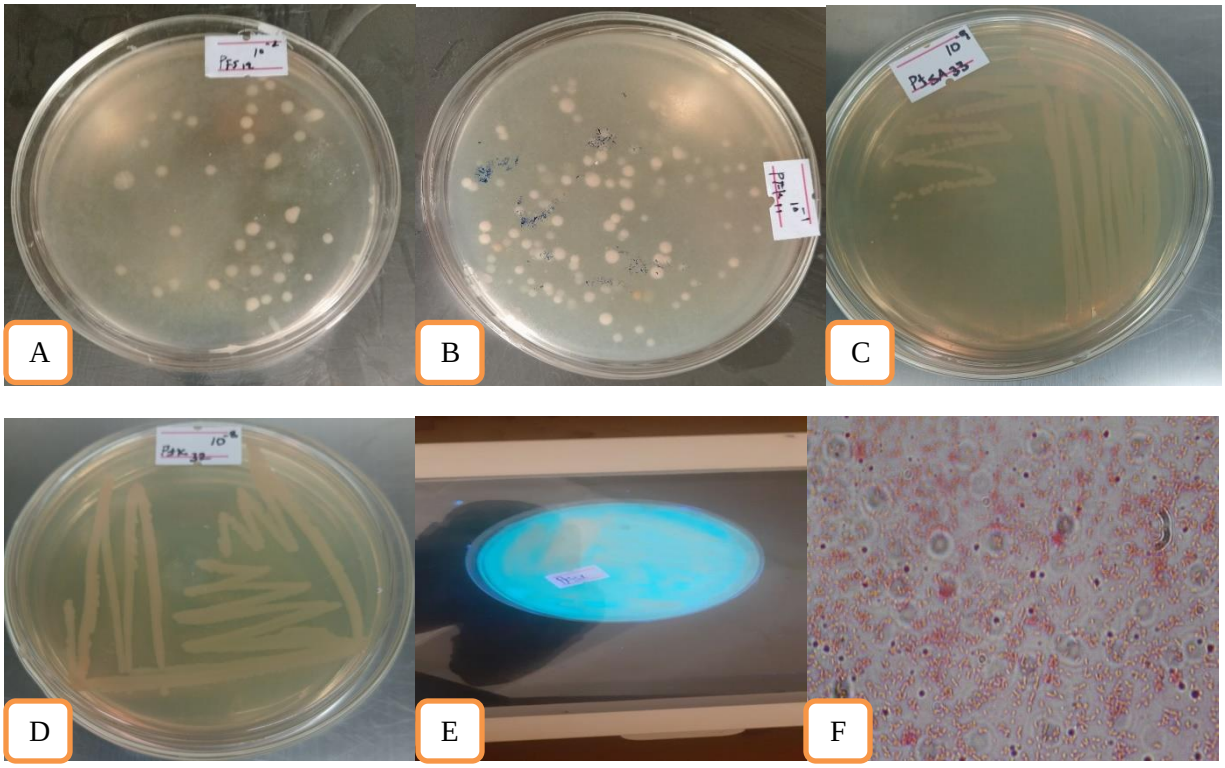
Appendix 7. 1 : Cultural and morphological characteristics of isolated *A.solani*

A and C: *Alternaria solani* seven day old colony (obverse), B and D: *Alternaria solani* ten day old colony (reverse), E and H: *Alternaria solani* hyphae (x40), F and G: *Alternaria solani* conidia (x40).



Appendix 7.2 : Early blight symptom on infected tomato leaf and health tomato leaf

A, B and C: healthy tomato leaf, D, E and F: early blight symptoms on infected tomato leaf



Appendix 7.3 : Cultural and morphological characteristics of isolated *P.fluorescens*

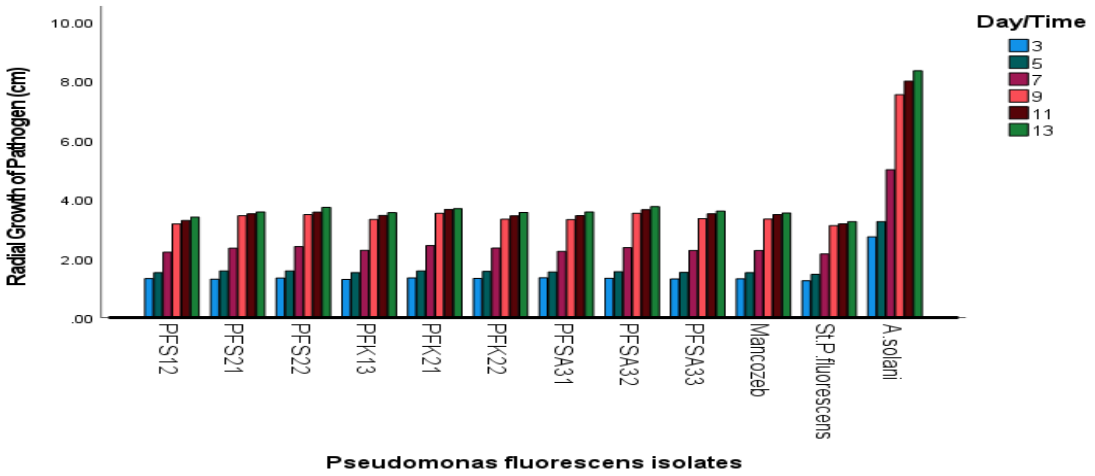
A and B: *Pseudomonas fluorescens* colonies on pseudomonas isolate agar medium, C and D: *Pseudomonas fluorescens* sub culturing on pseudomonas isolate agar medium, E: Isolated *Pseudomonas fluorescens* on pseudomonas isolate agar medium under ultraviolet light, F: Microscopic observation of *Pseudomonas fluorescens* on pseudomonas isolate agar medium.

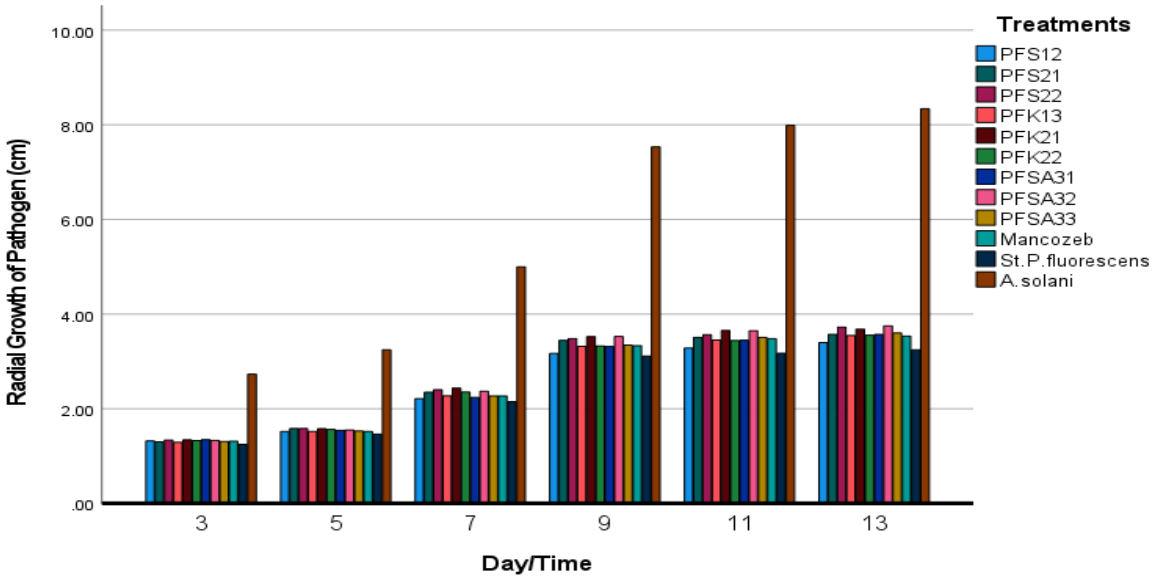
Appendix 7.4 : Different biochemical test of isolated *P.fluorescens*

A: Catalase test, B: Oxidase test, C: Starch hydrolysis test, D: *Fluorescens* pigmentation test, E: Citrate utilization test, F: Gelatin hydrolysis test, G: Indole test, H: motility test, I: TSI test.

Appendix 7.1 : Analysis of variance for the radial growth of pathogen in vitro condition

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	11	171.969	15.634	6344.17	<.001
Replication	2	0.005	0.0024	0.015	0.985
Day / Time	5	231.046	46.209	18751.92	<.001
Treatment*Day	55	30.436	0.5534	224.57	<.001
Residual /Error	142	0.350	0.0025		
Total	215	433.806			

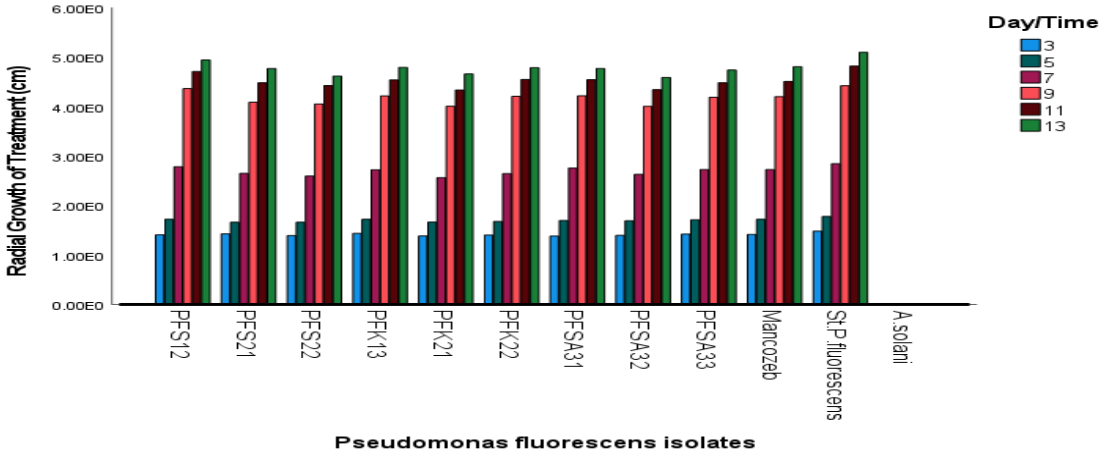


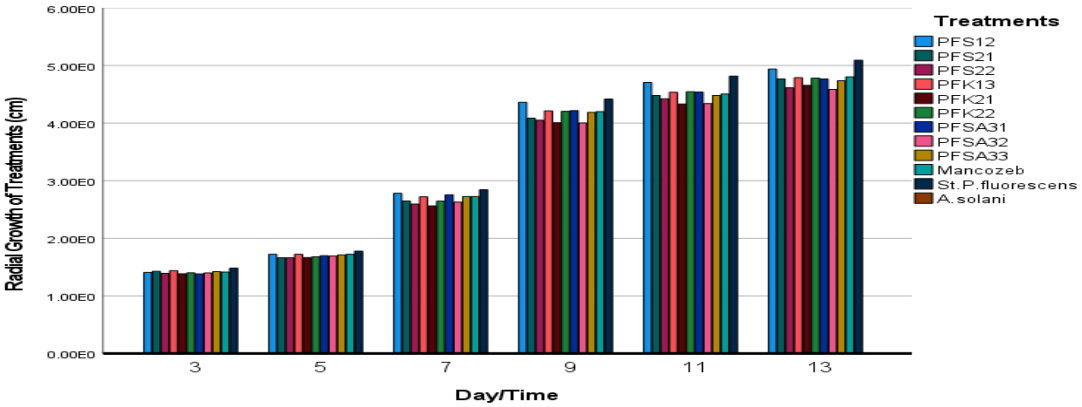


Appendix 7.5: Mean diameter of radial growth of pathogen in the presence of isolated *Pseudomonas fluorescens*

Appendix 7.2 : Analysis of variance for the radial growth of treatment in vitro condition

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	11	171.969	15.634	904.76	<.001
Replication	2	0.298	0.149	0.891	0.412
Day/Time	5	328.496	65.699	3802.20	<.001
Treatment*Day	55	30.436	0.5534	32.03	<.001
Residual / Error	142	2.454	0.0173		
Total	215	533.653			

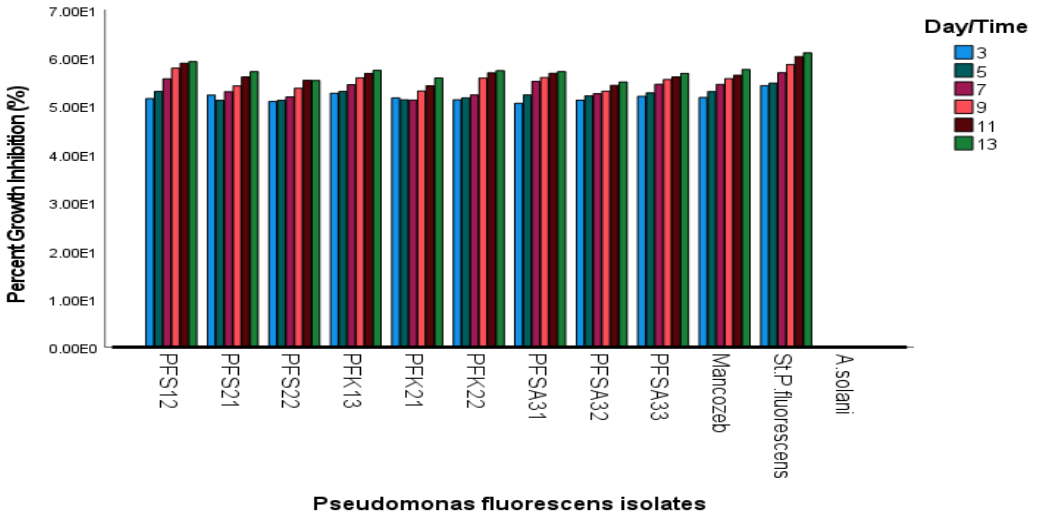


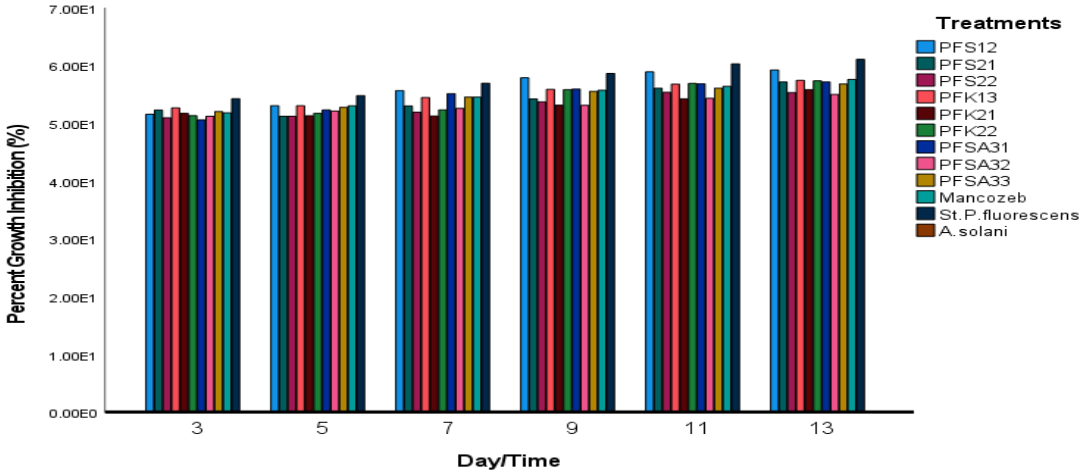


Appendix 7.6 : Mean diameter of radial growth of treatment in the presence of isolated *Pseudomonas fluorescens*

Appendix 7.3 : Analysis of variance for the percent growth inhibition in vitro condition

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	11	49,457.772	4,496.161	3362.84	<.001
Replication	2	15.184	7.592	4.399	0.014
Day /Time	5	740.134	148.027	110.71	<.001
Treatment*Day	55	150.1	2.73	2.04	0.0004
Residual / Error	142	189.9	1.34		
Total	215	50,553.066			

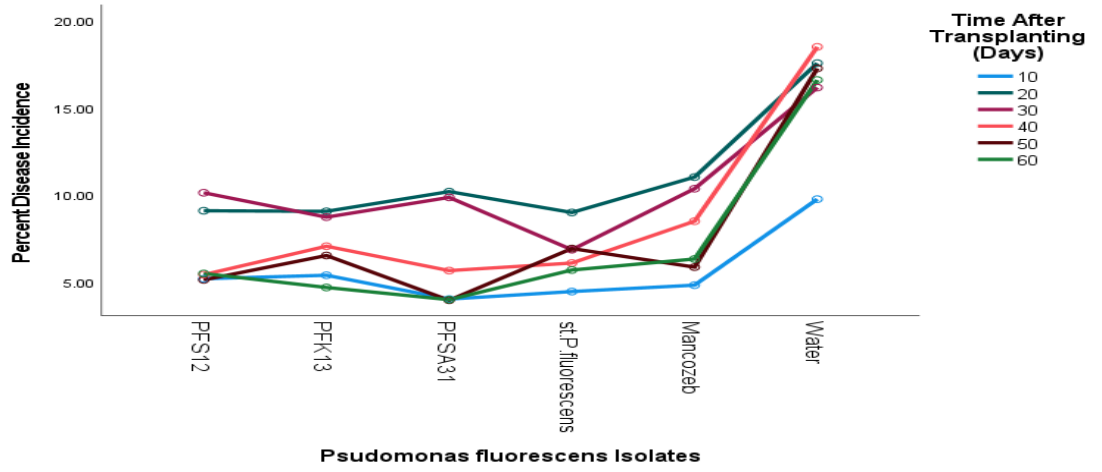


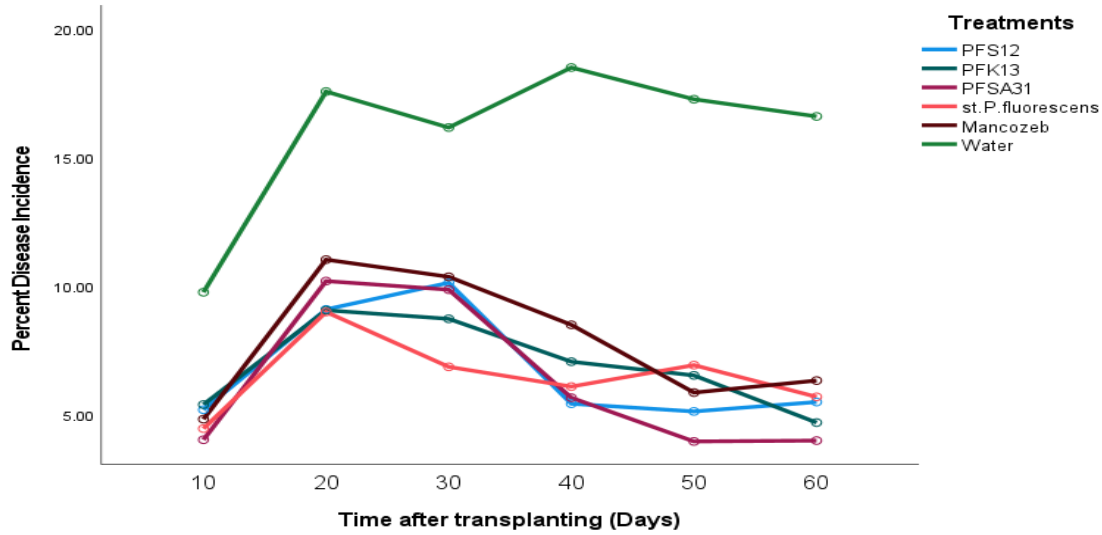


Appendix 7.7 : Mean diameter of radial growth of treatment in the presence of isolated *Pseudomonas fluorescens*

Appendix 7.4 : Analysis of variance for the percent disease incidence at greenhouse

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	5	1,272.62	254.524	337.01	<.001
Replication	2	0.37	0.184	0.080	0.923
Day / Time	5	369.44	73.887	97.83	<.001
Treatment*Day	25	164.76	6.590	8.73	<.001
Residual / Error	70	52.87	0.755		
Total	107	1,860.047			

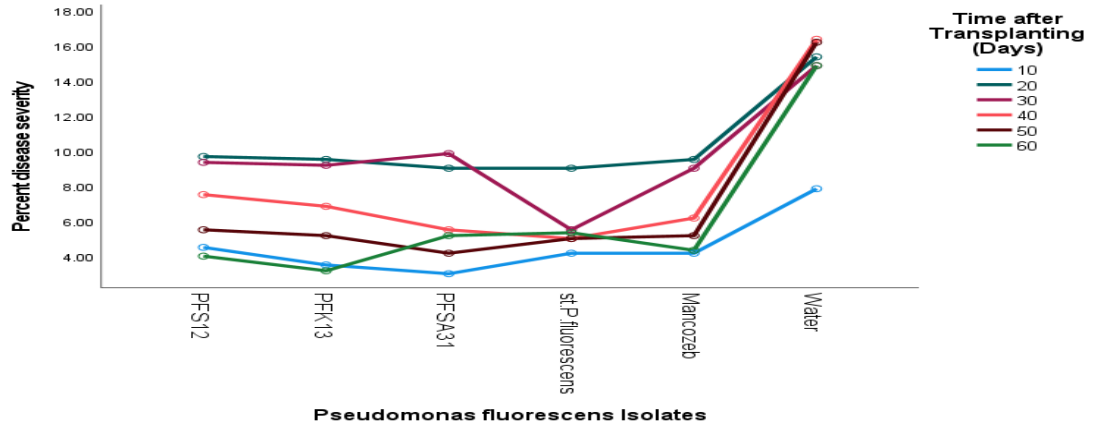


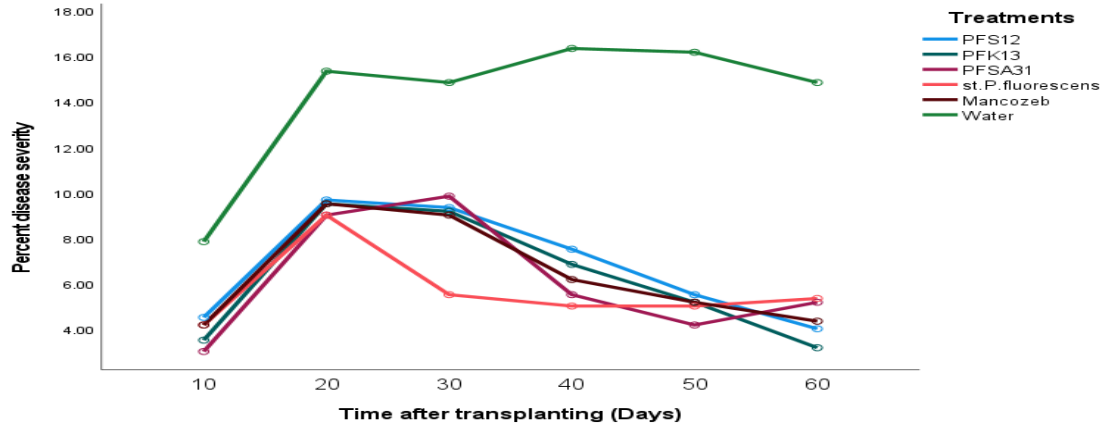


Appendix 7.8: Marginal mean of percent disease incidence for early blight of tomato at greenhouse

Appendix 7. 5 : Analysis of variance for the percent disease severity at greenhouse

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	5	969.96	193.991	278.18	<.001
Replication	2	8.69	4.343	1.933	0.150
Day / Time	5	426.76	85.352	122.39	<.001
Treatment*Day	25	164.64	6.586	9.44	<.001
Residual / Error	70	48.81	0.697		
Total	107	1,618.859			

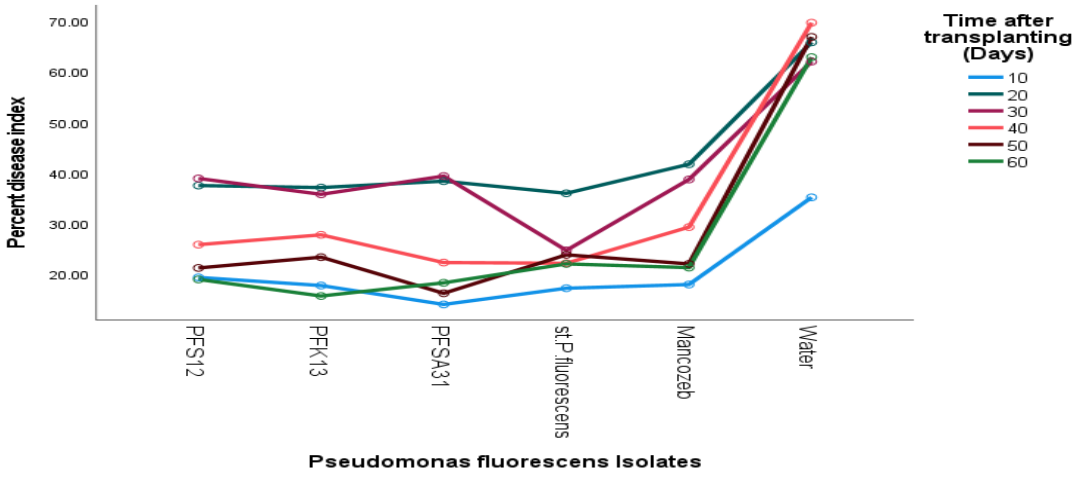


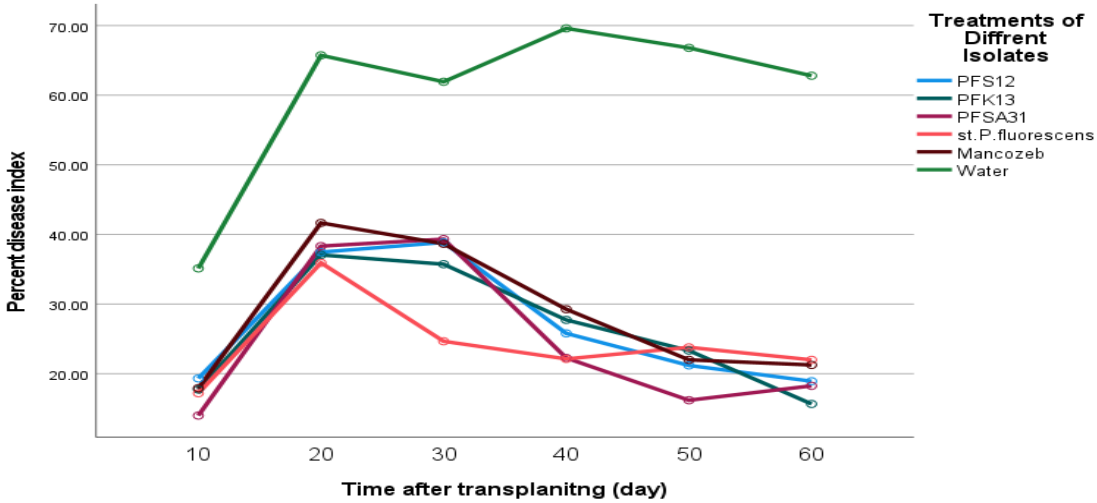


Appendix 7.9 : Marginal mean of percent disease severity for early blight of tomato at greenhouse

Appendix 7. 6 : Analysis of variance for the percent disease index at greenhouse

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	Sig.
Treatments	5	17757.6	3551.52	700.04	<.001
Replication	2	42.3	21.17	0.736	0.482
Day / Time	5	6400.9	1280.17	252.34	<.001
Treatment*Day	25	2376.1	95.04	18.73	<.001
Residual / Error	70	355.1	5.07		
Total	107	2,6932.037			





Appendix 7.10 : Marginal mean of percent disease index for early blight of tomato at greenhouse

Appendix 7.7 : Correlations between tomato early blight disease growth parameters

		Treatments of Different Isolates	Percent Disease Incidence	Percent Disease Severity	Percent Disease Index
Treatments of Different Isolates	Pearson Correlation	1	.577**	.472**	.536**
	Sig. (2-tailed)		.000	.000	.000
	N	108	108	108	108
Percent Disease Incidence	Pearson Correlation	.577**	1	.935**	.985**
	Sig. (2-tailed)	.000		.000	.000
	N	108	108	108	108
Percent Disease Severity	Pearson Correlation	.472**	.935**	1	.982**
	Sig. (2-tailed)	.000	.000		.000
	N	108	108	108	108
Percent Disease Index	Pearson Correlation	.536**	.985**	.982**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	108	108	108	108

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level