USE OF ENDOPHYTIC MICROORGANISMS IN CROP ADAPTATIONS TO INCREASED SALINITY AND RESISTANCE TO PESTS AND DISEASES

PRISCILLAR MUMO MUTUNGI

DOCTOR OF PHILOSOPHY

(Biotechnology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2025

Use of Endophytic Microorganisms in Crop Adaptations to Increased Salinity and Resistance to Pests and Diseases

Priscillar Mumo Mutungi

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology of the Jomo Kenyatta University of Agriculture and Technology

2025

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature……Date……

Priscillar Mumo Mutungi

This thesis has been submitted for examination with our approval as the University Supervisors

Signature……Date……

Prof. Justus Mong'are Onguso, PhD

JKUAT, Kenya

Signature……Date……

Prof. Hamadi Iddi Boga, PhD

JKUAT, Kenya

Signature……Date……

Dr. Vitalis Wafula Wekesa, PhD

Bioline Agrosciences Africa Limited, Kenya

Signature……Date……

Dr. Erustus Kanga, PhD

Kenya Wildlife Service, Kenya

DEDICATION

I dedicate this work to my entire family and my supervisors for their encouragement, understanding, support, financially, emotionally and spiritually throughout my journey to attain the degree.

ACKNOWLEDGEMENT

I thank the Almighty God for His care and protection throughout my academic journey. He granted me health and strength that was necessary to accomplish the task. May His name be glorified.

Special thanks to my supervisors, Prof. Hamadi Iddi Boga, Prof. Justus Onguso, Dr. Vitalis Wafula Wekesa and Dr. Erastus Kanga for their guidance, support, mentorship and encouragement all through from proposal development to the end of the project. This research would not have been possible without you. Thank you so much and may the Lord bless you as you continue to mentor and encourage many more young and upcoming scientists.

I am grateful to the National Commission for Science, Technology and Innovation (NACOSTI) for the grant of a research permit to allow me undertake the research in the country; and the Kenya Wildlife Service for the research Authorization to access the protected areas and facilitate sample collection; Institute for Biotechnology, Jomo Kenyatta University of Agriculture and Technology for laboratory and green house facilities, equipment and reagents that facilitated the research.

I also express my sincere gratitude to Prof. Bernard Nyende, former Director, IBR for his support and guidance during the critical time of my laboratory experiments, for permitting me access to the laboratory over the weekends and during holidays.

My profound appreciation to Dr. Steve So Baleba for his support during the preparation of the manuscripts, especially in running the data analysis programme and guidance in the interpretation of the analysis output. Thank you for dedicating your time, including weekends, to see to it that I did the work well. May the almighty God bless you.

Particular recognition goes to colleagues at the Institute for Biotechnology Research, Richard Rotich and Catherine Eyinda for providing a friendly working environment and for their support during the odd days and working hours to ensure my work went on smoothly.

Sincere gratitude to the Global Environment Facility (GEF) funded Soda lakes project through which the procurement of additional laboratory equipment was facilitated. The project also made it easy to navigate through the community hurdles in accessing the sampling sites.

Special thanks to my husband, Peter Mutisya and children, Dorcas Mutisya, Oliver Mutisya and Samuel Mutisya for their prayers, support, encouragement and understanding during the times I was away from them. My mother, Annah Mutungi, my brother Festus Mutungi and my sister sister Agnes Mutungi for providing a shoulder to lean on during tough times. May the Lord continue to keep us together in unity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CFU	Colony-Forming Unit
CM	Centimeter
CTAB	Cetyltrimethylammonium Bromide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FAO	Food and Agriculture Organization
FCL_3	Iron (iii) Chloride
GLM	Generalized Linear Model
GPS	Global Positioning System
HCL	Hydrochloric Acid
HCLO	Hypochlorous Acid
HCN	Hydrogen Cyanide
IAA	Indole-3-Acetic Acid
IPM	Integrated Pest Management
ITS	Internal Transcribe Spacer

K_2HPO_4	Potassium Hydrogen Phosphate
KH_2PO_4	Potassium Dihydrogen Phosphate
KWS	Kenya Wildlife Service
MEGA	Molecular Evolutionary Genetics Analysis
Ml	Milliliter
Mm	Millimeter
NA^+	Sodium Ion
NaCL	Sodium Chloride
NCBI	National Center for Biotechnology Information
OD	Optical Density
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
rRNA	Ribosomal Ribonucleic Acid
SE	Standard Error
SEM	Standard Error of Mean

SNK	Student's Newman-Keuls Test
TE	Tris EDTA
Tris-HCL	Trishydroxymethylaminomethane Hydrochloride
TSB	Tryptic Soy Broth
µL	Microliter
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
UV	Ultraviolet
V/V	Volume/Volume
W/W	Weight/Weight
WFP	World Food Programme

ABSTRACT

Understanding endophytic microorganisms associated with soda lakes shrubs and building a holistic Kenyan soda lake living library of microorganisms while exploring their potential applications in various industries is crucial in the current climate crisis. Lake Magadi and Lake Bogoria are saline alkaline endorheic lakes, in semi-arid regions with erratic rainfall. Vascular shrubs growing along the shores of these lakes must develop adaptive mechanisms to cope with high alkalinity, salinity and erratic environmental conditions. Previous studies on soda lake microorganisms have focused on isolations from water, soil and sediments, leaving behind the plant associated niche. Plant associated microorganisms from harsh environments have previously been shown to harbor diverse metabolic and genetic profiles that play an important role in plant growth, health and survival under stressful conditions. The aim of this study was to collect shrubs growing along the shores of Lake Bogoria and Lake Magadi, and isolate associated bacteria and fungi. The resultant isolates were identified using molecular techniques and consensus sequences aligned with highly homologous sequences in NCBI database. The isolates were screened for production of exo-enzymes, salinity tolerance and inhibition of bean root rot pathogen, *Fusarium solani*. The ability of the isolates to endophytically colonize bean and tomato seedlings was also evaluated. The effects of endophytic colonization on salinity stress, *Fusarium solani* and *Tetranycus urticae* were assessed in green house experiments. Endophytic bacteria were affiliated to three different phyla, *Firmicutes, Proteobacteria* and *Actinobacteria* while fungal endophytes were affiliated to two phyla, *Basidiomycetes* and A*scomycetes.* A larger number of bacterial isolates were more salt tolerant while only few fungal isolates indicated growth at 1M NaCl concentration. All tested fungal and bacterial isolates irrespective of the concentration or the method used, endophytically colonized tomato and bean seedlings respectively.. Two bacterial isolates; *Bacillus megaterium* and *Enterobacter hormaechei* subsp. *Xiangfangensis* showed more than 50% bean root rot pathogen, *Fusarium solani* mycelial inhibition in dual culture assays, completely inhibited germination of the spores in co-culture assays and resulted in its biocontrol in planta. All the four select fungal endophytes significantly enhanced the germination of tomato seeds by 23% under salinity stress. Endophytic colonization of tomato seedlings significantly decreased the quantity of hydrogen peroxide and enhanced total chlorophyll produced under salinity stress compared to non-inoculated seedlings. Exposure of tomato seedlings colonized with fungal endophytesdid not affect the number of eggs laid or the mortality of spider mites. The study gives a first insight into the bacterial and fungal endophytes associated with saline alkaline adapted shrubs. The isolates also provide the potential use of soda lakes derived microorganisms in the agricultural production, an application that has not been widely explored in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background

Global human population has been projected by the United Nations to reach 8.5 billion people by 2030 and 9 billion by 2050, a growth that is partly caused by the decreasing trends in mortality. The highest growth in population is projected to be in the African and Asian continents (United Nations, 2022). Food production will need to be increased to sustain the food and feed demand that is estimated to increase by 35% - 56% by 2050 (van Dijk *et al*., 2021). However, food production systems are currently facing a myriad of challenges, exacerbated by climate change, pests and diseases, depleting water resources and diminishing land available for agriculture. Worse still, the global demand and consumption is driven by a few crops, vegetables and fruits for food and nutrition, with cereals leading in demand (FAOSTAT 2023). Advances in technology and intensification of farming activities, especially increased use of irrigation, fertilizers, pesticides and high yielding varieties have been associated with an increase in food production (FAO 2023). However, despite these noted increase in food production, an estimated 309 million people were facing acute food insecurity in 2023 (WFP, 2024).

Expansion of land under agriculture has impacted negatively to forest lands, ecosystems and habitats alongside tremendous losses in biodiversity (Ma *et al*., 2023), which runs counter to the United Nations 2030 Agenda on sustainable development goals that sets ambitious targets to preserve nature by 2050. Expansions in irrigated agriculture has increased global crop production, but has created a wide spread stress on water resources, as much of global irrigation practices are expanding in already water stressed areas (Mehta *et al*., 2024), complicating the much-required climate adaptation (McDermid, 2024). In addition, use of ground water for irrigation has also been attributed to secondary salinization, which has resulted in an estimated 30% of land globally salinized (Hopmans *et al*., 2021). For example, in 2020, the Mekong delta, a prominent rice production hun in Southeast Asia was faced with unprecedented peak in salinity levels affecting 30,000ha of rice and 20,000ha of vegetables (Tarolli

et al. 2020). The PO river delta in Italy has been affected by low river discharge and ground water extraction leading to increase in soil salinity that has negatively impacted the deltas unique agroecosystem (Bellafiore *et al*., 2021). In Africa, the Nile delta has suffered a complex interplay of sea level rise, reduced sediment flow and anthropogenic activities exacerbating saline intrusion that has affected about 35% of cultivated land (El-Ramady *et al*., 2013). Kenya has also been affected by salinization of about 40% of arid and semi-arid lands as a result of irrigation with brackish water and increased ground water extraction (Wanjogu *et al*., 2013).

Impacts of climate change, including elevated levels of carbon dioxide, extreme temperatures and frequent droughts (water scarcity), regular episodes of diseases and pests, heat waves and erratic, unpredicted rains causing flooding have devastating impacts on crop productivity. Rising global temperatures as a result of increasing greenhouse gas emissions is a serious threat to global food production (Neupane *et al*., 2022). Increase in temperatures disrupts crop growth and development, pollen growth, fertilization, post fertilization development and germination as each crop has a cardinal range of temperature conditions required for optimum productivity (Liu *et al*., 2023; Soltani *et al*., 2019). Impacts of climate change on agriculture are not only limited to production outputs but also affect the nutritional value. For example, common bean is a good source of high-quality protein, micronutrients, antioxidants and vitamins. Its production is projected to be affected by the current global environmental challenges. A study to model the impacts of climate change induced drought stress on common bean yield and nutrition by 2050 across several countries in Southeastern Africa by Hummel *et al*., 2018 revealed reduced yields and reduced dietary iron. Sharps, 2024 modelled a loss of up to 27.5% of common bean production loses in Uganda as a result of ozone flux. Elevated levels of atmospheric carbon dioxide with concomitant increase in temperatures were found to decrease in zinc, iron, B vitamins and protein levels, and an increase in heavy metal concentration, raising the concern of heavy metal toxicity from grains (Ebi & Ziska, 2018; Jiaqing *et al*., 2020). Low nutrient concentrations have been associated with constrained seed germination and seedling vigour, affecting production (Jin *et al*., 2019). Increasing levels of carbon dioxide have also been attributed to an increase in sugars, phenols, flavonoids and calcium and a decrease in carotenoids, protein and micronutrients in vegetables (Dong *et al*., 2018).

Changing climatic parameters including increasing atmospheric carbon dioxide, elevated temperatures and precipitation patterns have also been linked to pest and disease dynamics, jeopardizing global food security. These include an expansion of geographic distribution, increased risk of invasion by migratory pests, shifts in pathogen range, crop disease outbreaks, increased survival and number of pest generations and increased incidences of insect-transmitted plant diseases *(*Skendžić *et al.*, 2021). Studies have indicated risks from pests, pathogens and weeds are expected to increase especially in Arctic, boreal, temperate and subtropical regions (IPPC, 2021). The rise in temperatures was noted to accelerate the life cycle of the spider mites by reducing the time it takes to move from egg to mature life stages thereby increasing the rate of reproduction and its spread (Ghazy *et al*., 2019). Modelling of future spread to project and compare the niche of some economically important crop pests and disease pathogen changes and risks have been undertaken. For example, the potato late blight pathogen, *Phytophthora infestans* is a pathogen whose origin has been traced to Central or South America and has now spread globally, causing devastating effects on a variety of Solanaceae crops (Goss *et al*., 2014). The pathogen is known to undergo population shifts in agricultural systems, especially under predisposing conditions of warm temperatures, flooding and drought which establish optimal conditions for its proliferation and crop infection *(*Cooke *et al.,* 2012*;* Angle *et al.,* 2023*)*. Changes in agricultural production systems and technological advancements, globalization and adoption of monocultures exacerbate the challenges of plant pests and diseases (Lamichhane *et al*., 2015). Precipitation and temperature variables too, play a key role in disease occurrence and severity especially for soil borne pathogens.

The plant pathogenic fungus, *Fusarium* sp., is an important mycotoxigenic genus responsible for blights, root and stem rots and wilts causing enormous economic losses to crops globally. The suitability of this fungus has been modelled and results predict regions currently suitable for the pathogen will lose suitability to even more than 100% by 2050 and vice versa (Ejaz *et al*., 2023). The bean root rot pathogen, *Fusarium solani* is projected to be more prevalent in regions experiencing drought stress, poor soil nutrition, water logged and in areas of long-term bean production, affecting the growing and production of beans (Macedo *et al*., 2017). Understanding and mapping

of plant diseases and future expectations is key to development of management strategies, including modifying integrated pest management strategies.

Crop losses are becoming more problematic to production as crops are affected by both biotic and abiotic factors, with climate change aggravating the problem. Current management options include use of chemical inputs and biological control methods, which are also limited to development of resistance and effects of changing climatic conditions. Therefore, additional methods to integrate into existing strategies are required to enhance efficiency and respond to the projected changes. The use of microbial endophytes could offer such an integration and ecofriendly strategy, as plants have, over time developed a multitrophic interaction with microorganisms, leading to development of specific functional traits by these microorganisms, which play a key role in their interaction and survival (Andreote *et al*., 2014a; Kusari *et al*., 2013). Domesticated crop plants are said to have lost important endophytic microorganisms as a result of continuous planting. Wild crop relatives are characterized by unique microbial endophytes which have evolved with the species providing for adaptability and survival in adverse environments, which, if harnessed could be important in inducing both biotic and abiotic stress tolerance in domesticated crops (Mili *et al*., 2021). Some endophytes are also known to confer habitat adapted characteristics acquired as a result of environmental conditions of the host plant (Rodriguez & Redman, 2008), and therefore confer the same benefits to crop plants. This study therefore focused on the isolation of endophytic fungi and bacteria from plants growing in hypersaline environments, along the shores of Kenyan soda lakes (Magadi and Bogoria), determine their diversity and evaluate their potential to confer salt tolerance, disease and pest resistance to tomato and bean crop plants.

1.2 Problem Statement

Food security is one of the greatest challenges of the world in the 21st century. Agricultural productivity must increase to meet the demands of an increasing population (FAO, 2011). Despite considerable efforts to increase productivity in major food crops, global food security has not been attained. Crop losses are becoming more problematic to production as crops are affected by both biotic and abiotic factors, and climate change exerting additional pressure. Among the abiotic stresses is salinity which inhibits overall plant growth through ionic imbalance, osmotic stress, oxidative stress, and reduced nutrient uptake. Mitigation measures include abandonment of the area, which is limited by availability of land and the development of salinity tolerant crop plants which is complicated by the complex inheritance pattern making it challenging (Atta *et al*., 2023).

Crop biotic stresses cause major reductions in yield. Pests are responsible for between 20% to 40% loss in global crop production annually. Tetranychid mites are a very important family of phytophagous mites worldwide, feeding on over 1100 plant species, constituting more than 140 crops families. They feed on the lower part of the leaves, sucking out the cell contents thereby impending photosynthesis and transpiration. They are also known to develop insecticide resistance very quickly which complicates their control (Legwaila *et al*., 2022). In addition to pests, global crop loss due to plant diseases is estimated annually to be $220 billion USD or 14.1% of total production. Diseases lower product quality and shelf-life of crops, decrease the nutritional value of vegetables and fruits, reduces crop yields, and diminish aesthetics, making some crops not to be marketable (CABI, 2022). Common bean is a major cereal crop whose production is constrained by various factors. Root rots are among the largest constraints, causing upto 100% crop losses under favorable environmental conditions. Root rots in bean production are caused by a number of different pathogens refered to as root rot complex. Among these pathogens, *Fusarium* spp. are the most common causal agents of root rots (Yu et al., 2023). Management options for root rots include crop rotation, use of fungicides and resistant cultivars. Crop rotation is limited by the broad host range of the pathogen, fungicides are associated with environmental pollution and specificity of their efficacy to specific strains of the fungus while

resistant cultivars are very few. Additional methods to cushion crop losses to both biotic and abiotic stresses are therefore required for integration into existing methods.

1.3 Justification

Current global changes in climatic conditions are increasingly exerting pressure on agricultural production with increasing outbreaks of plant diseases and pests, in addition to other biotic stresses. Measures to manage pests and diseases largely rely on chemical inputs, and to a lesser extent, applications of integrated pest and disease control measures. However, the global restrictions on the use of chemicals in agriculture are pushing researchers to explore alternative environmentally safe and integrated management options to these challenges, in line with sustainable development goals. Methods to enhance crop resistance to these biotic and abiotic stresses is a promising avenue to their management (Rodriguez-Saona *et al*., 2022). Endophytes, microorganisms living and colonizing healthy internal plant tissues asymptomatically, are known to confer host resistance to diseases and pests through signal transduction which induces systemic resistance (White *et al*., 2019) and protection against environmental stresses through production of phytohormones (Gupta *et al*., 2020). Plants have, over time developed a multitrophic interaction with microorganisms, leading to development of specific functional traits by these microorganisms, which play a key role in their interaction and survival (Kusari et al., 2012, Andreote et al., 2014). Endophytic fungi and bacteria have been shown to easily colonize the internal tissues of plants, playing a role, in the biosynthesis of phyto-hormones like salicylic acid and abscisic acid, which help the plant ameliorate stressful abiotic conditions (Latif et al., 2011). Other endophytes increase plant root and shoot growth by enhancing the plants ability in uptake of nutrients, resistance to plant pathogens and insect attacks and disease severity (Mei & Flinn, 2010). Studies have shown that halophilic plants, like other terrestrial plants harbor unique endophytic microorganisms that help the plant survive the harsh environment (Moghaddam *et al*., 2022; Zhang *et al*., 2019;*Sahu et al*., 2023). Kenya's soda lakes are characterized by high salt deposits, occasional droughts causing precipitation of salts; flooding which dilutes the salt concentrations, presenting unique and specific conditions. Halophilic plants at the edge of soda lakes have to withstand these periodic changes in

environmental conditions and therefore are believed to harbor unique and diverse endophytic microorganisms. Various studies on microbial diversity from Kenyan soda Lakes have been undertaken, mainly focusing on microorganisms from the soil, water and sediments (Kambura, 2016; Mwirichia, 2009; Nyakeri *et al*., 2018; Orwa *et al*., 2020). Besides, endophytic microorganisms have been isolated from several species of mangroves and other halophilic plants and no literature is available on endophytes from shrubs growing along the shores of Kenyan soda lakes. This study focuses on isolation of endophytic fungi and bacteria from halophilic plants growing along Kenya's Lake Magadi and Lake Bogoria, both of which are soda lakes, their characterization and potential application in crop protection against pests, diseases and salt tolerance.

1.4 Research Questions

a) Do shrubs growing along the shores of Kenya's soda lakes host bacterial and fungal endophytes?
b) Can endophytes from shrubs along the shores of Kenya's soda lakes colonize tomato and bean plants?
c) Can the endophytes confer fitness benefits to tomato seedlings under salt stress?
d) Do endophytes from these plants enhance tolerance to bean root rot and red spider mites in tomato?

1.5 Objectives

1.5.1 General Objective

To investigate the potential of endophytes from shrubs growing along the shores of Kenya's soda Lakes to confer tolerance to pests, diseases and increased salinity to tomato and bean plants.

1.5.2 Specific Objectives

a) To isolate fungal and bacterial endophytes from shrubs growing along the edges of Lakes Bogoria and Magadi;
b) To determine the ability of isolated endophytes to colonize tomato and bean seedlings.;
c) To assess the ability of endophytes to confer salinity stress tolerance in tomato seedlings.
d) To evaluate the ability of endophytes to confer tolerance to bean root rot and spider mites in tomato seedlings;

CHAPTER TWO

LITERATURE REVIEW

2.1 Endophytic Microorganisms

Endophytes have been defined by a number of researchers, for example Hallman *et al*., 1997 defined endophytic bacteria as "as those bacteria that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and that do dot visibly harm the plant" while Chitnis *et al*., 2020 defined endophytes as non-disease-causing microbes (bacteria and fungi) surviving in living tissues of plants. The most general definition of endophytes is microorganisms that live within plant cells without any signs of disease (Zivanovic & Rodgers, 2018). The association of plants and microorganisms has been hypothesized as an ancient association that enabled plants to colonize land, in a mutualistic partnership to facilitate survival and mitigate stress (Pirozynski & Malloch 1975). Endophytic microbial communities from bacterial, archaeal, fungal and protist taxa have been isolated from all plants in natural ecosystems (Hardoim *et al*., 2015), and from all of their stages in life, from germination to seed production, including seeds (Shahzad *et al*., 2018) and all plant parts. Since the discovery of endophytes, a lot of research has been carried out for over 100 years now, and still the ecological significance is not fully understood (Rodriguez *et al*., 2009). Endophytes have been used in various industries ranging from agriculture (Watts *et al*., 2023), in the pharmaceutical industry (Uzma *et al*., 2018) and food aroma and perfume industry (Khoyratty *et al*., 2015). Although the mechanisms used by these microorganisms are not always well understood, beneficial physiological effects include improved nutrient and water uptake, growth promotion, and alteration of plant hormonal status and metabolism. Improvement of salinity stress involve the alteration of crop hormonal status to decrease evolution of the growth-retarding and senescence-inducing hormone ethylene (or its precursor 1-aminocyclopropane-1-carboxylic acid), or to maintain source–sink relations, photosynthesis, and biomass production and allocation (by altering indole-3-acetic acid and cytokinin biosynthesis) by these microorganisms (Dodd and Pe´rez-Alfocea 2012). Root and leaf colonization by microorganisms have resulted in induced changes in the relative abundance of the

major groups of organic solutes with concomitant enhancement in growth under salinity stress (Ma *et al.,* 2020).

2.2 Bacterial Endophytes

A review on the structure and functions of bacterial microbiota of plants by Bulgarelli, 2013 attributes the phylogenetic composition of plant associated bacteria to a relatively small bacterial phylum of Bacteroidetes, Actinobacteria, Firmicutes and Proteobacteria. These endophytes are hypothesized to originate from the rhizosphere and their association with plants is shaped by both edaphic and host plant and microbe derived factors that affect the microbiota composition both at the rhizosphere and the plant endosphere (Bulgarelli *et al*., 2013; Hardoim *et al*., 2015). Studies have also revealed the transmission of bacterial endophytes as both horizontally, from the surrounding soil and vertically through seeds and pollen from one generation to the other (Hardoim *et al*., 2012). Vertical transmission of bacterial endophytes is believed to be transferred as a survival mechanism of the plant during germination and habitat adaptation (Abdelfattah *et al*., 2021; Ferreira *et al*., 2008). Rhizosphere microbiota consist of bacterial community shifts driven by rhizodeposition and plant genotype (Yue *et al*., 2023), while the host endosphere consist of highly specialized microbiota that is adapted to survival in the internal tissues of the plant (Romero & Pieckenstain, 2014). Studies have revealed the different processes that mediate subsequent bacterial endophytic colonization of the roots by rhizosphere bacteria. The Quorum sensing molecule, N-acyl homoserine lactone provides a pivotal role in bacterial endophyte colonization (Zúñiga *et al*., 2013) while some bacterial endophytes have been found to produce cellulolytic enzymes like cellulases, xylanases and endoglucanases that facilitate entry into plant cells (Carro & Menéndez, 2020). Both pathogenic and beneficial endophytes employ similar mechanisms to colonize and enter plant tissues, the host plant is known to select and allow the entrance of specific microorganism for its own benefit through fine-tuning of immune response, molecular cross talks and motility (Wippel, 2023).

2.3 Fungal Endophytes

Fungal endophytes have been divided into two groups, Class I endophytes also known as Clavicipitaceous fungi and Class II known as non-Clavicipitaceous fungi. This classification is based on phylogeny, life history traits, host range, colonization pattern, transmission and ecological functions (Rodriguez *et al*., 2009). Clavicipitaceous fungi are transmitted vertically from seeds to the new generation and their host range is restricted to grasses. Non-clavicipitaceous fungi have a wide biodiversity and distribution, being found in vascular plants, ferns, conifers and angiosperms. Despite the broad classification, these endophytes only belong to the sub-kingdom Dikarya and the class Ascomycota and Basidiomycota, with Ascomycota containing the vast majority of isolated species (Hamzah *et al*., 2018; Zivanovic & Rodgers, 2018). Fungal endophytes have been found ubiquitous in all living plants, in all ecosystems including in deserts (Moghaddam *et al*., 2021), marine (Debbab *et al*., 2013), terrestrial plants (Nasiruddin *et al*., 2020), Antarctic regions (Bertini *et al*., 2022) saline alkaline lakes (Kimbrough *et al*., 2019) and high alpine confers (Eo & Eom, 2022).

Fungal endophytes form symbiotic relationships with the host plant, deriving nutrients from the plant while protecting the plant from biotic and abiotic stress. Such associations in the plant endosphere are highly specific, being selected by the plant for its benefit (Van & Dufresne, 2015). Some endophytic fungi colonize plants and remain as latent and inactive pathogens until the host plant is exposed to stress conditions or the plant enters into a senescence state when they express their pathogenicity (Bamisile *et al*., 2018) or produce spores for proliferation. The population structure of endophytic fungi is hypothesised to be dependent on several factors. For example, environmental factors like temperature, sunshine, humidity and salinity/alkalinity gradient which are associated with the types and quantities of secondary metabolites from the plant species and consequently the endophytic structure (Jia *et al*., 2016).

2.4 Endophytes as Biofertilizers

2.4.1 Endophytes in Phosphate Solubilization

Plant interactions with endophytic microorganisms results in phyto-beneficial effects, positively influencing the growth of the plant. Advances in molecular techniques have aided the understanding of how these microorganisms influence plant growth promoting activities. Several mechanisms through which endophytes promote the growth of the host plant include the mobilization of nutrients from the soil to make them available for the plant to utilize. One of these nutrients is Phosphorus, an essential macronutrient in plant growth and plays a major role in energy transfer, photosynthesis, nutrient movement within the plant, transfer of genetic characteristics from one generation to the next (Lambers, 2022) and enhancing abiotic plant tolerance (Khan *et al*., 2023). Low phosphorus availability affects overall plant productivity. Due to its high reactive nature, phosphorus in soil does not occur in its pure form but in complex interactions with other chemical compounds and present as insoluble phosphorus, with less than 1% dissolved in soil solution (Ducousso-Détrez *et al*., 2022). Soil salinity and PH, complicate further the availability of phosphorus to plants. High sodium and chloride ions inhibit plants absorption of nutrients while at high PH, phosphorus precipitates with calcium and at low PH, it forms complexes with magnesium or iron, making it unavailable for plants to use (Dey *et al*., 2021). Increase in productivity in low or limited soil phosphorus conditions is a global concern. Currently, the use of inorganic fertilizers to supplement crop phosphorus requirements are widely used despite their ecological and environmental detriments (Ducousso-Détrez *et al*., 2022).

The use of phosphate solubilizing microorganisms is a viable and promising resource for sustainable agriculture. Phoshorus solubilizing bacteria are associated with enhanced crop biomass and the overall growth (Kiprotich *et al*., 2023). Although the potential of phosphate solubilizing microorganisms has been cited in literature in enhancing phosphorus availability in phosphorus deficient soils, their application is not yet widely adopted (da Silva *et al*., 2023). Several endophytic bacteria and fungi have been shown to solubilize insoluble phosphate to its soluble form and make it

available for plant absorption (Chen *et al*., 2021; Radhakrishnan *et al*., 2015). The mechanisms by which endophytes solubilize phosphate are varied and include production of organic and inorganic acids that chelate cations bound to the phosphate, reduce the pH or formation of complexes bound to the phosphorus thereby converting the phosphate to a form which can be absorbed by plants (Bononi *et al*., 2020). Release of protons from ammonia (NH_4^+) is also another method used by this plant associated microorganism to solubilize phosphate. An experiment by Gaind, 2016 resulted in dissolution of tricalcium phosphate with no detectable organic acids produced. He hypothesized that the results were linked to the release of protons during the process of NH_4^+ assimilation to synthesize amino acids. The conversion of NH_4^+ to NH_3^+ during assimilation results in the release of protons to the cytoplasm, and acidification of the medium surrounding the fungal cells, thus favouring dissolution of phosphorus.

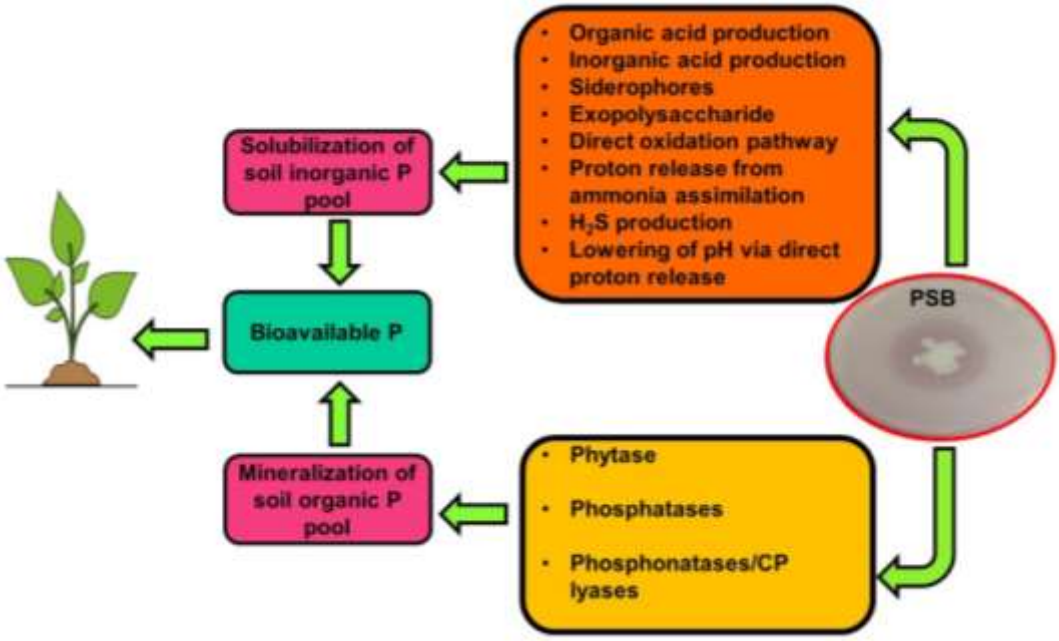

Figure 2.1: Schematic Presentation of Mechanisms Used by Phosphate Solubilizing Microorganisms

Source: Adopted from Rawat *et al*. (2021).

Production of exopolysaccharides by plant associated microorganisms, too have been shown to aid in phosphorus solubilization. These are high molecular weight

compounds secreted by microorganisms in stress conditions and aiding in bacterial biofilm formation and have a high affinity for metallic ions in soil, forming complexes (da Silva *et al*., 2023). A study on mechanisms of phosphate solubilization by a mangrove bacterium, *Bacillus marisflavi* FA7 by Prabhu, 2018 demonstrated the production of exopolysaccharides and solubilization of inorganic phosphate under alkaline conditions.

Phenotypic studies on solubilization of phosphate by endophytic microorganism have extensively been carried out under laboratory and greenhouse conditions and have verified their effectiveness in plant growth promotion and increased productivity through the solubilization of inorganic phosphate in several plant species (Bilal *et al*., 2018; Dahmani *et al*., 2020a; Varga *et al*., 2020). However, Silva, 2021 argued that the reproducibility of laboratory results in field conditions was still a difficult task that required to be overcome and proposed that an understanding of the modulation of microbial conditions under different cultivation conditions using metagenomic and genomic approaches could help predict the nature of phenotypic features and pathways required for plant growth. In his experiment, he revealed the presence of key gene copies including the alkaline phosphatase and PhoP required for resistance and the PstSCAB system for adaptation of the bacteria to low phosphate conditions. The best performing bacteria, *Bacillus megaterium*, had multiple copies of PhoP gene and alkaline phosphatase. Other researchers have also confirmed the presence of genes in their genomes for plant growth promotion (Dahmani *et al*., 2020a). The understanding of the molecular requirements would enhance the screening and application of phosphate solubilizing microorganisms in different environmental conditions.

2.4.2 Endophytes in Nitrogen Fixation

Nitrogen is an essential nutrient required by plants, termed as a primary yield determinant, and therefore should be available for crop production (Snapp *et al*., 2023). The growing population and diminishing land available for cultivation is pushing the demand for use of nitrogen fertilizers higher to increase food production. However, the current nitrogen use efficiency by crop plants, especially cereals has declined to approximately 30% (Tilman *et al*., 2002) and the rest of the applied nitrogen is lost in

the agricultural field leading to environmental pollution. This decline correlates with a decrease in production, and agriculture has to continuously increase production to meet the food and feed demands of the global growing population. Biological nitrogen fixation therefore becomes a feasible alternative to supply nitrogen to crop plants for enhanced production. Soumare *et al*., 2020 hypothesized more than 60% of the fixed nitrogen results from biological nitrogen fixation and this would provide a relief to the use of chemical fertilizers if well harnessed. Free living bacteria and endophytic bacteria and fungi are known to use the nitrogenase complex to fix atmospheric nitrogen to ammonia and release it to plants for growth and development (White *et al*., 2012). The nitrogenase enzyme, coded by the *Nif* genes is similar in most nitrogen fixing bacteria and has been hypothesized to be a result of horizontal gene transfer across phyla (Koirala & Brözel, 2021). Although the process of converting atmospheric nitrogen to ammonia is an energetically expensive process, microorganisms have evolved mechanisms to break the triple nitrogen bond under normal conditions using solar energy, and in symbiosis with plants (Soumare *et al*., 2020a). Developments in understanding further the process are underway, with a renewed interest as a result of the environmental and climate crisis to make agriculture more sustainable (Lindström & Mousavi, 2020). Currently, a database of all *NIFH* sequences of both free living and symbiotic nitrogen fixing bacteria, archaea and cyanobacteria is available in GenBank nucleotide databases (Soumare *et al*., 2020a).

Besides bacteria, archaea and cyanobacteria, fungal endophytes have been implicated in plant nitrogen processes. The fungal endophyte, *Phomopsis liquidambaris* improves nitrogen accumulation and nitrogen use efficiency in rice plants, and stimulates several genes involved in nitrogen uptake and metabolism in rice seedlings, although the mechanisms involved are not yet understood (Yang *et al*., 2015). This isolate also increased peanut yields, nodulation and nitrogen fixation even in the absence of nitrogen fertilizers (Xie *et al*., 2019). Whereas diazotrophs fix atmospheric nitrogen, fungal endophytes have various ways of providing nitrogen to plants. A study on five entomopathogenic *Metarhizium* and *Beauveria bassiana* fungal endophytes revealed the transfer of nitrogen from the insects to the plant and enhanced the growth and productivity of the plants (Behie & Bidochka, 2014).

Despite this plant microbial interactions and their supply of nitrogen to the plants, the levels are not adequate to support plant growth and development (Guo *et al*., 2023). In a bid to revolutionize agriculture to a more sustainable, low environment polluting sector, and increasing productivity, advances in synthetic biology are being considered in engineering the so-called Nitrogen self-fertilizing plants. These plants have enhanced associative interaction with nitrogen fixing microorganisms and are able to produce their own nitrogen nutrients (Guo *et al*., 2023). For example, a bacterial signalling rhizopine producing barley line was developed using the technology that improved the sensitivity for rhizopine receptor, and specifically induced the expression of nitrogenase in free living and associative bacteria (Haskett *et al*., 2022). Rice plants have also been engineered with increased flavonoid secretion, enhanced recruitment of nitrogen fixing bacteria and increased bacterial biofilm (Yan *et al*., 2022). Engineering nitrogen self-fertilizing crop plants is a novel technology, but has its limitations in the number of crop plants that can be engineered and the costs associated with the technological developments. Therefore, development of microbial biofertilizers using nitrogen fixing microorganisms still remain an easier and cheaper technology for enhanced crop production.

2.4.3 Endophytes in Phytohormone Production

Phytohormones are important plant growth regulators, impacting plant metabolism and stimulating response mechanisms against stress (Egamberdieva *et al*., 2017). Plants naturally produce their own phytohormones but several endophytic fungi and bacteria have been shown to reliably produce phytohormones and enhance plant growth and development and induced resistant to biotic and abiotic stress (Asaf *et al*., 2017; Bendaha & Belaouni, 2019; Khan *et al*., 2020). Plants produce hormones that regulate their growth and development, although at low quantities which are not sufficient for the plant, and their association with microorganisms help in supply of the additional phytohormone requirements (Egamberdieva *et al*., 2017). Due to their immense impact on plant growth, especially crop plants, microbial production of phytohormones have been intensely studied including their biosynthesis and signalling pathways for their production (Tang *et al*., 2023). Major phytohormones studied

include auxins, gibberellins, cytokinins, abscisic acid, salicylic acid, jasmonic acid, brassinosteroids and ethylene.

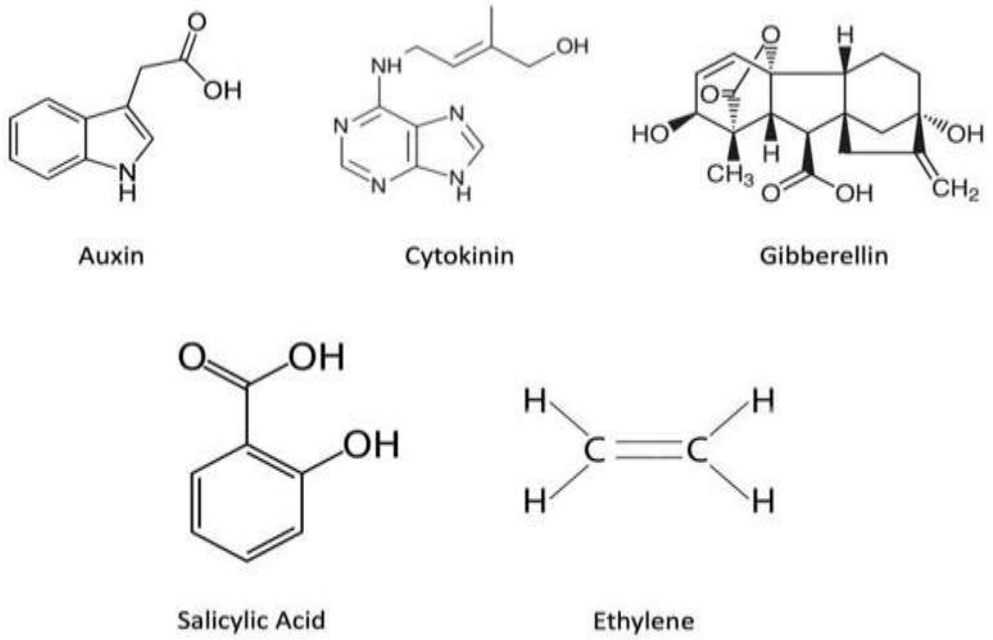

Figure 2.2: Chemical Structure of Major Plant Growth Promoting Bacterial-Synthesized Phytohormones.

Source: Adopted from Orozco-Mosqueda *et al. (*2023)

2.4.3.1 Auxins

Auxins are essential indole-derived phytohormones that regulate plant growth by stimulating cell elongation, root initiation, vascular tissue differentiation, bud and flower growth (Zhang *et al*., 2022). Biosynthesis of auxins is not only restricted to plants but is ubiquitous in all kingdoms of life (Krell *et al*., 2023). In microorganisms, they act as signalling molecules, playing a role in plant microbe interaction, adaptation and pathogenesis (Chanclud & Morel, 2016; Krell *et al*., 2023). Several biosynthetic pathways have been proposed both in plants and microorganisms, with two major pathways being the tryptophan dependent and tryptophan independent pathways (Tang *et al*., 2023). Although several different pathways for IAA biosynthesis have been proposed, Mashiguchi (2011) showed that two auxin production pathways, the Tryptophan Aminotransferase of Arabidopsis (TAA) and the YUC (flavin mono-

oxygenase like proteins), earlier perceived as two independent pathways were operating in the same pathway where TAA produced Indole Pyruvic Acid which was converted to Indole Acetic Acid by YUC. This pathway was also confirmed in *E. coli* in the same experiment.

Indole Acetic Acid (IAA) is one of the most important auxin hormones naturally occurring, regulating most functions of plant and microbial growth and development, including their interactions (Tang *et al*., 2023). Pathogenic microorganisms have evolved different mechanisms to manipulate host auxin production systems by either suppression or activation, depending on the type of interaction (Kunkel & Johnson, 2021). In these microorganisms, IAA acts as an invasion weapon during pathogenesis to facilitate pathogen entry, establishment and multiplication by regulating host gene expression, although plant response to pathogen IAA is not always the same. Some necrotic pathogens like *Alternaria* and viruses modify host auxin signalling to enhance susceptibility, while biotropic pathogens like *Pseudomonas* spp. stimulate auxin production to promote pathogenesis (Kunkel & Johnson, 2021). Moreover, the production of IAA in *Pseudomonas syringe* species complex was shown to be regulated by the iaaM/iaaH gene clusters which are horizontally transmitted and control the virulence of the plant pathogenic bacteria. The biosynthesis of IAA by the pathogens during infection activates the expression of virulence factors (Aragón *et al*., 2014).

To the contrary, in beneficial plant associated microorganisms, auxin production drives several aspects of plant development. The production of IAA is one of the methods that favour colonization of plant tissues without disrupting plant cells, consequently leading to enhanced growth of the host plant (Keswani *et al*., 2020). This hypothesis has been demonstrated in IAA producing endophytic microorganisms whose inoculation corresponded with enhanced growth and reproduction (Khan *et al*., 2016). Microbial produced IAA in plant associated microorganisms also plays a key role in disease suppression, and also are important in microbe-microbe interactions and competition within a niche, including quorum sensing (Keswani *et al*., 2020).

2.4.3.2 Gibberellins

Gibberellins are a class of plant hormones essential in enhancing plant stature, seed germination and flowering. In recent years, they have become vital components of research in their role in plant breeding process as part of the "green revolution" agricultural practices (Castro-Camba *et al*., 2022b). Gibberellins are produced by plants as well as microorganisms. Research on gibberellins was initiated in Japan in the mid-19th century through the study of a fungal rice disease caused by *Gibberella fujikuroi* later revised and named *Fusarium fujikuroi*. Infected rice plants were characterized by elongated stems and sterility which were attributed to overproduction of gibberellins by the fungus during infection (Castro-Camba *et al*., 2022b; Hedden & Sponsel, 2015). Further studies isolated various chemical compounds named gibberellic acid which were confirmed to rescue dwarf varieties and shorten the developmental phase (Yaxley *et al*., 2001). To date, rapid progress has been made on the role of gibberellins in crop growth and development, including their biosynthetic pathways (Shani *et al*., 2024), proteins involved (Iizuka *et al*., 2022), their roles in gene regulation (Jan & Komatsu, 2006) and in plant abiotic stress tolerance (Emamverdian *et al*., 2020). The hormone has been chemically synthesized and is commercially available for exogenous application in agriculture.

Gibberellins consist of a large group of tetracyclic diterpenoids produced by plants, fungi and bacteria that are associated with plants. Since the discovery of gibberellins from fungi, studies have focused on their production by fungi, revealing important functions in plant associated fungi. Although known for their role in plant growth promotion, gibberellins have been associated with enhancing susceptibility of plants to some pathogens (De Bruyne *et al*., 2014) and nematodes (Yimer *et al*., 2018). In rhizobacteria, gibberellin production has been associated with symbiosis, aiding the enlargement of nodules while increasing bacterial populations and decreasing the number of nodules. Thus, providing a selective advantage in plant association with gibberellin producing bacteria (Nett *et al*., 2022). Nodules also include a large diversity of non-rhizobial endophytic bacteria whose roles have not been fully understood (Dhole, 2018). Regulation of gibberellins is crucial in plant-pathogen interactions and elicitation of plant defence mechanisms, as plants have to balance

between growth enhancement and defence (Castro-Camba *et al*., 2022). It has been noted that despite nodules being packed with gibberellin producing bacteria, the cells remain active and do not develop defence reactions (Berrabah *et al*., 2019), and this could probably explain the association with a large diversity of non-rhizobia bacteria which would play a role in rhizosphere microbiota through modulation of root exudates and defence (Debnath *et al*., 2023; Dhole, 2018).

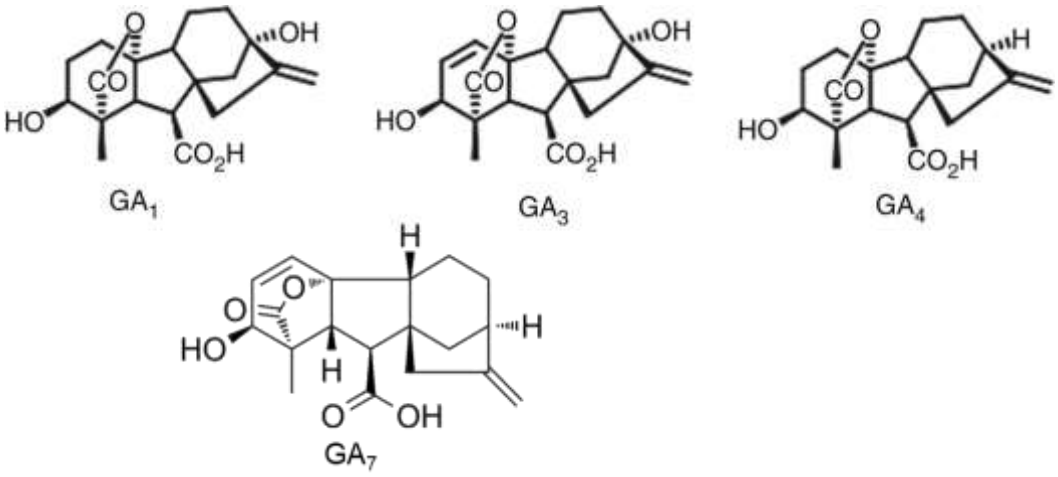

Figure 2.3: Chemical Structure of the Bioactive Gibberellins

Source: (Adopted from Keswani et al., 2022).

Gibberellins producing endophytic fungi and bacteria have been implicated in alleviation of abiotic stress tolerance in associated crop plants (Hamayun *et al*., 2017; Latif Khan *et al*., 2012). Gibberellin production pathway in microorganisms is regulated by other hormones and environmental stress signals, affecting its levels (Sun, 2008). The biosynthetic pathways in plants, bacteria and fungi were shown to have evolved independently, although the intermediates are the same (Keswani *et al*., 2022). More than 100 structurally different gibberellins have been identified, and only about 4 are biologically active and the rest are intermediaries in the final biosynthesis of the bioactive gibbrellins and others are implicated in species specific activities (Nett *et al*., 2017). For example, in *Fusarium fujikuroi*, the biosynthesis of GA_3, the main product of the fungi, follows a pathway that hydoxylates and oxygenates several GAs' catalysed by cytochrome P450 monooxygenases. Fungal GA biosynthetic pathways

seem to slightly differ, with some fungal species producing GA end products which are produced as precursors by other fungal species (Keswani *et al*., 2022). In bacteria, the biosynthetic pathway was shown to have evolved a route that is distinct from that used by plants and fungi, using a distinct set of enzymes to catalyse the relevant transformations (Nett *et al*., 2017).

2.4.3.3 Cytokinins

Microbial production of cytokinins is a well-documented phenomenon, particularly among plant-associated microorganisms such as endophytes, rhizobacteria, and certain fungi. Cytokinins are a class of plant hormones that regulate cell division, shoot and root development, break seed dormancy, enhance soil structure, degrade Xenobiotics and delay leaf senescence (Chaudhary et al., 2022). Several bacterial genera, including *Azospirillum*, *Agrobacterium*, *Pseudomonas*, and *Bacillus*, have been shown to synthesize cytokinins either through de novo biosynthesis or by converting plant-derived precursors. Microbially produced cytokinins can significantly influence plant development by enhancing shoot proliferation, improving nutrient uptake, and promoting resistance to environmental stresses such as drought and salinity (Chaudhary et al., 2022). Microbial inoculants capable of continuous cytokinin production are being explored as biofertilizers and plant growth-promoting agents to reduce reliance on chemical inputs and improve crop resilience under stress conditions.

2.4.3.4 Ethylene Regulation

Microbial management of ethylene production in plants is a key strategy used by certain beneficial microbes to enhance plant growth, especially under stress conditions such as salinity, drought, and heavy metal toxicity. Ethylene is a plant hormone that, while essential in small amounts for processes like fruit ripening and leaf abscission, can become harmful when overproduced during stress, leading to inhibited root elongation and premature senescence (Zhu et al., 2016). Many plant growth-promoting rhizobacteria (PGPR) and endophytes help manage ethylene levels through the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme breaks down ACC, the immediate precursor of ethylene in plants, into ammonia and α-ketobutyrate, thereby reducing ethylene synthesis. By lowering

ethylene levels, these microbes prevent stress-induced growth inhibition and promote root and shoot elongation and enhance plant resilience. Genera such as Pseudomonas, Bacillus, Enterobacter, Burkholderia and Azospirillum are well-known for ACC deaminase activity (Nascimento et al., 2016). By colonizing internal plant tissues without causing harm, these endophytes offer a sustained and localized means of ethylene regulation, making them valuable tools in sustainable agriculture and in the development of bio-inoculants for stress-prone environments.

2.4.3.5 Salicylic Acid

Salicylic acid (SA), a plant hormone and precursor to aspirin, can be produced by various microorganisms, primarily bacteria. Bacteria synthesize SA through different pathways compared to plants, often involving the enzyme isochorismate pyruvate lyase (IPL) and/or salicylate synthase (SAS). Some bacteria also utilize SA in the production of siderophores, iron-chelating molecules (Khan et al., 2015). In plants, salicylic acid involves the use of the shikimic acid pathway and the malonic acid pathway. These pathways convert simple carbohydrate precursors derived from glycolysis and pentose phosphate pathway to the aromatic amino acids including SA precursor, phenylalanine (Herrmann and Weaver, 1999). Microbial production of salicylic acid (SA) plays a crucial role in enhancing plant tolerance to abiotic stresses such as salinity, drought, heat, and heavy metal toxicity. Some microorganisms such as Pseudomonas, Bacillus, Azospirillum, Salmonella, Achromobacter, Vibrio, Yersinia, and Mycobacteria, have been reported to synthesize salicylates through the NRPS/PKS biosynthetic gene clusters. This bacterial salicylate production is often linked to the biosynthesis of small ferric-ion-chelating molecules, salicyl-derived siderophores (known as catecholate) under iron-limited conditions. Although bacteria possess entirely different biosynthetic pathways from plants, they share one common biosynthetic enzyme, isochorismate synthase, which converts chorismate to isochorismate, a common precursor for synthesizing SA (Islam et al., 2020). Certain plant-associated microbes, including endophytes and rhizobacteria, also synthesize salicylic acid and release it into the rhizosphere or directly into plant tissues (Mishra et al., 2021). Salicylic acid was reported to induce salinity tolerance and increased biomass of *Torreya grandis* as a result of enhanced chlorophyll content and the activity

of antioxidant enzymes that eventually activated the photosynthetic process and alleviated oxidative stress (Li et al., 2014).

2.4.4 Endophytes in Biotic and Abiotic Stress Management

Endophytic microorganisms form important partnerships with plants, playing a role in host fitness, hormone production, absorption of nutrients, hormone activation, production of secondary metabolites and antibiosis (Chaudhary *et al*., 2022). Both fungal and bacterial endophytes have been used in the biocontrol of plant diseases and pests. The modes of action of these endophytes are different, although the effects exerted remain more or less the same and include induction of systemic resistance, production of lytic enzymes to control the pathogen and quorum quenching or disruption of signalling molecules (Chaudhary *et al*., 2022). Pathogenic microbes, particularly bacteria use quorum sensing signalling molecules such as Acetylated Homoserine Lactones while producing virulence. Endophytic microorganisms produce quorum quenching enzymes which degrade signal molecules involved in quorum sensing to inhibit virulence factors (Chen *et al*., 2013).

Endophytic microbes induce exchangeable signals to the host plant at the onset of biotic or abiotic stress to help the plant acquire systemic resistance to the particular stress (Adeleke *et al*., 2022). Several methods are used by the endophytes to induce these changes to the host plant and include priming of defence related genes (Díaz-Valle *et al*., 2019), activation of signalling pathways for phytohormone production (Chen *et al*., 2018), and regulation of small RNAs (Niu *et al*., 2016).

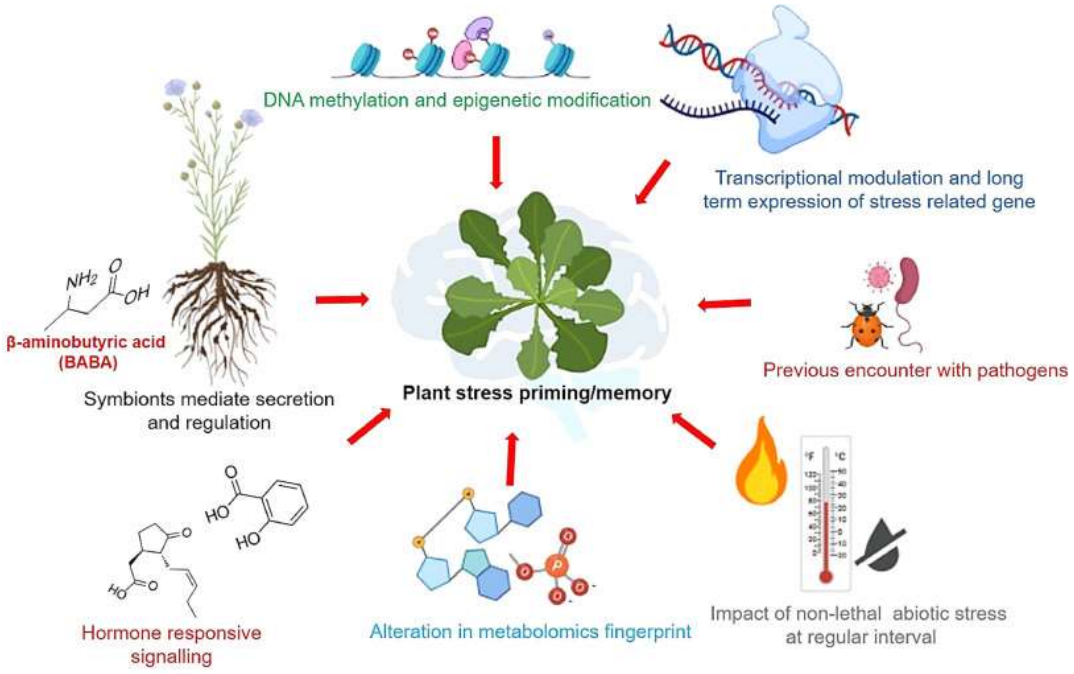

Figure 2.4: Diagrammatic Presentation of Microbe-Induced Plant Defense

Source: Adopted from Tiwari *et al*. (2022).

In some cases, the protective effect against biotic and abiotic stress mediated by endophytes can be transgenerational memory, exerting their protective effects to the next generation (Díaz-Valle *et al*., 2019). This kind of defence would be a great breakthrough in sustainable agriculture, especially for the low-income farmers who may not afford these inputs. These effects would be important in plant breeding and seed treatment of primed seeds, especially in plant growth promoting endophytic microorganisms that are transferred to seeds after inoculation. Current agricultural practices, especially monocropping have had negative effects on seeds, leading to the loss of important plant-microbe associations as witnessed by the loss of diversity in domesticated plants (Spor *et al*., 2020; Yue *et al*., 2023). The use of microbial endophytes can therefore compensate for this loss.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Research Authorization

Permission to conduct research in Kenya's soda lakes was granted by the Kenya Wildlife Service under research Authorization ref. KWS/BRM/5001 and National Commission for Science, Technology and Innovation, research permit number NACOSTI/P/17/22929/14802.

3.2 Description of Study Sites

Lake Bogoria is a saline-alkaline endorheic lake which lies in the half-graben basin to the east of the Kenyan Rift valley (Mulwa et al., 2010) on 0° 14′ 25.1700″ N, 36° 6 ′ 21.1716″ E. The Lake is believed to have been formed as a result of tectonic and volcanic activities during the Miocene era. It forms part of the Lake Bogoria National Reserve, a UNESCO world heritage site. The area is overlain by basalts, trachytes, and phonolites with volcanic soils (Mulwa *et al*., 2010). The region is semi-arid, with temperatures as high as 32°C and an erratic rainfall of about 500 mm per annum, spread in two seasons within a year; between March and May, representing the long rains and from October to November, representing the short rains. The vegetation is shaped by the lake's extreme environment, leading to a community of salt- and drought-tolerant plants. These plants form intricate and vital interactions with microorganisms, especially in the rhizosphere. These microbial-plant interactions are essential for nutrient cycling, stress tolerance, and ecosystem sustainability.

Lake Magadi is an internally drained saline alkaline lake (Deocampo *et al*., 2022) in the southern part of the Kenyan rift valley (2°S and 36°E), with an elevation of ~600 m. The lake is the most hypersaline of the East African Rift Valley lakes that were formed through tectonic and volcanic activities. It is situated in a hydrologically closed basin and is characterized by a thick trona deposit (Schagerl, 2016). The region is semiarid with temperatures ranging from 18 to 35°C. Due to these extreme conditions, certain plant communities have adapted and established around the lake margins, in

river deltas, and surrounding semi-arid areas. The vegetation is not continuous and it occurs in isolated patches where salinity is moderate and soil conditions allow plant growth. The vegetation often shows a gradient with highly salt-tolerant species growing closest to the lake shore, a mix of halophytes and shrubs at the transitional zone with moderate salinity and a typical savanna woodland and dryland shrub species further from the shore.

3.3 Sample Collection

Sample collection was done in both Lake Magadi (5 shrubs) and Lake Bogoria (8 shrubs). Healthy looking vascular shrubs growing along the shores, within 5 m from the shoreline, were collected in March and June 2016 (Lake Magadi and Lake Bogoria) respectively. For all the samples, duplicates were collected and GPS coordinates recorded. One set of plant samples was kept in plastic resealable bags in a cool box and the second set of plants was wrapped in newspapers, labeled and pressed in pieces of cartons and submitted for identification by a botanist at the National Museums of Kenya. Fungal and bacterial endophytes were isolated from these plants within 48 h of sample collection.

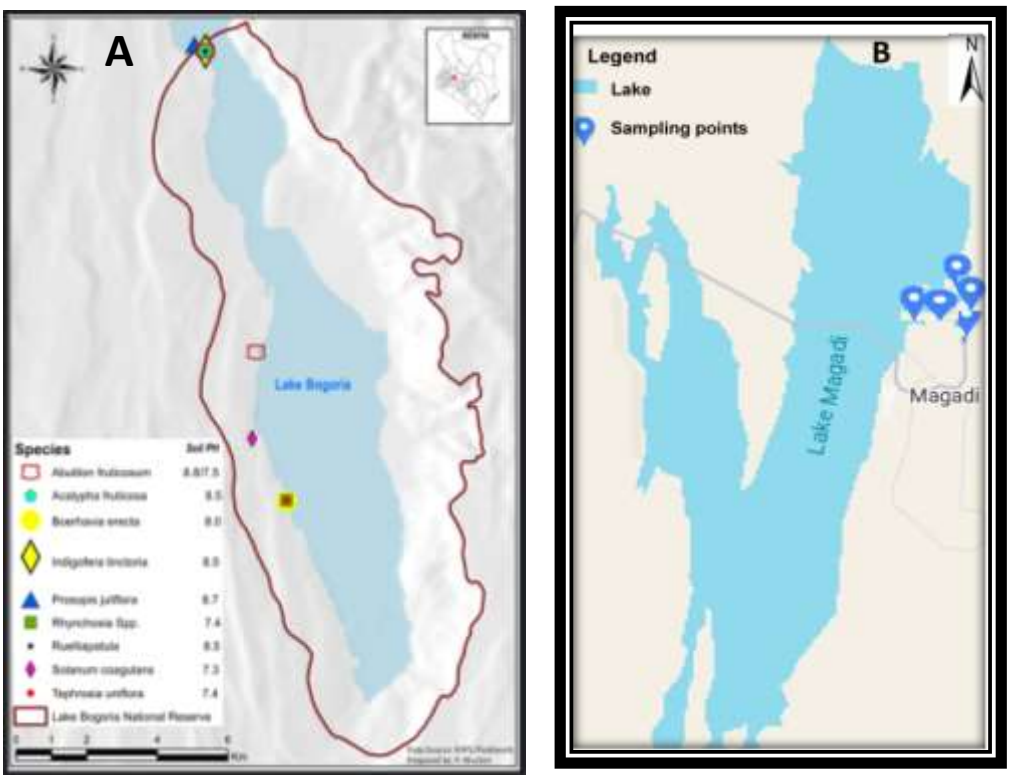

Figure 3.1 Showing Maps of Lake Bogoria and Lake Magadi Study Sites and Sampling Locations

3.3.1 Isolation of Endophytic Fungi

Isolation of endophytic fungi from shrubs collected in Lake Magadi followed the procedure described by Fouda *et al*., (2015), with some modifications. Briefly, the plant samples were separated into roots, stems and leaves and washed in running tap water to remove adhering soil and dust particles. Plants were then surface sterilized using 3% sodium hypochlorite for 3 min followed by 70% ethanol for 1 min, followed by several rinses of sterile distilled water. The last rinse water was plated out to confirm the sterilization process. Sterilized sections were asceptically cut into small pieces ~1-cm long with a sterile surgical blade and placed onto sterile filter paper. The sections were air dried under a clean bench for ~5 min and then they were placed onto freshly prepared Potato Dextrose Agar (PDA) medium (HiMedia, India) containing 50 µg/ml streptomycin sulfate and 0.25 M NaCl. The plates with plant pieces were then incubated at 28 ± 2◦C for 7–20 days with regular monitoring. Emerging fungal colonies

were isolated onto fresh PDA media and incubated under the same conditions.

3.3.2 Preservation of Fungal Cultures

Fungal cultures were preserved via agar slants and fungal spores for short- and long-term preservation, respectively. Short-term preservation followed the procedure described by Paul *et al*. (2015), with slight modifications. Slant cultures of pure isolates grown in PDA and incubated at 28°C for 4 days were overlaid with 15% v/v sterile glycerol and stored at 4◦C. Fungal spores for long-term preservation were collected from cultures grown in PDA for 2 weeks and then harvested in sterile 15% dimethyl sulfoxide (DMSO). One milliliter of the spore-DMSO mixture was transferred to a −80°C freezer, where the temperature was decreased slowly and at a controlled rate from room temperature to −80°C (Dahmen *et al*., 1983). The percentage of spore germination was calculated for each fungal culture before preservation, and only those with more than 90% spore germination were preserved.

3.3.3 Morphological Characterization of Fungal Endophytes

Sixty fungal endophytes were grouped into 18 groups based on morphological characteristics of the growing cultures as displayed on PDA. These characteristics included growth rate, colony morphology and pigmentation. Representative isolates from each morphological group were further characterized.

3.3.4 DNA extraction, Amplification and Sequence Analysis

Fungal DNA was extracted using the manual Cetyltrimethylammonium bromide (CTAB) extraction method, as described by Umesha *et al*., (2016). Pure fungal cultures were inoculated in PDA and incubated for 3 to 5 days. Growing mycelia were harvested using a sterile surgical blade and transferred into a sterile 1.5-ml Eppendorf microcentrifuge tube. The mycelia were ground with liquid nitrogen using a micropestle. Lysis buffer (800 µl of 0.1 M Tris-HCL, 50 mM EDTA, 2.5 M NaCl, 3% SDS and 3.5% CTAB) was added to the ground mycelia and the mixture was vortexed and incubated in a water bath at 65°C for 1 h with occasional shaking. The contents were centrifuged for 10 min at room temperature (25–27◦C). An equal volume of

phenol-chloroform-isoamyl alcohol (25:24:1) was added to 500 µl of the supernatant and mixed well. The mixture was centrifuged 10 000 × g for 10 min at room temperature, then the supernatant was carefully collected in a fresh tube and mixed with an equal volume of chloroform-isoamyl alcohol (24:1) and 30 µl of sodium acetate. The mixture was then centrifuged. An equal volume of ice-cold isopropanol was added, and the sample was kept at −20◦C for 2 h. The DNA was pelleted by centrifugation for 15 min at 13 000 × g at room temperature. Pelleted DNA was washed with 800 µl of 70% ethanol and air dried before dissolving in TE buffer (10 mM Tris-HCl PH 8, 1 mM EDTA). The purity of the DNA was checked on 0.8% agarose gel electrophoresis. The internal transcribed region (ITS1, 5.8S ITS2) of the ribosomal DNA was amplified by PCR using the primer set ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATGATGC-3′) (White *et al*. 1990). PCR was performed in a 50-µl reaction volume under the following conditions: 95◦C for 5 min for the initial denaturation and enzyme activation followed by 35 cycles of denaturation at 95◦C for 30 s, annealing at 55◦C for 1 min, elongation at 72◦C for 1 min, then a final elongation at 72◦C for 10 min. The PCR product was visualized under UV light on a 1.5% agarose gel stained with ethidium bromide. Thirty microliters of amplicons were submitted to macrogen-Europe for bidirectional sequencing.

3.3.5 Sequence Assembly and Phylogenetic Analysis

The resulting sequences were trimmed and edited using Chromas version 2.6.6 (www.technelysium.com.au/wp/chromas). Chromatogram viewing and editing, sequence assembly, ambiguity correction and double-pick mutation detection were performed using DNABaser version 4 (www. DNABaser.com). The resulting consensus sequences were matched to highly similar sequences in the National Institute for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLASTn) to infer evolutionary relationships. The MEGA11 (Molecular Evolutionary Genetic Analysis) program was used for phylogenetic analysis (Tamura *et al*., 2021). Sequences were aligned according to inferred evolutionary history using the maximum likelihood method with a bootstrap consensus of 1000 replicates. Evolutionary distance was inferred using the Tamura–Nei method

(Tamura & Nei, 1993), which considers the number of base substitutions per site and eliminates all positions with gaps and missing data.

3.3.6 Phenotypic Characterization

3.3.6.1 Amylase Activity

Amylase production of the isolates was screened using the plate culture technique as described by Sunitha *et al*., (2012), with slight modifications. Glucose yeast extract peptone agar (1 g/l glucose, 0.1 g/l yeast extract, 0.5 g/l peptone, 16 g/l agar) supplemented with 2% soluble starch and 50 µg/ml streptomycin was used to screen the isolates. First, an agar plug from a sporulating fungal plate was placed at the center of a glucose yeast extract peptone agar plate, and then the plate was incubated at 28°C for 3 days. Amylase production was detected by flooding the plates with Lugol's iodine solution (1 g of iodine crystals and 2 g of potassium iodide dissolved in 100 ml of distilled water). A clear zone around a fungal colony indicates amylase production.

3.3.6.2 Cellulase Activity

Cellulase production was tested by growing the isolates on yeast extract peptone agar supplemented with 0.5% carboxymethyl cellulose, as described by Carrasco, (2016). Agar plugs of uniform diameter were excised from a sporulating fungal plate using the end part of a 1ml pipet and placed at the center of a freshly prepared plate containing yeast extract peptone agar. The plate was incubated for 3 days at 28◦C, and then it was flooded with 0.2% Congo red and destained with 1 M NaCl. The development of a yellow ring around a fungal colony indicated the production of cellulases.

3.3.6.3 Protease Activity

Protease production was tested on fungal cultures inoculated on yeast extract peptone agar supplemented with 0.4% gelatin at pH 6 (Sharma *et al*., 2015). Agar plugs from a sporulating plate were excised using the end of a 1ml pipet and placed onto the yeast extract peptone agar. The plate was incubated at 28◦C for 3 days and then flooded with saturated aqueous ammonium sulfate, which was prepared by dissolving 541.8 g in one liter of distilled water (4.1 M) at 25◦C. Clear zones around the colonies indicated

protease activity.

3.3.7 Phosphate Solubilization

The ability of pure cultures of fungi to solubilize phosphate was tested in Pikovskaya agar (Hi Media, India) supplemented with 0.3% tricalcium phosphate. The sterilized medium was poured into 9-mm plastic Petri dishes and left to cool. Fungal mycelial plugs from actively growing cultures were placed onto the agar medium and incubated for 5–8 days. Clear zones around the fungal colonies indicate phosphate solubilization (Bilal *et al*., 2018).

3.3.8 Fungal Growth at Increasing Sodium Chloride Concentrations

The ability of fungal isolates to grow at increasing concentrations of sodium chloride was tested by growing them in plates of fresh PDA medium supplemented with 0 mM, 0.5 mM, 1 M and 1.5 M sodium chloride. An agar plug from a sporulating plate was placed at the center of plates containing the different sodium chloride concentrations. Three replicate plates per NaCl concentration were incubated for 14 days, and the radial growth of each culture was measured.

3.4 Isolation of Endophytic Bacteria

Isolation was carried out with shrub samples collected from Lake Bogoria, within 48 h of sample collection. Different plant parts i.e., the seeds, the roots, the stems, and the leaves were separately washed thoroughly under running tap water to remove adherent soil particles and dust; then they were superficially sterilized according to the procedure by Costa *et al*., (2012) with minor modifications. Plant parts were sequentially immersed in 3% sodium hypochlorite with 0.01% Tween 20 for 3 min, 70% ethanol for 3 min, and then rinsed 4 times with sterile distilled water. After sterilization, the different plant parts were aseptically placed on a sterile filter paper and left to drain excess water for about 5 min under a clean bench. Confirmation of sterilization was done by spreading 100 µl of the final rinse water on a nutrient agar plate. Sterilized plant samples were cut into small pieces of about 2 cm long and soft tissues (leaves and roots) were ground into a paste with a sterile normal saline *(Li et*

al., 2018), serially diluted ten-fold twice and 100 µl of the second dilution was plated onto freshly prepared nutrient agar plates supplemented with 0.25 M NaCl and incubated at 28°C for 3–10 days. The stem and seeds were cut into small pieces which were placed on nutrient agar plates. Emerging bacterial colonies were streaked onto fresh nutrient agar plates under the same conditions. Pure cultures were preserved in 30% glycerol and kept at −20◦C for immediate use and −80◦C for long-term preservation.

3.4.1 Morphological Characterization of Endophytic Bacteria

Pure endophytic bacterial colonies were grouped according to colony characteristics including colony edges, opacity, metabolite production into the agar media, colony color and representatives were chosen for further analysis.

3.4.1.1 Assay of Exo-Enzyme Production

Amylase activity was tested using spot inoculation on a mineral starch medium agar prepared using g/l 0.5 K_2HPO_4, 1 KH_2PO_4, 1 NH_4CL, 0.2 $MgSO_4$, 5 Starch, and 20 agar PH 8.0 (Al-Johani *et al*., 2017). Plugs of equal diameter were excised from a 24 h old plate and inoculated at the center of a freshly prepared starch agar plate. The plates were incubated at 28°C for 48 h after which grams iodine was flooded. Halo zone around the colonies indicated starch solubilization (Mishra & Behera, 2008).

Catalase reaction was tested with 3% V/V hydrogen peroxide (Reiner, 2010). A loopful of bacteria from actively growing bacterial cultures was scooped using a sterilized wooden pin, placed on a glass slide in a petri dish, and a drop of hydrogen peroxide was added to the bacterial scoop to observe immediate effervescence for catalase-positive bacteria.

Urease activity was evaluated using Stuart's urea broth containing the following in one liter: 20 g urea, 9.5 g K_2HPO, 9.1 g KH_2PO, 0.1 g yeast extract, and 0.01 g phenol red having the PH of 6.8. Ten milliliters of broth were distributed in test tubes and each test tube was inoculated with 10 µl of an 18 h culture. These were incubated at 35◦C for 48 h and the color change to bright pink indicated a positive urease reaction (Brink,

2016).

3.4.2 Plant Growth Promotion Assays

3.4.2.1 Phosphate Solubilization

Phosphate solubilization potential was tested according to the procedure by Goswami *et al*., (2014) on the selected bacterial isolates. Briefly, bacteria were spot inoculated at the center of a Pikovskaya agar plate and the plates were incubated at 27 ± 2°C for 5 days. Formation of a halo zone around the colony indicated solubilization of phosphate by the bacteria.

3.4.2.2 Production of Hydrogen Cyanide

Bacteria isolates were grown on a nutrient agar supplemented with 4.4 g/L of glycine (El-rahman & Shaheen, 2016). A sterile filter paper was soaked with 0.5% picric in 2% sodium carbonate and then placed on the cover of a plate. The plate was tightly sealed with parafilm and incubated at 28◦C for 3 days. Change of the filter paper from yellow to brick red indicated the production of hydrogen cyanide (HCN).

3.4.2.3 Indole Acetic Acid Production

Bacterial cultures were grown in Tryptic soy broth (TSB) supplemented with 0.2 mg/ml L-tryptophan and grown in a shaker at 28◦C for 72 h (Goswami *et al*., 2014). After incubation, the cultures were centrifuged at 12,000×g for 5 min. One milliliter of the supernatant was mixed with 2 ml of Salkowski reagent (0.5M $FeCl_3$ in 35% $HCLO_4$) in clean dry test tubes and incubated in the dark for 30 min (Orhan, 2016). The change of the solution to a reddish-brown color was an indication of the production of IAA by the respective isolate.

3.4.3 NaCl Tolerance

Endophytic bacterial salt tolerance was tested using a nutrient agar supplemented with 0, 0.5, 1 and 1.5 M NaCl and bacterial growth was observed after incubation at 28◦C for 3 days. Similar concentrations of NaCl were supplemented to nutrient broth and

the selected bacterial isolates were grown in an incubator shaker at 28◦C, 150 RPM for 48 h. The rate of growth, as turbidity, was determined with a spectrophotometer at OD_{600} with 4 replicates of each endophytic bacteria (Jiang *et al*., 2019).

3.4.4 Molecular Characterization

Bacterial DNA was extracted using commercial Quick DNA Fungal/Bacterial miniprep Kit (Zymo Research, CA, USA) following the instructions of the manufacturer. The integrity of the extracted DNA was visualized on 0.8% agarose gel. PCR amplification targeted the nearly full length bacterial 16S rRNA gene using the universal bacterial primer set 27F, 5′ -AGAGTTTGATCCTGGCTCAG-3′ and 1492R, 5′ - GGTTACCTTGTTACGACCT-3′ (Li *et al*., 2018). The PCR reaction mix in a 30 µl final reaction volume was comprised of each primer at 1 µl (10 pmol), 1 µl endophytic bacterial DNA, 15 µl One Taq 2x master mix (Biolabs, MA, USA), and 12 µl nuclease-free water. PCR amplifications (36 cycles) was done using the following conditions: an initial denaturation for 5 min at 95◦C, denaturation at 95◦C for 40 s, annealing at 55◦C for 45 s, and extension for 72◦C for 45 s followed by a final extension at 72◦C for 5 min. PCR amplicons were visualized on a 1.8% agarose gel stained with ethidium bromide. Twenty-five microliters of the amplicons were submitted to Inqaba, South Africa for bi-directional sequencing.

3.4.4.1 16S rRNA Sequence Analysis

Raw nucleotide sequences were viewed trimmed and edited using Chromas Version 2.6.6 (www.technelysium.com.au/wp/chromas). Assembly of contigs, contig ambiguity correction, and detection of double pick mutations were performed using DNABaser Version 4, Heracle Biosoft (www.DnaBaser.com). EZ BioCloud identification service (Yoon *et al*., 2017) and BLASTn (www.ncbi.nlm.nih.gov/ BLAST) search pipeline were used to compare the assembled sequences with highly homologous bacterial sequences in the databases. Gene sequences were deposited at the NCBI GenBank. Molecular phylogenetic analysis was performed using MEGA-X (Kumar *et al*., 2018). Sequences were aligned using ClustalW and evolutionary history of the different taxa were inferred using the Maximum likelihood method with a bootstrap consensus of 1,000 replicates. Evolutionary distance was computed using

the Tamura Nei model in the number of substitutions per site with complete elimination of the missing data and gaps. There were 1,524 positions in the final data set.

3.4.5 Data Analysis

All analyses were carried out in R statistical software. Comparison of means was performed on one-way ANOVA. To differentiate group means, post-hoc analysis using Student's Newman–Keuls (SNK) and Turkeys HSD tests were undertaken. Count data was fitted on a generalized linear model with Poisson distribution. All statistical analyses were conducted at a 95% confidence level.

CHAPTER FOUR

FUNGAL ENDOPHYTES FROM SALINE-ADAPTED SHRUBS INDUCE SALINITY STRESS TOLERANCE IN TOMATO SEEDLINGS

4.1 Introduction

Soil salinity is a major abiotic stress that affects individual plant growth and development and influences the diversity of plant species in affected soils, except those in salt-tolerant plant communities (Bandel *et al*., 2022). Soil salinity is caused either by natural processes, such as rock weathering and high evapotranspiration, or man-made processes such as irrigation using brackish water in farmlands (Jones *et al*. 2012) and continuous growth of shallow-rooted crops that raise the water table. The effects of salinity on plants are exacerbated by climate change that can seriously change water cycles through changing patterns of rainfall and prolonged droughts (FAO 2021).

Lands available for agriculture have declined by 22% over the last decade, while land under irrigation has almost doubled within the same period (FAO 2021). However, the expanded irrigated lands face challenges as more than one-third of the global irrigated land is already degraded by induced salinity, while most staple crops consumed by humans are sensitive to moderately tolerant to salt (Cheeseman, 2015). The Food and Agriculture Organization has estimated the need to increase agricultural productivity by 50% by 2050 to meet the demands of the growing population (FAO 2021).

Irrigated agriculture continues to play an important role in meeting the food needs of the world's population. Soil salinization, particularly resulting from irrigation and extreme weather conditions, is expected to increase and thereby continue to threaten food security in the future, especially in lands with arid and semiarid climates, where there is a rising demand for irrigation water to support agricultural production (Tnay, 2019).

Efforts have been put in place in the last three decades to understand the mechanisms of salt stress tolerance in plants, especially in halophytes (Zhao *et al*., 2020). Several physiological, metabolic and molecular mechanisms are used by plants to mitigate

salinity stress, and these can be used to engineer crops with enhanced salinity tolerance. However, crop engineering for salinity tolerance has been slow, expensive and challenging due to the many knowledge gaps regarding plant responses to salinity stress, especially at the organelle, transcriptional and expression levels (Zhao *et al*. 2020).

In addition to efforts to understand the mechanisms of plant salinity stress tolerance, dedicated and rigorous efforts have been made to mine the plant microbiome communities and study their interactions. Various studies on plant–microbe interactions have revealed the functions of endophytes in different plants growing in different environments, including saline, neutral, geothermal, desert and marine ecosystems (Andreote *et al*., 2014b; Berg *et al*., 2014;Kaul *et al*., 2016; Zhou *et al*., 2015; Rho *et al*., 2018; Verma *et al*., 2021). These microorganisms, especially fungi, form symbiotic relationships that confer fitness benefits to plants, such as biotic and abiotic stress tolerance and improved nutrient acquisition (Rodriguez & Redman, 2008). However, the ecological roles of endophytic fungi are not fully understood (Gonçalves *et al*., 2021).

Some benefits conferred by microorganisms are hypothesized to be related to habitat adaptation (Rodriguez & Redman 2008). For example, inoculation of an *Ampelomyces* sp. isolated from a plant growing under drought and poor nutrient conditions into tomato seedlings and grown for 8 days without water resulted in plant survival in the absence of water. Similarly, inoculation of *Penicillium chrysogenum* isolated from a plant growing in a salt-stressed environment into tomato seedlings and exposed to 300 mM NaCl resulted in plants that were healthier than uninoculated plants throughout the salinity exposure period (Morsy *et al*., 2020).

Therefore, collecting novel fungal endophytes from plants growing in extreme environments is of great biotechnological value for economically important crop plants because of the changing climatic conditions, especially in arid and semiarid regions.

Endophytes from extreme environments can confer tolerance to biotic and abiotic stresses on crop plants (Moghaddam *et al*., 2022; Redman *et al*., 2011; Morsy *et al*.

2020, Mutungi *et al*. 2021). Kenya is home to the East African Rift Valley System, which harbors several saline alkaline lakes (soda lakes) that are characterized by saline and alkaline conditions (Schagerl & Renaut 2016). Studies on fungal populations in these unique ecosystems in Kenya are sparse, and the few that have been conducted have mainly focused on the diversity of fungi in soil sediments and water (Orwa *et al*., 2020). Therefore, the current study focused on the isolation of fungal endophytes from five shrubs collected along the shores of the soda Lake Magadi in Kenya. The isolated fungal endophytes were assessed for their potential to enhance tomato seed germination and improve tomato growth under salinity stress in a greenhouse.

4.2 Materials and Methods

Study site, sample collection, isolation and characterization of endophytic fungi were carried out as outlined in chapter 3.

4.2.1 Seed Inoculation and Assessment of Endophytic Competence of the Isolates

4.2.1.1 Fungal Cultures

Four fungal isolates were selected for further in vitro experiments. The four isolates were chosen based on their ability to produce all the tested exo-enzymes and their growth in all the tested salt concentrations. Seeds were inoculated following the procedure described by (Jaber, 2018a). Fungal cultures were grown on PDA supplemented with 50 µg/ml streptomycin sulfate and incubated until sporulation (18–20 days). Each sporulating fungal culture was flooded with ~3 ml of sterile distilled water containing 1% Tween 80, and the conidia were harvested by gently scraping the surface using a sterile glass rod. The conidial suspension was then gently vortexed, and the conidial concentration was determined using a Neubauer hemocytometer (Electron Microscopy Sciences). Conidial viability was tested by plating 100 µl of spore suspension on a fresh PDA plate. A sterile coverslip was placed on top of the media and the plate was incubated for 24 h. Conidia with germ tubes longer than the length of the conidia were considered germinated. Only suspensions with more than 90% spore germination were considered for the experiment (Jaber 2018). Two concentrations of 10^6 and 10^8 conidia/ml were used to inoculate seeds to determine the

endophytic competence of the isolates in tomato seedlings.

4.2.1.2 Seed Inoculation

Solanum lycopersicum variety Cal J seeds were surface sterilized by washing them first in tap water followed by a 2-min wash in 3% sodium hypochlorite, followed by 2 min in 70% ethanol, then three rinses in sterile distilled water. The final rinse water was plated on PDA to confirm the effectiveness of surface sterilization (Muvea *et al*., 2014). Sterilized seeds were then air dried for 30 min on sterile filter paper and soaked in either 10^6 or 10^8 conidia/ml of each isolate overnight. Control seeds were soaked in sterile distilled water containing 1% Tween 80. Inoculated seeds were air dried for 30 min before being transferred to plastic pots containing sterile vermiculite moistened with half-strength Hoagland's solution. Three seeds were sown in each pot, and the pots were transferred to a growth chamber set at 27 ± 2◦C at a 12 h: 12 h light–dark cycle. The pots were arranged in a completely randomized block design with four replicates per treatment. Sterilized half-strength Hoagland's solution was added as necessary.

4.2.1.3 Assessment of Endophyte Colonization

Twenty-one days after germination, the seedlings were gently uprooted from the pots. Seedlings were washed in running tap water to remove any vermiculite adhering to the roots, and then each seedling was divided into roots, stems and leaves. Each of these plant parts was surface sterilized as described above. Six pieces of each plant part per conidial concentration per seedling were cut into ∼1-cm-long pieces using a sterile surgical blade under a laminar flow hood. The pieces were plated onto PDA plates supplemented with 50 μg/ml streptomycin sulfate and incubated in the dark at 25 ± 2◦C for 14 days. The growing fungal cultures were stained with lactophenol cotton blue stain. The morphological characteristics of the seedling-derived cultures were compared with those of the original isolates (Muvea *et al*. 2014).

4.2.2 Effect of Endophytic Fungi on Germination of Tomato Seeds Under Salinity Stress

The conidial concentration of 10^8 conidia/ml gave a higher endophyte recovery rate than 10^6 conia/ml and was therefore chosen for use in greenhouse experiments. Procedures for seed sterilization and colonization were performed as described above. Colonized seeds were transferred to 9-mm diameter Petri dishes containing sterile water agar supplemented with 0, 50, 75, 100 and 125mM sodium chloride. The seeds were incubated in the dark for up to 10 days while checking daily for germination. Two plates were set per isolate per salinity concentration and non-inoculated seeds served as controls.

4.2.3 Effect of Endophytic Fungi on Tomato Seedlings Under Salinity Stress in a Greenhouse

Solanum lycopersicum variety Cal J seeds were surface sterilized as described above and soaked overnight in 10^8 conidia/ml of each fungal isolate. Inoculated seeds were air dried on sterile filter paper under sterile conditions for 2 h before transferring them to 1% sterile water agar plates to avoid additional handling of seedlings in the course of the experiment. The seeds were germinated by incubating them in the dark at 25 ± 2◦C for 4 days, and then the germinated seeds were transferred to plastic pots (15 × 17 cm) containing a 5:1 mixture of sterilized forest soil and cattle manure, respectively. Before potting, the soil and cattle manure mixture were sterilized by autoclaving for 40 min at 121˚C, left to cool overnight, then autoclaved again. Approximately 1 kg of sterile soil was distributed in each pot, and two germinated seedlings per isolate were transplanted ∼2-cm deep into the soil. Uninoculated seedlings grown under salinity stress and no salinity stress served as controls. The seedlings were grown in a greenhouse and maintained under ambient conditions at 25–28˚C, arranged in a completely randomized design. The seedlings were watered with sterile tap water as required for 30 days with no additional fertilization, followed by watering with sterile tap water supplemented with 125 mM NaCl for 28 days. The following six treatments at 20 seedlings per treatment were compared: (i) F04 + 125 mM NaCl; (ii) F05 + 125 mM NaCl; (iii) F18 + 125 mM NaCl; (iv) F21 + 125 mM NaCl; (v) non-inoculated

seedlings (C + 125 mM NaCl); and (vi) non-inoculated (C with no NaCl). After the treatments, the seedlings were flooded with tap water overnight, uprooted and washed under running tap water to remove any adhering soil particles. The seedlings were then wrapped in a paper towel to remove excess water. Ten seedlings (one from each replicate) were selected per treatment for measurements of root and shoot wet and dry weights. Dry weight was measured by drying the seedlings in an oven at 68◦C for 48 hr (Balliu et al., 2015). Chlorophyll and carotenoid content were measured using the procedure described by Lichtenthaler & Buschmann (2001). Briefly, 1.5 g of fresh leaves were ground in the dark in 100% acetone and centrifuged at 10 000 × g for 10 min. The supernatant was collected for absorbance measurements using a microplate spectrophotometer (Versamax). The quantities of the pigments were calculated as follows:

Chlorophyll A: $12.25_{A662}–2.79_{A647}$

Chlorophyll B: $21.50_{A647}–5.10_{A662}$

Total chlorophyll: $20.2_{A647}–8.02_{A662}$, where A662 is the absorbance of the solution at 662 nm and A647 is the absorbance of the solution at 647 nm.

Hydrogen peroxide levels in the leaves were measured using the method of Junglee *et al.*, (2014), with slight modifications. Leaves were harvested from tomato seedlings and 500 mg were ground in liquid nitrogen using a mortar and pestle. Five milliliters of 1% TCA (w/v) was added to the ground powder and mixed well. The homogenate was then centrifuged at 12 000 × g for 15 min at 4◦C. The supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7) and 1 ml of 1 M potassium iodide. The absorbance of the mixture was measured at 390 nm. The mixture without the supernatant served as the control. A standard curve of hydrogen peroxide was developed by diluting 57 µl of 30% hydrogen peroxide to 100 µl with distilled water. Additional 10 x dilutions were prepared and the absorbances of the various dilutions and measured at 390 nm.

4.2.4 Statistical Analysis

The salinity tolerance of the isolates and seed germination rates were analyzed using one-way ANOVA ($P < 0.05$) and means compared using the Student's Newman–Keuls test. The effects of the endophytes on seedling biomass, chlorophyll content and hydrogen peroxide production were determined using the Kruskal–Wallis chi-square test. Post hoc analysis was performed using Dunn's test. Data on endophyte colonization and recovery rates were fitted to a generalized linear model with a Poisson distribution. The analysis was performed using R statistical software version 2.15.4.

4.3 Results

4.3.1 Isolation and Characterization of Fungal Endophytes

Five different shrubs were collected from the shores of Lake Magadi and used for the isolation of endophytic fungi. All sampled plants harbored fungal endophytes. Sixty fungal isolates were purified from the leaves, stems and roots of collected shrubs (Table 4.1). Grouping of the isolates based on the morphological characteristics of their growth on PDA resulted in 18 different groups. *Indigofera spinosa* Forssk generated the highest number of isolates, whereas *Commicarpus grandifloras* and *Lactuca inermis* Forssk generated the least number of isolates. Most of the fungal isolates were isolated from roots (48.3%), whereas stems and leaves produced 30% and 21.7%, respectively. Of the 60 isolates, 25, 30 and 27 were positive for amylase, protease and cellulase production, respectively. Thirty-two isolates solubilized phosphate (Table 4.1). Four isolates were selected for further experiments on the basis of the rate of growth, sporulation and production of exoenzymes. Two of these isolates, *Cephalotrichum cylindricum* (F04) and *Fusarium equiseti* (F05), were from the stem of *Commicarpus grandifloras*; and the other two, *Fusarium falciforme* (F18) and *Aspergilus puniceus* (F21), were from the roots of *Indigofera spinosa* Forssk (Fig. 4.1). All four isolates were able to grow on all tested NaCl concentrations; they were all positive for the production of amylase, cellulase and protease enzymes; and they all solubilized phosphate (Table 4.1).

Table 4.1: Endophytic Fungal Isolates, Their Respective Source Plants, and Their Physiological Characteristics

Isolate No.	Source plant name	Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
F01	*Commicarpus grandiflorus*	Stem	1˚59′00″S 36˚14′25″E 651M	++	-	-	-	+	+	-	+
F02	*Commicarpus grandifloras*	Root	1˚59′00″S 36˚14′25″E 651M	+++	+	-	-	+	-	+	+
F03	*Commicarpus grandifloras*	Stem	1˚59′00″S 36˚14′25″E 651M	++++	+	-	-	+	-	-	-
F04	*Commicarpus grandifloras*	Stem	1˚59′00″S 36˚14′25″E 651M	++	+++	++	+	+	+	+	+
F05	*Commicarpus grandifloras*	Stem	1˚59′00″S 36˚14′25″E 651M	++	+++	++	+	+	+	+	+
F06	*Commicarpus grandifloras*	Root	1˚59′00″S 36˚14′25″E 651M	++++	-	-	-	+	+	-	ND
F07	*Commicarpus grandifloras*	Root	1˚59′00″S 36˚14′25″E 651M	++++	+	-	-	-	-	-	-
F08	*Indigofera spinosa Forssk*	Root	1˚52′02″S 36˚14′46″E 587M	++++	+	-	-	ND	-	-	+
F09	*Indigofera spinosa Forssk*	Stem	1˚52′02″S 36˚14′46″E 587M	++++	++	+	-	-	+	-	ND
F10	*Indigofera spinosa Forssk*	Root	1˚52′02″S 36˚14′46″E 587M	+++	+	-	-	-	-	-	-

Isolate No.	Source plant name		Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
					0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
F11	*Indigofera Forssk*	*spinosa*	Leaves	1˚52′02″S 36˚14′46″E 587M	+++	-	-	-	-	-	-	+
F12	*Indigofera Forssk*	*spinosa*	Root	1˚52′02″S 36˚14′46″E 587M	++++	+	⁻	-	-	-	ND	+
F13	*Indigofera Forssk*	*spinosa*	Leaves	1˚52′02″S 36˚14′46″E 587M	++++	+	+	-	-	+	-	-
F14	*Indigofera Forssk*	*spinosa*	Stem	1˚52′02″S 36˚14′46″E 587M	+++	++	-	-	+	-	-	+
F15	*Indigofera Forssk*	*spinosa*	Root	1˚52′02″S 36˚14′46″E 587M	++++	++	-	ND	-	+	+	+
F16	*Indigofera Forssk*	*spinosa*	Root	1˚52′02″S 36˚14′46″E 587M	+++	++	-	-	-	-	-	+
F17	*Indigofera Forssk*	*spinosa*	Leaves	1˚52′02″S 36˚14′46″E 587M	++++	++	+	-	-	-	-	-
F18	*Indigofera Forssk*	*spinosa*	Root	1˚52′02″S 36˚14′46″E 587M	+++	++	+	+	+	+	+	+
F19	*Indigofera Forssk*	*spinosa*	Stem	1˚52′02″S 36˚14′46″E 587M	++++	-	-	-	-	+	ND	+
F20	*Indigofera Forssk*	*spinosa*	Leaves	1˚52′02″S 36˚14′46″E 587M	+++	+	-	-	-	-	+	-
F21	*Indigofera Forssk*	*spinosa*	Root	1˚52′02″S 36˚14′46″E 587M	+++	++	+	+	+	+	+	+

Isolate No.	Source plant name	Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
F22	*Indigofera spinosa Forssk*	Leaves	1˚52′02″S 36˚14′46″E 587M	++++	++	+	-	+	+	ND	+
F23	*Indigofera spinosa Forssk*	Leaves	1˚52′02″S 36˚14′46″E 587M	+++	+	+	-	-	+	+	-
F24	*Indigofera spinosa Forssk*	Stem	1˚52′02″S 36˚14′46″E 587M	++++	++	-	-	-	+	+	+
F25	*Indigofera spinosa Forssk*	Root	1˚52′02″S 36˚14′46″E 587M	++	-	-	-	+	-	+	-
F26	*Indigofera spinosa Forssk*	Root	1˚52′02″S 36˚14′46″E 587M	++++	+	++	+	+	+	-	-
F27	*Indigofera spinosa Forssk*	Root	1˚52′02″S 36˚14′46″E 587M	++++	++	+	-	+	-	+	+
F28	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	++	++	+	+	+	-	+	-
F29	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	++	++	-	-	-	-	-	+
F30	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	+++	+	-	-	-	-	-	+
F31	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	++	+	-	-	+	-	-	-
F32	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	+++	++	+	-	-	-	+	+

Isolate No.	Source plant name	Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
F33	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	++++	+	-	-	-	+	-	-
F34	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	++++	-	-	-	ND	-	-	ND
F35	*Tarchonanthus camphoratus*	Root	1˚53′41″S 36˚15′12″E 616M	+++	++	+	-	+	-	+	ND
F36	*Tarchonanthus camphoratus*	Stem	1˚53′41″S 36˚15′12″E 616M	+++	+	-	-	-	+	-	+
F37	*Tarchonanthus camphoratus*	Stem	1˚53′41″S 36˚15′12″E 616M	++	-	-	-	-	+	-	-
F38	*Tarchonanthus camphoratus*	Leaves	1˚53′41″S 36˚15′12″E 616M	++++	-	-	-	+	+	-	+
F39	*Tarchonanthus camphoratus*	Leaves	1˚53′41″S 36˚15′12″E 616M	++++	+	+	-	-	ND	-	-
F40	*Tarchonanthus camphoratus*	Leaves	1˚53′41″S 36˚15′12″E 616M	++++	++	+	+	-	+	+	+
F41	*Tarchonanthus camphoratus*	Leaves	1˚53′41″S 36˚15′12″E 616M	++++	+	-	-	+	-	+	-
F42	*Prosopis juliflora*	Stem	1˚56′52″S 36˚14′25″E 654M	++++	+	-	-	+	+	+	+
F43	*Prosopis juliflora*	Stem	1˚56′52″S 36˚14′25″E 654M	+++	-	-	-	-	-	-	-
F44	*Prosopis juliflora*	Root	1˚56′52″S	++	-	-	-	+	*ND*	-	-

Isolate No.	Source plant name	Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
			36˚14′25″E 654M								
F45	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	+++	-	-	-	-	+	+	ND
F46	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	++++	++	+	-	-	+	-	+
F47	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	+++	-	-	-	-	+	+	+
F48	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	+++	-	-	-	ND	+	-	-
F49	*Prosopis juliflora*	Stem	1˚56′52″S 36˚14′25″E 654M	++++	-	-	-	+	-	-	+
F50	*Prosopis juliflora*	Stem	1˚56′52″S 36˚14′25″E 654M	+++	++	+	+	-	-	+	-
F51	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	++	-	-	-	+	+	+	+
F52	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	+++	+	-	-	-	-	+	+
F53	*Prosopis juliflora*	Root	1˚56′52″S 36˚14′25″E 654M	++++	++	+	-	-	+	-	+
F54	*Lactuca inermis Forssk*	Root	$2^0$00′04″S $36^0$13′56″E 606M	++++	+	+	-	-	-	+	-
F55	*Lactuca inermis Forssk*	Root	$2^0$00′04″S $36^0$13′56″E	++	+	-	-	+	+	-	ND

Isolate No.	Source plant name		Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
					0M	0.5M	1.0M	1.5M	Amylase	Protease	Cellulase	
F56	*Lactuca Forssk*	*inermis*	Leaves	606M 2^{0}00′04″S 36^{0}13′56″E 606M	+++	-	-		-	-	+	-
F57	*Lactuca Forssk*	*inermis*	Stem	2^{0}00′04″S 36^{0}13′56″E 606M	++	+	-	-	+	+	+	+
F58	*Lactuca Forssk*	*inermis*	Stem	2^{0}00′04″S 36^{0}13′56″E 606M	+++	-	-	-	-	+	+	+
F59	*Lactuca Forssk*	*inermis*	Leaves	2^{0}00′04″S 36^{0}13′56″E 606M	++++	++	+	-	+	-	-	-
F60	*Lactuca Forssk*	*inermis*	Leaves	2^{0}00′04″S 36^{0}13′56″E 606M	++	+	-	-	-	+	+	+

Key: [a]Growth response to salt concentrations: -, no growth; +, Slight growth: ++, Low growth: +++, Moderate growth; ++++, Full growth. [b] Exo-enzyme production: -, No production: +, production, ND not tested. Lines in bold indicate the isolates and source plants that were used for further experiments.

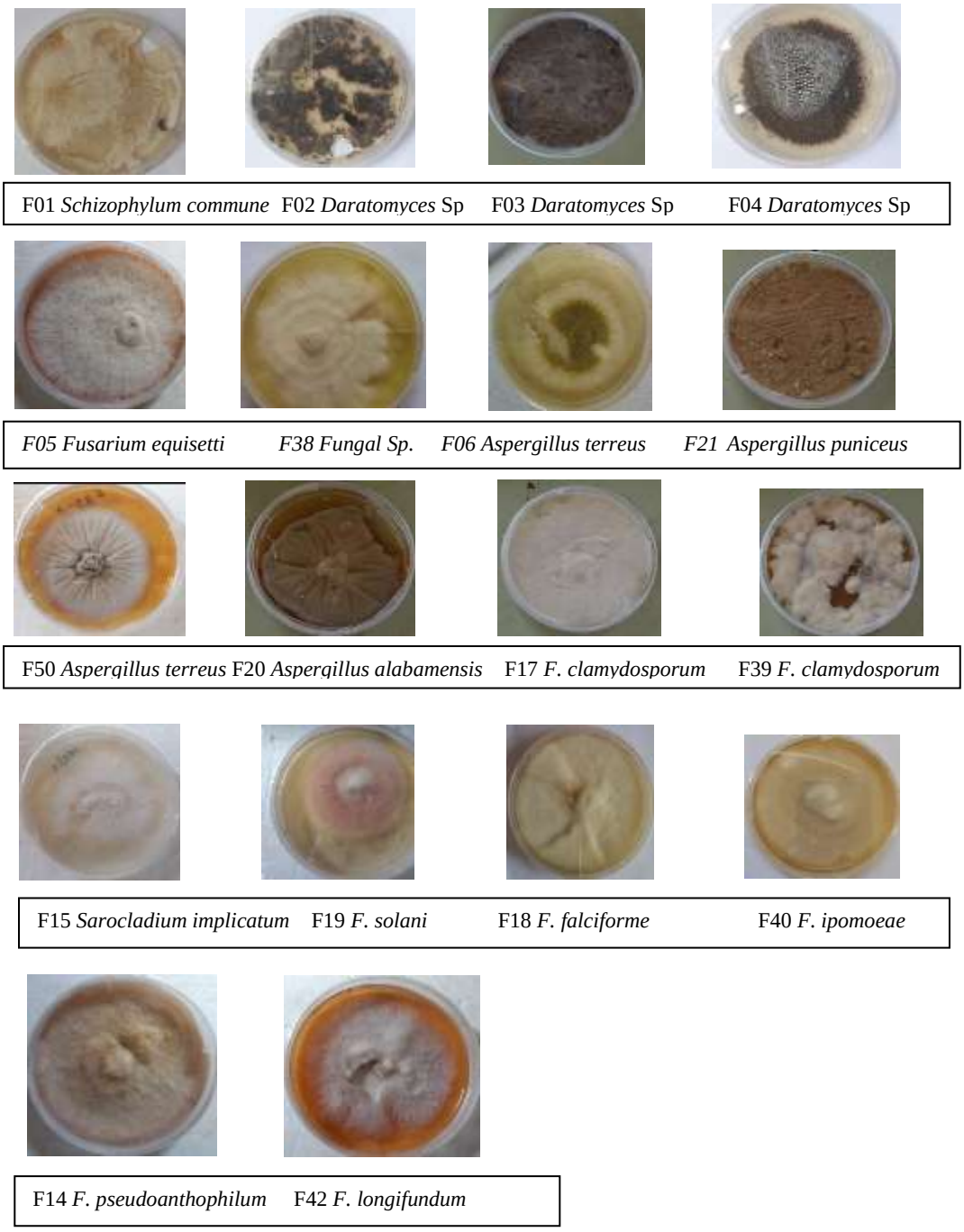

Figure 4.1: Morphological Characteristics of Representative Isolates for the Different Morphogroups

4.3.2 Molecular Identification

Analysis of the resulting consensus sequences and comparison with homologous sequences in the NCBI genebank database revealed that the genus *Fusarium* was isolated at the highest frequency and was represented by eight morphogroups; and these isolates represent seven different Fusarium species (*F. equiseti, F. pseudoathophilum, F. longifundum, F. falciforme, F. clamidosporum, F. solani* and *F. ipomea*). These morphogroups represented 28 of the 60 isolates. Species within the genus Aspergillus were the second most frequently isolated (*A. puniceus* and *A. terreus*), and these were represented by four morpho groups, to which 17 of the 60 isolates belonged. One species within the genus Cephalotricum (*C. cylindricum*) was in two morphogroups representing seven of the 60 isolates. The other identified genera (*Schizophyllum, Saracladium, Daratomyces* and Fungal species) were each represented by one morphogroup (Fig. 4.1). Ninety-five per cent of the isolates belonged to phylum Ascomycota and the remaining belonged to phylum Basidiomycota, both of which are in the subkingdom Dikarya. Isolates (three of the 60) classified under the phylum Basidiomycota all belonged to one morphogroup and to the genus Schizophyllum. The rest of the isolates belonged to the phylum Ascomycota. BLAST search results generated similarity matches ranging from 97% to 100% identity with known species. The distribution of the genus in isolation did not show any tissue or plant specificity.

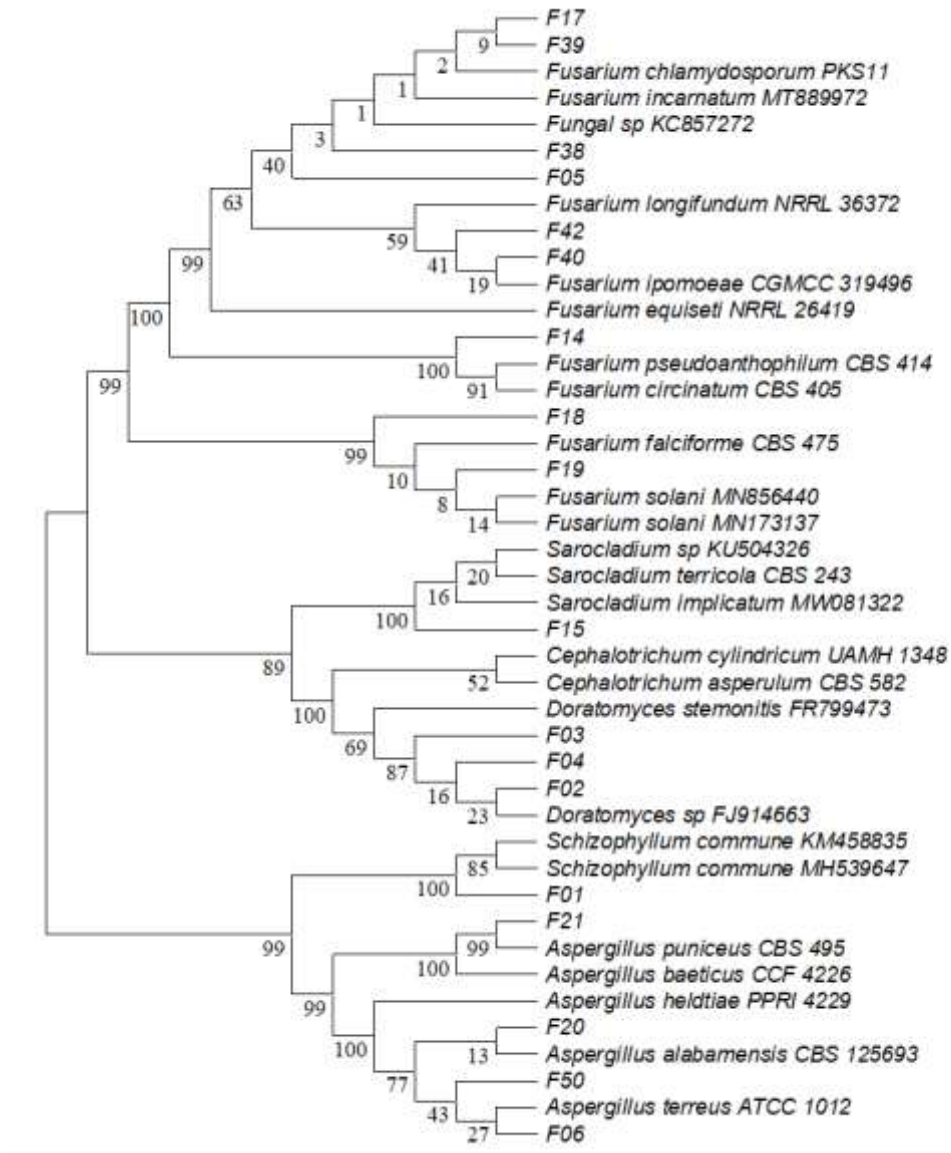

Figure 4.2: Unrooted Phylogenetic Tree of Fungal Endophytes Depicting the Evolutionary History of the Isolates Using the Maximum Likelihood Method with 1000 Bootstrap Replicates and Complete Elimination of Gaps and Missing Data

Phylogenetic analysis was performed in MEGA 11.The percentage of trees in which the associated taxa clustered together is shown below the branches.

4.3.3 Endophytic Competence of the Fungal Isolates

Two different concentrations (10^6 and 10^8 conidia/ml) of the isolates were tested for their endophytic competence in tomato seedlings grown on sterile vermiculite. The two conidial concentrations were recovered at significantly different rates (P = 0.0018). However, both concentrations of the four isolates were able to colonize all the tomato seedling parts (leaves, stems and roots) within 3 weeks (Fig. 4.2). A significant difference was detected in endophytic performance (P < 0.001). Specifically, isolate F21 was re-isolated at the highest rate at both concentrations. Although the four isolates were derived from the stem and root, no significant difference was found in fungal colonization for the different plant parts (P = 0.2492). No isolates were recovered from the control seedlings that were mock inoculated.

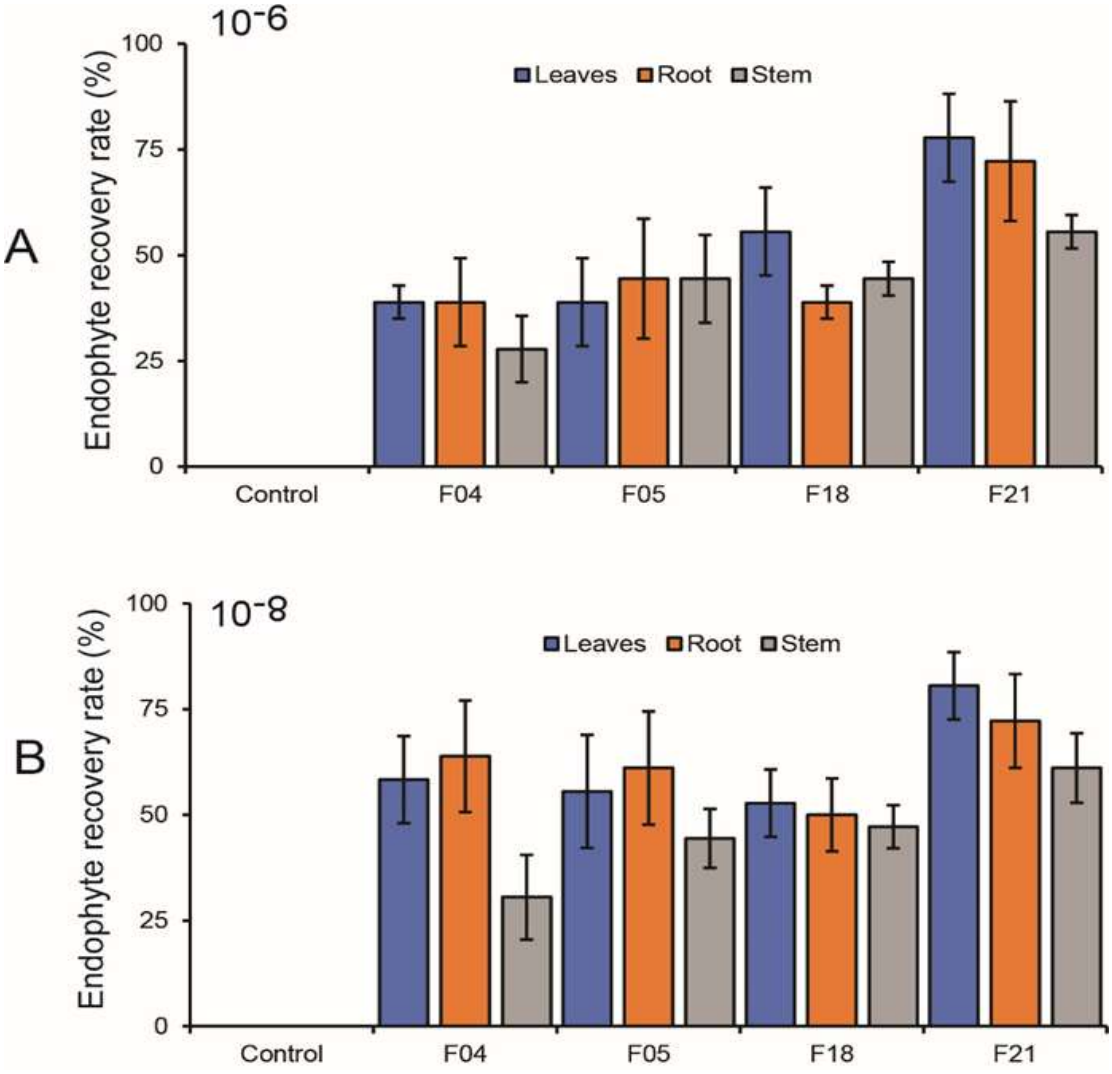

Figure 4.3: Fungal Endophyte Recovery Rates from Leaves, Stems and Roots of Tomato Seedlings Inoculated Via the Seed-Soaking Method

(A) and (B) show endophyte recovery rates using conidial concentrations of 10^6 and 10^8 Conidia/ml, respectively. Bars represent standard error of the mean (SEM).

4.3.4 Effect of Salinity on Fungal Endophytes and Seed Germination

Salinity significantly affected the radial growth of the isolates ($F_{15–32} = 169.2$, $P < 0.001$). The isolates significantly differed in their levels of salinity tolerance across the various sodium chloride concentrations tested ($P < 0.0001$) (Fig. 4.3A), with isolate F21 showing the largest radial growth across all concentrations. For example, at 1.5 M NaCl, the mean radial growth values of isolates F18 and F21 were 1.8 ± 0.1 and 2.7

± 0.2 cm, respectively. Increasing concentrations of sodium chloride significantly reduced the germination of seeds (F24–25 = 80.53, $P < 0.0001$) (Fig. 4.3B). In the controls, NaCl concentrations of 75 mM and above resulted in no germination. Inoculation of seeds with fungal endophytes significantly affected germination under salinity stress ($P < 0.0001$). In the presence of endophytes, seeds germinated at 100 mM NaCl, with isolate F21 showing the highest number of germinated seeds at all NaCl concentrations (Fig. 4.3B).

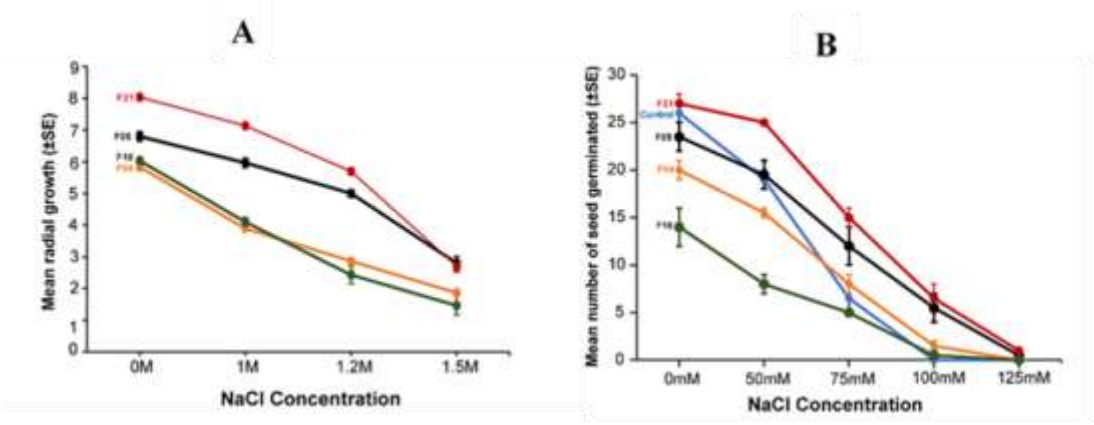

Figure 4.4: Effect of Salinity on Fungal Growth and Seed Germination

(A) Mean (±SE) radial growth of endophytes on PDA plates (n=3) supplemented with different concentrations of sodium chloride. (B) Effect of fungal endophytes on seed germination following seed soaking with 10^8 conidia/ml and incubation on PDA plates supplemented with 0 to 125 mM NaCl concentrations (n = 30).

4.3.5 Effect of Fungal Endophytes on Tomato Seedlings Under NaCl Stress

The effect of fungal endophytes on tomato was tested in seedlings grown in a greenhouse with sterile soil and a fungal spore concentration of 10^8 conidia/ml. Inoculation of tomato seedlings with the fungal isolates significantly affected both wet and dry weights ($\chi 2 = 21.193$, df = 5, $P = 0.00074$) of the seedlings compared with those of the controls with salt stress. Seed inoculation with isolate F04 resulted in the highest increases in both wet and dry weights compared with those of the controls with salt stress (Fig. 4.4). Growth of the seedlings symbiotically with fungal endophytes significantly ($P \leq 0.01$) increased the wet and dry weights of both roots and shoots

compared with those of the control plants deprived of the endophytes and exposed to salinity stress (Fig. 4.4). Isolates F05 and F18 had similar impacts on both root and shoot fresh weights. On average, the fresh weights of the roots and shoots of inoculated seedlings were 34% and 56% higher, respectively, than those of the control plants.

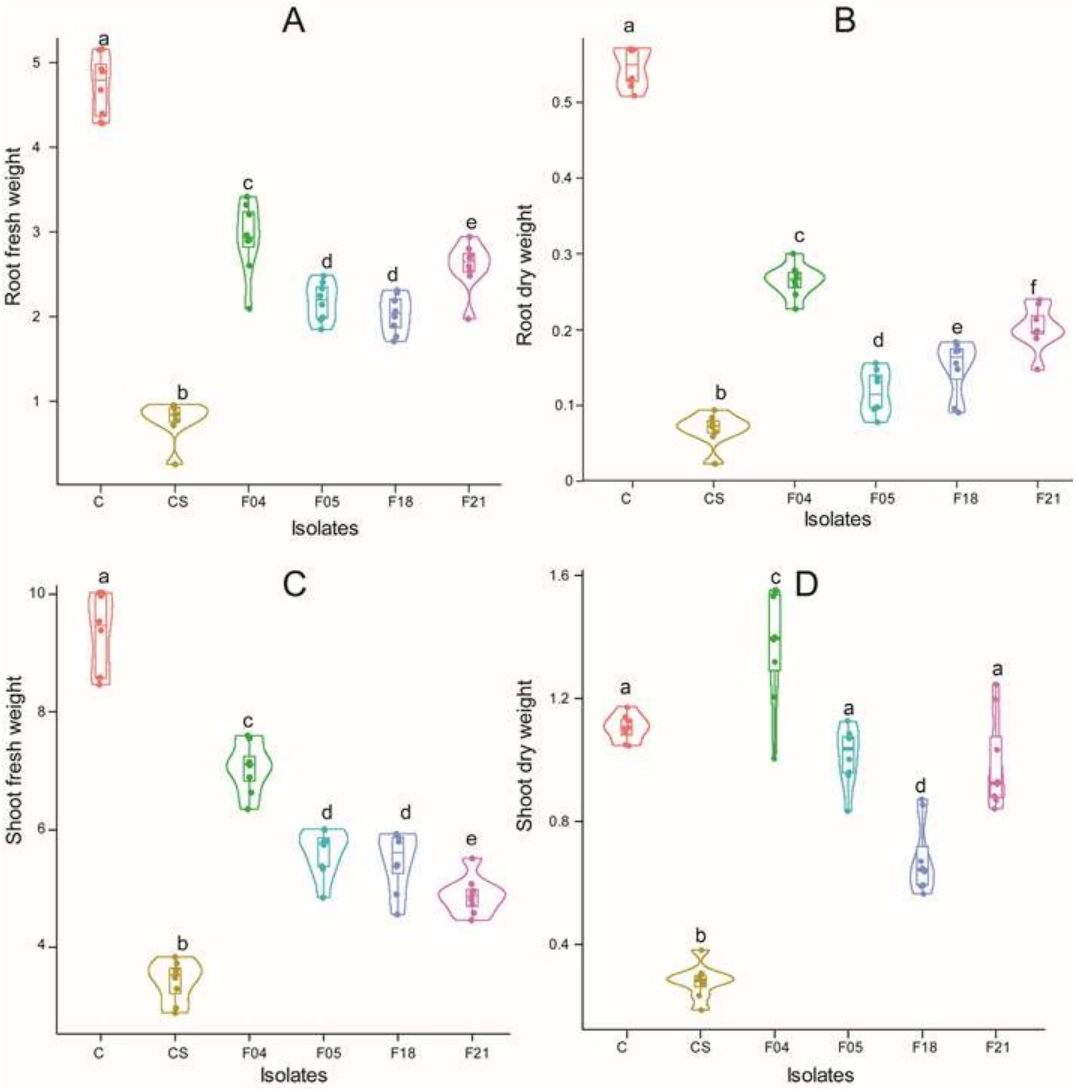

Figure 4.5: Violin Plot Representing the Effect of Different Fungal Endophytes on Seedling Growth in Terms of Root Fresh Weight

(A), root dry weight (B), shoot fresh weight (c), and shoot dry weight (D) after exposure of seedlings to salinity stress for 21 days. CS, uninoculated control seedlings exposed to salinity stress. C, uninoculated seedlings without exposure to salinity stress.

Treatments with different letters are significantly different from each other (ANOVA test followed by Student Newman–Keuls Test, $P = 0.05$)

Except for isolate F18, the symbiotic association of the isolates with tomato seedlings positively affected the biosynthesis of photosynthetic pigments (Fig. 4.5). Specifically, inoculation of the seedlings with fungal endophytes enhanced the content of chlorophyll a ($P < 0.0001$), chlorophyll b ($P < 0.001$) and total chlorophyll ($P < 0.0001$) compared with the controls. Plants inoculated with isolates F05 and F21 showed a higher percentage increase of chlorophyll b than chlorophyll a under salinity stress.

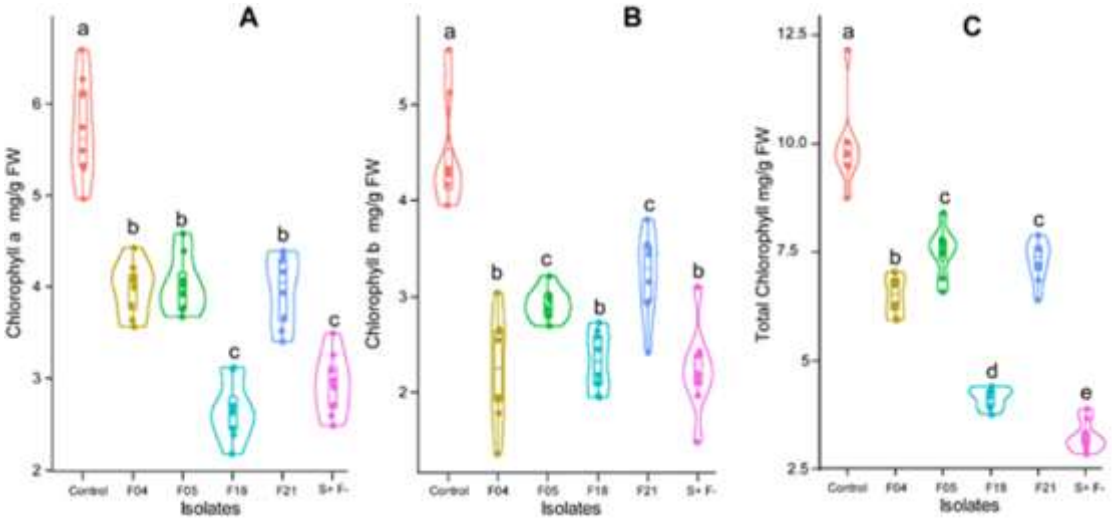

Figure 4.6: Effect of Endophytic Fungi on Chlorophyll.

Chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) contents of the leaves of tomato seedlings exposed to 125 mM NaCl for 28 days. The same letter indicates no significant difference in chlorophyll concentration. S+F-, uninoculated control seedlings exposed to salinity stress. Control refers to uninoculated seedlings with no exposure to salinity stress.

The endophytes significantly ($\chi 2 = 35.364$, df = 5, $P = 0.0001$) reduced the quantity of hydrogen peroxide produced by the seedlings under salinity stress compared with that of the controls (Fig. 4.6). Seedlings inoculated with isolate F18 exhibited the lowest tolerance to salinity stress in terms of hydrogen peroxide production, whereas those

inoculated with isolate F04 showed the best performance of the four isolates in reducing hydrogen peroxide. Isolates F05 and F21 did not differ significantly ($P \leq 0.05$) in their performance. Salinity increased the amount of hydrogen peroxide produced in the uninoculated control plants.

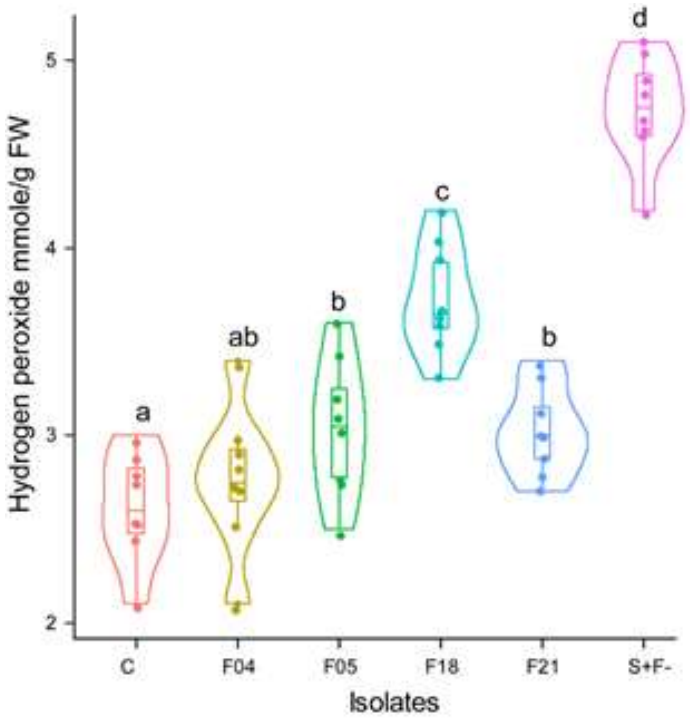

Figure 4.7: Effect of Fungal Endophytes on Hydrogen Peroxide Production by Tomato Seedlings Exposed to 125 mm NACL for 28 Days

Isolates followed by the same letter indicate no significant difference in hydrogen peroxide production. S + F-, uninoculated control seedlings exposed to salinity stress. C, uninoculated seedlings without exposure to salinity stress.

4.4 Discussion

Lake Magadi is an alkaline saline lake situated in a semiarid region in the southern part of the Kenyan rift valley. It is fed by ephemeral streams and has no outlet. Human activities, climate, geology, altitude and soil type shape and control the vegetation, soil macro communities and micro communities and habitats along the Magadi Natron

basin (Muiruri *et al*., 2021). The region's climate is changing from dry to even greater aridity, which, coupled with high evapotranspiration rates, creates high pH and alkalinity (Owen *et al*., 2019).These changes in environmental conditions have shaped the plant and microbial communities along the lake ecosystem to those more adapted to saline and alkaline conditions. This phenomenon has been noted by Maciá-Vicente *et al*., (2012), who reported a variable shift in endophytic and rhizosphere fungal communities along a spatially short salinity gradient in which halophytes harbor an endophytic assemblage of saline-adapted fungi.

Habitat-adapted microorganisms have been used to enable plants to adapt to biotic and abiotic stresses, enhance growth and increase reproductive success; some plants are unable to survive in their habitats without fungal symbiosis (Bouzouina *et al*., 2021; Redman *et al*., 2002; Moghaddam *et al*., 2022). Our results are consistent with these findings. Specifically, the results showed that selected endophytic isolates can tolerate and grow in salinity concentrations of up to 1.5 M NaCl. Endophytic fungi from halophilic plants are able to grow under high NaCl concentrations and impact positively on germination and biomass-related parameters upon salt stress, both in vitro and under glasshouse conditions (Majunatha et al., 2022). Moreover, our results complement the growing body of knowledge on the importance of microorganisms symbiotic to plants in stress environments and their applications in crop plants. Isolation and utilization of these habitat adapted microorganisms in agricultural systems offer an important, cheaper and more reliable solution than plant breeding, especially in saline soils.

Representatives of only two fungal phyla were isolated, Ascomycetes and Basidiomycetes, with a bias toward the former. Fungal endophyte communities are shaped by various factors, including host genotype, nutrient status around the plant and other environmental factors, although the plant is largely responsible for shaping the association (Bulgarelli *et al*., 2012; Wehner *et al*., 2014; Cheng *et al*., 2019) . Certain fungal phyla have been more frequently found as endophytes and in the soil rhizosphere than others, especially in abiotically stressed environments (Maciá-Vicente *et al*. 2012, Hamzah *et al*., 2018; Zhou *et al*., 2018; Khalil *et al*., 2021; Sahoo *et al*., 2021) . The ubiquity of Ascomycetes in soil can probably explain their

abundance as endophytes, which has been reported in previous studies (Hamzah et al., 2018).

In the current study, 46% (28) of the isolates were classified as *Fusarium*, based on the DNA internal transcribed spacer gene region. *Fusarium* species include both pathogenic and beneficial plant endophytes, and they are ubiquitous and economically important fungi that can cause diseases in plants. They can also produce mycotoxins that are passed on to animals when they feed on contaminated plants; and they can act as pathogens to humans (Ji *et al*., 2019; Srinivas *et al*., 2019). However, Fusarium endophytes in plants have been shown to lose their pathogenicity under stress conditions, and thus they become beneficial to the plant by inducing resistance to stress and enhancing growth (Ogbe *et al*., 2023; Pappas *et al*., 2018). The high frequency of *Fusarium* species isolated from endophytic communities of saline-adapted plants indicates that *Fusarium* may play a significant ecological role in plant adaptation to saline environments. This characteristic could be explained as a key reason why these plants symbiotically associate with *Fusarium* species.

Aspergillus was the second most frequently isolated genus of fungal endophytes in our study (17 isolates classified as either *A. terreus or A. puniceus*). The genus Aspergillus is a frequently isolated endophyte, as it is capable of growing in vital nutrient-depleted environments, including within plants growing in extreme environments (Kim *et al*., 2014; Sahoo *et al*. 2021). They have also been found to produce highly diverse secondary metabolites with various potential industrial applications (El-Hawary *et al*., 2020). They have been implicated in the production of endogenous plant hormones, amino acids and other soluble organic acids that help the plant mitigate stress and enhance growth (Waqas *et al*., 2015).

Establishing endophytism in non-host plants is especially important for beneficial endophytes, because they offer the possibility of conferring similar benefits to crop plants. In this study, the ability of four selected endophytic fungi was tested to competently colonize tomato plants growing in sterile vermiculite by seed inoculation using two different fungal spore concentrations. All isolates colonized tomato at both concentrations but differed in individual fungal performance and plant part. Similar

results were obtained by Akutse *et al*., (2013), as well as Jaber & Enkerli, (2016), who reported differences in colonization rates for different plant parts. Although isolation of specific taxa from the collected shrubs did not show any tissue or plant specificity, some studies have reported that certain fungal endophytes show tissue specificity and preference (Sun et al. 2012). Other studies have also inoculated seeds with a conidial concentration of 10^8 conidia/ml, resulting in successful post-inoculation recovery of the endophytes from all plant parts and effective performance on the test variable (Jaber, 2018b; Mutune *et al*., 2016). Several factors contribute to successful endophyte establishment in non-host plants, including the concentration of inoculum used, medium used (sterile or non-sterile) and method of inoculation (Bamisile *et al*., 2018). Using seed soaking and sterile vermiculite in our study, the concentration of conidia used correlated with the recovery rates, which is consistent with the results of other studies (Ownley *et al*., 2008).

The tested fungal endophytes enhanced the germination of seeds under salinity stress compared to non-inoculated seeds under the same stress conditions. This could be explained as as a result of modulating seed physiological and biochemical responses, including improved water uptake, production of growth-promoting hormones (such as indole-3-acetic acid), and lowering the synthesis of reactive Oxygen Species (Ali et al., 2022). These mechanisms help mitigate the toxic effects of salt stress, reduce oxidative damage, and support early seedling vigour, ultimately leading to improved germination rates under saline conditions. Salinity stress in plants can be a lethal factor that limits the normal functioning of plants and eventually affects growth and productivity. At elevated salinity levels, all growth stages (seed germination, seedling, vegetative growth and maturity), as well as the quality of the seeds/fruits, are negatively affected (Jafarzadeh & Aliasgharzad, 2007; Yao *et al*., 2022). Germination and seedling establishment are the most crucial stages in the plant life cycle. High salinity stress negatively affects the germination of seeds as it creates low water potential that disrupts cellular homeostasis and increases the production of reactive oxygen species (ROS), resulting in oxidative stress that tends to prolong the seed germination period and lower the germination rate (Dehnavi *et al*., 2020; Zhang & Mu, 2009). From the results from this study, it could be hypothesized that such mechanisms could have been responsible for the enhanced germination of inoculated seeds growing

at sodium chloride concentrations of 50, 75 and 100 mM. Although few studies have focused on the use of endophytes to improve seed germination under salinity stress, several studies have indicated that endophytes can be beneficial to seedling growth under salinity stress (Jogawat *et al*., 2016; Kumar & Verma, 2018; Molina-Montenegro *et al*., 2020; Verma *et al*., 2021). More studies on these positive effects of endophytes on seed germination, at the molecular and physiological levels, are essential to aid in the development of strategies to mitigate the impacts of climate change on food crops.

In this experiment, a commercial cultivar Cal J variety was used. This variety is locally known as Kamongo, and is popular in Kenya due to its high market value and long shelf life (Geoffrey *et al*., 2014). This variety was negatively affected at 125 mM NaCl, which markedly reduced leaf chlorophyll content and increased levels of hydrogen peroxide compared with those of control seedlings without NaCl. This reduction in chlorophyll could be associated with the plants' inability to manage ion toxicity caused by increased Na+ and Cl− ions in the plant tissues (Zhang & Mu 2009).

Symbiotic association with endophytic fungi significantly ($P < 0.001$) reduced the amount of hydrogen peroxide produced by the plants, increased their chlorophyll content and increased their dry weight compared with uninoculated control plants exposed to sodium chloride. These results indicate the endophytes-enhanced sodium chloride tolerance of the seedlings. The endophytic fungi in the tomato seeds and seedlings helped maintain the ionic balance in the plant cytosol, thereby preventing accumulation of toxic Na+ ions while enhancing photosynthesis in the seedlings under sodium chloride stress. Ionic homeostasis in plants, reduced ROS production and concomitant increases in shoot and root weight have been reported in several studies as mechanisms by which fungal endophytes alleviate salt stress (Ali *et al*., 2022; Bouzouina *et al*., 2021).

Of the 60 fungal isolates obtained, 62% solubilized inorganic phosphate, a finding that agrees with those of Ogbe *et al*., (2023). Fungal endophytes are prolific producers of extracellular enzymes and secondary metabolites (Debbab *et al*., 2013), which are important in the selection of beneficial microorganisms for use in agricultural

production.. Phosphorus is the second most important plant nutrient after nitrogen (Radhakrishnan *et al*., 2015). Although it is present at high concentrations in soil, plants are often starved for phosphorus because it occurs in a form that they cannot absorb (Castrillo *et al*., 2017). In soils with high salinity and pH, phosphorus forms stable complexes with other ions and becomes unavailable to plants (Penn & Camberato, 2019; Xie *et al*., 2022a). It has been suggested that to increase plant productivity, plants growing in such soils can select and symbiotically associate with microorganisms that help them alleviate environmental challenges such as nutrient deficiency (Bulgarelli *et al*., 2013). Therefore, the ability of a large number of our isolates to solubilize phosphate may be the result of the plant's natural selection during plant–microbiome evolution. Therefore, this study demonstrates the potential benefits of the endophytes from the shrubs growing along the shores of soda lakes and should be explored for use in sustainable agricultural production.

4.5 Conclusion

Fungal endophytes were isolated from all shrub samples collected and from all the plant parts (leaves, stems and roots), with a similar trend in the clustering of isolates to the two mostly isolated phyla (Ascomycetes and Basidiomycetes) as other studies from abiotically stressed environments (Maciá-Vicente *et al*. 2012). All the isolates were able to colonize tomato seedlings following seed soaking method and showed a positive effect on seed germination percentage, seedling shoot and root weight, chlorophyll and production of hydrogen peroxide under salinity stress. Isolate F04 (*Aspergillus puniceus*) exerted the greatest effect of the four tested isolates and therefore has a potential for development of a cheaper solution for use in climate resilient agriculture, especially in arid and semi-arid regions where crop plants are exposed to several biotic and abiotic stresses. Further studies, are necessary to understand the interactions of these endophytes and crop plants in the presence of other naturally existing soil microbiota under salinity stress and the success of seed inoculation method under field conditions. The study concludes that endophytic fungi from shrubs along the shores of saline alkaline lakes potentially harbor beneficial microorganisms which could be harnessed for sustainable agricultural production.

CHAPTER FIVE

ISOLATION OF BACTERIAL ENDOPHYTES AND THEIR ASSESSMENT OF ANTIFUNGAL POTENTIAL AGAINST *FUSARIUM SOLANI*

5.1 Introduction

Plants are sessile and therefore must acquire all their requirements for growth, development, and resistance to both biotic and abiotic stresses within the limited ecological unit. A host of organisms and other abiotic substances are found within an ecological unit, and plants continuously interact with these substances. Soil microorganisms play an important role in these plant interactions, establishing a symbiotic relationship with the plant thereby forming the plant microbiome. These plant–microbe interactions and their putative roles have been studied (Etesami *et al*., 2015; Potshangbam *et al*., 2017) and reviewed (Andreote *et al*., 2014a; Berg *et al*., 2014; Etesami & Beattie, 2018; Hassani *et al*., 2018). Currently, studies on the interaction of plants and microorganisms and their impacts on plant health and survival are ongoing. Association of plants and microorganisms is either mutualistic (Castrillo *et al*., 2017), providing and obtaining nutrients to and from the plant; commensal, assisting the plant in growth, pathogen control, and acquisition of nutrients (Bendaha & Belaouni, 2019; Omomowo & Babalola, 2019); or pathogenic (Lim *et al*., 2014; Wheeler & Wheeler, 2019). Plants have been hypothesized to shape their microbial associations in the rhizosphere (Bulgarelli *et al*., 2012). This function is mediated by the release of root exudates which attract or repel microorganisms and the physical and chemical properties of soil which determine the soil microbial communities (Shakya *et al*., 2013). Plant–microbe interactions are not only restricted to the rhizosphere but the phyllosphere and the endosphere also equally provide an environment for such associations (Bulgarelli *et al*., 2012). The endosphere represents a highly specialized and selected microbiota, which are adapted to survival in the internal plant tissues without causing disease symptoms while establishing as endophytes (Compant *et al*., 2010). The selection of endosphere microbiota is driven by various factors. Experiments by Wemheuer *et al*., (2017) on the leave endophytic bacteria from three different grass species grown in field conditions indicated that the

selection of endophytes by the plant may be functionally driven. Experiments on two different *Arabidopsis thaliana* ecotypes grown under controlled environmental conditions on soils with different geochemistry by Bulgarelli *et al*. (2012) concluded that the composition of root endophytes was influenced by soil type. In addition, Zúñiga (2013) and Schikora (2016) reported that quorum sensing ability of bacteria using the auto-inducer, N-acyl homoserine lactone in gram negative bacteria plays a key role in bacterial endophytic colonization.

Various reports have indicated the potential role of endophytic bacteria to help plants in amelioration of biotic and abiotic stresses (Rho *et al*., 2018). Salinity stress in plants is one of the major abiotic factors limiting growth and affecting productivity (Nia *et al*., 2012). Mangroves and other vascular plants and shrubs growing along the draw-down zones of saline lakes have to develop adaptive mechanisms to cope with high salinity and fluctuating environmental conditions (Harper *et al*., 2003). The use of microbial endophytes, one of the mechanisms employed by these plants, has been reported to be activating host plant stress response pathways in addition to the production of phytohormones and other secondary metabolites (Eaton *et al*., 2008; Khan *et al*., 2016;Li *et al*., 2018).

Inoculation of bacterial endophytes into crop plants confers similar benefits to the plants in ameliorating the stress conditions, thereby promoting growth and productivity. For example, inoculation of tomatoes with bacterial endophytes having indole acetic acid (IAA) and catalase production capabilities (Ali *et al*., 2017) from the mangrove, Avicennia marina resulted in reduced effects of salinity stress and increased biomass and chlorophyll content in *Solanum lycopersicum*. Bacterial endophytes from three different halophytes by Kearl, (2019) were able to enhance both root and shoot growth of alfalfa in the presence of salt.

Bacterial endophytes have also been used in the biocontrol of a vast majority of plant pathogenic fungi (Card *et al*., 2016). Biocontrol of fungal pathogens by endophytic bacteria is, in part, linked to the production of phytohormones (Devi *et al*., 2017; Li *et al*., 2018) that help the plant to overcome the deleterious effects of the pathogen while enhancing plant growth (Chernin & Glick, 2012; Mei & Flinn, 2010) and the

production of metabolites that are antagonistic to the fungi (Daungfu *et al*., 2019; Ludwig-müller, 2015). The success in the use of endophytic biocontrol bacteria can be a major stride in the management of plant fungal pathogens which are a cause of reduced yields especially in common bean production, which cause estimated annual losses of 221,000 metric tons in the sub-Saharan Africa (Paparu *et al*., 2018). Root rot pathogens are particularly difficult to control, as the pathogen can stay in the soil for long periods and in infected plant debris as clamydospores until conditions are favorable and a host plant is grown (Conner *et al*., 2014). The use of agrochemicals still remains as the major tactic in the control of these pathogens, but has persistently developed resistance in addition to environmental detriments. Seed coating with fungicides has been widely used in the management of root rots, but has produced little effect in the control of these pathogens (Xu & Kim, 2014). Biocontrol of root rot pathogens using endophytic microorganisms has been cited as a noble method as the endophytes occupy the same niche with the pathogen, produce antifungal metabolites, help the plants in the acquisition of nutrients and in the priming of plant defense (Muthukumar *et al*., 2017). However, to date, only a couple of endophytic biocontrol agents have been registered and are commercially available for use in sustainable agriculture, thereby calling for more studies in the search and development of biological control organisms, especially the use of endophytes.

Various studies on microbial diversity from Kenyan soda Lakes have been undertaken, mainly focusing on microorganisms from the soil, water, and sediments (Kambura, 2016; Mwirichia, 2009; Grant & Jones, 2016; Nyakeri *et al*., 2018; Orwa *et al*., 2020). Besides, endophytic microorganisms have been isolated from several species of mangroves and other halophilic plants and no literature is available on endophytes from shrubs growing along the draw-down zones of Kenyan soda lakes. Therefore, this study focuses on the isolation of bacterial endophyte communities associated with shrub vegetation growing along the draw-down zone of Lake Bogoria, a saline-alkaline lake along Kenya's rift valley, assessing their tolerance to salinity stress in vitro and screening their potential for the biocontrol of *Fusarium solani* in common beans.

5.2 Materials and Methods

Description of sampling site, sample collection, isolation of bacterial endophytes and their characterization are detailed in **chapter 3** above.

5.3 *In Vitro* Antifungal Assay

5.3.1 Effect of Endophytic Bacteria on Fungal Mycelia

Based on the 16S rRNA results and literature on antifungal activities of similar endophytic bacterial strains, 9 isolates were selected for further assessment of their biocontrol activities. The bean root rot plant pathogenic, *F. solani* used in the experiment was kindly supplied by Dudutech IPM Limited, Naivasha, Kenya. The strain was earlier isolated from infected field beans. Fungal cultures were grown on a potato dextrose agar (PDA) supplemented with 200 mg/L streptomycin sulfate and incubated in the dark at 28C ± 2 for 10 days prior to the experiment. Dual culture method (Xu & Kim, 2014) was used to test for the inhibition of *F. solani* mycelial growth by the endophytic bacteria. Bacterial cultures were grown on nutrient agar plates 24 h prior to the assay. A 3 mm diameter plug from a 10-day old culture plate of *F. solani* was excised and placed about 1 cm from the edge of a freshly prepared PDA plate. A loopful of the endophytic bacterial was spot inoculated at the edge on the opposite side of the fungal plug on the same plate. Control plates contained 3 mm diameter plugs of the fungus placed at the edge of the PDA plate. Plates were sealed with parafilm, incubated at 28◦C ± 2 and observed daily for 7 days. Three independent replicates were set for each pair of test bacteria and fungus dual cultures and control plates. The effect of the endophytic bacteria on the mycelial growth was evaluated after the seventh day by measuring the radial growth of the test fungi toward the bacteria (D1) and the radial growth of the test fungi in the control plate (D2). Antagonism of the endophytic bacteria was expressed as a percentage of mycelial inhibition on the test fungi plate calculated as D2-D1/D2 x 100.

5.3.2 *In Vitro* Bacterial Cell–Fungal Conidia Interaction in a Mixed Culture

The effect of endophytic bacterial cells on germination and hyphal development of *F.*

solani conidia was assessed using the method proposed by Toghueo *et al*., (2016) with minor modifications. Briefly, test bacterial endophytes were grown in a nutrient broth overnight. One milliliter of the overnight culture was centrifuged at 3,000g for 5 min, the supernatant was discarded, and the cells were adjusted with a sterile normal saline (0.85% NaCl) to an OD_{600} of 0.5 (~10^6 cfu/ml). *Fusarium solani* spores were harvested from a 10-day old culture grown on a PDA, filtered in 4 layers of sterile gauze, and adjusted to a concentration of 1×10^4 spores/ml in a sterile potato dextrose broth (PDB) using a Neubauer Chamber. In each treatment, 100 µl of the adjusted fungal spore suspension and 40 µl of the bacterial cell suspension were added, and the mixture was gently vortexed and incubated for 4 h at 28°C ± 2 to optimize cell–cell contact. All samples were set in triplicate. The control consisted of 100 µl of fungal spores and 40 µl of PDB. A 100µl of the bacterial-fungal mixture was then plated on freshly prepared PDA plates and a sterile coverslip was placed randomly on each plate. The plates were incubated overnight at 28°C ± 2. The effect of the bacterial cells on *F. solani* conidia germination, germ tube growth, and mycelium development were observed by direct microscopic observation without the use of a stain at 400× magnification.

5.4 Test for Endophytic Competence

Two methods were used to evaluate the competence of the selected bacterial endophytes in a colonizing common bean, Phaseolus vulgaris variety KAT B1 (yellow bean). For seed soaking procedure, the bean seeds were surface sterilized using 3% commercial bleach for 5 min followed by 70% ethanol for 3 min and 4 rinses with sterile distilled water, and air-dried for 4 h on a sterile filter paper under a clean bench (Toghueo *et al*., 2016). The final rinsed water was streaked on nutrient agar plates and incubated to confirm the effectiveness of sterilization. An overnight bacterial culture was centrifuged and the cells were adjusted to OD_{600} 0.5. The sterilized bean seeds were soaked in the bacterial suspension overnight, then transferred to pots containing sterilized vermiculite moistened with sterile half strength Hoagland's solution in a growth chamber at 27◦C ± 2 at a 12 h: 12 h light-dark cycle. At the true leaf stage, the seedlings were uprooted from the vermiculite, washed under running tap water, wrapped with a paper towel to remove excess water, and 1 g of each plant part (the

leaves, the stems, and the roots) was sterilized as the field plants. Sterile parts were ground in 9 ml sterile normal saline in sterile 2 ml tubes with a micro pestle and vortexed. The supernatant was serially diluted and plated on nutrient agar plates at triplicates of each plant part.

For drenching method, the bean seeds were sterilized as above, air dried, and then transferred to a sterilized vermiculite moistened with sterile half-strength Hoagland's media. Immediately after germination, the vermiculite around the root base of each seedling was gently removed and 1 ml of an overnight culture of the bacterial suspension was adjusted to OD_{600} of 0.5 with the sterile nutrient broth. The suspension was poured at the root base and then covered with a vermiculite. Two weeks after drenching, the seedlings were removed and assessment of colonization was undertaken as for seed coating. For each of the two methods, three bean seedlings per bacterial isolate were used for re-isolation of the endophytic bacteria. Biochemical characterization of the recovered bacterial endophytes from both, seed coating and drenching, were carried out to confirm the identity with the inoculated bacteria (Etesami *et al*., 2015).

5.5 *In Vivo* Biocontrol of *Fusarium Solani* in a Pot Experiment

Previous experiments by Whitaker & Bakker, (2019) indicated differences in the dual culture inhibition assay and the detached spikelet assay using bacterial endophytes on *Fusarium graminearum*. All the nine selected endophytic bacterial isolates were assessed for their biocontrol activities in planta. The experiment was carried out in a greenhouse in pots (15 × 17 cm). The details are mentioned in the following section.

5.5.1 Preparation of Potting Mix and Introduction of the Pathogen

A mixture of forest soil and cattle manure at a ratio of 5:1, respectively, served as the potting mixture (Mutune *et al*., 2016). The soil-cattle manure mixture was sterilized by autoclaving for 40 min at 121°C, left to cool, and then autoclaved again before use. Sterilized and cooled potting mixture was then distributed into pots at 1 kg per pot. Moistened soil in each pot was contaminated with 5 ml of 1.5×10^4 conidia/ml (Toghueo *et al*., 2016), mixed well, and covered with a foil paper to retain the moisture.

Control pots contained soil mixed with 5 ml of distilled water with no fungal conidia. The pots were then transferred to a greenhouse and left for 3 days before planting the bean seeds.

5.5.2 Inoculation of Bean Seeds and the Experimental Setup

The common bean, *P. vulgaris* variety KAT B1 (Yellow bean) was used in this experiment. The seeds were surface sterilized by washing in 3% of sodium hypochlorite for 1 min followed by 1 min of washing in 70% ethanol, and then rinsed 5 times in sterile distilled water. Bacterial endophytes were grown for 48 h in a nutrient broth and incubated at 28◦C ± 2 in a rotary shaker, centrifuged at 10,000 × g for 10 min, and the cells re-suspended in a normal saline solution. The cells were diluted at OD_{600} of 0.5 and the sterilized bean seeds were soaked for 24 h before sowing. The experiment was set up with 11 treatments: 9 treatments for the different bacterial endophytes in the soil contaminated with *F. solani; F. solani*-inoculated soil was used only as a control, and non-inoculated soil was used as a negative control. Each treatment consisted of 4 pots (15 × 17 cm) with 2 seeds per pot. The pots were kept in the greenhouse in a completely randomized design and the plants were watered 3 times a week until completion of the experiment after 4 weeks.

5.5.3 Assessment of *In Vivo* Biocontrol Performance

A day before the completion of the experiment, the plants were watered to loosen the soil around the roots for ease of getting the root system as intact as possible. All plants were destructively sampled, removed from the pots, and washed under running tap water to remove the adhering soil, and to enable visualization of disease lesions. A 5-point disease index on a scale of 0–4 was used to rate the severity of the disease on the roots and the hypocotyl. The ratings are as follows. 0: no disease symptom; 1: slightly brown coloration (50%) and 4: severe root pruning with dead or dying plants (Motallebi *et al*., 2015). Disease severity was assessed using the formula:

$$\%\text{Disease severity} = (\Sigma(c \times f)/n \times N) \times 100$$

Where: c is the disease class.

f is the frequency of disease class.

n is the number of observations.

N is the greatest value of the empirical scale used. In this case, N = 4.

The fresh and dry weights of the shoots and the roots of each plant was determined. Dry weights were obtained by drying the shoots and roots in an oven at 70◦C for 24 h.

5.5.4 Data Analysis

Plate assays were performed in triplicates. Tested parameters in pot experiments and data on anti-fungal activities and salinity tolerance were subjected to one-way analysis of variance and the means were compared using the Student Newman Kuels test. Data on endophyte re-isolation from bean plant parts were fitted on a generalized linear model with Poisson distribution. R statistical software version 2.15.4 was used for the analysis at 95% confidence interval.

5.6 Results

5.6.1 Isolation of Bacterial Endophytes

Sixty-nine bacterial cultures were isolated and purified from the leaves, stems, seeds, and roots of the eight different shrubs collected. *Solanum incanum* had the highest number of isolates while seeds from *Prosopis juliflora* gave the lowest number of isolates. Isolation from the stem tissues gave the highest number of isolates at 45.8% (31 isolates) followed by the roots (21 isolates) at 30%, the leaves at 20% (14 isolates), and then the seeds at 4% (3 isolates) (Table 5.1). The isolates were grouped into morph groups based on colony characteristics; exo-enzyme (Table 5.3), pigment production, and source plant. The grouping gave 33 isolates, which were used for further characterization using molecular techniques (Table 5.1).

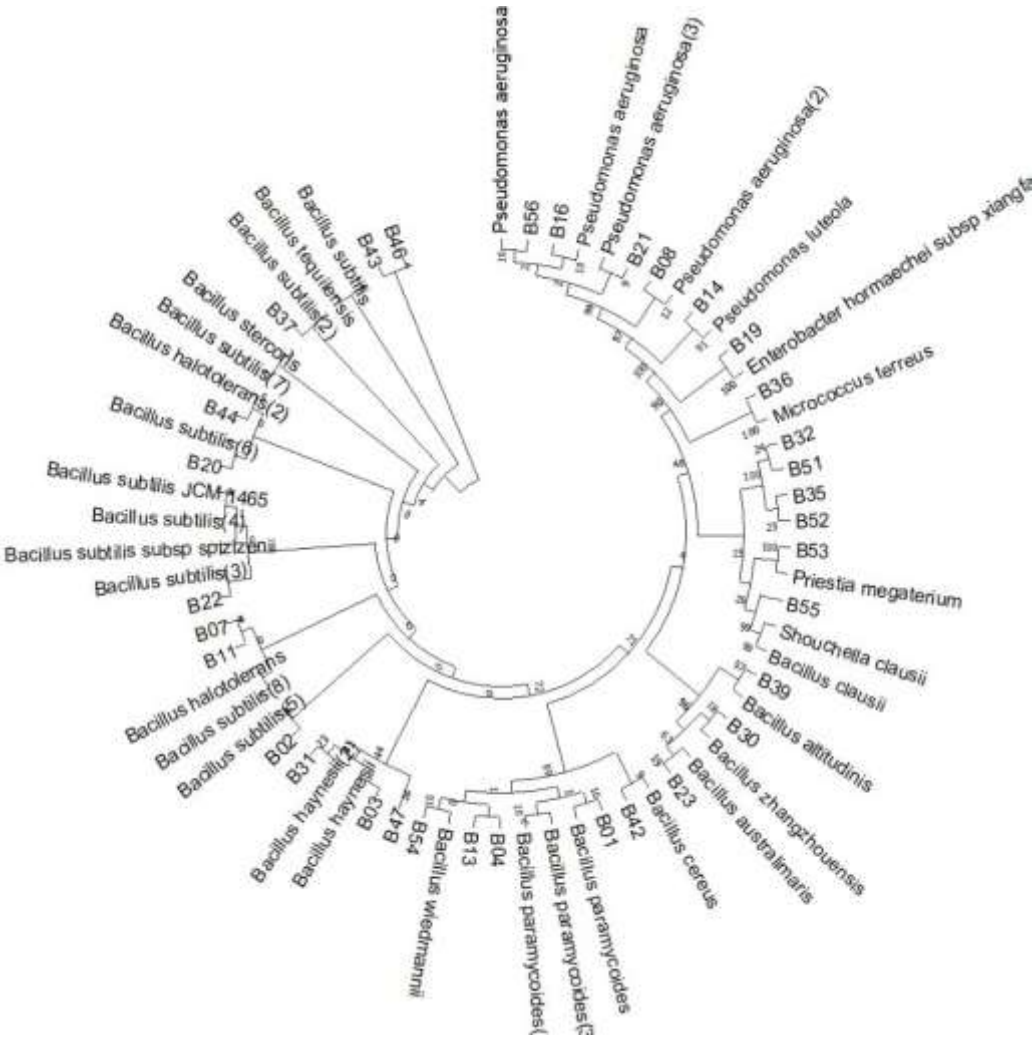

Figure 5.1: Unrooted Phylogenetic Tree of ENDOPHYTIC Bacterial 16s rRNA Sequences

Evolutionary history was inferred using the Maximum Likelihood method with 1000 bootstrap replicates in MEGA X software. Scale bar represents 0.05 nucleotide substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches

.

Table 5.1: List of Endophytic Bacteria and Their Closest Relatives in Gene Bank

Isolate code	Plant part	Name of source plant	Closest relative	Accession no.	% similarity
B01	Stem	*Solanum incanum*	*Bacillus cereus*	OK284403	100
B02	Root	*Abutilon fruticosum*	*Bacillus subtilis*	OK284374	99.78
B03	Leaves	*Boerhavia erecta*	*Bacillus haynesii*	OK284378	99.7
B04	Stem	*Solanum incanum*	*Bacillus cereus*	OK284402	100
B07	Root	*Solanum incanum*	*Bacillus subtilis*	OK284377	99.79
B08	Root	*Tephrosia uniflora*	*Pseudomonas aeruginosa*	OK284401	99.92
B11	Leaves	*Boerhavia erecta*	*Bacillus subtilis subsp. spezizenii*	OK284400	100
B13	Leaves	*Acalypha fruitcosa*	*Bacillus cereus*	OK284375	99.57
B14	Leaves	*Acalypha fruitcosa*	*Pseudomonas luteola*	OK284376	99.77
B16	Stem	*Rhynchosia Spp.*	*Pseudomonas aeruginosa*	OK284399	100
B19	Root	*Abutilon fruticosum*	*Enterobacter hormaechei subsp. Xiangfangensis*	OK284398	99.85
B20	Seed	*Prosopis juliflora*	*Bacillus subtilis*	OK284397	100
B21	Stem	*Rhynchosia Spp.*	*Pseudomanas aeruginosa*	OK284381	99.33
B22	Stem	*Acalypha fruitcosa*	*Bacillus subtilis*	OK284396	99.92
B23	Stem	*Tephrosia uniflora*	*Bacillus australimaris*	OK284386	100
B30	Leaves	*Solanum incanum*	*Bacillus australimaris*	OK284384	99.78
B31	Leaves	*Solanum incanum*	*Bacillus haynesii*	OK284385	99.79
B32	Leaves	*Acalypha fruitcosa*	*Bacillus velezensis*	OK284406	98.87
B44	Root	*Abutilon fruticosum*	*Bacillus subtilis*	OK284391	99.85
B52	Stem	*Abutilon fruticosum*	*Bacillus velezensis*	OK284404	100
B35	Stem	*Solanum incanum*	*Bacillus velezensis*	OK284389	99.87
B36	Leaves	*Boerhavia erecta*	*Micrococcus terreus*	OK284395	99.7
B37	Stem	*Ruelia patula*	*Bacillus tequilensis*	OK284394	99.92
B39	Leaves	*Solanum incanum*	*Bacillus aerius*	OK284393	99.71

Isolate code	Plant part	Name of source plant	Closest relative	Accession no.	% similarity
B42	Stem	*Ruelia patula*	*Bacillus paramycoides*	OK284392	99.93
B43	Stem	*Solanum incanum*	*Bacillus subtilis*	OK284387	100
B46	Root	*Rhynchosia Spp.*	*Bacillus halotolerans*	OK284382	98.01
B47	Stem	*Boerhavia erecta*	*Bacillus subtilis*	OK284379	99.83
B51	Root	*Rhynchosia Spp.*	*Bacillus velezensis*	OK284405	99.88
B53	Leaves	*Boerhavia erecta*	*Bacillus megaterium*	OK284388	99.93
B54	Stem	*Solanum incanum*	*Bacillus cereus*	OK284383	98.92
B55	Leaves	*Tephrosia uniflora*	*Alkalihalobacillus clausii*	OK284380	99.11
B56	Root	*Solanum incanum*	*Pseudomonas aeruginosa*	OK284390	99.93

5.6.2 Molecular Identification of Bacterial Endophytes

Analysis of the contigs and comparison with homologous sequences in both the EZBiocloud and the National Center for Biotechnology Information (NCBI) GenBank database indicated that the isolates belonged to the following three Phyla: Firmicutes, Proteobacteria, and Actinobacteria with 99 to 100% identities to gene bank sequences (Table 5.1). The Phylum, Firmicutes represented the highest number of isolates (26 isolates, 79%), followed by Proteobacteria (6 isolates, 18%), and Actinobacteria (I isolate, 3%) were distributed over 4 different genera: Bacillus (26 isolates), Enterobacter (1 isolate), Pseudomonas (5 isolates), and Micrococcus (1 isolate). The phylum, Firmicutes was represented by 11 different species isolated from all plant parts. Bacillus subtilis was the most frequently isolated species followed by *Bacillus velezenzis*. The Phylum, Proteobacteria was represented by two genera: Enterobacter and Pseudomonas. The genera, Pseudomonas had two species: *Pseudomonas luteola* and *Pseudomonas aeruginosa*, and were isolated from all plant parts i.e., the roots, the stems, and the leaves. The phylogenetic tree indicated two major clades (Figure 5.1), with the first clade comprising two subclades, one for Actinobacteria and the other for Firmicutes. The second clade comprised the Proteobacteria which was divided into two subclades, Pseudomonadales and Enterobacterales, confirming the identity of the bacterial endophytes.

5.6.3 Enzymatic Growth Promotion Activities and Salinity Stress of Select Isolates

Nine isolates were chosen based on their 16S rRNA sequence analysis and biochemical tests for further screening. The potential of the endophytic bacteria to produce amylase, catalase, urease, and IAA, were evaluated (Table 5.3).

Table 5.2: In Vitro Growth Promotion Assay and Biochemical Tests of the Test Bacteria

Isolates	Phosphate solubilisation	IAA	HCN	Catalase	Amylase	Urease
B23	-	+	-	+	+	+
B37	+	-	-	+	+	-
B19	+	+	+	+	+	-
B07	+	-	+	+	+	+
B35	+	-	-	+	+	-
B53	+	+	-	+	+	-
B11	+	-	-	+	-	-
B55	+	-	-	+	+	+
B39	+	-	+	+	-	-

Only three of the isolates (B23, B19, and B53) produced IAA (Figure 5.3) and one isolate, B23 was not able to solubilize phosphate while all isolates were catalase positive. The isolates, B19, B07, and B39 were the only isolates capable of producing HCN. All isolates except B39 and B11 were able to produce amylase while only three isolates, B23, B07, and B55 were positive for urease. Isolate B19 was positive for all biochemical tests except urease (Table 5.2). Majority of the isolates were able to grow moderately at 1.0 M NaCl. At 0.5 M NaCl, the isolates, B23, B19, B11, B39, and B35 exhibited maximum growth while the isolate, B53 exhibited maximum growth at 1.0 M NaCl (Figure 5.2). Only one isolate, B07 had maximum growth at 1.5 M NaCl. Isolates, B55, B37, B39, and B11 did not have any visible growth at 2.0 M NaCl and the isolate B55 exhibited the poorest growth at all tested concentrations. None of the isolates exhibited maximum growth at 0 M NaCl (Figure 5.2).

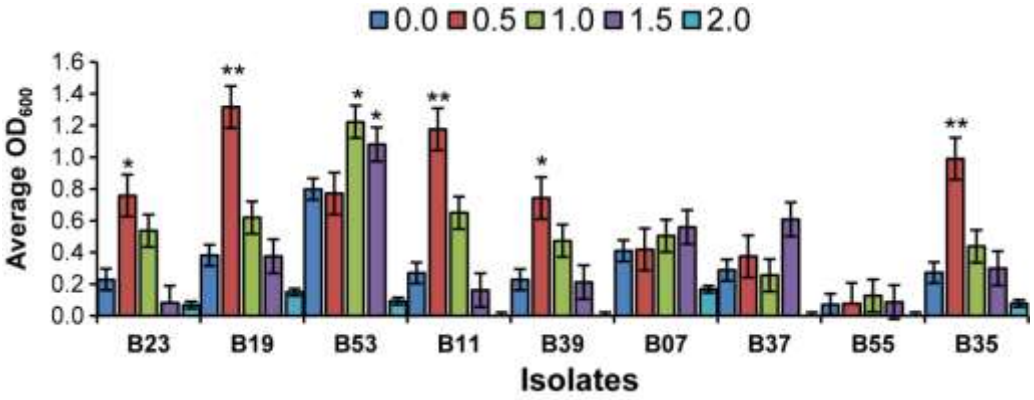

Figure 5.2: Effects of Salinity Stress on Selected Bacteria in Broth Cultures at OD_{600}

Bar chart represent means ±SE (standard error). Asterix on top of each error bar depict significant difference of the OD_{600} among the different salt concentration in a specific isolate (one-way ANOVA: *P<0.05, **P<0.01)

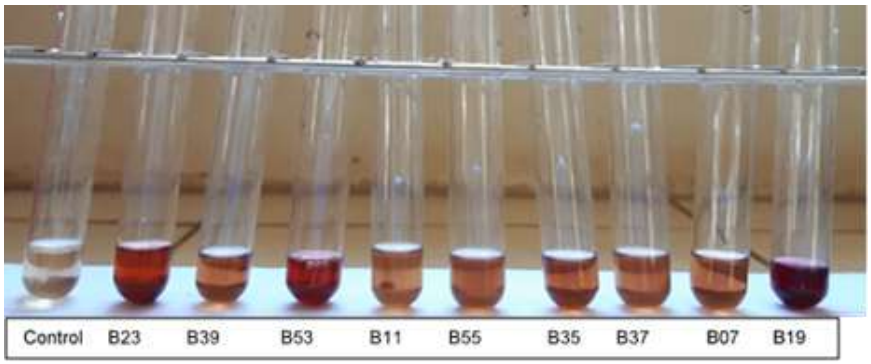

Figure 5.3: IAA Production by Bacterial Endophytes

Table 5.3: Phenotypic Characterization of Endophytic Bacterial Isolates

Isolate ID	Growth at 0.5M NaCl[a]	Growth at 1.5M NaCl[a]	Amylase Production[b]	Catalese production[b]	Urease Production[b]	Idole Acetic Acid[b]
B02	++++	+++	+	-	-	-
B19	+++	++	+	+	-	+
B44	++++	+++	+	+	-	-
B52	++++	+++	+	+	-	-
B13	++++	++	+	+	+	-
B14	++++	++	–	+	NT	NT
B22	++++	++	–	+	-	+
B32	++++	+++	-	+	-	+
B03	++++	+++	-	+	NT	NT
B11	+++	++	-	+	-	-
B36	++++	–	-	+	-	-
B47	+++	+	-	-	NT	NT
B53	+++	++	+	+	-	+
B20	+++	+++	–	+	NT	NT
B16	+++	+	–	+	NT	NT
B21	++++	+	–	+	+	-
B46	+++	++	+	-	NT	NT
B51	+++	+	–	+	-	-
B37	+	+	+	+	-	-
B42	+++	+++	–	+	+	-
B01	++++	++	-	+	+	-
B04	++++	+	–	+	+	-
B07	++	++	+	+	NT	NT
B35	+++	+	+	+	-	-
B43	-	+	-	-	-	-
B54	++++	–	+	+	+	-
B56	++++	+++	+	+	-	-
B30	++++	+++	–	+	NT	NT
B31	+++	-	+	-	-	-
B39	+++	++	-	+	-	-
B08	++++	–	–	+	-	-
B23	+++	++	+	+	+	+
B55	+	+	+	+	+	-

Key: [a]Growth response on salt concentrations: –, no growth; +, slight growth: ++, low growth: + + +, moderate growth; + + ++, full growth. [b]Exo-enzyme production: –, no production; +, production; NT, not tested.

5.6.4 Antifungal Tests

5.6.4.1 *In Vitro* Antifungal Tests

Nine bacterial isolates were selected for the evaluation of antifungal activities both in vitro and in vivo. The preference was guided by 16S rRNA analysis and by previous studies on the antifungal activities of similar bacterial isolates. The inhibitory activity of the isolates against *F. solani* had varying levels of inhibition of mycelial growth after incubation in a dual culture assay for 7 days as compared to the control plates (Figure 5.4A). Two of the isolates (B19 and B53) had the highest inhibitory activity of 67.8 and 51.9%, respectively, followed by B07 at 49.8% (Figure 5.4A). Isolates, B23 and B35 had the lowest mycelial inhibition rate at 24.3 and 24.3%, respectively.

Assessment of the effect of bacterial cells on *F. solani* spores was carried out in a mixed culture of fungal spores and bacterial cells. After an overnight incubation, the cells were observed under a microscope. Two isolates (B19 and B53) completely inhibited the germination of fungal spores of the pathogenic fungi upon interaction and made them appear to have undergone some morphological changes as compared to their state before incubation (Figure 5.4B). Isolates, B07 and B39 delayed the germination of the spores with germ tubes appearing to start emerging after overnight incubation in comparison to control plates which had a well-developed network of mycelia at the same time (Figure 5.4B). Isolates, B11 and B37 indicated a network of mycelia which appear to have morphological deformations unlike the straight mycelia of the control plate. Isolates, B55, B35, and B23 did not have any effect on the germination of the spores as compared to the control plate (Figure 5.4B).

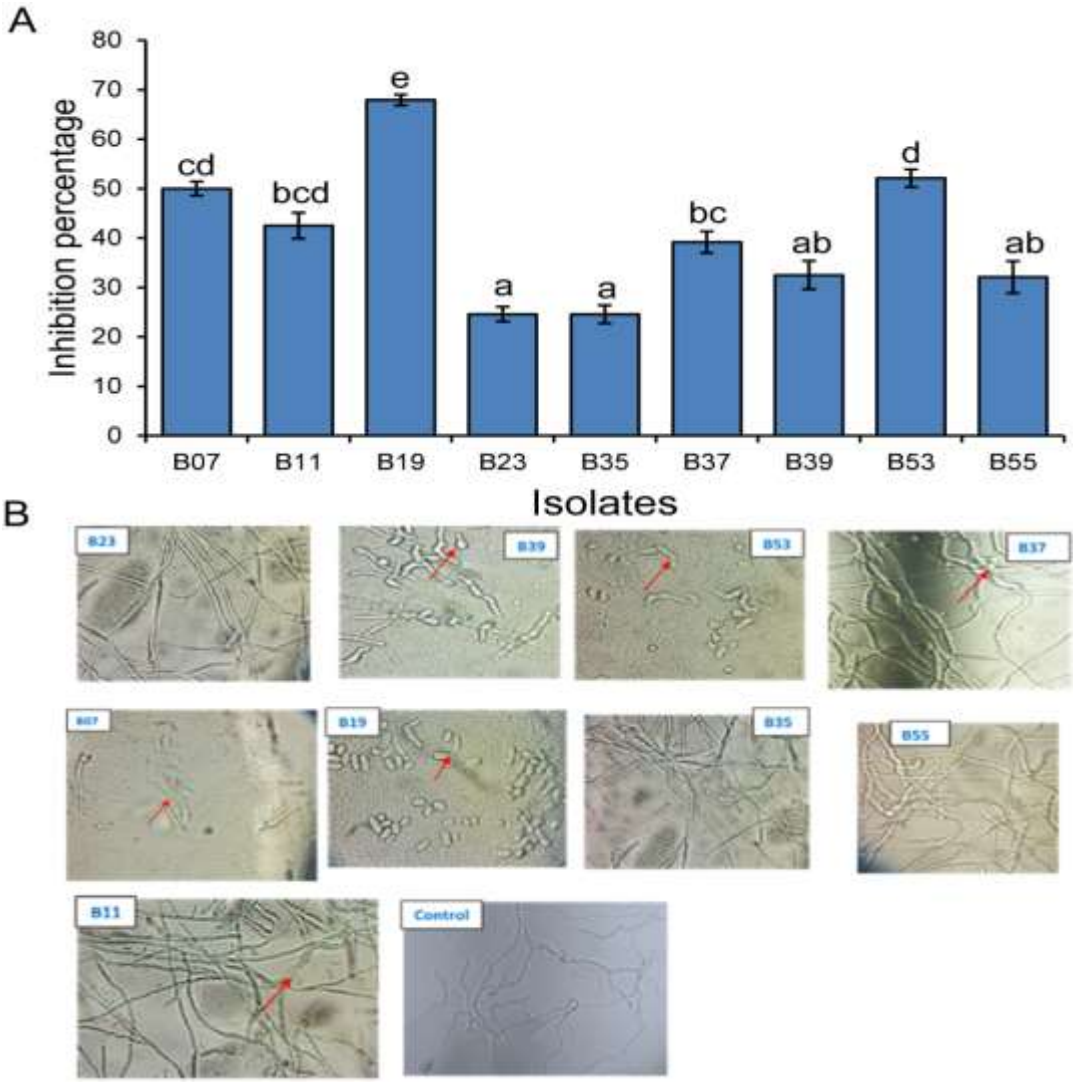

Figure 5.4: Inhibitory Effect of Bacterial Endophytes on Fusarium Solani Mycelial and Spores

(A) Bar plot shows the mean inhibition percentage of each isolate. Error bars represent SEM. Bars with different letters are significantly different from each other (GLM with binomial distribution followed by the analysis of deviance test, n = 3). (B) Pictures showing the effect of the interaction of Fusarium solani spores with bacterial endophyte cells after incubation in mixed culture plates for 24h. Arrows in B39 and B07 indicate delayed emergence of germ tubes from spores; B53 and B19 indicate non-germinated and morphological changes in spores; B11 and B37 indicate

morphological changes in the growing mycelium. Control plate contained only F. solani spores.

5.6.5 Endophytic Competence of Bacterial Endophytes on Beans

To test the potential of the endophytic establishment of the isolates in beans, two methods were used; seed soaking and drenching. During re-isolation of endophytes from bean plant parts, any isolate that appeared on agar plates and that did not have similar morphological features as that of the original isolate inoculated in the seeds or applied as drenching was not counted. All tested bacterial endophytes were able to establish endophytically in all bean parts using both seed soaking and drenching methods but at different rates (Figure 5.5). The performance of the isolates differed significantly in the two methods ($F_{1,160} = 460.98$; $P = 0.029$) and drenching

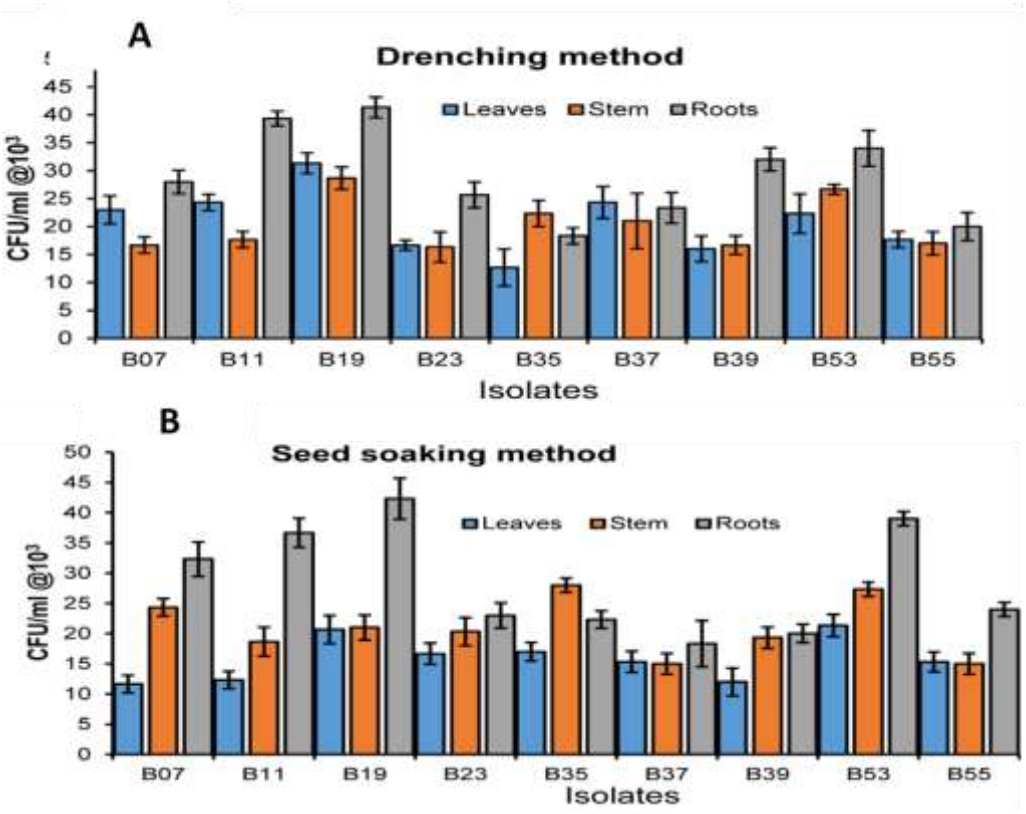

Figure 5.5: Recovery of Endophytic Bacteria from Bean Seedlings Inoculated Using Different Methods

Bacterial endophyte re-isolation from different bean plant parts following inoculation using (A) seed soaking method and (B) drenching method. Bars represent SE of the mean gave a higher bacterial recovery titer than seed soaking. Invariably, isolate B19 had the highest re-isolation titer in all plant parts using the drenching method with 4.1 $\times 10^4$ cfu/g in the roots, 3.1 $\times 10^4$ cfu/g in the leaves, and 2.9 $\times 10^4$ cfu/g in the stem. It was interesting to note that isolate B35, isolated from the stem, had a higher re-isolation titer in the stems than in the leaves and roots in both the methods. Endophytic colonization was higher in the roots than in the stems and leaves. In both methods, the roots had the highest re-isolation titer at 4.2 $\times 10^4$ cfu/ml. For seed soaking method, isolate B39 had the lowest titer in the leaves at 1.2 $\times 10^4$ cfu/g.

5.6.6 Biocontrol of *Fusarium solani* in a Pot Experiment

A pot experiment in a greenhouse was set up to evaluate the effect of endophytic bacteria on *F. solani* in planta. Bean seeds were bacterialized with the 9 selected endophytic bacteria and non-inoculated beans as controls. After 30 days of growth, treatment of bean seeds with bacterial endophytes significantly reduced the disease severity rating (Figure 5.6A) and the percentage of disease incidence (Figure 5.6B) when compared to the controls. Isolates, B53 and B19 did not show any symptoms of the disease at the end of the experiment and presented a well-developed root structure (Figure 5.6C). On the other hand, the treatment of bean seeds with B55 resulted in necrosis in the whole root system, yellowing, and eventual death of the seedlings similar to the control in fungus-infested soil (Figure 5.6C). Isolates B11, B07, B35, B37 and B23 indicated stagnated growth with poor and infected rooting system (Figure 5.6C).

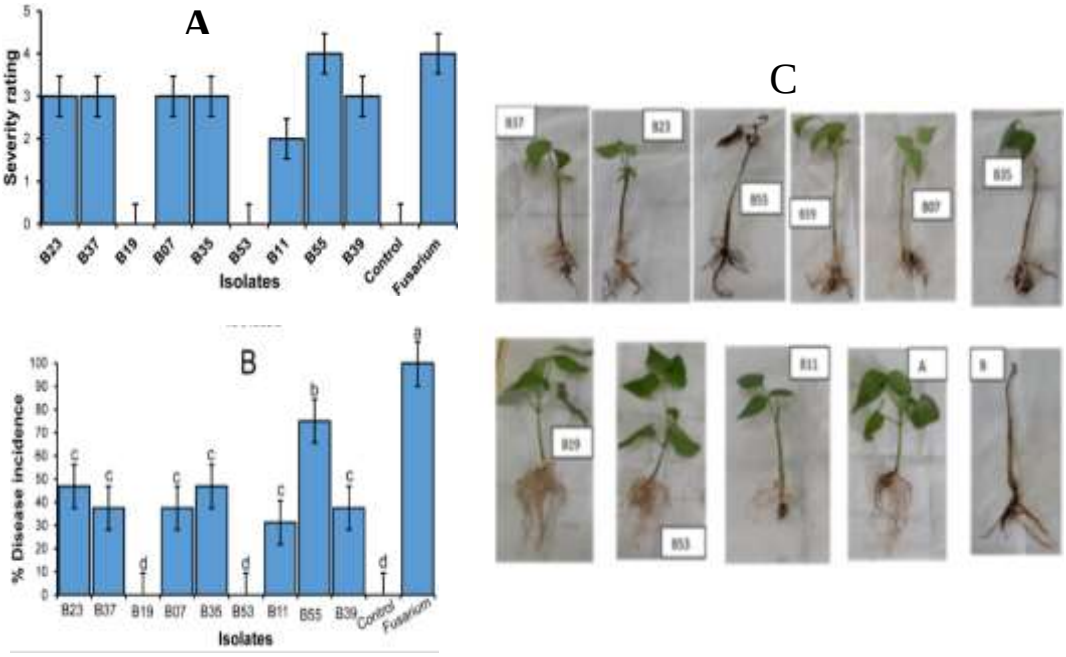

Figure 5.6: Effect of Endophytic Colonization of Beans by Bacterial Endophytes on *Fusarium solani*

A. severity of symptoms in bean root system in *F. solani* infected soil and endophytes B53 (*Bacillus megaterium*), B19 (*Enterobacter hormaechei subsp. Steigerwaltii*), B37 (*Bacillus tequilensis*), B07 (*Bacillus mojavensis*), B35 (Bacillus subtilis subsp. subtilis), B11 (*Bacillus subtilis subsp. stercoris*), B39 (*Bacillus altitudinis*), B23 (*Bacillus australimaris*) and B55 (*Bacillus clausii*). (A) Represents control seedling without endophyte and grown in un infested soil. (B) Represents control seedlings without endophytic bacteria and grown in *Fusarium solani* infested soil. All plants were grown in a greenhouse for 30 days. B. Percentage (± SEM) disease incidence. Bars with different letters indicate significant difference (ANOVA, then SNK post-hoc, $P < 0.05$). C. Diseases severity rating (± SEM)

The first true leaves of the seedlings had turned yellow and easily fell off while washing the excess soil around the roots. Conversely, bacterial endophytes significantly affected the root wet weight (Figure 5.7A; $F_{10–77} = 133.6$, $P < 0.0001$), root dry weight (Figure 5.7B; $F_{10–77} = 52.44$, $P < 0.0001$), and shoot dry weight (Figure 5.7D; $F_{10–77} = 184.3$, $P < 0.0001$). Isolates, B19 and B53 significantly increased the wet and dry weights of the roots and shoots.

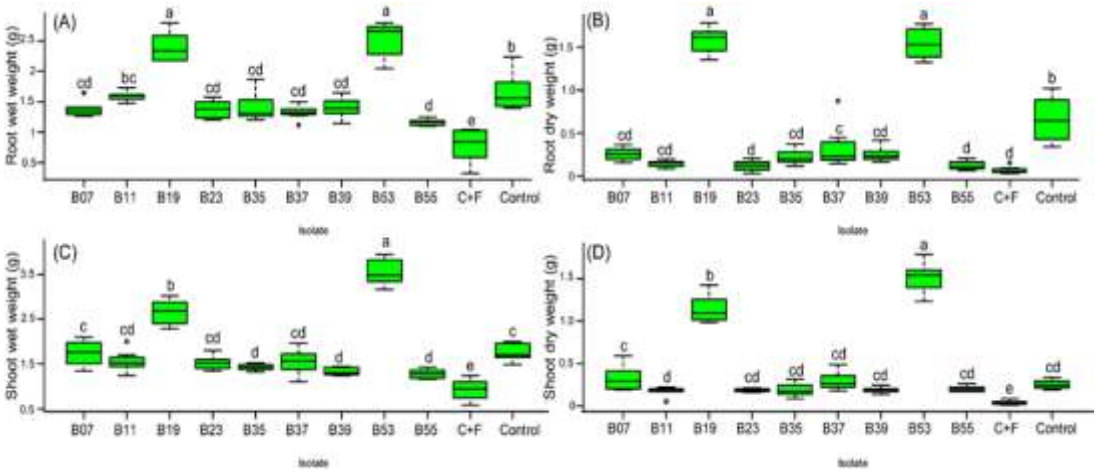

Figure 5.7: Box Plot Representing the Effect of Bacterial Endophytic Inoculation on Bean Seedlings in the Presence of *Fusarium solani* On Root and Shoot Fresh and Dry Weights

C+F represents a control with growth of seedlings without endophyte in the presence of fungal pathogen while control represents seedling growth without pathogen or endophyte in sterilized soil. Bars represent standard error of mean. Treatments with different letters are significantly different from each other (ANOVA test followed by SNK's *post-hoc* test, $P< 0.05$).

5.7 Discussion

Lake Bogoria, one of Kenya's rift valley lakes is characterized by high pH and high salt deposits, occasional droughts causing precipitation of salts, and flooding during the rainy season that creates different hydrological cycles and a unique and changing environment especially for the shrub vegetation growing off the lake shore. The Lake has undergone significant hydrological changes in the last 10,000 years (Olago *et al*., 2009) and is still experiencing such changes in periods of extreme drought and flooding which have been on-going, especially in the last 10 years. The vegetation thus has to adapt to these changes (Harper *et al*., 2003) and one of the known mechanisms of adaptation is through the recruitment of beneficial microorganisms that provide a selective advantage to the plants (Bulgarelli *et al*., 2013). These microorganisms are a storehouse of bioactive metabolites which help the plants tolerate both biotic and abiotic stresses (Fadiji & Babalola, 2020). Selim *et al*., (2012) hypothesized that plants

in unique ecological niches may contain endophytes with vast metabolic ability for applications in various industries. Endophytic bacteria isolated from arid land plants by Asaf (2017) were able to produce a variety of plant phytohormones and enhance the growth of soybean in a controlled environment. Similarly, Ali (2018) demonstrated the ability of a thermophilic fungal endophyte isolated from a hot desert plant to confer heat stress tolerance to cucumbers under field conditions. The present study therefore sought to explore the potential of bacterial endophytes from shrubs along the draw-down zone of Lake Bogoria for the biocontrol of bean root rot pathogen, *F. solani*.

Identification of the isolates based on their 16S rRNA gene sequence revealed a bias in the isolation of the genus, *Bacillus*. The tendency to isolate endophytic Bacillus was previously noted from a variety of plants (Ali *et al*., 2017; Mohamad *et al*., 2020; Sendi *et al*., 2020). Isolation of endophytic bacteria from two mangrove systems in Brazil by Castro (2014) also resulted in Bacilli as the most isolated genera followed by Gamma Proteobacteria, coinciding with our results. The rhizosphere is known to be the main source of endophytic microbiota (Andreote *et al*., 2014) and this could be the driving factor to this bias. Though there is limited information on rhizosphere microorganism from Lake Bogoria, other studies have indicated a similar abundance of the genera, *Bacillus* from both rhizosphere soil and plant endophytic bacteria. Besset-Manzoni *et al*., (2019) generated a culturable soil rhizosphere bacterial library, which resulted in *Bacillus* and *Pseudomonas* as the two most abundant genera. These genera have previously been isolated as endophytes. Their functional roles in plant growth promotion (Devi *et al*., 2017), biocontrol activities (Bendaha & Belaouni, 2019; Macedo-Raygoza *et al*., 2019), plant heat stress alleviation (Khan *et al*., 2020), and bioremediation (Wu *et al*., 2018) have been established.

A majority of the tested isolates were positive for plant growth promoting traits and enzymes, a trend also noted in other studies (Mohamad et al., 2020; Etesami et al., 2015). Production of hydrolytic enzymes, antimicrobial metabolites, and plant phytohormones are important traits associated with most endophytic microorganisms (Khan *et al*., 2016; Potshangbam *et al*., 2017). Hydrolytic enzymes are important in the endophytic lifestyle as they are used by the microorganism to gain entry into the plant and in the control of phytopathogens and in eliciting stress tolerance. Some

enzymes isolated from the endophytic bacteria include cellulases, proteases, lipases, pectinases, chitinases, and endoglucanases (Castro *et al*., 2014). Studies by *(Khan et al*., 2018) correlated the antifungal activity of *Bacillus simplex* and against *Fusarium oxysporum f. sp. Conglutinans* and *F. solani* to the production of hydrolytic enzymes which resulted in fungal hyphal thinning and reduced disease development. Our results reveal similar activities with the isolates that showed delayed or inhibition of spore germination and high mycelial inhibition producing at least two of the enzymes tested. Phytohormones are important in the regulation of plant growth and development. Indole-3-acetic acid is an auxin found both in the plants and microorganisms and plays a major role in plant cell differentiation, in pathogenesis, and in the promotion of plant growth and survival under stress conditions (Egamberdieva, 2009; Vurukonda *et al*., 2016). One of the objectives of this study was to screen the endophyte collection for beneficial traits including the production of enzymes, phosphate solubilization, and production of hydrocyanic acid in vitro.

From the results of the dual culture assay, a second set of in vitro antagonistic test was conducted to assess the effect of select bacterial cells on the spores of *F. solani* in co-cultures. Co-culturing of microorganisms provides cell to cell interactions that have been reported to facilitate the activation of cryptic pathways (Serrano *et al*., 2017), and hence the production of secondary metabolites that are not produced in axenic cultures that could potentially have antimicrobial activities (Bertrand *et al*., 2013). To test this concept on our isolates, we set up a cell spore direct interaction assay with each of the nine endophytic bacterial isolates with *F. solani* spores and incubated them overnight on a thin layer of PDA. The direct interaction of *F. solani* and the bacterial cells resulted in complete inhibition of germination of fungal spores by isolates B53 and B19 (Figure 5.4). This further confirms the potential of the two isolates in the biocontrol of the test fungi. Fungal spores incubated with isolates B39 and B07 showed delayed spore germination after an overnight incubation, with short germ tubes just starting to emerge. However, the two isolates did not control the fungus in planta, indicating loss of activity in vivo. Various studies on antagonist effects of two different strains through direct contact have been undertaken. (Nogueira *et al*., 2019), while studying the effect of interaction between *Klebsiella pneumoniae* and *Aspergillus fumigatus*, confirmed that antagonism and inhibition of fungal spore germination were

dependent on the direct contact of the two microorganisms that also affected biofilm formation. Further, Toghueo *et al*., (2016) indicated that the antagonistic activity of an endophytic *Trichoderma* spp. against *F.solani* in a spore–spore confrontation assay was concentration-dependent. In a natural environment, *Fusarium* spp. exist in both mycelia and clamydospores which infect crops when the conditions are favorable. Therefore, screening for the inhibition of mycelial and spore germination is an important factor to consider in the selection of an effective biocontrol agent in the field.

Fusarium solani is a soil-borne pathogen which persists in soil after crop harvest until conditions are favorable and another crop is planted (Naseri, 2014) and it is difficult to control it. Some of the methods employed in the control include agronomic practices which do not offer effective control, in addition to the diminishing availability of land. Thus, a better alternative is the use of biocontrol agents, which are mostly formulated for application as seed coating or sprays after seed and seedling germination (Bailly *et al*., 2020). However, the presence of fungal pathogens in soil significantly reduces the percentage of the germination of seeds (Botelho *et al*., 2013); therefore, there is need to protect seeds and seedlings before planting. Though not widely supported in the application of biocontrols (Bailly *et al*., 2020), seed and seedling treatment with antifungal microbial based biocontrol agents can limit the invasion of the pathogen and enhance the germination rate. Seed coating with microbial biocontrols provides an immediate avenue for the colonization of both the roots and shoots as they emerge, offering protection against root pests and pathogens (O'Callaghan, 2016). *Pereira et al*., (2011) showed that seed treatment with *Bacillus amyloliquefaciens* reduced *Fusarium verticillioides* in maize roots. Seed soaking by Mangmang *et al*., (2015) resulted in enhanced germination value, root and shoot growth, vigorous and more consistent beneficial effects on tomatoes as compared to the drenching method. In our experiments, both seed soaking and drenching methods of endophyte delivery were used. The two methods showed significantly different colonization rates, with drenching method indicating a higher re-isolation titer than the seed soaking method. However, due to the inhibitory activity of *F. solani* in the germination of seeds, seed soaking method for endophyte delivery was chosen in green house experiments.

The mode of action of biocontrol agents in vivo is diverse and include competition for nutrients with the pathogens, production of antagonistic secondary metabolites, induction of systemic resistance, and priming the defense system of the host plant. Some of the metabolites involved in the biocontrol have been well characterized and documented (Khan *et al*., 2018; Köhl *et al*., 2019). Previous studies by López-Bucio *et al*., (2007) revealed the enhancement of bean root system by a *Bacillus megaterium* isolate through IAA and ethylene independent mechanisms. Association of plants with IAA producing microorganisms results in the elevation of IAA in the host plant (Spaepen *et al*., 2007), thereby stimulating the anti-oxidant system of the plant in response to stress conditions (Xia *et al*., 2015). Our findings corroborate with those of Mannaa *et al*., (2017) and Dahmani *et al*., (2020), who indicated that *B. megaterium* produced volatile metabolites with anti-fungal and growth promoting activities. Bendaha & Belaouni (2019) too, reported significant protection against *Fusarium oxysporum f. sp. radicis lycopersi* and growth enhancement in tomato seedlings using *Enterobacter hormaechei subsp. Steigerwaltii* isolate EB8D. However, in some experiments, in vitro antagonistic activities do not always correlate with biocontrol in planta (Besset-Manzoni *et al*., 2019). This was observed in B07 which had a considerably higher mycelial inhibition (49.8%) in dual culture assay and indicated delayed emergence of germ tubes from the spores in co-cultures, but did not show considerable fungal biocontrol in the pot experiment. Association of microorganisms with plants can lead to either gain of function or loss of function by the microorganism. Vandernkuornhuyse *et al*. (2015) hypothesized that plant associated microorganisms are a consequence of adaptations and adjustments to environmental stress. These could probably explain the loss of antifungal activity in vivo by some isolates, and therefore predict other beneficial traits that could be associated with the bacterial endophytes from this study which were not screened.

In our selection, two isolates of *B. subtilis* were included in the screening (B11 - *B. subtilis subsp. spezizenii* and B07 - *B. subtilis*). Species of *B. subtilis* have been widely used in various industries including agriculture as biocontrol against plant pathogens and other industrial enzyme production processes (Su *et al*., 2020). However, our results indicate low levels of been rot protection against *F. solani* by the two B. subtilis isolates tested despite a considerable in vitro antagonism. In addition to industrial

applications, the species has been implicated in plant adaptations to stress, a function that was noted by Abd-Allah (2018) after inoculation of chickpea with endophytic *B. subtilis* isolate, BERA 71 under saline conditions which resulted in the modulation of the antioxidant system in the plants, reduction of reactive oxygen species (ROS), and significantly enhanced the growth of chickpeas. Association of microorganisms with plants has been noted as functionally driven (Wemheuer *et al*., 2017), and hence, based on the conditions of our sampling site, it is hypothesized that the two endophytic *B. subtilis* isolates, and the other isolates found to have weak inhibition of the tested fungal mycelia might have different functional roles and therefore proposed further screening especially on abiotic stress tolerance and the production of enzymes for other applications.

Several studies on the microbial populations from Lake Bogoria have focused on the microbial diversity in water, sediments, and soil, with a bias on archaeal diversity and applications in industrial enzyme production. Results from this study have indicated the potential application of endophytic bacteria associated with shrubs growing along the draw-down zone of Lake Bogoria, a niche that has not been explored in the research on the microorganisms of soda lakes in Kenya. Diverse endophytic bacteria were isolated, some of which are human opportunistic pathogens. For example, *Enterobacter hormaechei subsp. Xiangfangensis* has been implicated as a human pathogen and in this study, it has indicated a greater potential in the biocontrol of the bean root rot pathogen, *F. solani*, in addition to plant growth promotion. Several other Enterobacter strains have been isolated from environmental samples and others as endophytes and have been shown to be beneficial to plants with growth promotion and biocontrol activities against phytopathogenic fungi (Ullah *et al*., 2017; Bendaha & Belaouni, 2019; Macedo-Raygoza *et al*., 2019; Przemieniecki *et al*., 2019). The biocontrol and growth-promoting ability of endophytic *Enterobacter hormaechei* is associated with the presence of several gene clusters in the genome, responsible for nitrogen fixation, phytohormone production, and transcriptional regulation, which are important traits for survival in diverse environments and plant endophyte interaction (Ren *et al*., 2010). Other strains of bacterial opportunistic and enteric human pathogens have been isolated as endophytes from various plants from diverse environments and vegetables (Lim *et al*., 2014; Nithya & Babu, 2017; Etminani & Harighi, 2018).

However, Eberl & Vandamme, (2016) noted that based on the source of the *Burkholderia* isolate, phylogenetic classification does not always infer human pathogenicity of a strain, and therefore a detailed genetic analysis is important. In another study, further genetic analysis of *Burkholderia* species grouped the pathogenic and plant associated strains in different clades, with the plant beneficial endophytic species lacking the virulence-associated loci necessary for human pathogenesis (Angus *et al*., 2014). In addition, Kandel *et al*., (2017), through a comparative based genomic analysis of *Burkholderia* species, found that endophytic *Burkhoderia* strains lacked key mammalian pathogenesis-related gene clusters in their secretion system and recommended their use in agricultural systems. Further studies are therefore recommend to establish if these strains of endophytic *Enterobacter hormaechei subsp. Xiangfangensis* and *B. megaterium* contain human pathogenic properties. Further experiments to determine the endophytic localization of the bacteria in planta using fluorescent labeling and staining are necessary. In addition, further research should focus on the effectiveness of the two isolates in non-sterile soil and under field conditions.

5.8 Conclusion

Bacillus megaterium isolated from the leaves of *Boeravia erecta* and *Enterobacter hormaechei subsp., Xiangfangensis* isolated from the roots of *Abutilon frutcosum* significantly reduced the effect of *F. solani* and increased the shoot and root weight of the bean plants. *In vitro*, the isolates produced IAA, solubilized phosphate, and the enzymes, catalase and amylase which are important stress-mitigation features. The isolates also inhibited mycelial and spore germination in vitro, further confirming their ability to control *F. solani*. However, further experimental studies are required in future to better understand the efficacy of the two isolates under non-sterile soil and field conditions, including the genetic mechanisms involved in the synergistic interaction of the plants and the microorganisms. More genetic studies are necessary to determine if the endophytic *Enterobacter hormaechei subsp., Xiangfangensis* contain any human pathogenesis-related genes.

CHAPTER SIX

EFFECT OF FUNGAL ENDOPHYTE COLONIZATION IN TOMATO ON TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* (KOCH)

6.1 Introduction

Tetranychus urticae Koch (Two-spotted spidermite) is a cosmopolitan pest of global concern, causing economic losses to major agricultural crops in both closed and open systems (Daniels *et al*., 2022). They have a worldwide distribution, spread across the various climatic zones. The mites are polyphagous, feeding on an extremely large host range of plants, both cultivated and wild, ornamentals, fruit trees and shrubs (Jakubowska *et al*., 2022). Spider mites are mesophyll cell-content feeders, piercing the plant and sucking out the contents (Khamis Al-Zahrani *et al*., 2023), with dead cells appearing as yellow spots on the leaf, which eventually die out as a result of reduced photosynthesis. *Tetranycus urticae* Koch are known to have a high reproductive capacity and the most insecticide resistant among the arthropods (Daniels *et al*., 2022). Global losses from *T. urticae* in tomato plants have been estimated to more than 50% resulting from reduced quality of fruits, low productivity and poor markets (Meck *et al*., 2012).

Control of these mites in conventional agricultural systems is largely using acaricides, which have negative effects to the environment and people, coupled with the mite's high abilities to select for resistance (Daniels *et al*., 2022). The global call on use of alternatives to chemical methods of control has led to accelerated search for alternative methods of insect pests and disease control. Biological control agents, either parasitic insects or microbials have an advantage over chemical control methods as the agents do not develop insecticide resistance, have no harm to human health and non-target species (Hulot & Hiller, 2021). Of the biological control agents, microorganisms have, in addition to the management of the target pest and diseases been used in enhancing plant stress tolerance, plant growth (as biofertilizers) and in plant acquisition of nutrients (Singh *et al*., 2022). Despite more than 70 years and the dedicated efforts to search for useful microbial agents for use in sustainable agriculture, only a limited number has been commercialized (Pellan *et al*., 2020). The slow entry into the market

of microbial biological control agents has been partly caused by substantial or complete loss of activity in field environments, despite successful functioning in controlled environments; and the challenge of ensuring the agent is applied in the right quantities and is sustained long enough to control the particular pest or pathogen (Lahlali *et al*., 2022).

Some of the commercialized biocontrol fungi, especially entomopathogenic fungi have been found to colonize plants, and have also been isolated from wild and cultivated plants as endophytes (Bamisile *et al*., 2018a; Jaber & Ownley, 2018). Several experiments have successfully introduced these entomopathogenic fungi in different crop plants: tomato (Rasool *et al*., 2023); beans (Akutse *et al*., 2017); maize (Liu *et al*., 2022); potato (Tyurin *et al*., 2021); and wheat (Jaber, 2018a), resulting to biocontrol and additional benefits to the plant, especially on growth enhancement.

The search for additional novel endophytic beneficial fungi for use in sustainable agriculture as biocontrol products in pest and disease management and in crop stress tolerance is increasingly gaining attention, especially endophytes from plants in stressed environments. Endophytic microorganisms are known to have developed habitat acquired mechanisms to survive under the stress conditions and therefore provide an option for bioprospecting of novel biocontrol microbes. Moghaddam *et al*., 2021 showed that fungal endophytes from salt dominant plant species were able to induce salinity and drought stress tolerance and enhance growth of cucumber plants. A review by (Verma *et al*., 2022) reveals the immense potential fungal endophytes have in application to sustainable agriculture. The use of endophytic microorganisms in the control of herbivorous insects has revealed interesting interactions between the plant, the insect and the endophyte. Some endophytic fungi have been shown to prime the plants defense system against insect pests through production of plant volatiles (Sharifi *et al*., 2018) and defense compounds (Rasool *et al*., 2023). Other endophytes have been shown to increase the susceptibility of the plant to attack by the insect pests (Clifton *et al*., 2018) while others act by enhancing the growth of the plants.

The method used and treatment time in delivery of the endophyte to the plant has been noted to have an effect on establishment of the fungi as an endophyte in the plant and

the subsequent action in biocontrol (Jaber & Enkerli, 2016; Khamis Al-Zahrani *et al*., 2023; Sánchez-Rodríguez *et al*., 2018). The mechanisms of action of endophytic fungi and their interactions with the plant and insect pest have not been fully understood. To date, only a couple of entomopathogenic fungi as endophytes in the biocontrol of insect pests, especially *T. urticae* have been studied.

Seed treatment positions the microorganisms for early endophytic colonization of the radicle and plumule which are close to one another as the seed is germinating (Muvea et al., 2014), exposing the plant parts to the endophyte. This probably explains why it has, in almost all experiments conducted with seed treatment, achieved successful endophytic colonization. Commercial use of seed treatment with beneficial microorganisms as a method of endophyte delivery to the plant would be economical in crop production as it would reduce additional labour in fertilizer application (for growth enhancing endophytes) as well as pest and disease control. Recently, there has been growing interest in the use of beneficial microorganisms as alternatives to chemical inputs in agricultural production systems and their functional role as endophytes in plant protection as use in seed treatments (O'Callaghan, 2016). Seed inoculants have long been used commercially with Rhizobia, especially for legume crops (O'Callaghan, 2016). However, there are very little seed inoculants, either single strain or a combination, commercially available for use in endophyte colonization and eventual improvement of plant performance for agriculture markets. The current study therefore sought to screen endophytic fungi isolated from shrub plants growing along the shores of Lake Magadi as potential endophytes in the management of *T. urticae* in tomatoes under a greenhouse experiment.

6.2 Materials and Methods

Description of sampling site, sample collection, isolation of bacterial endophytes and their characterization are detailed in **chapter 3** above.

6.2.1 Preparation of Plant Materials

Tomato seeds (variety Cal J) were surface sterilized by washing first in tap water followed by a 2 min wash in 3% sodium hypochlorite, 2 min in 70% ethanol and three

times rinse in sterile distilled water. The final rinse water was plated on PDA to confirm the effectiveness of surface sterilization (Muvea *et al*., 2014). Sterilized seeds were air-dried for about 45 minutes before soaking them overnight in a conidial concentration of $1x10^8$ of each isolate. This conidial concentration had previously been used in other experiments and has successfully shown systemic colonization of fungal endophytes in all plant parts (Jaber & Enkerli, 2016). Four fungal isolates (*Cephalotrichum cylidrichum* F04, *Fusarium equiseti* F05, *Fusarium falciforme* F18 and *Aspergilus puniceus* F21) were selected for the assay. Test for endophytic competence of the isolates was earlier done in section 4.2.1 above. Inoculated seeds were sown directly into the potting mixture containing sterilized soil and cattle manure and maintained in a greenhouse for 4 weeks and watered as necessary with sterilized tap water. One week after germination, the seedlings were thinned to two seedlings per pot.

6.2.2 Rearing of the Mites

The two spotted spider mites, *Tetranychus urticae* were provided by *icipe* from a rearing colony maintained on tomato plants. The rearing to obtain synchronized age adult mites for the experiment followed the procedure by (Pappas *et al*., 2018) with some slight modifications. One hundred and fifty adult mites from the colony were transferred to non-endophyte inoculated 3-week-old potted tomato plant leaves. Each leave was infested with 10 adult females and 5 adult males. The pots were kept in a growth chamber at 25 ± 2^{o}C and 12:12 LD and left for 48hours to allow the adults to lay eggs. The adult mites were then removed and the plants maintained at the same conditions for about two weeks to allow emergence of mites from the eggs.

6.2.3 Assessment of Endophyte Colonization

The plants were assessed for colonization rates at 4 weeks at the time of introduction of *T. urticae*. Seedlings were washed in running tap water to remove any adhering soil before sectioning them into roots, stems and leaves. Each of these segments was surface sterilized as above. Six pieces of each plant part (root stem and leaves) per seedling from each pot were cut into approximately 1cm long using a sterile surgical blade under a laminar flow hood, plated onto PDA plates supplemented with 50µg/ml

streptomycin sulphate and incubated in the dark at 25^{o}C ± 2 for 14days to observe any fungal outgrowth. The fungal outgrowth from the plant samples were stained with lactophenol cotton blue to compare the morphological characteristics with the original isolate (Muvea *et al*., 2014). Growth of other fungal cultures not inoculated was noted and this were not considered in the analysis. Percentage colonization of the inoculated fungus was scored using the formulae: number of plant pieces colonized /number of plant pieces plated x 100 (Jaber & Enkerli, 2016).

6.2.4 Endophyte Performance on *T. urticae*

At 4 weeks, the seedlings were separated, one seedling was used for assessment of endophyte colonization and one left in the pot for the mite assay. Endophyte inoculated and control plants were infested with 2-4 days old adult female mites: 15 mites per leaf, 3 leaves per plant and 4 plants per isolate. After 4 days, infested leaves were assessed for the number of eggs laid (fecundity) and mortality of the mites. Cadavers of *T. urticae* were placed onto petri dishes with sterile moist filter paper and incubated for 10 days to check for mycosis.

6.2.5 Data Analysis

Fungal endophyte colonization data at 28 days was fitted on a generalized linear model with Poisson distribution using the R software version 4.2.3. Data on the number of eggs laid and mortality was analysed using one-way ANOVA and means compared using Turkeys HSD *post Hoc test*.

6.3 Results

6.3.1 Colonization of Tomato Seedlings with Endophytic Fungi

Endophytic establishment of the isolates in the various plant parts (leaves, stem and roots) was assessed at 28 days post seed inoculation. Two additional fungal colonies noted in control and F18 seedlings which were morphologically different from the test fungi were disregarded in the analysis. These were noted as airborne contaminants that could have colonized the plants in the greenhouse during the 4-week growth period of the plants.

The isolates used in the experiment had earlier been assessed for endophytic colonization in tomato seedlings using seed soaking method at 21 days post inoculation and indicated colonization at all plant parts but with differences in the rates of colonization of each isolate ($P < 0.001$), but no significant difference in colonization of the plant parts ($P = 0.2492$). Assessment at 28 days indicated colonization at all plant parts, with significant differences across the isolates ($P = 0.016$). The recovery rates of leaves, stem and roots for isolate F04 was 55.6 ±11.1%, 16.7 ± 9.6% and 61.1 ± 11.1% respectively, while isolate F21 had 50 ± 9.4%, 50 ± 9.5% and 83 ± 8.2% respectively (Fig.1). Recovery of the endophytes was highest at the roots across the isolates except for isolate F18 (50 ± 9.2%). No mycoses were observed in any of the dead insects after 10 days of incubation.

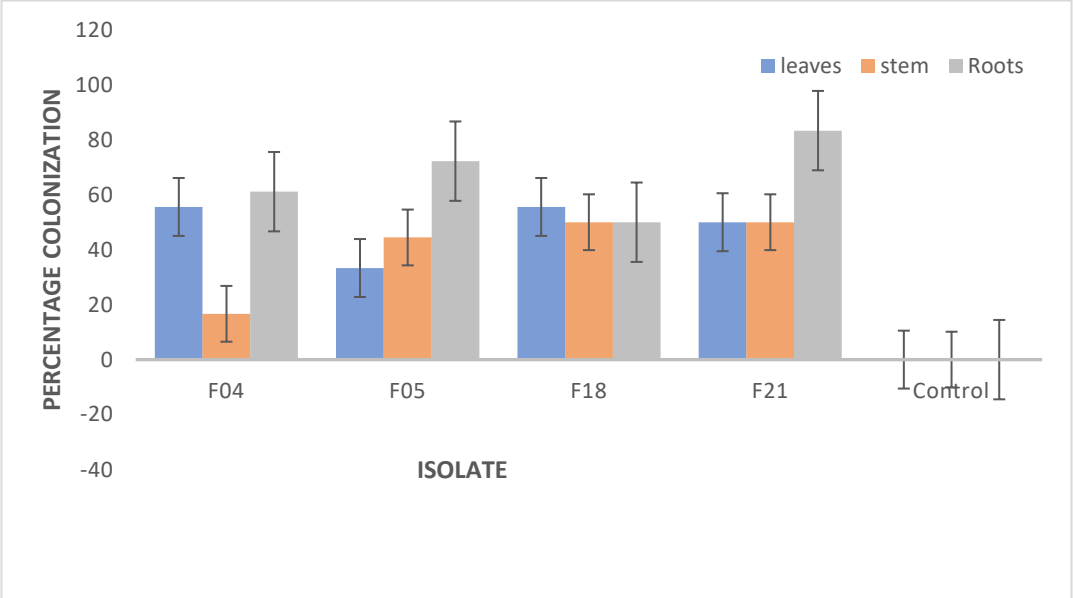

Figure 6.1: Endophytic Colonization of the Different Plant Parts by the Isolates at 28 Days Post Seed Inoculation and Growth of Seedlings in a Green House

6.3.2 Effect of Endophytically Colonized Tomato Leaves on *T. urticae* Oviposition and Mortality

Endophytic colonization of the leaves with the test fungi did not significantly affect the number of eggs laid across all the isolates as compared to the control ($F = 2.025$, $df = 4$, $P = 0.104$), although colonization of the isolates differed (Fig. 2). Similarly,

there was no significant difference in mortality of the insects observed both at the endophytically colonized and control plants (F = 0.762, df = 4, P = 0.554).

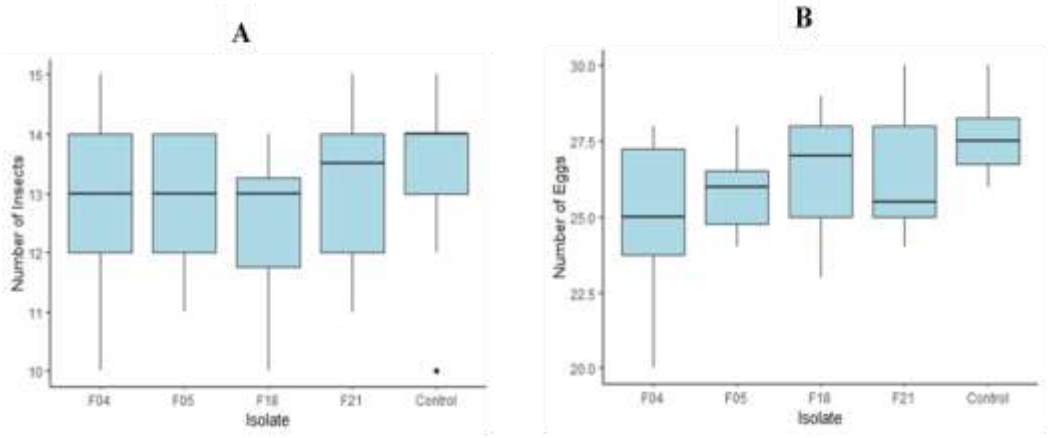

Figure 6.2: Box Plot Indicating the Effects of Tomato Leaf Colonization by Fungal Endophytes

C. cylidrichum (F04), *F. equiseti* (F05), *F. falciforme* (F18) and *A. puniceus* (F21) on (A) mortality rates and (B) number of eggs laid of two spotted spider mites (*T. urticae*) and a control with no endophyte

6.4 Discussion

The results of our study show the success of endophyte introduction into the plant tissues using seed soaking method. Several methods have been used to achieve endophytic colonization of desired fungal and bacterial strains into plant tissues including foliar application (Bamisile *et al*., 2019); soil drench (Bhagyasree *et al*., 2020; Rasool *et al*., 2023); seed treatment (Canassa *et al*., 2019; Jaber & Enkerli, 2016) and root dipping (Sahu *et al*., 2023). These methods have been well documented and reviewed (Bamisile *et al*., 2018b). Delivery of endophytes can be systemic or non-systemic depending on the method of application used, and in some cases endophytic colonization is temporary, lasting only a few weeks post inoculation, especially in foliar spray (Bamisile *et al*., 2019). Treatment duration has been found to influence the rates of endophyte colonization in inoculated plants. The current study used an overnight seed treatment method of endophyte inoculation and resulted in systemic colonization of all plant parts by all the tested fungal isolates, and re-isolated at high

rates of up to 80% in some tissues 4 weeks post inoculation. Similar results were also obtained by other projects with varying treatment durations (Jaber & Enkerli, 2016) while using *Beauveria bassiana* and *Metarhizium brunneum* in *Vicia faba* and (Muvea *et al*., 2018) in onion plants using endophytic *Hypocrea lixii* F3ST1, 50 days post seed treatment.

Incubation of the dead *T. urticae* insects from both the endophyte inoculated and control plants did not result to any mycoses of the cadavers. In several experiments, endophyte inoculated plants have not resulted in mycosis of insect cadavers (Sánchez-Rodríguez *et al*., 2018; Sani *et al*., 2023). The mechanisms underlying the pathogenicity of endophytic microorganisms is not yet fully understood. It would be expected that after exposure of insects to endophyte inoculated plants, the insect would ingest the insect-pathogenic microorganism as it feeds and therefore cause growth and development of the microbe inside the insect, eventually killing it and an outgrowth observed as mycosis in the cadaver. However, mycoses are often observed in cadavers after direct spray of insect-pathogenic microorganisms (Elhakim *et al*., 2020). In endophyte colonization, the effects on insects is either as a result of toxic metabolites produced from the plant-endophyte interaction or effects of plant defence system (Samaras *et al*., 2023). Plants induce direct and indirect defense strategies against insect attacks which include glandular trichomes in tomato and protease inhibitors that constrain digestion of the herbivore (Pappas et al., 2017). In such occasions where plant defense system is activated during insect attack, no mycoses would be observed from dead insects. Although the lack of mycoses cannot simply be pointed out as a result of plant defense induction or the lack of performance of the endophytic microbials used, their impact on enhanced growth of the seedlings in insect herbivory would be of interest for further investigation. Therefore, the lack of mycoses in *T. urticae* cadavers in our experiment could probably be as a result of a lack of direct interaction between the fungus and the insect.

Despite the successful colonization of tomato leaves by all the four tested endophytic fungi, none of the endophytes indicated any effect on the number of eggs laid and mortality of *T. urticae* as compared to control plants without endophyte inoculation, an indication that these endophytes might not be lethal to the mites. In plant endophyte

associations, the plant provides nutrients to the endophyte while the endophytes have direct effect on the plant, including influencing nutrient status, inducing production of secondary metabolites and volatile compounds that are lethal to herbivorous insects to the induction of systemic resistance to biotic and abiotic stress (Pirttilä *et al*., 2023). Some of the plant volatiles act as cues for attracting insects, making the plants more susceptible to insect herbivores and pathogens (Sharifi *et al*., 2018). Studies have shown a reduction in herbivorous insect oviposition and high mortality rates after exposure to endophytically colonized insecticidal fungal endophytes, including endophytic fungal entomopathogens (Bamisile *et al*., 2019; Pappas *et al*., 2021 ; Muvea *et al*., 2014). These negative effects of endophyte colonized plants on insect herbivores are hypothesized to be attributed to a strengthened and constitutive induced defence mechanisms, priming of plants to growth promotion (Pappas *et al*., 2018) and volatile production which are lethal to the insect herbivores (Ballhorn, *et al*., 2013).

Some studies have noted that not all endophyte-plant associations can protect plants against insect herbivores. In some cases, the presence of the endophyte makes the plant more susceptible to pests as seen with endomycorrhiza (Mueller *et al*., 2005). Our results are consistent with those of Clifton *et al*., 2018 while using endophytic *Metarhizium brunneum* following inoculation in bean plants. The experiment resulted in an overall abundance of *Aphis glycine* populations as compared to control plants without the endophyte. The researchers argued that the endophyte may have potentially reduced the efficacy of the general plant defense, or improved the host plant quality in favour of the insect, or the plants may have recognized the endophyte as a pathogen, responding to the pathogen defense salicylic acid mediated pathway and the subsequent crosstalk between salicylic acid and Jasmonic acid pathway, thereby becoming less capable of defense against aphid attack stress. Similarly, (Khaitov *et al*., 2015) noted increased two-spotted spider mite (*Tetranycus urticae*) reproduction and increased growth in reproductive plant tissues in *Glomus mosseae* inoculated common bean (*Phaseolus vulgaris*) plants as compared to control. In this case, it was hypothesized that since the fungi enhanced the nutritional value of the plant, the increase in mite population was because of a trade-off between the positive effects due to improved quality and quantity of host plant, and the negative effects due to induced resistance in the plant-microbe interaction.

In our earlier experiment, the 4 endophytes indicated induction of resistance to salinity stress while increasing chlorophyll content and biomass, enhanced seed germination and decreased hydrogen peroxide content in tomato seedlings. These results indicate the potential effect of the endophytes in priming tomato plants to induced systemic resistance, which could be effective both on biotic and abiotic stress. It is therefore concluded that, the positive effects noted on the two-spotted spider mites (*T. urticae*) on their reproduction and mortality could be because of the net effect of induced stress resistance and growth enhancement resulting from the endophyte-plant interaction. It is worth noting that in our experiment, all plants were grown on sterilized soil and therefore the effect of interaction of the endophytic plant with other soil microbiota in a natural experiment were not evaluated. Further experiments are therefore recommend to establish the impacts of the endophytes on tomato plants in the presence of general soil microbiota, high throughput genomic analysis to understand the upstream and downstream pathways involved in the endophyte functions and any volatile compounds that may be produced as a result of the plant-endophyte interaction.

6.5 Conclusion

Our results reveal successful endophyte establishment of the tested fungi and the ability of tomato plants to sustain the endophytic colonization up to 28 days post inoculation. This is an important characteristic that makes them potential candidates for use in agricultural systems, coupled with their potency to help tomato seedlings resistant to insect herbivory. However, additional studies would be required to understand the mode of action in inducing stress tolerance. In addition, it would be important to understand how they would perform in a mixed inoculation with insect pathogenic microorganisms, and therefore further studies are required.

CHAPTER SEVEN

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

7.1 Discussions

The main goal of this study was to isolate endophytic fungi and bacteria from shrubs growing along the edges of Lake Magadi and Lake Bogoria and their assessment for potential induction of biotic and abiotic stress tolerance in crop plants. Both Lakes Magadi and Bogoria are saline alkaline lakes along the East African Rift system. Saline alkaline lakes are found in arid and semi-arid regions of the world and are the most productive ecosystems on earth. Their origin and geochemistry are linked to tectonic forces and volcanic bedrocks and have been referred to as sentinels of climate change (Schagerl & Renaut 2016). The waters of the lakes are highly saline and alkaline, resulting from the weathering of lake surrounding volcanic rocks and climatic conditions, coupled with their endorheic nature. Although Grant & Jones (2016) argued that the diversity of Bacteria and Archaea is extremely high in these lakes, and a low diversity for other groups because of the extreme conditions, a study by Luo (2013) revealed a much larger hidden diversity. The microbial community found in these ecosystems are highly and specially adapted at the molecular level through the synthesis and accumulation of high concentrations of osmoprotectants without impending metabolism (Schagerl & Renaut 2016). Plants and shrubs growing in the drawdown and littoral zones of soda lakes harbour a large diversity of halotolerant, alkalitolerant and growth promoting endophytic and rhizosphere microorganisms to help them survive in these harsh environments (Luo *et al*., 2021). The ecosystem around soda lakes are exposed to periodic changes in salinity and alkalinity resulting from changes in rain and dry season. The rhizosphere microorganisms shift in relation to changes in edaphic factors and their adaptation is more or less determined by the type of dominant anion (Felföldi, 2020). The rhizosphere is the main source of plant endophytic microbiota, and are often recruited by the plant in a well-structured and selective process for the benefit of the plant.

In this study, bacterial and fungal endophytes isolated from shrubs growing along the drawdown zones of Lake Bogoria and Lake Magadi were characterized using both

phenotypic and 16S rDNA for bacterial isolates and the ITS region of the fungal isolates. This resulted in bacterial isolates in three phyla; Firmicutes, Proteobacteria and Actinobacteria and two fungal phyla of Basidiomycetes and Ascomycetes. Firmicutes and Ascomycetes dominated the isolation for bacteria and fungi respectfully. The isolates were able to grow in NaCl concentrations as high as 1.5M and produced various extracellular metabolites.

This study also involved the screening of the endophytic bacteria and fungi on plant growth promoting activities including phosphorus solubilization and Indole acetic acid production. Phosphorus is a major nutrient required by plants and takes up to about 0.2% of the plants dry weight, and is second to nitrogen among the minerals that greatly limit plant growth, development and production (Alori *et al*., 2017). The development of highly concentrated phosphorus fertilizers marked a revolution in agriculture, providing synthetic phosphorus to farmers. Availability of phosphorus to plants is also affected by the soil conditions including salinity and PH, which aggravate its reaction with other soil substances to form insoluble phosphate which is unavailable to plants. Several of the tested isolates solubilized inorganic phosphorus and were able in enhance the growth of tomato and bean plants. The advent of the use of rhizobia that fix atmospheric nitrogen and make it available for plants and the advances in isolation and screening of phosphate solubilizing, other nitrogen fixing and phytohormone producing microorganisms that enhance plant growth, coupled with their successful establishment in plant tissues as endophytes and expression of these functions *inplanta* has become a great milestone in plant fertilization processes. Currently, efforts are being put in place to engineer crop plants that are self-fertilizing using nitrogen fixing and phosphorus solubilizing microorganisms with the aim of providing sustainable alternatives to application of artificial fertilizers (Haskett *et al*., 2022). Selective isolation using nitrogen free media and screening using insoluble phosphate have also been employed. Although studies on genes responsible for phosphate solubilization is still limited to the functional validation and the metabolic mechanisms of the upstream and downstream is still relatively limited (Pan & Cai, 2023), recent advances in synthetic biology make it a promising cause, amidst the challenges (Rogers & Oldroyd, 2014).

Microbial colonization of plants as endophytes in nature follows a close association that is facilitated by several factors. Artificial inoculation methods have been used to attain successful endophytic colonization in non-host plants, and the distribution of the endophyte in the different plant tissues, though at different rates for the various plant tissues (Saragih *et al*., 2019). The variations in colonization rates is sometimes tissue specific and depends on the adaptation of the endophyte to particular conditions in the plant part (Jaber & Enkerli, 2016). Colonization of the different plant parts by artificially inoculated microorganisms was also noted in this study. The distribution of the endophyte across the plant is hypothesized to be an adaptation mechanism to enhance efficiency in performance.

7.2 Conclusion

Continuous crop cultivation, including monocropping are a known cause of loss of important endophytic microbial diversity associated with crop plants. These practices, including the intensified use of chemical fertilizers and pesticides have an effect of microbial diversity in agricultural farmlands which also impact on crop plant microbial associations. These impacts have been projected to be a source of low productivity and an urgent solution is required for an ecologically stable and sustainable agriculture. Therefore, increased use of microbial solutions in farming practices are required to enhance soil health and increase productivity.

Studies in microbial communities of soda lakes were not explored until the 1980s, when systematic search took root in Kenyan soda lakes, but have since remained more confined to bacteria, archaea and phytoplanktons from lake waters and sediments and their potential economic importance, especially industrial applications. The current study focused on endophytic microorganisms from the shrubs growing along the lake shores of Lakes Bogoria and Magadi, and has revealed a new source of microbial populations from Kenyan soda lakes with potential applications in salinity stress tolerance and induction of resistance to fungal diseases of beans, a different industry from the traditionally known applications in industrial enzymes. The isolates solubilized insoluble phosphate, grew well at high NaCl concentrations of up to 1.5M and produced indole acetic acid, this screening procedures would be important for

adoption in screening for useful endophytes in agriculture.

Screening for microbial inhibitions of pathogens majorly relies on dual culture assays and metabolite extracts. The results of the current study reveal an additional screening method of spore-cell co-culturing method that can be adopted for quick identification of potential biocontrol isolates. In this study, all isolates established endophytic associations with beans and tomato seedlings, and were able to enhance stress tolerance as endophytes. Seed treatment method of microbial application can be adopted for mitigation of the challenges in formulations of biocontrol organisms, especially for enhancement of seed germination in saline soils and control of soil borne pathogens.

7.3 Recommendations

1. Kenya is endowed with several soda lakes and therefore the opportunities exist to undertake microbial endophytic studies. This would help in building a living library of microorganisms, and continue to enrich the global genebank with Kenyas microbial datasets. These living library of endophytic can be utilized in the various fields of agriculture, including as biofertilizers, biopesticides and salinity management as observed in this study,, while responding to the global call on sustainable agriculture and reductions in the use of chemical farm inputs to more organic farming practices.
2. Previous studies on soda lakes microorganisms have focused on isolation of bacteria, archaea, algae and to a little extend on bacteriophages. Isolation of endophytic microorganisms from shrubs growing along the shores of these lakes would provide a living library of previously unknown soda lakes microbial functions.
3. This work has shown that most microorganisms attain functional roles and characteristics based on habitat adaptation, more dedicated, all year-round studies are necessary that focus on isolation and screening of endophytic microorganisms from plants and shrub species growing along the littoral and drawdown zones of Kenyan soda lakes.

4. Use of microbial agents in biocontrol and as biofertilizers has focused majorly on field applications after crop establishment. This study reveals an additional method of microbial applications as endophytes that can be used to enhance seed germination in saline soils as noted with isolates F04, F05, F18 and F21 and soils already infested with pathogens as shown with isolates B19 and B53 in this study.
5. Endophytic microorganisms contain both human pathogenic and non-pathogenic microorganisms. A number of human pathogenic microorganisms have been isolated as endophytes with great potentials for use in agricultural systems as shown in this study with isolate B19. Although some studies have revealed that some human pathogenic species isolated as endophytes do not contain human pathogenic genes, it is necessary to undertake further genetic characterization studies including whole genome sequences of the isolates to understand the species further.
6. The soda lakes of East Africa are unique and fragile ecosystems, threatened by anthropogenic and climatic changes and could potentially lead to a great shift or loss in plant and shrub populations, and therefore they require protection. Data on agriculturally and industrially useful microorganisms from these soda lakes would help policy makers and conservation managers in enhancing the balance required in the conservation of the soda lakes.

REFERENCES

Abd-Allah, E. F., Alqarawi, A. A., Hashem, A., Radhakrishnan, R., Al-Huqail, A. A., Al-Otibi, F. O. N., Malik, J. A., Alharbi, R. I., & Egamberdieva, D. (2018). Endophytic bacterium Bacillus subtilis (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *Journal of Plant Interactions*, *13*(1), 37–44. https://doi.org/10.1080/17429145.2017.1414321

Abdalla, O., Mohamad, A., Liu, Y., Zhang, D., Hua, S., Bhute, S., Hedlund, B. P., Li, W., & Li, L. (2020). *Bene fi cial Endophytic Bacterial Populations Associated With Medicinal Plant Thymus vulgaris Alleviate Salt Stress and Confer Resistance to Fusarium oxysporum*. *11*(February), 1–17. https://doi.org/10.3389/fpls.2020.00047

Abdelfattah, A., Wisniewski, M., Schena, L., & Tack, A. J. M. (2021). Experimental evidence of microbial inheritance in plants and transmission routes from seed to phyllosphere and root. *Environmental Microbiology*, *23*(4), 2199–2214. https://doi.org/10.1111/1462-2920.15392

Abdelshafy Mohamad, O. A., Ma, J. B., Liu, Y. H., Zhang, D., Hua, S., Bhute, S., Hedlund, B. P., Li, W. J., & Li, L. (2020). Beneficial Endophytic Bacterial Populations Associated With Medicinal Plant Thymus vulgaris Alleviate Salt Stress and Confer Resistance to Fusarium oxysporum. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00047

Adeleke, B. S., Ayilara, M. S., Akinola, S. A., & Babalola, O. O. (2022). Biocontrol mechanisms of endophytic fungi. In *Egyptian Journal of Biological Pest Control* (Vol. 32, Issue 1). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1186/s41938-022-00547-1

Akutse, K. S., Maniania, N. K., Fiaboe, K. K. M., & Berg, J. Van Den. (2017). *Endophytic colonization of Vicia faba and Phaseolus vulgaris (Fabaceae) by fungal pathogens and their effects on the life-history parameters of Liriomyza*

huidobrensis (Diptera : Agr ... August 2013. https://doi.org/10.1016/j.funeco.2013.01.003

Akutse, K. S., Maniania, N. K., Fiaboe, K. K. M., Van den Berg, J., & Ekesi, S. (2013). Endophytic colonization of Vicia faba and Phaseolus vulgaris (Fabaceae) by fungal pathogens and their effects on the life-history parameters of Liriomyza huidobrensis (Diptera: Agromyzidae). *Fungal Ecology*, *6*(4), 293–301. https://doi.org/10.1016/j.funeco.2013.01.003

Ali, A., Shahzad, R., Khan, A. L., Halo, B. A., Al-Yahyai, R., Al-Harrasi, A., Al-Rawahi, A., & Lee, I. J. (2017). Endophytic bacterial diversity of avicennia marina helps to confer resistance against salinity stress in solanum lycopersicum. *Journal of Plant Interactions*, *12*(1), 312–322. https://doi.org/10.1080/17429145.2017.1362051

Ali, R., Gul, H., Rauf, M., Arif, M., Hamayun, M., Husna, Khilji, S. A., Ud-Din, A., Sajid, Z. A., & Lee, I. J. (2022). Growth-Promoting Endophytic Fungus (Stemphylium lycopersici) Ameliorates Salt Stress Tolerance in Maize by Balancing Ionic and Metabolic Status. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.890565

Al-Johani, N. B., Al-Seeni, M. N., & Ahmed, Y. M. (2017). Optimization of alkaline α-amylase production by thermophilic *Bacillus subtilis*. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, *14*(1), 288–301. https://doi.org/10.21010/ajtcam.v14i1.31

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. In *Frontiers in Microbiology* (Vol. 8, Issue JUN). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2017.00971

Andreote, F. D., Gumiere, T., & Durrer, A. (2014a). Exploring interactions of plant microbiomes. *Scientia Agricola*, *71*(6), 528–539. https://doi.org/10.1590/0103-9016-2014-0195

Andreote, F. D., Gumiere, T., & Durrer, A. (2014b). Exploring interactions of plant microbiomes. *Scientia Agricola*, *71*(6), 528–539. https://doi.org/10.1590/0103-9016-2014-0195

Angus, A. A., Agapakis, C. M., Fong, S., Yerrapragada, S., Estrada-de Los Santos, P., Yang, P., Song, N., Kano, S., Caballero-Mellado, J., De Faria, S. M., Dakora, F. D., Weinstock, G., & Hirsch, A. M. (2014). Plant-associated symbiotic Burkholderia species lack hallmark strategies required in mammalian pathogenesis. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0083779

Aragón, I. M., Pérez-Martínez, I., Moreno-Pérez, A., Cerezo, M., & Ramos, C. (2014). New insights into the role of indole-3-acetic acid in the virulence of Pseudomonas savastanoi pv. savastanoi. *FEMS Microbiology Letters*, *356*(2), 184–192. https://doi.org/10.1111/1574-6968.12413

Asaf, S., Khan, M. A., Khan, A. L., Waqas, M., Shahzad, R., Kim, A. Y., Kang, S. M., & Lee, I. J. (2017). Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants: An example of Sphingomonas sp. and Serratia marcescens. *Journal of Plant Interactions*, *12*(1), 31–38. https://doi.org/10.1080/17429145.2016.1274060

Bailly C., Bousquet A., Braun V., Buitink J., Desbois-vimont C., Durand-Tardif M., Fougereux J., Gaillard A., Gouleau A., Grappin P., Hily Y., Lejard F., Malausa T., Marchi M., Multeau C., Orgeur G., Piovan R., Poisson A. -s., Profiz C., Rajjou L., R. X. and S. M. (2020). Towards seed protection using biocontrol strategies. *Biocontrole*, *Position p*(June), 1–12. papers3://publication/uuid/7C65BDC6-6DB5-434A-8424-57F5743D85C6

Balliu, A., Sallaku, G., & Rewald, B. (2015). AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability*. 15967–15981. https://doi.org/10.3390/su71215799

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., Afolabi, O. G., Hussain, M., Qasim, M., & Wang, L. (2018a). Prospects of endophytic fungal

entomopathogens as biocontrol and plant growth promoting agents: An insight on how artificial inoculation methods affect endophytic colonization of host plants. In *Microbiological Research* (Vol. 217, pp. 34–50). Elsevier GmbH. https://doi.org/10.1016/j.micres.2018.08.016

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., Afolabi, O. G., Hussain, M., Qasim, M., & Wang, L. (2018b). Prospects of endophytic fungal entomopathogens as biocontrol and plant growth promoting agents: An insight on how artificial inoculation methods affect endophytic colonization of host plants. In *Microbiological Research* (Vol. 217, pp. 34–50). Elsevier GmbH. https://doi.org/10.1016/j.micres.2018.08.016

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., & Wang, L. (2018). Fungal endophytes: Beyond herbivore management. In *Frontiers in Microbiology* (Vol. 9, Issue MAR). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2018.00544

Bamisile, B. S., Dash, C. K., Akutse, K. S., Qasim, M., Aguila, L. C. R., Wang, F., Keppanan, R., & Wang, L. (2019). Endophytic *Beauveria bassiana* in foliar-treated citrus limon plants acting as a growth suppressor to three successive generations of diaphorina citri kuwayama (Hemiptera: Liviidae). *Insects*, *10*(6). https://doi.org/10.3390/insects10060176

Bandel, K., Salameh, E. M., Al-Gharaibeh, M., Alhejoj, I., Salameh, E., & Ibbini, J. (2022). Plants of Saline Coastal and Inland Sabkha Areas as Indicators of Environmental Conditions in Southern Jordan Article. In *Jordan Journal of Earth and Environmental Sciences*.Retrieved from https://www.researchgate.net/publication/363157268

Behie, S. W., & Bidochka, M. J. (2014). Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: An additional branch of the soil nitrogen cycle. *Applied and Environmental Microbiology*, *80*(5), 1553–1560. https://doi.org/10.1128/AEM.03338-13

Bendaha, M. E. A., & Belaouni, H. A. (2019). Tomato growth and resistance promotion by Enterobacter hormaechei subsp. steigerwaltii EB8D. *Archives of Phytopathology and Plant Protection*, *52*(3–4), 318–332. https://doi.org/10.1080/03235408.2019.1620511

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). Unraveling the plant microbiome: Looking back and future perspectives. *Frontiers in Microbiology*, *5*(JUN), 1–7. https://doi.org/10.3389/fmicb.2014.00148

Berrabah, F., Ratet, P., & Gourion, B. (2019). Legume nodules: Massive infection in the absence of defense induction. In *Molecular Plant-Microbe Interactions* (Vol. 32, Issue 1, pp. 35–44). American Phytopathological Society. https://doi.org/10.1094/MPMI-07-18-0205-FI

Bertini, L., Perazzolli, M., Proietti, S., Capaldi, G., Savatin, D. V., Bigini, V., Longa, C. M. O., Basaglia, M., Favaro, L., Casella, S., Fongaro, B., Polverino de Laureto, P., & Caruso, C. (2022). Biodiversity and Bioprospecting of Fungal Endophytes from the Antarctic Plant Colobanthus quitensis. *Journal of Fungi*, *8*(9). https://doi.org/10.3390/jof8090979

Bertrand, S., Schumpp, O., Bohni, N., Monod, M., Gindro, K., & Wolfender, J. L. (2013). De novo production of metabolites by fungal co-culture of trichophyton rubrum and bionectria ochroleuca. *Journal of Natural Products*, *76*(6), 1157–1165. https://doi.org/10.1021/np400258f

Besset-Manzoni, Y., Joly, P., Brutel, A., Gerin, F., Soudière, O., Langin, T., & Prigent-Combaret, C. (2019). Does in vitro selection of biocontrol agents guarantee success in planta? A study case of wheat protection against Fusarium seedling blight by soil bacteria. *PLoS ONE*, *14*(12), 1–18. https://doi.org/10.1371/journal.pone.0225655

Bhagyasree, S. N., Suroshe, S. S., & Kalia, V. K. (2020). Effect of endophytic colonisation of entomopathogenic *Beauveria bassiana* (Balsamo) *Tetranychus*

urticae. *Indian Journal of Entomology*, *82*(4), 716–719. https://doi.org/10.5958/0974-8172.2020.00163.7

Bilal, L., Asaf, S., Hamayun, M., Gul, H., Iqbal, A., Ullah, I., Lee, I. J., & Hussain, A. (2018). Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. *Symbiosis*, *76*(2), 117–127. https://doi.org/10.1007/s13199-018-0545-4

Bononi, L., Chiaramonte, J. B., Pansa, C. C., Moitinho, M. A., & Melo, I. S. (2020). Phosphorus-solubilizing Trichoderma spp. from Amazon soils improve soybean plant growth. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-59793-8

Botelho, S., Luis, W., Zancan, A., Machado, C., & Barrocas, E. N. (2013). Performance of common bean seeds infected by pelo fungo *Sclerotinia sclerotiorum*. *Journal of Seed Science*, *35*(2), 153–160.

Bouzouina, M., Kouadria, R., & Lotmani, B. (2021). Fungal endophytes alleviate salt stress in wheat in terms of growth, ion homeostasis and osmoregulation. *Journal of Applied Microbiology*, *130*(3), 913–925. https://doi.org/10.1111/jam.14804

Brink, B. (2016). Urease Test Protocol. *November 2010*, 1–7.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., & Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91–95. https://doi.org/10.1038/nature11336

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Canassa, F., Tall, S., Moral, R. A., Lara, I. A. R. de, Delalibera, I., & Meyling, N. V. (2019). Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite populations and behavior of predatory mites. *Biological Control*, *132*, 199–208. https://doi.org/10.1016/j.biocontrol.2019.02.003

Card, S., Johnson, L., Teasdale, S., & Caradus, J. (2016). *Deciphering endophyte behaviour : the link between endophyte biology and efficacious biological control agents*. *February*, 1–19. https://doi.org/10.1093/femsec/fiw114

Carrasco, M., Villarreal, P., Barahona, S., Alcaíno, J., Cifuentes, V., & Baeza, M. (2016). Screening and characterization of amylase and cellulase activities in psychrotolerant yeasts. *BMC Microbiology*, *16*(1). https://doi.org/10.1186/s12866-016-0640-8

Carro, L., & Menéndez, E. (2020). Knock, knock-let the bacteria in: enzymatic potential of plant associated bacteria. In *Molecular Aspects of Plant Beneficial Microbes in Agriculture* (pp. 169–178). Elsevier. https://doi.org/10.1016/B978-0-12-818469-1.00014-6

Castrillo, G., Teixeira, P. J. P. L., Paredes, S. H., Law, T. F., De Lorenzo, L., Feltcher, M. E., Finkel, O. M., Breakfield, N. W., Mieczkowski, P., Jones, C. D., Paz-Ares, J., & Dangl, J. L. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature*, *543*(7646), 513–518. https://doi.org/10.1038/nature21417

Castro-Camba, R., Sánchez, C., Vidal, N., & Vielba, J. M. (2022a). Interactions of Gibberellins with Phytohormones and Their Role in Stress Responses. In *Horticulturae* (Vol. 8, Issue 3). MDPI. https://doi.org/10.3390/horticulturae8030241

Castro-Camba, R., Sánchez, C., Vidal, N., & Vielba, J. M. (2022b). Plant Development and Crop Yield: The Role of Gibberellins. In *Plants* (Vol. 11, Issue 19). MDPI. https://doi.org/10.3390/plants11192650

Castro, R. A., Quecine, M. C., Lacava, P. T., Batista, B. D., Luvizotto, D. M., Marcon, J., Ferreira, A., Melo, I. S., & Azevedo, J. L. (2014). *Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem*. 1–9.

Chanclud, E., & Morel, J. B. (2016). Plant hormones: a fungal point of view. In *Molecular plant pathology* (Vol. 17, Issue 8, pp. 1289–1297). Blackwell Publishing Ltd. https://doi.org/10.1111/mpp.12393

Chaudhary, P., Agri, U., Chaudhary, A., Kumar, A., & Kumar, G. (2022). Endophytes and their potential in biotic stress management and crop production. In *Frontiers in Microbiology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2022.933017

Cheeseman, J. M. (2015). The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist*, *206*(2), 557–570. https://doi.org/10.1111/nph.13217

Chen, F., Gao, Y., Chen, X., Yu, Z., & Li, X. (2013). Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. In *International Journal of Molecular Sciences* (Vol. 14, Issue 9, pp. 17477–17500). https://doi.org/10.3390/ijms140917477

Chen, G., Escobar-Bravo, R., Kim, H. K., Leiss, K. A., & Klinkhamer, P. G. L. (2018). Induced resistance against western flower thrips by the pseudomonas syringae-derived defense elicitors in tomato. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01417

Cheng, Y. T., Zhang, L., & He, S. Y. (2019). Plant-Microbe Interactions Facing Environmental Challenge. In *Cell Host and Microbe* (Vol. 26, Issue 2, pp. 183–192). Cell Press. https://doi.org/10.1016/j.chom.2019.07.009

Chen, J., Zhao, G., Wei, Y., Dong, Y., Hou, L., & Jiao, R. (2021). Isolation and screening of multifunctional phosphate solubilizing bacteria and its growth-

promoting effect on Chinese fir seedlings. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-88635-4

Chernin, L., & Glick, B. R. (2012). The Use of ACC Deaminase to Increase the Tolerance of Plants to Various Phytopathogens. D.K. Maheshwari (ed.), Bacteria in Agrobiology: Stress Management,

DOI 10.1007/978-3-642-23465-1_14, Springer-Verlag Berlin Heidelberg 2012 279–299. https://doi.org/10.1007/978-3-642-23465-1

Chitnis, V. R., Suryanarayanan, T. S., Nataraja, K. N., Prasad, S. R., Oelmüller, R., & Shaanker, R. U. (2020). Fungal Endophyte-Mediated Crop Improvement: The Way Ahead. In *Frontiers in Plant Science* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fpls.2020.561007

Clifton, E. H., Jaronski, S. T., Coates, B. S., Hodgson, E. W., & Gassmann, A. J. (2018). Effects of endophytic entomopathogenic fungi on soybean aphid and identification of Metarhizium isolates from agricultural fields. *PLoS ONE*, *13*(3). https://doi.org/10.1371/journal.pone.0194815

Conner, R. L., Hou, A., Balasubramanian, P., McLaren, D. L., Henriquez, M. A., Chang, K. F., & McRae, K. B. (2014). Reaction of dry bean cultivars grown in western Canada to root rot inoculation. *Canadian Journal of Plant Science*, *94*(7), 1219–1230. https://doi.org/10.4141/CJPS2013-416

Cooke, D. E. L., Cano, L. M., Raffaele, S., Bain, R. A., Cooke, L. R., Etherington, G. J., Deahl, K. L., Farrer, R. A., Gilroy, E. M., Goss, E. M., Grünwald, N. J., Hein, I., MacLean, D., McNicol, J. W., Randall, E., Oliva, R. F., Pel, M. A., Shaw, D. S., Squires, J. N., … Kamoun, S. (2012). Genome Analyses of an Aggressive and Invasive Lineage of the Irish Potato Famine Pathogen. *PLoS Pathogens*, *8*(10). https://doi.org/10.1371/journal.ppat.1002940

Costa, L. E. D. O., de Queiroz, M. V., Borges, A. C., de Moraes, C. A., & de Araújo, E. F. (2012). Isolation and Characterization of Endophytic Bacteria isolated from the leaves of *Brazilian Journal of Microbiology*, *43*(4), 1562–1575.

Dahmani, M. A., Desrut, A., Moumen, B., Verdon, J., Mermouri, L., Kacem, M., Coutos-Thévenot, P., Kaid-Harche, M., Bergès, T., & Vriet, C. (2020a). Unearthing the Plant Growth-Promoting Traits of *Bacillus megaterium* RmBm31, an endophytic Bacterium isolated from root rodules of *Retama monosperma*. *Frontiers in Plant Science*, *11*(February), 1–15. https://doi.org/10.3389/mfpls.2020.00124

Dahmen, H., Staub, T., & Schwinn, F. J. (1983). Technique for Long-Term Preservation of Phytopathogenic Fungi in Liquid Nitrogen. *Phytopathology*. DOI:10.1094/PHYTO-73-241

Daniels A., Maharaj, G., Ram, M., Lakenarine, R. (2023). Biological Control Methods for Agricultural Mites: A Review. Agricultural Reviews. *44*(1): 12-21. doi: 10.18805/ag. RF-247

Daungfu, O., Youpensuk, S., & Lumyong, S. (2019). Endophytic bacteria isolated from citrus plants for biological control of citrus canker in lime plants. *Tropical Life Sciences Research*, *30*(1), 73–88. https://doi.org/10.21315/tlsr2019.30.1.5

Debbab, A., Aly, A. H., & Proksch, P. (2013). Mangrove derived fungal endophytes - A chemical and biological perception. In *Fungal Diversity*. https://doi.org/10.1007/s13225-013-0243-8

Debnath, S., Chakraborty, S., Langthasa, M., Choure, K., Agnihotri, V., Srivastava, A., Rai, P. K., Tilwari, A., Maheshwari, D. K., & Pandey, P. (2023). Non-rhizobial nodule endophytes improve nodulation, change root exudation pattern and promote the growth of lentil, for prospective application in fallow soil. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1152875

De Bruyne, L., Höfte, M., & De Vleesschauwer, D. (2014). Connecting growth and defense: The emerging roles of brassinosteroids and gibberellins in plant innate immunity. In *Molecular Plant* (Vol. 7, Issue 6, pp. 943–959). Oxford University Press. https://doi.org/10.1093/mp/ssu050

Dehnavi, A. R., Zahedi, M., Ludwiczak, A., Perez, S. C., & Piernik, A. (2020). Effect of salinity on seed germination and seedling development of sorghum (Sorghum bicolor (L.) Moench) genotypes. *Agronomy*, *10*(6). https://doi.org/10.3390/agronomy10060859

Deocampo, D. M., Owen, R. B., Lowenstein, T. K., Renaut, R. W., Rabideaux, N. M., Billingsley, A., Cohen, A., Deino, A. L., Sier, M. J., Luo, S., Shen, C. C., Gebregiorgis, D., Campisano, C., & Mbuthia, A. (2022). Orbital control of Pleistocene euxinia in Lake Magadi, Kenya. *Geology*, *50*(1), 42–47. https://doi.org/10.1130/G49140.1

Devi, K. A., Pandey, G., Rawat, A. K. S., Sharma, G. D., & Pandey, P. (2017). The endophytic symbiont-*Pseudomonas aeruginosa* stimulates the antioxidant activity and growth of Achyranthes aspera L. *Frontiers in Microbiology*, *8*(SEP). https://doi.org/10.3389/fmicb.2017.01897

Dey, G., Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., Huang, Y. H., Huang, H. Bin, & Chen, C. Y. (2021). Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—a review. In *Agronomy* (Vol. 11, Issue 8). MDPI AG. https://doi.org/10.3390/agronomy11081552

Dhole, A. (2018). Non-rhizobial endophytes in root nodules. *MOJ Biology and Medicine*, *3*(1). https://doi.org/10.15406/mojbm.2018.03.00064

Díaz-Valle, A., López-Calleja, A. C., & Alvarez-Venegas, R. (2019). Enhancement of Pathogen Resistance in common bean plants by inoculation with Rhizobium etli. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01317

Dodd I. C., and Pe´rez-Alfocea F., (2012). Microbial amelioration of crop salinity stress Journal of Experimental Botany, Vol. 63, No. 9, pp. 3415–3428, doi:10.1093/jxb/ers033

Dong, J., Gruda, N., Lam, S. K., Li, X., & Duan, Z. (2018). Effects of elevated CO2 on nutritional quality of vegetables: A review. In *Frontiers in Plant Science* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fpls.2018.00924

Ducousso-Détrez, A., Fontaine, J., Sahraoui, A. L. H., & Hijri, M. (2022). Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes. In *Microorganisms* (Vol. 10, Issue 3). MDPI. https://doi.org/10.3390/microorganisms10030609

Eaton, C. J., Jourdain, I., Foster, S. J., Hyams, J. S., & Scott, B. (2008). Functional analysis of a fungal endophyte stress-activated MAP kinase. *Current Genetics*, *53*(3), 163–174. https://doi.org/10.1007/s00294-007-0174-6

Eberl, L., & Vandamme, P. (2016). Members of the genus Burkholderia: Good and bad guys [version 1; referees: 3 approved]. *F1000Research*, *5*(May). https://doi.org/10.12688/F1000RESEARCH.8221.1

Ebi, K. L., & Ziska, L. H. (2018). Increases in atmospheric carbon dioxide: Anticipated negative effects on food quality. *PLoS Medicine*, *15*(7). https://doi.org/10.1371/journal.pmed.1002600

Ebrahim, M. K. H., & Saleem, A. R. (2017). Alleviating salt stress in tomato inoculated with mycorrhizae: Photosynthetic performance and enzymatic antioxidants. *Journal of Taibah University for Science*, *11*(6), 850–860. https://doi.org/10.1016/j.jtusci.2017.02.002

Egamberdieva, D. (2009). *Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat*. 861–864. https://doi.org/10.1007/s11738-009-0297-0

Egamberdieva, D., Davranov, K., Wirth, S., Hashem, A., & Abd_Allah, E. F. (2017). Impact of soil salinity on the plant-growth – promoting and biological control abilities of root associated bacteria. *Saudi Journal of Biological Sciences*, *24*(7), 1601–1608. https://doi.org/10.1016/j.sjbs.2017.07.004

Ejaz, M. R., Jaoua, S., Ahmadi, M., & Shabani, F. (2023). An examination of how climate change could affect the future spread of Fusarium spp. around the world, using correlative models to model the changes. *Environmental Technology and Innovation*, *31*. https://doi.org/10.1016/j.eti.2023.103177

Elhakim, E., Mohamed, O., & Elazouni, I. (2020). Virulence and proteolytic activity of entomopathogenic fungi against the two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae). *Egyptian Journal of Biological Pest Control*, *30*(1). https://doi.org/10.1186/s41938-020-00227-y

El-Hawary, S. S., Moawad, A. S., Bahr, H. S., Abdelmohsen, U. R., & Mohammed, R. (2020). Natural product diversity from the endophytic fungi of the genusAspergillus. *RSC Advances*, *10*(37), 22058–22079. https://doi.org/10.1039/d0ra04290k

El-rahman, A., & Shaheen, H. A. (2016). *Biological Control of the Brown Rot of Potato , Ralstonia solanacearum and Effect of Bacterization with Antagonists on Promotion of Potato Growth*. *26*(4), 733–739.

Emamverdian, A., Ding, Y., & Mokhberdoran, F. (2020). The role of salicylic acid and gibberellin signaling in plant responses to abiotic stress with an emphasis on heavy metals. In *Plant Signaling and Behavior* (Vol. 15, Issue 7). Taylor and Francis Inc. https://doi.org/10.1080/15592324.2020.1777372

Eo, J. K., & Eom, A. H. (2022). Community of Endophytic Fungi from Alpine Conifers on Mt. Seorak. *Mycobiology*, *50*(5), 317–325. https://doi.org/10.1080/12298093.2022.2135832

Etesami, H., Alikhani, H. A., & Hosseini, H. M. (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX*, *2*, 72–78. https://doi.org/10.1016/j.mex.2015.02.008

Etesami, H., & Beattie, G. A. (2018). Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology*, *9*(FEB). https://doi.org/10.3389/fmicb.2018.00148

Etminani, F., & Harighi, B. (2018). *Isolation and Identification of Endophytic Bacteria with Plant Growth Promoting Activity and Biocontrol Potential from Wild Pistachio Trees*. *34*(3), 208–217.

Fadiji, A. E., & Babalola, O. O. (2020). Elucidating Mechanisms of Endophytes Used in Plant Protection and Other Bioactivities with Multifunctional Prospects. In *Frontiers in Bioengineering and Biotechnology* (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2020.00467

Felföldi, T. (2020). Microbial communities of soda lakes and pans in the Carpathian Basin: a review. In *Biologia Futura* (Vol. 71, Issue 4, pp. 393–404). Akademiai Kiado Rt. https://doi.org/10.1007/s42977-020-00034-4

Ferreira, A., Quecine, M. C., Lacava, P. T., Oda, S., Azevedo, J. L., & Araújo, W. L. (2008). Diversity of endophytic bacteria from Eucalyptus species seeds and colonization of seedlings by Pantoea agglomerans. *FEMS Microbiology Letters*, *287*(1), 8–14. https://doi.org/10.1111/j.1574-6968.2008.01258.x

Fouda, A. H., Hassan, S. E.-D., Eid, A. M., & Ewais, E. E.-D. (2015). Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). *Annals of Agricultural Sciences*, *60*(1), 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Gaind, S. (2016). Phosphate dissolving fungi: Mechanism and application in alleviation of salt stress in wheat. *Microbiological Research*, *193*, 94–102. https://doi.org/10.1016/j.micres.2016.09.005

Geoffrey, S. K., Hillary, N. K., Antony, K. M., Mariam, M., & Mary, M. C. (2014). Challenges and Strategies to Improve Tomato Competitiveness along the Tomato Value Chain in Kenya. *International Journal of Business and Management*, *9*(9), 205–212. https://doi.org/10.5539/ijbm.v9n9p205

Goss, E. M., Tabima, J. F., Cooke, D. E. L., Restrepo, S., Frye, W. E., Forbes, G. A., Fieland, V. J., Cardenas, M., & Grünwald, N. J. (2014). The Irish potato famine pathogen Phytophthora infestans originated in central Mexico rather than the Andes. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(24), 8791–8796. https://doi.org/10.1073/pnas.1401884111

Goswami, D., Dhandhukia, P., Patel, P., & Thakker, J. N. (2014). Screening of PGPR from saline desert of Kutch : Growth promotion in Arachis hypogea by Bacillus licheniformis A2 ☪. *Microbiological Research*, *169*(1), 66–75. https://doi.org/ 10.1016/ j.micres.2013.07.004

Guo, K., Yang, J., Yu, N., Luo, L., & Wang, E. (2023). Biological nitrogen fixation in cereal crops: Progress, strategies, and perspectives. In *Plant Communications* (Vol. 4, Issue 2). Cell Press. https://doi.org/10.1016/j.xplc.2022.100499

Gupta, S., Schillaci, M., Walker, R., Smith, P. M. C., Watt, M., & Roessner, U. (2020). Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. *Plant and Soil*, *2050*. https://doi.org/10.1007/s11104-020-04618-w

Hamayun, M., Hussain, A., Khan, S. A., Kim, H. Y., Khan, A. L., Waqas, M., Irshad, M., Iqbal, A., Rehman, G., Jan, S., & Lee, I. J. (2017). Gibberellins producing endophytic fungus Porostereum spadiceum AGH786 rescues growth of salt affected soybean. *Frontiers in Microbiology*, *8*(APR). https://doi.org/10.3389/ fmicb.2017.00686

Hamzah, T. N. T., Lee, S. Y., Hidayat, A., Terhem, R., Faridah-Hanum, I., & Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, Rhizophora mucronata, and identification of potential antagonists against the soil-borne fungus, Fusarium solani. *Frontiers in Microbiology*, *9*(JUL). https://doi.org/10.3389/fmicb.2018.01707

Hardoim, P. R., Hardoim, C. C. P., van Overbeek, L. S., & van Elsas, J. D. (2012). Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0030438

Hardoim, P. R., Overbeek, L. S. van, Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3). https://doi.org/10.1128/MMBR.00050-14

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293–320. https://doi.org/10.1128/mmbr.00050-14

Harper, D. M., Childress, R. B., Harper, M. M., Boar, R. R., Hickley, P., Mills, S. C., Otieno, N., Drane, T., Vareschi, E., Nasirwa, O., Mwatha, W. E., Darlington, J. P. E. C., & Escuté-Gasulla, X. (2003). Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia*, *500*, 259–276. https://doi.org/10.1023/A:1024722821407

Haskett, T. L., Paramasivan, P., Mendes, M. D., Green, P., Geddes, B. A., Knights, H. E., Jorrin, B., Ryu, M.-H., Brett, P., Voigt, C. A., Oldroyd, G. E. D., Poole, P. S., Kondorosi, E., Performed, P. B., & Contributed, G. E. D. O. (2022). *Engineered plant control of associative nitrogen fixation*. https://doi.org/10.1073/pnas

Hassani, M. A., Durán, P., & Hacquard, S. (2018). Microbial interactions within the plant holobiont. In *Microbiome* (Vol. 6, Issue 1, p. 58). NLM (Medline). https://doi.org/10.1186/s40168-018-0445-0

Hedden, P., & Sponsel, V. (2015). A Century of Gibberellin Research. In *Journal of Plant Growth Regulation* (Vol. 34, Issue 4, pp. 740–760). Springer New York LLC. https://doi.org/10.1007/s00344-015-9546-1

Hossain, M. A., & Tran, L. P. (2015). *Hydrogen peroxide priming modulates abiotic oxidative stress tolerance : insights from ROS detoxification and scavenging*. *6*(June), 1–19. https://doi.org/10.3389/fpls.2015.00420

Hosseyni Moghaddam, M. S., Safaie, N., Rahimlou, S., & Hagh-Doust, N. (2022). Inducing Tolerance to Abiotic Stress in Hordeum vulgare L. by Halotolerant Endophytic Fungi Associated With Salt Lake Plants. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.906365

Hosseyni Moghaddam, M. S., Safaie, N., Soltani, J., & Hagh-Doust, N. (2021). Desert-adapted fungal endophytes induce salinity and drought stress resistance in model crops. *Plant Physiology and Biochemistry*, *160*, 225–238. https://doi.org/10.1016/j.plaphy.2021.01.022

Hummel, M., Hallahan, B. F., Brychkova, G., Ramirez-Villegas, J., Guwela, V., Chataika, B., Curley, E., McKeown, P. C., Morrison, L., Talsma, E. F., Beebe, S., Jarvis, A., Chirwa, R., & Spillane, C. (2018). Reduction in nutritional quality and growing area suitability of common bean under climate change induced drought stress in Africa. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-33952-4

H, S. V. (2012). Amylase Production by Endophytic fungi cylindrocephalum sp. isolated from medicinal plant alpinia calcarata (HAW.) ROSCOE. *Brazilian Journal of Microbiology*, 1213–1221.

Iizuka, T., Barre, A., Rougé, P., Charpin, D., Scala, E., Baudin, B., Aizawa, T., Sénéchal, H., & Poncet, P. (2022). Gibberellin-regulated proteins: Emergent allergens. *Frontiers in Allergy*, *3*. https://doi.org/10.3389/falgy.2022.877553

Islam, M.N., Ali, M.S., Choi, S.-J., Park, Y.-I., Baek, K. H (2020). Salicylic Acid-Producing Endophytic Bacteria Increase Nicotine Accumulation and Resistance against Wildfire Disease in Tobacco Plants. Microorganisms 8: 31. https://doi.org/10.3390/microorganisms8010031

Jaber, L. R. (2018a). *caused by Fusarium culmorum in wheat*. *November*. https://doi.org/10.1007/s00425-018-2991-x

Jaber, L. R. (2018b). Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by Fusarium culmorum in wheat. *Planta*, *248*(6). https://doi.org/10.1007/s00425-018-2991-x

Jaber, L. R., & Enkerli, J. (2016). Effect of seed treatment duration on growth and colonization of Vicia faba by endophytic Beauveria bassiana and Metarhizium brunneum. *Biological Control*, *103*, 187–195. https://doi.org/10.1016/j.biocontrol.2016.09.008

Jaber, L. R., & Ownley, B. H. (2018). Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? In *Biological Control* (Vol. 116). https://doi.org/10.1016/j.biocontrol.2017.01.018

Jafarzadeh, A. A., & Aliasgharzad, N. (2007). Salinity and salt composition effects on seed germination and root length of four sugar beet cultivars. *Biologia*, *62*(5), 562–564. https://doi.org/10.2478/s11756-007-0111-7

Jakubowska, M., Dobosz, R., Zawada, D., & Kowalska, J. (2022). A Review of Crop Protection Methods against the Twospotted Spider Mite—Tetranychus urticae Koch (Acari: Tetranychidae)—with Special Reference to Alternative Methods. In *Agriculture (Switzerland)* (Vol. 12, Issue 7). MDPI. https://doi.org/10.3390/agriculture12070898

Jan, A., & Komatsu, S. (2006). Functional Characterization of Gibberellin-Regulated Genes in Rice Using Microarray System. In *Genomics, Proteomics and Bioinformatics* (Vol. 4, Issue 3, pp. 137–144). https://doi.org/10.1016/S1672-0229(06)60026-0

Jia, M., Chen, L., Xin, H. L., Zheng, C. J., Rahman, K., Han, T., & Qin, L. P. (2016). A friendly relationship between endophytic fungi and medicinal plants: A systematic review. In *Frontiers in Microbiology* (Vol. 7, Issue JUN). Frontiers Research Foundation. https://doi.org/10.3389/fmicb.2016.00906

Jiang, H., Qi, P., Wang, T., Chi, X., Wang, M., Chen, M., Chen, N., & Pan, L. (2019). *Role of halotolerant phosphate-solubilising bacteria on growth promotion of peanut (Arachis hypogaea) under saline soil*. *2018*(October 2018), 20–30. https://doi.org/10.1111/aab.12473

Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J., & Shi, J. (2019). Occurrence, toxicity, production and detection of Fusarium mycotoxin: a review. In *Food Production, Processing and Nutrition* (Vol. 1, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s43014-019-0007-2

Jogawat, A., Vadassery, J., Verma, N., Oelmüller, R., Dua, M., Nevo, E., & Johri, A. K. (2016). PiHOG1, a stress regulator MAP kinase from the root endophyte fungus Piriformospora indica, confers salinity stress tolerance in rice plants. *Scientific Reports*, *6*. https://doi.org/10.1038/srep36765

Junglee, S., Urban, L., Sallanon, H., & Lopez-Lauri, F. (2014). Optimized Assay for Hydrogen Peroxide Determination in Plant Tissue Using Potassium Iodide. *American Journal of Analytical Chemistry*, *05*(11), 730–736. https://doi.org/10.4236/ajac.2014.511081

Kambura, A. K. (2016). *Metagenomic and Metatranscriptomic Analysis of Bacterial, Archaeal and Fungal Communities within the Hot Springs of Lake Magadi in Kenya*.

Kandel, S., Joubert, P., & Doty, S. (2017). Bacterial Endophyte Colonization and Distribution within Plants. *Microorganisms*, *5*(4), 77. https://doi.org/10.3390/microorganisms5040077

Kaul, S., Sharma, T., & K. Dhar, M. (2016). "Omics" Tools for Better Understanding the Plant–Endophyte Interactions. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00955

Keswani, C., Singh, S. P., Cueto, L., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., Borriss, R., Singh, S. P., Blázquez, M. A., & Sansinenea, E. (2020). Auxins of microbial origin and their use in agriculture. In *Applied Microbiology and*

Biotechnology (Vol. 104, Issue 20, pp. 8549–8565). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00253-020-10890-8

Keswani, C., Singh, S. P., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., Borriss, R., Rajput, V. D., Minkina, T. M., Ortiz, A., & Sansinenea, E. (2022). Biosynthesis and beneficial effects of microbial gibberellins on crops for sustainable agriculture. In *Journal of Applied Microbiology* (Vol. 132, Issue 3, pp. 1597–1615). Oxford University Press. https://doi.org/10.1111/jam.15348

Khaitov, B., Patiño-Ruiz, J. D., Pina, T., & Schausberger, P. (2015). Interrelated effects of mycorrhiza and free-living nitrogen fixers cascade up to aboveground herbivores. *Ecology and Evolution*, *5*(17), 3756–3768. https://doi.org/10.1002/ece3. 1654

Khalil, A. M. A., Hassan, S. E. D., Alsharif, S. M., Eid, A. M., Ewais, E. E. D., Azab, E., Gobouri, A. A., Elkelish, A., & Fouda, A. (2021). Isolation and characterization of fungal endophytes isolated from medicinal plant ephedra pachyclada as plant growth-promoting. *Biomolecules*, *11*(2), 1–18. https://doi.org/10.3390/biom11020140

Khamis Al-Zahrani, J., Hassan Al-Abdalall, A., Aly Osman, M., Aldakheel, L. A., Faisal AlAhmady, N., Aldakeel, S. A., AbdulAzeez, S., Francis Borgio, J., ElNaggar, M. A., Alabdallah, N. M., & Almustafa, M. M. (2023). Entomopathogenic fungi and their biological control of Tetranychus urticae: Two-spotted spider mites. *Journal of King Saud University - Science*, *35*(8). https://doi.org/10.1016/j.jksus.2023.102910

Khan, A. L., Halo, B. A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A., & Lee, I. J. (2016). Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of Solanum lycopersicum. *Electronic Journal of Biotechnology*. https://doi.org/10.1016/j.ejbt.2016.02.001

Khan, F., Siddique, A. B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. In *Plants* (Vol. 12, Issue 15). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/plants12152861

Khan, M. A., Asaf, S., Khan, A. L., Jan, R., Kang, S. M., Kim, K. M., & Lee, I. J. (2020). Thermotolerance effect of plant growth-promoting Bacillus cereus SA1 on soybean during heat stress. *BMC Microbiology*, *20*(1), 1–14. https://doi.org/10.1186/s12866-020-01822-7

Khan, N., Martínez-Hidalgo, P., Ice, T. A., Maymon, M., Humm, E. A., Nejat, N., Sanders, E. R., Kaplan, D., & Hirsch, A. M. (2018). Antifungal activity of bacillus species against fusarium and analysis of the potential mechanisms used in biocontrol. *Frontiers in Microbiology*, *9*(OCT), 1–12. https://doi.org/10.3389/fmicb.2018.02363

Khoyratty, S., Dupont, J., Lacoste, S., Palama, L. T., Choi, H. Y., Kim, K. H., Payet, B., Grisoni, M., Fouillaud, M., Verpoorte, R., & Kodja, H. (2015). Fungal endophytes of Vanilla planifolia across Réunion Island: Isolation, distribution and biotransformation. *BMC Plant Biology*, *15*(1). https://doi.org/10.1186/s12870-015-0522-5

Kimbrough, E. R., Berlow, M. L., & Van Bael, S. A. (2019). Water Level and Salinity Drive Community Structure of Culturable Baldcypress (Taxodium distichum) Endophytes in Southern Louisiana. *Wetlands*, *39*(2), 329–335. https://doi.org/10.1007/s13157-018-1098-2

Kim, H., You, Y. H., Yoon, H., Seo, Y., Kim, Y. E., Choo, Y. S., Lee, I. J., Shin, J. H., & Kim, J. G. (2014). Culturable fungal endophytes isolated from the roots of coastal plants inhabiting Korean east coast. *Mycobiology*, *42*(2), 100–108. https://doi.org/10.5941/MYCO.2014.42.2.100

Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy.

Frontiers in Plant Science, *10*(July), 1–19. https://doi.org/10.3389/fpls.2019.00845

Koirala, A., & Brözel, V. S. (2021). Phylogeny of nitrogenase structural and assembly components reveals new insights into the origin and distribution of nitrogen fixation across bacteria and archaea. *Microorganisms*, *9*(8). https://doi.org/10.3390/microorganisms9081662

Krell, T., Gavira, J. A., Roca, A., & Matilla, M. A. (2023). The emerging role of auxins as bacterial signal molecules: Potential biotechnological applications. *Microbial Biotechnology*, *16*(8), 1611–1615. https://doi.org/10.1111/1751-7915.14235

Kumar, A., & Verma, J. P. (2018). Does plant—Microbe interaction confer stress tolerance in plants: A review? *Microbiological Research*, *207*(November 2017), 41–52. https://doi.org/10.1016/j.micres.2017.11.004

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). *MEGA X : Molecular Evolutionary Genetics Analysis across Computing Platforms*. *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kunkel, B. N., & Johnson, J. M. B. (2021). Auxin Plays Multiple Roles during Plant-Pathogen Interactions. In *Cold Spring Harbor perspectives in biology* (Vol. 13, Issue 9). NLM (Medline). https://doi.org/10.1101/cshperspect.a040022

Kusari, S., Pandey, S. P., & Spiteller, M. (2013). Untapped mutualistic paradigms linking host plant and endophytic fungal production of similar bioactive secondary metabolites. *Phytochemistry*, *91*. https://doi.org/10.1016/j.phytochem.2012.07.021

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., & Barka, E. A. (2022). Biological Control of Plant Pathogens: A Global Perspective. In *Microorganisms* (Vol. 10, Issue 3). MDPI. https://doi.org/10.3390/microorganisms10030596

Lambers, H. (2022). *Annual Review of Plant Biology Phosphorus Acquisition and Utilization in Plants*. https://doi.org/10.1146/annurev-arplant-102720

Lamichhane, J. R., Barzman, M., Booij, K., Boonekamp, P., Desneux, N., Huber, L., Kudsk, P., Langrell, S. R. H., Ratnadass, A., Ricci, P., Sarah, J. L., & Messéan, A. (2015). Robust cropping systems to tackle pests under climate change. A review. In *Agronomy for Sustainable Development* (Vol. 35, Issue 2, pp. 443–459). Springer-Verlag France. https://doi.org/10.1007/s13593-014-0275-9

Latif Khan, A., Hamayun, M., Kang, S.-M., Kim, Y.-H., Jung, H.-Y., Lee, J.-H., & Lee, I.-J. (2012). *Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of Paecilomyces formosus LHL10*. http://www.biomedcentral.com/1471-2180/12/3

Lichtenthaler, H. K., & Determination, C. (2001). *1. Chlorophylls Carotenoiddetermination HK.Lichte*. *8*(Supplement 1).

Li, H., Guan, Y., Dong, Y., Zhao, L., Rong, S., Chen, W., Lv, M., Xu, H., Gao, X., Chen, R., Li, L., & Xu, Z. (2018). Isolation and evaluation of endophytic Bacillus tequilensis GYLH001 with potential application for biological control of Magnaporthe oryzae. *PLoS ONE*, *13*(10), 1–18. https://doi.org/10.1371/journal.pone.0203505

Lim, J. A., Lee, D. H., & Heu, S. (2014). The interaction of human enteric pathogens with plants. *Plant Pathology Journal*, *30*(2), 109–116. https://doi.org/10.5423/PPJ.RW.04.2014.0036

Lindström, K., & Mousavi, S. A. (2020). Effectiveness of nitrogen fixation in rhizobia. In *Microbial Biotechnology* (Vol. 13, Issue 5, pp. 1314–1335). John Wiley and Sons Ltd. https://doi.org/10.1111/1751-7915.13517

Liu, X., Xiao, Y., Zi, J., Yan, J., Li, C., Du, C., Wan, J., Wu, H., Zheng, B., Wang, S., & Liang, Q. (2023). Differential effects of low and high temperature stress on pollen germination and tube length of mango (Mangifera indica L.) genotypes. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-27917-5

Liu, Y., Yang, Y., & Wang, B. (2022). Entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae play roles of maize (Zea mays) growth promoter. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-19899-7

López-Bucio, J., Campos-Cuevas, J. C., Hernández-Calderón, E., Velásquez-Becerra, C., Farías-Rodríguez, R., Macías-Rodríguez, L. I., & Valencia-Cantero, E. (2007). Bacillus megaterium rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana. *Molecular Plant-Microbe Interactions*, *20*(2), 207–217. https://doi.org/10.1094/MPMI-20-2-0207

Ludwig-müller, J. (2015). Plants and endophytes : equal partners in secondary metabolite production ? Plants and endophytes : equal partners in secondary metabolite production ? *Biotechnology Letters*, *37*(7), 1325–1334. https://doi.org/10.1007/s10529-015-1814-4

Luo, J., Zhang, Z., Hou, Y., Diao, F., Hao, B., Bao, Z., Wang, L., & Guo, W. (2021). Exploring Microbial Resource of Different Rhizocompartments of Dominant Plants Along the Salinity Gradient Around the Hypersaline Lake Ejinur. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.698479

Luo, W., Kotut, K., & Krienitz, L. (2013). Hidden diversity of eukaryotic plankton in the soda lake Nakuru, Kenya, during a phase of low salinity revealed by a SSU rRNA gene clone library. *Hydrobiologia*, *702*(1), 95–103. https://doi.org/10.1007/s10750-012-1310-y

Ma Y, Dias MC and Freitas H (2020). Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. Front. Plant Sci. 11:591911. doi: 10.3389/fpls.2020.591911

Macedo-Raygoza, G. M., Valdez-Salas, B., Prado, F. M., Prieto, K. R., Yamaguchi, L. F., Kato, M. J., Canto-Canché, B. B., Carrillo-Beltrán, M., Di Mascio, P., White, J. F., & Beltrán-García, M. J. (2019). Enterobacter cloacae, an Endophyte That Establishes a Nutrient-Transfer Symbiosis With Banana Plants and Protects

Against the Black Sigatoka Pathogen. *Frontiers in Microbiology*, *10*(3), 1–15. https://doi.org/10.3389/fmicb.2019.00804

Macedo, R., Sales, L. P., Yoshida, F., Silva-Abud, L. L., & Lobo, M. (2017). Potential worldwide distribution of Fusarium dry root rot in common beans based on the optimal environment for disease occurrence. *PLoS ONE*, *12*(11), 1–19. https://doi.org/10.1371/journal.pone.0187770

Maciá-Vicente, J. G., Ferraro, V., Burruano, S., & Lopez-Llorca, L. V. (2012). Fungal Assemblages Associated with Roots of Halophytic and Non-halophytic Plant Species Vary Differentially Along a Salinity Gradient. *Microbial Ecology*, *64*(3), 668–679. https://doi.org/10.1007/s00248-012-0066-2

Mangmang, J. S., Deaker, R., & Rogers, G. (2015). Early seedling growth response of lettuce, tomato and cucumber to Azospirillum brasilense inoculated by soaking and drenching. *Horticultural Science*, *42*(1), 37–46. https://doi.org/10.17221/159/2014-HORTSCI

Mannaa, M., Oh, J. Y., & Kim, K. D. (2017). Biocontrol activity of volatile-producing Bacillus megaterium and Pseudomonas protegens against Aspergillus flavus and Aflatoxin production on stored rice grains. *Mycobiology*, *45*(3), 213–219. https://doi.org/10.5941/MYCO.2017.45.3.213

Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, H., McSteen, P., Zhao, Y., Hayashi, K. I., Kamiya, Y., & Kasahara, H. (2011). The main auxin biosynthesis pathway in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(45), 18512–18517. https://doi.org/10.1073/pnas.1108434108

McDermid, S. S. (2024). Agricultural water demand is taxing regional water supplies. *Nature Water*, *2*(3), 225–227. https://doi.org/10.1038/s44221-024-00215-8

Mehta, P., Siebert, S., Kummu, M., Deng, Q., Ali, T., Marston, L., Xie, W., & Davis, K. F. (2024). Half of twenty-first century global irrigation expansion has been in

water-stressed regions. *Nature Water*, *2*(3), 254–261. https://doi.org/10.1038/s44221-024-00206-9

Mei, C., & Flinn, B. S. (2010). The Use of Beneficial Microbial Endophytes for Plant Biomass and Stress Tolerance Improvement. *Recent Patents on Biotechnology*, *4*, 81–95.

Mili, C., Roy, S., & Tayung, K. (2021). Endophytic Fungi of Wild and Domesticated Crop Plants and Their Prospect for Applications in Sustainable Agriculture. In *Endophytes* (pp. 21–35). Springer Singapore. https://doi.org/10.1007/978-981-15-9371-0_2

Mishra, S., & Behera, N. (2008). *Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes*. *May 2014*.

Molina-Montenegro, M. A., Acuña-Rodríguez, I. S., Torres-Díaz, C., Gundel, P. E., & Dreyer, I. (2020). Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na+ sequestration. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-62544-4

Morsy, M., Cleckler, B., & Armuelles-Millican, H. (2020). Fungal endophytes promote tomato growth and enhance drought and salt tolerance. *Plants*, *9*(7), 1–18. https://doi.org/10.3390/plants9070877

Motallebi, P., Alkadri, D., Pisi, A., & Nipoti, P. (2015). *Pathogenicity and mycotoxin chemotypes of Iranian Fusarium culmorum isolates on durum wheat, and comparisons with Italian and Syrian isolates*. *January 2016*. https://doi.org/10.14601/Phytopathol

Mueller, R. C., Sthultz, C. M., Martinez, T., Gehring, C. A., & Whitham, T. G. (2005). The relationship between stem-galling wasps and mycorrhizal colonization of Quercus turbinella. *Canadian Journal of Botany*, *83*(10), 1349–1353. https://doi.org/10.1139/b05-105

Muiruri, V. M., Owen, R. B., Lowenstein, T. K., Renaut, R. W., Marchant, R., Rucina, S. M., Cohen, A., Deino, A. L., Sier, M. J., Luo, S., Leet, K., Campisano, C., Rabideaux, N. M., Deocampo, D., Shen, C. C., Mbuthia, A., Davis, B. C., Aldossari, W., & Wang, C. (2021). A million year vegetation history and palaeoenvironmental record from the Lake Magadi Basin, Kenya Rift Valley. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *567*. https://doi.org/ 10.1016/ j.palaeo.2021.110247

Mulwa, J., Barongo, J., Fairhead, D., Mariita, N., & Patel, J. (2010). *Integrated Geophysical Study of Lake Bogoria Basin , Kenya : Implications for Geothermal*. *4*(April), 2–5.

Muthukumar, A., Regunathan, U., & Ramasamy, N. (2017). *Role of Bacterial Endophytes in Plant Disease Control Chapter 7 Role of Bacterial Endophytes in Plant Disease Control*. *December 2018*. https://doi.org/10.1007/978-3-319-66544-3

Mutune, B., Ekesi, S., Matiru, V., Bii, C., & Maniania, N. K. (2016). Ophiomyia phaseoli on beans Phaseolus vulgaris. *Journal of Pest Science*, *Ii*. https://doi.org/10.1007/s10340-015-0725-4

Muvea, A. M., Meyhöfer, R., Subramanian, S., Poehling, H. M., Ekesi, S., & Maniania, N. K. (2014a). Colonization of onions by endophytic fungi and their impacts on the biology of thrips tabaci. *PLoS ONE*, *9*(9), 1–7. https://doi.org/10.1371/journal.pone.0108242

Muvea, A. M., Meyhöfer, R., Subramanian, S., Poehling, H. M., Ekesi, S., & Maniania, N. K. (2014b). Colonization of onions by endophytic fungi and their impacts on the biology of thrips tabaci. *PLoS ONE*, *9*(9). https://doi.org/10.1371/journal.pone.0108242

Muvea, A. M., Subramanian, S., Maniania, N. K., Poehling, H.-M., Ekesi, S., & Meyhöfer, R. (2018). Endophytic Colonization of Onions Induces Resistance

Against Viruliferous Thrips and Virus Replication. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01785

Mwirichia, R. (2009). Microbial Diversity of Lake Elmenteita, Kenya. (Dissertation/PhD thesis). University of Embu. Retrieved from: http://repository.embuni.ac.ke/handle/123456789/316

Nascimento, F.X., Rossi, M.J., Glick, B.R. (2016). Role of ACC Deaminase in Stress Control of Leguminous Plants. In: Subramaniam, G., Arumugam, S., Rajendran, V. (eds) Plant Growth Promoting Actinobacteria. Springer, Singapore. https://doi.org/10.1007/978-981-10-0707-1_11

Naseri, B. (2014). Bean production and fusarium root rot in diverse soil environments in Iran. *Journal of Soil Science and Plant Nutrition*, *14*(1), 177–188. https://doi.org/10.4067/S0718-95162014005000014

Nasiruddin, N., Chen, G., Zhangxin, Y., & Zhao, T. (2020). Endophytes of Terrestrial Plants: A Potential Source of Bioactive Secondary Metabolites. *Journal of Food and Nutrition Research*, *8*(7), 362–377. https://doi.org/10.12691/jfnr-8-7-8

Nett, R. S., Bender, K. S., & Peters, R. J. (2022). Production of the plant hormone gibberellin by rhizobia increases host legume nodule size. *ISME Journal*, *16*(7), 1809–1817. https://doi.org/10.1038/s41396-022-01236-5

Nett, R. S., Montanares, M., Marcassa, A., Lu, X., Nagel, R., Charles, T. C., Hedden, P., Rojas, M. C., & Peters, R. J. (2017). Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. *Nature Chemical Biology*, *13*(1), 69–74. https://doi.org/10.1038/nchembio.2232

Neupane, D., Adhikari, P., Bhattarai, D., Rana, B., Ahmed, Z., Sharma, U., & Adhikari, D. (2022). Does Climate Change Affect the Yield of the Top Three Cereals and Food Security in the World? In *Earth (Switzerland)* (Vol. 3, Issue 1, pp. 45–71). MDPI. https://doi.org/10.3390/earth3010004

Nia, S. H., Zarea, M. J., Rejali, F., & Varma, A. (2012). Yield and yield components of wheat as affected by salinity and inoculation with Azospirillum strains from saline or non-saline soil. *Journal of the Saudi Society of Agricultural Sciences*, *11*(2), 113–121. https://doi.org/10.1016/j.jssas.2012.02.001

Nithya, A., & Babu, S. (2017). *Prevalence of plant beneficial and human pathogenic bacteria isolated from salad vegetables in India*. 1–16. https://doi.org/10.1186/s12866-017-0974-x

Niu, D., Xia, J., Jiang, C., Qi, B., Ling, X., Lin, S., Zhang, W., Guo, J., Jin, H., & Zhao, H. (2016). Bacillus cereus AR156 primes induced systemic resistance by suppressing miR825/825* and activating defense-related genes in Arabidopsis. *Journal of Integrative Plant Biology*, *58*(4), 426–439. https://doi.org/10.1111/jipb.12446

Niu, S., Gao, Y., Zi, H., Liu, Y., Liu, X., Xiong, X., Yao, Q., Qin, Z., Chen, N., Guo, L., Yang, Y., Qin, P., Lin, J., & Zhu, Y. (2022). The osmolyte-producing endophyte Streptomyces albidoflavus OsiLf-2 induces drought and salt tolerance in rice via a multi-level mechanism. *Crop Journal*, *10*(2), 375–386. https://doi.org/10.1016/j.cj.2021.06.008

Nogueira, M. F., Pereira, L., Jenull, S., Kuchler, K., & Lion, T. (2019). Klebsiella pneumoniae prevents spore germination and hyphal development of Aspergillus species. *Scientific Reports*, *9*(1), 1–15. https://doi.org/10.1038/s41598-018-36524-8

Nyakeri, E. M., Mwirichia, R., & Boga, H. (2018). Isolation and characterization of enzyme producing bacteria from Lake Magadi, an extreme soda lake in Kenya. *Journal of Microbiology & Experimentation*, *6*(2), 57–68. https://doi.org/10.15406/jmen.2018.06.00189

O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. In *Applied Microbiology and*

Biotechnology (Vol. 100, Issue 13, pp. 5729–5746). Springer Verlag. https://doi.org/10.1007/s00253-016-7590-9

Ogbe, A. A., Gupta, S., Stirk, W. A., Finnie, J. F., & Van Staden, J. (2023). Growth-Promoting Characteristics of Fungal and Bacterial Endophytes Isolated from a Drought-Tolerant Mint Species Endostemon obtusifolius (E. Mey. ex Benth.) N. E. Br. *Plants*, *12*(3). https://doi.org/10.3390/plants12030638

Olago, D., Opere, A., & Barongo, J. (2009). Holocene palaeohydrology, groundwater and climate change in the lake basins of the Central Kenya Rift. *Hydrological Sciences Journal*, *54*(4), 765–780. https://doi.org/10.1623/hysj.54.4.765

Omomowo, O. I., & Babalola, O. O. (2019). Bacterial and fungal endophytes: Tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity. *Microorganisms*, *7*(11). https://doi.org/10.3390/microorganisms7110481

Orhan, F. (2016). Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (Triticum aestivum). *Brazilian Journal of Microbiology*, *47*(3), 621–627. https://doi.org/10.1016/j.bjm.2016.04.001

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science*. Mwirichia, *144*(1):31-43. doi:10.1017/S0021859605005708

Orozco-Mosqueda, M. del C., Santoyo, G., & Glick, B. R. (2023). Recent Advances in the Bacterial Phytohormone Modulation of Plant Growth. *Plants*, *12*(3). https://doi.org/10.3390/plants12030606

Orwa, P., Mugambi, G., Wekesa, V., & Mwirichia, R. (2020). Heliyon Isolation of haloalkaliphilic fungi from Lake Magadi in Kenya. *Heliyon*, *6*(November 2019), e02823. https://doi.org/10.1016/j.heliyon.2019.e02823

Owen B., R., Renaut, R. W., Muiruri, V. M., Rabideaux, N. M., Lowenstein, T. K., McNulty, E. P., Leet, K., Deocampo, D., Luo, S., Deino, A. L., Cohen, A., Sier, M. J., Campisano, C., Shen, C. C., Billingsley, A., Mbuthia, A., & Stockhecke,

M. (2019). Quaternary history of the Lake Magadi Basin, southern Kenya Rift: Tectonic and climatic controls. Palaeogeography, Palaeoclimatology, Palaeoecology, 518, 97–118. https://doi.org/10.1016/j.palaeo.2019.01.017

Ownley, B. H., Griffin, M. R., Klingeman, W. E., Gwinn, K. D., Moulton, J. K., & Pereira, R. M. (2008). Beauveria bassiana: Endophytic colonization and plant disease control. *Journal of Invertebrate Pathology*, *98*(3), 267–270. https://doi.org/10.1016/j.jip.2008.01.010

Pan, L., & Cai, B. (2023). Phosphate-Solubilizing Bacteria: Advances in Their Physiology, Molecular Mechanisms and Microbial Community Effects. In *Microorganisms* (Vol. 11, Issue 12). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/microorganisms11122904

Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Musoke, S., Nkalubo, S., & Mukankusi, C. (2018). PREVALENCE and INCIDENCE of FOUR COMMON BEAN ROOT ROTS in UGANDA. *Experimental Agriculture*, *54*(6), 888–900. https://doi.org/10.1017/S0014479717000461

Pappas, M. L., Liapoura, M., Papantoniou, D., Avramidou, M., Kavroulakis, N., Weinhold, A., Broufas, G. D., & Papadopoulou, K. K. (2018). The beneficial endophytic fungus fusarium solani strain K alters tomato responses against spider mites to the benefit of the plant. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01603

Pappas, M. L., Samaras, K., Koufakis, I., & Broufas, G. D. (2021). Beneficial soil microbes negatively affect spider mites and aphids in pepper. *Agronomy*, *11*(9). https://doi.org/10.3390/agronomy11091831

Pellan, L., Durand, N., Martinez, V., Fontana, A., Schorr-Galindo, S., & Strub, C. (2020). Commercial biocontrol agents reveal contrasting comportments against two mycotoxigenic fungi in cereals: Fusarium graminearum and fusarium verticillioides. *Toxins*, *12*(3). https://doi.org/10.3390/toxins12030152

Penn, C. J., & Camberato, J. J. (2019). A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. *Agriculture (Switzerland)*, *9*(6). https://doi.org/10.3390/agriculture9060120

Pereira, P., Ibáñez, S. G., Agostini, E., & Etcheverry, M. (2011). Effects of maize inoculation with Fusarium verticillioides and with two bacterial biocontrol agents on seedlings growth and antioxidative enzymatic activities. *Applied Soil Ecology*, *51*(1), 52–59. https://doi.org/10.1016/j.apsoil.2011.08.007

Pirttilä, A. M., Brusila, V., Koskimäki, J. J., Wäli, P. R., Ruotsalainen, A. L., Mutanen, M., & Markkola, A. M. (2023). Exchange of Microbiomes in Plant-Insect Herbivore Interactions. In *mBio* (Vol. 14, Issue 2). American Society for Microbiology. https://doi.org/10.1128/mbio.03210-22

Potshangbam, M., Indira Devi, S., Sahoo, D., & Strobel, G. A. (2017). Functional characterization of endophytic fungal community associated with Oryza sativa L. and Zea mays L. *Frontiers in Microbiology*, *8*(MAR). https://doi.org/10.3389/fmicb.2017.00325

Prabhu, N., Borkar, S., & Garg, S. (2018). Current Science Association Phosphate solubilization mechanisms in alkaliphilic bacterium Bacillus marisflavi FA7. In *Source: Current Science* (Vol. 114, Issue 4). https://about.jstor.org/terms

Przemieniecki, S. W., Kurowski, T. P., Kotlarz, K., Krawczyk, K., Damszel, M., Pszczółkowska, A., Kacprzak-Siuda, K., Chareńska, A., & Mastalerz, J. (2019). Bacteria Isolated from Treated Wastewater for Biofertilization and Crop Protection Against Fusarium spp. Pathogens. *Journal of Soil Science and Plant Nutrition*, *19*(1), 1–11. https://doi.org/10.1007/s42729-018-0001-9

Radhakrishnan, R., Khan, A. L., Kang, S. M., & Lee, I. J. (2015). A comparative study of phosphate solubilization and the host plant growth promotion ability of Fusarium verticillioides RK01 and Humicola sp. KNU01 under salt stress. *Annals of Microbiology*, *65*(1), 585–593. https://doi.org/10.1007/s13213-014-0894-z

Rasool, S., Markou, A., Hannula, S. E., & Biere, A. (2023). Effects of tomato inoculation with the entomopathogenic fungus Metarhizium brunneum on spider mite resistance and the rhizosphere microbial community. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1197770

Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021a). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. In *Journal of Soil Science and Plant Nutrition* (Vol. 21, Issue 1, pp. 49–68). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s42729-020-00342-7

Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021b). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. In *Journal of Soil Science and Plant Nutrition* (Vol. 21, Issue 1, pp. 49–68). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s42729-020-00342-7

Redman, R. S., Kim, Y. O., Woodward, C. J. D. A., Greer, C., Espino, L., Doty, S. L., & Rodriguez, R. J. (2011). Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. *PLoS ONE*, *6*(7). https://doi.org/10.1371/journal.pone.0014823

Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., & Henson, J. M. (2002). Thermotolerance generated by plant/fungal symbiosis. In *Science* (Vol. 298, Issue 5598, p. 1581). https://doi.org/10.1126/science.1072191

Reiner, K. (2010). Catalase Test Protocol. *American Society for Microbiology. Barlett Publishers, Inc., Sudbury, MA., November 2010*, 1–9.

Reis Gonçalves, D., Pena, R., Zotz, G., & Albach, D. C. (n.d.). *Effects of fungal inoculation on the growth of Salicornia (Amaranthaceae) under different salinity conditions*. https://doi.org/10.1007/s13199-021-00783-3/Published

Ren, Y., Ren, Y., Zhou, Z., Guo, X., Li, Y., Feng, L., & Wang, L. (2010). Complete genome sequence of Enterobacter cloacae subsp. cloacae type strain ATCC

13047. *Journal of Bacteriology*, *192*(9), 2463–2464. https://doi.org/10.1128/JB.00067-10

Rho, H., Hsieh, M., Kandel, S. L., Cantillo, J., Doty, S. L., & Kim, S. H. (2018). Do Endophytes Promote Growth of Host Plants Under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes. *Microbial Ecology*, *75*(2). https://doi.org/10.1007/s00248-017-1054-3

Rodriguez, R. J., White, J. F., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: Diversity and functional roles: Tansley review. In *New Phytologist* (Vol. 182, Issue 2, pp. 314–330). https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rodriguez, R., & Redman, R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany*, *59*(5), 1109–1114. https://doi.org/10.1093/jxb/erm342

Rodriguez-Saona, C., de Lange, E., & Dara, S. K. (2022). Editorial: Advances in Crop Resistance for Insect Pest Control. In *Frontiers in Agronomy* (Vol. 4). Frontiers Media S.A. https://doi.org/10.3389/fagro.2022.845961

Rogers, C., & Oldroyd, G. E. D. (2014). Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. In *Journal of Experimental Botany* (Vol. 65, Issue 8, pp. 1939–1946). Oxford University Press. https://doi.org/10.1093/jxb/eru098

Romero, F. M., & Pieckenstain, F. L. (2014). *The communities of tomato (Solanum lycopersicum L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing*. *351*, 187–194. https://doi.org/10.1111/1574-6968.12377

Sahoo, S., Subban, K., & Chelliah, J. (2021). Diversity of Marine Macro-Algicolous Endophytic Fungi and Cytotoxic Potential of Biscogniauxia petrensis Metabolites Against Cancer Cell Lines. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/ fmicb.2021.650177

Sahu, P. K., Shafi, Z., Singh, S., Ojha, K., Jayalakshmi, K., Tilgam, J., Manzar, N., Sharma, P. K., & Srivastava, A. K. (2023). Colonization potential of endophytes from halophytic plants growing in the “Runn of Kutch” salt marshes and their contribution to mitigating salt stress in tomato cultivation. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1226149

Samaras, K., Mourtiadou, S., Arampatzis, T., Kakagianni, M., Feka, M., Wäckers, F., Papadopoulou, K. K., Broufas, G. D., & Pappas, M. L. (2023). Plant-Mediated Effects of Beneficial Microbes and a Plant Strengthener against Spider Mites in Tomato. *Plants*, *12*(4). https://doi.org/10.3390/plants12040938

Sánchez-Rodríguez, A. R., Raya-Díaz, S., Zamarreño, Á. M., García-Mina, J. M., del Campillo, M. C., & Quesada-Moraga, E. (2018). An endophytic Beauveria bassiana strain increases spike production in bread and durum wheat plants and effectively controls cotton leafworm (Spodoptera littoralis) larvae. *Biological Control*, *116*, 90–102. https://doi.org/10.1016/J.BIOCONTROL.2017.01.012

Sani, I., Jamian, S., Saad, N., Abdullah, S., Hata, E. M., Jalinas, J., & Ismail, S. I. (2023). Inoculation and colonization of the entomopathogenic fungi, Isaria javanica and Purpureocillium lilacinum, in tomato plants, and their effect on seedling growth, mortality and adult emergence of Bemisia tabaci (Gennadius). *PLoS ONE*, *18*(5 May). https://doi.org/10.1371/journal.pone.0285666

Saragih, M., Trizelia, Nurbailis, & Yusniwati. (2019). Endophytic Colonization and Plant Growth Promoting Effect by Entomopathogenic fungus, Beauveria bassiana to Red Chili (Capsicum annuum L.) with Different Inoculation Methods. *IOP Conference Series: Earth and Environmental Science*, *305*(1). https://doi.org/10.1088/1755-1315/305/1/012070

Sarhan, M. S., Hamza, M. A., Youssef, H. H., Patz, S., Becker, M., ElSawey, H., Nemr, R., Daanaa, H. S. A., Mourad, E. F., Morsi, A. T., Abdelfadeel, M. R., Abbas, M. T., Fayez, M., Ruppel, S., & Hegazi, N. A. (2019). Culturomics of the plant prokaryotic microbiome and the dawn of plant-based culture media – A

review. In *Journal of Advanced Research* (Vol. 19, pp. 15–27). Elsevier B.V. https://doi.org/10.1016/j.jare.2019.04.002

Schagerl, M. (2016). *Soda Lakes of East Africa* (M. Schagerl, Ed.). Springer International Publishing Switzerland.

Scientific review of the impact of climate change on plant pests. (2021). In *Scientific review of the impact of climate change on plant pests*. FAO on behalf of the IPPC Secretariat. https://doi.org/10.4060/cb4769en

Sendi, Y., Pfeiffer, T., Koch, E., Mhadhbi, H., & Mrabet, M. (2020). Potential of common bean (Phaseolus vulgaris L.) root microbiome in the biocontrol of root rot disease and traits of performance. *Journal of Plant Diseases and Protection*, *127*(4), 453–462. https://doi.org/10.1007/s41348-020-00338-6

Serrano, R., González-Menéndez, V., Rodríguez, L., Martín, J., Tormo, J. R., & Genilloud, O. (2017). Co-culturing of fungal strains against Botrytis cinerea as a model for the induction of chemical diversity and therapeutic agents. *Frontiers in Microbiology*, *8*(APR), 1–15. https://doi.org/10.3389/fmicb.2017.00649

Shahzad, R., Khan, A. L., Bilal, S., Asaf, S., & Lee, I.-J. (2018). What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.00024

Shakya, M., Gottel, N., Castro, H., Yang, Z. K., Gunter, L., Labbé, J., Muchero, W., Bonito, G., Vilgalys, R., Tuskan, G., Podar, M., & Schadt, C. W. (2013). A Multifactor Analysis of Fungal and Bacterial Community Structure in the Root Microbiome of Mature Populus deltoides Trees. *PLoS ONE*, *8*(10). https://doi.org/10.1371/journal.pone.0076382

Shani, E., Hedden, P., & Sun, T. P. (2024). Highlights in gibberellin research: A tale of the dwarf and the slender. In *Plant Physiology* (Vol. 195, Issue 1, pp. 111–134). American Society of Plant Biologists. https://doi.org/10.1093/plphys/kiae044

Sharifi, R., Lee, S. M., & Ryu, C. M. (2018). Microbe-induced plant volatiles. In *New Phytologist* (Vol. 220, Issue 3, pp. 684–691). Blackwell Publishing Ltd. https://doi.org/10.1111/nph.14955

Sharma, A. K., Sharma, V., Saxena, J., Yadav, B., Alam, A., & Prakash, A. (2015). Isolation and Screening of Extracellular Protease Enzyme from Bacterial and Fungal Isolates of Soil. *International Journal of Scientific Research in Environmental Sciences*, *3*(9), 334–340. https://doi.org/10.12983/ijsres-2015-p0334-0340

Sharps, K., Foster, J., Vieno, M., Beck, R., & Hayes, F. (2024). Ozone pollution contributes to the yield gap for beans in Uganda, East Africa, and is co-located with other agricultural stresses. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-58144-1

Silva, L. I. da, Pereira, M. C., Carvalho, A. M. X. de, Buttrós, V. H., Pasqual, M., & Dória, J. (2023). Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. In *Agriculture (Switzerland)* (Vol. 13, Issue 2). MDPI. https://doi.org/10.3390/agriculture13020462

Silva, U. C., Cuadros-Orellana, S., Silva, D. R. C., Freitas-Júnior, L. F., Fernandes, A. C., Leite, L. R., Oliveira, C. A., & Dos Santos, V. L. (2021). Genomic and Phenotypic Insights

into the Potential of Rock Phosphate Solubilizing Bacteria to Promote Millet Growth in vivo. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.574550

Singh, S. K., Wu, X., Shao, C., & Zhang, H. (2022). Microbial enhancement of plant nutrient acquisition. In *Stress Biology* (Vol. 2, Issue 1). Springer. https://doi.org/10.1007/s44154-021-00027-w

Skendžić, S., Zovko, M., Živković, I. P., Lešić, V., & Lemić, D. (2021). The impact of climate change on agricultural insect pests. In *Insects* (Vol. 12, Issue 5). MDPI AG. https://doi.org/10.3390/insects12050440

Snapp, S., Sapkota, T. B., Chamberlin, J., Cox, C. M., Gameda, S., Jat, M. L., Marenya, P., Mottaleb, K. A., Negra, C., Senthilkumar, K., Sida, T. S., Singh, U., Stewart, Z. P., Tesfaye, K., & Govaerts, B. (2023). Spatially differentiated nitrogen supply is key in a global food–fertilizer price crisis. *Nature Sustainability*, *6*(10), 1268–1278. https://doi.org/10.1038/s41893-023-01166-w

Soltani, A., Weraduwage, S. M., Sharkey, T. D., & Lowry, D. B. (2019). Elevated temperatures cause loss of seed set in common bean (Phaseolus vulgaris L.) potentially through the disruption of source-sink relationships. *BMC Genomics*, *20*(1). https://doi.org/10.1186/s12864-019-5669-2

Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. (2020a). Exploiting biological nitrogen fixation: A route towards a sustainable agriculture. In *Plants* (Vol. 9, Issue 8, pp. 1–22). MDPI AG. https://doi.org/10.3390/plants9081011

Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. (2020b). Exploiting biological nitrogen fixation: A route towards a sustainable agriculture. In *Plants* (Vol. 9, Issue 8, pp. 1–22). MDPI AG. https://doi.org/10.3390/plants9081011

Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*, *31*(4), 425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Spor, A., Roucou, A., Mounier, A., Bru, D., Breuil, M. C., Fort, F., Vile, D., Roumet, P., Philippot, L., & Violle, C. (2020). Domestication-driven changes in plant traits associated with changes in the assembly of the rhizosphere microbiota in tetraploid wheat. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-69175-9

Srinivas, C., Nirmala Devi, D., Narasimha Murthy, K., Mohan, C. D., Lakshmeesha, T. R., Singh, B. P., Kalagatur, N. K., Niranjana, S. R., Hashem, A., Alqarawi, A. A., Tabassum, B., Abd_Allah, E. F., & Chandra Nayaka, S. (2019). Fusarium

oxysporum f. sp. lycopersici causal agent of vascular wilt disease of tomato: Biology to diversity– A review. In *Saudi Journal of Biological Sciences* (Vol. 26, Issue 7, pp. 1315–1324). Elsevier B.V. https://doi.org/10.1016/j.sjbs.2019.06.002

Sun, T. (2008). Gibberellin Metabolism, Perception and Signaling Pathways in Arabidopsis. *The Arabidopsis Book*, *6*, e0103. https://doi.org/10.1199/tab.0103

Su, Y., Liu, C., Fang, H., & Zhang, D. (2020). Bacillus subtilis: A universal cell factory for industry, agriculture, biomaterials and medicine. *Microbial Cell Factories*, *19*(1), 1–12. https://doi.org/10.1186/s12934-020-01436-8

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tang, J., Li, Y., Zhang, L., Mu, J., Jiang, Y., Fu, H., Zhang, Y., Cui, H., Yu, X., & Ye, Z. (2023). Biosynthetic Pathways and Functions of Indole-3-Acetic Acid in Microorganisms. In *Microorganisms* (Vol. 11, Issue 8). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/microorganisms11082077

Tiwari, M., Pati, D., Mohapatra, R., Sahu, B. B., & Singh, P. (2022). The Impact of Microbes in Plant Immunity and Priming Induced Inheritance: A Sustainable Approach for Crop protection. In *Plant Stress* (Vol. 4). Elsevier B.V. https://doi.org/10.1016/j.stress.2022.100072

Tnay, G. (2019). *Too Much Salt: The Growing Threat that Salinity Poses to Global Food Production*.

Toghueo, R. M. K., Eke, P., Zabalgogeazcoa, Í., de Aldana, B. R. V., Nana, L. W., & Boyom, F. F. (2016). Biocontrol and growth enhancement potential of two

endophytic Trichoderma spp. from Terminalia catappa against the causative agent of Common Bean Root Rot (Fusarium solani). *Biological Control*, *96*(January 2016), 8–20. https://doi.org/10.1016/j.biocontrol.2016.01.008

Tyurin, M., Kabilov, M. R., Smirnova, N., Tomilova, O. G., Yaroslavtseva, O., Alikina, T., Glupov, V. V., & Kryukov, V. Y. (2021). Can potato plants be colonized with the fungi metarhizium and beauveria under their natural load in agrosystems? *Microorganisms*, *9*(7). https://doi.org/10.3390/microorganisms 9071373

Ullah, A., Mushtaq, H., Fahad, S., Hakim, Shah, A., & Chaudhary, H. J. (2017). Plant growth promoting potential of bacterial endophytes in novel association with Olea ferruginea and Withania coagulans. *Microbiology (Russian Federation)*, *86*(1), 119–127. https://doi.org/10.1134/S0026261717010155

Umesha, S., Manukumar, H. M., & Raghava, S. (2016). A rapid method for isolation of genomic DNA from food-borne fungal pathogens. *3 Biotech*, *6*(2). https://doi.org/10.1007/s13205-016-0436-4

Uzma, F., Mohan, C. D., Hashem, A., Konappa, N. M., Rangappa, S., Kamath, P. V., Singh, B. P., Mudili, V., Gupta, V. K., Siddaiah, C. N., Chowdappa, S., Alqarawi, A. A., & Abd-Allah, E. F. (2018). Endophytic fungi-alternative sources of cytotoxic compounds: A review. In *Frontiers in Pharmacology* (Vol. 9, Issue APR). Frontiers Media S.A. https://doi.org/10.3389/fphar.2018.00309

van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, *2*(7), 494–501. https://doi.org/10.1038/s43016-021-00322-9

Van, L., & Dufresne, A. (2015). *Tansley review The importance of the microbiome of the plant holobiont*.

Varga, T., Hixson, K. K., Ahkami, A. H., Sher, A. W., Barnes, M. E., Chu, R. K., Battu, A. K., Nicora, C. D., Winkler, T. E., Reno, L. R., Fakra, S. C., Antipova,

O., Parkinson, D. Y., Hall, J. R., & Doty, S. L. (2020). Endophyte-Promoted Phosphorus Solubilization in Populus. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.567918

Verma, A., Shameem, N., Jatav, H. S., Sathyanarayana, E., Parray, J. A., Poczai, P., & Sayyed, R. Z. (2022). Fungal Endophytes to Combat Biotic and Abiotic Stresses for Climate-Smart and Sustainable Agriculture. In *Frontiers in Plant Science* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fpls.2022.953836

Verma, H., Kumar, D., Kumar, V., Kumari, M., Singh, S. K., Sharma, V. K., Droby, S., Santoyo, G., White, J. F., & Kumar, A. (2021). The potential application of endophytes in management of stress from drought and salinity in crop plants. *Microorganisms*, *9*(8). https://doi.org/10.3390/microorganisms9081729

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., & SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*, *184*, 13–24. https://doi.org/10.1016/j.micres.2015.12.003

Waqas, M., Khan, A. L., Hamayun, M., Shahzad, R., Kim, Y. H., Choi, K. S., & Lee, I. J. (2015). Endophytic infection alleviates biotic stress in sunflower through regulation of defence hormones, antioxidants and functional amino acids. *European Journal of Plant Pathology*, *141*(4), 803–824. https://doi.org/10.1007/s10658-014-0581-8

Watts, D., Palombo, E. A., Jaimes Castillo, A., & Zaferanloo, B. (2023). Endophytes in Agriculture: Potential to Improve Yields and Tolerances of Agricultural Crops. In *Microorganisms* (Vol. 11, Issue 5). MDPI. https://doi.org/10.3390/microorganisms11051276

Wehner, J., Powell, J. R., Muller, L. A. H., Caruso, T., Veresoglou, S. D., Hempel, S., & Rillig, M. C. (2014). Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland. *Journal of Ecology*, *102*(2), 425–436. https://doi.org/10.1111/1365-2745.12197

Wemheuer, F., Kaiser, K., Karlovsky, P., Daniel, R., Vidal, S., & Wemheuer, B. (2017). Bacterial endophyte communities of three agricultural important grass species differ in their response towards management regimes. *Scientific Reports*, *7*(October 2016), 1–13. https://doi.org/10.1038/srep40914

Wheeler, D. L., & Wheeler, D. L. (2019). *From pathogen to endophyte : an endophytic population of Verticillium dahliae evolved from a sympatric pathogenic population*. 497–510. https://doi.org/10.1111/nph.15567

Whitaker, B. K., & Bakker, M. G. (2019). Bacterial endophyte antagonism toward a fungal pathogen in vitro does not predict protection in live plant tissue. *FEMS Microbiology Ecology*, *95*(2), 1–11. https://doi.org/10.1093/femsec/fiy237

White, J. F., Crawford, H., Torres, M. S., Mattera, R., Irizarry, I., & Bergen, M. (2012). A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. *Symbiosis*, *57*(3), 161–171. https://doi.org/10.1007/s13199-012-0189-8

White, J. F., Kingsley, K. L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M. T., Verma, S. K., Gond, S. K., & Kowalski, K. P. (2019). Review: Endophytic microbes and their potential applications in crop management. In *Pest Management Science* (Vol. 75, Issue 10, pp. 2558–2565). John Wiley and Sons Ltd. https://doi.org/10.1002/ps.5527

Wippel, K. (2023). Plant and microbial features governing an endophytic lifestyle. In *Current Opinion in Plant Biology* (Vol. 76). Elsevier Ltd. https://doi.org/10.1016/j.pbi.2023.102483

Wu, T., Xu, J., Xie, W., Yao, Z., Yang, H., Sun, C., & Li, X. (2018). Pseudomonas aeruginosa L10: A hydrocarbon-degrading, biosurfactant-producing, and plant-growth-promoting endophytic bacterium isolated from a Reed (Phragmites australis). *Frontiers in Microbiology*, *9*(MAY), 1–12. https://doi.org/10.3389/fmicb.2018.01087

Xia, X. J., Zhou, Y. H., Shi, K., Zhou, J., Foyer, C. H., & Yu, J. Q. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *Journal of Experimental Botany*, *66*(10), 2839–2856. https://doi.org/10.1093/jxb/erv089

Xie, W., Yang, J., Gao, S., Yao, R., & Wang, X. (2022a). The Effect and Influence Mechanism of Soil Salinity on Phosphorus Availability in Coastal Salt-Affected Soils. *Water (Switzerland)*, *14*(18). https://doi.org/10.3390/w14182804

Xie, W., Yang, J., Gao, S., Yao, R., & Wang, X. (2022b). The Effect and Influence Mechanism of Soil Salinity on Phosphorus Availability in Coastal Salt-Affected Soils. *Water (Switzerland)*, *14*(18). https://doi.org/10.3390/w14182804

Xie, X. G., Zhang, F. M., Yang, T., Chen, Y., Li, X. G., & Dai, C. C. (2019). Endophytic fungus drives nodulation and N2 fixation attributable to specific root exudates. *MBio*, *10*(4). https://doi.org/10.1128/mBio.00728-19

Xu, S. J., & Kim, B. S. (2014). *Biocontrol of Fusarium Crown and Root Rot and Promotion of Growth of Tomato by Paenibacillus Strains Isolated from Soil*. *October*. https://doi.org/10.5941/MYCO.2014.42.2.158

Yan, D., Tajima, H., Cline, L. C., Fong, R. Y., Ottaviani, J. I., Shapiro, H. Y., & Blumwald, E. (2022). Genetic modification of flavone biosynthesis in rice enhances biofilm formation of soil diazotrophic bacteria and biological nitrogen fixation. *Plant Biotechnology Journal*, *20*(11), 2135–2148. https://doi.org/ 10.1111/ pbi.13894

Yang, B., Wang, X. M., Ma, H. Y., Yang, T., Jia, Y., Zhou, J., & Dai, C. C. (2015). Fungal endophyte Phomopsis liquidambari affects nitrogen transformation processes and related microorganisms in the rice rhizosphere. *Frontiers in Microbiology*, *6*(SEP). https://doi.org/10.3389/fmicb.2015.00982

Yao, D., Wu, J., Luo, Q., Zhang, D., Zhuang, W., Xiao, G., Deng, Q., & Bai, B. (2022). Effects of Salinity Stress at Reproductive Growth Stage on Rice (Oryza sativa L.)

Composition, Starch Structure, and Physicochemical Properties. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.926217

Yaxley, J. R., Ross, J. J., Sherriff, L. J., & Reid, J. B. (2001). *Gibberellin Biosynthesis Mutations and Root Development in Pea*. www.plantphysiol.org

Yimer, H. Z., Nahar, K., Kyndt, T., Haeck, A., Van Meulebroek, L., Vanhaecke, L., Demeestere, K., Höfte, M., & Gheysen, G. (2018). Gibberellin antagonizes jasmonate-induced defense against Meloidogyne graminicola in rice. *New Phytologist*, *218*(2), 646–660. https://doi.org/10.1111/nph.15046

Yoon, S., Ha, S., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). *Introducing EzBioCloud : a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies*. 1613–1617. https://doi.org/10.1099/ijsem.0.001755

Yue, H., Yue, W., Jiao, S., Kim, H., Lee, Y. H., Wei, G., Song, W., & Shu, D. (2023). Plant domestication shapes rhizosphere microbiome assembly and metabolic functions. *Microbiome*, *11*(1). https://doi.org/10.1186/s40168-023-01513-1

Zhang, J. T., & Mu, C. S. (2009). Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage Lathyrus quinquenervius. *Soil Science and Plant Nutrition*, *55*(5), 685–697. https://doi.org/10.1111/j.1747-0765.2009.00411.x

Zhang, Q., Acuña, J. J., Inostroza, N. G., Mora, M. L., Radic, S., Sadowsky, M. J., & Jorquera, M. A. (2019). Endophytic Bacterial Communities Associated with Roots and Leaves of Plants Growing in Chilean Extreme Environments. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-41160-x

Zhang, Q., Gong, M., Xu, X., Li, H., & Deng, W. (2022). Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. In *Cells* (Vol. 11, Issue 17). MDPI. https://doi.org/10.3390/cells11172761

Zhao, C., Zhang, H., Song, C., Zhu, J. K., & Shabala, S. (2020). Mechanisms of Plant Responses and Adaptation to Soil Salinity. *Innovation*, *1*(1), 100017. https://doi.org/10.1016/j.xinn.2020.100017

Zhou, J., Diao, X., Wang, T., Chen, G., Lin, Q., Yang, X., & Xu, J. (2018). Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species Rhizophora stylosa and R. mucronata in the South China Sea. *PLoS ONE*, *13*(6). https://doi.org/10.1371/journal.pone.0197359

Zhou, W., White, J. F., Soares, M. A., & Torres, M. S. (2015). *Diversity of fungi associated with plants growing in geothermal ecosystems and evaluation of their capacities to enhance thermotolerance of host plants*. *9145*. https://doi.org/10.1080/17429145.2015.1101495

Zhu, T., Deng, X., Zhou, X., Zhu, L., Zou, L., & Li, P. (2016). Ethylene and hydrogen peroxide are involved in brassinosteroid- induced salt tolerance in tomato. *Nature Publishing Group*, *October*, 1–15. https://doi.org/10.1038/srep35392

Zivanovic, A., & Rodgers, L. (2018). *The role of fungal endophytes in plant pathogen resistance*. *89*(4), 192–197. https://doi.org/10.2307/26789679

Zúñiga, A., Poupin, M. J., Donoso, R., Ledger, T., Guiliani, N., Gutiérrez, R. A., & González, B. (2013). Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of arabidopsis thaliana by burkholderia phytofirmans PsJN. *Molecular Plant-Microbe Interactions*, *26*(5), 546–553. https://doi.org/10.1094/MPMI-10-12-0241-R

APPENDICES

Appendix I: Analysis of Deviance Table from the Generalised Linear Model with Poisson Distribution Showing the Effect of Methods, Isolates, Plant Parts and Their Respective Interactions on Fungal Re-Isolation

	D.f.	Residual deviance	Deviance	P
Concentrations	1	9.7	281.95	**0.0018**
Plant parts	2	8.1	273.82	**0.017**
Isolates	4	171.0	102.87	**0.001**
Concentrations ×Plant parts	2	3.8	99.10	0.2492
Concentrations ×Isolates	4	4.6	94.48	0.33
Plant parts × Isolates	8	5.5	89.00	0.70
Concentration × Plant parts× Isolates	8	5.4	83.56	0.71

Appendix II: Analysis of Deviance Table from the Generalised Linear Model with Poisson Distribution Showing the Effect of Methods, Isolates, Plant Parts and Their Respective Interactions on Bacterial Re-Isolation

	DF	Deviance	Resid. DF	Resid. Dev	Pr(>Chi)
NULL			161	465.73	
Methods	1	4.751	160	460.98	P=0.029
Isolates	8	123.224	152	337.76	P<0.0001
Plant parts	2	141.66	150	196.1	P<0.0001
Method×Isolates	8	24.095	142	172	P=0.0022
Method×Plant parts	2	14.891	140	157.11	P<0.001
Isolates× Plant parts	16	52.72	124	104.39	P<0.0001
Methods×Isolates×Plant parts	16	28.106	108	76.29	P=0.031

Appendix III: Tukey's HSD Post Hoc Test on the Effect of Endophytic Fungi on Spidermite Mortality

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate	4	4.93	1.233	0.762	0.554
Residuals	55	89.00	1.618		

Appendix IV: Turkey's HSD Post Hoc Test on the Number of Eggs Laid per Isolate

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate	4	33.73	8.433	2.025	0.104
Residuals	55	229.00	4.164		