Characterization of *Bacillus subtilis* and *Paenibacillus polymxa strains* to reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions

Urgessa Ensermu

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

November, 2022

Adama, Ethiopia

Characterization of *Bacillus subtilis* and *Paenibacillus polymxa* strains to reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions

Urgessa Ensermu

Main Advisor: Zerihun Belay (PhD)

Co-advisor: Mulissa Jida (PhD)

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

November, 2022

Adama, Ethiopia

DECLARATION

I hereby declare that this master thesis entitled "**Characterization of *Bacillus subtilis* and *Paenibacillus polymxa strains to* reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions** is my original work It has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

_____	_____	_____
Name of the student	Signature	Date

RECOMMENDATION

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Characterization of Bacillus subtilis and Paenibacillus polymxa strains to reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions**" prepared under our guidance by **Urgessa Ensermu** submitted in partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-Advisor	Signature	Date

APPROVAL PAGE

We, the advisors of the thesis entitled "**Characterization of *Bacillus subtilis* and *Paenibacillus polymxa* strains to reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions"**developed by **Urgessa Ensermu**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Urgessa Ensermu have read and evaluated the thesis entitled **"Characterization of *Bacillus subtilis* and *Paenibacillus polymxa* strains to reduce disease incidence of Fusarium Wilt Disease and enhance production of resistant and susceptible Chickpea varieties under greenhouse conditions"**and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Biotechnology.

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

_____	_____	_____
Department Head	Signature	Date
_____	_____	_____
School Dean	Signature	Date
_____	_____	_____
Office of Postgraduate	Signature	Date

ACKNOWLEDGEMENT

First and foremost, I would like to express my heartfelt gratitude to my Major Advisor Dr. Zerihun Belay for his continuous encouragement and help starting from the beginning to the end of this research work. I am very grateful to him for his critical and constructive comments during preparation of the manuscript. I would also appreciate his fatherly approach, inspiring advice, critical analysis of the current issues and friendship during my study period.

I would also like to express my deepest appreciation to Dr.Mulisa Jida, Bio and Emerging Technology Institute, Environmental Biotechnology Director for accepting me to work in his project on chickpea Wilt disease bio-control and giving me pure culture of Antagonistic rhizobacteria strains and pure culture of fungal pathogens from his previous work.

I would also like to extend my appreciation to Debre Zeit Agricultural Research Center for facilitating my research work by providing me different chickpea genotypes and allowing me to work in their greenhouse. I would like to thank Dr. Asnake Fikre, EIAR, and Debere Zeit for his support and showing willingness to provide me Chickpea genotypes and his brotherly approaches while I was working there.

I am very much grateful to Dr. Tulu Degefu, EIAR, and Debere Zeit for his provision of greenhouse testing pots. I would also like to thank Mr. Asrat Zewede and Mr.Tolosa Bedada EIAR, Debere Zeit Agricultural Research Center for their brotherly approaches and kindness in follow up of the greenhouse works and for their provision of assistances with the disease assessment and continuous follow up during my absence.

I would like to thank Mr. Dereje and Mr. Arega for their assistance during laboratory works and greenhouse experiments at Debere Zeit Agricultural Research Center. I thanks my friend Yeabsira Worku for her voluntary preparation of maps of the study area. I would also like to express my special thanks to my Father **Ensermu Diro** who has passed without seeing my final and my Mother **Arfase Gedefa** for nurturing me with love and inspirations.

I would like to express my heartfelt appreciations to my wife Shitaye Shaka Gudeta for encouraging me to join the MSc study, shouldering all family affairs and taking care of our kids. Last, but not least I would like to thank my beloved daughter **Amarti Urgessa** for tolerating loss of my attentions which you deserve during your early age.

TABLE OF CONTENTS

Contents Pages

DECLARATION ..... **i**

RECOMMENDATION ..... **ii**

APPROVAL PAGE ..... **iii**

ACKNOWLEDGEMENT ..... **iv**

TABLE OF CONTENTS ..... **v**

LISTS OF TABLES ..... **ix**

LISTS OF FIGURES ..... **x**

LISTS OF ACRONYMS AND ABBREVIATIONS ..... **xi**

ABSTRACT ..... **xii**

CHAPTER ONE **1**

1. INTRODUCTION **1**

1.1. Background to the study 1

1.2. Statement of the problem 2

1.3. Research Questions 3

1.4. Objectives of the Study 3

1.4.1. General Objective 3

1.4.2. Specific objectives 3

2. LITERATURE REVIEW **4**

2.1 Botanical description of chickpea 4

2.2 Taxonomy 4

2.3 Climate and Growth Conditions 4

2.4 Chickpea production in Ethiopia 5

2.5 Types of Chickpea 6

2.5.1 *Desi chickpea* 7
2.5.2 *Kabuli chickpea* 7
2.6 Agronomic and economic importance of Chickpea 8
2.6.1 Nodulation and nitrogen fixation 8
2.6.2 Nutritional value of chickpea 8
2.7 Major Constraints of Chickpea Production 8
2.8 Fusariums wilt 9
2.8.1 Symptoms of Fusarium oxysporum causing wilt of chickpea 9
2.9 Epidemiological Fusarium Wilt in Ethiopia 10
2.10 Chickpea root rot disease management strategy 11
2.10.1 Cultural practices 12
2.10.2 Mechanical control 12
2.10.3 Chemical control 12
2.10.4 Biological control 13
2.11 Mechanisms of plant Growth Promoting Rhizobacteria and its importance 14
2.11.1 Direct Antagonism 14
2.12. Indirect antagonism 16
2.12.1. Production of antibiotics 16
2.12.2. Secretion of lytic enzymes 17
2.12.3. Hydrogen cyanide (HCN) production 18
2.12.4. Induced Systemic Host Resistance (ISR) 18
2.12.5. Competition for niche and nutrients 19
CHAPTER THREE 20
3. MATERIALS AND METHODS 20
3.1 Description of the study area and growth condition 20

3.2 Study Design and Period 20
3.3 Sources of Antagonistic rhizobacteria and pathogen strains 21
3.4 Validation of *in vitro* Antagonism Activities 22
3.5 Inoculum preparation and evaluation of carrier 22
3.6 Eco-Physiological characterization 23
3.6.1 Carbon sources utilization pattern of the isolates 23
3.6.2 Nitrogen sources utilization pattern of the isolates 23
3.6.3 Intrinsic Antibiotic Resistance (IAR) 23
3.6.4 Intrinsic heavy metal resistance of the isolates 24
3.6.5 P^H Tolerance 24
3.6.6 Temperature Tolerance 24
3.6.7 Salt Tolerance 24
3.7 Evaluation of Rhizobacteria isolates to fungicide 25
3.8 Mass multiplication of the Rhizobacteria for inoculation 25
3.9 Mass multiplication of the fungal pathogens 25
3.10 Green house experiment 25
3.11 Assessment of disease incidence 26
3.12 Statistical Analysis 27
CHAPTER FOUR 28
4. RESULTS AND DISCUSSIONS 28
4.1 In vitro Screening *Bacillus subtilis and Paenobacillus polymyxa* 28
4.2 Fungicides tolerance by PGPR isolates 29
4.3 Carbon sources utilization pattern of the isolates 29
4.4 Nitrogen sources utilization pattern of the isolates 30
4.5 Effects of salinity, PH and temperature on growth of Bacillus subtilis strains 30

4.6 Intrinsic antibiotics (IAR) and heavy metals resistance (HR)....32
4.7 Determination of shelf life....33
4.8 Assessment of disease incidence....34
4.9 Effects of selected *Bacillus* strains on growth of different chickpea genotypes....36
4.9.1 Effect on Dhera genotypes....36
4.9.2 Effect on JG-62 chickpea genotype....38
4.9.3. Effects on Eshete chickpea genotype....40
4.9.4 Effects of Rhizobacteria inoculation on Habru chickpea genotype....42
5. CONCLUSION....45
6. RECOMMENDATIONS....46
Research Funding Acknowledgment....47
REFERENCES....48
APPENDIXES....70

LISTS OF TABLES

Tables **Pages**

Table 1: Detail information of antagonistic Rhizobacteria. 21
Table 2: Invitro Fungicides tolerance test by Bacillus subtilis and paenibacilluspolymayxa 29
Table 3: Carbon source utilization pattern 29
Table 4: Nitrogen source utilization by *Bacillus subtilis* strains 30
Table 5: Salt tolerance.....31
Table 6: PH and Temperatures tolerance 32
Table 7: Patterns of Antibiotic resistance (µg/ml) of *Bacillus subtilis* strains 32
Table 8: Patterns of intrinsic Heavy metal resistance (µg/ml).....33
Table 9: Shelf life of bacillus subtilis and Paenibacillus polymyxa on different carriers 34
Table 10: Results of percentages of diseases incidence 36
Table 11: Effect of *Bacillus subtilis*, strains on growth of Dhera chickpea genotype 38
Table 12: Pearson correlation for Dhera chickpea genotype after 45 days 38
Table 13: Effect of *Bacillus subtilis*, strains on growth of JG-62 chickpea genotype 39
Table 14: Pearson correlation for Gj-62 Chickpea after 45 day 40
Table 15: Effect of *Bacillus subtilis*, strains on growth of Eshete chickpea genotype 41
Table 16: Pearson correlation for Eshete chickpea after 45 day.....41
Table 17: Effect of *Bacillus subtilis*, strains on growth of Habru genotype.....43
Table 18: Pearson correlation for Habru chickpea after 45 day 44

LISTS OF FIGURES

Figures **Pages**

Figure 1: Trend Area harvested production and productivity of chickpea in Ethiopia. 6
Figure 2: Types of chickpea genotypes cultivated on the world 7
Figure 3: Symptoms of fusarium wilt 10
Figure 4: Map of the study area. 20
Figure 5: In-vitro inhibition of Fusarium oxysporium ceceri by selected rhizobacterial isolates.....28

LISTS OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CSA	Central Statistical Agency
DZARC	Debrezeit Agricultural Research Center
FAO	Food and Agricultural Organization
FOC	Fusarium Oxysporum Ceceri
GAI	Growth in Antagonists Inoculated
GC	Growth in Control
NA	Nutrient Agar
PDA	Potato Dextrose Agar
PGPR	Plant Growth Promoting Rhizobacteria
PI	Percent of Inhibition
PIRG	Percent Inhibition of Radial Growth
PSB	Phosphate Solubilizing Bacteria
RCBD	Randomized Complete Block Design
SDW	Shoot dry weight
SH	Shoot Height
SPSS	Statistical Package for Social Science

ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) are one of the major components of the soil microbial populations, which play significant roles in improving plant growth and protecting plants from disease of which spore forming bacteria such as Bacillus and Paenibacillus are widely known as main contributors to disease management by induction of host defenses and biological control of pathogens. Therefore, the aim of the present study was to characterize and evaluate the potential of selected four Bacillus and one Paenibcillus strains to inhibit Fusarium oxysporum, decrease wilt diseases and improve production of four chickpea genotypes;i.e Dhera, JG-62, Eshete and Habru .The experimental design was a randomized complete block design with three replication. The greenhouse treatment includes five strains of paenibacillus polymxa (ALCR-42), and Bacillus subtilus (ALCR-45, ALCR-46, ARC-262 and ARC-358) strains against Apronstar Fungicides (positive control) and non-bioprimed seeds (negative control).The ecophyisiological characteristics like temperatures, salinity, pH tolerance, carbon, nitrogen source utilization pattern, fungicide resistance, shelf life of the isolates on different carriers were also assessed to select best formulation that favours for long storage period. The ability of the isolates in promoting plant growth parameters was measured using agronomic parameter and the data were analyzed by one way ANOVA. The result showed that the bacillus and Paenibacillus strains exhibited 53–65 % growth inhibition against Fusarium oxysporum, of which ARC-358, ALCR-46, ALCR-262 and ALCR-45 exerted their higher inhibitory effect of 65%, 64.11%, 63.7 % and 61.69% against F. oxysporum ceceri, respectively. In addition, the greenhouse study also indicated that the highest percent wilt disease incidences were observed in JG-62 treated with ALCR-42 (55%) and minimum percent of wilt diseases incidence was observed in JG-62 treated with ARC-262 (22%). The results from growth parameters indicated that Bacillus subtilis strains ARC-262 and ARC-358 induced the highest biomass on all chickpea genotypes. In general, the results suggested the application of bioinoculants of rhizobacteria under a competitive environment can be explored as bio-protectants for improving productivity and suppression of Fusarium wilt in chickpea. However, the application of present findings required further trials under field conditions.

Key words: Antagonistic; Biological control; Fusarium wilt; Rhizobacteria.

CHAPTER ONE

1. INTRODUCTION

1.1. Background to the study

Legumes play an important role in agriculture and are a major source of dietary protein for many people. Chickpea (*Cicer arientinum* L.) is an important annual legume crop in Ethiopia and other countries. It is one of the primitive crops of the world originated in Turkish Kurdistan about 8000-9000 years ago from its wild ancestor *C. reticulatus* (Sunkad *et al*., 2019). It is being extensively grown as major winter crop in more than 50 tropical and sub-tropical countries in the world (Patil *et al*., 2017).

Globally, dry bean is the most widely cultivated grain legume with a total area of 34.5 million ha and 30.4 million tones productions followed by chickpea (17.8 million ha) and cowpea (dry) (12.5 million ha) (Byasik *et al*., 2022). Chickpea is grown in more than 50 countries (89.7% area in Asia, 5% in Africa, 2.6% in Oceania, 2.9% in Americas and 0.4% in Europe). Globally, it is cultivated on over 17.8 million ha of land with production of 9.7 million tons of grain (FAOSTAT, 2018). The crop is widely grown in Turkey, India, Pakistan, Iran, Mexico, Australia, Myanmar, Canada, the United States and Ethiopia (FAOSTAT, 2019).

African countries accounted for 4.55% of global production of which Ethiopia contributed to70.51% (FAOSTAT 2018), and, Ethiopian farmers cultivated chickpea on 242,703 hectares, yielding 499,426 tons of grain (CSA 2018). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production during 1994-2008 (Tebkew and Chris. 2016). The national average yields of chickpea in Ethiopia under farmers' production condition remains less than 2.0 tons per hectare (CSA, 2018).

The low average productivity of chickpea in Ethiopia is low due to various abiotic and biotic factors that adversely affect the chickpea productivity around the globe (Caballo *et al.,* 2019). Among the biotic stresses, the most important limiting factor in crop production are fusarium wilt, ascochyta blight, pod borer, and cutworm, of which fusarium wilt was the most devastating pathogen of chickpea. Fusarium wilt is caused by a fungus pathogen Fusarium oxysporum is a major constraint to chickpea cultivation throughout the world and especially in Indian subcontinent where chickpea is a commonly grown pulse crop causing up to 100% yield loss annually (Sunkad *et al.,* 2019: Muhammad Rafiq *et al.,* 2020). It is a vascular

pathogen that perpetuates in seed and soil and hence is difficult to manage by the use of single control method (Barlow *et al*., 2012).

In Ethiopia, Fusarium wilt/root rot diseases prevalent in almost all the surveyed chickpea-growing areas of Ethiopia (Sultman Mohammad *et al* 2018; Dagnachew Bekele *et al* (2021).The recent survey on selected fields of chickpea growing kebeles among Ambo and Dendi districts of West Shewa indicated prevalence and incidence of the disease of 92.9%, and 35.09%, respectively (Daniel Assfaw and Tilahun Negash,2020).The higher prevalence and incidence of the disease was recorded in Ambo district with 40.96% and 93.5%, respectively while, in Dendi district it was 29.10% and 92.3%, respectively. Dagnachew Bekele et al (2021) reported the occurrence of Fusarium wilt and root rot diseases throughout chickpea cultivated regions in Ethiopia. Average disease incidence was highest throughout Amhara (30.98%) and Oromiya (22.83%) regions, and lower in SNNP (15.18%), although there was significant variation among zones in each region.

1.2. Statement of the problem

Ethiopian farmers cultivated chickpea on 242,703 hectares, yielding 499,426 tons of grain (CSA 2018). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production during 1994-2008 (Tebkew and Chris. 2016). The national average yields of chickpea in Ethiopia under farmers' production condition remains less than 2.0 tons per hectare (CSA, 2018).The low average productivity of chickpea in Ethiopia is low due to various abiotic and biotic factors that adversely affect the chickpea productivity around the globe (Caballo *et al*., 2019).

In Ethiopia 30% yield loss occurs due to Fusarium wilt (Meki et al. 2008). For many years now, different studies were undertaken on BNF and plant growth promoting properties of leguminous crops from different parts of Ethiopia. For instance, different research were conducted on chick pea and lentil (Mulisa Jida and Fassil Assefa, 2012), soyabean (Diriba Temesgen and Fassil Assefa, 2017), and cowpea (Girmaye Kenasa and Fassil Assefa, 2017). The studies showed these legumes harbored different effective PSB with multiple growth promotion properties that enhanced plant production but in Ethiopia Limited research efforts have been made to find out effective and appropriate biocontrol strategies in managing Fusarium wilt/root rot diseases using *Bacillus subtilis* and *paenibacillus polymayxa* is not widely exploited.

1.3. Research Questions

This research project is designed to answer the following questions:

- What are the potential roles of *Bacillus subtilis strains* as biological control of fusarium wilt root rot?
- What are the effects of the different *Bacillus subtilis* strains on growth parameters of chickpea genotypes?
- Which chickpea genotypes are more benefited from *Bacillus subtilis* inoculation?
- Which carrier materials support maximum shelf life for longer time?

1.4. Objectives of the Study

1.4.1. General Objective

To evaluate the anatagonistic potential of Bacillus subtilis strains_as biocontrol agent against fusarium wilt disease under greenhouse condition using resistant and susceptible chickpea varieties in Ethiopia.

1.4.2. Specific objectives

- To determine Eco physiological characterstics of the *Bacillus subtilis* isolates
- To evaluate the biocontrol potential of *Bacillus subtilis and paenobacllus poymayxa* against fusarium wilt disease under greenhouse conditions using resistant and susceptible varieties
- To determine shelf life of the formulated Bacillus subtilis inoculants.
- To identify relevant carrier materials and formulation for selected *Bacillus subtilis* inoculants.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Botanical description of chickpea

Cultivated chickpea is a self-pollinated, diploid (2n= 2x =16) annual pulse crop with a relatively small genome size of 738Mb (Varshney *et al.*, 2013, Sharma *et al.*, 2010). The crop is an herbaceous, a small bush with diffused spreading branches from the base, which reach a height of 20–150 cm depending on cultivar and suitability of the growing environment. Its stem is mostly erect, branched and solid and has strong deep taproot system which makes it relatively drought tolerant. The crop has an indeterminate growth habit which continues to produce vegetative growth whenever soil moisture, temperature and other environmental factors are favorable (Akkopru *et al*., 2011).

2.2 Taxonomy

Chickpea is classified as, Kingdom Plantae, Family Fabaceae, Division Magnoliophyta, Subfamily Faboideae, Class Magnoliopsida, Genus Cicer, Order Fabales and Species C. arietinum .The genus Cicer L. (Leguminosae, Cicereae) comprises 9 annual and 35 perennial species that have a center of diversity in south-western Asia, with remote, endemic species found in Morocco and the Canary Islands. Out of 9 annual species, chickpea (Cicer arietinum L.) is the only cultivated species. The eight other annual species of chickpea are wild and include: Cicer reticulatum, Cicer echinospermum, Cicer pinnatifidum, Cicer judaicum, Cicer bijugum, Cicer cuneatum, Cicer chorassanicum and Cicer yamashitae (Singh *et al*., 2008).

2.3 Climate and Growth Conditions

It is grown under wide agro climatic conditions around the world. It is grown between 20◦N and 40◦N in the northern hemisphere and is also cultivated on a small scale between 10◦N and 20◦N in India and Ethiopia at relatively higher elevations (Berger *et al*., 2006). Growing regions of chickpea can be broadly divided into two, non- tropical dry areas and semiarid tropics (Imtiaz *et al*., 2011). Chickpea is usually grown on black vertisol, Such kind of soil is known for excess water and drainage problem during the main rainy period (June-August) Hence, to come out of this complication farmers grown chickpea during the post-rainy season in the season (September- October) commonly on residual moisture to escape waterlogging

conditions and major diseases associated with high humidity during the rainy season (Legesse Dadi *et al*., 2005; Sisay Guta, 2018; Lijalem Korbu *et al*., 2020).

2.4 Chickpea production in Ethiopia

Ethiopia is the leading chickpea producer in Africa, producing more than 500,000 metric tons per year from an area of ~243,000 ha of smallholder farms (CSA, 2019; FAOSTAT, 2019), accounting for over 90% of grain production in sub-Saharan Africa (Verkaart *et al*., 2017). In Ethiopia, chickpea is grown primarily on heavy black soil (vertisols), within an altitudinal range of 1,500 to 2,800 m above sea level from mid-August to First week of September on residual moisture where the mean annual rainfall ranges from 700 to 2000mm (Geletu Bejiga and Million Eshete, 1996; Dagnachew Bekele *et al*., 2021). Vertisols are heavy clay soils with a high proportion of smectites that are formed from parent materials of fine grained basic nature (Eyasu, 2016; Dagnachew Bekele *et al*., 2021). Smectite clays reversibly expand and contract upon wetting and drying. During the dry season, the clay shrinks, hardens and forms deep, wide cracks, and the surface soil becomes granular. During the wet season, the previously dry clay becomes wet and expands to form a sticky, plastic substrate (Eyasu, 2016; Dagnachew Bekele *et al.*, 2021).

The usual periods for harvesting in most growing areas takes place 4-5 Months after emergence where pods turn to golden brown. The usual period of harvesting is known to be from December to January. Most of the time harvesting is performed manually by pulling the entire plant from the soil, and then drying and threshing by beating with stick or driving oxen n the heaps (Tebikew *et al*., 2009).

Ethiopia is a secondary center of genetic diversity for chickpea, where the wild relative of cultivated chickpea, Cicer cuneatum L., is found in the Tigray region of Ethiopia (Yadeta and Geletu, 2002; Gemechu *et al*., 2012). It is also the seventh largest producer worldwide and contributes about 3% to the total world chickpea production (Menale *et al.*, 2009; Abate *et al*., 2011). Ethiopia has diverse agro ecologies with high potential for chickpea production (Gemechu Keneni *et al*., 2012;Asnake Fikre *et al*., 2018; Lijalem Korbu *et al*., 2022), making it one of the world's leading countries in terms of productivity per unit area (FAO, 2018).

Although chickpea is widely grown in Ethiopia; the major producing areas are concentrated in the two regional states i.e., Amara and Oromia. These two regions cover more than 90% of the entire chickpea area and constitute about 92% of the total chickpea production (CSA, 2020).

From Amhara region, the major producing zones are North Gonder, South Gonder, North Shewa, East Gojam, South Wello, North Wello, West Gojam, (Gonder Zuria) account for about 80% of the country's chickpea production(CSA,2020). In the Oromia region, the major producing zones are in West Shewa, East Shewa and North Shewa, which account for about 85% of the total area and production in this regional, state (Menale *et al*., 2009, Merkuz *et al*., 2010). Chickpea is mainly grown in Amhara (52.5%), Oromia (40.5%), SNNP (3.5%) and Tigray (3%) (Asnake Fikre, 2014, CSA, 2016). According to CSA (1995–2017), chickpea area, production, and productivity in Ethiopia have been increased between 1995- 2017, respectively (Figure1).

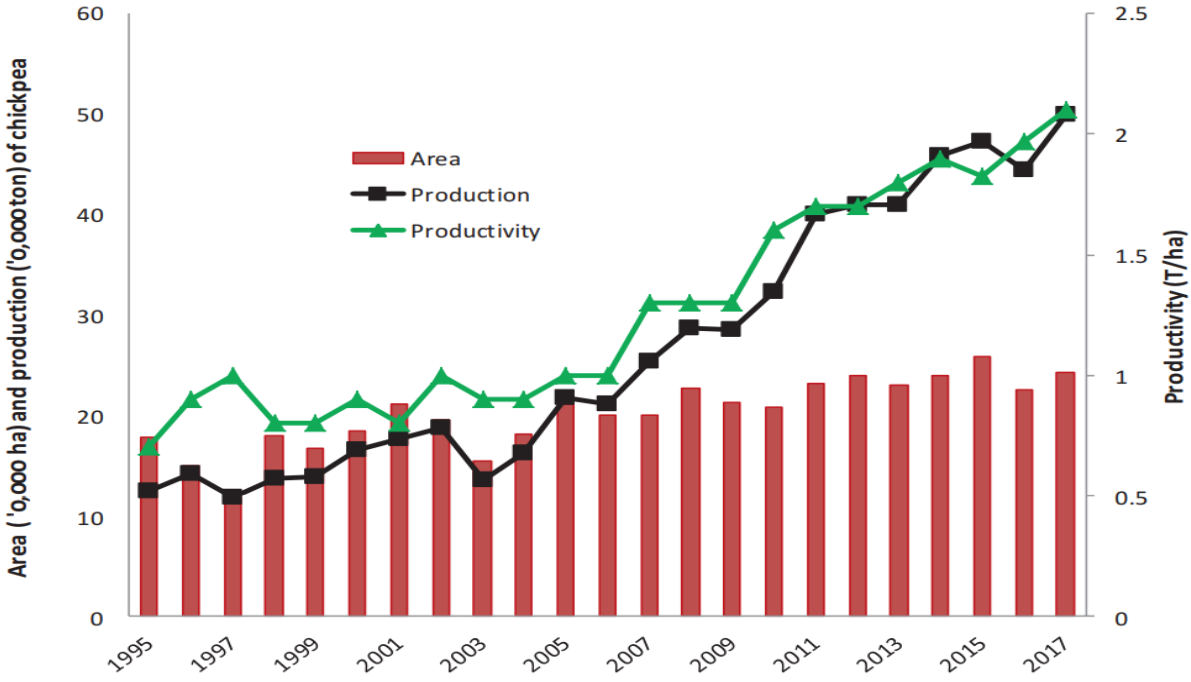


Figure 1: Trend Area harvested production and productivity of chickpea in Ethiopia.

Source: CSA (1995–2017).

2.5 Types of Chickpea

There are two distinct types of cultivated chickpea (Desi and Kabuli) based on morphological characters mainly seed size, shape and color, plant growth habit, pigmentation of vegetative floral part (Gaur *et al*., 2010). About 95% of Ethiopian chickpea production is predominated by Desi type chickpea (Castro *et al*., 2012). However, recently there has been an increase in the interest of farmers in growing large seeded Kabuli varieties due to their higher price in the

market (CSA, 2016). The two types represent different genetic background in disease resistance and important agronomic traits, such as cold tolerance and growth habit (Menale kassie *et al.*, 2009).

Therefore, there is a higher economic incentive for farmers to shift from Desi to Kabuli production due to its high price in world. Moreover,Ethiopia chickpea has enjoyed by the Kabuli types joined since the mid-1980s and accelerates over the Desi types due to some peculiar traits (AB-resistance, taste, seed texture, etc.) liked by the market and consumers (Asnake and Dagnachew,2020).

Figure 2: Types of chickpea genotypes cultivated on the world (*Source*: Gaur et al., 2010)

2.5.1 Desi chickpea

Chickpeas with colored and thick seed coat are called Desi type. The common seed colors include various shades and combinations of brown, yellow, green and black. The seeds are generally small and angular with a rough surface (Wood *et al.*, 2012). The flowers are generally pink and the plants show various degrees of anthocyanin pigmentation, although some Desi types have white flowers and no anthocyanin pigmentation on the stem. The Desi types account for 80- 85% of chickpea area in Ethiopia (Gaur *et al.*, 2012).

2.5.2 *Kabuli chickpea*

The Kabuli type chickpeas are characterized by white or beige-colored seed with ram's head shape, thin seed coat, smooth seed surface, white flowers, and lack of anthocyanin pigmentation on the stem (Jukanti *et al*., 2012). As compared to Desi types, the Kabuli types have higher levels of sucrose and lower levels of fiber. The Kabuli types generally have large sized seeds and receive higher market price than Desi types (Gaur *et al.*, 2010).

2.6 Agronomic and economic importance of Chickpea

2.6.1 Nodulation and nitrogen fixation

The biological nitrogen fixation is a process converting inert atmospheric dinitrogen (N_2) to biologically available form ammonia (NH_3) by prokaryotic micro-organisms. Prokaryotic organisms (bacteria and the archaea) (diazotrophs) have the enzymatic nitrogenase, encoded by the nif gene; to break the strong triple bond between the two N atoms and make them reactive with hydrogen atoms to form ammonia (Kumar *et al.*,2019). BNF is categorized into two categories; non symbiotic nitrogen fixing group that are either free living, associative or endophytic and endo-symbiotic nitrogen fixation. A symbiotic nitrogen fixation is mediated by bacteria such as *Cyanobacteria, Azotobacter, Azospirillum, Acetobacter diazotrophicus, Azoarcus* (Ipek *et al.* 2019). The second category includes; nodule forming bacteria (Rhizobiaceae family) with Leguminous plants and the actinomycete Frankia with non-leguminous plants. It is estimated that Biological nitrogen fixation (BNF) contributes ~139 to 170×10^6 tons of nitrogen per year to the terrestrial ecosystem compared to the ~65×10^6 tons of nitrogen added in the form of synthetic fertilizer per year (Khan *et al.*,2019).

2.6.2 Nutritional value of chickpea

It is an excellent source of protein, fiber, complex carbohydrates, vitamins, and minerals, as a cash crop for smallholder producers. It also increases livestock productivity as the residue is rich in digestible crude protein content compared to cereals (Menale Kassie *et al.*, 2009). Chickpea seed contains highly digestible protein content (23%) and also rich in carbohydrates (64%) starch (47%), fiber (6%), and minerals (phosphorus, calcium, magnesium, iron and zinc). Its Lipid fraction is also high in unsaturated fatty acids (Jukanti *et al.*, 2012). According to Mamta *et al.* (2021), chickpea seed contain 22.1 -24.42% proteins, 0.15-1.25 sulphur-containing amino acid and 0.63-1.38% Tryptophan.

2.7 Major Constraints of Chickpea Production

Despite its large cultivation area and considerable economic importance, chickpea's productivity in Ethiopia remains as low as 2 tons/ha (CSA 2018; Dagnachew Bekele *et al*., 2021) and in many regions is declining. Decreasing productivity derives in large part from constraints of diverse biotic and abiotic stresses that are exacerbated by insufficient crop management practices which reduces seed yields. Nearly 172 pathogens (About 67 fungi, 22 viruses, 3 bacteria and 80 nematodes) have been reported so far that infect chickpea in

different parts of the world (Gurjar *et al*., 2010)), but only few of these cause economically important diseases (Haware, 1998).

Among fungal diseases impacting chickpea are *Fusarium wilt, dry root rot, black root rot, wet root rot, collar rot and Ascochyta blight,* caused respectively by *Fusarium oxysporum, Rhizoctonia bataticola, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Ascochyta rabiei* (Ahmed and Melkamu 2006; Navas Cortes *et al.* 2008;Dagnachew Bekele *et al*., 2021). Ascochyta blight and fusarium wilt are the most devastating diseases affecting chickpea in temperate and tropical regions, respectively; while in the Mediterranean countries, ascochyta blight, fusarium wilt, grey mold, stem rot, stunt and root rot are the most commonly occurring diseases (Gurjar *et al*., 2010).

According to Yimer et al. (2018), in major chickpea growing areas of Ethiopia the dominant root infecting pathogen is *Fusarium oxysporum f.sp. ciceris*, followed in importance by *Fusarium solani*. Fusarium wilt disease can cause yield losses approaching 100% in highly infested fields (Sharma *et al.* 2016; Upasani *et al*. 2017).

2.8 Fusariums wilt

Fusariums wilt, caused by Fusarium oxysporum f.sp. Ciceris (Foc, Padwick) is one of the most important serious soil or seed borne disease of chickpea (Hossain *et al*., 2013; Merkuz *et al*., 2011). The average annual yield losses due to wilt have been estimated to 10 to 90% and sometimes increased to 100% when the relative humidity is greater than 60% and temperature ranges between 10 and 25°C (Cortes *et al.*, 2012; Kumar *et al*., 2012; Sunkad *et al.*, 2019: Muhammad Rafiq *et al*., 2020). *Fusarium oxysporum f.sp. Ciceris* is a vascular pathogen that perpetuates in seed and soil and hence is difficult to manage by the use of single control method (Barlow *et al.*, 2012).

2.8.1 Symptoms of Fusarium oxysporum causing wilt of chickpea

Wilt induced by an important soil borne pathogen Fusarium oxysporum f. sp. Ciceris is regarded as the most deadly and widespread disease among the fungus that harm chickpea (Jiménez-Díaz *et al*., 2015). Wilt is a vascular disease that causes xylem browning or blackening due to melanin pigment and disrupts water and nutrient transfer, causing the plant to wilt or die Different chickpea cultivars exhibit wilting at different phases of growth, resulting in varying degrees of yield loss. Under extreme circumstances, the wilt infection can entirely destroy the crop, resulting in a 100% yield loss (Jiménez-Díaz *et al.*, 2015).

Symptoms can be developed at any stage of plant growth, and affected plants may be grouped in patches or appear spread across a field (Jimenez-Diaz *et al*., 2015). The wilt can be observed in susceptible genotypes within 25 days after sowing in the field (designated "early wilt"). However, symptoms are usually more visible in the early stages of flowering, 6 to 8 weeks after sowing and can also appear up to podding stage ("late wilt"). Late wilted plants exhibit drooping of the petioles, rachis and leaflets, followed by yellowing and necrosis of foliage (Jiménez-Díaz *et al*., 2015).

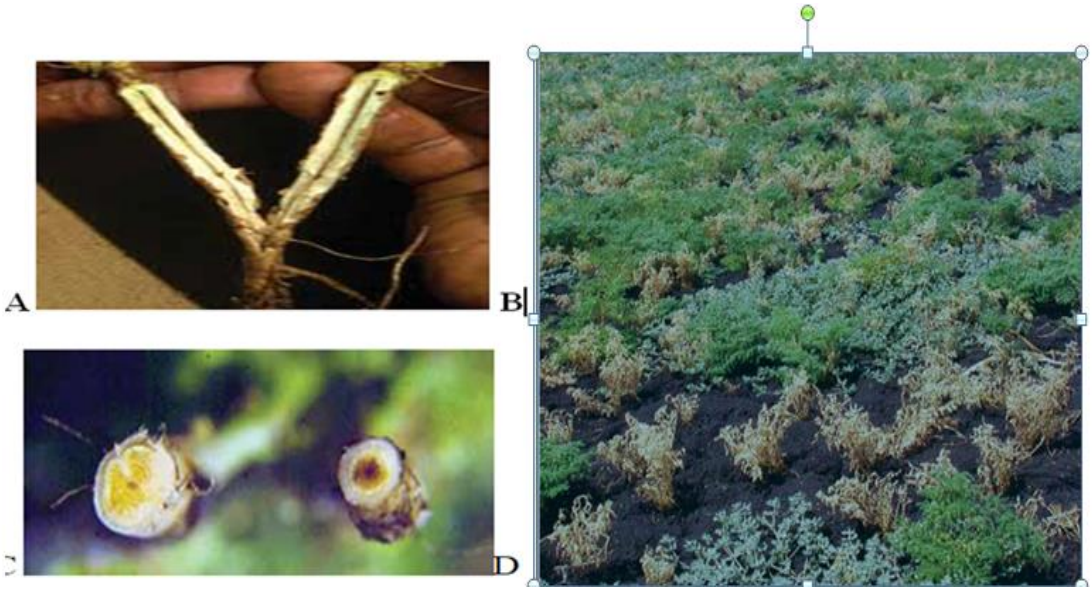


Figure 3: Symptoms of fusarium wilt (Nene *et al*., 2012)

(A); Internal blackening of the stem caused by Fusarium wilt(B); Drooping of Fusarium wilt affected plant (C) and a transverses cut of stem showing xylem blackening caused by Fusarium wilt (D). This adopted from Nene *et al*. (2012).

2.9 Epidemiological Fusarium Wilt in Ethiopia

Fusarium wilt caused by *F. oxysporum f.sp. ciceris* is a major yield-limiting factor wherever the crop is grown in Ethiopia (Nigussie 1996). Nigussie (1995) reported that wilt and root rot are the major diseases of chickpea and lentil grown on black soils of Ethiopia. He found that the chickpea wilt and root rot incidence ranged from 29% to 68% in Shewa, 40% to 53% in Wollo, 26% to 52% in Tigray, 3% to 67% in Gonder and 29% to 65% in Gojam. Out of the samples collected from these five regions, *F. oxysporum f.sp. Ciceris* was isolated from 50% of the disease samples.

Daniel As and Tilahun Negash (2020) reported the occurrence of chickpea fusarium wilt in selected fields of chickpea growing kebeles among Ambo and Dendi districts of West Shewa, Ethiopia found that, among 70 chickpea fields surveyed in both districts, the overall mean prevalence and incidence of the disease were 92.9%, and 35.09%, respectively. The higher prevalence and incidence of the disease was recorded in Ambo district with 40.96% and 93.5%, respectively while, in Dendi district it was 29.10% and 92.3%, respectively.

Global positioning system (GPS) based field surveys conducted in 2015 and 2016, in Oromiya, Amhara and SNNP regions; including 52 districts in 17 diverse chickpea growing zones revealing that Fusarium oxysporum f. sp. ciceris (Foc) wilt diseases were widely distributed in all surveyed areas. Across all surveyed sites, Fusarium oxysporum f. sp. ciceris (Foc) was the predominant species encountered among fungi cultured from plant tissue, representing 69.4% of total isolates indicating that Fusarium wilt and root rot diseases are ubiquitous throughout chickpea cultivated regions in Ethiopia. The Average disease incidence was highest throughout Amhara (30.98%) and Oromiya (22.83%) regions, and lower in SNNP (15.18%), although there was significant variation among zones in each region (Dagnachew *et al*., 2021).

2.10 Chickpea root rot disease management strategy

Several strategies are used by chickpea growers to manage Fusarium wilt/root rot complex in different countries that include crop rotation or application of chemicals are there, but it is difficult to manage fusarium wilt by either of these, because of soil nature persistence and its capacity to survive for long time even in the absence of host (Moradi *et al*., 2012). Fusarium wilt of chickpea can be managed using cultural practices (diversified crop rotation and adjusting sowing dates), growing resistant cultivars, fungicide seed treatment, and biological control (Sampaio *et al*. 2020; Amine Elbouazaouia *et al*., 2022). The use of wilt-resistant chickpea varieties and adjustment of sowing dates are potentially cheap and easily adoptable methods in managing chickpea wilt. Developing and releasing wilt/root rot-resistant cultivars is the major objective of the national chickpea improvement programs and chickpea varieties having resistance to wilt/root rot have been released for cultivation in Ethiopia (Awoke Ayana *et al*., 2019).

2.10.1 Cultural practices

Research on cultural control was focused only on date and depth of sowing and manipulation of agronomic practices. Merkuz *et al*. (2011) reported that Fusarium wilt incidence was reduced with different doses of green manure and dried plant residue and none of the treatments showed complete disease suppression. Cultivation methods involving destruction of inoculum pool in un-decomposed fresh organic matter is an excellent medium for root rot causing pathogen at the time of seed bed preparation gives effective results in control of root rot disease (Ahmed and Ayalew, 2006;Daniel Asfaw,2018).

In addition since only chickpeas are susceptible several cultural practices such as rotation with non-host crops, not growing chickpeas more frequently than every 3-4 years, and not planting new crops near previous blighted fields, the use of disease free seeds and destruction of plant diseased debris, will all help to reduce inoculums level and inhibit severe epidemics. Tillage practices like burial of infected residue and controlling volunteer chickpeas will also be beneficial (Daniel Asfaw, 2018).

2.10.2 Mechanical control

Deep ploughing over summer and removal of infected trash can reduce inoculum levels of fusarium wilt of chickpea. Solarization of soil by covering the soil with transparent polythylene sheeting for 6-8 weeks during the summer months has been shown to effectively control fusarium wilt of chickpea and improve plant growth and yield (Chauhan *et al.* 1988). However, this method of control is not practical for broad-acre farming systems.

2.10.3 Chemical control

For chickpea obtaining useful levels of fusarium wilt control with seed-applied fungicides can be considered effective. Fungicides are powerful chemicals which may have a significant impact on the environment, in either a positive or negative way. The fungicides like Thiram and Apron star offers a good protection against wilt (DZARC, 2005). Some of the advantages of Fungicides are that they are fast acting, they can control large infestations, they are easy to obtain and apply, and they may increase crop production by reducing crop losses (Yonas Urbanos, 2010). Some of the disadvantages of pesticides are that high usage of chemical fungicides may causes changes in soil microbiome, damage and/or accumulate within the environment, environmental pollution, they may kill non-target species, pathogens may also develop fungicidal resistance, suppress host defense mechanisms, increased risk to human and

animal health and creates an imbalance in the microbial community in soil (Jimenez-Diaz *et al.*, 2015; Anusha *et al.*, 2019; Sankar *et al.*, 2019).

Moreover, due to the soil borne nature of these pathogens, chemical control is generally in efective (Jimenez Diaz *et al.* 2015).So, the alternative possible progress for management of the wilt disease is through biocontrol agents specifically rhizobacteria otherwise called as "Plant growth promoting rhizobacteria" (PGPR) (Datta *et al*., 2011; Sankar *et al.*, 2019).

2.10.4 Biological control

The application of antagonistic microbes to a host plant that inhibits pathogen growth is referred to as a biological control (Selim *et al*., 2017;Osei *et al*., 2022). Biological Control is the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists. The biocontrol potential of rhizobacteria in controlling the growth of various phytopathogens can be attributed to their ability to colonize plant tissues in different ways; they compete for space and nutrients, produce secondary metabolite secretions such as bacteriocins, lytic enzymes, antibiotics, hydrogen cyanide, and exopolysaccharide, and stimulate novel genes involved in secretion systems and (Adeleke *et al*. 2022). Similarly, antagonistic bacteria deprive the pathogen of iron by producing siderophores and ultimately exclude the pathogen from the slot (Beneduzi *et al*. 2012). Biological control provides an alternative to the use of synthetic pesticides with the advantage of greater public acceptance and reduced environmental impact (Reino *et al*., 2008).

Moreover, Bio-inoculants are preferred over synthetic chemicals due to their eco-friendly and cost-effective nature for plant growth promotion and suppression of disease (Sharon Nagpal *et al.*, 2020**)**. Environment friendly species of Rhizobium, Bacillus, Pseudomonas, Azotobacter and Burkholderia have been widely exploited as alternatives to the deleterious chemical agents to control disease (Das et al. 2017). In this regard, antagonistic bacteria and fungi are widely used to control plant diseases (*Shahzad et al*., 2018).

At present, biological control of soil and seed borne plant pathogenic fungi has been addressed mainly by using bacterial and fungal antagonists. The strains of *Trichoderma sp*ecies and nonpathogenic isolates of *Fusarium oxysporum* and some rhizobacteria especially *Pseudomonas spp., Bacillus spp., Azospirillum, Serratia, Pythium* and *Coniothyrium* isolated

from the rhizospheres of crop plants, are reported to be effective not only to control plant pathogens but also help the plants to mobilize and acquire nutrients (Gopalakrishnan *et al*., 2011;Heidarzadeh & Baghaee Ravari 2015). When integrated with moderately susceptible or resistant cultivars Trichoderma species are more effective in controlling Fusarium wilt by 30–46% (Meki *et al*., 2011).

Moreover the use of biocontrol agents is much safer and is presumed to be less polluting to the environment than the chemical pesticides (Sumeet and Mukerji, 2000).The drawback of this management option was the short life span of the micro-organisms and the over reliance on conducive environmental conditions which might not always be possible (Kirui Kipngetich Rickson, 2016).

2.11 Mechanisms of plant Growth Promoting Rhizobacteria and its importance

2.11.1 Direct Antagonism

Direct plant growth promotion occurs when PGPR increase plant growth by supplying growth factors such as nutrients and hormones to plants (Lugtenberg and Kamilova ., 2009; Rufus J. Akinrinlola *et al.*, 2018).Direct antagonism results from the physical contact and / or high degree of selectivity for the pathogens by bio control agent. This includes:

2.11.1.1 *Nitrogen fixation*

Nitrogen is one of the important nutrients essential for the growth of all living organisms including plants and bacteria. The observation of nitrogen deficiency in soil has led to the use of large amounts of nitrogenous fertilizers in order to make up for the necessary plant requirements to achieve maximum plant yield in most soils (Zhang *et al*. 2015). Despite nitrogen's abundance in the earth's atmosphere, about 78%, this form of gaseous nitrogen N_2 (g) is not readily accessible to most organisms, i.e. it is not suitable for plant assimilation until it is first converted to ammonia (Baas *et al.* 2014). This conversion to ammonia requires a significant amount of energy because of the stability of the triple bond in N (g). This energy may be provided at the expense of fossil fuels, through biological nitrogen fixation at the expense of ATP and through other mechanisms of nitrogen input in terrestrial systems.

The free-living nitrogen-fixing PGPR include *Azotobacter, Azospirillum, Herbaspirillum, Bacillus, Burkholderia, and Paenibacillus,* which have been shown to attach to the root and efficiently colonize root surfaces (Aloo *et al.,* 2019).

2.11.1.2 *Phosphate solubilization*

Phosphorus (P) is the second most important plant growth-limiting nutrient after nitrogen. The majority of phosphorus is in its insoluble form, whilst the plants can only absorb phosphorus when it is bonded with oxygen as in monobasic (H_2PO4^-) and dibasic forms ($HPO_4^-)_2$. A low abundance of phosphorus is typical in many agricultural soils, where phosphates make complexes with soil constituents, making them unavailable to many organisms (Lambers *et al.*, 2013). To overcome phosphate deficiency, phosphate dense fertilizers are applied to crops regularly. However, plants can only absorb limited amount of phosphates and the rest is rapidly converted into insoluble P. There is also an extensive loss of phosphates in agricultural lands via run off and much of the phosphate ends up in water reservoirs. Bacteria that solubilize phosphorus are referred to as phosphate solubilizing bacteria (Alori *et al.* 2017).The most powerful phosphate solubilizers are bacteria of the genera *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratiaare* reported as the most effective PSB are known to solubilize P from substrates and make it available to plants, and hence are a possible alternative to phosphate rich fertilizers (Bhattacharyya *et al*., 2012).

The principal mechanisms of inorganic phosphate solubilization by Phosphate-solubilizing bacteria employ different strategies to convert unavailable forms of phosphate into available forms. In most bacteria, the production of organic acids is shown to be related to the dissolution of mineral glucose to gluconic acid (GA) as the foremost mechanism for mineral phosphate solubilization in Gram-negative bacteria. Organic acids released by microorganisms act as good chelators of divalent cations of Ca^{2+} coupled with the release of phosphates from insoluble complexes (Sharma *et al.* 2017)). The organic acids which are either gluconic or keto gluconic acids are produced and excreted by phosphate solubilizing bacteria resulting reduce the pH medium either by H+ extrusion or by secretion of various organic acids (Sharma *et al.* 2017) resulting in the acidification of the microbial cells and their surroundings, hence, phosphate ions are released (Khosro 2012). Many of the phosphate-solubilizing microorganisms cause a reduction in the pH of the medium.

2.11.1.3. ***Phytohormone production***

PGPR also produce multitudes of phytohormones such as auxins (Indole acetic acid; IAA), gibberellins, cytokinins, abscisic acid and ethylene ammonia (Kour *et al.*,2019). Indole acetic

acid (IAA) is a common product of L- tryptophan metabolism produced by PGPRs helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Mohite,2013). It has been estimated that more than 80% of the soil bacteria are able to produce auxins, especially IAA, indolebutyric acid or similar compounds derived from tryptophan metabolism (Vargas *et al.,* 2010). The naturally occurring auxin indole-3-acetic acid (IAA) promote root elongation lateral root development and is involved in the early steps of nodule organogenesis (Coba de la Pena and Pueyo,2012). Cytokinins mediate the rhizobial infection in legumes and increase chlorophyll content.

Abscisic acid (ABA) is an important plant hormone related to plant response to drought; Abscisic acid could inhibit nodulation, rhizobial infection and gene expression of several nodule associated genes (Valentine *et al*.,2011). The production of gibberellins stimulates the root system and few bacterial species such as *Bacillus pumilus* and *Bacillus licheniformis* are capable of producing the hormone (Jha and Saraf, 2015).

2.12. Indirect antagonism

Bio control agents can reduce the disease incidence of crops by increasing their growth at least during the early stages of the life cycle. The best example of this is the resistance of damping off of Solanaceous crops with advance of age. Indirect effects include antibiotics production, nutritional competition, parasitism, pathogen toxin inhibition, and induced resistance (Elnahal *et al*., 2022).

2.12.1. Production of antibiotics

Many PGPR have the ability to produce peptide antibiotics. Antibiotic production is the prominent mechanism to suppress the growth of phytopathogens (Sindhu *et al.* 2016).Many antibiotics have been derived from bacteria of the genera *Bacillus* and *Pseudomonas.* They produce a variety of metabolites which serve as antifungal, antibacterial, antihelminthic, antiviral, antimicrobial, phytotoxic, antioxidant, cytotoxic, and antitumor agents. For Bacillus, they are either derived from the ribosome or the non-ribosomal peptide and/or polyketide synthetases (NRPSs/PKS). Examples include Tas A, sublancin, subtilosin, bacilysin, chlorotetain, subtilin, bacillaene, surfactin, iturin, and fengycin while from Pseu domonas such as Ecomycins, 2,4-Diacetyl Phloroglucinol (DAPG), Pseudomonic acid, Phenazine-1-carboxylic acid (PCA), Pyoluteorin, Pyrrolnitrin, OomycinA, Cepaciamide A, Viscosinamide,

Butyrolactones, Zwittermycin A, Aerugine, Azomycin, Rhamnolipids, Cepafungins, Kanosamine, and Karalicin have reported (Goswami *et al*. 2016;Olanrewaju *et al*.,2017).

These are oligopeptides that inhibit synthesis of pathogens cell walls, influence membrane structures of cells, and inhibit the formation of initiation complex on small subunit of ribosomes (Kundan *et al.*, 2015).Some studies showed an active influence of bacterial antibiotics in the regulation of defense system of the plant. It was revealed that *Bacillus subtilis* surfacine is able to stimulate induced systemic resistance by activation of components like lipoxygenases, lipid peroxidases and the formation of reactive oxygen species (Satyavir *et al.*, 2009). Antibiotics fengycin and iturins were produced by *Bacillus XTICECT8661 strain* which inhibited the growth of fungus Podosphaera fusca (Toral *et al*., 2018).

2.12.2. Secretion of lytic enzymes

The production of lytic enzymes by rhizospheric bacteria involved in the control mechanisms against plant root pathogens including *Fusarium oxysporium* and *Rhizoctonia solani* (Labusinagne *et al*., 2010). Hydrolytic enzymes act as agents for prevention of plant diseases by causing lysis of deleterious microbes in the close vicinity of the plant as they secrete increased level of cell wall lytic enzymes (chitinases, glucanases and proteases) (Figueiredo *et al*.,2016a).

Some PGPR strains have been found to produce enzymes that can lyse fungal cell walls. Cell walls of most of the phytopathogenic fungi (except Oomycetes) are made up of chitin, Chitinase which hydrolyse this polymer are produced by various organisms and have been implicated in the control of fungal diseases. Inactivation of genes involved in their biosynthesis has been used to provide evidence for their contribution in biocontrol in plant (Kundan *et al*., 2015).

The soil borne *fluorescent Pseudomonas* has received particular attention because of their capacity to produce a wide range of enzymes and metabolites. Antagonistic or biocontrol activity of *fluorescent Pseudomonas* is due to the production of different types of cell wall degrading enzymes like chitinase, protease/elastase and β-1, 3 glucanase. These enzymes are supposed to degrade the cell wall of various bacterial and fungal plant pathogens. Interestingly, some allelochemicals produced by PGPB are used as experimental pharmaceuticals, and this group can serve as additional resources to deal with the alarming ascent of multidrug-resistant human pathogenic bacteria (Premachandra *et al*., 2016**).**

2.12.3. Hydrogen cyanide (HCN) production

Hydrogen cyanide (HCN) is secondary metabolite produced by many rhizosphere bacteria and plays a significant role in biological control of the pathogens. It is likely to inhibit electron transport chain and energy supply to cell, leading to death of cells. It also seems that PGPR inhibit proper functioning of enzymes and natural receptors reversible mechanism of inhibition and also known to inhibit the action of cytochrome oxidase (Behargavi *et al*., 2016). Recently, many different bacterial genera have shown to be capable of producing HCN.These bacterial genera including species of *Alcaligenes, Aeromonas, Bacillus, Pseudomonas* and *Rhizobium.* Various studies attribute a disease protective effect to HCN, in the suppression of "root-knot" and black rot in tomato and tobacco root caused by the nematodes *Meloidogyne javanica* and *Thielaviopsis basicota*, respectively (Satyavir *et al.,* 2009).Suppression of tomato root knot disease caused by *Meloidogyne javanica* have been attributed to the effect of HCN (Siddiqui et al. 2006) as well as the control of *Odontotermes obesus,* a crop pest in India (Kumar *et al*. 2015).

2.12.4. Induced Systemic Host Resistance (ISR)

Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses. Biocontrol agents induce a sustained change in the plant; increasing its tolerance to infection by a pathogen and this phenomenon is called induced systemic resistance (Satyavir *et al.,* 2009).Moreover, ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses against a variety of plant pathogens (Glick, 2012).

Bioagents increased the defensive system of plants by increasing the antioxidants genes, such as superoxide dismutase, peroxidase (POD) and catalase (CAT), and eventually increased the plant growth and yield production (Osei *et al*., 2022). Fungal proteins such as xylanase, cellulases, endochitinase secreted by *Trichoderma species* can also enhance defense, probably through induction of plant defense related proteins. Moreover, various non-pathogenic Pseudomonas rhizobacteria, *Bacillus spp* also have the ability to induce a state of systemic resistance in plants, which provides protection against a broad spectrum of phytopathogenic organisms including fungi, bacteria, and viruses (Satyavir *et al.,* 2009).

2.12.5. Competition for niche and nutrients

From the microbial perspective, soils and living plant surfaces are nutrient limited environments. Both the bio control agents and the pathogens compete with one another for the nutrients and space to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio control agent whereby the pathogens are excluded by the depletion of food base and by physical occupation of site (Pal *et al*., 2006). *Bacillus sp*. inhibited the growth of pathogenic fungi Botrytis cinerea by creating competition for nutrients (Rabosto *et al.,* 2006).Similar, biocontrol activity of antagonistic bacteria was reported against fungal pathogen *Botrytis cinerea* by creating competitive environments (Haidar *et al*., 2016). Siderophore production by rhizobacteria is very important attribute to provide competitive environment for uptake of iron in the rhizosphere. Siderophores produced by rhizosphere microorganisms suppress the growth of phytopathogens by chelating the iron from the soil and make it unavailable to the phytopathogens (Sahu *et al*., 2011; Yu *et al.,* 2011).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area and growth condition

The Greenhouse experiment was conducted at Debrezeit Agricultural Research Center (DZARC), Bishoftu located at a distance of 47 km south east of Addis Abeba in Oromia Regional State, Ethiopia. The town has an elevation of 1,920m (6,300ft) above sea level, with temperature condition from 13^{o}c-25^{o}c (average annual temperature of 18.5^{o}c). Culture activation, sub culturing, and other tests were conducted at Adama Science and Technology University (ASTU) in molecular and Microbiology Laboratory.

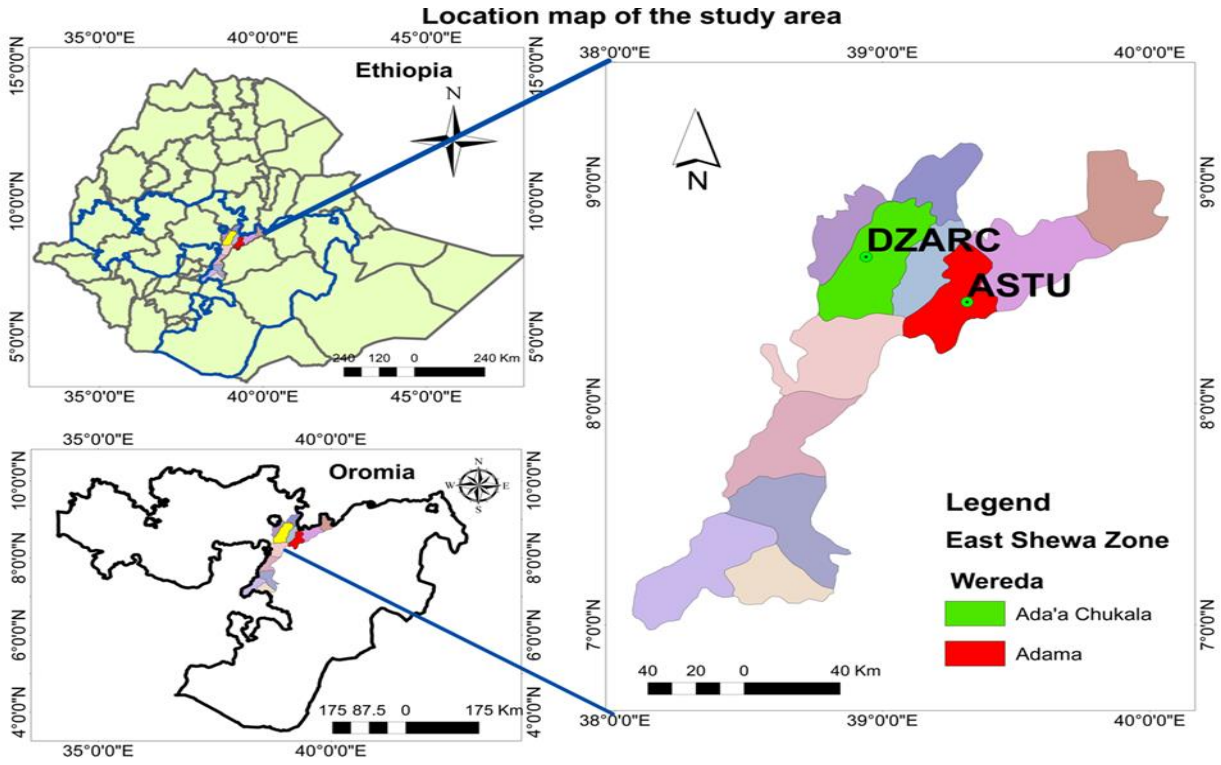


Figure 4: Map of the study area.

3.2 Study Design and Period

The study design was completely randomized block design. The study employed laboratory and green house based experiment aimed at characterizing rhizobacteria at *in vitro* and *in vivo*

testing of antagonistic activity against *Fusarium oxysporum*. All experiments were conducted in triplicate. This study was conducted from February 2021 to March 2022.

3.3 Sources of Antagonistic rhizobacteria and pathogen strains

Pure culture of antagonistic rhizobacteria isolates labeled with stain code ALCR- 42, ALCR-45, ALCR-46, ARC-262, ARC 358 and fungal pathogen were provided from culture collections from the Addis Ababa University/ Ethiopian Emerging technology Institute, from his previous work done on chickpea and identified as plant growth-promoting and phosphate solubilizer bacteria (Mulissa Jida *et al*., 2016). The isolates were registered in National Centre for Biotechnology Information (NCBI) Gen Bank Gen Bank with accession numbers of KJ381101, KJ 381114, KJ 381129 and KJ381145, respectively (Table 1)

Table 1: Detail information of antagonistic Rhizobacteria.

Strain codes	Accession number	Organism name
ALCR 42	KJ381098	*Paenobacillus polymyxa*
ALCR 45	KJ 381101	*Bacillus subtilis*
ALCR 46	_	*Bacillus subtilis*
ARC 262	KJ 381129	*Bacillus subtilis*
ARC 358	KJ 381145	*Bacillus Subtilis*

Sources: NCBI data.

3.4 Validation of *in vitro* Antagonism Activities

The inhibitory effect of the *Bacillus subtilis* isolates against the radial mycelia growth of the *F. oxysporium ceceri* was evaluated using the dual culture technique according to Zivkovic *et al.* (2010). One loop ful of 24 h old culture of the strains(10^9cfu/ml) at the exponential growth phase were spoted on Waksman Agar medium as spot band (making a spot) approx. 2 cm away from the center of Petri dishes (90 mm diameter) in triplicates followed by incubation at 28±2ºCfor 48hrs. After 48 hour of incubation, 5 mm mycelia disc from 7-days old culture of the *Fusarium oxysporum* ceceri was inoculated at the center on the same plate and were incubated until the control plates were fully covered by the growth of *fusarium oxysporium ceceri*.

In all antagonistic studies, a plate inoculated only with the test fungus placed at the center served as a control. Petri plates were incubated at 28 ± 2 °C for 7 days. The assay was performed in triplicates and the percentage fungal radial growth inhibition and percent inhibition of radial growth (PIRG) was calculated by using the following formula (Zivkovic *et al*., 2010). % Inhibition (R−r) /R × 100.

Where, R is the radial growth of fungus in control, and r is the radial growth of fungus in dual culture after 7 days of incubation.

3.5 Inoculum preparation and evaluation of carrier

The formulations were developed by using a mixture of 1g of carboxymethyl cellulose and 100gm of carrier (Talc, Lignite and fly ash obtained from Addis Abeba University and Wonji Sugar factory respectively. The carriers were autoclaved for 30 min on each of two consecutive days. Rhizobacteria strains (Bacillus Subtilis) were grown on nutrient broth poured in 250ml conical flask for 48 h at 150 rpm (25±2°C). A 40 ml of the bacterial suspensions, containing 1×109 colony forming units (CFU) per ml were added to 100g of sterilized carriers' material and mixed, then packed in sterilized polythene bags, sealed and stored at room temperature (25±2oC) and, in each carrier, the population of bacteria was estimated at monthly interval for 6 months, i.e 0th, 30th, 60th, 90th, 120th, 150th and 180th day by using serial dilution techniques on nutrient agar.

3.6 Eco-Physiological characterization

3.6.1 Carbon sources utilization pattern of the isolates

The ability of isolates to utilize different carbon sources was tested on modified basal medium containing different carbon sources. The carbohydrates were prepared at 1% (w/v) in place of manitol and the yeast extract was replaced by NH4Cl (0.1%, w/v) according to Mohamed et al. (2000). The heat stable carbohydrates (lactose, D- manitol, and sucrose and Starch) were sterilized together with the modified basal medium (gl/1): K_2HPO4, 1; KH_2PO4, 1; FeCl $3.6H_2O$, 0.01, NH_4SO_4, 1NaCl*2*, 0.1; $MgSO_4.7H_2O$, 0.2; and agar, 15, whereas the rest heat labile (Galactose, D-maltose, glucose) were filter sterilized using 0.2 µm membrane filter of 0.2nm and added to sterilized basal medium.Colony growths was determined qualitatively as (+) for positive growth and (-) for no growth(Amarger *et al.*, 1997).

3.6.2 Nitrogen sources utilization pattern of the isolates

Isolates were also tested for their ability to grow on different nitrogen sources on modified basal medium containing (gL-1): K_2HPO4, 1; KH_2PO_4, 1; $FeCl_3$. $6H_2O$, 0.01; $MgSO_4.7H_2O$,0.2; $CaCl_2$, 0.1; NH_4 $(SO_4)_2$, 1; and agar, 15; from which $(NH_4)2(SO_4)$ was omitted and mannitol was added after autoclaving and filter sterilized amino acids like, thymine hydrochloride, L- aspargine, alanine, L.proline, L.Tryptophane, Ammonium acetate, Urea, Aniline and glycine were added as nitrogen sources. The N sources were prepared as 0.5g L-1(w/v) and where yeast extract was reduced to 50 mg/l (Amarger *et al.*, 1997).Colony growths was determined qualitatively as (+) presence of growth but (-) absence of growth.

3.6.3 Intrinsic Antibiotic Resistance (IAR)

The intrinsic antibiotic resistance of the isolates were tested on nutrient agar plates consisting different concentration of the following antibiotics (µg ml-1), Amoxicillin (5&10), Chloramphenicol (5&10), Ciproflaxin (5&10), Erythromycin (5&10), penicillin (5&10), Tetracycline (5&10), Streptomycin (5&10), Erythromycin was dissolved in ethanol, whereas the other four were dissolved in sterilized water. The stock solution of each antibiotic was prepared by dissolving 2g of each antibiotic in 100ml of water. The required concentration was aseptically added to the media using a single pipette for each antibiotic. The antibiotic solutions were filter sterilized using 0.2 µm mesh sized sterile filter papers and added to the

medium after the medium was autoclaved and cooled to approximately 50^0C, the final concentrations of 5 and 10 µg /ml, which is 25 and 50µl of antibiotic solution per 100 ml medium, respectively, and finally poured separately in to plates (Somasegaran and Hoben, 1994).

3.6.4 Intrinsic heavy metal resistance of the isolates

The selected bacterial strains were tested for their resistance to heavy metals by agar dilution method. Freshly prepared agar plates were amended with various filtered sterilized heavy metals (at the concentrations µg ml-1 in the parenthesis): Mercury Chloride (5&10), Manganese sulphate (50), and Zinc Sulphate(50), Lead Acetate(50), and Potassium Dichromate(50).The NA media adjusted at pH 6.8 and inoculated with overnight grown cultures (Maatallah et al., 2002). Heavy metal tolerance was determined by the appearance of bacterial growth qualitatively as (+) presence of growth and (-) absence of growth after incubating the plates at room temperature for 24-48h.

3.6.5 P^H Tolerance

The ability of isolates to grow on acidic and alkaline media was checked by inoculating the bacterial culture in 100 ml of Nutrient Broth Medium in 250 ml flasks and adjusted to pH levels of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 by using pH meter with addition of diluted HCL (Bernal and Graham, 2001).Colony growths was determined qualitatively as (+) presence of growth but (-) absence of growth

3.6.6 Temperature Tolerance

The temperature tolerance of bacterial isolates was tested by inoculating them on Nutrient Agar Medium. They were incubated at different temperatures of 4^0c, 25^0c, 37^0c, and 40^0c, for 3 days (Rhitu *et al*., 2012).Colony growths was determined qualitatively as (+) presence of growth but (-) absence of growth (Laranjo and Oliveira, 2011).

3.6.7 Salt Tolerance

Tolerance of isolates was also assessed by observing their growth on Nutrient agar medium supplemented with 1.0, 2.0, 3.0, 4.0, 5.0, 6.0,7,8,9,10 % (w/v) NaCl. The colony growth was recorded after 3 days of incubation at 28±2^{o}C (Laranjo and Oliveira, 2011).

3.7 Evaluation of Rhizobacteria isolates to fungicide

Fungicides resistant pattern of the Rhizobacteria isolates were tested on Nutrient agar Medium supplemented with different concentration of Fungicides (Apron star and Equation) with concentration of (50ppm, 100ppm and 200ppm). The medium were inoculated with screened isolates and incubated at 30^0c for 24-48hrs. After 48hr the results was recorded qualitatively as (+) for presence of growth and (-) for no growth.

3.8 Mass multiplication of the Rhizobacteria for inoculation

The rhizobacterial strains selected from the stock culture were streaked on NA media and incubated at 30^0C for 3 days. A loopful of bacterial biomass of each *Bacillus strains* was transferred from slant culture into 250 mL of nutrient broth (NB). The flasks were incubated at 30^0C on orbital shaker (Gollen hamp, England)) at 120 rpm until the cell concentrations reach approximately 10^8- 10^9 CFU/ml (viable plate count method and optical density measurement using spectrophotometer (SM-1600 Double Beam UV/VIS Spectrophotometer) at 600 nm = 1.0.

3.9 Mass multiplication of the fungal pathogens

Inoculum of the fungus was prepared by soaking sorghum grains in water overnight and then as surface dried by spreading on paper towels in laboratory (Iqbal *et al.*, 2010). Surface dried seeds were put into conical flasks at 250g. The flasks were inoculated with two to three agar discs of each of the pathogens isolate using sterile cork borer (6mm in diameter). After plugging, these flasks were incubated at 25^0C for 7 days. At the time of inoculation, each of the flasks containing inoculum pathogens fusarium ceceri adjusted to $1x10^6$/ml using Haemocytometer was mixed in 10g kg-1 of soil (30g 3kg-1 of soil), which was put in the plastic pots (20 x 15 cm) (Iqbal et al., 2010). The pathogens were allowed to become established in the infested soil/ for one week before sowing of chickpea (Iqbal *et al.*, 2010).

3.10 Green house experiment

The experiment was arranged in a completely randomized design (CRD) with 21 treatments in triplicates resulting in 84 experimental units. The Vertisoil was brought from chickpea cultivation field, Debrezeit Agricultural Research center farm and was properly mixed, sieved

(0. 2mm), sterilized air–dried, mixed with sterilized sand in a ratio of 2:1 filled in 4 kg capacity plastic pots. The pots were arranged in complete random design with each block consisting of negative control (no PGPR) and positive control (Fungicides treated seeds). All the pots were watered every three days.

The seventeen treatments were: ALCR-42 (T1), ii. ALCR-45 (T2), iii. ALCR-46 (T3), iv. ARC-262 (T4), v. ARC-358 (T5), VI. Positive control (Apron star) (T6), vii. Negative control (neither Rhizobacteria nor Fungicide) (T7).

The pots were inoculated with 10gm/kg wilt pathogens (FOC) adjusted to $1x10^6$/ml using Haemocytometer and allowed to grow in the medium (sand: soil mixture) for seven days. Chickpea seeds (Dhera, JG-62, Eshete and Habru Cultivars) were surface sterilized with 95% (v/v) ethanol and 3% (v/v) sodium hypochlorite solution for 10 s and 3 min, respectively. The seeds were rinsed five times with sterilized distilled water (Somasagaren and Hoben, 1994) and seeds were soaked in liquid medium for 1- 2 h using 10% (v/v) CMC as adhesive to deliver approximately 10^8 cells seed−1 (Oves *et al.* 2013). The seeds were removed and air dried overnight under laminar air flow cabinets and allowed to germinate on water agar (1%) surface for three days at 25°C. Five pre-germinated bioprimed and coated seeds were sown in to each pot innoculated with *Fusarium oxysporium ceceri* and later thinned down to three after one week of germination. Then, two weeks later each seedling was inoculated with one ml of *Bacillus subtilis* isolate grown for 72 hrs. in Nutrient broth (OD 600=1.0)

3.11 Assessment of disease incidence

Disease score for Fusarium wilt incidence following the standard procedure (Neupane *et al.,* 2007) was done from the 21th DAS to 45 days. Disease incidence was recorded by counting the number of infected plants and dividing it with the total number of plants assessed in each treatment and the data was tabulated. The results were interpreted as resistant (less than 10% incidence), moderately resistant (11-20% incidence), susceptible (21- 50% incidence) and highly susceptible (over 50% incidence) (Neupane *et al.*, 2007).

After 45 days of planting (DAP), soil was carefully washed off the plant roots under low running tap water and data of growth parameters such as Shoot Height, Root height, shoot Fresh weight, Root Fresh Weight were measured and data of shoot and root cell dry weights

were determined after drying for 3 days at 70°C per a single plant (Somasegaran and Hoben, 1994). The result obtained was converted to percentage using the formula; Percent of disease reduction was obtained by the formula below (Haruna *et al.*, 2012):

$$Percentdiseaseincidence = \frac{Numberofsymptomatic}{Totalnumberofplants} \times 100$$

3.12 Statistical Analysis

The data of Carbon and N-source Utilization, Eco-physiological characterization of isolates, Antagonistic tests and compatibility tests were qualitatively Expressed as (+) and (-). Data of greenhouse Experiments were analyzed and interpreted using ANOVA. The experimental treatment means were compared and contrasted against their control and with each other following Duncan's multiple range test (DMRT) at significance level of $t < 0.05$. The correlation between different greenhouse data was evaluated by Pearson correlation coefficient using SPSS v.26.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1 In vitro Screening *Bacillus subtilis and Paenobacillus polymyxa*

The results of in vitro tests of antagonism toward the plant pathogen Fusarium oxysporium ceceri were shows in figure 7. Inhibition was clearly discerned by very limited growth of fungal mycelium in the inhibition zone surrounding a bacterial colony. The antagonistic effects of bacillus subtilis isolates against fusarium oxysporium ceceri were in the range of 53-65 %. ARC358 gave the maximum inhibition about 65 %, followed by ARC 262 (64 %). While, minimum inhibition of about (53%) showed by ALCR-42 (paenobacillus polymayxa). Control plates not treated with Bacillus subilis and paenobacillus isolates were completely covered by the Fusarium oxysporium ceceri showing no inhibition. The result showed significantly higher mycelial inhibition of 53% exhibited **by** *Bacillus* spp (*BS4, B, KPB5, KK, BSD3, C1, G1, and B2*) (Karthick et al., 2017) and *Bacillus* spp B28 (Karimi et al. 2012) against *F. oxysporum* f. sp. Ciceri. In a similar study, Sahu *et al.*, (2019) showed inhibition percentage of 50 to 70%on soil-borne fungal pathogens by potent bacterial endophytes.

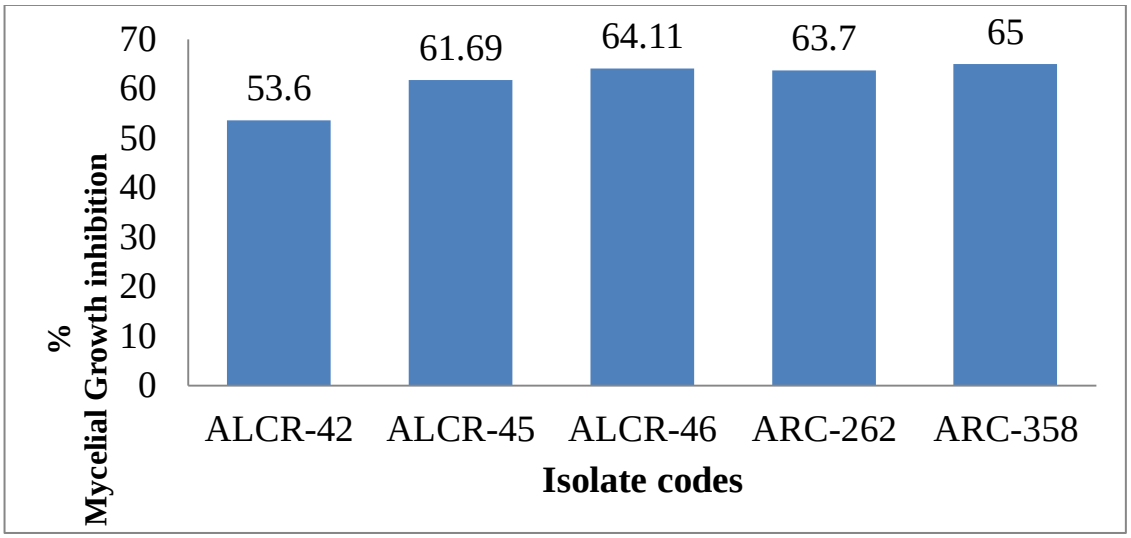


Figure 5: In-vitro inhibition of Fusarium oxysporium ceceri by selected rhizobacterial isolates.

4.2 Fungicides tolerance by PGPR isolates

The highest percent fungicide tolerance % was recorded by *Bacillus subtilis* strains treated with Apronstar at concentration of 50ppm, 100ppm and 200ppm by the isolate ALCR-45 and ALCR-46 while the minimum percent of compatibility of 33.33 % was recorded by ARC-358 (Table 3). On the same way, the highest compatibility of 100% was recorded by all *Bacillus* strains treated with 50ppm, 100ppm and 200ppm of Equation wdp Fungicides (table 2).

Table 2: Invitro Fungicides tolerance test by Bacillus subtilis and paenibacillus polymayxa

Fungicides	Con.	Bacillus strain codes				
		ALCR-42	AlCR-45	ALCR-46	ARC-262	ARC-358
Apron star	50ppm	+	+	+	+	+
	100ppm	+	+	+	+	-
	200ppm	-	+	+	-	-
	% growth	66.66	100	100	66.66	33.33
Equation WDp	50ppm	+	+	+	+	+
	100ppm	+	+	+	+	+
	200ppm	+	+	+	+	+
	% growth	100	100	100	100	100

\+ implies growth, - implies no growth

4.3 Carbon sources utilization pattern of the isolates

Table 3: Carbon source utilization pattern

Isolates	Carbon source						
	D-Glu	Gala	Fru	Malt	Suc	Man	Lact
ALCR42	+	+	+	+	+	+	+
ALCR45	+	+	+	+	+	+	+
ALCR 46	+	+	+	+	+	+	+
ARC262	+	+	+	+	+	+	+
ARC358	+	+	+	+	+	+	+

Note: D-Glu-D-Glucose, Gala-Galactose, Fru-Fructose, Malt-Maltose, Suc-Sucrose, man-Mannitol, Lact-Lactose

Note: + slightly utilized

4.4 Nitrogen sources utilization pattern of the isolates

All the *Bacillus subtilis* strains were grown on Tryptophan, Proline, Ammonium sulphate and Urea but failed to grow on Thiamine and Aniline (Table 4). However, they showed variations in utilizing different nitrogen source as substrates. Thus, ALCR-42 and ALCR-45did not utilize glycine whereas ACL45 failed to utilize Asparagine.

Table 4: Nitrogen source utilization by *Bacillus subtilis* strains

Isolate	Gly	Tryp	Asp	Thia	proline	$(NH4)_2So_4$	Urea	Alanine
ALCR42	-	+	+	-	+	+	+	-
ALCR45	-	+	-	-	+	+	+	-
ALCR 46	+	+	+	-	+	+	+	-
ARC262	+	+	+	-	+	+	+	-
ARC358	+	+	+	-	+	+	+	-
	60	100	80	0	100	100	100	0

Note: Gly - Glycine, Tryp - tryptophan, Asp - Aparagine, Thia - Thiamine.

'+' means utilized, '-' means not utilized

4.5 Effects of salinity, PH and temperature on growth of Bacillus subtilis strains

All bacterial isolates were characterized for abiotic stress tolerance activities such as (1% NaCl, 2% NaCl, 3% NaCl,4%,5%, 6%, 7 %, 8%,9 and 10% NaCl (w/v), pH ranges (4, 5,6, 7,8, 9 and 10, and temperature ranges 4, 25, 30, 35, 40 and 45^{o}c (Laranjo and Oliveira, 2011) . Almost all strains (100%) were able to grow at 1-6% NaCl concentration, whereas, 33 % and 16 % of bacterial strains were able to survive at 7-9 %and 10 % NaCl w/v, respectively (Table 5). Studies in Ethiopia (Mulisa Jida and Fassil Assefa, 2012) showed 11% of the tested chickpea isolates were tolerant to 5% NaCl and other isolates were even tolerant to higher NaCl concentration of (6%) (Daniel Muleta and Fassil Assefa, 2015). Study done by Jabborova *et al. (*2020) showed that B. subtilis 1 strain tolerated 8% NaCl in medium.

This result is comparable to previous finding in Ethiopia that indicates a wide range of variation in salt tolerance to 1-5% (w/v) NaCl concentration (Atsede Muleta *et al.*, 2020). According to Zahran (1999) salt tolerant rhizobia are endowed with the capacity to tolerate

osmotic stress that is mainly associated with their capacity to accumulate low molecular weight organic solutes in their cells.

Table 5: Salt tolerance

Isolates	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
ALCR-42	+	+	+	+	+	+	±	±	±	±
ALCR-45	+	+	+	+	+	+	±	±	±	±
ACR-46	+	+	+	+	+	+	±	±	±	±
ARC-262	+	+	+	+	+	+	±	±	±	±
ARC-358	+	+	+	+	+	+	±	±	±	±

Note: + growth, ± no growth.

All isolates were characterized with respect to their growth response to pH (Results are presented in Table 6). The results of pH tolerance indicated that all isolates tolerate pH range 6-9, but only 16 % of bacterial were able to grow at pH-10. None of the bacterial strains tested were able to grow in a medium of pH-4 and 5 (Table 6).The results were more or less in line with previous studies of Mulissa and Fassil (2012) found the tolerance of all isolates from chickpea at pH 5.5-8 and 25% of isolates also found to grow at pH 9.5.Previous studies in Ethiopia showed that chickpea rhizobial isolates grew in moderately acidic pH5 to alkaline pH 9; but sensitive to pH4 (Mulisa Jida and Fassil Assefa, 2012; Wubayehu Gebremedhin et al., 2018; Tassew Sirage and Fassil Assefa,2018).

However, Kucuk and Kivanc, 2008) showed that almost all chickpea rhizobial isolates from Turkey were tolerant to pH 4.Moreover, studies in Ethiopia (Zehara Mohammed *et al.*, 2020) showed more strains of chickpea rhizobacteria grew at pH 10 (85%) than at pH 5 (60%), but fewer strains were tolerant to pH 4 (25%) which is in contrary with the present findings. In contrast to this finding Bhattacharya *et al.* (2013) reported that none of their isolates from Pisum sativum (pea plant) grew at pH 4.0 and 9.0.Temperature tolerance of the strains showed the isolates were tolerated a temperature ranges between 25- 45^{o}C but only 33.33 % of tested isolates could able to grow at 4^{o}C (Table 6). Maâtallah et al. (2002) reported that C. arietinum rhizobial isolates showed growth within a wide temperature range (5 °C to 45 °C).

Table 6: PH and Temperatures tolerance

Isolates	pH tolerance							Temperature tolerance(^{o}c)			
	4	5	6	7	8	9	10	4	25	37	40
ALCR-42	-	-	+	+	+	+	-	+	+	++	+
ALCR-45	-	-	+	+	+	+	-	-	+	++	+
ACR-46	-	-	+	+	+	-	-	-	+	++	+
ARC-262	-	-	+	+	+	+	-	+	+	++	+
ARC-358	-	-	+	+	+	+	+	-	+	++	+
% growth	0	0	100	100	100	80	16.33	33.33	100	100	100

4.6 Intrinsic antibiotics (IAR) and heavy metals resistance (HR)

The *Bacillus subtilis* and *Paenibacillus polymxa* strains showed variations in inherent antibiotic resistance; ALCR-45 showed resistance against ciproflaxin, Streptomycin sulphate. Strain ALCR-46 showed resistance against tetracycline and Streptomycin sulphate. In addition, Strain ARC-358 had also exhibited resistance against penicillin and Erythromycin. Most of the strains were sensitive to the majority of tested antibiotics (Table 7). Moreover, Strain ALCR-42, ARC-262 and ARC358 showed sensitivity against tetracycline and streptomycin sulphate. In line with the recent finding, Dipta B. and Kausha R. (2018) assay on antibiotic revealed that all Bacillus strains were sensitive to amoxicillin (30 mcg), ampicillin (10 mcg), erythromycin (5 mcg), gentamycin (50 mcg) and tetracycline (30 mcg).

Table 7: Patterns of Antibiotic resistance (µg/ml) of *Bacillus subtilis* strains

Isolates	Amox		Chlora		Cipro		Tet		Strep		Pen	
	5	10	5	10	5	10	5	10	5	10	5	10
ALCR-42	-	-	-	-	-	-	-	-	-	-	-	-
ALCR-45	-	-	-	-	+	+	-	-	+	+	-	-
ALCR-46	-	-	-	-	-	-	+	+	+	+	-	-
ARC-262	-	-	-	-	-	-	-	-	-	-	-	-
ARC-358	+	+	-	-	-	-	-	-	-	-	+	+
% IAR	20		0		20		20		40		20	

Note: Amox-Amoxacillin, Chlora-Chloramphenicol, Cipro-Ciprofloxacillin, Tet-Tetracycline, Strep-Sterptomycin, Pen-Penicilline, Ery-Erytromycine

The *Bacillus* strains showed different response to heavy metals (Table 8). Accordingly, all strains failed to grow on $Hgcl_2$, whereas most strains (80-100%) were tolerant to lead, zinc (80%), manganese (100%) (Table 8). In the present study it is evident that the Bacillus species showed more resistance to heavy metals except Mercury Chloride. In line with the precent findings, Sevim Elif & Sevim Ali (2015) indicated that the isolated Bacillus strains (especially *Bacillus sp. B3, B. thuringiensis B11* and B. *thuringiensis* B12) were found to be the most resistance against many heavy metals and the lowest heavy metal resistance in all Bacillus strains has been identified as mercury. These heavy metal resistant Bacillus strains could be a potential agent for bioremediation of heavy metals in many polluted environments.

Table 8: Patterns of intrinsic Heavy metal resistance (µg/ml)

Isolates	$Hgcl_2$	Pb(CH3COOH)2	$Znso_4$	$Nicl_2$	$K_2c_{r2}o_7$	$Mnso_4$	% of Resistance
	5	10	50	50	50	50	
ALCR-42	-	+	+	+	+	+	87.5
ALCR-45	-	+	+	+	+	+	75
ALCR-46	-	+	-	+	+	+	75
ARC-262	-	+	+	+	+	+	87.5
ARC-358	-	+	+	+	+	+	87.5
Resistance %	0	100	80	100	100	100	

4.7 Determination of shelf life

The results of Shelf life study of the *Bacillus subtilis* strains on Talc, Liginites and fly-ash as carrier material showed a decline in CFUcount after six (6) months in Talc and five (5) months in liginite and four (4) months in fly-ash based bio-formulations. Among carrier tested talc based formulation supports maximum growth of almost all strains for six months (Table 9). Similarly, El-Hassan and Gowen (2006) reported viabilities in talc-based formulations after 180 days of storage at room temperature for *B. subtilis* (78.13 %) and Omer (2010) reported the viability B. megaterium after 180 day is (68 %) indicates that number of viable bacteria was decreased after six month storage periods. This is mainly because, it has very low moisture equilibrium, relative hydrophobicity and reduced moisture absorption; in addition, it prevents the formation of hydrate bridges, favoring long-term storage (Martínez-Álvarez *et al*., 2016).Vidhyasekaran and Muthamilan (1995) had found that in talc-based and peat-based

formulations, the bacteria even survived up to 240 days of storage although the population declined after 30 days.

In the present study, different carrier materials were used to prepare commercial formulation of B. subtilis and paenibacilluspolymayxa. All the different carrier materials tested supported the multiplication of bacteria. When cfu count was observed after 0,30,60,90,120,150 and 180 days of incubation, talc based formulation was the best with highest log 10 cfu count of greater than 6.477 at 180 days followed by lignite as a carrier material with log10 cfu count of 6.3 whereas, least growth was observed in Fly ash (10g10 cfu count of 5.3). Similar studies were carried out by Nekkeeran et al. (2006) and they reported that talc, FYM, vermiculite and lignite as a carrier material supported B. subtilis multiplication.

Table 9: Shelf life of bacillus subtilis and Paenibacillus polymyxa on different carriers

Talc based formulation		**log10 CFU/g**					
Months	0	1	2	3	4	5	6
ALCR 42	8.296	8.264	8.176	8.0253	7.97	6.903	6.819
ALCR 45	8.292	8.262	8.23	8.278	8.25	7.198	7.204
ALCR 46	8.301	8.236	8.318	8.252	8.19	7.255	6.477
ACR262	8.29	8.267	8.245	8.206	8.0961	8.068	6.968
ACR 358	8.287	8.26	8.206	8.149	8.1205	9.996	6.889
Fly ash based formulation							
Months	0	1	2	3	4	5	6
ALCR 42	8.296	8.113	9.012	8.93	8.86	7.02	6.518
ALCR 45	8.292	8.127	9.117	8	8.75	7.837	5.3
ALCR 46	8.301	9.2	9.181	8.1367	8.086	7.822	6.301
ACR262	8.29	9.068	9.1818	8.103	8.079		6.778
ACR 358	8.287	9.1205	9.0606	8.123	8	7.264	6.653
Lignite based Formulation							
Months	0	1	2	3	4	5	6
ALCR 42	8.296	8.23	9.146	8.079	9.064	7.75	6.518
ALCR 45	8.292	8.033	9.155	8.176	8.064	8.079	6.725
ALCR 46	8.301	9.264	9.217	8.204	8.13	7.929	6.301
ACR262	8.29	9.139	9.103	8.041	8.113	7.565	6.778
ACR 358	8.287	9.1003	9.071	8	8	7.7741	6.653

4.8 Assessment of disease incidence

Treatment of different the chickpea varieties with Bacillus strains showed variations in percent wilt incidence in a greenhouse experiment (Table 10). Thus, the moderately susceptible Dhera variety did not show any disease symptopms when treated with AlCR-45, ALCR-46, and ARC-262 strains; whereas those treated with AlCR-42 and ARC-358 showed disease incidence of 11%.

Regarding the highly resistant variety, Eshete treated with *Bacillus* strains did not show anydisease incidence compared to 11% incidenceof non-treated (negative control) chickpea plants. on the contrary,Habruchickpea variety treated with ARC-262 and ARC-358 did not show any disease incidence compared to disease incidence of 11%, 33%and 44%with the ones treated withALCR-46,ALCR-45andALCR-42, respectively. Likewise, the highly susceptible JG-62to root rot wilt diseases showed variation in percent of disease resistance (Table 10). Thus, But which is highly susceptible variety when treated with *Bacillus* strains AlCR-45, ALCR-46, ARC-262 did not exhibit disease incidence whereas those that were treated with strains AlCR-42, and ARC-358 showed disease incidence of 33.33% and ,22.22% , respectively compared to Negative control with disease incidence of (22-55%).

In general, the result showed that wilt root rot disease incidence showed variation among different chickpea genotypes. The maximum disease incidence reduction was observed in Habru genotype treated with ALCR-42 (44.44%) and the minimum was observed in Dhera genotypes treated with ALCR-42 and ARC-358 (11%). However, the maximum disease incidence occurrence in negative control was observed in Habru genotype (66.66%) and the minimum was observed in Eshete genotypes (0%) this implies the diseases incidence ranges between 0-66.66percent (Table 10).

Similar results were reported by Ayana *et al*. (2019), where disease incidence ranged between 7 to 73% from the Desi-type chickpea. Mirzapour et al. (2014) evaluated 18 genotypes/cultivars against chickpea wilt with rhizobcteria treatment and observed disease incidence of 0 % to 46.6% at the seedling stage, and it varied from 0 to100% at the reproductive stage. Patil *et al*. (2003) also indicated that the biological control agents (*Bacillus spp*) were more effective and economical when applied as seed treatment than as soil treatment as observed in the present study. Moreover, our finding is in accordance with the results of Landa et al. (2004) who showed that Soil treatment with *Bacillus megaterium* and *Paenibacillus* macerates inhibited Fusarium wilt in chickpeas (Cicer arietinum).Similarly, application of biocontrol agents including *Bacillus subtilis* is reported to effectively reduce root rot caused by soil borne pathogens in several crops (Senthilkumar *et al*., 2009; Sanjeevkumar *et al*., 2019).

Table 10: Results of percentages of diseases incidence

strain code	Variety	% DI in treatment	% DI in Control
ALCR42	Dhera	11	22.22
	Gj-62	33.33	55.55
	Eshete	0	11
	Habru	44.44	66.66
ALCR 45	Dhera	0	11
	Gj-62	0	33.33
	Eshete	0	11
	Habru	33.33	44.44
ALCR 46	Dhera	0	22.22
	Gj-62	0	33.33
	Eshete	0	0
	Habru	11	33.33
ARC 262	Dhera	0	0
	Gj-62	0	22.22
	Eshete	0	0
	Habru	0	44.44
ARC 358	Dhera	11	11
	Gj-62	22.22	44.44
	Eshete	0	11
	Habru	0	33.33

4.9 Effects of selected *Bacillus* strains on growth of different chickpea genotypes

4.9.1 Effect on Dhera genotypes

The results of greenhouse testing on dhera genotypes revealed that isolate ARC-358 gave the best results with a shoot length of 30 cm and shoot dry weight of 2.53g and Isolate ALCR-46 showed shoot height of 30.33cm and a shoot dry weight of 3.23g (Table 11). Regarding root length, all isolates were able to significantly increase root length in comparison to the control ($p \leq 0.05$), with ARC-262 giving the highest length of 17.3 cm followed by ALCR-46 with a length of 14.67 cm. Only isolate ARC 262 was able to significantly increase root dry weight in comparison to the control but other isolates showed less root dry biomass than positive control ($p \leq 0.05$).

There was no significant difference between root dry weights of genotypes treated with isolates ALCR-46, ARC-262 and ARC-358(1.37, 1.33 and 1.4g) respectively. However, plants treated with isolate ALCR-45 registered the lowest root dry weight (0.7) followed by ALCR-

42 showed the least root dry weight (0.4 g) which are less than both positive and negative control (1.14 and 0.81g) respectively (Table 11).

The Pearson correlation of Dhera chickpea showed strong positive correlation in parameter like shoot height with, shoot fresh weight (0.494**) and Root dry weight (0.437**). Root height with, Root fresh weight (0.417**), Root dry weight (0.694**) and the correlation is significant at the 0.01 level (2 tailed). Moreover Root length have moderately correlated with shoot fresh weight (0.313*) and the Correlation is significant at the 0.05 level. Shoot fresh weight with shoot height (0.494**), Shoot dry weight (0.714**) have strongly correlated and the **Correlation is significant at p= 0.01 level.

Thus, the pot inoculated with ALCR-46, ARC-262 and ARC-358 strains had considerable effect on shoot height, shoot fresh weight, root length, shoot biomass, shoot dry mass, root biomass, and root dry mass of Dhera chickpea genotype. Mulisa Jida *et al*.(2016) showed B. flexus (PSBC17) was the most effective ones in terms of increasing shoot dry weight of chickpea with an increase in shoot dry weigh 0.92+0.02** over the uninoculated control (0.75+0.02**).

Similar results Karimi *et al*.,(2012) showed in their experiment on chickpea fusarium wilt diseases biocontrol using bacillus spp as antagonists and found that there was no significant difference between shoot height of genotypes treated with isolates B1B6,B28, (33.11, 33.77 and 35.44cm) respectively. However, plants treated with isolate B40,B99 and non-infected control has showed shoot height of (32.5,32.44and 31.44cm).The maximum root length, Shoot dry weight and root dry weight was showed by plant treated with B-40 18.22cm, 0.86g and 0.24g respectively. The minimum root length, Shoot dry weight and root dry weight was showed by plant treated with B108 (12.33cm, 0.52g and 0.11gm respectively.

Table 11: Effect of *Bacillus subtilis*, strains on growth of Dhera chickpea genotype

Isolate	Above ground growth parameter			Belowground growth parameter		
	SH (cm)	SFW(g)	SDW(g)	RL(cm)	RFW(g/p)	RDW(g/p)
ALCR 42	25±3.0a	7.5±3.65b	1.77±0.57ab	11±1.73ab	5.27±1.67a	**0.4±0.17a**
ALCR 45	24.3±5.51a	7.4333±3.44b	1.87±0.058a	11.67±3.06a	7.43±3.44a	**0.7±0.17a**
ALCR 46	30.33±4.041a	10.6±6.065ab	3.23±0.42a	14.67±0.58ab	8.13±3.66a	1.37±0.15a
ARC 262	29.67±0.58a	13.53±6.66ab	1.87±0.25a	17±3.00b	14.7±3.96b	1.33±0.12a
ARC 358	30.67±3.21a	16.63±0.42a	2.53±0.21a	12.33±1.53ab	4.13±0.31a	1.4±0.2a
PC	31.17±4.47a	10.24±3.63ab	2.21±0.67a	13.6±3.66ab	9.02±4.81a	1.14±0.63ab
NC	26.77±5.79a	13.47±3.46ab	1.22±4.43ab	10.46±3.45ab	4.45±3.27a	0.81±0.53a

Data are means of three replicates

Means in the column followed by different letters indicate significant differences among treatments at $P \leq 0.05$ according to DMRT

SH= shoot height, RL= length of root, SDW= dry weight of shoot, RDW= dry weight of root

Table 12: Pearson correlation for Dhera chickpea genotype after 45 days

parameters	RL	SH	SFW	SDW	RFW	RDW
RL	1	0.111	0.313*	0.127	0.417**	0.694**
SH	0.111	1	0.494**	0.437**	0.108	0.191
SFW	0.313*	.494**	1	0.714**	0.158	0.243
SDW	0.127	.437**	0.714**	1	0.044	0.139
RFW	0.417**	0.108	0.158	0.044	1	0.406**
RDW	0.694**	0.191	0.243	0.139	0.406**	1

SH= Shoot Height, RL= Root length, SFW=Shoot Fresh weight, SDW= shoot dry weight, RFW= Root Fresh Weight, RDW =Root Dry weight.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

4.9.2 Effect on JG-62 chickpea genotype

All Isolates ALCR 42,ALCR 45,ALCR 46,ACR262 and ACR 358 showed significantly higher shoot length over control (26cm, 27.8cm,25.83, 26.33and 26.33, respectively) (Table 13). The result also showed that the highest shoot length 27.8 cm plant-1 was recorded in

chickpea treated with ALCR-45 followed by ACR-262and ACR-358 (26.33cm). Likewise, ACR-262 treated plant showed the highest root length (13.33cm/plant) followed by ALCR-45 and ACR-358 (12.33cm and 11.33cm, respectively) in comparison to control (9.05 for PC) and other isolates. Consequently, there was significant difference amongst the inoculants. The highest shoot dry matter (2.4 g plant-1) was recorded by isolateACR-358 followed by statistically at par values due to ALCR-45 (2.2 g) and ACR-262 (1.9 g). Root dry weight was significantly increased (P< 0.01) in chickpea inoculated with selected PSB isolates, and the highest was recorded by ACR-358 (0.30g/plant) and ACR-262and ALCR-45 (0.27) followed by pc (0.26g/plant).

Table 13: Effect of Bacillus *subtilis*, strains on growth of **JG-62** chickpea genotype

Isolate	Above ground growth parameter			Belowground growth parameter		
code	SH (cm)	SFW(g)	SDW(g)	RL(cm)	RFW(g)	RDW(g)
ALCR-42	26±3.61b	5.67±1.50b	1.3±0.46b	7.83±2.25c	0.43±0.11cd	0.13±0.06a
ALCR- 45	27.8±4.07a	7.2±4.33ab	2.2±1.47ab	12.33±2.52ab	0.87±0.31abc	0.27±.12a
ALCR- 46	25.83±2.84ab	3.87±0.57b	1.33±0.06b	10.5±1.32abc	0.37±0.06d	0.17±0.06a
ARC-262	26.33±5.03ab	7.7±2.51ab	1.9±0.61ab	13.33±0.76a	0.97±0.57ab	0.27±0.12a
ARC- 358	26.33±1.89ab	9.93±4.35a	2.4±1a	11.33±1.60ab	1.10±.44a	0.30±0.10a
PC	23.76±3.68ab	4.96±1.77b	1.37±0.52ab	9.85±2.56abc	0.55±0.2abc	0.26±0.18a
NC	21.7±3.60a	6.15±2.83b	1.17±0.58ab	9.00±2.44bc	0.60±0.28bcd	0.21±.08a

Note: Values are expressed as a mean of triplicates ± SE. Mean values followed by same letter are not significantly different (P< 0.05). * Significant at 1%.

Table 14: Pearson correlation for Gj-62 Chickpea after 45 day

parameters	RL	SH	RFW	SFW	RDW	SDW
RL	1	0.462**	0.478**	0.294*	0.221	0.387**
SH	0.462**	1	0.274	0.381**	0.209	0.596**
RFW	0.478**	0.274	1	0.675**	0.425**	0.626**
SFW	0.294*	0.381**	0.675**	1	0.319*	0.726**
RDW	0.221	0.209	0.425**	0.319*	1	0.309*
SDW	0.387**	0.596**	0.626**	0.726**	0.309*	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Correlation test results indicated a significant positive correlation among most parameters (Table 14). Shoot height (SH) showed a highly significant positive correlation with Root height (Person's r=0.462), shoot dry weight (Person's r=0.596), shoot fresh weight (Person's r=0.381). There was also a highly significant positive correlation between Root length (RL) and Shoot height (Person's r=0.462), Root fresh weight (Person's r=0.478), shoot dry weight (Person's r=0.387), there was also a strong positive correlation between Root Fresh weight with Shoot fresh Weight (r=0.675), Shoot dry Weight (r=0.626), and there was a significant correlation between root fresh weight with Root length (r=0.478) Root dry weight (r=0.425). Moreover, the data also showed strong positive correlation between Shoot dry weight(r=0.596), Root fresh weight(r=0.626), Shoot fresh weight (r=0.726).

4.9.3. Effects on Eshete chickpea genotype

As Stated in (Table 15) all the selected isolates promotes plant growth with a range of 36.33 – 30 cm. Thus, the highest shoot length (36.33 cm/p) was recorded from plants inoculated with isolate ACR-358 (Bacillus species) that was followed by a shoot length of 36.3 cm/p from plants treated with ACR- 45(Bacillus species). Whereas, the shortest shoot length (30 cm/p) was recorded by plants inoculated with isolate ALCR- 42.

The highest shoot dry weight (3.57g/p) was recorded from plants inoculated with isolates ACR-358 (Bacillus species) followed 3.03g/p and 2.8g recorded from plants inoculated with isolates ARC -262 (Bacillus species) and ALCR-45 (Bacillus species). Plants treated with Bacillus and *Paenibacillus* strains accumulated a shoot dry weight greater than the both positive and negative controls. The fact that all bacterial treated plants accumulated a shoot dry weight greater than the negative control plants indicated inoculation improved the growth of plants. It is interesting to note that inoculated plants with isolates ALCR-42, 45, 46, ARC-262 and ARC-358 accumulated shoot dry weight higher than the Negative control plants. This

increment in growth might be due to increased activity of plant growth promoting rhizobacteria (PGPR) through multiple mechanisms of action i.e. biological fixation of atmospheric nitrogen by the tested free living entophytes such as *bacillus subtilis spp* and *paenibacillus polymayxa*.

Table 15: Effect of *Bacillus subtilis*, strains on growth of Eshete chickpea genotype

Isolates	Above ground growth parameter			Belowground growth parameter		
	SH(cm)	SFW(g)	SDW(g)	RL(cm)	RFW(g)	RDW(g)
ALCR 42	30±7.21a	5.07±2.40b	1.17±0.58b	9.67±1.15a	1±.10b	0.2±.00a
ALCR45	36.3±2.08a	10.9±2.29ab	2.87±0.45ab	12.7±3.79a	3.17±2.37ab	0.63±.31a
ALCR 46	34.0±4.58a	9.23±2.90ab	2.53±0.83ab	10.67±0.58a	4.43±1.23ab	0.83±0.23a
ACR 262	33.67±5.51a	11.7±8.45ab	3.03±2.36ab	13±4.36a	7.5±5.44a	1.1±0.95a
ACR 358	36.33±4.04a	16.03±6.18a	3.57±1.43a	11.3±2.31a	4.2±0.90ab	0.83±0.06a
PC	34.37±4.31a	9.53±3.59ab	2.11±0.89ab	11.8±2.65a	5.55±4.74ab	0.89±0.73a
NC	32.53±7.07	9.86±4.94	1.29±1.37	13.33±3.14	2.74±2.56	0.6±0.42

Data are means of three replicates ± SE. The means followed by different letters are significantly different at $P < 0.01$ (Duncan test) - SPSS 26.0 version. SH=Shoot Height, RH=Root Height, SFW= shoot Fresh Weight, SDW= Shoot dry weight, RFW= Root Fresh weight, RDW= Root dry weight.

Table 16: Pearson correlation for Eshete chickpea after 45 day

Parameters	RL	SH	RFW	SFW	RDW	SDW
RL	1	0.235	0.513**	0.373*	0.391**	0.421**
SH	0.235	1	0.322*	0.736**	0.462**	0.730**
RFW	0.513**	0.322*	1	0.435**	0.537**	0.468**
SFW	0.373*	0.736**	0.435**	1	0.547**	0.898**
RDW	0.391**	0.462**	0.537**	0.547**	1	0.410**
SDW	0.419**	0.723**	0.470**	0.899**	0.407**	1

**** Correlation is significant at the 0.01 level (2-tailed).**

*** Correlation is significant at the 0.05 level (2-tailed).**

Correlation test results indicated a significant positive correlation among most parameters (Table 16). Shoot height (SH) showed a highly significant positive correlation with shoot fresh weight (r=0.736), Root dry weight (r =0.462), shoot dry weight (r=0.73), Root fresh weight

showed strong correlation with root height (r=0.513), Root dry weight(r=0.537),shoot dry weight(r=0.468). There was also a strong positive correlation between Shoot fresh Weight with Shoot dry weight (r=0.898), shoot height (r=0.736), root dry weight (r=0.547) and there was a significant correlation between Root dry weight with shoot height (r=0.462) Root fresh weight (r=0.537), shoot fresh weight (0.547).

4.9.4 Effects of Rhizobacteria inoculation on Habru chickpea genotype

Analysis of greenhouse experiment data revealed significant increase ($P<0.05$) compared to control in at least five parameters due to the effect of bacterial treatment (Table 17). Among the isolates, ACR 262 showed the highest mean valves in parameters such as Shoot height (38cm), shoot fresh weight (15.7g), Shoot dry weight (4.43g) as compared to control and others isolates. Isolate ALCR-42 exhibited best effect on root height whereas ALCR-45 and ACR-262 showed best effect on root dry weights of 0.93g and 0.83g, respectively. The least root dry biomass was showed by ALCR-42(0.4g) which is smaller than negative control (0.56g).

From this result it is possible to judge strain ALCR42 can be used as antagonistic strains against fusarium oxysporium ceceri wilt disease and there must be excluded from the result if tried to test the effects of the others on field trials since it fails both in in vitro and green testing. Among the tested isolates, isolates (ACR-262, ALCR-46 and ACR-358) showed best performance with mean value of 38, 32 and 28cm on shoot height respectively. Furthermore, isolate ALCR-42 exhibited the best effect on root length (16cm) compared to positive control (12.9 cm) and negative control (11.07cm). However, isolate ARC-358 did not have a positive effect on root length compared to control since the mean value (9.01cm) was smaller than mean valve of negative control (11.07cm).

Yadv et al. (2010) reported that inoculation of PGPR like, *B. subtilis* BHUPSB13, and *B. boronophillus* BHUPSB19 isolates resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings. Yadav et al. (2010) showed that shoot , root length and dry matter of chickpea (Cicer arietinum L.) increases when chickpea seedling was inoculated by *Bacillus subtilis, Paenibacillus polymyxa* and *Bacillus boronophillus* in under in Vitro conditions.

The comparative study regarding wilt root rot diseases resistance among chickpea germ plasm showed great variation, where JG-62 which is Desi germplasm landrace type showed

susceptibility and (Dhera) the Kabul germplasm (advanced genotypes) showed wilt resistance. Similarly, Asrat and Tolasa (2020) revealed that considerable variations were recorded for resistance in desi and Kabuli chickpea against *Fusarium* wilt diseases and they are found that Kabuli germplasm had been proved to be a better resistance source than the Desi material. Likewise, Parasappa *et al.* (2017) observed a high level of resistance in advanced genotypes compared to landraces.

Dagnachew *et al.* (2021) showed the occurrences of Fusarium wilt and root rot diseases are ubiquitous throughout chickpea cultivated regions in Ethiopia. Average disease incidence was highest throughout Amhara (30.98 %) and Oromiya (22.83 %) regions, and lower in SNNP (15.18%). The resistant check (Dhera) and susceptible check (JG-62) exhibited 0 % and 100 % disease incidence, respectively which is in line with the recent study that showed JG-62 was susceptible.

Table 17: Effect of *Bacillus subtilis*, strains on growth of Habru genotype

Isolates	Above ground growth parameter			Belowground growth parameter		
	SH(cm)	SFW(g)	SDW(g)	RL(cm)	RFW(g)	RDW(g)
ALCR 42	26.33±1.53c	6.87±1.83b	1.43±0.32b	11±2.65a	3.43±0.42a	0.4±0.10a
ALCR 45	26.67±1.53c	10.10±4.90b	2.27±0.27b	11.67±2.52bc	7.68±10.07a	0.93±0.64a
ALCR 46	32.67±4.04b	9.8±1.68b	2.3±0.17b	13.33±2.08ab	6.3±4.00a	0.60±0.20a
ARC 262	38 ±4.36a	15.7±7.75a	4.43±2.41a	13.33±3.06ab	4.23±4.05a	0.83±0.61a
ARC358	28 ±1.0bc	9.0±1.11b	2.02±0.104b	9.0±1.00c	4.53±3.59a	0.45±0.25a
PC	27.27±3.86c	7.81±2.63b	1.87±0.54b	12.93±1.94ab	3.70±2.23a	1.01±0.79a
NC	27.23±2.83c	7.63±2.34b	1.82±0.62b	11.07±2.98bc	2.61±2.15a	0.56±0.39a

Data are presented as mean value ± standard division of three replicates, and each replicate contains three plants. Values with different letters within each column indicate significant difference at $p < 0.05$.

Table 18: Pearson correlation for Habru chickpea after 45 day

Treatments	SH	RL	SFW	SDW	RFW	RDW
SH	1	0.371*	0.409**	0.585**	0.310*	0.013
RL	0.371*	1	0.039	0.383**	0.371*	0.430**
SFW	0.409**	0.039	1	0.272	0.504*	0.482**
SDW	0.585**	0.383**	0.272	1	0.403**	0.493**
RFW	0.310*	0.371*	0.504**	0.403**	1	0.467**
RDW	0.562**	0.307*	0.372*	0.943**	0.457**	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed

5. CONCLUSION

Therefore, from the present findings, it can be concluded that application of (Bacillus sp.) demonstrate could significantly improve the growth by inhibiting plant pathogens and reducing disease incidence of chickpea varieties In this regard, plants bioprimed with rhizobacteria showed significant increase in many parameters including height, fresh and dry plant biomass. In addition, seeds bioprimed with Rhizobacteria also showed a significant increase in disease incidence reduction as compared to the negative controls. For examples strains labeled as ARC-262 and ARC-358 showed maximum reduction of the fusarium wilt disease in in vitro and the green house test of the susceptible chickpea genotypes like Gj-62 and Habru.

6. RECOMMENDATIONS

During this study, in vitro and greenhouse the potential of rhizobacteria on growth promotion and pathogen resistance of chickpea plant were evaluated only. However, further study is necessary to evaluate their effectiveness under different environmental conditions and against different plant pathogens, both in the glass house and in field trials. Moreover, single application of the PGPR showed increases in all growth parameters and disease suppression, however during this experiment no investigation was carried out concerning the co-inoculation effect of different PGPR in diseases suppression and plant growth promotion. Therefore, in the future the i) co-inoculations effects of PGPR on both growth and pathogen control parameters compare the effectiveness of the native inoculums with commercial microbial inoculants and iii) scale up and validated them in extensive trials at farmer's fields are recommended.

Research Funding Acknowledgment

This research project is funded by Adama Science and Technology University under the grant number ASTU/SM-R/339/21, Adama, Ethiopia.

REFERENCES

Abate, T., Shiferaw, B., Gebeyehu, S., Amsalu, B., Negash, K., Assefa, K., Eshete, M.,Aliye, S. & Hagmann J. (2011). A systems and partnership approach to agricultural research and development – lessons from Ethiopia. Outlook on Agriculture **40**(3), 213-220.

Abera Mnalku and and Getachew Mitiku (2019). Response of chickpea (Cicer arietinum L.) to indigenous rhizobial isolates inoculation on Vertisol of central Ethiopian highland. *Ethiop. J. Agric. Sci.*; **29**(2):109-117.

Adeleke Saanu, Bartholomew, Ayilara Stella Modupe, Akinola Adekunle Saheed and Babalola Oluranti Olubukola (2022). A review article on Bio control mechanisms of endophytic fungi. *Egyptian Journal of Biological Pest Control*. **32**:46.

Aggarwal, H., Singh, J., Khaket, T. P and Chhokar, V. (2013). Genetic diversity in chickpea using various molecular markers: first step towards molecular breeding. *International Journal of Advanced Research* **1**: 393-398.

Ahamad, S. (2014). Fungicidal management of yellow rust diseases of wheat in Jammu. *Res. Environ. Life Sci.* **7** (4) 319- 322

Ahemad M, Kibret M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ. Sci*.**26**: 1-20.

Ahmad, F.; Ahmad, I.; Khan, M.S (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res*., **163**, 173–181.

Ahmad, M.; Zahir, Z.A.; Khalid, M. (2013). Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiol. Biochem. 2013, 63, 170–176.

Ahmed S, Melkamu A (2006) Chickpea, lentil, grass pea, fenugreek and lupine disease research in Ethiopia. In: Ali K, Keneni G, Ahmed S, Malhorta R, Beniwal S, Makkouk K, Halila MH (eds) Food and forage legumes of Ethiopia: progress and prospects. Proceedings of the Workshop on Food and Forage Legume, pp 215–220.

Akköprü, A., Erman, M., Demir, S., Ocak, E., Tüfenkçi, Ş. & Oğuz, F. (2011). Effects of Rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (Cicer arietinum L.) under irrigated and rain fed conditions 1—Yield, yield components, nodulation and AMF colonization. Field Crops Research, 122(1), 14-24.

Alacho F., BergaLemaga G. T., Hakiza J. J., and Kakuhenzire R.(2010) .Past, present and future strategies for integrated management of chickpea fusarium wilt disease in Uganda," *African Potato Association Conference Proceedings*, **5**:353–359,.

Aloo BN, Makumba BA, Mbega ER (2019).The potential of bacilli rhizobacteria for sustainable crop production and environmental sustainability. *Microbiol Res*. **219**:26–39.

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol **8**:971.

Amber p, Akram A, Qureshi R, Akram Z. (2012). Hplc analysis for secondary metabolites detection in Sclerotium rolfsii isolated from chickpea. *Pak J Bot* .**44:** 417–422

Amin K. S., Ghanekar P., A. M., Nene Y. L., Reddy M. and Haware V., M. (2011).Field Diagnosis of Chickpea Diseases and their Control," Information Bulletin No. 28, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad.

Amine Elbouazaoui, Badreddine Sijilmassi, Ilyass Maafa, Douira Allal & Seid Ahmed (2022). Biocontrol activity of Bacillus, Paenibacillus and Pseudomonas against Fusarium wilt of chickpea in Morocco, Acta Agriculture Scandinavica, Section B — Soil & Plant Science, **72**:1, 847-859

Anmol Singh Yadav, Supriya Yadav and Ramesh Singh (2018). Screening of fungicides in vitro and seed treatment against fusarium wilt of chickpea caused by Fusarium oxysporum F. sp. Ciceris. *Journal of Pharmacognosy and Phyto chemistry***7**(3): 2908-2911.

Anusha B, Gopalakrishnan S, Naik M, Sharma M. (2019). Evaluation of Streptomyces spp. and Bacillus spp. for bio control of Fusarium wilt in chickpea (*cicer arietinum L.*). *Arch. Phytopathol. Plant Prot*. **52**:1–26.

Anusha B, Gopalakrishnan S, Naik M, Sharma M. 2019. Evaluation of Streptomyces spp. and Bacillus spp. for bio control of Fusarium wilt in chickpea (cicer arietinum L.). *Arch. Phytopathology. Plant Prot*. **52**:1–26.

Anwar, R., Ali, S., Zeshan, M. A., Ghani, M. U., Ali, A., Iftikhar, Y., & Bakhsh, A. (2022). Effect of Fusarium wilts disease on varied Chickpea germplasm and its management. *Journal of Pure and Applied Agriculture*, **7**(1), 1-9.

Asnake Fikre (2018).Improving chickpea production to create wider welfare impact by leveraging technology and seed system development. *Bulletin of Tropical Legumes*.

Asnake Fikre and Dagnachew Bekele (2020). Chickpea breeding and crop improvement in Ethiopia: past, present, and the future. *Universal Journal of Agricultural Research*, 8(2): 33-40.

Asnake Fikre, Assefa Funga., Lijalem Korbu.,Million Eshete, Asrat Zewudie , Nugusse Girma Dagnachew Bekele, Redwan Muhamed, Ketema Daba, and C. O. Ojiewo (2018). Stability analysis in chickpea genotype sets as tool for breeding germplasm structuring strategy and adaptability scoping. Ethiopian Journal of Crop Science, 6(2), 19–37.

Asnake Fikre, (2014).An overview of chickpea improvement research program in Ethiopia. *Journal of the International Legume Society*, (3)34:41.

Asrat Zewde (2015). Epidemiology and management of ascochyta blight (didymella rabiei) on chickpea in central rift valley, Ethiopia *M.Sc. submitted to Haromya university*.

Asrat Zewdie and Tolasa Bedasa (2020). Source of resistance to chickpea Fusarium wilt (Fusarium oxysporum f. sp. ciceris) under field conditions in Ethiopia. *African Journal of Agricultural Research*, **15**(1): 85-89.

Assfaw, D., & Negash, T. (2020).Spatial Distribution and Association of Chickpea Wilt/Root Rots epidemics with biophysical factors at West Shewa, Oromia Regional State, *Ethiopia. Journal of Plant Pathology and Microbiology*, **11**(9), 1-6.

Atsede Muleta. (2020). Genomic diversity, plant growth promoting properties and symbiotic effectiveness of Mesorhizobum spp. nodulating chickpea (*Cicer arietinum L.*) On acidic soils of Ethiopia: Implication for inoculant development to enhance production under low inputs Agriculture. PhD thesis, Addis Ababa University, Ethiopia (pp 1-178).

Awoke Ayana, Negash Hailu and Wondimeneh Taye (2019).Screening Desi and Kabuli Chick Pea Varieties against Fusarium Wilt Resistance in West Gojam, Northwestern Ethiopia. *J Plant Pathol Microbiol.*

Baas P, Mohan JE, Markewitz D, Knoepp JD (2014) Assessing heterogeneity in soil nitrogen cycling: a plot-scale approach. *Soil Sci Soc Am J***78**:237–247

Barlow E., Cook, D., and Sequeira L. (2012). Genetic diversity of Fusariumoxysporum f. sp. ciceris: detection of restriction fragment length polymorphisms with DNA probes that specify virulence and the hypersensitive response," Molecular Plant-Microbe Interactions, vol. **2**:113–121,

Bayaa and Erskine (1998).Screening lentil for resistance to fusarium wilt methodology and diseases of lentil. In: Allen, D. J. and Lenne, J. M. (eds), The Pathology of Food and Pasture Legumes (Wallingford: CAB International), pp. 423 - 471.

Beneduzi A., Ambrosini A., Passaglia L.M. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and bio control agents. Genetic and Molecule Biology**, 35**: 1044–1051.

Berger J.D., Ali M., Basu P.S., Chaudhary B.D., Chaturvedi S.K., Deshmukh P.S., Dharmaraj P.S., Dwivedi S.K., Gangadhar G.C., Gaur P.M., Kumar J., Pannu R.K., Siddique K.H.M., Singh D.N., Singh D.P., Singh S.J., Turner N.C., Yadava H.S. and Yadav S.S. (2006).Genotype by environment studies demonstrates the critical role of phenology in adaptation of chickpea (Cicer arietinum L.) to high and low yielding environments of India. Field Crops Research 98: 230–244.

Bhargavi, V.G., and .Tallapragada, P. (2016). Bacillus sp as potential plant growth promoting rhizobacteria. *Int .J .Advanced life Sciences*.9 (1): 29- 36.

Bhattacharya, C., Deshpande, B., & Pandey, B.(2013).Isolation and characterization of Rhizobium sp. form root of legume plant (Pisum sativum) and its antibacterial activity against different bacterial strains. International Journal of Agricultural and Food Science, 3(4), 138-141.

Bhattacharyya, P.N. and Jha, D.K (2012). Plant growth promoting rhizobacteria (PGPR): Emergence in agriculture. *World. J. Microbiol. Biotechnol*. **28**: 1327-1350.

Caballo C, Castro P, Gil J, Millan T, et al. (2019).Candidate genes expression profiling during wilting in chickpea caused by Fusarium oxysporum f. sp. ciceris race 5. PLoS One 14: e0224212. https://doi.org/10.1371/journal.pone.0224212

Cappucino and Sherman, "Microbiology: A Laboratory Manual," 3rd Edition, Benjamin/Cumming Pub. Co., New York, 1992.

Castro P, Rubio J, Millán T, Gil J, Cobos MJ (2012) Fusarium wilt in chickpea: General aspect and molecular breeding. In Fusarium: Epidemiology, Environmental Sources and Prevention; Rios, T.F., Ortega, E.R., Eds. Nova Science Publishers: New York, NY, USA pp: 101-122.

Chaube, H. S., Mishra, D. S., Varshney, S. and Singh, U. S. (2003). Bio control of plant pathogens by fungal antagonists: a historical background, present status and future prospects. *Ann. Rev. Plant pathol.* **2**: 1-42.

Chaudhry, M.A. Ilyas, M.B. Muhammad, F. and Ghazanfar, M. U. (2007). Sources of resistance in chickpea germplasm against fusarium wilt. *Mycopath*. 5(1): 17-21.

Chauhan, Y.S., Nene, Y.L., Johansen, C, Haware, M.P., Saxena, N.P., Sardar Singh, Sharma, SB... Sahrawat,K.L., Burford, JR., Rupcla, OP., Kumar Rao, J.V.D.K., and Sithanantham, S (1988). Effects of Soil Solarization on Pigeonpea and Chickpea. *Research Bulletin* no. **11**. 502-324.

Chhokar, V., Aggarwal, H., Singh, J., Khaket, T.P. (2013). Genetic diversity in chickpea using various molecular markers: first step towards molecular breeding. *Int J Adv Res***1:** 393–398.

Chobe, D. R., Gupta, O., and Pawar, M. (2016). Radiation induced mutation for resistance against races/pathotypes of Fusarium oxysporum f.sp ciceris in chickpea (Cicer arietinum L.). *IndianPhytopath*.**69**:260–265.

Coba de la Pena, J.J. T. José J. Pueyo J. J. (2012). Legumes in the reclamation of marginal soils, from cultivar and inoculant selection to transgenic approaches Agronomy. Sustainable. Development. **32**:65-91. DOI 10.1007/s13593-011-0024-2

Cortes J. A, Hav. N., B., and Jimenez-Diaz R. M. (2012.) .Yield loss in chickpea in relation to development of Fusarium wilt epidemics," Phytopathology.**11**; 1269–1278

CSA (Central Statistical Agency of Ethiopia (2019). Agricultural sample survey 2018/19 (2011 E.C.) *Report on area and production of major crops*.

CSA (Central Statistical Agency) (1995–2017). *Agricultural Sample Survey, Report on area and production of crops, Private peasant holdings, Maher season*.

CSA (Central Statistical Agency) (2018). Agricultural sample survey 2017/2018. Report on area and production of major crops.Statistical Bulletin no. **5** 85. Addis Ababa: The Federal Democratic Republic of Ethiopia.

CSA (Central Statistical Authority) (2015). Report on area and production of major crops (private peasant holdings, Maher season). Statistical Bulletin. Addis Ababa, Ethiopia. Volume I. pp. 13.

Dagnachew Bekele ;Kassahun Tesfaye; Asnake Fikre; Douglas R. Cook (2021). The extent and association of chickpea Fusarium wilt and root rot disease pressure with major biophysical factors in Ethiopia. *Journal of Plant Pathology*.**103**:409–419.

Daniel Assfaw Mekonene (2018). Assessment of chickpea wilt/root rots intensity, pathogen identification and evaluation of chickpea varieties for resistance against the pathogens in west Shewa, Oromia regional state, Ethiopia. A thesis submitted to School of Graduate Studies, Ambo University. Unpublished

Daniel Muletta and Fassil Assefa (2015). Screening for symbiotically effective and ecologically competitive chickpea rhizobia inoculants from Ethiopian soils. *Ethiopian. Journal. Biological. Science*. (14): 1-18.

Das, K., Prasanna, R., & Saxena, A. (2017). Rhizobia: A potential bio control agent for soil borne fungal pathogens. Folia Microbiologic, **62**, 425–435.

Datta, A., Choudhary, R. G., Shamim, Md., Singh, R. K., Dhar, V. (2011). Molecular diversity in Indian isolates of Fusarium oxysporum f.sp. Lentis inciting wilt disease in lentil (Lens culinaris Medik). *African Journal of Biotechnology.* **10**(38): 7314-7323.

Dean Ralph, Van Kan Jan A L, Pretorius Zacharias A, Hammond-Kosack Kim E, Di Pietro Antonio, Spanu Pietro D, Rudd Jason J, Dickman Marty, Kahmann Regine, Jeff Ellis, Foster Gary D (2012). The Top 10 fungal pathogens in molecular plant pathology.Areview paper. *Mol Plant Pathol*. ; **13**(4):414-30.

Dipta Bhawna and Kausha Rajesh (2018). Assessment of antibiotic and fungicide resistance by indigenous Bacillus strains of cauliflower (Brassica oleracea var. botrytis L.). *The Pharma Innovation Journal;* 7(5): 26-28

Dipta, B., & Kaushal, R. (2018). Assessment of antibiotic and fungicide resistance by indigenous Bacillus strains of cauliflower (Brassica oleracea var. botrytis L.). *The Pharma Innovation*, **7(**5, Part A), 26.

Diriba Temesgen Dagaga (2017). Genetic diversity of Rhizobia and Rhizobacteria from Soybean [Glycine max (L) Merr.]: Implication for the Commercial Production and Application to Enhance Soybean Production under Low Input Agriculture in Ethiopia. A PhD Dissertation, School of Graduate studies the Addis Ababa University

DZARC (Debre Zeit Agricultural Research Center) (2005). Annual Research Report 2004/05. Debre Zeit, Ethiopia.

EEPA (Ethiopian Export Promotion Agency) (2004) Ethiopian pulses profile. Product Development and Market Research Directorate, Addis Ababa, Ethiopia.

Elnahal, A. S., El-Saadony, M. T., Saad, A. M., Desoky, E. S. M., El-Tahan, A. M., Rady, M. M., et al. (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: a review. *Eur. J. Plant Pathol*. **162**, 759–792.

Eyasu E (2016). S*oils of the Ethiopian Highlands: Geomorphology and Properties. CASCAPE Project,* ALTERA, Wageningen University and Research Centre (Wageningen UR), the Netherlands, p 385

FAO. (2018). World food and agriculture: Statistical pocketbook 2018. FAO.

FAO. (2019).FAOSTATdata base. Retrieved October 2021 from https: //www.fao.org/faostat/en/

FAOSTAT (Food and Agriculture Organization of the United Nations Statistical Database) (2018) FAOSTAT Production Statistics of Crops.

Figueiredo, M. D. V. B., Bonifacio, A., Rodrigues, A. C., & de Araujo, F. F. (2016a). Plant Growth-Promoting Rhizobacteria: Key Mechanisms of Action. *In Microbial-Mediated Induced Systemic Resistance in Plants* (pp. 23–37).

Food and Agricultural Organization of the United Nations. FASOSTAT. (2013).

Gaur MP, Aravind KJ and Rajeev KV (2012). A review of impact of Genomic technologies on chickpea breeding strategies. Agronomy, **2**: 200-203, India.

Geletu Bejiga and Million Eshete (1996). Chickpea in Ethiopia. In: (Saxena N.P., Saxena M.C., Johansen C., Virmani S.M. and Harris H. (Eds.). Adaptation of Chickpea in the West Asia and North Africa Region. pp. 137-153, ICRISAT/ACARDA.

Geletu Bejiga and Vander Maesen, L. J. G.(2006). Cicer arietinum L In: Brink, M and Belay Gurja (Eds.). PROTA 1: Cereals and pulses, PROTA, Wageningen, Netherlands.

Geletu Bejiga, Milion Eshetu and Yadeta Anbese (1996).Improved cultivars and production technology of chickpea in Ethiopia. Research Bulleti No. 2.DebreZeit, Ethiopia: *DebreZeit Agricultural Research Center*, Alemaya Univ. of Agri. P, 60.

Gemechu Keneni, Endashaw Bekele, Fassil Assefa, Muhammad Imtiaz, Tolessa Debele, Tolessa Debele, Kifle Dagne and Emana Getu (2012).Phenotypic diversity for symbio-agronomic characters in Ethiopian chickpea (Cicer arietinum L.) germplasm accessions. *African Journal of biotechnology* 11(63): 12634-12651

Girmaye Kenasa and Fassil Assefa (2017). Diversity, Plant Growth Promoting and Symbiotic Properties of Cowpea (Vigna unguiculata L. walp.) Root Nodule Bacteria from Ethiopian Soils. PhD Dissertation, School of Graduate Studies, Addis Ababa University.

Glick, B. R., Cheng, Z., Czarny, J. and Duan,J. (2007).Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur. J. Plant Pathol.**119**: 329-339.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B.K., Sandeep, D.,Vidya, Deepthi, M. S.K., Rupela, O. (2011). Evaluation of actinomycete isolates obtained

from herbal vermicomposting for the biological control of Fusarium wilt of chickpea. *Crop Prot*. **30**: 1070-1078.

Goswami D, Thakker JN, Dhandhukia PC, Tejada Moral M (2016).Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agric* 2:1127500

Gul R., Khan H., Bibi M., Ain Q. U. and Imran B. (2013).Genetic analysis and interrelationship of yield attributing traits in chickpea (Cicer arietinum L.). *The Journal of Animal & Plant Science* 23 (2): 521-526.

Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., & Singh, V. (2015). Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *J Microb Biochem Technol*, 7(2), 096-102.

Gurjar, G., Mishra, M., Kotkar, H., Upasani, M., Soni, P., Tamhane, V., Kadoo, N., Giri, A.,& Gupta, V.,(2010).Major biotic stresses of chickpea and strategies for their control. Pests and Pathogens: Management Strategies. BS Publications, ISBN: 978-81-7800-227-9

Haas, D. & Défago, G.(2005).Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol*. **3**: 307–319

Haidar, R.; Fermaud, M.; Calvo-Garrido, C.; Roudet, J. & Deschamps, A. (2016).Modes of action for biological control of Botrytis cinerea by antagonistic bacteria. *Phytopathol.Mediterr*. 55(3), 301–322.

Hajibarat, Z., A. Saidi, Z. Hajibarat and R.Talebi (2014. Genetic diversity and population structure analysis of landrace and improved chickpea (Cicer arietinum) genotypes using morphological and microsatellite markers. *Environmental and Experimental Biology* **12**: 161–166.

Hameed Ab, Harini G, Rupela Op, Rao Jvdk, Reddy G. (2010). Biological controls of chickpea collar rot by co-inoculation of antagonistic bacteria and compatible rhizobia. *Indian J Microbiol* **50**: 419–424.

Haq I, Jamil FF. (1995).Comparison of vascular discoloration and growth of Fusarium oxysporum in various segments of chickpea grown in 'wilt sick plot' in Faisalabad, Pakistan. *International Chickpea and Pigeon pea Newsletter*, **2**:30–32

Harman, G.E., Howell, C.R.,Viterbo, A., Chet, I. and Lorito, M. (2004).Trichoderma species opportunistic, a virulent plant symbionts. *Nat. Rev. Microbiol*.**2**: 43-4.

Harshita, A. Sinha, J.B. Khan, S. Trivedi, A. Verma and S.G. Rao (2018).Compatibility of Fungal and Bacterial Bio-Agents and their Antagonistic Effect against Fusarium oxysporum f. Sp. Lycopersici. *Int.J.Curr.Microbiol.App.Sci*. **7**(7): 2305-2316

Haruna, S. G., Adebitan, S. A., & Gurama, A. U. (2012). Field evaluation of compost extracts for suppression of Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici. *Int J Agr Res*, 2(7).

Hassan, M.I., Mohsan, M., Mohy-ud-Din, G. & Zia, A. (2012). Evaluation of chickpea germplasm lines against Ascochyta blight. *Pak. J. Phytopathol*., **24**, 117-121.

Hassen, A.I.; Labuschagne, N.(2010). Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. *World J. Microbiol. Biotechnol.***26**,1837–1846.

Haware MP, Nene YL and Natarajan M.(1996).Survival of F. oxysporum f.sp. ciceris. *Plant Disease*, **66**: 809-810.

Haware, M. P. (1990). Fusarium wilt and other important diseases of chickpea in the Mediterranean area. *Options Mediterranian Series*, **9**: 63-166.

Haware, M.P and Nene, Y.L. (1982).Races of Fusarium oxysporum f. sp. ciceris. *Plant Disease*. **66**: 809-10.

Haware, M.P.(1998). Diseases of chickpea. In The pathology of food and pasture legumes'. (Eds DJ Allen, JM Lenne) pp. 473–516.

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010). Soil benefcial bacteria and their role in plant growth promotion: a review. *Ann microbiol*.**60** (4):579–98.

Hossain, M.M., Hossain, N., Sultana, F., Islam, S.M.N., Islam, M.S. Bhuiyan, M.K.A. (2013). Integrated management of Fusarium wilt of chickpea (Cicer arietinum L.) caused by

Fusarium oxysporum f. sp. ciceris with microbial antagonist, botanical extract and fungicide. *Afr. J. Microbiol*. **29**: 4699-4706

Imtiaz, M., Malhotra, S. R and Yadav, S. S. (2011). Genetic Adjustment to Changing Climates: Chickpea In: Yadav, S. S., Redden J. R.., Hatfield L.J., Lotze-Campen H. and Hall E. A. (Eds.). *Crop Adaptation to Climate Change*. pp: 251-268

Ipek, M., Arikan, S., Pirlak, L. and Essitken, A. (2019). Sustainability of Crop Production by PGPR under Abiotic Stress Conditions. In: Kumar, A. and Vijay Singh Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp 292-314. Springer Nature Singapore Pte Ltd.

Iqbal, A., Ateeq, N., Khalil, I. A., Perveen, S., Saleemullah, S.(2006). Physicochemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *J. Food Service* **17**, 94-101.

Iqbal, M.S., G. Abdul, B. Ahmad, A. Iftkhar, and S. Altaf (2010). Identification of resistant resources for multiple disease resistance in chickpea. *Pakistan J. Phytopathology*, 22(2), 89-94.

Iqbal, S. M. (2005).Screening of chickpea genotypes for resistance against Fusarium wilt. *Mycopath (Pakistan*), **3**(1-2), 1-5

Iqbl, M.J., K. Iftikhar and M.B. Ilyas.(1993).Evaluation of the chickpea germplasm for resistance against wilt disease. J. Agric. Res. **314**(4):449-453.

Jha, C. K. and Saraf, M. (2015). Plant growth promoting Rhizobacteria (PGPR): a review. E3 *Journal of Agricultural Research and Development*.5 (2):0108-011

Jiménez-Díaz RM, Castillo P, del Mar Jiménez-Gasco M, Landa BB, Navas-Cortés (2015). Journal Agriculture Fusariumwilt of chickpeas: Biology, ecology and management. *Crop Protection*. **73**:16-27.

Jukanti A.K., Gaur P.M., Gowda C.L.L. and Chibbar R.N. (2012). Nutritional Quality and Health Benefits of Chickpea (Cicer arietinum L.). A review. *British Journal of Nutrition*, **108**: 12-26.

Karimi K, Amini J, Harighi B and Bahramnejad B (2012) Evaluation of biocontrol potential of Pseudomonas and Bacillus spp. against Fusarium wilt of chickpea. Australian Journal of Crop Sciences 6(4):695-703

Karthick, M., Gopalakrishnan, C., Rajeswari, E., & Pandi, V. K. (2017). In vitro Efficacy of Bacillus spp. against Fusarium oxysporum f. sp. ciceri, the Causal Agent of Fusarium wilts of Chickpea. *Int. J. Curr. Microbiol. App. Sci*, **6**(11), 2751-2756.

Khan, M. N., Ijaz, M., Ali, Q., Ul-Allah, S., Sattar, A. and Ahmad, S. (2019). Biological Nitrogen Fixation in Nutrient Management. In: Hasanuzzaman, M. (ed.), Agronomic Crops, pp. 127-148.

Khosro M (2012) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Resour Environ **2**:80–85.

Killani, A.S.; Abaidoo, R.C.; Akintokun, A.K. and Abiala, M.A. (2011). Antagonistic Effect of Indigenous Bacillus subtilis on Root-/Soil borne Fungal Pathogens of Cowpea. *Researcher*. **3**: 11-18.

Kirui Kipngetich Rickson (2016). Screening chickpea (*cicer arietinum l.)* Genotypes for fusarium wilt resistance and its management using fungicides, in njoro kenya. *A thesis submitted to the graduate school of Egerton university.* (Unpublished)

Kour, D., Rana, K. L., Yadav, N., Yadav, A. N., Kumar, A., Meena, V. S., Singh, B., Chauhan, V. S., Dhaliwal, H.S. and Saxena, A. K. (2019). Rhizospheric Micro biomes: Biodiversity, Mechanisms of Plant Growth Promotion and Biotechnological Applications for Sustainable Agriculture. In: Kumar, A. and Vijay Singh Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp19-66. *Springer Nature Singapore Pte Ltd*

Küçük, Ç., & Kivanc, M. (2008). Preliminary characterization of Rhizobium strains isolated from chickpea nodules. *African Journal of Biotechnology*, 7(6).

Kumar A, Bahadur I, Maurya B, Raghuwanshi R, Meena V, Singh D, Dixit J (2015). Does a plant growth promoting rhizobacteria enhance agricultural sustainability? *J Pure Appl Microbiol* **9**:715–724

Kumar A, Lal HC, Akhtar J (2012).Morphological and pathogenic characterization of Fusarium oxysporum f. sp. ciceris causing wilt of chickpea. *Indian Phytopathology* **65**(1):64-66

Kumar, A., Meena, V. S., Roy, P., Vandana and Kumari, R. (2019). Role of Rhizobia for Sustainable Agriculture: Lab to Land. In: Kumar, A. and Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, PP 129-149.

Kumar, B.S.D. (1999). Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chick pea growing in soils infested with Fusarium oxysporum f. sp. ciceris. *Biol. Fertil. Soils* **29**: 87–91

Kumar, S., Kumar, J., Singh, S., Seid Ahmed, Chaudhary, R.G. and Sarker, A. (2010). Vascular Wilt Disease of Lentil : *A Review.*3 p.

Kumari, S. and Khanna, V. (2014). Effect of antagonistic rhizobacteria inoculated with Mesorhizobium ciceris on control of fusarium wilt in chickpea (Cicer arietinum L.). *Afr. J. Micro. Res*. **8**(12): 1255- 1265.

Kundan, R., Pant, G., Jadon, N. and Agrawal, P.K. (2015) .Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. *J. Fertil. Pestic*. **6**: 155-163.

Lambers, H., Ahmedi, I., Berkowitz, O., Dunne, C., Finnegan, P.M. (2013) .Phosphorus nutrition of phosphorus-sensitive Australian native plants: threats to plant communities in a global biodiversity hotspot. *Conserv Physiol*.**1**: 1-21.

Landa B. B, Navas-Cortés, J. A. and Jiménez-Díaz R. M (2004). Influence of temperature on plant–rhizobacteria interactions related to biocontrol potential for suppression of fusarium wilt of chickpea. *Plant Pathology*. **53**, 341– 352.

Laranjo. M., Alexandre, A. and Oliveira, S. (2014). Legume growth-promoting rhizobia: An overview on the Mesorhizobium genus. *Microbiological Research*. **169**: 2-17.

Legesse Dadi, Senait Regassa, Asnake Fikre, Demissie Mitiku, Gaur, P. M., Gowda, C. L. L and Bantilan, M. C. S. (2005). Adoption studies on improved chickpea verities in Ethiopia. Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia

Lijalem Korbu, Asnake Fikre, Kassahun Tesfaye, Assefa Funga, Dagnachew Bekele and Chris O. Ojiewo (2022). Response of chickpea to varying moisture stress conditions in Ethiopia. Response of chickpea to varying moisture stress conditions in Ethiopia. Agrosyst Geosci Environ,5:e20234.https://doi.org/10.1002/agg2.20234.

Lugtenberg B. and Kamilova, F (2009). "Plant growth-promoting rhizobacteria," *Ann Review Microbiology,* **63**, 541–556.

Maatallah , J., Berraho, E. B., Sanjuan, J. and Lunch, C.(2002). Phenotypic characterization of rhizobia isolates from chick pea (Cicer arrientium) growing in Morroccan soils. Agronomic. **22**: 321-329.

Mamta R., Prakash H.G., Shashi B. (2021). Evaluation of the Nutritional Quality and Health Benefits of Chickpea by Using New Technology in Agriculture (NIRS). *Asian Journal of Dairy and Food Research*, **40**:123-126.

Meki, S., Ahmed S. and Sakhuja P.K. (2009).Control of chickpea wilt (Fusarium oxysporum f.sp. ciceris) using Trichoderma spp. in Ethiopia. Arch. Phytopathol. Pl. Protec. **44,** 5.

Menale Kassie, Bekele Shiferaw, Solomon Asfaw, Tsedeke Abate, Geoffrey Muricho, Stotaw Ferede, Million Eshete and Kebebew Assefa, (2009). Current situation and Future Outlooks of the chickpea sub-sector in Ethiopia. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi Ethiopia Institute of Agricultural Research (EIAR), Debre Zeit Agricultural Research Center, Debre Zeit Ethiopia,pP, 1-43.

Merkuz A, Getachew A (2012). Influence of chickpea Fusarium wilt (Fusarium oxysporum f.sp. ciceris) on Desi and Kabuli-type of chickpea in integrated disease management option at wilt sick plot in North Western Ethiopia. *Inter J Curr Res* **4**: 046-052

Merkuz A., Seid A., Chemeda F., Sakhuja P.K. and Getachew A. (2011). Effects of mustard green manure and dried plant residue on chickpea wilt (Fusarium oxysporum f.sp. ciceris). *Arch. Phytopathol. Pl. Protec*. **44**: 821 – 831

Merkuz Abera, Chemeda Fininsa, P.K. Sakhuja and Seid Ahmed (2010).Epidemiology and Management of Chickpea Fusarium Wilt (Fusarium Oxysporum F.Sp Ciceris) in Northwestern Ethiopia.

Merkuz, A. and Getachew, A.(2012a). Influence of chickpea fusarium wilt (Fusarium oxysporum f.sp.ciceris), on Desi and Kabuli-type of chickpea in integrated disease management option at wilt sick plot in north western Ethiopia. *International Journal of Current Research*, 4 (04): 45-46.

Millan T, Clarke H, Siddique K, Buhariwalla H, Gaur P, et al. (2006) Chickpea molecular breeding: New tools and concepts. *Euphytica* **147**: 81-103.

Mirzapour, S., Darvishnia, M., Bazgir, E. and Goodarzi, D. (2014). Identification of resistant sources in chickpea against Fusarium wilts under greenhouse condition. *International Journal of Farming and Allied Sciences*, **3**: 772-776.

Mitiku Demissie (2011). Marketing of kabuli and desi chickpeas by smallholder farmers in Eastern Shewa Zone. M.Sc. thesis submitted to Haramaya University, Ethiopia.Mnalku, A., & Mitiku, G.(2019). Response of chickpea (Cicer arietinum L.) to indigenous rhizobia isolates inoculation on vertisol of Central Ethiopian Highland. Ethiopian *Journal of Agricultural Sciences,***29**(2), 109-117.

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of Soil Science and Plant Nutrition. 13(3): 638-649.doi:10.4067/s0718-9516201300500005

Moradi, H., Bahramnejad, B., Amini, Siosemarde, A., and Allahverdipoor, K.H. (2012). Suppression of chickpea (*Cicer arietinum L*.) Fusarium wilt by *Bacillus subtillis* and *Trichoderma harzianum*. *Pl. Omics J*. **5**(2): 68-74.

Muhammad Anis, M.; Waseem, A. and. Javed, Z.M. (2010).Bio efficacy of microbial antagonists against Macrophomina phaseolina on sunflower. *Pak. J. Bot.*, **42**(4): 2935-2940.

Muhammad Rafiq, Muhammad Tariq Mahmood, Mushtaq Ahmad, Imtiaz Ali, Sadia Kaukab, Muhammad Shafiq, Muhammad Saleem, Umer Iqbal (2020). A review on Fusarium wilt's pathogenic studies and disease management.

Mulissa Jida and Fassil Assefa (2012). Phenotypic diversity and plant growth promoting characteristics of Mesorhizobium species isolated from chickpea (Cicer arietin/ {IJI L.) growing areas of Ethiopia. *Ajar-. J. Biotechnol*. **11**: 7483-7493.

Mulissa Jida, Löscher, C. R., Schmitz, R. A. and Fassil Assefa (2016). Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea (Ciceraeritinum L.) producing areas of Ethiopia. *Afr J Biotechnol*. **15**(35): 1899-1912.

Muzquiz M. and Wood J.A. (2007). Chickpea breeding and management. *Oxford University Press, New York, USA*. pp. 101-142.

Navas Cortes JA, Landa BB, Rodriguez Lopez J, Jimenez Diaz RM, Castillo P (2008) Infection by Meloidogyne eartiellia does not break down resistance to races 0, 1A, and 2 of *Fusarium oxysporum f. sp Ciceris* in chickpea genotypes. *Phytopathology*, **98**:709–718.

Nazir, M., Khan, M., & Ali, S. (2012). Evaluation of national and international chickpea germplasm for resistance against Fusarium wilt (Fusarium oxysporum f. sp. ciceris) in Pakistan. Pakistan. *Journal of Phytopathology*, **24**(2), 149-151.

Nene YL, Reddy MV, Haware MP, Ghanekar AM, Amin KS, Pande S, Sharma M (2012). Field diagnosis of chickpea diseases and their control. Information bulletin No. 28 (revised). *International Crops Research Institute for the Semi-arid Tropics, Patancheru*

Neupane, R.K., Sharma M., Jha, P., Narayana, R.J., Rao B.V., Gaur, P.M. and Pande, S. (2007). Evaluation of chickpea genotypes for resistance to Fusarium wilt in Nepal. *An open access Journal by ICRISAT*

Nico, L., Pretorius, T. and Idris,A.H. (2010).Plant Growth Promoting Rhizobacteri as Biocontrol Agents against Soil-Borne Plant Disease. In: *Plant Growth and Health Promoting Bacteria*, pp 221-230.

Nigussie, T. (1995). ICC 6045: A kabuli chickpea resistant to wilt and root rots in Ethiopia. *International chickpea and pigeon pea Newsletter, 2:17-19.*

Osei R., Yang C., Cui L., Wei L., Jin M., Wei X. (2022): Antagonistic bio agent mechanisms of controlling potato soft rot. *Plant Protect*. Sci., **58**: 18–30.

Oves, M., Khan, M. S., & Zaidi, A.(2013). Chromium reducing and plant growth promoting novel strain Pseudomonas aeruginosa OSG41 enhance chickpea growth in chromium amended soils. European Journal of Soil Biology, 56, 72–83

Pal, K. K. and Gardener, B. McS. (2006).Biological Control of Plant Pathogens. *The Plant Health Instructor*. **10**:1117-1142.

Parasappa, R. S., Mishra, R. K. and Chaturvedi, S. K. (2017). New sources of resistance in land races and advance germplasm against Fusarium oxysporum f. sp. ciceris race 2 causal agent of chickpea wilt. *Legume Research*, 40(2): 364-368.

Patil, P.P.; Raut, B.T.; Minakshi, P.; Ambhore, A.K. and Sonavane, R.S. (2003). Efficacy of different bio control agents and chemical agents against seed/ seedling/root rot of sunflower. *Ann. Plant Physiol*., **17**(2): 187-189.

Premachandra, D., Hudek, L. and Brau, L., (2016) Bacterial Modes of Action for Enhancing of Plant Growth. *J. Biotechnol. Biomater*. **6**: 236-244.

Qiao, J.; Yu, X.; Liang, X.; Liu, Y.; Borriss, R.; Liu, Y. (2017). Addition of plant-growth-promoting Bacillus subtilis PTS-394 on tomato rhizosphere has no durable impact on composition of root micro biome. *BMC Microbiol*. **17,**131.

Rabosto, X.; Carrau, M.; Paz, A.; Boido, E.; Dellacassa, E. & Carrau, F.M. (2006).Grapes and vineyard soils as sources of microorganisms for biological control of Botrytis cinerea. *Am. J. Enol. Viticult.* **57**(3), 332-338

Raju, G.P., Rao, S.V.R., Gopal, K. (2008). In vitro evaluation of antagonists and fungicides against the red gram wilt pathogen Fusarium oxysporum f. sp. udum (Butler) Snyder and Hansen. *Legume Research*. **31**(2): 133-135.

Ranjana Joshi, Gururaj Sunkad, Amaresh Y.S. and Mallikarjun Kenganal (2022). Biological management of Fusarium oxysporum f. sp. ciceris an incitant of chickpea wilts by consortium of Trichoderma asperellum and Pseudomonas fluorescens under glasshouse conditions. Biological Forum – *An International Journal*, **14**(2): 346-349.

Reino, L.R, Raul, F., Herna´ndez-Gala´n, G.R., Collado, I.G (2008). Secondary metabolites from species of the biocontrol agent Trichoderma. *Phytochem. Rev* **7**: 89-123.

Rhitu R, Prasanta KD, Trilochan M., and Aqbal S. (2012). Phenotypic and molecular characterization of indigenous rhizobia nodulating chickpea in India. *Indian J. Extal.Biol.* **50**:340-350.

Rufus J. Akinrinlola, Gary Y. Yuen, Rhae A. Drijber, and Anthony O. Adesemoye (2018). Evaluation of Bacillus Strains for Plant Growth Promotion and Predictability of Efficacy by In Vitro Physiological Traits. *Hindawi International Journal of Microbiology*.

Sachin, S., Govind, G., Ekta, K., Behal, K. K., & Arora, N. K. (2014). Effect of enrichment material on the shelf life and field efficiency of bio formulation of Rhizobium sp. and P-solubilizing Pseudomonas fluorescens. *Science Research Reporter*, **4**(1), 44-50.

Sahu, G.K. & Sindhu, S.S (2011).Disease control and plant growth promotion of green gram by siderophore producing Pseudomonas sp. *Res. J. Microbiol*. **6**(10), 735–749.

Sahu, P. K., Singh, S., Gupta, A., Singh, U. B., Brahmaprakash, G. P., and Saxena, A. K. (2019). Antagonistic potential of bacterial endophytes and induction of systemic resistance against collar rot pathogen Sclerotium rolfsii in tomato. *Biol. Control* **137**:104014.

Sampaio AM, Araújo SdS, Rubiales D, Vaz Patto MC. (2020) .Fusarium wilt management in legume crops. *Agronomy*.**10**:1073

Sanjeevkumar, K., Balabaskar, P., Siva Kumar, T., & Renganathan, P. (2019). Effect of Bacterial Antagonists against Root Rot of Cowpea Caused by Macrophomina Phaseolina (Tassi.) Goid. *Plant Archives,* **19** (2), 2430-2435.

Satyavir, S.S., Yuvraj, S.,Rakshiya, G. S. (2009). Biological control of soil borne plant pathogens with rhizosphere bacteria. Pest Technology. **3**:10-21.

Selim H.M.M., Gomaa N.M., Essa A.M.M. (2017): Application of endophytic bacteria for the bio control of Rhizoctonia solani (Cantharellales: Ceratobasidiaceae) damping-off disease in cotton seedlings. *Bio control Science and Technology*, **27**: 81–95.

Senthilkumar, M.; Swarnlakshmi, K.; Govindasamy, V.; Lee, Y.K. and Annapurna, K. (2009). Bio control potential of soybean bacterial endophytes against charcoal rot fungus *Rhizoctonia bataticola*. *Curr Microbiol.*,**58**: 288–93.

Sevim, E., & Sevim, A. (2015). Plasmid mediated antibiotic and heavy metal resistance in Bacillus strains isolated from soils in Rize, Turkey. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, **19**(2), 133-141.

Shahzad S, Rajput AQ, Khanzada MA. (2018).Effect of different organic substrates and carbon and nitrogen sources on growth and shelf life of trichoderma harzianum. *J Agr Sci Tech* 16: 731-745.

Sharma M, Sengupta A, Ghosh R, Agarwal G, Tarafdar A, Nagavardhini A, Pande S, Varshney RK (2016). Genome wide transcriptome profiling of Fusarium oxysporum f sp. Ciceris conidial germination reveals new insights into infection related genes. *Sci Rep* **6**:37353.

Sharma S, Kumar V, Tripathi RB (2017) Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J Microbiol Biotechnol Res***1**:90–95.

Sharma, K.D.andF.J. Muehlbauer. (2007). Fusarium wilt of chickpea: physiological specialization, genetics of resistance and resistance gene tagging. *Euphytica.***157**:1–14.

Sharon Nagpal, Poonam Sharma, Asmita Sirari &Gupta R.K. (2020). Coordination of Mesorhizobium sp. and endophytic bacteria as elicitor of bio control against Fusarium wilts in chickpea. *Eur JPlant Pathol*. DOI 10.1007/s10658-020-02062-1

Shiferaw Bekele, Jones R, Silim S, Tekelewold Haile Mariam and Gwata E (2007). Analysis of production costs, market opportunities and competitiveness of desi and kabuli chickpea in Ethiopia. IMPS (Improving productivity and market Success) of Ethiopian Farmers Project Working Paper 3. *ILRI (International Livestock Research Institute), Nairobi, Kenya*, pp. 48.

Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006). Role of cyanide production by Pseudomonas fluorescens CHA0 in the suppression of root-knot nematode, Meloidogyne javanica in tomato. *World J Microbiol Biotechnol* **22:**641–650.

Sindhu, S.S.; Sehrawat, A.; Sharma, R. & Dahiya, A. (2016). Bio pesticides: Use of rhizosphere bacteria for biological control of plant pathogens. *Def. Life Sci. J.* **1**(2), 135–148.

Singh B. P, Saikia R, Yadav M, Singh R, Chauhan VS and Arora DK. (2006). Molecular characterization of F. oxysporum f. sp. Ciceris causing wilt of chickpea. *African Journal of Biotechnology*, **5**(6), 497-502.

Singh G, Chen W, Rubiales D, Moore K, et al. (2007) Diseases and their management. In Chickpea Breeding and Management (Eds Yadav, Redden, Chen and Sharma). *CAB International, pp.* 497-519. https://doi.org/10.1079/9781845932138.024

Singh, R., Sharma, R., Varshney, R. K., Sharma, S. K and Singh, N. K. (2008). Chickpea improvement: Role of wild species and genetic markers. *Biotechnology and Genetic Engineering Reviews* **25**: 267-314.

Sisay Guta (2018). Isolation And Characterization of Chickpea (Cicer Arietinum L) root endophytic rhizobacteria antagonistic to Fusarium oxysporum(Chickpea Wilt). *A thesis Submitted Bahir Dar University* (Un Published Source).

Somasegaran, P.and Hoben, H.J. (1994). Handbook for Rhizobia: Methods in Legume Rhizobium Technology. *Springer-Verlag New York, USA*, pp. 415.

Sultan Mohammed Yimer, Seid Ahmed, Chemeda Fininsa, NegussieTadesse, Aladdin Hamwieh, Douglas R.Cook (2018). Distribution and factors influencing chickpea wilt and root rot epidemics in Ethiopia. *Crop Protection*, ***106***, 150-155.

Suman Kumari and Veena Khanna (2018). Biological Management of Vascular Wilt of Chickpea (Cicer arietinum L.) Incited by Fusarium oxysporum f. sp. ciceris by Antagonistic Rhizobacteria Co-Inoculated with Native Mesorhizobium. *Int.J.Curr.Microbiol.App.Sci*. **7**(01): 920-941.

Sumeet and Mukerji,(2000). Bio control potential and its exploitation in sustainable agriculture: Volume 1. Crop diseases, Weeds, and Nematodes. *Kluwer Academy plenum, New York.* Pp. 39-48.

Sunkad G, Deepa H, Shruthi TH, Singh D (2019). Chickpea wilt: status, diagnostics and management. Indian Phytopathol **72**: 619-627.

Tebikew Damite, Asnake Fikre, Kibebew Assefa and Lijalem Korbu (2009). Improved Chickpea (*Cicer arietinum L*.) Technologies and seed production in Ethiopia. Ethiopia Agricultural Research Center Institute (EIARC), *Research bulletin, Debre Zeit, Ethiopia*

Tebkew Damte and Chris O. Ojiewo. (2016). Current status of wilt/root rot diseases in major chickpea growing areas of Ethiopia, Archives of Phytopathology and Plant Protection, **49** 222-238.

Tewari, A. K and Mukhopadhyay, A. N. (2003). Management of chickpea root rot and collar rot by integration of biological and chemical seed treatment. *Indian Phytopathology* **56:**39- 42.

Toral, L.; Rodríguez, M.; Béjar, V. & Sampedro, I. (2018). Antifungal activity of lipopeptides from Bacillus XT1 CECT 8661 against Botrytis cinerea. *Front. Microbiol.* **9**: 1315

Upadhyaya, H.D., Gowda, G.L.L. and Sastry, DVSSR. (2008). Management of Germplasm Collections and Enhancing their Use by Mini Core and Molecular Approaches. Workshop Capacity Building for risk Management Systems, Genet Resources. Pp., 35-70.

Upasani ML, Limaye BM, Gurjar GS, Kasibhatla SM, Joshi RR, Kadoo NY, Gupta VS (2017). Chickpea Fusarium oxysporum interaction transcriptome reveals differential modulation of plant defense strategies. Sci Rep **7**:7746.

Valentine, A. J., Benedito, A. and Kang, Y. (2011). Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. *Annual Plant Reviews*. 42: 207- 248

Vargas, L.K., Lisboa, B. B., Giongo, A., Beneduzi, A. and Pereira Passaglia, M. M. (2010). Potential of Rhizobia as Plant Growth Promoting Rhizobacteria. In: Khan, M. S., Zaidi, A. and Musarrat, J. (ed.), Microbes for Legume Improvement, pp 137- 155. SpringerWienNewYork

Varshney, R.K.; Song, C.; Saxena, R.K.; Azam, S.; Yu, S.; Sharpe, A.; Cannon, S.; Baek, J.; Rosen, B.D.; Taran, B.; et al.(2013).Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat. Biotechnol.* ***31***, 240–246.

Wood, J.A, Knights, E.J. Harden, S. and Choct, M (2012). Milling Performance and other Quality Traits affected by Seed Shape in Isogenic Lines of Desi Chickpea (Cicer arietinum L.). *Journal of Agricultural Science*. **4** (10), 244 – 252.

Yadav Janardan, Verma Jay Prakash and Tiwari Nath Kavindra (2010). Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (Cicer arietinum L.) under in Vitro conditions. *An International Journal*, **2**(2): 15-18.

Yadeta A and Geletu B (2002). Evaluation of Ethiopian chickpea landraces for tolerance to drought. *Genetic Resources and Crop Evolution* **49:** 557-564.

Yaqub F, shahzad S. (2011).Efficacy and persistence of microbial antagonists against Sclerotium rolfsii under field conditions. *Pak J Bot* **43**: 2627–2634

Yonas Urbanos (2010). Characterization of antagonistic Trichoderma species against Coffee Wilt Disease (Fusarium xylarioides). A Thesis submitted to the School of Graduate Studies of Addis Ababa University (Unpublished source).

Yu, X.; Ai, C.; Xin, L. & Zhou, G.(2011).The siderophore-producing bacterium, Bacillus subtilis CAS15, has a bio control effect on Fusarium wilt and promotes the growth of pepper. *Eur. J. Soil Biol*. **47**(2), 138–145.

Zahran, H.H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. **63**: 968-989.

Zaim S, Belabid L, Bellahcene M. (2013). Bio control of chickpea Fusarium wilt by Bacillus spp. rhizobacteria. *Journal of Plant Protection Research*. **53**(2):177–183.

Zehara Mohammed Damtew., Douglas R Cook., Greenlon A., Asnake Fikre., von Wettberg E., Marques E., Kassahun Tesfaye., Carrasquilla-Garcia N and Fassil Assefa (2020). Ecological Competence, Plant Growth Promoting and Symbiotic Characteristics of Different Mesorhizobium Strains Nodulating Chickpea (Cicer arietinum L.) from Ethiopia. *Journal of Plant Pathology and Microbiology*. 11(8): 1-11.

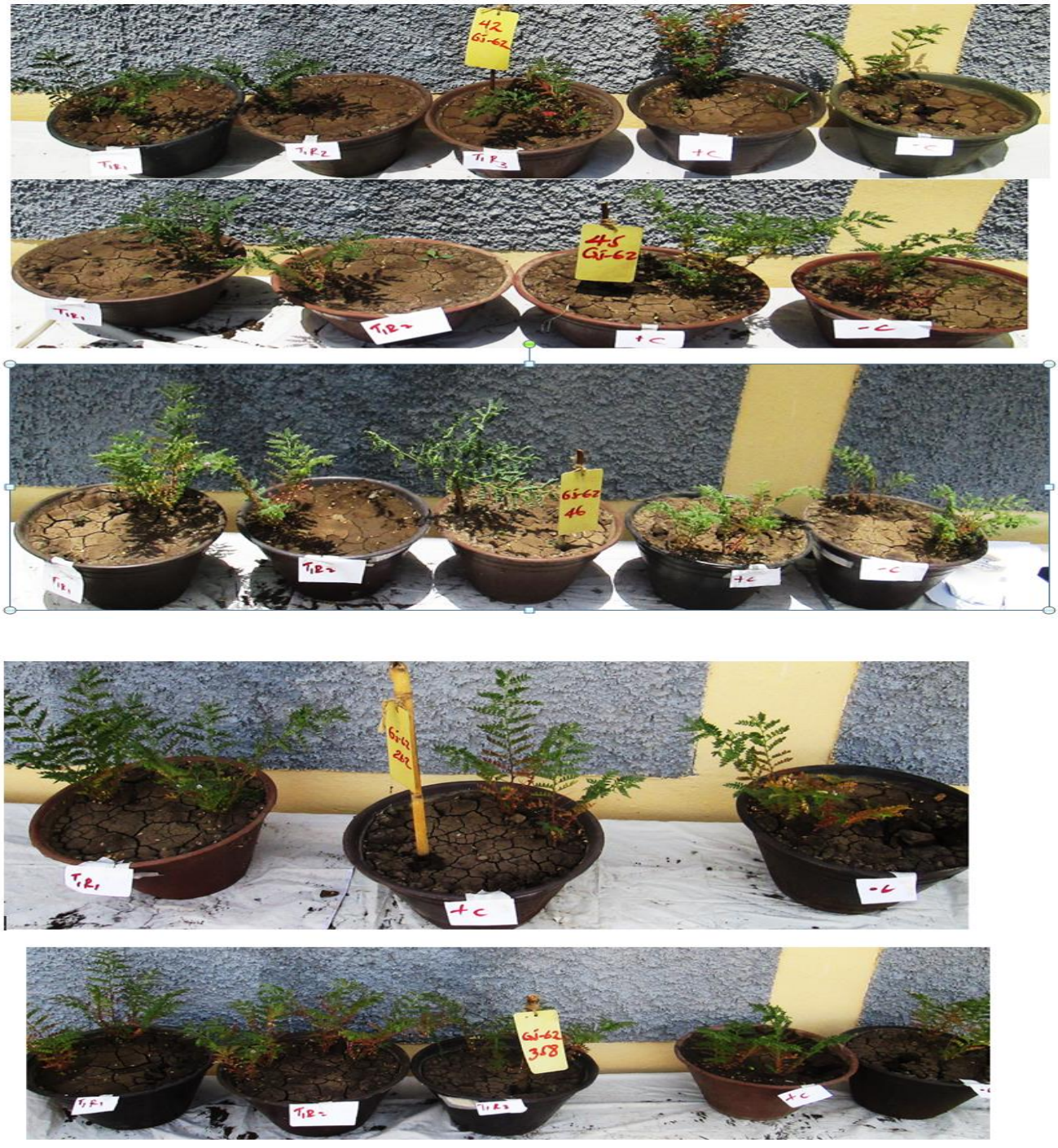
APPENDIXES

Appendix1: Effects of Rhizobacteria inoculation of growth of Dhera chickpea genotypes


T1R1
Dhara 262
+C
-C

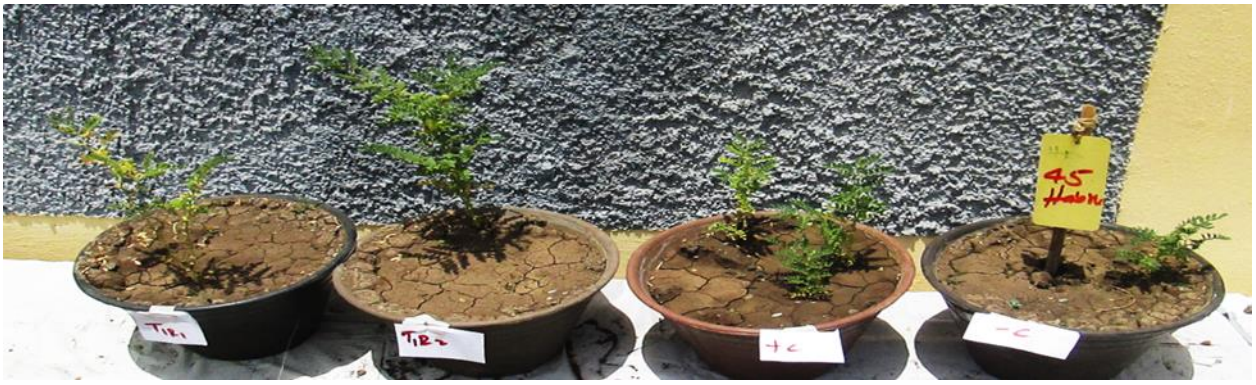

T1R1
T1R2
Dhara 358
+C
-C

Appendix 2: Effects of Rhizobacteria inoculation on JG-62 chickpea genotype



Appendix 3: Effects of PGPR inoculation on growth parameters of Eshete chickpea genotypes.

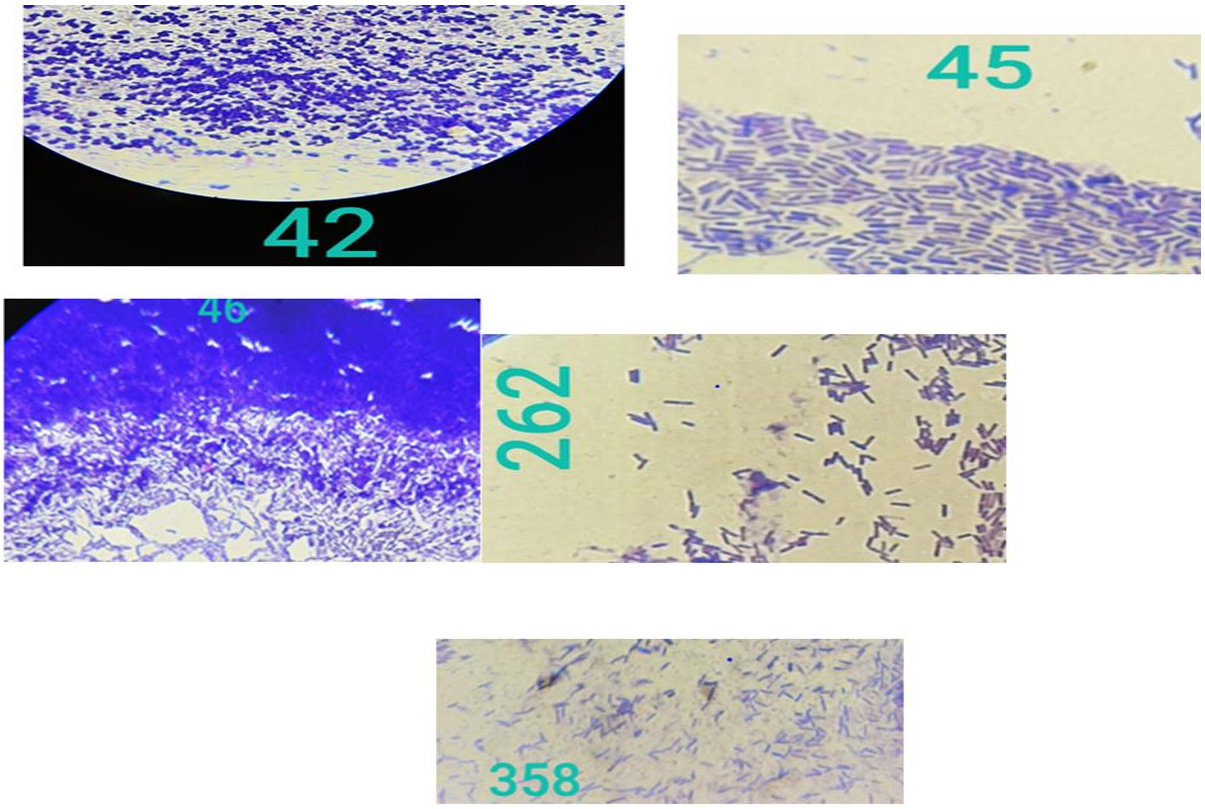
Appendix 4 : Effects of PGPR inoculation on growth parameters of Habru chickpea genotypes




T1R1
T1R2
+C
T1R3
+C
-C


T1R1
T1R2
-C
+C

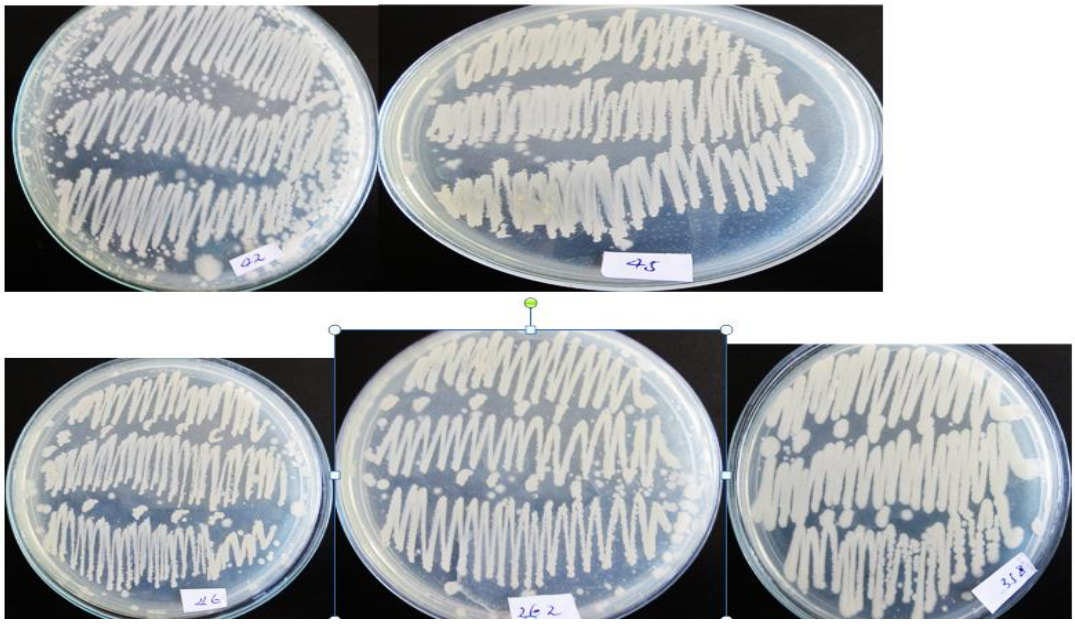
Appendix 5: Gram staining images of Isolates



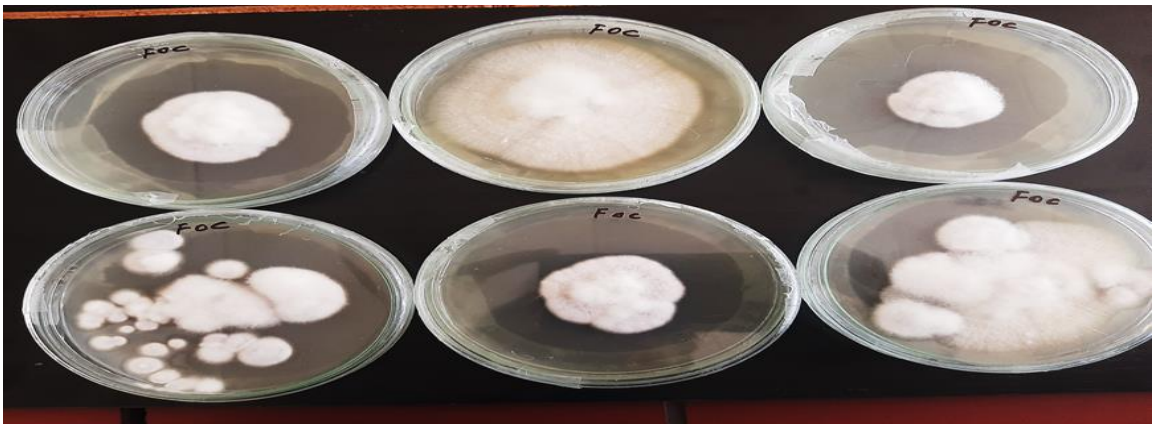
Appendix 6: Culture of *Bacillus subtilis* and *paenobacillus polymayxa* grown for seed bio-priming

Appendix 7: Bio-primed chickpea seeds allowed to air dry

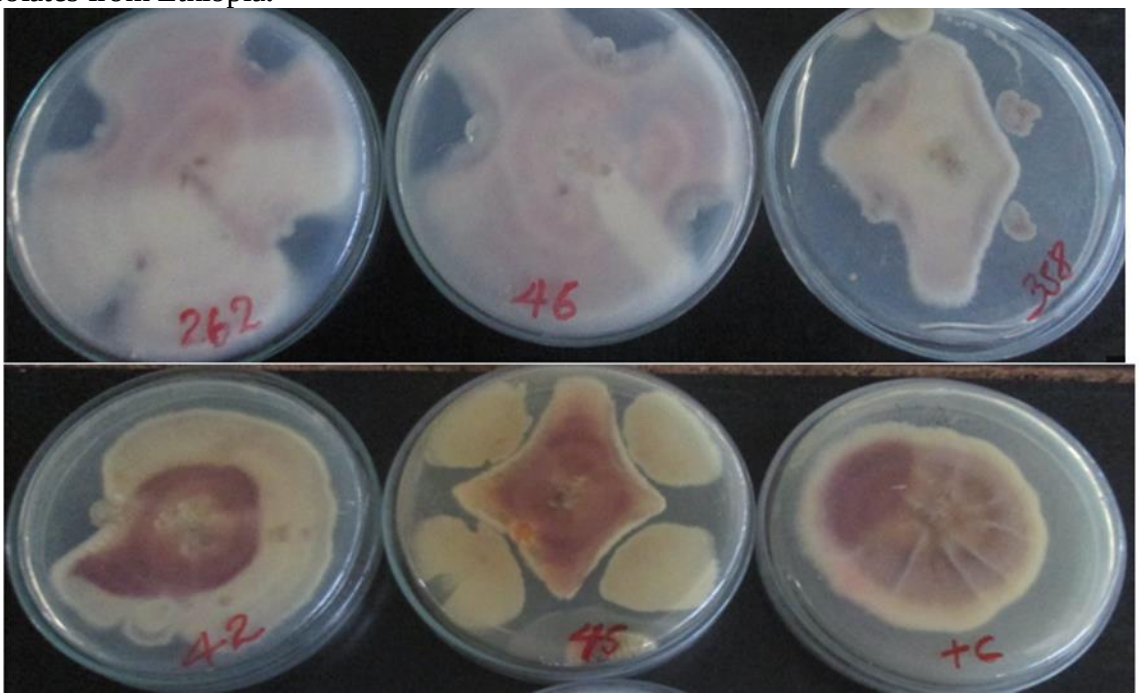
Appendix 8 : Colony morphology of pure culture of Rhizobacteria isolated from chickpea Rhizosphere on NA.



Appendix 10: Pure Culture of Fusarium Oxysporium ceceri on Waxyman Agar



Appendix11. In-vitro inhibition of Fusarium oxysporium ceceri by selected rhizobacterial isolates from Ethiopia**.**



Appendix 12: Green House Experiments partial view

Appendix 13: 16SRNA partial sequence of the isolates

Paenibacillus polymyxa strain ALCR42 16S ribosomal RNA gene, partial sequence

GenBank: KJ381098.1

FASTA Graphics PopSet

LOCUS KJ381098 807 bp DNA linear BCT 23-APR-2014
DEFINITION Paenibacillus polymyxa strain ALCR42 16S ribosomal RNA gene, partial sequence.
ACCESSION KJ381098
VERSION KJ381098.1
KEYWORDS .
SOURCE Paenibacillus polymyxa
ORGANISM *Paenibacillus polymyxa*
Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae;
Paenibacillus.
REFERENCE 1 (bases 1 to 807)
AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F. TITLE Diversity and plant growth promoting characteristics of antagonistic rhizobacteria isolated from chickpea and lentil rhizosphere JOURNAL Unpublished
REFERENCE 2 (bases 1 to 807)
AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.
TITLE Direct Submission
JOURNAL Submitted (31-JAN-2014) Microbial, Cellular and Molecular Biology,
Addis Ababa University, College of Natural Sciences, Addis Ababa
1176, Ethiopia
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..807
/organism="Paenibacillus polymyxa"

/mol_type="genomic DNA"
/strain="ALCR42"
/isolation_source="rhizosphere of lentil"
/db_xref="taxon:1406"
/country="Ethiopia"
/collection_date="Oct-2010"
/note="PCR_primers=fwd_name: 27f, rev_name: 1492r"
rRNA <1..>807
/product="16S ribosomal RNA"
ORIGIN
1 atactgcaag ttcgagccgg ggttatttag aagcttgctt ctaaataacc tagcggcgga
61 cgggtgagta acacgtaggc aacctgccca caagacaggg ataactaccg gaaacggtag
121 ctaatacccg atacatcctt ttcctgcatg ggcgaaggag gaaagacgga gcaatctgtc
181 acttgtggat gggcctgcgg cgcattagct agttggtggg gtaaaggcct accaaggcga
241 cgatgcgtag ccgacctgag agggtgatcg gccacactgg gactgagaca cggcccagac
301 tcctacggga ggcagcagta gggaatcttc cgcaatgggc gaaagcctga cggagcaacg
361 ccgcgtgagt gatgaaggtt ttcggatcgt aaagctctgt tgccagggaa gaacgtcttg
421 tagagtaact gctacaagag tgacggtacc tgagaagaaa gccccggcta actacgtgcc
481 agcagccgcg gtaatacgta gggggcaagc gttgtccgga attattgggc gtaaagcgcg
541 cgcaggcggc tctttaagtc tggtgtttaa tcccgaggct caacttcggg tcgcactgga
601 aactggggag cttgagtgca gaagaggaga gtggaattcc acgtgtagcg gtgaaatgcg
661 tagagatgtg gaggaacacc agtggcgaag gcgactctct gggctgtaac tgacgctgag
721 gcgcgaaagc gtggggagca aacaggatta gataccctgg tagtccacgc cgtaacgatg
781 aatgctaggt gttaggggtt cgatacc
//

Bacillus subtilis strain ALCR45 16S ribosomal RNA gene, partial sequence

GenBank: KJ381101.1

FASTA Graphics PopSet

Go to:

LOCUS KJ381101 742 bp DNA linear BCT 23-APR-2014

DEFINITION Bacillus subtilis strain ALCR45 16S ribosomal RNA gene, partial sequence.

ACCESSION KJ381101

VERSION KJ381101.1

KEYWORDS .

SOURCE Bacillus subtilis

ORGANISM *Bacillus subtilis*

Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.

REFERENCE 1 (bases 1 to 742)

AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.

TITLE Diversity and plant growth promoting characteristics of antagonistic rhizobacteria isolated from chickpea and lentil rhizosphere

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 742)

AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.

TITLE Direct Submission

JOURNAL Submitted (31-JAN-2014) Microbial, Cellular and Molecular Biology, Addis Ababa University, College of Natural Sciences, Addis Ababa 1176, Ethiopia

COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..742
/organism="Bacillus subtilis"
/mol_type="genomic DNA"
/strain="ALCR45"

/isolation_source="rhizosphere of lentil"

/db_xref="taxon:1423"

/country="Ethiopia"

/collection_date="Oct-2010"

/note="PCR_primers=fwd_name: 27f, rev_name: 1492r"

rRNA <1..>742

/product="16S ribosomal RNA"

ORIGIN

```
      1 agcttgctcc ctgatgttag cggcggacgg gtgagtaaca cgtgggtaac ctgcctgtaa
     61 gactgggata actccgggaa accggggcta ataccggatg gttgtttgaa ccgcatggtt
    121 caaacataaa aggtggcttc ggctaccact tacagatgga cccgcggcgc attagctagt
    181 tggtgaggta acggctcacc aaggcgacga tgcgtagccg acctgagagg gtgatcggcc
    241 acactgggac tgagacacgg cccagactcc tacgggaggc agcagtaggg aatcttccgc
    301 aatggacgaa agtctgacgg agcaacgccg cgtgagtgat gaaggttttc ggatcgtaaa
    361 gctctgttgt tagggaagaa caagtaccgt tcgaataggg cggtaccttg acggtaccta
    421 accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg tggcaagcgt
    481 tgtccggaat tattgggcgt aaagggctcg caggcggttt cttaagtctg atgtgaaagc
    541 ccccggctca accggggagg gtcattggaa actggggaac ttgagtgcag aagaggagag
    601 tggaattcca cgtgtagcgg tgaaatgcgt agagatgtgg aggaacacca gtggcgaagg
    661 cgactctctg gtctgtaact gacgctgagg agcgaaagcg tgggagcgaa caggattaga
    721 taccctggta gtccacgccg cg
```

//

Bacillus subtilis strain ARC262 16S ribosomal RNA gene, partial sequence

GenBank: KJ381129.1

FASTA Graphics PopSet

LOCUS KJ381129 694 bp DNA linear BCT 23-APR-2014
DEFINITION Bacillus subtilis strain ARC262 16S ribosomal RNA gene, partial sequence.
ACCESSION KJ381129
VERSION KJ381129.1
KEYWORDS .
SOURCE Bacillus subtilis
ORGANISM *Bacillus subtilis*
Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 694)
AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F. TITLE Diversity and plant growth promoting characteristics of antagonistic rhizobacteria isolated from chickpea and lentil rhizosphere JOURNAL Unpublished
REFERENCE 2 (bases 1 to 694)
AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.
TITLE Direct Submission
JOURNAL Submitted (31-JAN-2014) Microbial, Cellular and Molecular Biology, Addis Ababa University, College of Natural Sciences, Addis Ababa 1176, Ethiopia
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..694
/organism="Bacillus subtilis"
/mol_type="genomic DNA"
/strain="ARC262"
/isolation_source="rhizosphere of chickpea"
/db_xref="taxon:1423"

/country="Ethiopia"

/collection_date="Oct-2010"

/note="PCR_primers=fwd_name: 27f, rev_name: 1492r"

rRNA <1..>694

/product="16S ribosomal RNA"

ORIGIN

1 agcttgctcc ctgatgttag cggcggacgg gtgagtaaca cgtgggtaac ctgcctgtaa
61 gactgggata actccgggaa accggggcta ataccggatg gttgtttgaa ccgcatggtt
121 caaacataaa aggtggcttc ggctaccact tacagatgga cccgcggcgc attagctagt
181 tggtgaggta acggctcacc aaggcgacga tgcgtagccg acctgagagg gtgatcggcc
241 acactgggac tgagacacgg cccagactcc tacgggaggc agcagtaggg aatcttccgc
301 aatggacgaa agtctgacgg agcaacgccg cgtgagtgat gaaggttttc ggatcgtaaa
361 gctctgttgt tagggaagaa caagtaccgt tcgaataggg cggtaccttg acggtaccta
421 accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg tggcaagcgt
481 tgtccggaat tattgggcgt aaagggctcg caggcggttt cttaagtctg atgtgaaagc
541 ccccggctca accggggagg gtcattggaa actggggaac ttgagtgcag aagaggagag
601 tggaattcca cgtgtagcgg tgaaatgcgt aaagatgtgg aggaacacca gtggcgaagc
661 gactctctgg tctgtactga cctgagagca aagc

Bacillus subtilis strain ARC358 16S ribosomal RNA gene, partial sequence

GenBank: KJ381144.1

FASTA Graphics PopSet

LOCUS KJ381144 683 bp DNA linear BCT 23-APR-2014
DEFINITION Bacillus subtilis strain ARC358 16S ribosomal RNA gene, partial sequence.
ACCESSION KJ381144
VERSION KJ381144.1
KEYWORDS .
SOURCE Bacillus subtilis
ORGANISM Bacillus subtilis
Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 683)
AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.

TITLE Diversity and plant growth promoting characteristics of antagonistic rhizobacteria isolated from chickpea and lentil rhizosphere

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 683)

AUTHORS Jida,M., Loscher,C.R., Schmitz-Streit,R.A. and Assefa,F.
TITLE Direct Submission
JOURNAL Submitted (31-JAN-2014) Microbial, Cellular and Molecular Biology, Addis Ababa University, College of Natural Sciences, Addis Ababa 1176, Ethiopia
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..683
/organism="Bacillus subtilis"
/mol_type="genomic DNA"
/strain="ARC358"
/isolation_source="rhizosphere of chickpea"
/db_xref="taxon:1423"
/country="Ethiopia"
/collection_date="Oct-2010"
/note="PCR_primers=fwd_name: 27f, rev_name: 1492r"
rRNA <1..>683
/product="16S ribosomal RNA"
ORIGIN
1 ggagcttgct ccctgatgtt agcggcggac gggtgagtaa cacgtgggta acctgcctgt
61 aagactggga taactccggg aaaccggggc taataccgga tggttgtttg aaccgcatgg
121 ttcaaacata aaaggtggct tcggctacca cttacagatg gacccgcggc gcattagcta
181 gttggtgagg taacggctca ccaaggcaac gatgcgtagc cgacctgaga gggtgatcgg
241 ccacactggg actgagacac ggcccagact cctacgggag gcagcagtag ggaatcttcc
301 gcaatggacg aaagtctgac ggagcaacgc cgcgtgagtg atgaaggttt tcggatcgta
361 aagctctgtt gttagggaag aacaagtacc gttcgaatag ggcggtacct tgacggtacc
421 taaccagaaa gccacggcta actacgtgcc agcagccgcg gtaatacgta ggtggcaagc
481 gttgtccgga attattgggc gtaaagggct cgcaggcggt ttcttaagtc tgatgtgaaa
541 gcccccggct caaccgggga gggtcattgg aaactgggga acttgagtgc agaagaggag
601 agtggaattc cacgtgtagc ggtgaaatgc gtagagatgt ggaggaacac cagtggcgaa
661 ggcgactctc tggtctgtag taa//

Appendix14: One-way ANOVA result analysis output of the plant growth parameters in each chickpea genotypes from SPSS software Dhera, Gj-62, Eshete and Habru, respectively

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Plant height	Between Groups	470.544	6	78.424	2.250	.059
	Within Groups	1324.533	38	34.856		
	Total	1795.078	44			
Shoot fresh weight	Between Groups	197.840	6	32.973	1.865	.112
	Within Groups	671.680	38	17.676		
	Total	869.520	44			
Shoot dry weight	Between Groups	5.434	6	.906	1.137	.360
	Within Groups	30.272	38	.797		
	Total	35.706	44			
Root fresh Weight	Between Groups	374.152	6	62.359	4.218	.002
	Within Groups	561.788	38	14.784		
	Total	935.940	44			
Root Dry weight	Between Groups	3.420	6	.570	2.206	.064
	Within Groups	9.819	38	.258		
	Total	13.239	44			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
root height	Between Groups	90.450	6	15.075	3.223	.012
	Within Groups	177.713	38	4.677		
	Total	268.163	44			
Shoot Height	Between Groups	119.978	6	19.996	2.243	.060
	Within Groups	338.833	38	8.917		
	Total	458.811	44			
Shoot Fresh Weight	Between Groups	95.527	6	15.921	2.619	.032
	Within Groups	231.005	38	6.079		
	Total	326.532	44			
Shoot Dry Weight	Between Groups	5.079	6	.847	2.152	.070
	Within Groups	14.949	38	.393		
	Total	20.028	44			
Root Fresh Weight	Between Groups	1.569	6	.262	3.290	.010
	Within Groups	3.021	38	.080		
	Total	4.591	44			
Root Dry Weight	Between Groups	.077	6	.013	.774	.595
	Within Groups	.633	38	.017		
	Total	.711	44			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Plant Height	Between Groups	162.078	6	27.013	.553	.764
	Within Groups	1855.733	38	48.835		
	Total	2017.811	44			
Root Height	Between Groups	52.800	6	8.800	1.052	.408
	Within Groups	317.900	38	8.366		
	Total	370.700	44			
Shoot Height	Between Groups	109.944	6	18.324	.576	.747
	Within Groups	1207.967	38	31.789		
	Total	1317.911	44			
Root Fresh Weight	Between Groups	122.456	6	20.409	1.591	.177
	Within Groups	487.533	38	12.830		
	Total	609.990	44			
Shoot Fresh Weight	Between Groups	198.639	6	33.106	1.615	.170
	Within Groups	779.009	38	20.500		
	Total	977.648	44			
Root Dry Weight	Between Groups	1.939	6	.323	.980	.452
	Within Groups	12.527	38	.330		
	Total	14.466	44			
Shoot Dry Weight	Between Groups	11.333	6	1.889	1.310	.276
	Within Groups	54.795	38	1.442		
	Total	66.128	44			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Plant Height	Between Groups	577.700	6	96.283	3.850	.004
	Within Groups	950.300	38	25.008		
	Total	1528.000	44			
Root Height	Between Groups	112.267	6	18.711	2.927	.019
	Within Groups	242.953	38	6.394		
	Total	355.220	44			
Shoot Height	Between Groups	392.411	6	65.402	6.161	.000
	Within Groups	403.367	38	10.615		
	Total	795.778	44			
Root Fresh Weight	Between Groups	87.263	6	14.544	1.285	.287
	Within Groups	430.108	38	11.319		
	Total	517.371	44			
Shoot Fresh Weight	Between Groups	247.065	6	41.178	4.936	.001
	Within Groups	316.987	38	8.342		
	Total	564.052	44			
Root Dry Weight	Between Groups	2.348	6	.391	1.173	.341
	Within Groups	12.684	38	.334		
	Total	15.032	44			