ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF INDIGENOUS BIOCONTROL AGENTS

Rifat Ara Sultana

DEPARTMENT OF PLANT PATHOLOGY

FACULTY OF AGRICULTURE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA-1207

JUNE, 2022

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF INDIGENOUS BIOCONTROL AGENTS

BY
RIFAT ARA SULTANA

REGISTRATION NO. 15-06617

A Thesis
Submitted to the faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the Degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JANUARY- JUNE, 2022

Approved by:

Dr. Nazneen Sultana
Professor
Department of Plant Pathology
Sher-e-Bangl3a Agricultural University,
Dhaka 1207
Supervisor

Abu Noman Faruq Ahmmed
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University,
Dhaka 1207
Co-Supervisor

Prof. Abu Noman Faruq Ahmmed
Chairman
Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka 1207

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9
Fax: +88029112649
Web site: www.sau.edu.bd

CERTIFICATE

This is to certify that the thesis entitled, "**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF INDIGENOUS BIOCONTROL AGENTS**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in PLANT PATHOLOGY**, embodies the result of a piece of bona-fide research work carried out by **RIFAT ARA SULTANA**, Registration no. **15-06617** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

Professor Dr. Nazneen Sultana
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

DEDICATED TO MY BELOVED PARENTS AND BROTHER

ACKNOWLEDGEMENTS

The author likes to express her praise and gratefulness to the almighty Allah for owing mercy upon her and for imbibing confidence in her to complete the research work and to prepare this manuscript for the degree of Master of Science (M.S.) in Plant Pathology. It is a great pleasure to articulate the author's gratitude to her respected parents, for their blessings and the constant support that they have been for her in prosecuting her studies, thereby receiving proper education.

The author would like to express her utmost gratitude, and indebtedness to her research supervisor ***Professor Dr. Nazneen Sultana****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for her persistent encouragement, precious suggestions, constant inspiration, and timely advice along with providing an atmosphere of freedom in the research work and for taking care in preparing this manuscript. The author had the opportunity to share her academic insights, and benefit from her professional skill in carrying out the research.*

The author expresses her vast appreciation, heartfelt gratitude, and indebtedness to the respected Co-Supervisor and Chairman of the department ***Prof. Abu Noman Faruq Ahmmed****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for his cooperation, criticisms on the manuscript, and helpful suggestions for the successful completion of the research work.*

The author would like to convey her gratitude and thanks to ***Prof. Dr. Md. Belal Hossain****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, who supported her in whole journey of research by allowing her entrance into his established Laboratory for working and spontaneous response to all her situations.*

The author would like to convey her gratitude and thanks to ***Golam Sharowar*** *and* ***Raihan Ferdous****, batchmate, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka who cordially helped her a lot during the entire period of the study and the lab work, the author is grateful for that.*

There is also a group of special people; the author's friends- ***Md. Shamim Reza, Atkiya Rahman, Rubaya Binte Rezaul, Tohidur Rahman*** *and* ***Yobaida Afrin Ara****; also the loving juniors who inspired throughout her study period in Sher-e-Bangla Agricultural University, Dhaka. The author is also thankful to them.*

Finally, the author found no words to thank her beloved Father ***Late F. F. Md. Rahmat Ali*** *Mother* ***Mrs. Runa Ali****, Brother* ***Md. Rabby Alam Shishir*** *and Sister-in-law* ***Sumaya Ferdous*** *for their love and continuous support, constant encouragement, their sacrifice, never-ending affection, immense strength and their faith in her which always kept her focused on her objectives and helped to achieve her goals.*

June, 2022 *The Author*
SAU, Dhaka

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF INDIGENOUS BIOCONTROL AGENTS

ABSTRACT

A study was performed to isolate, identify and characterize the potential indigenous bio control agents and test them against pathogenic fungi from the rhizosphere soil of crop from different location (viz. Dhaka, Gazipur, Manikganj, Noakhali, Joypurhat and Rajshahi) and mushroom substrate (Savar). The experiment was conducted in the MS Laboratory of Department of the Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during July 2021 to June 2022. Nineteen different indigenous bacterial strains (*Rhizobium sp., Mesorhizobium* sp*., Azospirillum* sp*., Azotobacter* sp*., Agrobacterium* sp*., Streptomyces* sp*., Burkholderia* sp*., Xanthomonas* sp*., Acidovorax* sp*., Pectobacterium* sp. *Erwinia* sp*., Ralstonia* sp*., Salinico roseus, Pseudomonas aeruginosa, Bacillus* sp*., Bacillus cereus Lactobacillus* sp*., Thermomonospora* sp. and *E. coli*) and fifteen indegenous fungal species (*Sclerotium* sp*., Rhizoctonia* sp*., Cunninghamela* sp*., Chaetomium* sp*., Alternaria* sp*., Curvularia* sp*., Penicillium* sp., *Trichoderma harzianum, T. viride, T. hamatum, Aspergillus niger, A. flavus, A. terreus, Fusarium oxysporum and Fusarium* sp.) were isolated by dilution plate method using 10^8 and 10^4 dilution in case of bacteria and fungi, respectively. Bacteria were identified and characterized by their cultural characteristics; observing 10 biochemical tests response and selective media growth. In case of fungal species morphological and microscopical observation were done. The bio-efficacy of the identified indigenous bacteria and fungi were tested and among the isolated fungi 3 fungal genera were selected as soil borne pathogenic fungi (*Sclerotium, Rhizoctonia* and *Fusarium oxysporum*) for trial in dual culture technique. Only *Trichoderma harzianum*, *T. viride*, *T. hamatum*, *Bacillus cereus* and *Pseudomonas aeruginosa* were found effective against the pathogenic fungi in dual culture technique. In case of fungi the inhibition of *Sclerotium* sp. was 86.48% by *T. harzianum*, 89.18% by *T. viride and* 48.64 % by *T. hamatum*; inhibition of *Rhizoctonia* sp. was 50% by *T. harzianum*, 62.5% *by T. viride* and 80% by *T. hamatum*; and the inhibition of *Fusarium oxysporum* was 68.42% by *T. harzianum*, 70.52% by *T. viride and* 81.5%. by *T. hamatum.* In case of bacteria the inhibition of *Sclerotium* sp. by *Bacillus cereus* was 72.98% and by *Pseudomonas aeruginosa* was 62.16%.; inhibition of *Rhizoctona* sp*. by Bacillus cereus* was 70% and by *P. aeruginosa* was 50%; inhibition of *Fusarium oxysporum* by *Bacillus cereus* was 66.67% and by *P. aeuginosa* was 60%. This study filters the potential indigenous biocontrol agents those can be used as bio-pesticide to develop crop production and reduce the chemical dependency.

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LISTS OF ABBREVIATIONS

et al. =	And others	PGPR=	Plant Growth
i.e. =	That is		Promoting
eg. =	Example		Rhizobacteria
etc. =	Etcetera	PDA=	Potato Dextrose Agar
viz. =	Namely	NA =	Nutrient Agar
Sl No. =	Serial Number	BCA=	Bacillus Cereus Agar
% =	Percentage	CA=	Cetrimide Agar
< =	Less than	EMB =	Eosin Methylene Blue
ml =	Mili Liter		
gm/g =	Gram	CFU =	Colony Forming Unit
cm =	Centimeter	WHO =	World Health
Ibs =	Pounds		Organization
°C =	Degree Celsius	BBS =	Bangladesh Bureau of
hr. =	Hour		Statistics
sp. =	Species		
spp. =	Species	FAO =	Food and Agricultural
BCA=	Biological control		Organization
	agents	FDA =	Food and Drug
H_2S =	Hydrogen Sulfide		Administration
KOH =	Potassium Hydroxide	UK =	United Kingdom
		USA =	United States of
H_2O_2 =	Hydrogen Peroxide		America
J. =	Journal		
ISR=	Inducing Systemic		
	Resistance		

CHAPTER I
INTRODUCTION

The need to create eco-friendly, long-lasting techniques to increase agricultural productivity is urgently felt throughout the world. A major threat to healthy ecosystems and global food supply is posed by microbial diseases of plants. According to Wu *et al*., (2015), plant pathogenic bacteria are projected to reduce crop yield globally by about 25% annually. Additionally, the human population has doubled over the past 50 years, and by 2050, it is anticipated to reach over nine billion people (Boyd *et al*., 2013). A significant increase in agricultural productivity is needed to meet the rising population's demand for food. Meeting the growing population's demand for food is negatively impacted by changing environmental conditions and the loss of agricultural farmlands (Berin *et al*., 2018). Additionally, agrochemicals like pesticides and fertilizers, which have negative impacts on both the environment and human health, are frequently utilized in modern agriculture (Sharma *et al*., 2019). Numerous studies have looked for novel bacterial strains that can be utilized as biocontrol and/or biofertilizer substitutes in agriculture in an effort to replace chemical agents (Marian et al., 2019). The demand for biocontrol agents is expected to reach USD 10.6 billion by 2027, up from a market value of USD 4.0 billion in 2020 (Collinge *et al*., 2022).

The effectiveness of *Trichoderma* against various pathogens can be attributed to a wide range of biochemical attributes released by these fungi, which may be constitutively present or induced in response to pathogen presence (Aarti and Meenu, 2015; Rawat and Tewari, 2011). The understanding and utilization of these biochemicals hold significant importance for harnessing the suppressive potential of *Trichoderma* species against diverse phytopathogens. Integrated management strategies that incorporate the use of Biological Control Agents (BCAs) like *Trichoderma* offer sustainable alternatives for disease control while promoting plant growth and overall agricultural productivity.

Managing plant diseases caused by soil-borne pathogens presents significant challenges in agriculture. Various fungal species belonging to the *Fusarium* genus have been associated with detrimental conditions such as crown rot, stem rot, and head blight in wheat (Nourozian *et al.,* 2006). *Fusarium oxysporum*, on the other hand, affects more than 100 plant species, leading to vascular wilt and root rot (Li *et al.,* 2015). Traditionally, the control of soil-borne diseases has relied on the use of fungicides and the cultivation of

resistant crop varieties. However, developing resistant varieties without a dominant gene can be difficult, and the application of chemical fungicides poses environmental and human health risks (Shanmugam and Kanoujia, 2011; Dufour *et al.,* 2011). Addressing soil-borne diseases requires alternative approaches that are more sustainable and environmentally friendly. Exploring biological disease management methods, such as employing plant growth-promoting rhizobacteria (PGPRs) or other biocontrol agents, offers potential solutions for controlling these challenging pathogens. By harnessing the beneficial interactions between plants and microbes, it becomes possible to develop more resilient and effective strategies for managing soil-borne diseases, reducing reliance on chemical treatments, and promoting sustainable agricultural practices.

Previous research has indicated that chemical control methods can disrupt microbial communities, leading to imbalances that may reduce the activity of beneficial organisms and even promote the development of pathogen strains resistant to these chemicals (Shanmugam and Kanoujia, 2011). This approach represents a shift towards a more holistic and integrated approach to disease management in agriculture.

Pseudomonas spp. and *Bacillus* spp. are notable for their ability to colonize the rhizosphere of various crops and produce metabolites that inhibit enzymes or membrane proteins, thus exhibiting antagonistic effects. These microorganisms have demonstrated success as biocontrol agents in managing plant pathogens (Schippers *et al.,* 1987). Additionally, nonpathogenic bacteria, fungi, and Actinomycetes from genera such as *Trichoderma*, *Fusarium, Streptomyces*, and *Serratia* have also been employed as biocontrol agents to combat damping-off, root rot pathogens, and other soil-borne diseases (Someya *et al.,* 2003; Minerdi *et al.,* 2009; Khabbaz and Abbasi, 2014). These organisms offer potential solutions for disease management in agricultural systems, demonstrating the broad range of microorganisms that can be harnessed for biocontrol purposes.

The rhizosphere harbors a diverse array of microbial species, ranging from hundreds to millions, leading to specialized interactions between roots and soil bacteria shaped by co-evolutionary pressures (Duffy *et al.,* 2004; Morrissey *et al.,* 2004). This specialized interaction zone, the rhizosphere, plays a crucial role in important ecosystem processes such as carbon sequestration and nutrient cycling, highlighting the significance of plant-microbe interactions (Singh *et al.,* 2004). Beneficial plant-microbe interactions are exemplified by plant symbioses with plant-growth-promoting rhizobacteria (PGPR),

epiphytes, and mycorrhizal fungi, which contribute to enhanced plant growth and health. These symbiotic relationships between plants and microbes further underscore the importance of understanding and harnessing the potential of plant-microbe interactions for sustainable agriculture and ecosystem functioning.

Interactions between plants and soil microbes have been found to have numerous positive effects on plants, including disease suppression (Mendes *et al.,* 2011), enhanced nutrient availability and absorption, and increased immunity to abiotic stresses (Zolla *et al.,* 2013). These interactions play a crucial role in boosting plant productivity under various biotic stresses (Zamioudis and Pieterse, 2012).

The rhizosphere is the region of soil surrounding living roots that is influenced by plant root exudates. It is considered one of the most important microbial hotspots in the soil due to its higher process rates and intense interactions. The rhizosphere microbiome refers to the diverse microbial population inhabiting the rhizosphere. This microbial consortium consists of various microorganisms, including bacteria, algae, and fungi, which reside in close proximity to plant roots. The rhizosphere provides a favorable environment for significant and intricate interactions among plants, soil, microbes, and soil microfauna, primarily due to the availability of carbon sources. Plants possess the ability to attract specific beneficial rhizosphere bacteria, which can help reduce disease activity and enhance the plant's resistance to environmental stressors. These interactions in the rhizosphere contribute to the overall health and well-being of plants and play a crucial role in pest and disease control.

Research projects focusing on the role of rhizosphere microorganisms in pest and disease control have experienced significant growth between 2000 and 2019. A Google Scholar search using the keywords "microorganisms," "control," "pest," and "diseases," along with the addition of the term "rhizosphere," revealed an increase in the number of documents obtained. The count rose from approximately 5,000 documents in the period of 2000-2005 to 8,500 documents in the period of 2006-2010, and further escalated to over 20,000 documents in the period of 2011-2019. However, the number of records decreased to around 15,000 in the most recent era when the term "rhizosphere" was not included. To conduct a comprehensive analysis and establish a network of papers on biocontrol agents with a global distribution, bibliometric data has been obtained from the current review.

Mushroom substrate a left-over product of mushroom industry is generated after several cycles ofmushroom production. It is organically a rich substrate produced in large quantity i.e. around five times of thefresh mushrooms produced. It can adversely affect the environment if not disposed-off timely and properly asthe leachate from it contaminates the runoff water leading to microbial growth and oxygen depletion in waterbodies. Its casual disposal may also spread nuisance and releases hazardous gases in the environment duecontinual degradation process. However, if it is handled properly it has the potential to supplement the nutritionaldemand of the crop plants along with protecting them from different insect-pests and diseases. It also possessesthe properties to bio-remediate the soils that are contaminated with over dose of pesticides and heavy metals.The ability of SMS in management of different plant diseases exist in its physical, chemical and microbialproperties. This way it has the potential to protect plant by multipronged approach, where its nutritional richnesssupport plant growth, while the chemical constituents andmicrobial components either induce the plant defensesystem or exert antagonism to the plant pathogenic microorganisms and even kill them. The recycling back of SMS for use in agriculture is one of the examples of circular economy, where the left-over part of one activityis used for supporting the next activity leading to sustainable. growth of agriculture economy.

Objectives

- To isolate and identify the biocontrol agents from the rhizosphere and mushroom substrate
- To characterize their efficacy against soil borne pathogens

CHAPTER II
REVIEW OF LITERATURE

2.1 Bio controlling microorganisms

In 2020 Sood *et al.,* explained that, Trichoderma was first isolated from soil in 1794 and it has been a well-known biocontrol resource since then. Trichoderma is considered an efficient biocontrol agent due to low development cost, strong adaptability, broad-spectrum efficiency and ecological friendliness, and broad application prospects.

Oso *et al.,* discovered in 2019 that many *Pseudomonas* spp. are efficient colonizers of the plant surface (rhizosphere and phyllosphere) and the endosphere. They can use many plant exudates as nutrients and have a high growth rate, which are prerequisites to efficiently compete with other microorganisms for space and nutrients in the plant environment.

Hyakumachi *et. al.,* (2013) opined that Treatment of tomato roots with *B. thuringiensis* culture followed by challenge inoculation with *Ralstonia solanacearum* suppressed the development of wilt symptoms to less than one third of the control. This disease suppression in tomato plants was reproduced by pretreating their roots with a cell-free filtrate (CF) that had been fractionated from *B. thuringiensis* culture by centrifugation and filtration. In tomato plants challenge-inoculated with *R. solanacearum* after pretreatment with CF, the growth of R. solanacearum in stem tissues clearly decreased, and expression of defense-related genes such as PR-1, acidic chitinase, and β-1,3-glucanase was induced in stem and leaf tissues. Furthermore, the stem tissues of tomato plants with their roots pretreated with CF exhibited resistance against direct inoculation with *R. solanacearum*. Taken together, these results suggest that treatment of tomato roots with the CF of *B. thuringiensis* systemically suppresses bacterial wilt through systemic activation of the plant defense system.

According to Anita (2015), *Trichoderma* spp are free living filamentous fungi. They are cosmopolitan and versatile in nature. They have the potential to produce several enzymes that can degrade the cell wall materials. Also, they release a number of fungi toxic substances that can inhibit the g33rowth of fungal pathogens. Many mechanisms have been described on how Trichoderma exert beneficial effects on plants as a bio-control agent.

In 2013 Calderón *et al.,* said that Di alkylresorcinols exhibit antifungal and antibacterial activities such as the compound 2-hexyl-5-propyl resorcinol produced by *P. chlororaphis* PCL 1606 is responsible for the biocontrol of *R. necatrix*.

Abriouel in 2011 said that bacteriocins and compounds that like them are peptides produced by the ribosome that attack target cells by preventing the production of the cell wall or by creating gaps in the cell membrane. Several bacteriocins having antimicrobial activity, including amylolysin, amylocyclicin, amysin, subtilin, subtilosin A, and subtilosin B, are produced by Bacillus species. Several of them have experience in plant pathogen biocontrol. For instance, Agrobacterium tumefaciens is active against Bac-GM_17 generated by B. clausii GM_17.

In 2010 Raaijmakers *et al.,* invented that cyclic lipopeptides (CLPs), which are extensively distributed in Bacillus species, are non-ribosomally synthesized amphiphilic molecules made up of a fatty acid tail connected to a short oligopeptide that forms a macrocyclic ring structure. Iturins, fengicins, and surfactins are three of the most significant CLPs made by Bacillus. They interact with target pathogens' cell membranes, creating holes and disrupting transmembrane ion fluxes. For which it acts as a bio control agent.

In 2006 Marra *et al.,* described that, Trichoderma is asexual filamentous fungi who share its anamorph with the genus Hypocreae. They are facultative anaerobes. They are versatile, highly rhizosphere competent, profuse root colonizers and cosmopolitan in nature. They are also opportunistic avirulent plant symbionts. They are known for prolific production of extracellular proteins and fungitoxic substances. They have been utilized extensively as model microorganisms to analyze and improve the understanding of the role that these antagonistic fungi have been playing in biological interactions, for instance with crop plants and phytopathogens.

In 2004 Gardener experienced that the most frequently used beneficial bacteria as biopesticides are those of the genus *Bacillus*. They have a wide range of physiological abilities and the capacity to generate endospores, which confers resistance to unfavorable climatic conditions. They are extensively distributed in many environments, including soil and plant surfaces. They can create antagonistic relationships with a variety of bacterial and fungi that cause plant diseases. The ability of Bacillus spp. to create a wide range of bioactive chemicals useful for agricultural applications, including metabolites with

antibacterial activity, surface-active, and linked to the generation of plant defense responses, is the species' most notable characteristic.

In the year of 2003 Howell described that several fungitoxic CWDEs and peptaibol antibiotics are produced by the Trichoderma induced by the cell wall materials of the target fungus. This induces a cascade of physiological changes within the fungus. Due to action of these CWDES holes are produced in the hyphae of target fungus near the site of appressoria. The antagonistic hyphae grow along the host hyphae and secrete different lytic enzymes such as glucanase, chitinase and pectinase that are involved in mycoparasitism. This ultimately leads to the degeneration of the target fungus.

In 2003 Chin-A-Woeng *et al.,* found another relevant trait of *Pseudomonas* spp. That is they are major producers of bioactive metabolites, such as antibiotics, cyclic peptides, or enzymes that play important ecological roles. Specifically, they produce different antimicrobial compounds such as phenazines, phloroglucinols, dialkylresorcinols, pyoluteorin, and pyrrolnitrin, whose involvement as a mechanism of action in biological control has been well documented.

Trichoderma was first isolated from soil in 1794 and it has been a well-known biocontrol resource since then. Trichoderma is considered an efficient biocontrol agent due to low development cost, strong adaptability, broad-spectrum efficiency and ecological friendliness, and broad application prospects (Sood *et al.,* 2020)

2.2 Mode of action of BCA in controlling diseases

Priya *et al.,* in 2022 obseverved that Mycoparasitic effect of *T. longibrachiatum* was carried out by dual plate technique against agriculturally important plant pathogens *viz*., *Macrophomina phaseolina, Phytopthora infestans, Collectrotrichum gleosporides* (Mango) and *Colletotrichum falcatum.* The pathogen inhibition effect of *T. longibrachiatum* isolate was in between 50% to 81.94%. As per the investigation, *Trichoderma longibrachiatum* (TL-RD-01) could be deployed as a biocontrol agent and a biostimulant.

Zerihan *et al.,* in 2019 found in his study that 15.5% isolates (*Pseudomonas fluorescent* biotype G, Pseudomonas aeruginosa, Enterobacter cloacae ss disolvens, *Flavobacterium mizutai, Klebsiella oxytoca* and *Bacillus cereus*/ pseudomycoide) PGPR identified from

the rhizosphere were positive for organic acid production and enhance crop yield and grain quality.

In 2018 Ghorbanpour *et al.,* said that MBCAs may also interact directly with the pathogen by hyperparasitism or antibiosis. Hyperparasites invade and kill mycelium, spores, and resting structures of fungal pathogens and cells of bacterial pathogens.

According to Conrath *et al.,* (2015) microbial biological control agents protect crops from damage by diseases via different modes of action. They may induce resistance or prime enhanced resistance against infections by a pathogen in plant tissues without direct antagonistic interaction with the pathogen.

According to Raaijmakers and Mazzola (2012) production of antimicrobial secondary metabolites with inhibiting effects against pathogens is another direct mode of action.

Köhl *et al.,* investigated in the year of 2011 that Pathogen populations thus can be limited by antagonistic microorganisms in very different ways. The nature of the mode(s) of action does not only determine how a pathogen population is affected by the antagonist. Also the characteristics of the MBCA depend on the exploited mode of action. Possible risks for humans or the environment, risks for resistance development against the biocontrol agent, its pathogen specificity and its dependency on environmental conditions and crop physiology may differ between different modes of action. Preferences for certain modes of action for an envisaged application of a biocontrol agent will also have impact on the screening methods used to select new antagonists.

Segarra *et al.,* (2010) said iron competition is also the mode of action of several fungal antagonists. For example, *Trichoderma asperellum* producing iron-binding siderophores controls Fusarium wilt.

In 2008 Pilego *et al.,* said, the activity of *P. fluorescens* EPS62e and *P. pseudoalcaligenes* AVO110 in the reduction of *Erwinia amylovora* or *Rosellinia necatrix* infections, respectively, is based on their strong fitness in colonizing plant tissues as they have higher growth potential and nutrient use efficiency than the target pathogens.

In 2001 Sindhu *et al* found that, Pseudomonads can also produce lytic extracellular enzymes such as chitinases, β-1, 3 glucanases, cellulases that have important roles in biocontrol activity by their degradative activities of cell wall compounds, such as chitin, glucan, and glucosidic bridges. For example, hydrolytic enzymes produced

by *Pseudomonas* sp. have in vitro antifungal activity against *Pythium aphanidermatum* and *Rhizoctonia solani* and promote growth in chickpea.

Elle *et al.,* (1999) explained that competition for limited nutrients has been described as an important mechanism of Pseudomonas spp., but it is only relevant when the concentration of a given limited nutrients is low, such as in the biological control of *Pythium ultimum* by *P. fluorescens* 54/96.

In 1995 Raaijmakers *et al.,* said that microbial strains with the ability to produce high amounts of siderophores with high affinity to iron play an important role in disease suppression and can be selected for biological control through competition for iron with pathogens that produce lesser amounts of siderophores with lower affinity for iron This mechanism has been investigated in particular for isolates of Pseudomonas spp. and it has been demonstrated that siderophore mediated iron competition result in reduced pathogen populations in rhizospheres

In 1994 Duijff *et al.,* said, in the case of siderophore-mediated competition for iron in the reduction of *Fusarium* wilt of carnation by *P. putida* WCS358

Seddon and Edwards suggested in 1993 that competitive antagonists may modulate growth conditions for the pathogen in the targeted niche not only through nutrient depletion but also by other mechanisms. Application of *Bacillus brevis* resulted in fast drying of leaf surfaces and reduced *B. cinerea* by 68% similar to the application of a standard fungicide in Chinese cabbage.

In 1980 Elad *et al.,* discovered that the potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930s and in subsequent years, control of many diseases has been added to the list This has culminated in the commercial production of several *Trichoderma* species for the protection and growth enhancement of a number of crops in the United States and in the production of *Trichoderma* species and mixtures of species in India, Israel, New Zealand, and Sweden.

2.3 Isolation of bio agents from rhizosphere soil and mushroom substrate

In the year 2022 Lopez *et al.,* worked and isolated Twenty-five native strains from the avocado cultivation orchards, principally from soil and root. More native strains of *Trichoderma* were isolated in Michoacan than in the state of Jalisco. The micro and

macroscopic characteristics of the colonies were considered to establish the identification with traditional techniques of *Trichoderma* spp. Regarding the microscopic characterization, all samples showed a similar shape (proliferating with conidiation predominantly effuse, appearing granular or powdery due to dense conidiation, rapidly turning yellowish-green to dark green, or producing tufts or pustules fringed by sterile white mycelium), size (6 cm in 96 h), phialides (ampulliform to lageniform, usually 3–4 verticillate, occasionally paired, mostly 3.5–7.5 × 2.5–3.8 µm, terminal phialides up to 10 µm long), and conidia (globose to obovoid, mostly 2.5–3.5 × 2.1–3.0 µm, smooth-walled, subhyaline to pale green).

Suhaida investigated in 2022 that a total of 24 isolates of *Trichoderma* have been successfully recovered from cocoa rhizosphere soil collected from Raub, Pahang. They were primarily selected based on the pigmentation and colony features on THSM after three days of incubation. The lumpy growth of colony with yellowish to greenish color were subcultured on PDA media to get a pure culture of *Trichoderma* spp.

In 2020 Liu *et al.,* did research in order to obtain the *Trichoderma* resource against powdery mildew of *S. oblata*. 16 soil samples were collected from the rhizosphere of *S. oblata* at eight districts of Harbin City, China. Trichoderma strains were then isolated and identified. Furthermore, the *Trichoderma* biofertilizer was made to research the biocontrol effect on inhibiting powdery mildew and promoting growth of *S. oblata* seedlings. To study the growth promotion effect of *Trichoderma* biofertilizer to seedlings of *S. oblata*, the content of chlorophyll, the expression of chlorophyll synthesis genes and the changes in the lateral root number of *S. oblata* seedlings were determined.

In 2020 Roudriguez *et al.,* isolated a total of 90 bacteria from tomato roots and rhizosphere (40 and 50, respectively). Then, they were screened for N_2 fixation ability and from them, 36 isolated were able to grow in N-free media (50% from rhizosphere and 50% from roots). The majority of the bacterial isolates showed bacillus shape (75%) and Gram negative (72%).

In 2018 Naher *et al.,* found a total of 355 diverse colonies of *Trichoderma*, grown from the soil suspension culture. Based on the macro morphological characteristic of *Trichoderma* species, the pigmentation of colony was the primary selection for isolation of *Trichoderma* colony from soils. Trichoderma species had been found from all rhizosphere soil.

Sixteen samples were collected by Kale *et al.,* 2018 from rhizosphere soil of tomato crop of eight (8) districts of marathwada region. From these soil samples 16 isolates were isolated on PDA medium. This rhizosphere soil was isolated by using 10-4 to 10-5 dilution by dilution plate technique. Out of 16, only 8 rhizosphere soil samples had the population of Trichoderma spp. By visual observation *Trichoderma* spp. were identified as *Trichoderma viride* isolates, *T. harzianum* isolates and *T. hamatum* isolates.

In 2016 Shaikhul *et al.,* found that PGPR colonizing the surface or inner part of roots play important beneficial roles that directly or indirectly influence plant growth and development. They classified 10 PGPR as *Pseudomonas stutzeri* (PPB1), *B. subtilis* (PPB2, 5, 8, 9, and 11), *S. maltophilia* (PPB3), and *B. amyloliquefaciens* (PPB4, 10, and 12) were isolated from the rhizosphere of cucumber plants.

In 2015 Majid *et al.,* reported that the PGP potential of Rhizobacteria isolated from rhizosphere and endo-rhizosphere of wheat grown in the mountain region (not previously explored) and was examined and characterized through polyphasic approach. Based on morphological observations, they found two putative *Bacillus* sp. strains including one rhizosphere isolate AJK-4 and one endophytic isolate AJIK-8.

In 2014 Woo *et al.,* find that Trichoderma is efficient in improving vegetative growth of plants and nutrient content of soil through decomposition and biodegradation. Active substance such as fungal spores is applied as foliar sprays and pre and post-planting treatments, during watering and transplanting. Trichoderma-based products are marketed worldwide and applied in fields, nurseries, and horticulture for management of fungal soil-borne pathogens such as *Pythium* and *Rhizoctonia*. It is a safe and environmentally friendly method to reduce the detrimental effects of chemical pesticides.

Mantja and associates found *Trichoderma* isolates in all soil samples of maize rhizosphere from Maros, Takalar, and Jeneponto regencies in 2013. Cultural and morphological characteristic of Maros and Takalar isolates showed the similitude, while Jeneponto isolates indicated the difference. It mean apparently that isolates of Maros and Takalar have same species and isolate of Jeneponto classified in other species.

Zhang and Wang obtained many *Trichoderma* spp. Isolates from the potato soils in the middle areas of Gansu Province, China in 2012. The 10 antagonistic isolates were identified via morphology and genomic sequences. The 10 isolates were identified in 4 species: 4 isolates belonged to *Trichoderma hazianum*, 2 isolates belonged to *Trichoderma*

longibrachiatum, 2 isolates belonged to *Trichoderma atroviride* and 2 isolates belonged to *Trichoderma virens*.

Wahyudi *et al.,* in 2011 explored the existence of any indigenously *Pseudomonas* sp. isolated from l and of Indonesia that may broadly used at crop field as potential inoculants. The isolation of *Pseudomonas* sp. from rhizosphere soil was yielded 115 isolates with various morphological appearances. All those isolates were subsequently analyzed for plant growth promoting attributes. Sixty three *Bacillus* strains previously isolated from the rhizosphere of native varieties of potato (*Solanum tuberosum*) growing in Huancavelica and Puno, two Andean regions of Peru, were used.

Farah Ahmad *et al.,* 2008 isolated a total of 72 bacteria belonging to *Azotobacter, Pseudomonas fluorescent*, *Mesorhizobium* and *Bacillus* were from different rhizosphere soil and plant root nodules in the vicinity of Aligarh and determined the production of IAA and observed that more than 80% of the isolates of *Azotobacter, Pseudomonas fluorescent* and *Mesorhizobium* cicero produced IAA, whereas only 20% of *Bacillus* isolates was IAA producer.

2.4 Future aspect of BCA

In 2022 Liu *et al.,* experimented that, 86 isolates of *Trichoderma* spp. were assessed against *Phytophthora nicotianae, P. capsici, Pythium vexans, P. ultimum*, and *P. dissotocum* through dual culture assay. Furthermore, the antagonistic effect of selected isolates was studied against tobacco black shank disease and damping-off of cucumber seedlings in the greenhouse. The relative control effect of the three antagonistic *Trichoderma* strains AR-4, Tv-1, and ST4-1 on tobacco black shank was more than 60%, which was not significantly different from 6.88 gl^{-1} fluopicolide–propamocarb. Whereas, the relative control effect of *Trichoderma* AR-4 and ST4-1 on damping-off of cucumber seedlings was 80.33% and 82.67%, respectively, which were significantly higher than *Trichoderma* Tv-1 (35.49%) and fluopicolide–propamocarb (47.82%). According to the morphological and molecular characterization, the fungal strains AR-4, Tv-1, and ST4-1 were identified as *Trichoderma koningiopsis, T. asperellum*, and *T. gamsii*, respectively. So the strains exhibited a strong antagonistic effect against oomycete pathogens and can be integrated into disease management strategies.

In 2021 Ferreira *et al.,* suggested that, *Trichoderma* can be handled that will have influence on biocontrol efficacy such as preventive treatments, frequency of applications and its delivery methods. Strategies useful to improve the antagonistic performance such as the use of native strains, protoplast fusion, formulation, growth on pathogen cell wall medium and combination with other antagonists in integrated treatments can also be performed.

According to Robin *et al.,* (2019), In EU there are 13 bacterial-based biocontrol agents (BCA) registered as biopesticides for the control of bacterial and fungal diseases (*Bacillus amyloliquefaciens* strains: QST 713, AH2, MBI 600, FZB24 and IT 45, *Bacillus amyloliquefaciens* subsp. *plantarum* strain D747, *Bacillus firmus* I-1582, *Bacillus pumilus* strain QST 2808, *Bacillus subtilis* strain IAB/BS03, *Pseudomonas* sp. strain DSMZ 13134, *Pseudomonas chlororaphis* strain MA 342, *Streptomyces* K61 and *Streptomyces lydicus* strain WYEC 108). However, efforts are still required to increase the commercially available microbial biopesticides for plant disease management.

Chowdhury *et al.,* mentioned in 2015 that various *Bacillus* spp. strains can elicit ISR in different plants and confer an enhanced defense mechanism against a range of pathogens. Several studies have shown that VOCs and CLPs, such as surfactin and fengycin, are involved in the immune response of plants elicitation. For example, *B. amyloliquefaciens* FZB42 produced secondary metabolites (surfactin, fengycin, and bacillomycin D) that trigger plant defense gene expression and contribute to lettuce bottom rot reduction.

Sharon *et al.,* (2011) described that, several Trichoderma species and isolates have been evaluated as biocontrol agents against the nematodes with various crops and experimental conditions. Significant results of nematode control and plants growth were achieved. Aiming to improve the biocontrol process, modes of action of the fungus against the root-knot nematodes have been investigated.

CHAPTER III
MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted in the MS laboratory of the Department of Plant Pathology situated at Dr. M. A Wazed Miah Central laboratory building, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207.

3.2 Experimental Period

Duration of the experiment was about one year. It was conducted from July 2021 to June 2022.

3.3 Collection of Samples

Twelve samples of rhizosphere soil and one sample of mushroom substrate have been taken during July and August 2021. The soil samples were collected from six different locations of Bangladesh. These locations were Dhaka (SAU campus), Gazipur (BRRI), Manikganj, Rajshahi, Noakhali, Joypurhat. From each location two working samples were taken from the rhizosphere of two different standing crops. And the mushroom substrate was collected from the Mushroom Development Institute, Savar and examined before and after harvest effects. An insulated box with ice and a sterile zipper bag was used to collect all of the samples, which were then thoroughly examined and analyzed within 48 hours of being collected.

3.4 Labeling of the Samples

As there were multiple samples it was required to label them carefully with appropriate location and crop name with dates. So the samples were labeled with permanent marker on the zipper bags.

3.5 Preservation of Samples

The collected soil and substrate samples were placed in sterile zipper bags and kept in the refrigerator at 4° C temperature for further research and evaluation.

3.6 Selection of crop and mushroom substrate

For this experiment the 12 soil samples were collected from the rhizosphere of standing herbs in the fields. And the mushroom substrate was made of rice straw carrying the spawn of oyester mushroom. This is the most common substrate in Bangladesh which is used for mushroom production. Description of the samples were given in table 1.

Table 1. List of collected rhizosphere soil and mushroom substrate sample with date

Sl. No.	Location	Symbol	Crop	Type of Sample	Sample Colour	Date of Collection
1	Dhaka (SAU Agro. field)	**D_1**	Quinoa	Rhizosphere soil	Dark brown	22.01.2021
2	Dhaka (SAU Hort. Farm)	**D_2**	Cabbage	Rhizos. Soil	Brown	22.01.2021
3	Gazipur (BRRI)	**G_1**	BRRI dhan28	Rhizos. Soil	Dark brown	28. 01.2021
4	Gazipur (BRRI)	**G_2**	BRRI dhan29	Rhizos. Soil	Dark brown	28.01.2021
5	Manikganj	**M_1**	Mustard	Rhizos. Soil	Brown	05.01.2021
6	Manikganj	**M_2**	Tomato	Rhizos. Soil	Blackish brown	05.01.2021
7	Noakhali	**N_1**	Wheat	Rhizos. Soil	Grey	18.02.2021
8	Noakhali	**N_2**	Soyabean	Rhizos. Soil	Red	18.02.2021
9	Joypurhat	**J_1**	Okra	Rhizos. Soil	Brown	01.02.2021
10	Joypurhat	**J_2**	Chilli	Rhizos. Soil	Brown	01.02.2021
11	Rajshahi	**R_1**	Lentil	Rhizos. Soil	Black	11.02.2021
12	Rajshahi	**R_2**	Brinjal	Rhizos. Soil	Black	11.02.2021
13	Mushroom Development Institute, Savar	**S_1**	Oyester mushroom	Rice straw substrate Before harvest	Golden brown	01.03.2021
14	Mushroom Development Institute, Savar	**S_2**	Oyster mushroom	Rice straw substrate After harvest	Darkish brown	28.03.2021

Plate 1. Sample collection; A. collecting soil from the crop rhizosphere, B. soil samples in the zipper bag for better preservation, C. Mushroom substrate packet, D. collecting substrate from the packet before and after harvesting and E. substrate in the zipper bag for better preservation

3.7 Isolation of bacteria and fungus in soil and substrate

For isolation of bacteria and fungus from soil and substrate the following steps are to be followed:

3.7.1 Preparation of stock solution

From each sample 1g soil was taken and mixed with 10ml distilled water in a sterile test tube labeled as per sample symbol. The test tube was shaken well with the help of a vortex mixture to make a more even mixture. By this the stock solution was prepared.

3.7.2 Dilution of the stock solution

8 sterile testubes were taken labeled as 1: 10, 1: 10^2, 1: 10^3, 1: 10^4, 1: 10^5, 1: 10^6, 1:10^7 and 1: 10^8 for each sample. Total 12 soil samples and 2 substrate sample were diluted. From the stock solution 1ml solution was transferred to a test tube containing 9 ml of sterile distilled water by using a micropipette. Here the solution got diluted by 10times. Again, by the micropipette from the second test tube 1ml solution was transferred to another test tube containing 10ml of sterile distilled water. By this action a 100 times diluted solution then the stock was achieved. The procedure was replicated 8 times to achieve a solution that was 10^8 times diluted from the stock solution. Each time a sterile pipette tube was used for transferring for preventing contamination and each of the test tubes was shaken well by a vortex mixture to have an even solution before transferring.

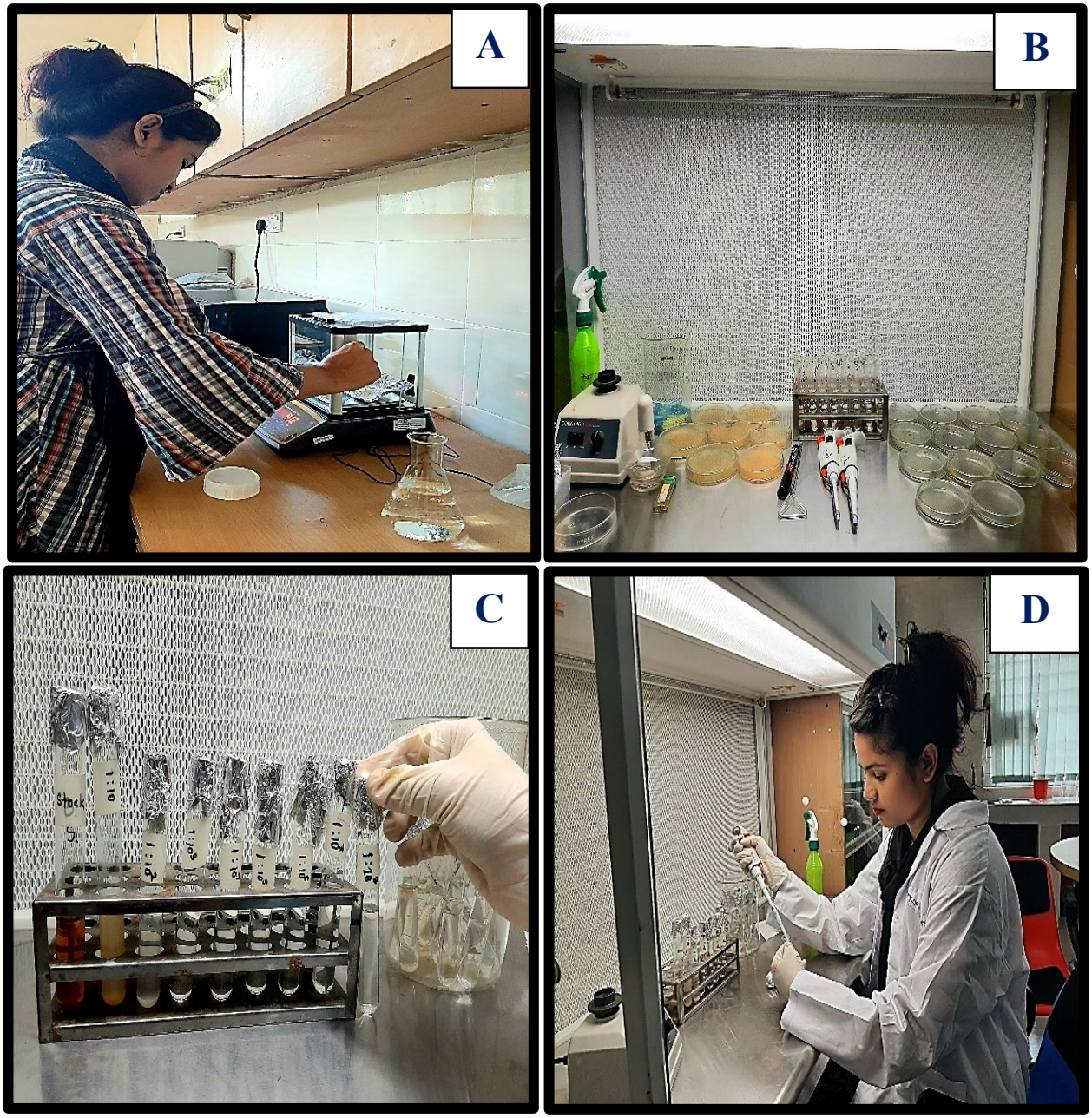


Plate 2. Stock solution preparation and sample dilution for bacteria and fungus; A. Measuring sample for stock preparation, B. Preparation for dilution into the Laminar, C. Dilution tubes with stock solution, D. Performing dilution plate method

3.8 In case of bacterial isolation

3.8.1 NA media preparation

For bacterial isolation NA (Nutrient Agar) media was prepared. The NA media preparation took 1000ml of lukewarm distilled water in a sterile conical flask. In the water 30gm of NA media powder was dissolved by agitating. After that the flask was sealed by cotton and aluminium foil. And then autoclaved for 15 minits at 121^{o} C temperature 15 lbs pressure for further sterilization. After that media was poured on 9 cm sterile petri dish for 14 samples inside the laminar air flow cabinet those were marked as D_1, D_2, G_1, G_2, M_1, M_2, N_1, N_2, J_1, J_2, R_1, R_2, S_1, S_2. For each sample 3 plates were prepared.

3.8.2 Sample placing

In the laminar air flow cabinet when the NA media in the plates are cold and settled down to solidification, from each sample 10^8 dilution solution was taken in a micropipette in a volume of 0.05ml. And poured on the media plates. By a sterile 'L' shaped glass rod the solution was spreaded well on the plates. Each time the glass rod was used it was dipped into 70% ethanol and burned by the spirit lamp. For each sample 3 plates were prepared with the respective sample solution.

3.8.3 Incubation

After placing the samples the plates were covered by their lids and sealed with suitable stickers for example: Scotch taps, para film or transparent taps. And then the plates are placed upside down in the incubator setting the temperature at 30^{o}C for 24hrs.

3.8.4 Observation

After 24hrs growth of bacterial colonies were visualized. As the dilution was high, the colonies were clear and countable. The colony morphological characters were studied and noted down in a notebook. Different colony characters were seen.

3.8.5 Preparation of bacterial pure culture by streaking method

For each different character found in the dilution plate a petri dish with NA media was prepared following the above mentioned technique. By a sterile inoculating loop a colony was taken and placed on the media following the streak plate method. In this method to obtain pure colonies from each individual sample, the one colony that had expanded over

the NA plate was chosen to streak on a new NA plate. A sterile loop was used to perform the streaking. First, a flame was used to sterilize the inoculation loop. When a single colony was selected from the NA plate culture and distributed throughout the first quarter (about 1/4 of the plate) using closely spaced parallel streaks after the loop had cooled. The inoculation loop was immediately back and forth streaked very gently over the first quadrant of the plate. The plate was then rotated 90 degrees while the loop was re-sterilized. The loop was moved to extend the streaks into the second quadrant of the plate, beginning in the previously streaked area. The operation was then repeated in the third and center fourth halves of the plate once the loop had been once more thoroughly sterilized. Then the plate was sealed and incubated upside down for 24hrs. For each different colonies same technique was followed. Each time the loop was used it was burned in the flame of a spirit lamp until it was red then cold down and used for further streak. Whole process of streaking was done inside the laminar air flow cabinet. From all the samples a total of 55 isolates were streaked to make pure and chemical analysis based on their morphological characters.

3.8.6 Preservation of isolates for future tests and uses

For preservation of bacteria NA slants were prepared. First in a beaker 1L distilled water was taken and the beaker was placed on a stove. After the water was warm 30gm of NA powder was dissolved in that warm water. After some time the mixture started boiling and after 5-7 minutes of boiling a transparent solution was ready. Then that hot transparent solution was poured immediately in sterile test tubes. After that they were autoclaved. When the autoclaving is done test tubes are taken out from the machine and placed in a 25-30^{o} angle in the laminar air flow cabinet to settle down. When the slants were cold and coagulated the bacterial isolated are placed in a zigzag pattern or in a straight line. And kept for incubation.

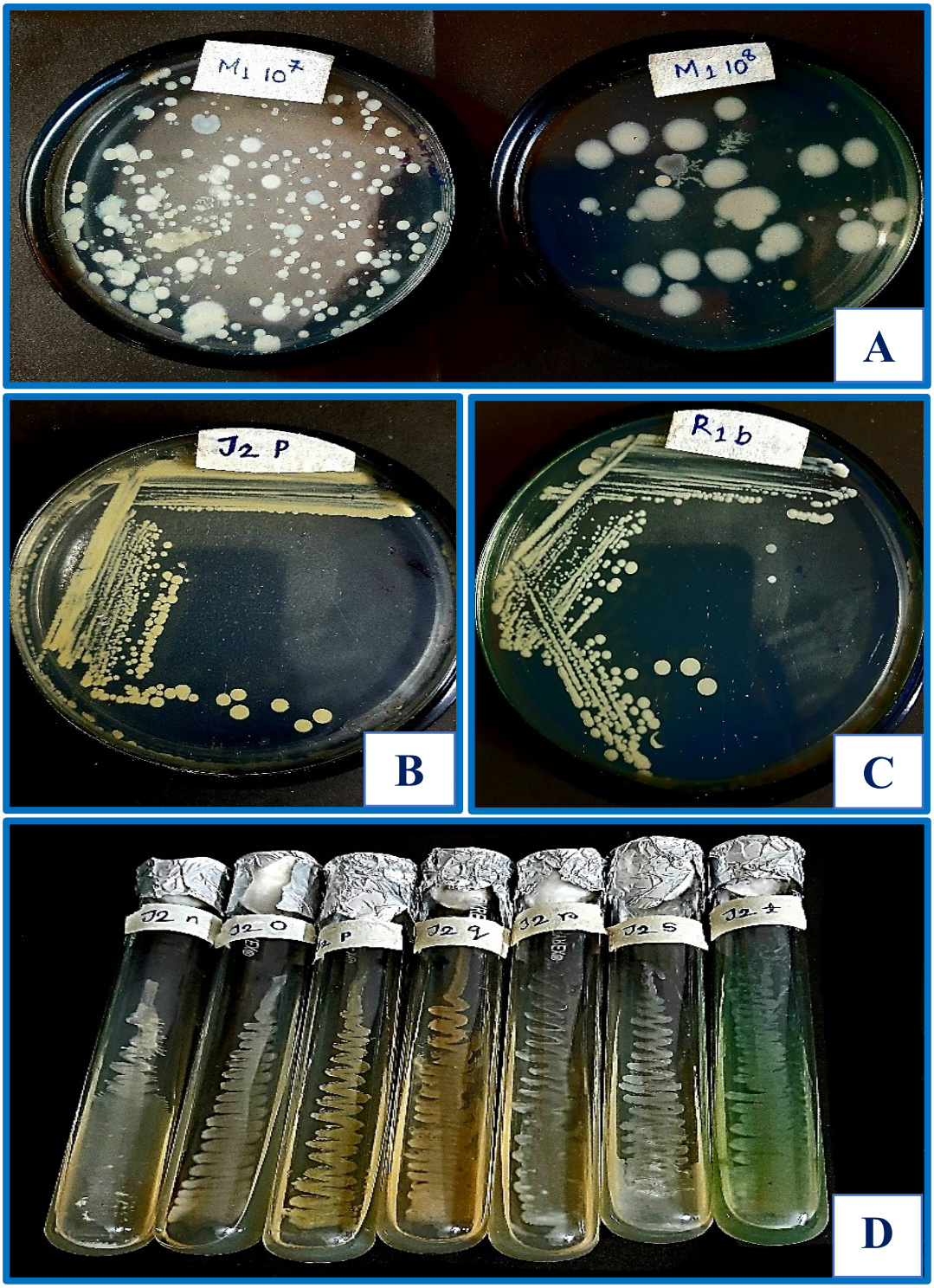


Plate 3. Bacterial isolation; A. Dilution plates after 24hrs. B & C. Pure culture of Bacteria by streaking method and D. Slants of isolated bacteria for preservation

3.9 In case of Fungal Isolation

3.9.1 PDA media preparation

For fungal isolation PDA (potato dextrose agar) media was prepared. In the preparation of PDA media (potato dextrose agar) 200g. of potato was peeled and sliced for 1000 ml of media. Now 1000ml distilled water was taken on a pan and the potato slices was taken in the water after that the pan was kept on a stove for boiling. After 15 minutes the water was filled with the starch of the potato slices that was taken on a conical flask. On that 20g agar powder and 20g dextrose powder were mixed with it and sealed with cotton and foil paper. After that autoclaved for 45 minutes under 121^0 C temperature 15 psi pressure for further sterilization. After that 5ml lactic acid was added to the media for acidification of it and then the media was poured on 9cm sterile petri dish for each sample inside the laminar air flow cabinet. Those were marked as D_1, D_2, G_1, G_2, M_1, M_2, N_1, N_2, J_1, J_2, R_1, R_2, S_1, S_2. For each sample 3 plates were prepared.

3.9.2 Sample placing

In the laminar air flow cabinet when the PDA media in the plates are cold and settled down to solidification, from each sample $1:10^3$ dilution solution was taken in a micropipette in a volume of 0.05ml. And poured on the media plates. By a sterile 'L' shaped glass rod the solution was spreaded well on the plates. Each time the glass rod was used it was dipped into 70% ethanol and burned by the spirit lamp. For each sample 3 plates were prepared with the respective sample solution.

3.9.3 Incubation

After placing the samples the plates were covered by their lids and sealed with suitable stickers for example: Scotch taps, para film or transparent taps. And then the plates are placed upside down in the incubator setting the temperature at 25 ± 2^0C for 5-7 days.

3.9.4 Observation

After 3days growth of fungal mycelia were visualized. As the dilution was appropriate, the fungus which were in great amount in the soil started growing and they were clear and countable. They were more visible and understandable at 5^{th} and 7^{th} day. The fungal characters were studied and noted down in a notebook. Fungus of different morphology were seen.

3.9.5 Preparation of fungal pure culture by picking method

After 7 days of incubation, for each different fungus found in the dilution plate a petri dish with PDA media was prepared following the above mentioned technique. By a sterile inoculating needle mycelia or conidia of a fungal character was taken and placed on the media. Then the plate was sealed and incubated upside down for 5-7days. For each different fungus same technique was followed. Each time the needle was used it was burned in the flame of a spirit lamp until it was red then cold down and used for further inoculation. The whole process of inoculation was done inside the laminar air flow cabinet. From all the samples a total of 51 isolates were picked to make pure and morphological analysis.

3.9.6 Preservation of isolates for future tests and uses

For preservation of fungus PDA slants were prepared. First in a beaker 1L distilled water and 200gm of peeled sliced potato was taken and the beaker was placed on a stove. After the water boiled for 15minits it was filtered to remove the potato slices. Then again the filtered water was placed on the stove. On this step 20gm agar and 20gm dextrose was added to it and again boiled until the mix got transparent in color. Then that hot transparent solution was poured immediately in sterile test tubes. As per the number of fungal isolates prepared, sterile test tubes were taken. Then the openings of test tubes were sealed with cotton and foil paper. After autoclaving test tubes were placed in a 25-30^{o} angle in the laminar air flow cabinet to settle down. The fungal isolates were placed in the middle of the tube by a needle by picking technique. And incubation for 5-7 days at 25±2^{0} C was provided. Then preserved at 40 C for about 6 months to 1 year.

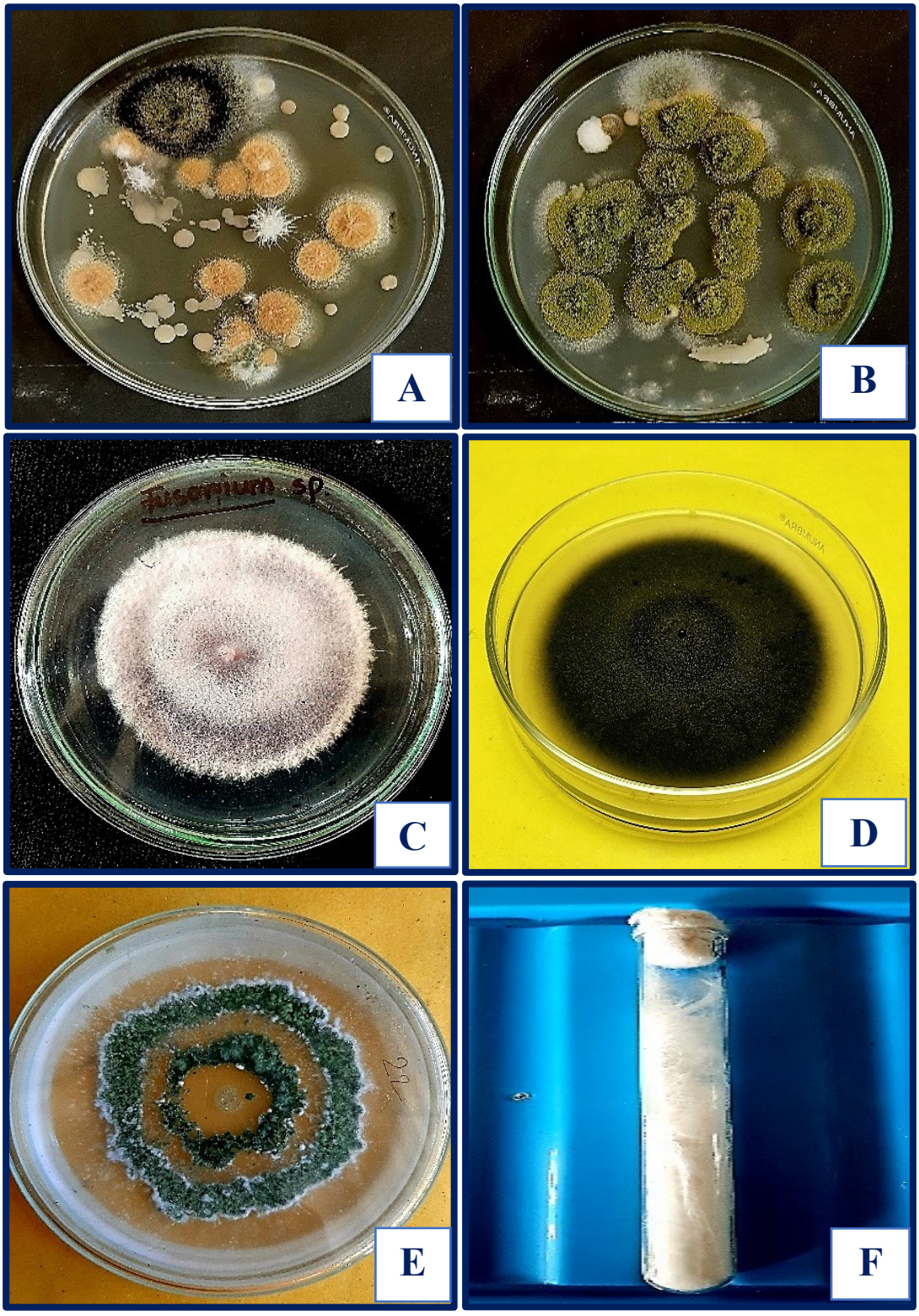


Plate 4. Isolation of fungus; A & B. Growth on dilution plates, C, D & E. Pure cultures of fungus grown on dilution plates and F. fungal slant for preservation

3.10 Symbols of Indication

The isolated bacteria and fungus from the sample were indicated with defined symbols according to their location of collection and given in table 2.

Table 2. Symbols for proper indication of the isolated bacteria and fungus according to sample location

Location	Sample symbol	Bacterial isolate	Sample symbol	Bacterial isolate	Symbol	Fungal isolate	Symbol	Fungal isolate
Dhaka	**D_1**	D_1d_1 D_1e_1	**D_2**	D_2a_2 D_2b_2 D_2c_2 D_2d_2	**D_1**	1 2 3 4	**D_2**	5 6 7 8
Gazipur	**G_1**	G_1 t_1 G_1u_1 G_1v_1 G_1w_1	**G_2**	G_2 p_1 G_2 q_1 G_2 s_1 G_2 s_1	**G_1**	9 10 11	**G_2**	12 13 14 15 16
Manikganj	**M_1**	M_1 i_1 M_1 m_1 M_1 N_1 M_1 o_1	**M_2**	M_2 g M_2 i M_2 j M_2 k M_2 l M_2 m	**M_1**	17 18 19 20 21	**M_2**	22 23 24
Noakhali	**N_1**	N_1 f_1 N_1 g_1 N_1 h_1 N_1 i_1 N_1 J_1	**N_2**	N_2 x1 N_2 y1 N_2 z1	**N_1**	25 26 27 28	**N_2**	29 30 31 32 33
Joypurhat	**J_1**	J_1 y J_1 a_1 J_1 b_1 J_1 c_1	**J_2**	J_2 n J_2 o J_2 p J_2 q J_2 r J_2 s J_2 t	**J_1**	34 35 36	**J_2**	37 38 39
Rajshahi	**R_1**	R_1 a R_1 b R_1 c R_1 d R_1 e R_1 f	**R_2**	R_2 u R_2 v R_2 w R_2 x	**R_1**	40 41 42 43	**R_2**	44 45 46 47
Savar	**S_1**	S_1 b_1 S_1 d_1	**S_2**	S_2 a_2 S_2 b_2 S_2 d_2	**S_1**	48 49 50	**S_2**	51 52 53 54

3.11 Biochemical Test for bacterial identificati3on

3.11.1 Preparation of Young Culture from Slant Culture

Young cultures were prepared on the fresh new NA plates from the preserved slants of bacteria. Pure inoculum was taken from the slant and streaking was done with the help of a sterile loop. Every NA plate contained multiple isolates of pure cultures. After growing young colonies of 24-48 hours they were taken to perform the biochemical tests for further identification (Plate 5).

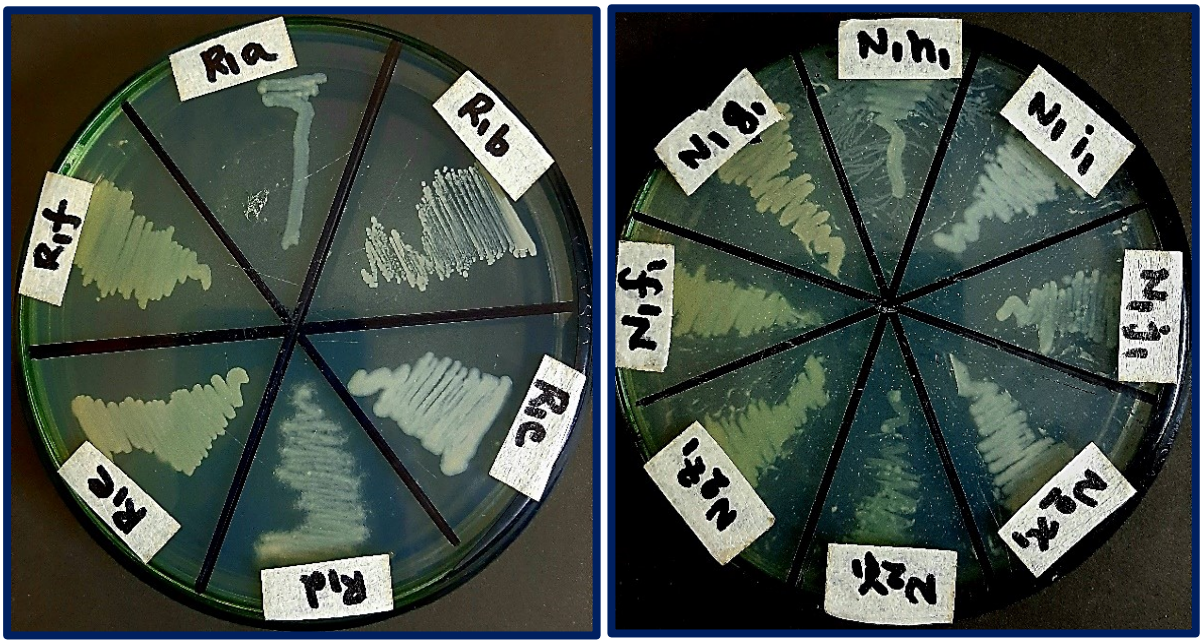


Plate 5. Prepared 24hrs young culture of bacteria for performing the biochemical tests

A series of biochemical tests were performed:

3.11.2 Gram's Staining Test

Slide preparation:

For the gram staining procedure at first the slide was prepared. A clean sterile slide was taken on that a clean sterile loop was used to place a small amount of bacterial isolate accompained with a drop of clean water. By moving the loop round and round on the slide a thin layer of bacterial smear was prepared.

Chemical preparation:

1. Crystal violet dye: To prepare 0.5% (aq) solution of crystal violet in a glass container 100ml distilled water was taken. In that water 0.5gm of crystal violet powder was mixed.

2. Safranin preparation: To prepare 0.5% (aq) solution of safranin in a glass container 100ml distilld water was taken and in that 0.5 gm safranin powder was mixed.

3. Iodine solution: Lugol's Iodine solution was taken

4. Dicolorizing agent: For dicolorization 70% ethanol was used.

Procedure of gram staining:

On the bacterial smear prepared before, 0.5% crystal violet was poured in a flooding amount. After 1minit the slide was rinsed under running tap water. Then iodine solution was poured again in flooding amount, kept for 1minit and washed under running tap water. Next the 70% ethanol was poured for dicoloring and kept for 30seconds. Again washed under tap water. Now for 0.5% safranin was poured for counter staining, kept for 30seconds and washed under tap water. Then the slide was dried by the burner by passing the slide over the flame in a very speedy motion 2-4 times. In the next step, a drop of immersion oil was taken on the stained bacterial smear and observed under compound microscope through the 100X magnification. Here the slide was taken so close to the lense that it was dipped into the oil of the slide and made the bacteria visible. By observing the slides gram positive (violet) and negative (red) bacteria was recorded.

3.11.3 KOH Solubility Test

It is a quick method for testing weather the bacteria is gram positive or negative. It is a method invented by Suslow in the year 1982. For this method a clean sterile slide was taken, on those 2 drops of 3% KOH solution was dropped. Now by a sterile loop a small amount of 18-24hrs old bacterial isolate was macerated for homogenization. It took about 10-15seconds. After that when the loop was put upside from the slide a thin, slimy, viscous thread was formed for bacteria who were gram negative and gave a positive test. (Vice versa).

3.11.4 Catalase Test

For this test 3% H_2O_2 solution was prepared. In 10ml distilled water 0.3ml H_2O_2 was mixed and prepared in a sterile screw cap test tube. On a sterile glass slide a pinch of bacterial isolate was rubbed with a sterile loop. After that a drop of prepared H_2O_2 solution was dropped. Two results were seen one is forming bubbles (positive results) and another is no bubble formed (Negative results).

3.11.5 Oxidase Test

For this test 1% N, N, N′, N′-Tetramethyl-p-Phenylenediamine dihydrochloride solution was prepared. In 10ml of sterile distilled water 0.1gm of N, N, N′, N′-Tetramethyl-p-Phenylenediamine dihydrochloride was dissolved. On a sterile petridish a sterile filter paper was placed and the just ready N, N, N′, N′-Tetramethyl-p-Phenylenediamine dihydrochloride solution was sprayed on it to make it wet with the chemical. On that notice with the help of a sterile tooth pick the bacterial isolates were rubbed on the paper. In case of some isolates after 10-15seconds the rubbed area turned blue color (positive results) and the remaining didn't give any color change (negative results).

(**N.B**. This solution is very much unstable, so it should be taken care intensively. The filter paper, petri dish, the screw cap test tube and the water must be sterilized. It should be used right after it preparation otherwise chemical breakdown happens due to light sensitivity and accurate results are not observed.)

3.11.6 Casein hydrolysis Test

In this test the casein media is prepared. For this in a sterile 1L conical flask following ingredients are mixed with 1L distilled water:

1. Casein 28.0gm
2. Peptone 5gm
3. Dextrose 1gm
4. Agar 15gm
5. Yeast extract 2.50gm

All the ingredients are mixed well and the flask was sealed with cotton and foil paper. After that the flask was autoclaved as per conditions of autoclaving for sterilization. A number of sterile petri dish were taken in the laminar air flow cabinet and the ready media was poured on them for solidification. After the casein media was solidified the bacterial isolated were placed in a straight line with the help of a sterile inoculating loop. Then the plates were sealed and kept upside down for incubation for about 24-72hrs at 30^{o}C temperature. After incubation some bacteria showed a transparent zone around the inoculated line (positive results due to the hydrolysis of casein around the bacterial line). And some bacteria didn't show any transparent zone (negative results).

3.11.7 Levan production Test

For this test with normal NA media solution 5% of sucrose was mixed. That means in 1L distilled water 30gm NA media and 50gm of sucrose was mixed with the solution and autoclaved well under conditions of autoclaving for further sterilization. In the laminar air flow cabinet after the media solidified in the plates with the help of a sterile inoculating loop the isolates were inoculated in a zigzag patteren. After that the plates were sealed and kept upside down for incubation of 24-48 hrs. When observed the growth of the bacterial colony was dome or raised then normal height. The isolates those showed the dome colony was Levan positive and those didn't show any raised growth were Levan negative.

3.11.8 Starch hydrolysis Test

In this test soluble starch was used. With 30gm NA media is used soluble starch was also dissolved in the media and the amount of starch was 2% of NA media. Both were dissolved in 1L distilled water in a sterile conical flask. The flask was sealed as before and autoclaved for sterilization of the media. Sterile petri plates were taken. Inside the laminar the sterilized media was poured into the sterile petri dishes. After cooling and solidification of the media the young culture of isolates were placed on the plates in a straight line by the sterile inoculating loop. And after proper labelling of placed isolates plates were properly

sealed and incubated for 24-48hrs keeping upside down at 30oC temperature. After incubation the Lugol's Iodine solution was poured in a flooding amount on the newly grown culture and the whole plate turned black. After few seconds some isolate showed a clear transparent zone around the growth line (positive result). It was due to the bacteria hydrolyzed the starch. And some were black as the iodine solution color, no clearing around the growth was seen (negative results).

All the process after incubation was done inside the laminar air flow cabinet.

3.11.9 Gelatin Liquefaction Test

In this test whether the bacteria could liquefy the gelatin or not was seen. For the gelatin preparation firstly 1L distilled water was taken in a beaker and kept to warm on the stove. After a while 120gm gelatin, 3g beef extract, 5g peptone was poured into the warm water and stirred by a sterile glass rod for dissolving. After some time the mixture started boiling and after 5-7 minutes of boiling a hazy solution was ready. Then that hot solution was poured immediately in sterile test tubes at a height of half. As per the number of bacterial isolates prepared, sterile test tubes were taken. Then the opening of test tubes were sealed with cotton and foil paper. After that they were autoclaved. When the autoclaving is done test tubes are taken out from the machine and placed straight in a test tube holder in the laminar air flow cabinet to cold down. When cold down a jelly media was obtained. In the laminar with the help of a long sterile needle the bacterial isolates were stabbed one by one. By proper sealing and labelling they were incubated for 48hrs at 30ºC temperature. After incubation the tubes were kept at 4ºC for 15minits. After that when the tubes were taken out of the refrigerator some tubes were totally solidified and some were still showing jelly like movement when tilted. The solid tubes gives negative results of bacteria which couldn't liquefy the gelatin. And the liquids were positive.

3.11.10 Motility Test

This test is done to observe weather the bacterial isolate is motile of non-motile. In this test a number of sterile test tubes were taken according to the total bacterial isolate number. First in a beaker 1L distilled water was taken and the beaker was placed on a stove. After the water was warm 30gm of SIM (Sulfide Indole Motility) media powder was dissolved in that warm water by stirring with a glass rod. After some time the mixture started boiling and after 5-7 minutes of boiling a transparent solution was ready. Then that hot transparent

solution was poured immediately in sterile test tubes at a height of half. As per the number of bacterial isolates prepared, sterile test tubes were taken. Then the opening of test tubes were sealed with cotton and foil paper. After that they were autoclaved. When the autoclaving is done test tubes are taken out from the machine and placed straight in a test tube holder in the laminar air flow cabinet to cold down. When cold a golden semi solid media was ready. After that the bacterial isolates were placed with the help of a long needle by stabbing into the media. A long sterile needle was taken and a small amount of culture was touched with it and stabbed in the semi solid media into the test tube. The tubes were properly labeled. And incubation for 4 days was provided at 30^{o} C temperature. After incubation few drops of tri phenyl tetrazolium chloride was dropped (10ml distilled water+0.1gmTTC) into the test tubes, after 15minits red marks were visible. In the media where the bacterial growth was existing reaction happened and a red sign was formed. The non-motile bacteria gave only a straight line following there growth area of stabbing (negative) and the motile bacteria turned the whole media red as the bacteria moved all around the media (positive).

3.11.11 Simmon's Citrate Agar Test

First in a beaker 1L distilled water was taken and the beaker was placed on a stove. After the water was warm 24.28gm of Simon's Citrate Agar powder was dissolved in that warm water that immediately turned green. After some time the mixture started boiling and after 5-7 minutes of boiling a green solution was ready. Then that hot solution was poured immediately in sterile test tubes. As per the number of bacterial isolates prepared, sterile test tubes were taken. Then the opening of test tubes were seal with cotton and foil paper. After that they were autoclaved. When the autoclaving is done test tubes are taken out from the machine and placed in a 25-30^{o} angle in the laminar air flow cabinet to cold and solidify. When the slants are cold and coagulated the bacterial isolated are placed in a zigzag pattern or in a straight line with the help of a sterile loop. Here also the inoculating loop was sterilized after each use. And incubation for 4days was provided. After incubation it was seen that some slants turned into blue color from green. It was because the bacteria grew, absorbed the citrate of the media (Positive result). Where the citrate is not absorbed the color remained green as before (negative results).

3.12 Growing bacteria on selective media

3.12.1 Bacillus cereus agar media

Selective *Bacillus cereus* the highly specific for *Bacillus cereus* agar is its foundation (Holbrook and Anderson, 1980). With the exception of *Bacillus thuringiensis*, these characteristics set *Bacillus cereus* apart from other *Bacillus* spp.

Media preparation: 20.5gm of Bacillus Cereus Agar was suspended in 475ml distilled water in a conical flask, placed over a stove and heat to boil. Boiling was done to dissolve the agar in the water completely. Then sealing the mouth of the flask it was taken to sterilize by autoclaving at 15 lbs pressure, 121^0C temperature for 15miniutes and cold down to 45-50^0C. Aseptically rehydrated contents of one vial Polymixin B Selective Supplement was added. 25ml of sterile egg yolk Emulsion was also added. All the ingredients were mixed well and poured on the sterile petridishes and solidified (Plate 7; A).

Inoculation: When the media is solidified with the help of sterile loop the isolates were placed on the media. With proper labelling the plates were sealed and kept for incubation at 30^0C temperature for 24hrs. Then the growth was observed.

3.12.2 Cetrimide Agar

Pseudomonas aeruginosa can be isolated and identified using Cetrimide Agar as a selective medium. King *et al.*, (1954) modified Tech Agar to create Cetrimide Agar, which increased the production of pyocyanin by *Pseudomonas* species. Later, the selective agent cetrimide, which only selects for *Pseudomonas aeruginosa*, was added to this formulation.

Media preparation: For preparing 1L of media in 1L distilled water 46.7gm of Cetrimide Agar Base was added with 10ml of glycerin in a conical flask. Over a stove the mixer was heated and boiled. After boiling the ingredients were dissolved completely. Then the solution was autoclaved for sterilization. Cold down to 45-50ºC and poured into sterile petri dishes (Plate 7; B).

Inoculation: When the media is solidified with the help of sterile loop the isolates were placed on the media. With proper labelling the plates were sealed and kept for incubation at 30^0C temperature for 24hrs. Then the growth was observed.

3.12.3 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar is a differential medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment the lactose (e.g., E. coli) and those that do not such as Salmonella (Levine, 1918).

Media preparation: For preparing suspend 18 grams of EMB Agar was mixed in 500 ml of purified distilled water. The mixture was shaken to boiling to dissolve the medium completely. Then the media was sterilized by autoclaving at 15 lbs. pressure and 121ºC for 15 minutes. After that, it was cooled to 45-50°C and shook the medium to oxidize the methylene blue (i.e., to restore its blue color) and suspend the flocculent precipitate. Then the medium was poured into sterile petri plates (Plate 7; C).

Inoculation: Bacteria were streaked with a sterile loop for isolation and incubated the plates aerobically at 35-37ºC for 18-24 hours and protected from light. The plates were examined the next day for colony morphology and growth characteristics.

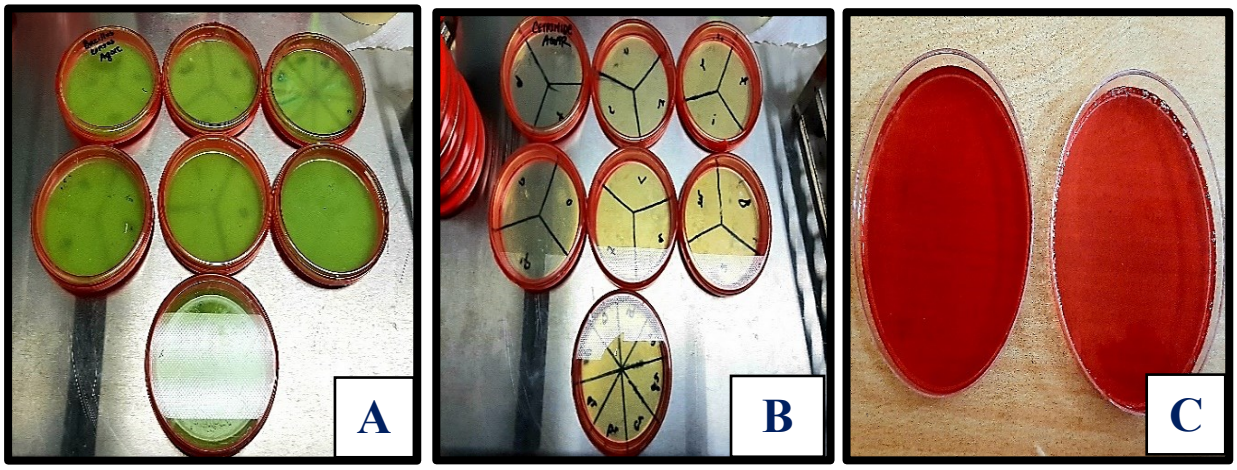


Plate 6. Selective media for bacterial identification; A. Bacillus cereus Agar, B. Cetrimide agar and C. Eosin Methylene Blue (EMB)

3.13 Assessment of Bacterial Colony Forming Unit

The colony forming units were counted on the dilution plate on the next day of inoculation. For each sample the counting was done. The counting was done by observing the single colonies. As for bacteria high dilution was performed the colonies were clear enough to be recorded. By using a marker the colonies were marked by putting dots on the colonies visual from the bottom side of the plates. In some plates colonies were lower in number in those plates all of them were counted and in others where the concentration was a little high the plates were divided into 2 or 4 even sections and the colonies of one section was counted and then multiplied by 2 or 4 respectively (Plate 8).

CFU was estimated by using the following formula:

$$CFU/ml = \frac{\text{No of colonies} \times \text{Total dilution factor}}{\text{Volume of culture plated in ml}}$$

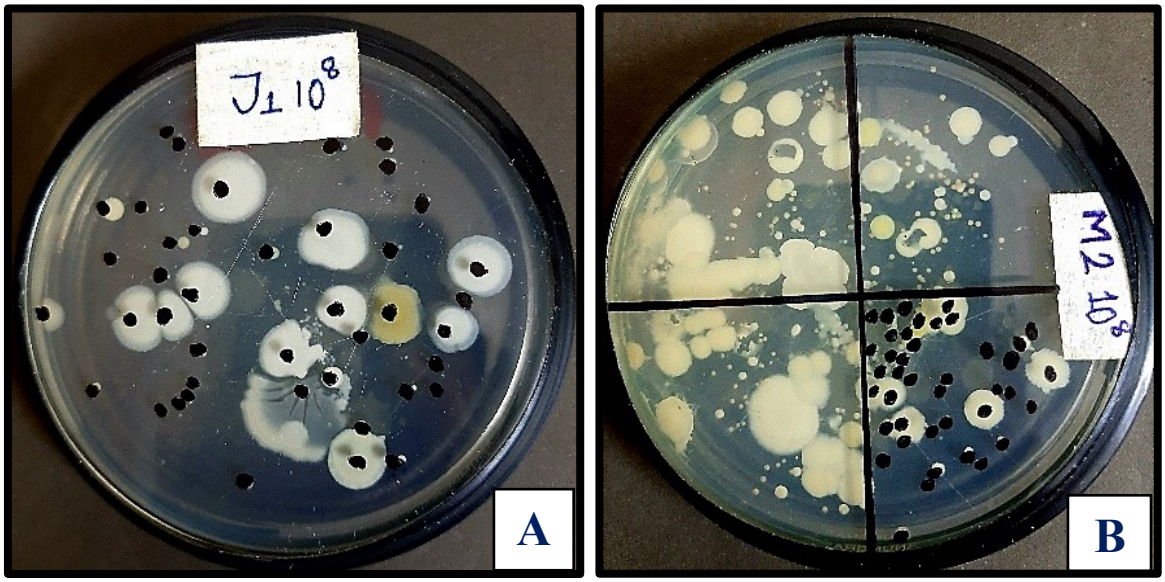


Plate 7. Count of CFU; A. Count the whole part of a plate, B. Count the quadrant part of a plate

3.14 Identification of fungal isolates

The fungal isolates were purified by making pure cultures from the dilution plates. Which is to be purified was decided by studying the fungal morphological characters of the fungus on the dilution plate. Fungus of different colors and textures were taken to be purified. And for similar looking fungi slides were prepared using needle and forceps also transparent tapes and placed under the lense of compound microscope. The slides were observed through the 10X lense and for more detailed observation it was observed under the 40X. On the pure plates the mycelium and conidial characters were observed more specifically under the microscope and by these morphological data the fungus were identified.

3.15 Trial for bio controlling agents on pathogenic fungus

Bioassay or the trial for bio controlling agents was studied by dual culture technique as per Ramanathan et al. (2002). On a PDA media plate (same procedure for preparation mentioned before) mycelia of pathogenic fungus was placed. Five millimeters mycelial discs were cut from young growing edge of the fungus from seven days old culture with a sterilized cork borer and placed at one side of a Petri plate. And the trial fungus was placed on the middle of the other side by a block cutter or a needle with the same measurement, maintaining 15-20mm distance between them. In case of bacteria the isolates were streaked aseptically parallel to the pathogenic fungus at a distance of 15-20 mm and incubated at 28 ± 2ºC for 7 days. And after inoculation the plates were sealed well and kept upside down to incubate for about 7 days at 25±2^{0}C temperature. After the incubation the growth of both organisms were recorded and the suppression was observed. The inhibition zone between the two cultures was measured in the nearest millimeter. After 7 days incubations the percent inhibition of the fungus was calculated by using the formula:

$$\text{Growth inhibition (\%)} = \frac{R1\text{-}R2}{R1} \times 100$$

Where,

R_1 = Radius (mm) of the fungus from the center of the colony towards the center of the plate in the absence of antagonistic microorganism

R_2 = Radius (mm) of the fungus from center of the colony towards the antagonistic microorganism

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Enumeration of Colony Forming Units of Rhizosphere soil and mushroom substrate

CFU stands for "Colony Forming Units," which is a measure of the number of viable bacteria or other microorganisms in a sample. All the collected samples of Rhizosphere soil and mushroom substrate were diluted up to 1:10^8 dilutions before plating on the NA plates and observed the bacterial colony of each collected sample where a variety of bacterial colonies were grown on every plate.

4.1.1 CFU of different Rhizosphere soil and mushroom substrate

Among 14 collected samples, the highest mean of CFU was observed in the rhizosphere soil of Manikganj district and the lowest mean of CFU was observed in the soil of Joypurhat district. Whereas the substrate has lower CFU then all the soil samples. The CFU counts of different location is shown in table 3.

Table 3. Bacterial CFU of different rhizosphere soil and mushroom substrate

Location	Sample no and type	Plate no	CFU/plate	CFU/ml	Mean
Dhaka	1(R.S)	D_1	71	7.1×10^9	**6.2×10^{10}**
	2(R.S)	D_2	53	5.3×10^9	
Gazipur	3(R.S)	G_1	152	15.2×10^9	**9.15×10^{10}**
	4(R.S)	G_2	31	3.1×10^9	
Manikganj	5(R.S)	M_1	46	4.6×10^9	**10.5×10^{10}**
	6(R.S)	M_2	164	16.4×10^9	
Noakhali	7(R.S)	N_1	62	6.2×10^9	**6.25×10^{10}**
	8(R.S)	N_2	63	6.3×10^9	
Joypurhat	9(R.S)	J_1	42	4.2×10^9	**4×10^{10}**
	10(R.S)	J_2	38	3.8×10^9	
Rajshahi	11(R.S)	R_1	63	6.3×10^9	**10.3×10^{10}**
	12(R.S)	R_2	244	14.4×10^9	
Savar	13(M.S)	S_1	26	2.6×10^9	**3.25×10^{10}**
	14(M.S)	S_2	39	3.9×10^9	

*D=Dhaka, G=Gazipur, M= Manikganj, N= Noakhali, J= Joypurhat, R=Rajshahi, S=Savar. R.S= Rhizosphere Soil, M.S= Mushroom substrate.

4.2 Isolation and Identification of bacterial isolates

From the dilution of total 14 samples (Plate 8) 59 bacterial isolates were isolated by Dilution and streak plate method. After isolation 19 different bacteria were identified depending on their morphological, biochemical characteristics and growth on selective media. Among them *Rhizobium sp., Mesorhizobium* sp*., Azospirillum* sp*., Azotobacter* sp*., Agrobacterium* sp*., Streptomyces* sp*., Burkholderia* sp*., Xanthomonas* sp*., Acidovorax* sp*., Pectobacterium* sp. *Erwinia* sp*., Ralstonia* sp*., Salinico roseus, Pseudomonas aeruginosa, Bacillus* sp*., Bacillus cereus and Lactobacillus* sp. were identified from the crop rhizosphere soil and *Bacillus cereus, Thermomonospora* sp. and *E. coli* were identified from the mushroom substrate (Table 4).

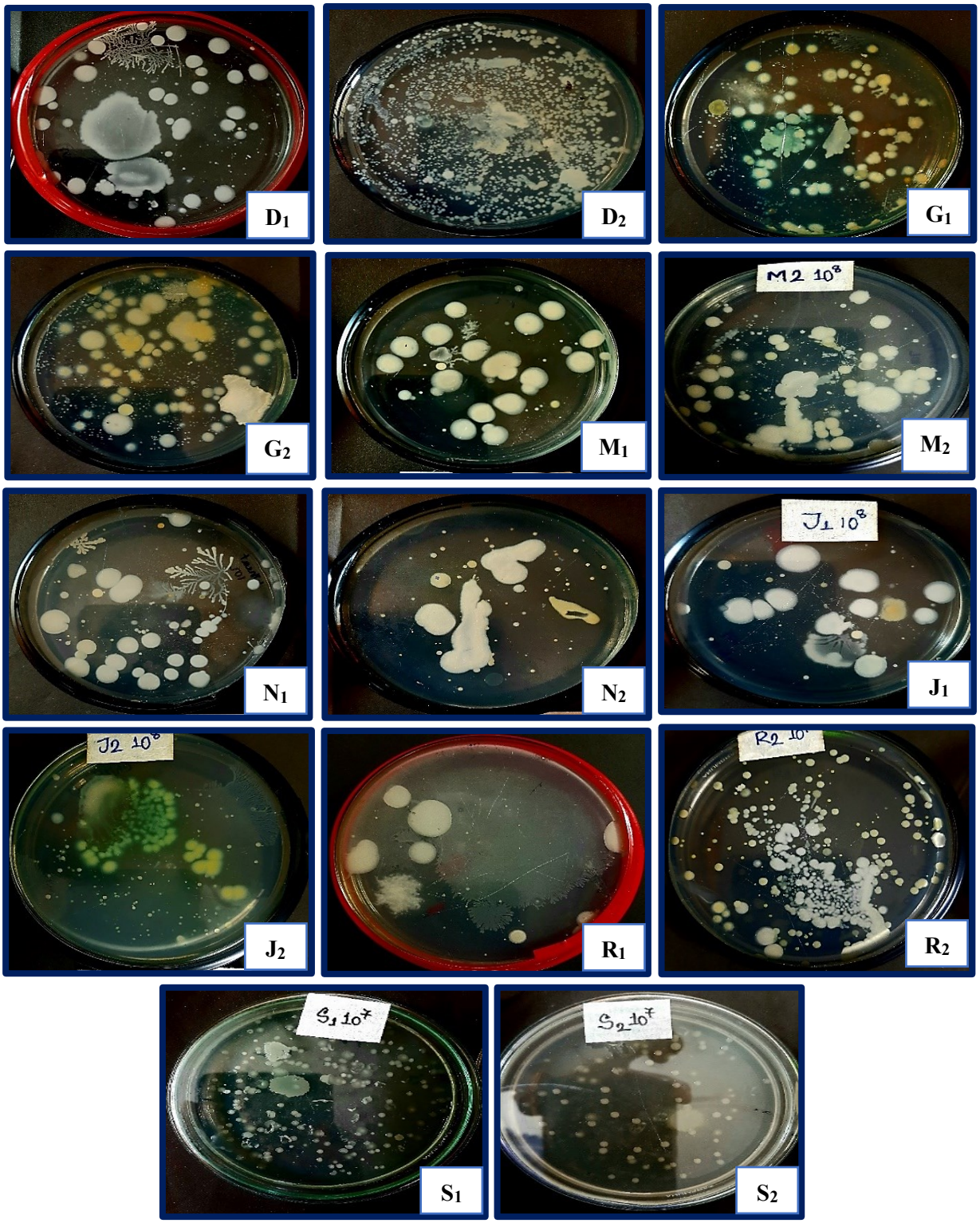


Plate 8. Growth of bacteria on NA media at 10^8 dilution of the respective samples D_1, D_2, G_1, G_2, M_1, M_2, N_1, N_2, J_1, J_2, R_1, R_2, S_1 and S_2

Table 4. Identified bacterial isolates from the sample D_1, D_2, G_1, G_2, M_1, M_2, N_1, N_2, J_1, J_2, R_1, R_2, S_1 and S_2

Location	Sample symbol	Bacterial isolate	Identified bacteria
Dhaka	D_1	D_1d_1	*Acidovorax* sp.
		D_1e_1	*Bacillus cereus*
	D_2	D_2a_2	*Bacillus cereus*
		D_2b_2	*Azospirillum* sp
		D_2c_2	*Agrobacterium* sp.
		D_2d_2	*Streptomyces* sp.
Gazipur	G_1	G_1 t_1	*Acidovorax* sp.
		G_1u_1	*Pseudomonas aeruginosa*
		G_1v_1	*Azotobacter* sp
		G_1w_1	*Bacillus cereus*
	G_2	G_2 p_1	*Pseudomonas aeruginosa*
		G_2 q_1	*Acidovorax* sp.
		G_2 r_1	*Bacillus cereus*
		G_2 S_1	*Streptomyces* sp.
Noakhali	N_1	N_1 f_1	*Xanthomonas* sp.
		N_1 g_1	*Rhizobium* sp.
		N_1 h_1	*Azotobacter* sp
		N_1 i_1	*Bacillus cereus*
		N_1 J_1	*Acidovorax* sp.
	N_2	N_2 x_1	*Bacillus cereus*
		N_2 y_1	*Burkholderia* sp
		N_2 z_1	*Erwinia* sp.
Savar	S_1	S_1 b_1	*Thermomonospora* sp.
		S_1 d_1	*Bacillus cereus*
	S_2	S_2 a_2	*Thermomonospora* sp.
		S_2 b_2	*Escherichia coli*
		S_2 d_2	*Bacillus cereus*

Location	Sample symbol	Bacterial isolate	Identified bacteria
Manikganj	M_1	M_1 l_1	*Bacillus cereus*
		M_1 m_1	*Acidovorax* sp.
		M_1 n_1	*Ralstonia* sp.
		M_1 o_1	*Rhizobium* sp.
	M_2	M_2 g	*Ralstonia* sp.
		M_2 i	*Acidovorax* sp.
		M_2 j	*Rhizobium* sp.
		M_2 k	*Bacillus cereus*
		M_2 l	*Azospirillum* sp
		M_2 m	*Agrobacterium* sp.
Joypurhat	J_1	J_1 y	*Xanthomonas* sp.
		J_1 a_1	*Mesorhizobium* sp.
		J_1 b_1	*Bacillus cereus*
		J_1 c_1	*Pseudomonas aeruginosa*
	J_2	J_2 n	*Azotobacter* sp
		J_2 o	*Bacillus cereus*
		J_2 p	*Rhizobium* sp.
		J_2 q	*Salinico roseus*
		J_2 r	*Acidovorax* sp.
		J_2 s	*Lactobacillus* sp.
		J_2 t	*Pseudomonas aeruginosa*
Rajshahi	R_1	R_1 a	*Azotobacter* sp
		R_1 b	*Ralstonia* sp.
		R_1 c	*Bacillus* sp.
		R_1 d	*Bacillus cereus*
		R_1 e	*Acidovorax* sp.
		R_1 f	*Erwinia* sp.
	R_2	R_2 u	*Xanthomonas* sp.
		R_2 v	*Mesorhizobium* sp.
		R_2 w	*Pectobacterium* sp.
		R_2 x	*Bacillus sp.*

The isolated indigenous bacterial strains from the crop rhizosphere soil and mushroom substrate were identified. Many researchers previously exploited the rhizosphere microorganisms, the results of the present study correlates with them. Rhizosphere is the root zone of plant that contains types of microorganisms. Bacteria are very common in the root zone some are pathogenic some are beneficial. The beneficial microbes provides benefits to plants by establishing symbiotic relation with plant or by suppressing the pathogenic microbes. Rhizobium species are well-known symbiotic nitrogen fixers in soil, but diazotrophic PGPR, such as, *Azospirillum, Pseudomonas* and *Azotobacter* are non-symbiotic nitrogen fixers (Umar *et al.,* 2020). Also there are harmful bacteria in the rhizosphere that creates problems for plants such as diseases and physiological issues. In the rhizosphere, competent *Mesorhizobium loti* MP6 induces root hair curling (Chandra *et al.,* 2007). *Streptomyces* spp. have been referred to as plant growth-promoting bacteria because of their advantageous effects in producing lytic enzymes (Prapagdee *et al.,* 2008), that is helpful in interacting with the plant rhizosphere. Various bacterial genera including *Burkholderia*, *Pseudomonas*, *Ralstonia* contain root-associated strains that can encounter bivalent interactions with both plant and human hosts (Berg *et al.,* 2005). *Salinico roseus* is a bacteria that is found mostly in abattoir soil. It can be in the rhizosphere by the runoff water coming from the slaughter house to crop fields. It is a bacteria that is used to produce bio pigments for its attractive orange color (Usman *et al.,* 2018). *Pectobacterium, Erwinia* are the bacteria of same family are found in the rhizosphere that causes soft rot disease of potato tubers also in onion (Alinejad *et al.,* 2020). In the lettuce field among the rhizosphere microbiome *Acidovorax* can be in bulk. It is enriched in the rhizosphere of lettuce (Schreiter *et al.,* 2014). *Lactobacillus* strains survives in the presence of phenols, acidic environment (pH 2–3) in the rhizosphere but it is a rare bacteria to be found in the rhizosphere soil (Singhal *et al.,* 2021). From all individual samples *Bacillus cereus* was identified. The strains of them have the colonizining ability in the rhizosphere which is excellent (Du *et al.,* 2022). Also rhizosphere competent *Pseudomonas aeruginosa* GRC1 produces characteristic siderophore (Pandey *et al.,* 2005). On the other hand substrate of mushroom contains thermophilic actinobacter like *Thermomonospora* sp. and *Bacillus* sp. (Vajna *et al.,* 2012). Bacterial disease of edible mushrooms and financial loss might result from the presence of possible pathogens such *Bacillus* sp*., E. coli*. These findings indicates that the identified bacteria in this study are highly associated with the sources.

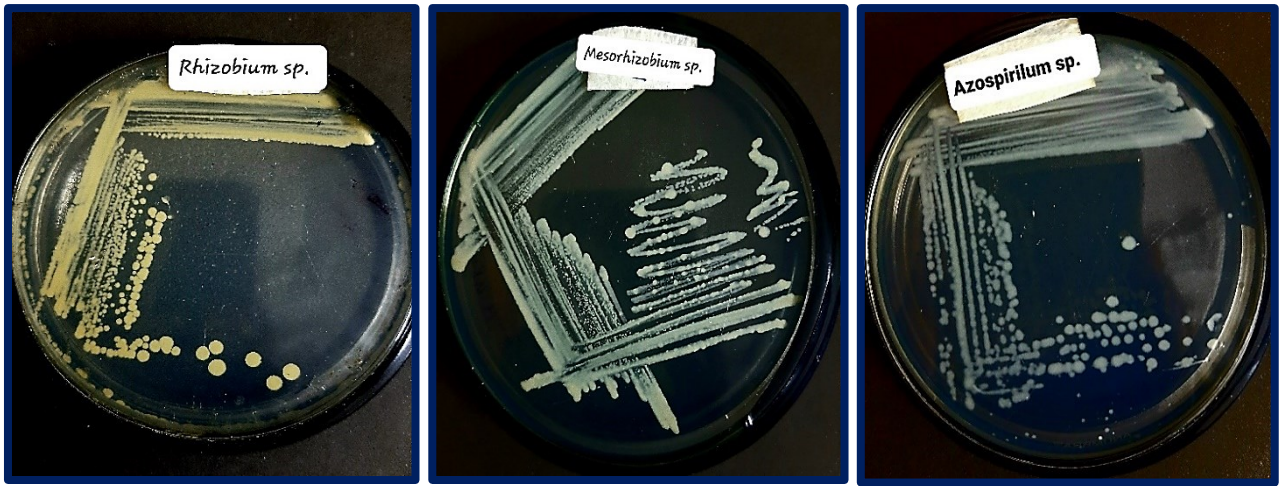
4.3 Cultural characteristics of identified bacteria

The morphology of different bacteria was analyzed depending on their appearance (shape, size, margin, texture, color and elevation) on the Nutrient agar plate (Plate 10 and 11). In (table 5) the results from observation of the morphology of the identified bacteria is given.

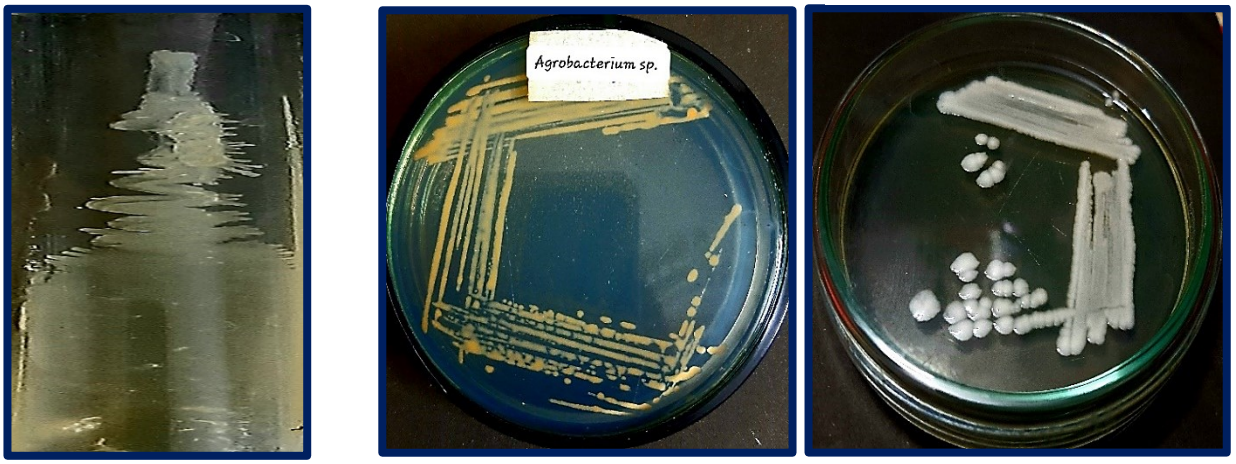
Table 5. Cultural characters of the identified bacteria

Name of the bacteria	Shape	Size	Margin	Texture	Color	Elevation
***Rhizobium* sp.**	Round	Medium	Smooth	Moist	Yellowish	Raised
***Mesorhizobium* sp.**	Round	Medium	Smooth	Moist	White opaque	Slightly Dome
***Azospirillum* sp.**	Round	Medium	Wrinkled	Outer dry inter water	White	Raised
***Azotobacter* sp.**	Rhizoid	Branched	smooth	Dry-Diffused	Creamy white, off-white	flat
***Agrobacterium* sp.**	Round	Small	Smooth	Moist	Orange brown	Convex
***Streptomyces* sp.**	Filamentous	Irregular	Cottony	Dry leathery	White	Raised
***Burkholderia* sp.**	Round	Small	Smooth	Dry	Pale yellow	Raised
***Xanthomonas* sp.**	Round	Small	Smooth	mucoid	Yellow	Raised
***Acidovorax* sp.**	Round	Tiny	Smooth	Mucoid	White cream	Raised
***Pectobacterium* sp.**	Oval	Small	Smooth	Slimy	Cream	Raised
***Erwinia* sp.**	Round	Small	Smooth	Shiny	creamy white	Raised
***Ralstonia* sp.**	Round	Medium	Smooth	Moist	White	Convex
Salinico roseus	Irregular	Medium	Wavy	Moist	Orange	Flat
Pseudomonas aeruginosa	Round	Medium	Smooth	Mucoid	Greenish yellow	Convex
***Bacillus* cereus**	Round	Medium	Smooth	Dry	White	Flat
Bacillus sp.	Filamentous	Irregular	Thread like	Dry	White	Flat
***Lactobacillus* sp.**	Round to oval	Medium	Smooth	Extremely slimy	Transparent	Raised
***Thermomonospora* sp.**	Round	Medium	Spiny	Moist	White	Smooth Convex
E. coli	Round	Small	Smooth	Moist	Grayish White	Convex

The morphological characters of the identified bacteria from rhizosphere soil and mushroom substrate were shown above (table 5). Previously the morphology of the rhizosphere bacteria was explained by many researchers. *Bacillus cereus* and *E. coli* produce round off white entire colonies of large and small size; flat and raised elevation; dry and moist texture respectively (Aneja *et al.,* 2015). *Pseudomonas aeruginosa* shows round, smooth, greenish yellow colonies with entire margins and convex elevations (Ningthoujam and Shovarani, 2008). *Salinico roseus* produces irregular, moist, orange, medium colonies (Ventosa *et al.,* 1993). *Burkholderia* and *Xanthomonas* produce Round, Small, Smooth and raised colonies of pale yellow and yellow colors respectively (Eram *et al.,* 2004; Kim *et al.,* 2021). *Pectobacteria* and *Erwinia* are the bacteria of same family; produces small, smooth, slimy colonies of cream and creamy white color respectively (Cinisli *et al.,* 2019; Ali *et al.,* 2014), *Streptomyces* produces filamentous, irregular, cottony, white, raised colonies (Gupta *et al.,* 2009), *Azotobacter* produces rhizoid, branched, smooth, creamy white or off-white colonies (Kasa *et al.,* 2015). *Rhizobium* and *Mesorhizobium* produce round, medium, smooth, moist colonies of yellowish and white opaque in color respectively (Ondieki *et al.,* 2017; Ahemad *et al.,* 2012). *Azospirillum* and *Ralstonia* produces round, medium sized colonies of white colour (Sharma *et al.,* 2015; Rasool *et al.,* 2019); *Agrobacterium* and *Acidovorax* produce round small sized colonies of orange brown and white color respectively (LaMOVŠEK *et al.,* 2014; Fontana *et al.,* 2013). These findings indicated that the identification of the bacteria in this study based on their morphology was appropriate.



***Rhizobium* sp.** ***Mesorhizobium* sp.** ***Azospirillum* sp.**

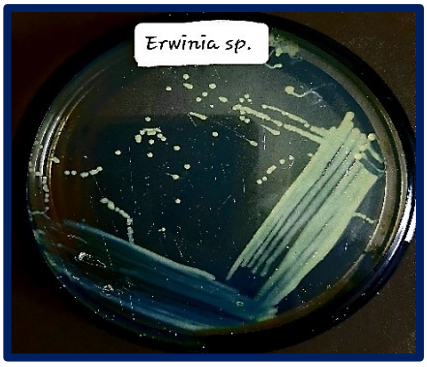


***Azotobacter* sp.** ***Agrobacterium* sp.** ***Streptomyces* sp.**

***Burkholderia* sp.** ***Xanthomonas* sp.** ***Acidovorax* sp.**

Plate 9. Pure culture plates of identified bacteria

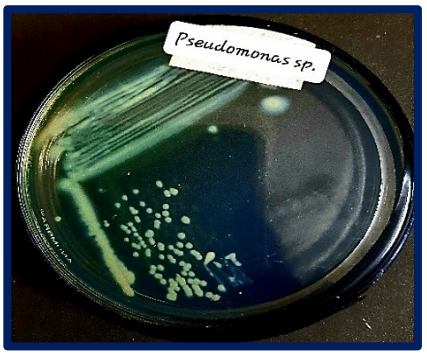
***Pectobacterium* sp.**



***Erwinia* sp.**

***Ralstonia* sp.**

Salinico roseus



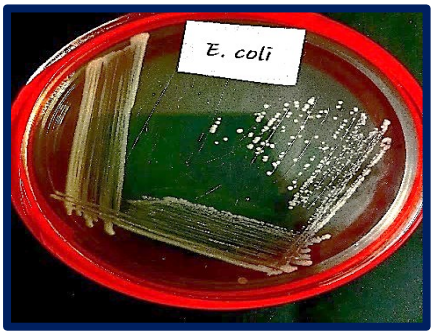
Pseudomonas aeruginosa

***Thermomonospora* sp.**

***Bacillus* sp.**

Bacillus cereus

***Lactobacillus* sp.**



Escherichia coli

Plate 10. Pure culture plates of identified bacteri

4.4 Biochemical characters of the identified bacterial strains

Total 10 tests were performed. Biochemical characters of the identified bacteria were observed and analyzed according to their positive and negative response to the tests. And the bacteria were identified according to the points of Bergeys Manual of Systematic Bacteriology (Bergey *et al.,* 1989).

4.4.1 Gram Staining Test of the Bacterial Isolates

Gram staining test was performed on all 59 bacterial isolates to determine their cell wall characteristics as well as the shape of bacterial cells. The Potassium hydroxide test (KOH test) was also performed which is an alternative way to identify gram-negative bacteria where a KOH-positive result refers to gram-negative bacteria and vice versa. The results showed that 19 isolates (33%) were gram-positive and 40 isolates (67%) were gram negative bacteria (Table 6). Among them *Streptomyces* sp. *Bacillus* sp., *Bacillus cereus, Lactobacillus* sp. and *Thermomonospora* sp. were gram-positive retaining violate color whereas *Rhizobium*, *Xanthomonas* sp., *Agrobacterium* sp. *Pseudomonas* sp. and the remaining were gram negative bacteria showing red color (Plate 12).

4.4.2 Catalase test of the Bacterial Isolates

Catalase test was performed on all 59 bacterial isolates. The results showed that 58 isolates (98%) were catalase positive and 1 isolate (2%) was catalase negative (Table 6). Except *Lactobacillus* all the bacteria formed bubbles on the slides of hydrogen peroxide. That means *Lactobacillus* doesn't carry the catalase enzyme.

4.4.3. Oxidase test of the Bacterial Isolates

Oxidase test was performed on all 59 bacterial isolates. The results showed that 33 isolates (55%) were oxidase positive and 26 isolates (45%) were oxidase negative (Table 6). *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Agrobacterium* sp., *Streptomyces* sp., *Ralstonia* sp., *Salinico roseus*, *Bacillus cereus* and *Bacillus* sp. were oxidase positive. *Mesorhizobium* sp., *Burkholderia* sp., *Xanthomonas* sp., *Acidovora* sp., *Pectobacterium* sp., *Erwinia* sp., *Pseudomonas aeruginosa*, *Lactobacillus* sp., *Thermomonospora* sp. and *E. coli* were oxidase negative bacteria.

4.4.4. Motility test of the Bacterial Isolates

Motility test was performed on all 59 bacterial isolates. The results showed that 52 isolates (88%) were motility positive and 7 isolates (12%) were motility negative (Table 6). *Rhizobium* sp., *Mesorhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Agrobacterium* sp., *Streptomyces* sp., *Xanthomonas* sp., *Acidovorax* sp., *Pectobacterium* sp., *Ralstonia* sp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus* sp. and *E. coli* were motility positive. *Burkholderia* sp., *Erwinia* sp., *Salinico roseus*, *Lactobacillus* sp. and *Thermomonospora* sp. were motility negative bacteria.

4.4.5. Simmon's citrate agar test of the Bacterial Isolates

Simmon's citrate agar test was performed on all 59 bacterial isolates. The results showed that 43 isolates (72%) were positive green and 16 isolates (28%) were negative blue (Table 6). *Azospirillum* sp., *Streptomyces* sp., *Burkholderia* sp., *Xanthomonas* sp., *Acidovorax* sp., *Pectobacterium* sp., *Erwinia* sp., *Ralstonia* sp., *Salinico roseus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus* sp. showed positive green color. *Rhizobium* sp., *Mesorhizobium* sp., *Azotobacter* sp., *Agrobacterium* sp., *Lactobacillus* sp., *Thermomonospora* sp. and *E. coli* showed negative blue color in this test.

4.4.6. Levan production test of the Bacterial Isolates

Levan production test was performed on all 59 bacterial isolates. The results showed that 36 isolates (61%) were Levan positive and 23 isolates (39%) were Levan negative (Table 6). *Rhizobium* sp., *Azotobacter* sp, *Xanthomonas* sp, *Acidovorax* sp., *Bacillus cereus* , *Bacillus* sp., *Lactobacillus* sp. and *E. coli* were Levan positive. *Mesorhizobium* sp., *Azospirillum* sp., *Agrobacterium* sp., *Streptomyces* sp., *Burkholderia* sp, *Pectobacterium* sp., *Erwinia* sp., *Ralstonia* sp., *Salinico roseus*, *Pseudomonas aeruginosa* and *Thermomonospora* sp. were Levan negative in this test.

4.4.7. Casein Hydrolysis test of the Bacterial Isolates

Casein Hydrolysis test was performed on all 59 bacterial isolates. The results showed that 32 isolates (54%) were casein positive and 27 isolates (46%) were casein negative (Table 6). *Azospirillum* sp., *Streptomyces* sp, *Burkholderia* sp., *Xanthomonas* sp., *Pectobacterium* sp., *Salinico roseus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus* sp., *Lactobacillus* sp. and *E. coli* were casein positive. *Rhizobium* sp., *Mesorhizobium* sp,

Azotobacter sp., *Agrobacterium* sp., *Acidovorax* sp, *Erwinia* sp., *Ralstonia* sp. and *Thermomonospora* sp. were casein negative in this test.

4.4.8. Starch Hydrolysis test of the Bacterial Isolates

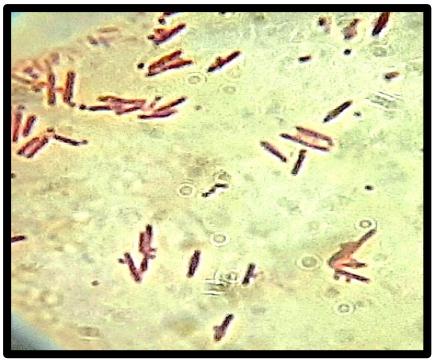
Starch hydrolysis test was performed on all 59 bacterial isolates. The results showed that 32 isolates (54%) were starch positive and 27 isolates (46%) were starch negative (Table 6). *Mesorhizobium* sp, *Azospirillum* sp., *Azotobacter* sp., *Streptomyces* sp, *Burkholderia* sp, *Xanthomonas* sp, *Acidovorax* sp., *Erwinia* sp, *Salinico roseus*, *Bacillus cereus*, *Bacillus* sp, *Lactobacillus* sp, and *Thermomonospora* sp. were starch positive. *Rhizobium* sp., *Agrobacterium* sp., *Pectobacterium* sp., *Ralstonia* sp., *Pseudomonas aeruginosa* and *E. coli* were starch negative in this test.

4.4.9. Gelatin Liquefaction test of the Bacterial Isolates

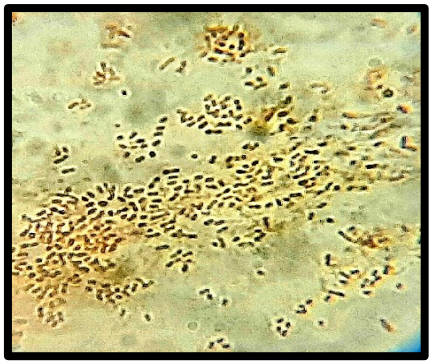
Gelatin liquefaction test was performed on all 59 bacterial isolates. The results showed that 34 isolates (58%) were tested positive and 25 isolates (42%) were tested negative (Table 6). *Agrobacterium* sp., *Streptomyces* sp., *Burkholderia* sp., *Xanthomonas* sp., *Pectobacterium* sp *Erwinia* sp., *Salinico roseus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus* sp., *Lactobacillus* sp. and *E. coli* were positive. *Rhizobium* sp., *Mesorhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Acidovorax* sp., *Ralstonia* sp. and *Thermomonospora* sp. were negative in this test.

Table 6. Gram stain and KOH solubility test and cell shape of the identified bacterial from the samples

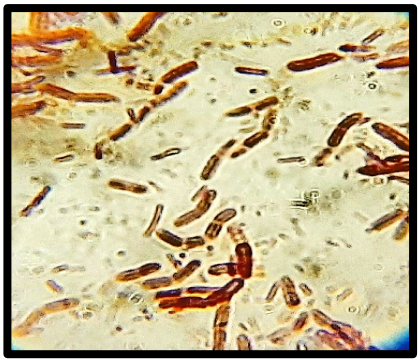
Sl no.	Name of the bacteria	Total isolates	Gram	KOH solubility	Color after Gram stain	Cell shape
1	***Rhizobium* sp.**	4	-	+	Red	Rod
2	***Mesorhizobium* sp.**	2	-	+	Red	Rod
3	***Azospirillum* sp.**	2	-	+	Red	Spiral Rods
4	***Azotobacter* sp.**	4	-	+	Red	Rod
5	***Agrobacterium* sp.**	2	-	+	Red	Rod
6	***Streptomyces* sp.**	2	+	-	Violet	Rod
7	***Burkholderia* sp.**	1	-	+	Red	Rod
8	***Xanthomonas* sp.**	3	-	+	Red	Rod
9	***Acidovorax* sp.**	8	-	+	Red	Rod
10	***Pectobacterium* sp.**	2	-	+	Red	Rod
11	***Erwinia* sp.**	2	-	+	Red	Chain of small rods
12	***Ralstonia* sp.**	3	-	+	Red	Rod
13	***Salinico roseus***	1	-	+	Red	Cocci
14	***Pseudomonas aeruginosa***	4	-	+	Red	Rod
15	***Bacillus cereus***	14	+	-	Violet	Chain of Rods
16	***Bacillus* sp.**	1	+	-	Violet	Long chain of Rods
17	***Lactobacillus* sp.**	1	+	-	Violet	Rod
18	***Thermomonospora* sp.**	2	+	-	Violet	Cocci
19	***E coli***	1	-	+	Red	Small Rod



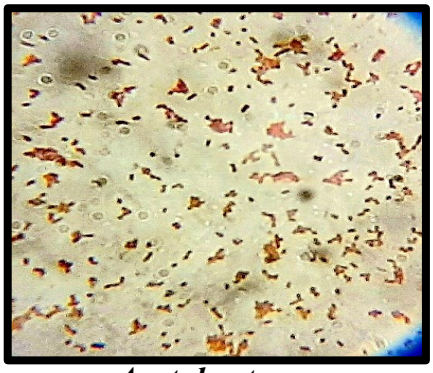
***Rhizobium* sp.**



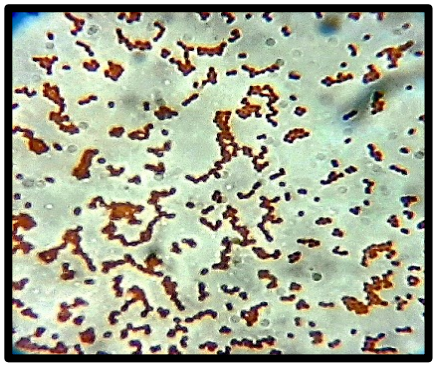
***Mesorhizobium* sp.**



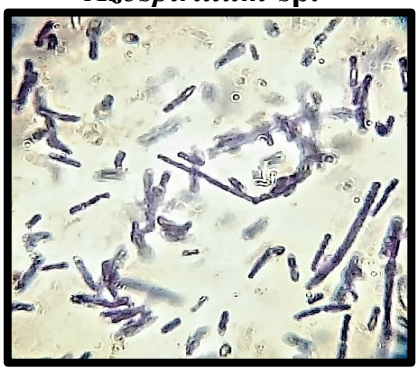
***Azospirillum* sp.**



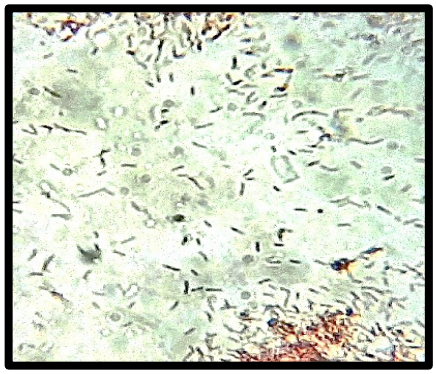
***Azotobacter* sp.**



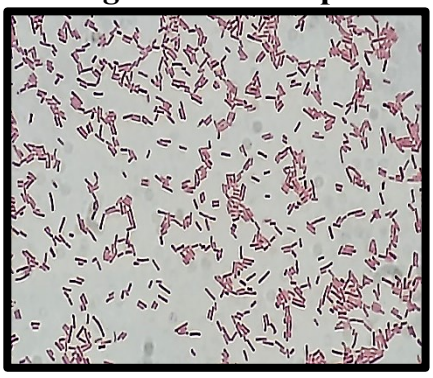
***Agrobacterium* sp.**



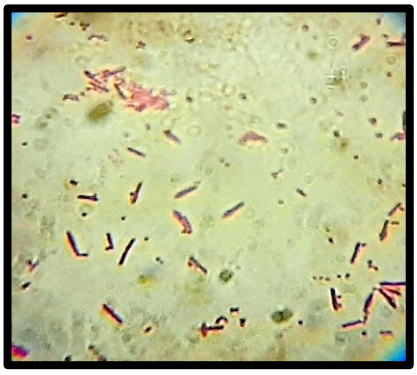
***Streptomyces* sp.**



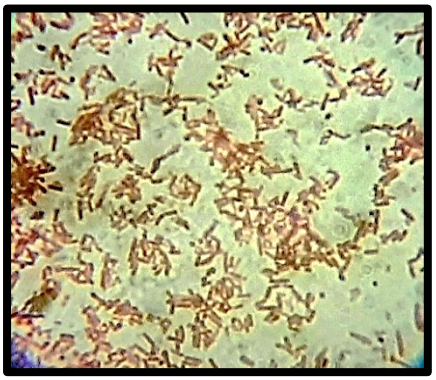
***Burkholderia* sp.**



***Acidovorax* sp.**

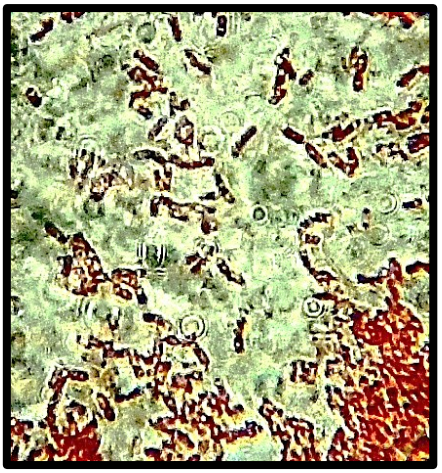


***Xanthomonas* sp.**

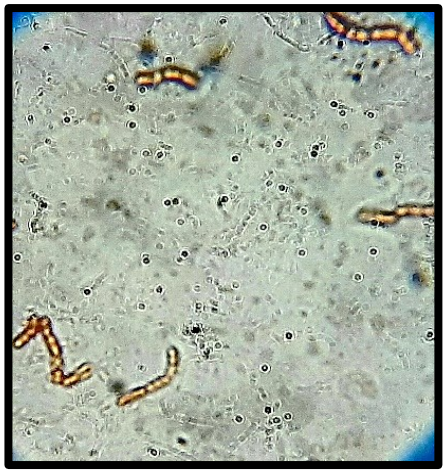


***Pectobacterium* sp.**

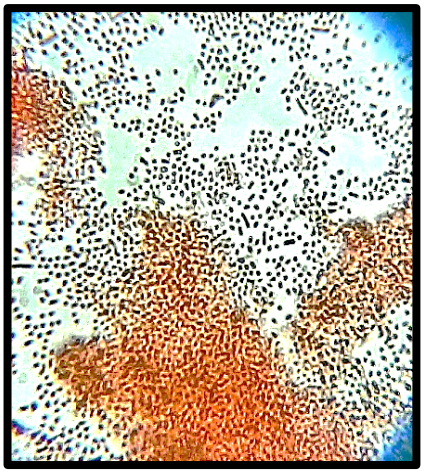
Plate 11. Shape and color of identified bacteria in gram staining test



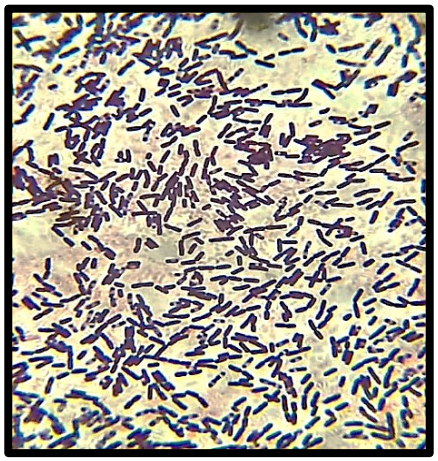
***Erwinia* sp.**



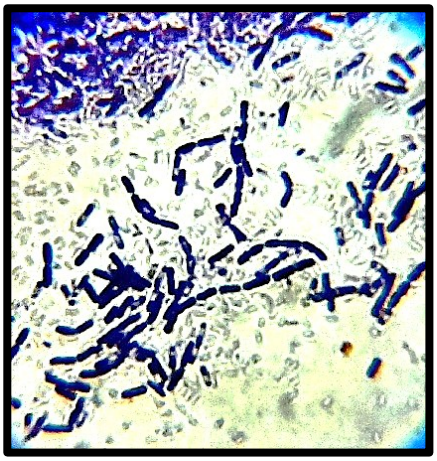
***Ralstonia* sp.**



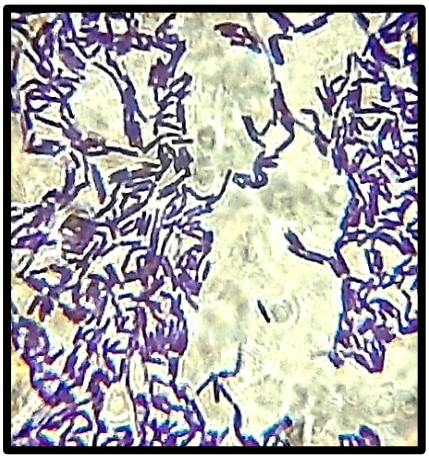
Salinico roseus



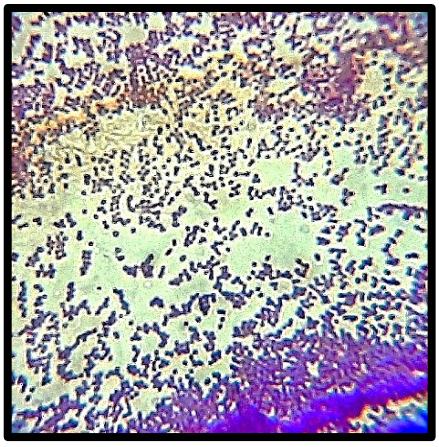
Bacillus cereus



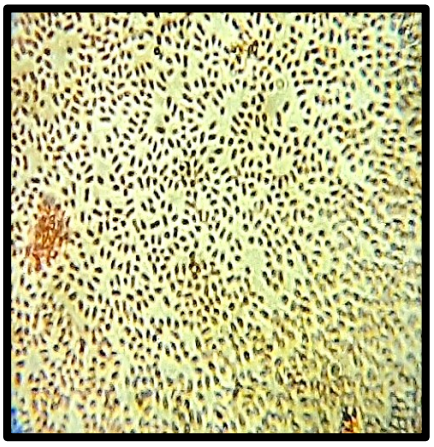
***Bacillus* sp.**



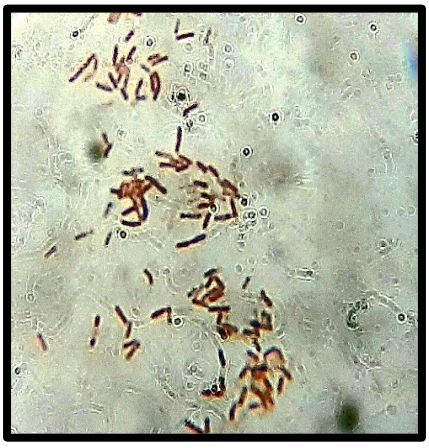
***Lactobacillus* sp.**



***Thermomonospora* sp.**



Escherichia coli



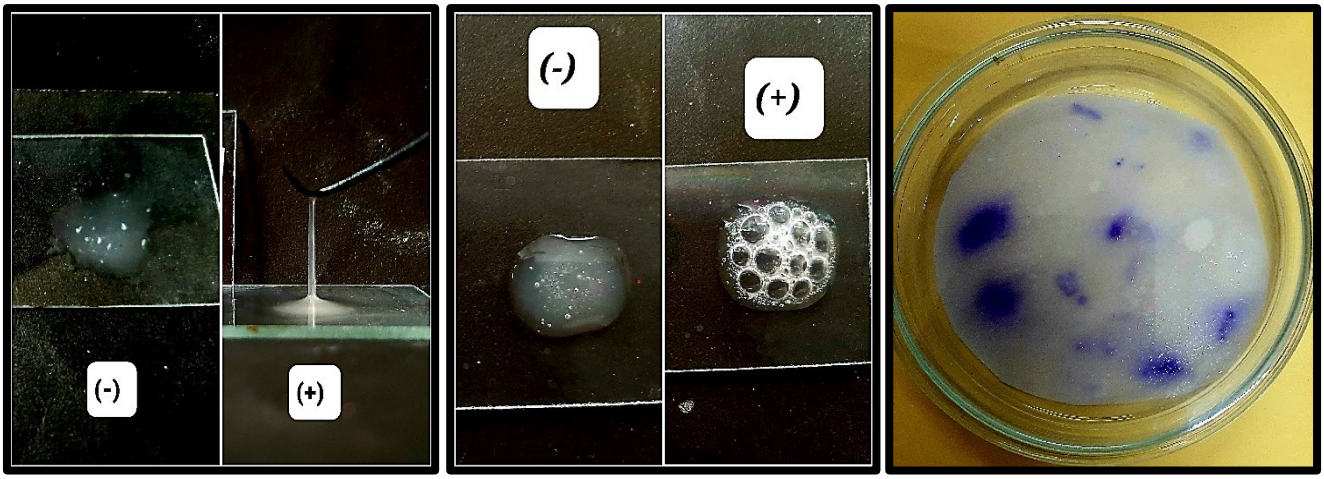
Pseudomonas aeruginosa

Plate 12. Shape and color of identified bacteria in gram staining test

Table 7. Biochemical characters of the identified bacterial isolates from the samples

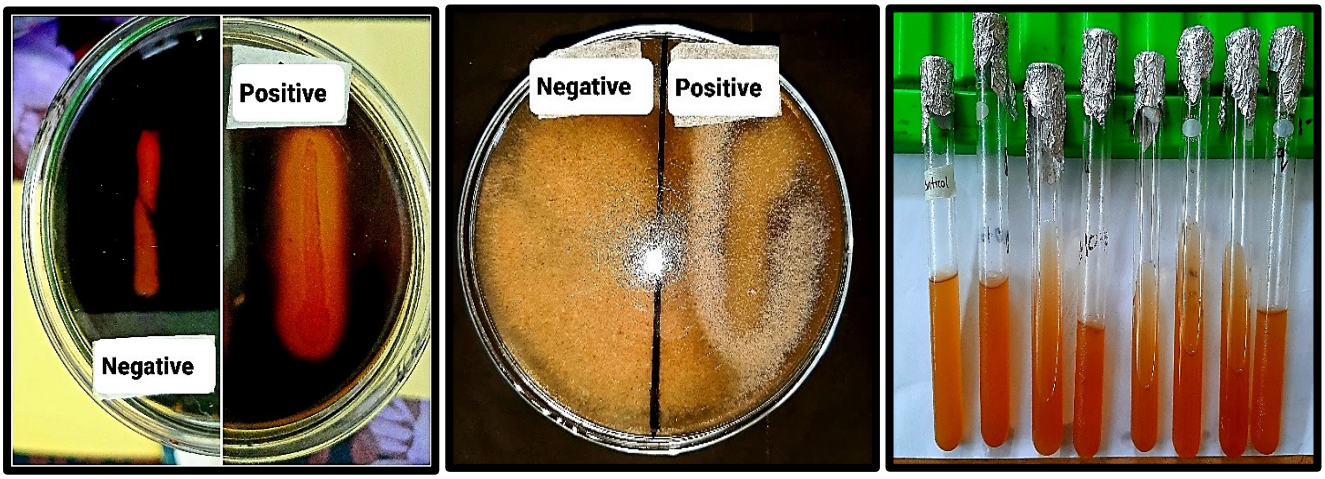
Name of the bacteria	Total isolate	Catalase	Oxidase	SIM (Motility)	Simmon`s citrate	Levan production	Casein	Starch hydrolysis	Gelatin Liquefaction
***Rhizobium* sp.**	4	+	+	+	-	+	-	-	-
***Mesorhizobium* sp.**	2	+	-	+	-	-	-	+	-
***Azospirillum* sp.**	2	+	+	+	+	-	+	+	-
***Azotobacter* sp.**	4	+	+	+	-	+	-	+	-
***Agrobacterium* sp.**	2	+	+	+	-	-	-	-	+
***Streptomyces* sp.**	2	+	+	+	+	-	+	+	+
***Burkholderia* sp.**	1	+	-	-	+	-	+	+	+
***Xanthomonas* sp.**	3	+	-	+	+	+	+	+	+
***Acidovorax* sp.**	8	+	-	+	+	+	-	+	-
***Pectobacterium* sp.**	2	+	-	+	+	-	+	-	+
***Erwinia* sp.**	2	+	-	-	+	-	-	+	+
***Ralstonia* sp.**	3	+	+	+	+	-	-	-	-
Salinico roseus	1	+	+	-	+	-	+	+	+
Pseudomonas aeruginosa	4	+	-	+	+	-	+	-	+
Bacillus cereus	14	+	+	+	+	+	+	+	+
***Bacillus* sp.**	1	+	+	+	+	+	+	+	+
***Lactobacillus* sp.**	1	-	-	-	-	+	+	+	+
***Thermomonospora* sp.**	2	+	-	-	-	-	-	+	-
E. coli	1	+	-	+	-	+	+	-	+

The biochemical response of the identified bacteria. Perhaps Bergeys Manual of Systematic bacteriology the test results were similar with the findings of many other expertise. In catalase test all the bacterial strain showed positive results except *Lactobacillus. Lactobacillus* gives negative results to catalase test (Sagar aryal, 2022). Bacillus cereus showed similar results with the present study in these biochemical tests previously (Rajesh *et al.,* 2017; Hadi *et al.,* 2019; Ghosh *et al.,* 2002). *Xanthomonas* sp. *Acidovorax* sp. *Pectobacterium* sp. *Erwinia* sp. *Ralstonia* sp. *Salinico roseus, Pseudomonas* sp. all showed positive green colour in Simmon's Citrate Agar test. *Burkholderia* sp., *Erwinia* sp., *Salinico roseus, Lactobacillus* sp., *Thermomonospora* sp. showed negative straight red line in motility test. These are the non-motile bacteria (Eram *et al.,* 2004; Ventosa *et al.,* 1993; Rajesh *et al.,* 2017). Also, *Psudomonas aeruginosa* biochemically response similar with the present findings (Sagar Aryal. 2022). In case of starch hydrolysis test *Mesorhizobium* sp., *Azospirillum* sp., *Azotobacter* sp, *Streptomyces* sp, *Burkholderia* sp, *Xanthomonas* sp, *Acidovorax* sp, *Erwinia* sp, *Salinico roseus*, *Bacillus cereus, Bacillus* sp, *Lactobacillus* sp and *Thermomonospora* sp showed positive clear transparent zone around the bacteria. E. coli showed negative result (Fishman, 1984). In gelatin liquefaction test *Rhizobium* sp., *Mesorhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Acidovorax* sp., *Ralstonia* sp., *Thermomonospora* sp. couldn't liquefy the gel that means they showed negative results. Many other scientists (eg. Islam *et al.,* 2020; Kasa *et al.,* 2015; Hossain *et al.,* 2014; Rashid *et al.,* 2013; Hussnain *et al.,* 2011; Jambena *et al.,* 2011; Bahar *et al.,* 2010; Rahman *et al.,* 2010; Alippi *et al.,* 2009;) find same results in the biochemical analysis of bacteria mentioned in table 7.



KOH solubility test | Catalase test | Oxidase test

Motility Test | Simmon's Citrate Agar Test | Levan Production Test



Starch Hydrolysis Test | Casein Hydrolysis Test | Gelatin Hydrolysis Test

Plate 13. Biochemical test of bacteria showing positive and negative results

4.5 Bacterial growth on specific media

Three different types of selective media were used to identify different bacteria by observing their growth on the media and their colony color (Table 8 and plate 9).

Table 8. Analysis of colony growth and color of bacterial isolates on selective media

Sl no.	Name of the bacteria	Total isolates	BCA	CA	EMB
1	***Rhizobium* sp.**	4	-	-	-
2	***Mesorhizobium* sp.**	2	-	-	-
3	***Azospirillum* sp.**	2	-	-	-
4	***Azotobacter* sp.**	4	-	-	-
5	***Agrobacterium* sp.**	2	-	-	-
6	***Streptomyces* sp.**	2	-	-	-
7	***Burkholderia* sp.**	1	-	-	-
8	***Xanthomonas* sp.**	3	-	-	-
9	***Acidovorax* sp.**	8	-	-	-
10	***Pectobacterium* sp.**	2	-	-	-
11	***Erwinia* sp.**	2	-	-	-
12	***Ralstonia* sp.**	3	-	-	-
13	***Salinico roseus***	1	-	-	-
14	***Pseudomonas aeruginosa*.**	4	-	Cream	-
15	***Bacillus cereus***	14	Blue	-	-
16	***Bacillus* sp.**	1	-	-	-
17	***Lactobacillus* sp.**	1	-	-	-
18	***Thermomonospora* sp.**	2	-	-	-
19	***E coli***	1	-	-	Metal green

*BCA= Bacillus Cereus Agar, CA= Cetrimide Agar, EMB= Eosin Methylene Blue * (-) = no color

Here in table 8 it is shown that 14 isolates exhibit blue color on Bacillus cereus Agar, it means those were Bacillus *cereus*, 4 isolates created cream color on Cetrimide Agar plate, it means those were *Pseudomonas aeruginosa* and 1 isolate produced metal green color on Eosin Methylene Blue plate, it means that was E. coli. BCA is a selective media for the growth of Bacillus cereus (BASE, C. B. F. S. A. Dehydrated Culture Media), Cetrimide Agar is the selective media for identification of Pseudomonas aeruginosa (Lowbury *et al.,* 1955) and EMB is the sective and differential media, *E. coli* gives metal green color in this media (Levine, 1918).

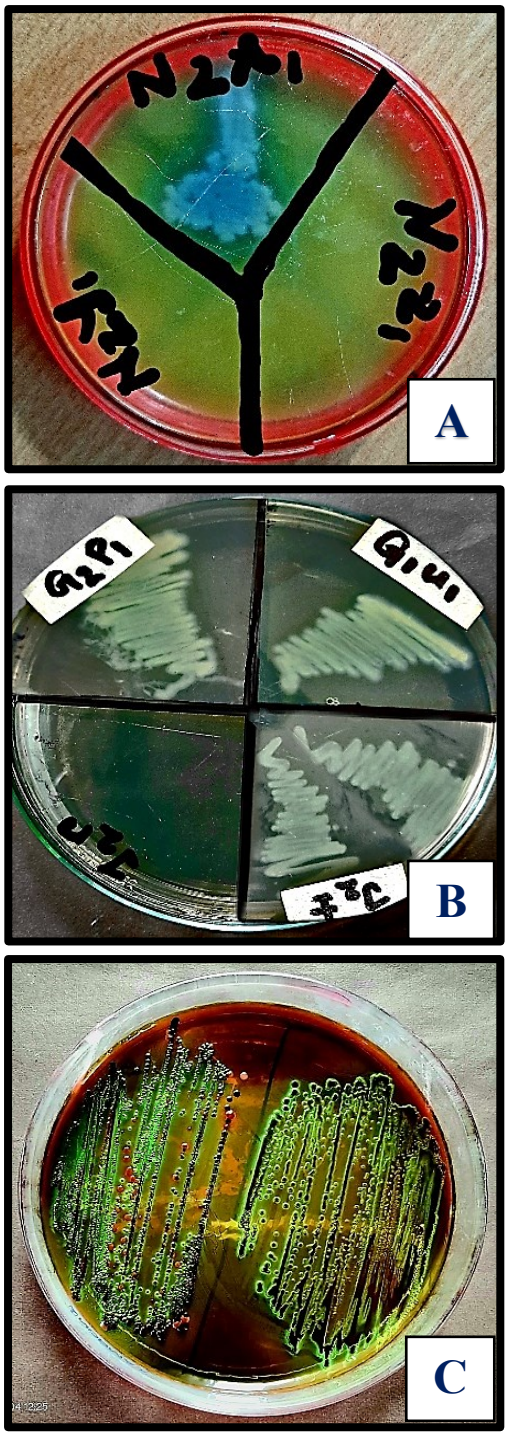


Plate 14. Growth of *Bacillus cereus*, *Pseudomonas aeruginosa* and *E. coli* bacteria on specific media; Here A = BCA (Bacillus Cereus Agar), B =CA (Cetrimide Agar), C= EMB (Eosin Methylene Blue) media

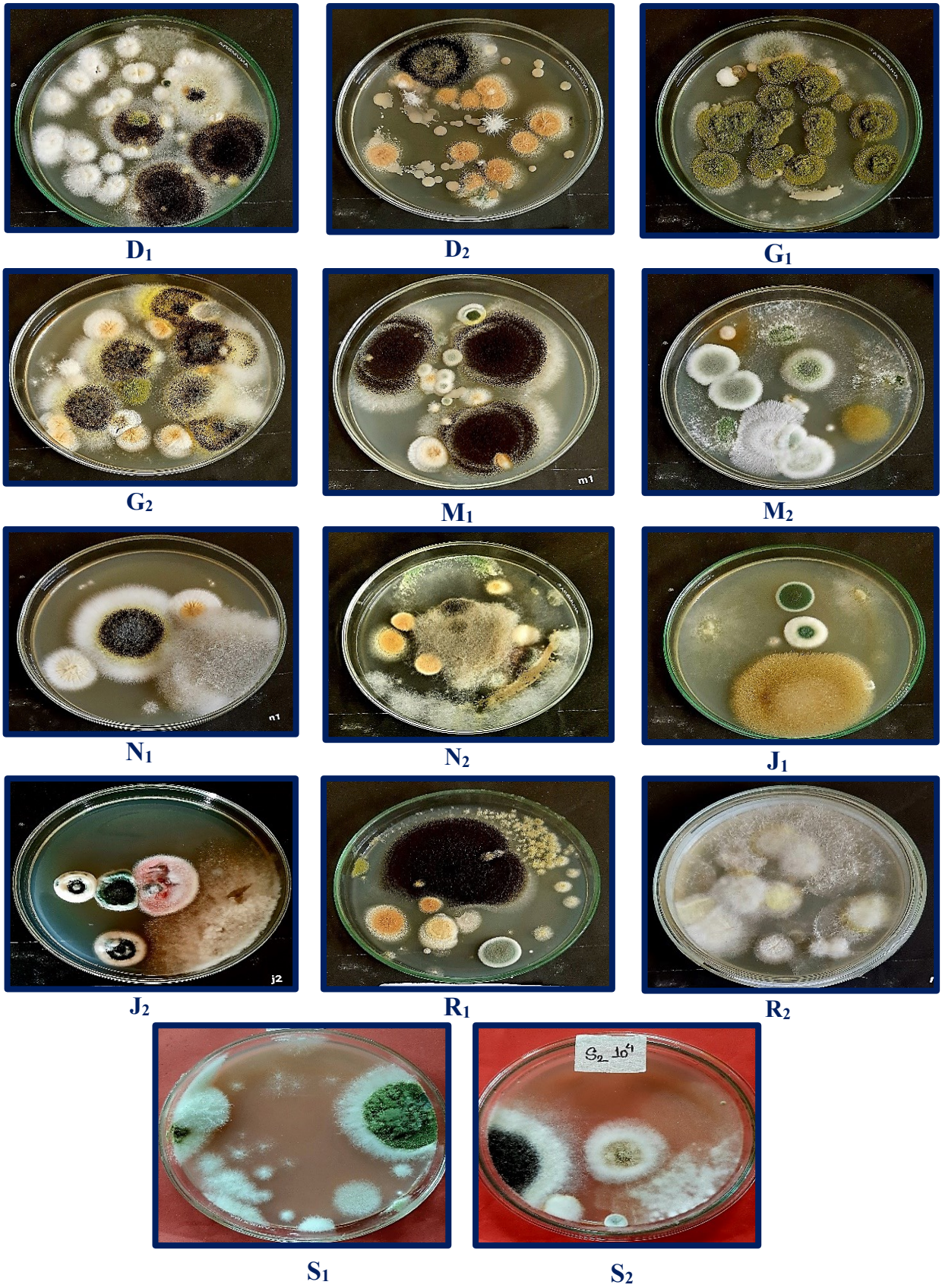


Plate 15. Growth of fungus on PDA media at 10^4 dilution of the respective samples D_1, D_2, G_1, G_2, M_1, M_2, N_1, N_2, J_1, J_2, R_1, R_2, S_1 and S_2

Table 9. Identified fungal isolates from sample D_1, G_1, M_1, N_1, J_1, R_1, S_1, D_2, G_2, M_2, N_2, J_2, R_2 and S_2

Sample symbol	Isolate no.	Bacterial isolate
D_1 (Cabbage)	1	*T. harzianum*
	2	*A. niger*
	3	*Fusarium* sp.
	4	*Sclerotium* sp.
G_1 (BRRI dhan28)	9	*A. flavus*
	10	*Fusarium oxysporum*
	11	*T. harzianum*
M_1 (Mustard)	17	*A. niger*
	18	*Penicillium* sp.
	19	*Fusarium* sp.
	20	*T. viride*
	21	*Sclerotium* sp.
N_1 (Wheat)	25	*A. niger*
	26	*A. terreus*
	27	*T. harzianum*
	28	*Cunninghamela* sp.
J_1 (Okra)	34	*Cunninghamela* sp.
	35	*T. viride*
	36	*Penicillium* sp.
R_1 (Lentil)	40	*Alternaria* sp.
	41	*Sclerotium* sp.
	42	*Penicillium* sp.
	43	*A. terreus*
S_1 (Before Harvest)	48	*Fusarium* sp.
	49	*T. hamatum*
	50	*A. flavus*

Sample symbol	Isolate no	Bacterial isolate
D_2 (Quinoa)	5	*A. niger*
	6	*T. harzianum*
	7	*Fusarium* sp.
	8	*A. terreus*
G_2 (BRRI dhan 29)	12	*Fusarium* sp
	13	*A. niger*
	14	*A. flavus*
	15	*Sclerotium* sp.
	16	*A. terreus*
M_2 (Tomato)	22	*T. harzianum*
	23	*Sclerotium* sp.
	24	*Penicillium* sp.
N_2 (Soybean)	29	*T. viride*
	30	*A. terreus*
	31	*Fusarium* sp.
	32	*Curvularia* sp
	33	*Rhizoctonia* sp.
J_2 (Chilli)	37	*Fusarium oxysporum*
	38	*Penicillium* sp.
	39	*T. harzianum*
R_2 (Brinjal)	44	*Fusarium oxysporum*
	45	*T. harzianum*
	46	*Sclerotium* sp.
	47	*Rhizoctonia* sp.
S_2 (After Harvest)	51	*Chaetomium* sp.
	52	*Fusarium* sp.
	53	*T. hamatum*
	54	*Penicillium* sp.

4.6 Isolation and Identification of fungal isolates

From the dilution of total 14 samples (Plate 15) 54 bacterial isolates were isolated by making pure cultures on PDA media. After studying their morphological and microscopic characteristics 15 different fungi were identified. Among them *Sclerotium* sp*., Rhizoctonia* sp*., Cunninghamela* sp*., Chaetomium* sp*., Alternaria* sp*., Curvularia* sp*., Penicillium* sp*.*, *Trichoderma harzianum, T. viride, , Aspergillus niger, A. flavus, A. terreus, Fusarium oxysporum* and *Fusarium* sp. were identified from the crop rhizosphere soil and *T. hamatum, Fusarium* sp., *A. flavus* and *Penicillium* sp. were identified from mushroom substrate (Table 9).

The identified fungus from the rhizosphere and mushroom substrate from this study were mentioned (Table 9). From the rhizosphere of cabbage, quinoa and rice *T. harzianum*, *A. niger*, *Fusarium* sp*.*, *Sclerotium* sp*.*, *A. flavus*, *Fusarium oxysporum* and *A. terreus* this fungus were found. Rhizosphere of mustard, tomato, wheat, soybean, okra and chilli contained *A. niger*, *Penicillium* sp*.*, *Fusarium* sp*.*, *T. viride*, *Sclerotium* sp, *A. niger*, *A. terreus*, *T. harzianum*, *Cunninghamela* sp, *Curvularia* sp, *Rhizoctonia* sp. and *Fusarium oxysporum. Again from the rhizosphere soil of lentil and brinjal the isolated fungi were Alternaria* sp., *Sclerotium* sp, *Penicillium* sp, *A. terreus*, *Fusarium oxysporum*, *T. harzianum* and *Rhizoctonia* sp. From the mushroom substrate of before and after harvest condition the isolated fungi were *Fusarium* sp*.*, *T. hamatum*, *A. flavus*, *T. hamatum* and *Penicillium* sp. In case of isolated fungi the *Sclerotium, Rhizoctonia, Fusarium* are soil borne pathogenic fungi (Khan and Rao, 2019; Coulibaly *et al.,* 2022; Dusengemungu, 2021). Fusarium, Penicillium were also found in the rhizosphere (Larekeng *et al.,* 2019). Like present study Hafez also found that A*spergillus* was the most dominant in both rhizosphere and nonrhizosphere soils and was represented by species: *A. niger*, *A. terreus*, *A. flavus*, and *A. ustus* in 2012. *Cunninghamella* sp. in the rhizosphere reduce symptoms of salinity, drought and heavy metal stresses in tomato plants (Kazerooni *et al.,* 2022). Curvularin and its derivatives are produced by rhizosphere fungal communities belonging to genera *Curvularia, Aspergillus, Alternaria,* and *Penicillium* (Pattnaik *et al.,* 2019). In the substrate Ascomycota, specifically the thermophilic fungi *Chaetomium*, promotes the growth of mushroom (Salar and Aneja, 2007).

Table 10. Cultural and microscopical characters of the identified fungal isolates from the samples

Name of the fungus	Cultural characteristics			Microscopical Characteristics		
	Color	**Growth**	**Texture**	**Mycelia**	**Conidiophore**	**Spore**
Trichoderma harzianum	Green	Fast	Forms in concentric ring	Septate	Short Branched	Ellipsoidal
T. viride	Green yellow	Fast	Forms through out	Septate	Short Branched	Globose
T. hamatum	Green	Fast	Forms making clusters	Septate	Long Branched	Oval
***Fusarium* sp.**	White	Slow	Cottony	Septate	Sporodochium (With thin elongated phialides on it)	Sickle shape
Fusarium oxysporum	Pink	Slow	Cottony	Septate	Sporodochium (With thin elongated phialides on it)	Sickle shape
***Sclerotium* sp.**	White	Fast	Fluffy	Septate	Absent	Mustard seed like sclerotia
***Rhizoctonia* sp.**	White to brown	Rapid	Thread like	Septate forming right angle (90°)	Absent	Irregular shape dark brown sclerotia is formed
Aspergillus niger	Black	Medium	Mold	Septate	Long and Straight	Chain of globose conidia on vesicle
A. flavus	Light Green	Medium	Mold	Septate	Long and Straight	Chain of globose conidia on vesicle
A. terreus	Brown	Medium	Mold	Septate	Long and Straight	Chain of globose conidia on vesicle
***Cunninghamela* sp.**	Light Gray to brown	Fast	Leathery	Aseptate	Simple sporangiophore holding a swollen vesicle	Globose
***Curvularia* sp.**	Gray to black	Medium	Velutinous	Septate	Branched	Septate, Swollen middle cell
***Chaetomium* sp.**	Black	Medium	Produces ascocarp	Septate	Dichotomously branched	Dark globose ascospores
***Alternaria* sp.**	Brown to dark brown	Medium	Wooly	Septate	Branched	Muriform, small beaked
***Penicillium* sp.**	Green	Medium	Mold	Septate	Long broom like	Chain of round conidia

4.7 Morphological features of the identified fungus

Morphology of fungus recorded by observing the color, growth and texture of the fungus on the PDA media plate. *Trichoderma harzianum, T. viride* and *T. hamatum* were fast growing and produced green sporulation on the PDA media plate. The pattern of the sporulation was different for each of them viz. *T. harzianum* formed concentric rings of spores on the plate, T. viride grew throughout plate spreading all over and *T. hamatum* spores grew in clusters. *Aspergillus niger*, *A. flavus* and *A. terreus* all of them grew in medium speed and in same pattern, evenly spread on the plate, coarse in texture but in different colors. *A. niger, A. flavus* and *A. terreus* produced black, yellow green and brown molds, respectively. *Sclerotium* and *Rhizoctonia* both were very fast in growth, white in color but soft fluffy and rough leathery in texture, respectively. Both formed dark brown colored sclerotia after 10 days. Sclerotia of *Sclerotium* sp. was mustard seed like and sclerotia of *Rhizoctonia* was irregular in shape but bigger than *Sclerotium* sp. *Cunninghamella* sp was rapid in growth, dispersed mycelium, rough in texture and light grey to brown in color. *Alternaria, Curvularia* were slow in growth, superficial and flat with the media, brown to dark brown and grey to black in color, respectively. *Chaetomium* was slow in growth, white to black in color and flat with the media. *Fusarium oxysporum* was cottony, slow growing, pink in center and the other species of *Fusarium* was coarse in texture, white in color also slow growing. *Penicillium* sp. showed dark bluish green color, having a white border outside, spores were like powder, very dry and fine in texture. These morphological characters are shown in (Table 10).

4.7.1 Microscopic features of the identified fungus

Microscopic features of the fungus were recorded by observing mycelia, conidiophore and conidia of the fungus under the 4X, 10X and 40X lense of compound microscope. Whether the mycelium was septate or not. All the identified fungus showed septation in the mycelium that means they all were higher fungi except *Cunninghamela*. Following the keys of "Illustrated genera of imperfect fungi" (Hunter and Barnett; 1998) the isolated fungi in this study were identified. *Trichoderma harzianum* showed hyaline short conidiophores, branched, each branches carrying 3 phialides for spore production, phialides are small in size and spores were ellipsoidal in shape. *T. viride* also appeared with hyaline short, branched, conidiophores, branches made right angle to each other, carrying 3 phialides but these phialides were in higher length then *T. harzianum* and the

spores were globose in shape. The *Trichoderma hamatum* had long branched conidiophores carrying 3 phialides producing right angle with each other and spores are oval in shape. In case of *A. niger* conidiophores are long and smooth, spore covers the vesicle. The conidiophores of *A. flavus* is variable in length and the vesicles were not fully covered by spores, *A. terreus* produced conidia of too small size which were columner with the vesicle. The microscopic character of *Cunninghamella* was observed and pyriform vesicle, straight and erect sporangiophore, globose spores were found. *Fusarium oxysporum* showed hyaline septate mycelium, sporodochium (with thin elongated phialides on it), sickle shape macroconidia with 5-6 cells and oval shaped microconidia. The other species of *Fusaium* was found to have hyaline septate mycelium, Sporodochium (With thin elongated phialides on it), elongated sickle shape macroconidia with 7-8 cells and small globose shaped microconidia. Under the microscope the *Rhizoctonia* sp. was visualized as septate mycelium forming right angle. *Alternaria* formed brown colored muriform conidial chain, *Curvularia* formed brown colored tetrad of curved conidia which had the big sized middle cell. *Penicillium* formed conidiophore and containing chains of round conidia on head. Altogether it looked like a broom. Chaetomium produced oval shaped thick walled dark colored spores (ascospores) also it produced ascocarp that was perithecium (flask shaped) (Table 10).

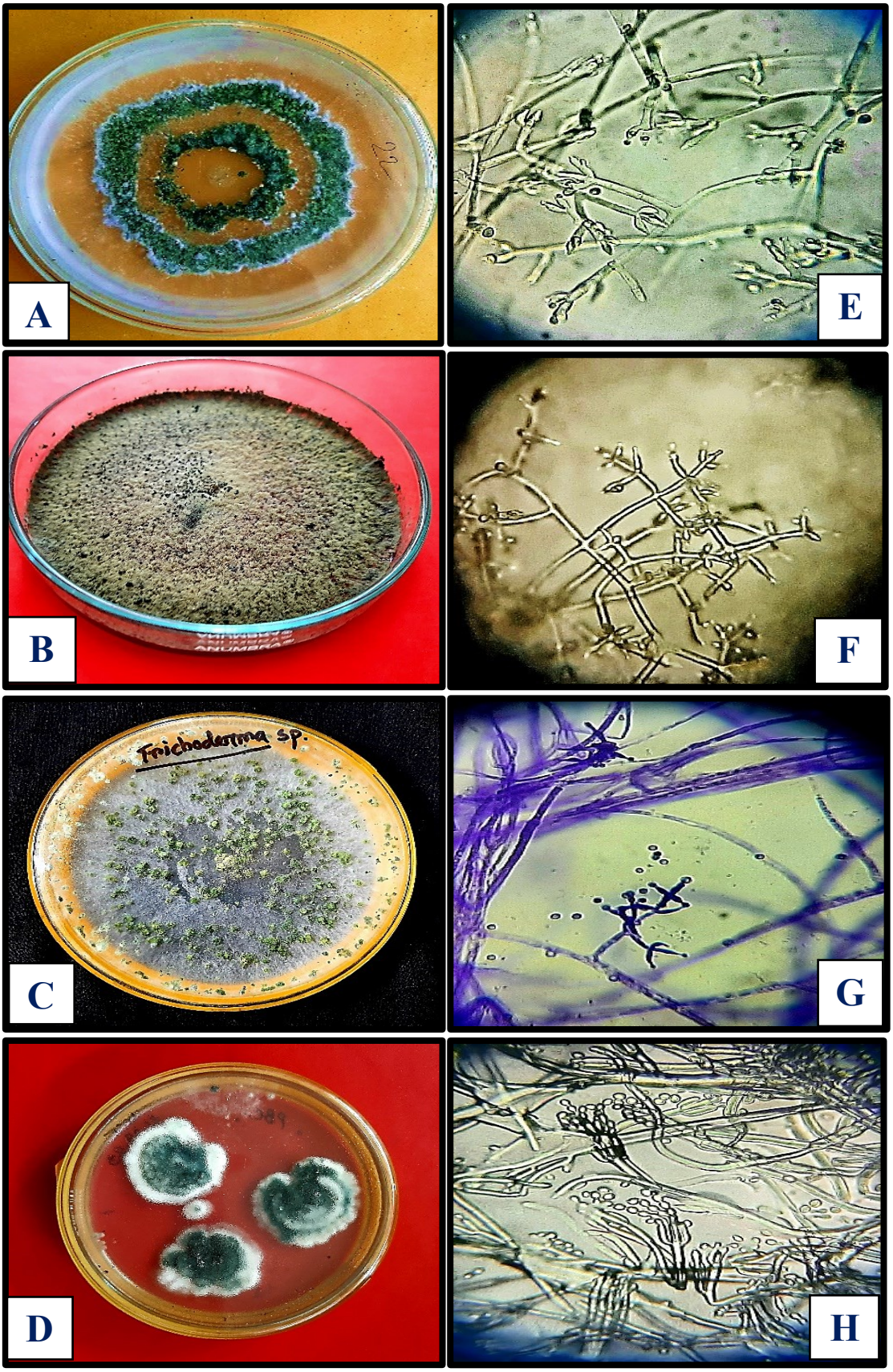


Plate 16. Morphology of the identified fungus from the samples; A, B, C and D are the pure cultures of *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma hamatum* and *Penicillium* sp. respectively and E, F, G and H are the microscopic view of the respective fungi

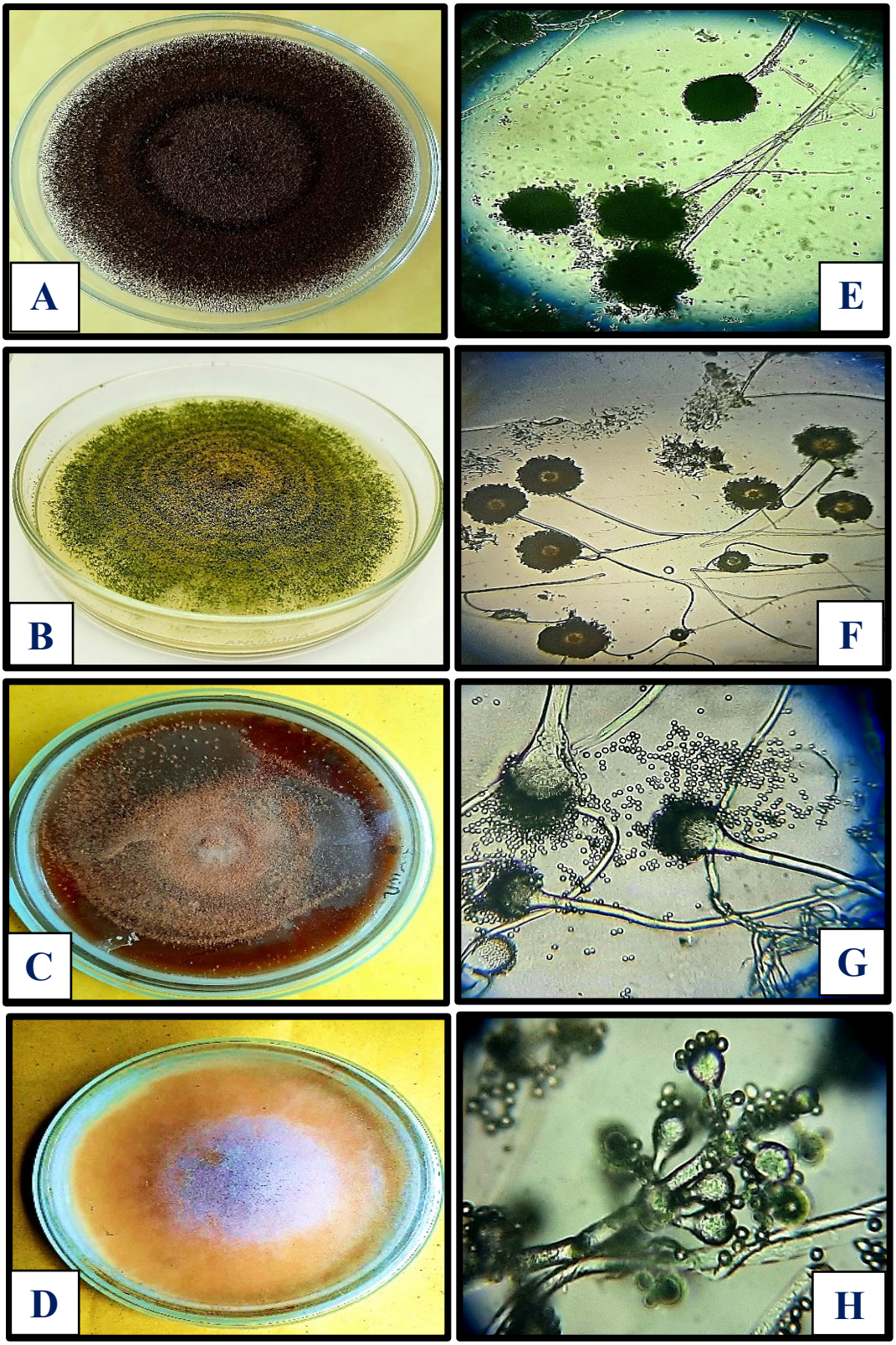


Plate 17. Morphology of the identified fungus from the samples; A, B, C and D are the pure cultures of *Aspergillus niger, Aspergillus flavus, Aspergillus terreus* and *Cunninghamella* sp. respectively and E, F, G and H are the microscopic view (40X) of the respective fungi

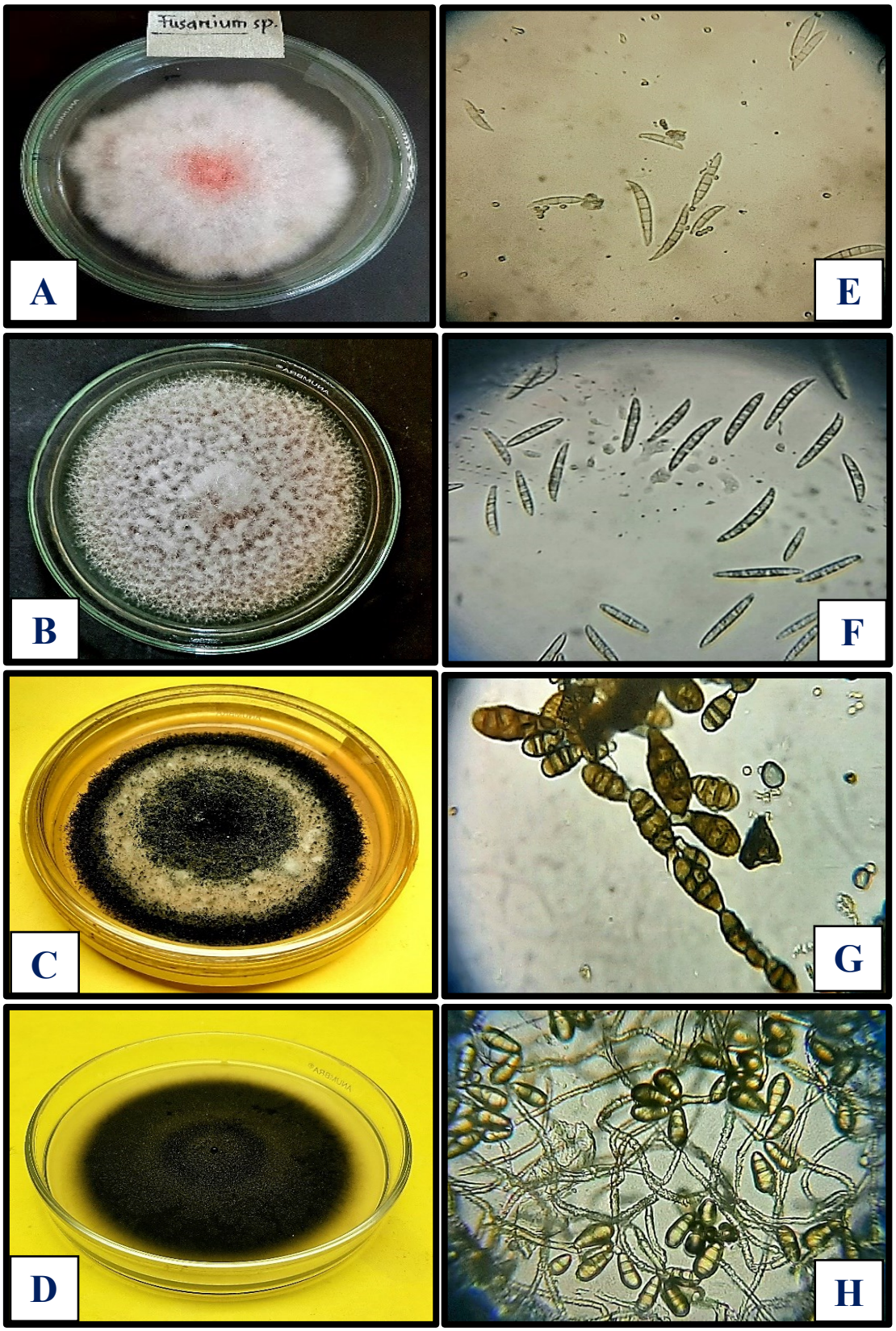


Plate 18. Morphology of the identified fungus from the samples; A, B, C and D are the pure cultures of *Fusarium oxysporum*, (Pink), *Fusarium* sp. (White), *Alternaria* sp. and *Curvularia* sp. respectively and E, F, G and H are the microscopic view (40X) of the respective fungi

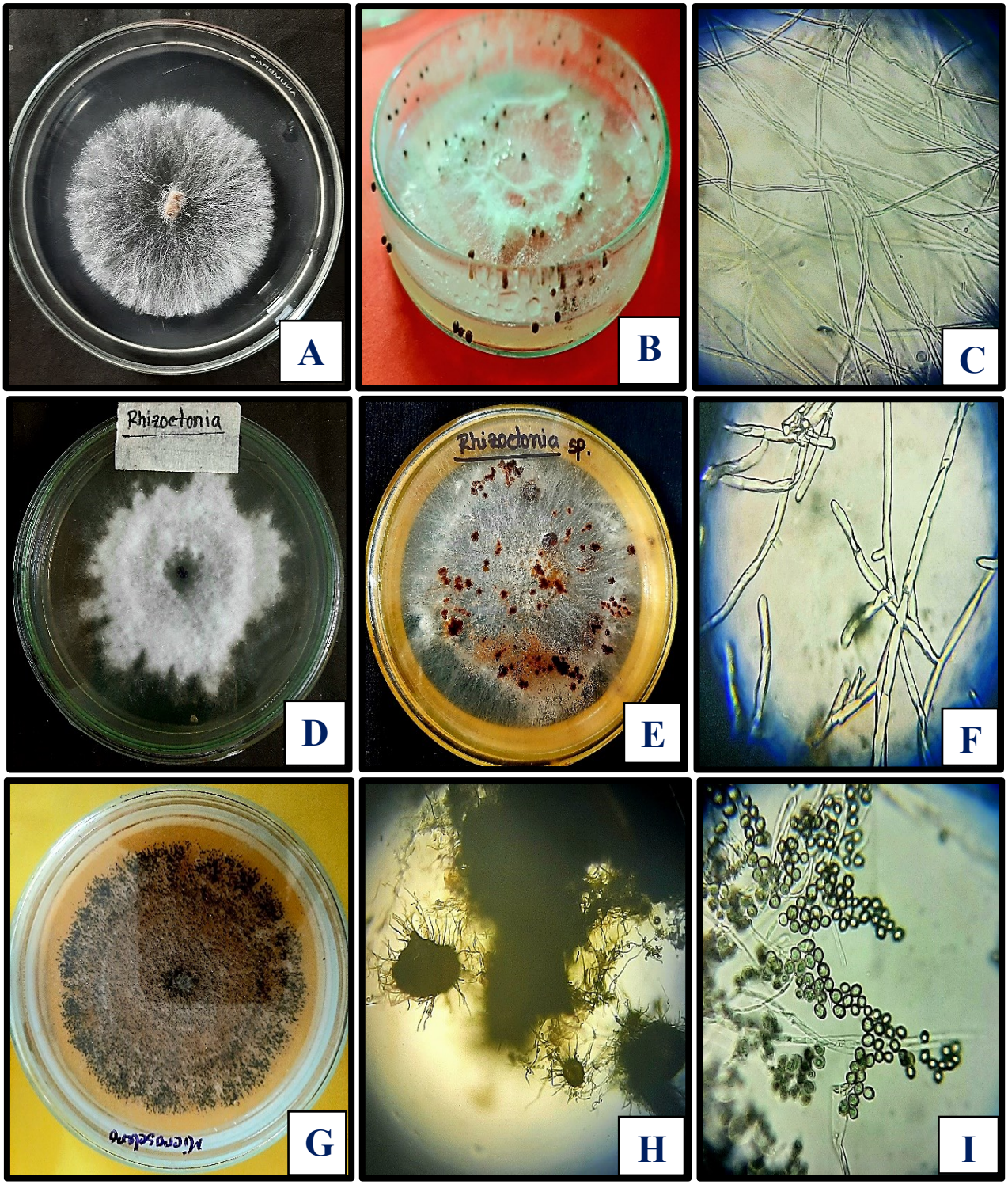


Plate 19. Morphology of the identified fungus from the samples; Here A, B, C are the Pure culture, mustard seed like sclerotia and compound microscopic view of septate mycelium of *Sclerotium* sp. respectively; D, E, F are the Pure culture, irregularly shaped sclerotia and compound microscopic view of mycelium forming right angles (90^0) of *Rhizoctonia* sp. respectively and G, H, I are the pure culture, microscopic view of the ascocarp (perithecium) and microscopic view of the ascospores of *Chaetomium* sp. respectively

4.8 Bio-efficacy test of the identified fungus and bacteria

This study demonstrates rhizosphere soil of different crop contain naturally occurring indigenous bacteria and fungi with antagonistic activities towards soil borne pathogenic fungi as effective bio control agents. Among the 15 identified fungal species in this study there were soil borne pathogenic fungi like *Sclerotium, Rhizoctonia* and *Fusarium oxysporum*. *Rhizoctonia* is the causal agent of stem and root rot of various plants also *Fusarium oxysporum* is the causal agent of Fusarium wilt (Wilhelm and Nelson, 1973). Also *Sclerotium* causes stem rot of vegetable and ornamental plants (Kator *et al.,* 2015). These are the devastating diseases of plants resulting in huge economic and nutritional loss. That must be brought under control. So against these pathogenic fungi the bio-efficacy of other identified bacteria and fungi was tested by dual culture technique (Table 11 and 12; Plate 20-24).

Table 11. Bio-efficacy of isolated fungi against *Sclerotium* sp. *Rhizoctonia* sp. and *Fusarium oxysporum* in dual culture method

Fungus	*Sclerotium* sp.			*Rhizoctonia* sp.			*Fusarium oxysporum*		
	Co (mm)	GD (mm)	ZI (%)	Co (mm)	GD (mm)	ZI (%)	Co (mm)	GD (mm)	ZI (%)
A. flavus	3.7	3.7	0	4	4	0	1.9	1.9	0
A. terreus	3.7	3.7	0	4	4	0	1.9	1.9	0
A. niger	3.7	3.7	0	4	4	0	1.9	1.9	0
***Alternaria* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Chaetomium* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Cunninghamela* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Curvularia* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
Fusarium oxysporum	3.7	3.7	0	4	4	0	1.9	1.9	0
***Fusarium* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Penicillium* sp**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Rhizoctonia* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
***Sclerotium* sp.**	3.7	3.7	0	4	4	0	1.9	1.9	0
T. hamatum	3.7	1.9	48.64	4	0.8	80	1.9	0.35	81.5
T. viride	3.7	0.4	89.18	4	1.5	62.5	1.9	0.56	70.52
T. harzianum	3.7	0.5	86.48	4	2	50	1.9	0.6	68.42

*Here GD= Growth on Dual culture plate after 7 Days, Co= Control plate after 7 Days, ZI= Zone of inhibition (%)

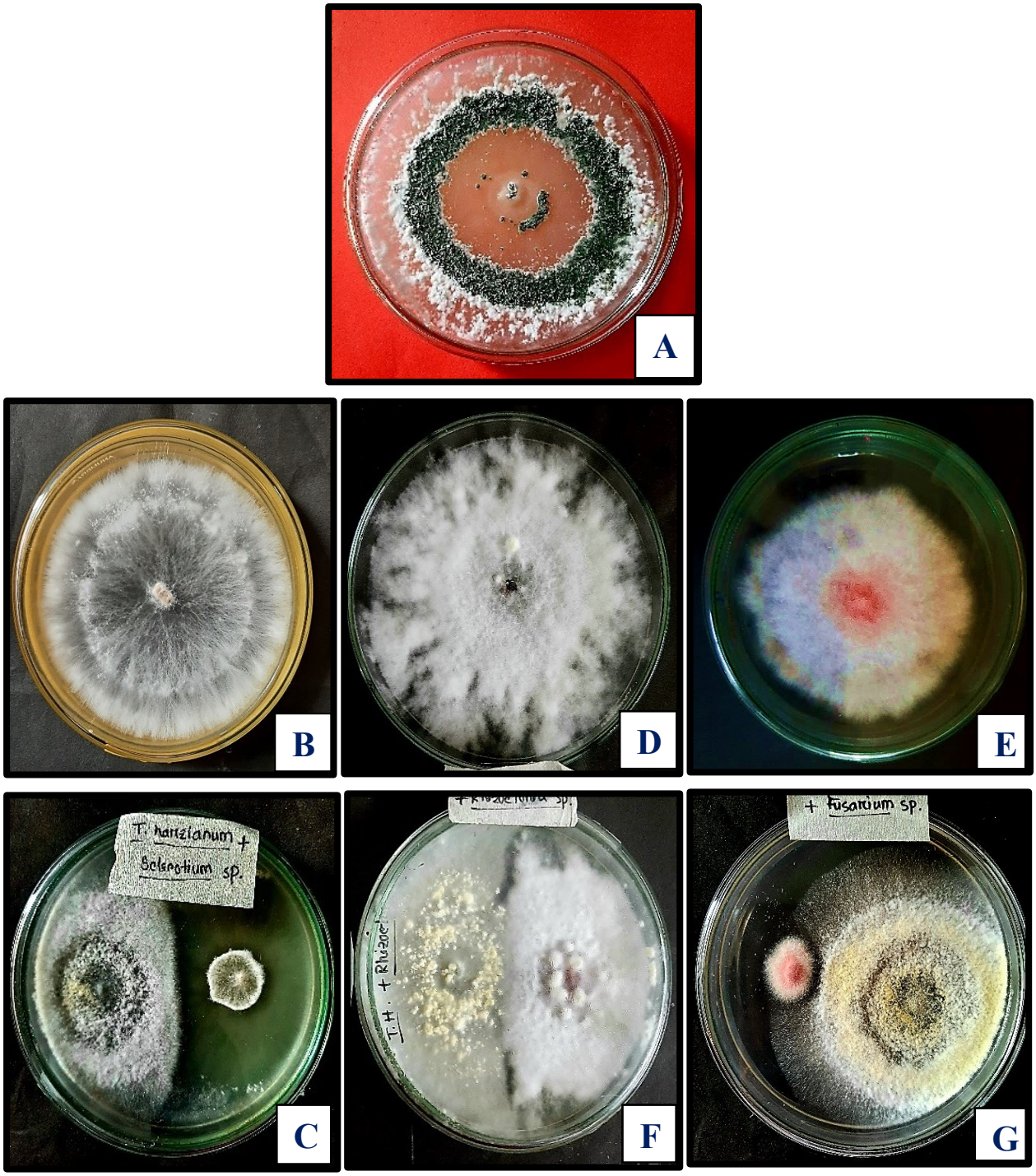


Plate 20. Bio-efficacy test of *Trichoderma harzianum* biocontrol activity, suppressing pathogenic fungi. A. Pure culture of *T. harzianum*, B. Pure culture of *Sclerotium* sp., C. *T. harzianum* suppressing *Sclerotium* sp., D. Pure culture of *Rhizoctonia* sp. E. *T. harzianum* suppressing *Rhizoctonia* sp. F. Pure culture of *Fusarium oxysporum*. G. *T. harzianum* suppressing *Fusarium oxysporum*

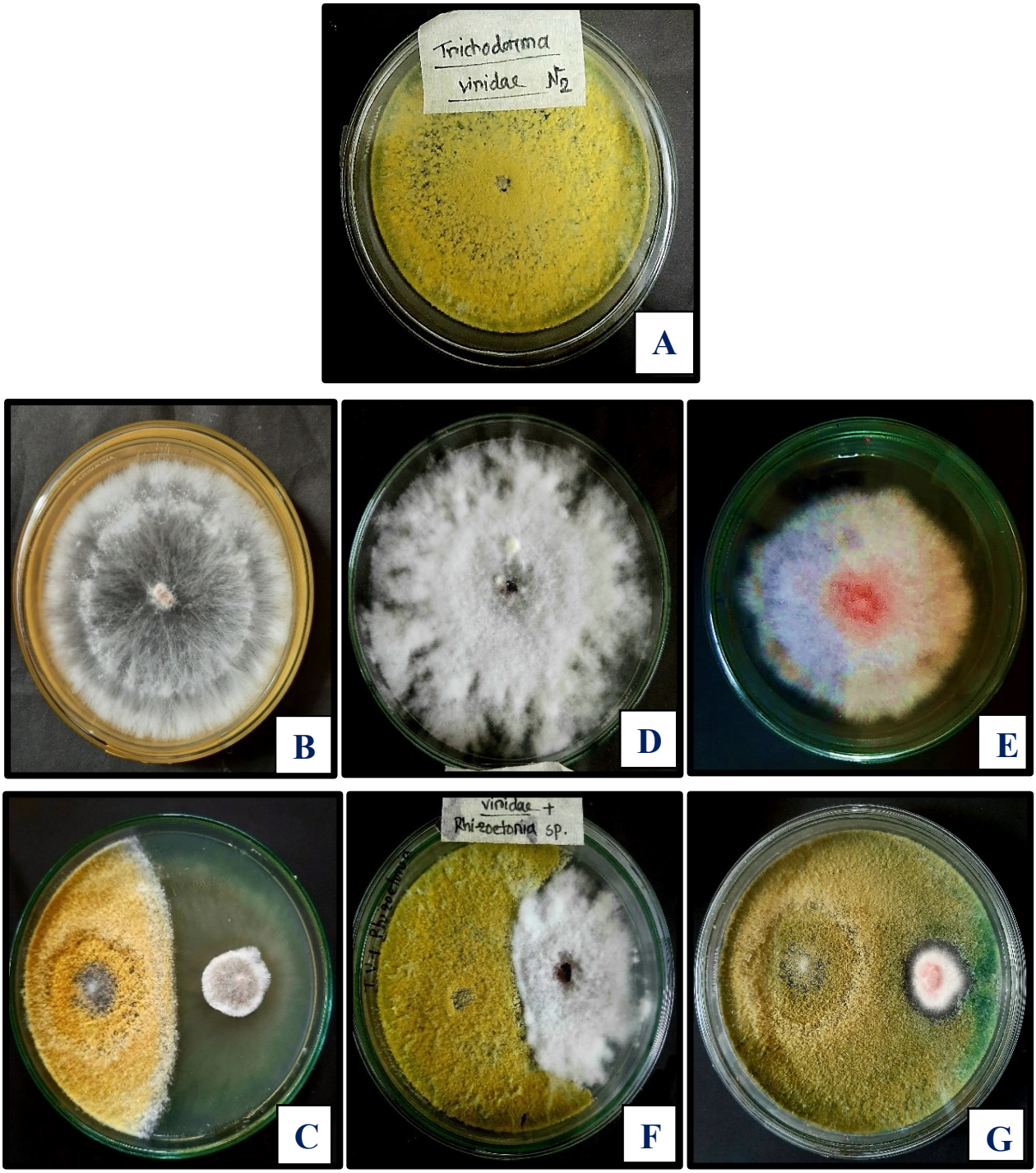


Plate 21. Bio-efficacy test of *Trichoderma viride* biocontrol activity, suppressing pathogenic fungi. A. Pure culture of *T. viride*, B. Pure culture of *Sclerotium* sp., C. *T. viride* suppressing *Sclerotium* sp., D. Pure culture of *Rhizoctonia* sp. E. *T. viride* suppressing *Rhizoctonia* sp. F. Pure culture of *Fusarium oxysporum*. G. *T. viride* suppressing *Fusarium oxysporum*

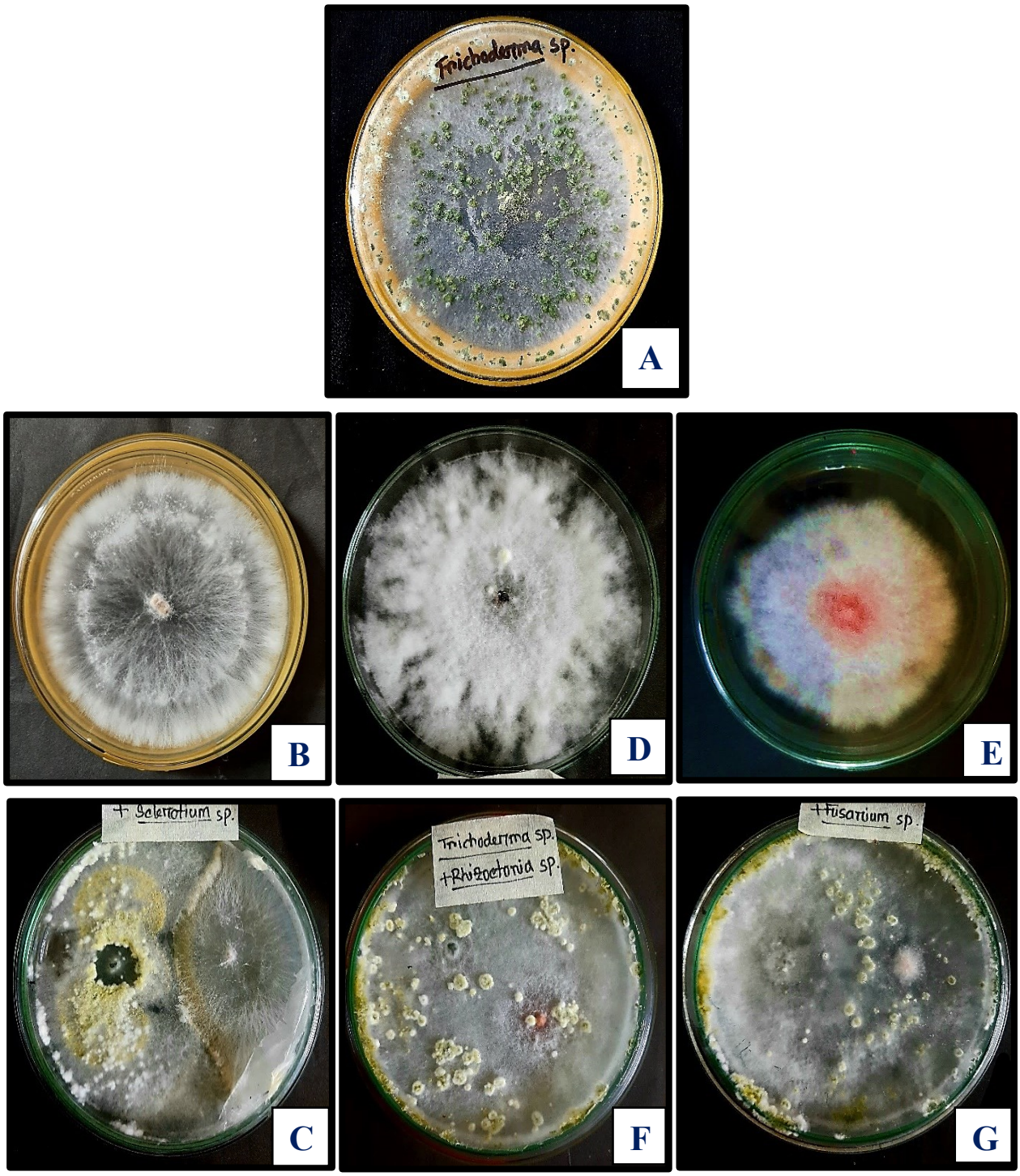


Plate 22. Bio-efficacy test of *Trichoderma hamatum* biocontrol activity, suppressing pathogenic fungi. A. Pure culture of *T. hamatum*, B. Pure culture of *Sclerotium* sp., C. *T. hamatum* suppressing *Sclerotium* sp., D. Pure culture of *Rhizoctonia* sp. E. *T. hamatum* suppressing *Rhizoctonia* sp. F. Pure culture of *Fusarium oxysporum*. G. *T. hamatum* suppressing *Fusarium oxysporum*

Table 12. Bio-efficacy of isolated bacteria against *Sclerotium* sp. *Rhizoctonia* sp. and *Fusarium oxysporum* in dual culture method

Bacteria	*Sclerotium* sp.			*Rhizoctonia* sp.			*Fusarium oxysporum*		
	Co (mm)	GD (mm)	ZI (%)	Co (mm)	GD (mm)	ZI (%)	Co (mm)	GD (mm)	ZI (%)
***Rhizobium* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Mesorhizobium* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Azospirillum* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Azotobacter* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Agrobacterium* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Streptomyces* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Burkholderia* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Xanthomonas* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Acidovorax* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Pectobacterium* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Erwinia* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Ralstonia* sp.**	3.7	3.7	0	4	4	0	3	3	0
Salinico roseus	3.7	3.7	0	4	4	0	3	3	0
Pseudomonas aeruginosa	3.7	1.4	62.16	4	1.8	55	3	1.2	60
Bacillus cereus	3.7	1	72.98	4	1.2	70	3	1	66.67
***Bacillus* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Lactobacillus* sp.**	3.7	3.7	0	4	4	0	3	3	0
***Thermomonospora* sp.**	3.7	3.7	0	4	4	0	3	3	0
E. coli	3.7	3.7	0	4	4	0	3	3	0

*Here GD= Growth on Dual culture plate after 7 Days, Co= Control plate after 7 Days, ZI= Zone of inhibition (%)

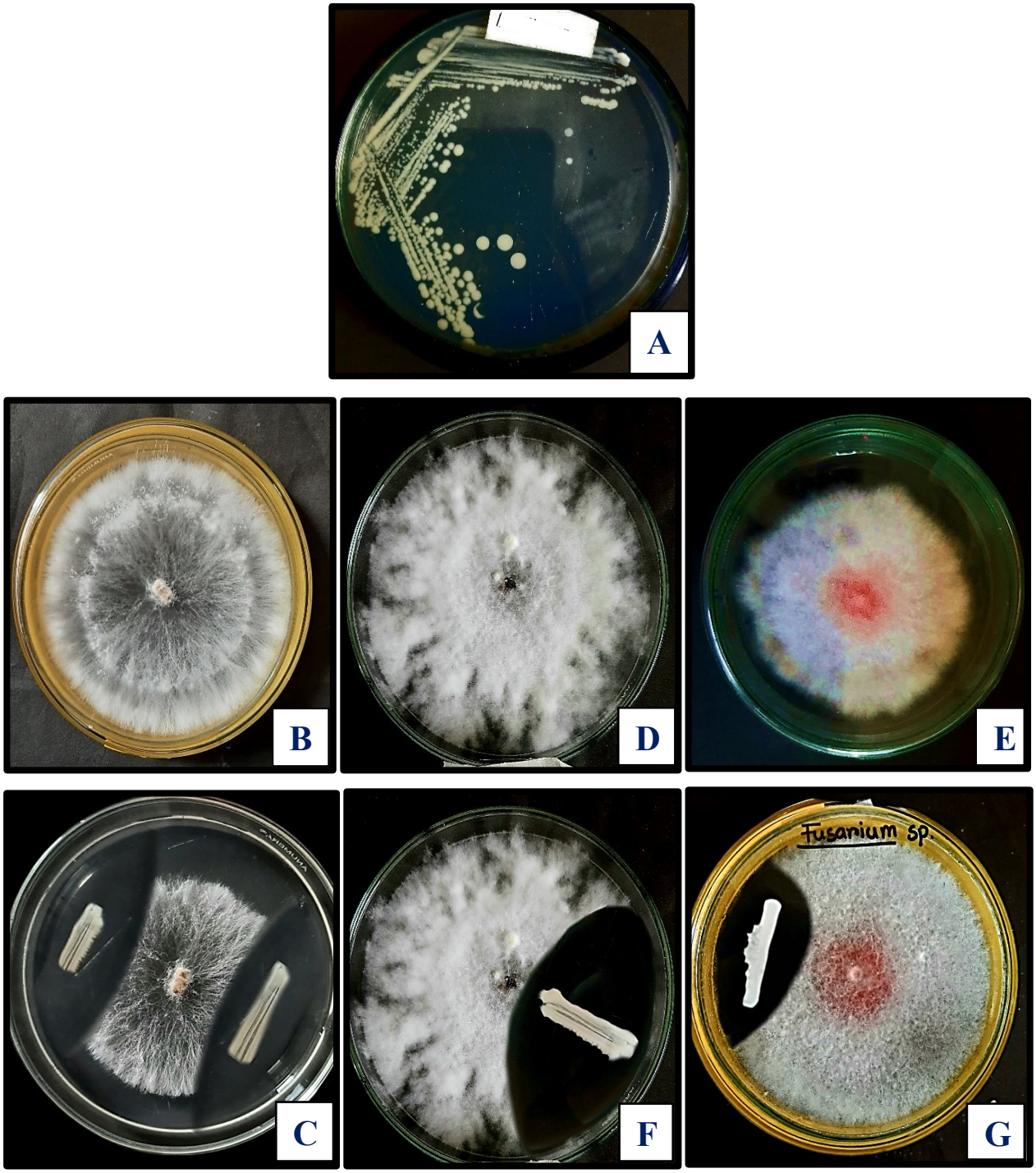


Plate 23. Bio-efficacy test of *Bacillus cereus* biocontrol activity, suppressing pathogenic fungi. A. Pure culture of *Bacillus cereus*, B. Pure culture of *Sclerotium* sp., C. *B. cereus* suppressing *Sclerotium* sp., D. Pure culture of *Rhizoctonia* sp. E. *B. cereus* suppressing *Rhizoctonia* sp. F. Pure culture of *Fusarium oxysporum*. G. *B. cereus* suppressing *Fusarium oxysporum*

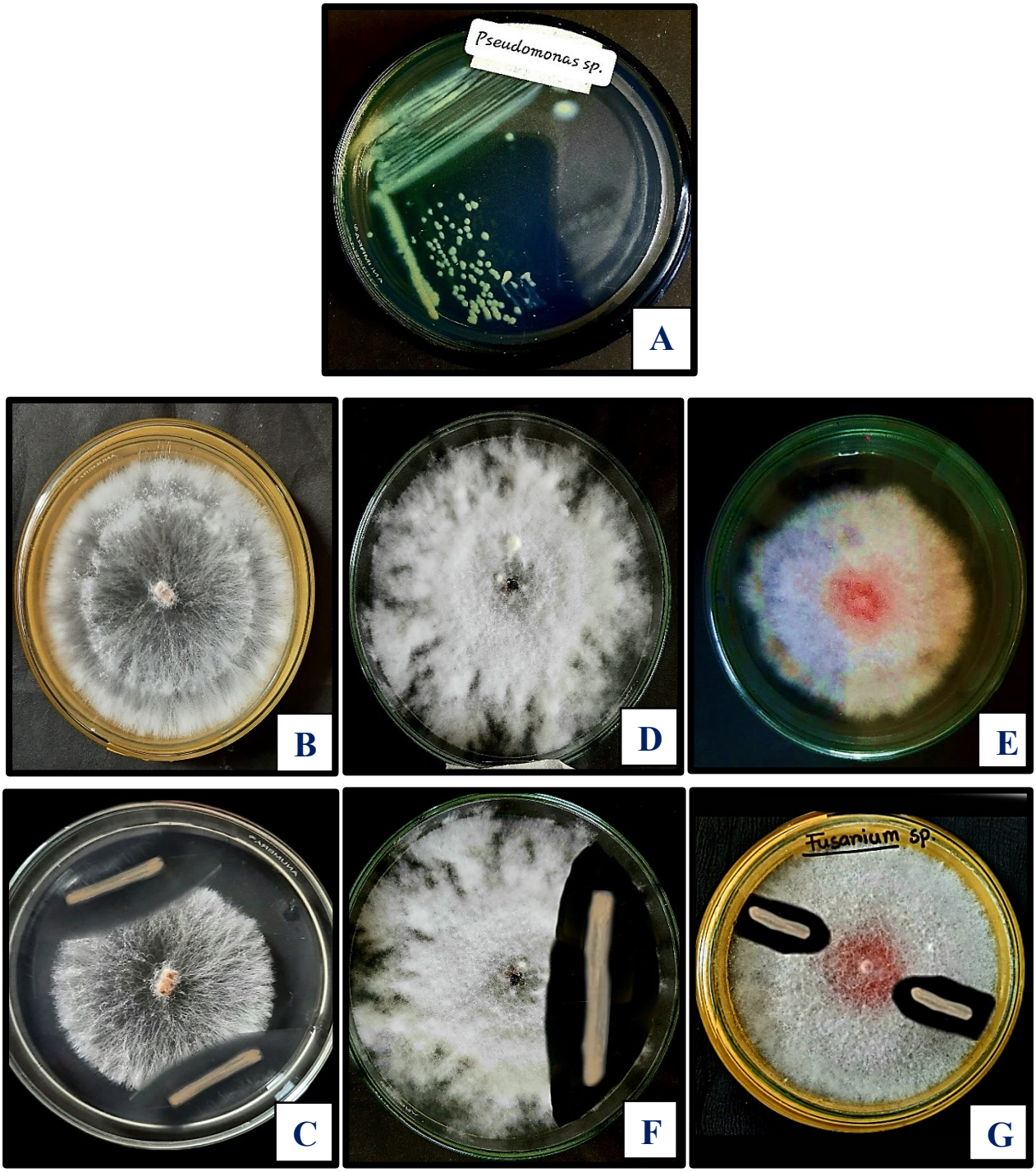


Plate 24. Bio-efficacy test of *Pseudomonas aeruginosa*. Bio-control activity, suppressing pathogenic fungi. A. Pure culture of *Pseudomonas aeruginosa*, B. Pure culture of *Sclerotium* sp., C. *Pseudomonas aeruginosa* suppressing *Sclerotium* sp., D. Pure culture of *Rhizoctonia* sp. E. *Pseudomonas aeruginosa* suppressing *Rhizoctonia* sp. F. Pure culture of *Fusarium oxysporum*. G. *Pseudomonas aeruginosa* suppressing *Fusarium oxysporum*

After analysis it was found that only the species of *Trichoderma, Bacillus cereus* and *Pseudomonas aeruginosa* were able to inhibit the growth of soil borne pathogenic fungi. In the rhizosphere *Trichoderma* is a dominating fungi as it works as the bio control agents. In this study *Trichoderma, Bacillus cereus* and *Pseudomonas aeruginosa* species were the identified bio agents from rhizosphere and substrate. They suppressed the growth of the disease causing fungi *Sclerotium, Rhizoctonia* and *Fusarium oxysporum*. The inhibition percentage for the isolated *Trichoderma viride* was 89.18%, 62.5% and 70.52% on *Sclerotium, Rhizoctonia, Fusarium oxysporum,* respectively which was higher than *Trichoderma harzianum* that showed 86.48%, 50% and 68.42% inhibition on the same target. On the other hand *Trichoderma hamatum* showed 48.64%, 80% and 81.5% inhibition against *Sclerotium*, *Rhizoctonia* and *Fusarium oxysporum,* respectively. In case of Bacteria, *Bacillus cereus* showed 72.98%, 70% and 66.67% inhibition on *Sclerotium, Rhizoctonia, Fusarium oxysporum* respectively which was higher than *Pseudomonas aeruginosa* that showed 62.16%, 55% and 60% inhibition on similar target. *Trichoderma* sp. *Bacillus cereus* and *Pseudomonas aeruginosa* are the strong biocontrol agents in the rhizosphere (Bastakoti *et al.,* 2017; Ramírez *et al.,* 2022; Wang *et al.,* 2020). It has been found that *Bacillus cereus* has antagonistic activity that reduces the soil borne pathogenic (Banerjee *et al.,* 2018). Also Pseudomonas aeruginosa is a potential suppressor on soil borne microorganisms and has shown effective results before (Durairaj *et al*., 2017). And the present study also declares *Trichoderma* sp. (*T. harzianum, T. viride* and *T. hamatum*), *Bacillus cereus* and *Pseudomonas aeruginosa* are the efficient bio agents.

CHAPTER V

SUMMARY AND CONCLUSION

The present study was performed to isolate, identify and characterize the indigenous bio controlling fungus and bacteria associated with rhizosphere soil of different crops and mushroom substrate. The experiment was conducted in the MS laboratory of Department of the Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period from July 2021 to June 2022. The rhizosphere soil samples were collected from Dhaka (Cabbage and Quinoa), Gazipur (BRRI dhan28 and 29), Manikganj (Mustard and Tomato), Noakhali (Wheat and Soybean), Joypurhat (Okra and Chilli) and Rajshahi (Lentil and Brinjal) on the other hand the mushroom substrate was collected from Mushroom Development Institute, Savar (Oyester mushroom). 59 bacteria were isolated from the rhizosphere and mushroom substrate in this study where they were grown by dilution plate and streak plate method on NA media. Then they were taken to perform 10 different biochemical tests viz. Gram stain, KOH solubility test, catalase test, oxidase test, motility test, Simmon's citrate agar test, Levan production test, casein hydrolysis test, starch hydrolysis test and gelatin liquefaction test. For species confirmation of some strains, bacteria were grown in some selective and differential media. After all these procedures 19 bacterial strains were identified viz. *Rhizobium sp., Mesorhizobium* sp*., Azospirillum* sp*., Azotobacter* sp*., Agrobacterium* sp*., Streptomyces* sp*., Burkholderia* sp*., Xanthomonas* sp*., Acidovorax* sp*., Pectobacterium* sp. *Erwinia* sp*., Ralstonia* sp*., Salinico roseus, Pseudomonas* sp*., Bacillus* sp*., Bacillus cereus Lactobacillus* sp*., Thermomonospora* sp. and *E. coli*. At the same time 54 fungi were isolated from the same sample by dilution plate method growing on PDA media. By studying their morphology and microscopic characteristics 14 fungal genera were identified viz. were *Sclerotium* sp*., Rhizoctonia* sp*., Cunninghamela* sp*., Chaetomium* sp*., Alternaria* sp*., Curvularia* sp*., Penicillium* sp., *Trichoderma harzianum, T. viride, T. hamatum, Aspergillus niger, A. flavus, A. terreus*, *Fusarium oxysporum and Fusarium sp.* The results indicate that rhizosphere soil and mushroom substrate can be a potential source of bacterial and fungal occurrence and contamination.

After isolation, identification and characterization of these bacteria and fungi their bio efficacy was tested against 3 soil borne pathogenic fungi *Sclerotium* sp. *Rhizoctonia* sp. and *Fusarium oxysporum* by Dual culture technique. Among these microorganisms *Trichoderma harzianum, T viride, T. hamatum Bacillus cereus, Pseudomonas aeruginosa* showed strong bio control activity against *Sclerotium, Rhizoctonia* and *Fusarium oxysporum*. From each location bio control agents were found. They suppressed the growth of the disease causing fungi *Sclerotium* sp., *Rhizoctonia* sp. and *Fusarium oxysporum*. In case of fungi the inhibition of *Sclerotium* sp. was 86.48% by *T. harzianum*, 89.18% by *T. viride and* 48.64 % by *T. hamatum*; inhibition of *Rhizoctonia* sp. was 50% by *T. harzianum*, 62.5% *by T. viride* and 80% by *T. hamatum*; and the inhibition of *Fusarium oxysporum* was 68.42% by *T. harzianum*, 70.52% by *T. viride and* 81.5%. by *T. hamatum.* In case of bacteria the inhibition of *Sclerotium* sp. by *Bacillus cereus* was 72.98% and by *Pseudomonas aeruginosa* was 62.16%.; inhibition of *Rhizoctona* sp. *by Bacillus cereus* was 70% and by *P. aeruginosa* was 50%; inhibition of *Fusarium oxysporum* by *Bacillus cereus* was 66.67% and by *P. aeuginosa* was 60%. So the present study declares that *Trichoderma* sp. (*T. harzianum, T. viride* and *T. hamatum*), *Bacillus cereus* and *Pseudomonas aeruginosa* are the efficient bio agents.

Potential indegenous bio control agents from the crop rhizosphere and mushroom substrate were isolated, identified and characterize in this study, which were *Trichoderma harzianum, T. viride, T. hamatum*, *Bacillus cereus* and *Pseudomonas aeruginosa.* Their bio-efficacy against soil borne pathogenic fungi was commendable. Further studies are needed to assess the potential of these isolates to be used as an inoculant for bio control of pathogenic fungi in soil. These are able to reduce the infestation of disease causing agents. So, if we can multiply and incorporate the bio agents into our growth media it can be huge for organic production, which will prevent our environment from being ruined. It will increase our production and economy as well.

CHAPTER VI

REFERENCES

Aarti, T and Meenu, S. (2015). Role of volatile metabolites from *Trichoderma citrinoviride* in biocontrol of phytopathogens. *Int. J. Res. Chem. Environ.* **5**: 86-95.

Abdel-Azeem, A. M., Salem, F. M., Abdel-Azeem, M. A., Nafady, N. A., Mohesien, M. T., and Soliman, E. A. (2016). Biodiversity of the genus Aspergillus in different habitats. In New and future developments in microbial biotechnology and bioengineering. 3-28. Elsevier.

Abd-Elgawad, M.M.M. and Askary, T.H. (2020). Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. *Egypt. J. Biol. Pest Control*. **30**: 17.

Abriouel, H., Franz, C.M.A.P., Omar, N.B. and Gálvez, A. (2011). Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiol. Rev.* **35**: 201–232.

Ahemad, M., and Khan, M. S. (2012). Effects of pesticides on plant growth promoting traits of *Mesorhizobium* strain MRC4. *J. Saudi Society Agril. Sci*. **11**(1): 63-71.

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

Ajit, N. S., Verma, R., & Shanmugam, V. (2006). Extracellular chitinases of fluorescent pseudomonads antifungal to Fusarium oxysporum f. sp. dianthi causing carnation wilt. *Current Microbiology*. **52:** 310-316.

Ali, H. F., Bibi, A., Ahmad, M., Junaid, M., Ali, A., Hussain, S. and Alam, S. A. R. T. A. J. (2014). Characterization of the causal organism of blackleg and soft rot of potato and management of the disease with balanced fertilization. *Pak. J. Bot*. **46**(6): 2277-2284.

Alinejad, F., Shahryari, F., Eini, O., Sarafraz-Niko, F., Shekari, A., and Setareh, M. (2020). Screening of quorum-quenching bacteria associated with rhizosphere as biocontrol agents of *Pectobacterium carotovorum* subsp. c*arotovorum*. Archives of Phytopathology and Plant Protection. **53**(11-12): 509-523.

Alippi, A. M. and López, A. C. (2009). First Report of *Pectobacterium carotovorum* subsp. *carotovorum* on *Spathiphyllum wallisii* in Argentina. *Plant Disease*. **93**(8): 842-842.

Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. (2014). Microbes associated with freshly prepared juices of citrus and carrots. *Int. J. Food Sci. 2014*.

Angelopoulou, D.J., Naska, E.J., Paplomatas, E.J. and Tjamos, S.E. (2014). Biological control agents (BCAs) of verticillium wilt: Influence of application rates and delivery method on plant protection, triggering of host defence mechanisms and rhizosphere populations of BCAs. *Plant Pathol.* **63**: 1062–1069.

Anita Puyam. (2016). Advent of Trichoderma as a bio-control agent. *J. Appl. Natural Sci.* **8** (2): 1100 – 1109.

Arthurs, S. and Dara, S.K. (2019). Microbial biopesticides for invertebrate pests and their markets in the United States. *J. Invertebr. Pathol. 165*: 13–21.

Bahar, O., De La Fuente, L., and Burdman, S. (2010). Assessing adhesion, biofilm formation and motility of *Acidovorax citrulli* using microfluidic flow chambers. *FEMS Microbiol. Letters*. **312(**1): 33-39.

Bastakoti, S., Belbase, S., Manandhar, S., & Arjyal, C. (2017). Trichoderma species as biocontrol agent against soil borne fungal pathogens. *Nepal J. Biotechnol.* **5**(1): 39-45.

Bcere levan- Rajesh, M., Samundeeswari, M., and Archana, B. (2017). Available online at Isolation of Biosurfactant Producing Bacteria from Garbage Soil. *J. Appl. Environ. Microbiol.* **5**(2): 74-78

Benítez, T., Rincon, A.M., Limon, M.C. and Codon, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.* **7**: 249-260.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* **84**: 11–18.

Berg, G. and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* **68**: 1-13.

Berg, G., Eberl, L., and Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ. microbiol.* **7**(11): 1673-1685.

Berini, F., Katz, C., Gruzdev, N., Casartelli, M., Tettamanti, G.and Marinelli, F. (2018). Microbial and viral chitinases: Attractive biopesticides for integrated pest management. *Biotechnol. Adv*. **36:** 818–838.

Boyd, L.A., Ridout, C., O'Sullivan, D.M., Leach, J.E. and Leung, H. (2013). Plant-pathogen interactions: Disease resistance in modern agriculture. *Trends Genet*. **29**: 233–240.

Calderón, C.E., Pérez-García, A., de Vicente, A. and Cazorla, F.M. (2013). The dar genes of *Pseudomonas chlororaphis* PCL1606 are crucial for biocontrol activity via production of the antifungal compound 2-hexyl, 5-propyl resorcinol. *Mol. Plant Microbe Interact.* **26**: 554–565.

Calvo, P., Ormeño-Orrillo, E., Martínez-Romero, E. and Zúñiga, D. (2010). Characterization of Bacillus isolates of potato rhizosphere from andean soils of Peru and their potential PGPR characteristics. *Braz J Microbiol*. **41**(4): 899-906.

Cavalcante, R.S., Lima, H.L.S., Pinto, G.A.S., Gava, C.A.T. and Rodrigues, S. (2008). Effect of moisture on *Trichoderma* conidia production on corn and wheat bran by solid state fermentation. *Food Bioprocess Technol.* **1**: 100-104.

Chadha, N., Prasad, R. and Varma, A. (2015). Plant promoting activities of fungal endophytes associated with tomato roots from central himalya, India and their interaction with *Piriformosporaindica*. *Int. J. Pharma Bio Sci.* **6**: 333 – 343.

Chakraborty, A.P, Chakraborty, B.N. and Chakraborty, U. (2015). *Bacillus megaterium* from tea rhizosphere promotes growth and induces systemic resistance in tea against *Sclerotium rolfsii*. *Indian Phytopath*. **68**(3): 237-247.

Chin-A-Woeng, T.F., Bloemberg, G.V. and Lugtenberg, B.J.J. (2003). Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol.* **157**: 503–523.

Chowdhury, S.P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, R. and Hartmann, A. (2015). Cyclic Lipopeptides of *Bacillus amyloliquefaciens* subsp. *Plantarum* colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen *Rhizoctonia solani*. *Mol. Plant Microbe Interact.* **28**:984–995.

Ciancio, A., Pieterse, C.M.J. and Mercado-blanco, J. (2019). Editorial: Harnessing Useful Rhizosphere Microorganisms for Pathogen and Pest Biocontrol—Second Edition. *Front. Microbiol.* **10**: 1935.

Cinisli, K. T., Kiliç, S. M., Uçar, S., & Canca, E. (2019). Isolation of *Pectobacterium Carotovorum*, Identification With 16s Rrna, Phytase Activity And Characterization Of The Bacteria.

Collinge, D.B., Jensen, D.F., Rabiey, M., Sarrocco, S., Shaw, M.W. and Shaw, R.H. (2022). Biological control of plant diseases—What has been achieved and what is the direction? *Plant Pathol*. **71**: 1024–1047.

Compant, S., Duffy, B., Nowak, J., Clément, C. and Barka, E. (2005) Biocontrol of plant diseases using plant growth-promoting bacteria (PGPB): Principles, mechanisms of action and future prospects. *Appl. Environ. Microbiol.* **71**: 4951–4959.

Coombs, J. T., and Franco, C. M. (2003). Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Appl. Environ. Microbiol.* **69**: 5603–5608.

Coulibaly, A. E., Pakora, G. A., Ako, A. B. A., Dadé, G. E. L. N. O., N'Guessan, C. A., Kouabenan, A., and Djaman, J. A. (2022). Diversity of Sclerotium rolfsii antagonist fungi isolated from soils of the rhizosphere of tomato crops and identification of some antifungal compounds. *Heliyon*. **8**(2). e08943.

Du, E., Chen, Y., Li, Y., Sun, Z., & Gui, F. (2022). Rhizosphere Bacillus-facilitated effects on the growth and competitive ability of the invasive plant Ageratina adenophora. *Front. Plant Sci*. **13**

Dubey, S.C., Suresh, M. and Singh, B. (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. *Biol. Cont.* **40**: 118-127.

Duffy, B., Keel, C. and Defago, G. (2004). Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. *Appl. Env. Microbiol.* **70**(3): 1836-1842.

Dufour, M. C., Fontaine, S., Montarry, J., & Corio-Costet, M. F. (2011). Assessment of fungicide resistance and pathogen diversity in Erysiphe necator using quantitative real-time PCR assays. *Pest Manage. Sci.* **67**(1), 60-69.

Duijff, B.J., Bakker, P.A.H.M. and Schippers, B. (1994). Suppression of fusarium wilt of carnation by Pseudomonas putida WCS358 at different levels of disease incidence and iron availability. *Biocontrol. Sci. Technol*. **4**:279–288.

Dusengemungu, L. (2021). Fusarium Soilborne Pathogen. In *Fusarium-An Overview of the Genus*. IntechOpen.

Elad, Y., Chet, I. and Katan, J., (1980). Trichoderma harzianum: A biocontrol agent of Sclerotium rolfsii and Rhizoctonia solani. *Phytopathol*. **70**: 119-121.

Ellis, R.J., Timms-Wilson, T.M., Beringer, J.E., Rhodes, D., Renwick, A., Stevenson, L. and Bailey, M.J. (1999). Ecological basis for biocontrol of damping-off disease by *Pseudomonas fluorescens* 54/96. *J. Appl. Microbiol.* **87**:454–463.

Eram, S. M., Behzadiannezhad, G., Khatami, G. R., & Nafissi, N. (2004). Detection of *Burkholderia cepacia* complex in patients with cystic fibrosis.

Ferreira, F.V. and Musumeci, M.A. (2021). *Trichoderma* as biological control agent: scope and prospects to improve efficacy. *World J. Microbiol Biotechnol*. **37**: 90

Fishman, L. D. (1984). *The effect of commercial starch blockers on starch hydrolysis in vitro* (Master's thesis, California State University, Northridge).

Fontana, P. D., Rago, A. M., Fontana, C. A., Vignolo, G. M., Cocconcelli, P. S., and Mariotti, J. A. (2013). Isolation and genetic characterization of *Acidovorax avenae* from red stripe infected sugarcane in Northwestern Argentina. *European J. Plant Path.***137**: 525-534.

Ghosh, K., Sen, S. K., and Ray, A. K. (2002). Characterization of Bacilli isolated from the gut of rohu, Labeo rohita, fingerlings and its significance in digestion. *J. Appl.Aqua.* **12**(3): 33-42.

Gopalakrishnan, S., Humayun, P., Kiran, B. K., Kannan, I. G. K., Vidya, M. S., Deepthi, K., & Rupela, O. (2011). Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. *World J. Microbiol. Biotechnol. 27*, 1313-1321.

Gupta, N., Mishra, S., and Basak, U. C. (2009). Occurrence of Different Morphotypes of *Streptomyces exfoliatus* in Mangrove Ecosystems of Bhitarkanika, Orissa, India. *J. Culture Collections.* **6**(1): 21-27.

Hadi, H. W. (2019). Genetic study on *Bacillus cereus. Res. J. Pharm. Tech.* **12**(3): 1301-1306**.**

Holbrook, R., and Anderson, J. (1980). An improved selective and diagnostic medium for the isolation and enumeration of *Bacillus cereus* in foods. *Can. J. Microbiol*. **26**(7): 753-759

Hossain, N., and & Rahman, M. (2014). Antagonistic activity of antibiotic producing *Streptomyces* sp. against fish and human pathogenic bacteria. *Brazilian Arch. Biol. Tech.* **57**: 233-237.

Hyakumachi, M., Nishimura, M., Arakawa, T., Asano, S., Yoshida, S., Tsushima, S., and Takahashi, H. (2013). *Bacillus thuringiensis* suppresses bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. *Microbes Environ*. **28**(1):128-34.

Islam, R., Imran, M. A. S., and Mahmud, S. N. (2020). Isolation, Identification and Charact *Cicer Arietinum*. *J. Curr. Res.* **12**(4): 10322-10325.

Islam, S., Akanda, M.A., Prova, A., Islam, M.T., and Hossain, M.M. (2016). Isolation and Identification of Plant Growth Promoting Rhizobacteria from Cucumber Rhizosphere and Their Effect on Plant Growth Promotion and Disease Suppression. *Front. Microbiol*. **6.**

Jain, A., Chatterjee, A., and Das, S. (2020). Synergistic consortium of beneficial microorganisms in rice rhizosphere promotes host defense to blight-causing *Xanthomonas oryzae* pv. *oryzae*. *Planta*. **252**: 1-25.

Jambenal, S., Ravikumar, M. R., and Hiremani, N. (2011). Basic studies on *Xanthomonas campestris.* Pv. *viticola* causing bacterial leaf spot of grape and evaluated in-vitro efficacy of different chemicals and bioagents against its growth. *Int. J. Plant Protection*. **4**(2): 397-401.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M., Coleman, D.C. and Whitman, W.B. (2008). Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biol. Biochem*. **40**: 2843-2853.

Jetiyanon, K., & Kloepper, J. W. (2002). Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological control*. **24**(3): 285-291.

Kale, G.J., Rewale, K.A., Sahane, S.P. and Magar, S.J. (2018) Isolation of *Trichoderma* spp. from the rhizosphere soils of tomato crop grown in Marathwada region. *J. Pharmacognosy Phytochem.* **7**(3): 3360-3362.

Kamilova, F., Leveau, J. H., & Lugtenberg, B. (2007). *Collimonas fungivorans*, an unpredicted in vitro but efficient in vivo biocontrol agent for the suppression of tomato foot and root rot. *Environ. Microbiol.* **9**(6): 1597-1603.

Kasa, P., Modugapalem, H., and Battini, K. (2015). Isolation, screening, and molecular characterization of plant growth promoting rhizobacteria isolates of *Azotobacter* and *Trichoderma* and their beneficial activities. *J. Natural Sci. Biol. Med.* **6**(2): 360.

Kator, L., Hosea, Z. Y. and Oche, O. D. (2015). *Sclerotium rolfsii*: Causative organism of southern blight, stem rot, white mold and sclerotia rot disease. *Annals Biol. Res*. **6**(11): 78-89.

Kazerooni, E. A., Maharachchikumbura, S. S., Al-Sadi, A. M., Rashid, U., Kim, I. D., Kang, S. M., and Lee, I. J. (2022). Effects of the rhizosphere fungus

Cunninghamella bertholletiae on the *Solanum lycopersicum* response to diverse abiotic stresses. *Int. J. Mol. Sci.* **23**(16): 8909.

Khabbaz, S. E., & Abbasi, P. A. (2014). Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Canadian J. microbiol.* **60**(1): 25-33.

Khan, A., and Rao, T. S. (2019). Molecular evolution of xenobiotic degrading genes and mobile DNA elements in soil bacteria. Microbial diversity in the genomic era. 657-678. Academic Press.

Kim, H. S., Cheon, W., Lee, Y., Kwon, H. T., Seo, S. T., Balaraju, K., & Jeon, Y. (2021). Identification and characterization of *Xanthomonas arboricola* pv. *juglandis* causing bacterial blight of walnuts in Korea. *The Plant Pathology Journal*. **37**(2): 137.

King, E.O., Ward, M.K., and Raney, E.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *J Lab Clin Medicine*. **44**: 301.

Kloepper, J. W., & Beauchamp, C. J. (1992). A review of issues related to measuring colonization of plant roots by bacteria. *Canadian J. microbial.* **38**(12): 1219-1232.

Kogel, K.H., Franken, P. and Hückelhoven, R. (2006). Endophyte or parasite – what decides? *Curr. Opin. Plant Biol*. **9**: 358–363.

Köhl, J., Kolnaar, R. and Ravensberg, W.J. (2019). Mode of Action of Microbial Biological Control Agents against Plant Diseases: Relevance Beyond Efficacy. *Front. Plant Sci.* **10:** 845.

Kushwaha, A., Baily, S. B., Maxton, A., and Ram, G. D. (2013). Isolation and characterization of PGPR associated with cauliflower roots and its effect on plant growth. *The Bioscan*. **8**(1): 95-99.

Kuzyakov, Y. and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept and review. *Soil Biol. Biochem.* **83**: 184–199.

Larekeng, S. H., Restu, M., Tunggal, A., and Susilowati, A. (2019, October). Isolation and identification of rhizosphere fungus under Mahoni (*Swietenia mahagoni*) stands and its ability to produce IAA (Indole Acetid Acid) hormones. In IOP

Conference Series: Earth and Environment Science. **343** (1): 012-051. IOP Publishing.

Levine, M (1918). Differentiation of *B. coli* and *B. aerogenes* on a simplified eosin-methylene blue agar. *J Infect. Dis*. **23**: 43–47

Li, E., Ling, J., Wang, G., Xiao, J., Yang, Y., Mao, Z., & Xie, B. (2015). Comparative proteomics analyses of two races of *Fusarium oxysporum* f. sp. *conglutinans* that differ in pathogenicity. *Scientific Reports*. **5**(1): 13663.

Liu, B., Ji, S., Zhang, H., Wang, Y. and Liu, Z. (2020). Isolation of Trichoderma in the rhizosphere soil of *Syringa oblata* from Harbin and their biocontrol and growth promotion function. *Microbiol. Res*. **235**:126445.

Liu, Y., He, P., He, P., Munir, S., Ahmed, A., Wu, Y., Yang, Y., Lu, J., Wang, J., Yang, J., Pan, X., Tian, Y. and He, Y. (2022). Potential biocontrol efficiency of *Trichoderma* species against oomycete pathogens. *Front Microbiol*. **13**:974024.

Lo, C.T., Nelson, E.B., Hayes, C.K. and Harman, G.E. (1998). Ecological Studies of Transformed *Trichoderma harzianum* Strain 1295-22 in the Rhizosphere and on the Phylloplane of Creeping Bentgrass. *Phytopath.* **88**: 129-136.

López-López, M.E., Del-Toro-Sánchez, C.L., Gutiérrez-Lomelí, M., Ochoa-Ascencio, S., Aguilar-López, J.A., Robles-García, M.A., Plascencia-Jatomea, M., Bernal-Mercado, A.T., Martínez-Cruz, O., Ávila-Novoa, M.G., González-Gómez, J.P. and Guerrero-Medina, P.J. (2022). Isolation and Characterization of *Trichoderma* spp. for Antagonistic Activity against Avocado (*Persea americana* Mill) Fruit Pathogens. *Horticulturae*. **8**: 714.

Lugtenberg, B., and Kamilova, F. (2009) Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol*. **63**: 541–556.

Ma, Z., Geudens, N., Kieu, N.P., Sinnaeve, D., Ongena, M., Martins, J.C. and Höfte, M. (2016). Biosynthesis, chemical structure, andstructure-activity relationship of orfamide lipopeptides produced by *Pseudomonas protegens* and related species. *Front. Microbiol*. **7**:382.

Marian, M. and Shimizu, M. (2019). Improving performance of microbial biocontrol agents against plant diseases. *J. Gen. Plant Pathol*. **85**: 329–336.

McSpaddenand Gardener, B.B. (2004). Ecology of *Bacillus* and *Paenibacillus* spp. In agricultural systems. *Phytopathology.* **94**:1252–1258.

Mendes, R., Kruijt, M., Bruijn, I., Dekkers, E., Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A. and Raaijmakers, J.M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science.* **332**(6033): 1097-1100.

Minerdi, D., Bossi, S., Gullino, M. L., & Garibaldi, A. (2009). Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of *Fusarium oxysporum* strain MSA 35. *Environ. Microbiology*. **11**(4): 844-854.

Mohanram, S. and Kumar, P. (2019). Rhizosphere microbiome: Revisiting the synergy of plant-microbe interactions. *Ann. Microbiol. 69*: 307–320.

Morrissey, J.P., Dow, J.M., Mark, G.L. and O'Gara, F. (2004). Are microbes at the root of a solution to world food production? *EMBO Rep*. **5**(10): 922-926.

Naher, L., Syawani, N., Amieza, N., Kamarudin, A.B. and Karim, S.M.R. (2019). *Trichoderma* species diversity in rhizosphere soils and potential antagonism with *Fusarium oxysporum*. *Biosci. J.* [online]. **35**(1):13–26.

Naz, I., Bano, A., Rehman, B., Pervaiz, S., Iqbal, M., Sarwar, A., and Yasmin, F. (2012). Potential of *Azotobacter vinelandii* Khsr1 as bio-inoculant. *African J. Biotech.* **11**(45): 10368-10372.

Ningthoujam D. S. and Shovarani N. (2008). Isolation and Characterization of a Pseudomonas aeruginosa Strain DN1 Degrading p-Nitrophenol. *Res. J. Microbiol*. **3**(5): 345-351

Nourozian, J., Etebarian, H. R., & Khodakaramian, G. (2006). Biological control of Fusarium graminearum on wheat by antagonistic bacteria. *Songklanakarin J. Sci. Technol*. **28**(Suppl 1): 29-38.

Omokaro, O., and Ogechi, A. A. (2013). Cultivation of mushroom (Pleurotus ostreatus) and the microorganisms associated with the substrate used. *E- J. Sci. Technol*. **8**(4).

Ondieki, D. K., Nyaboga, E. N., Wagacha, J. M., & Mwaura, F. B. (2017). Morphological and genetic diversity of Rhizobia nodulating cowpea (*Vigna unguiculata* L.) from agricultural soils of lower eastern Kenya. *Int. J. Microbiol.* 2017.

Ondieki, D. K., Nyaboga, E. N., Wagacha, J. M., and Mwaura, F. B. (2017). Morphological and genetic diversity of Rhizobia nodulating cowpea (*Vigna unguiculata* L.) from agricultural soils of lower eastern Kenya. *Int. J. microbial.*

Oso, S., Walters, M., Schlechter, R.O., Remus-Emsermann, M.N.P. (2019). Utilisation of hydrocarbons and production of surfactants by bacteria isolated from plant leaf surfaces. *FEMS Microbiol. Lett.* **366**: fnz061

Pandey, P., Kang, S. C., Gupta, C. P., & Maheshwari, D. K. (2005). Rhizosphere competent *Pseudomonas aeruginosa* GRC 1 produces characteristic siderophore and enhances growth of Indian mustard (*Brassica campestris*). *Current microbiology*. **51**: 303-309

Pattnaik, S. S., and Busi, S. (2019). Rhizosphere fungi: diversity and potential biotechnological applications. Recent Advancement in White Biotechnology Through Fungi:Diversity and Enzymes Perspectives. **1**: 63-84.

Pérez-García, A., Romero, D. and De Vicente, A. (2011). Plant protection and growth stimulation by microorganisms: Biotechnological applications of Bacilli in agriculture. *Curr. Opin. Biotechnol.* **22**: 187–193.

Pérez-Rodriguez, M.M., Piccoli, P. and Anzuay, M.S. (2020). Native bacteria isolated from roots and rhizosphere of *Solanum lycopersicum L*. increase tomato seedling growth under a reduced fertilization regime. *Sci Rep* .**10**:15642.

Pliego, C., de Weert, S., Lamers, G., de Vicente, A., Bloemberg, G.V., Cazorla, F.M., Ramos, C. (2008). Two similar enhanced root colonizing *Pseudomonas* strains differ largely in their colonization strategies of avocado roots and *Rosellinia necatrix* hyphae. *Environ. Microbiol.* **10**: 3295–3304.

Prapagdee, B., Kuekulvong, C., and Mongkolsuk, S. (2008). Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. *Int. J. Biol. Sci.* **4**: 330–337.

Priya, R., Balachandar, S., Murugesan, N. V., Prabhakaran, N., RadheshKrishnan, S. and Latha, Kb. (2022). Characterization and biocontrol potential of Trichoderma longibrachiatum TL-RD-01 against plant pathogens. Archives of Phytopathology and Plant Protection. **55**(18): 2111-2129

Raaijmakers, J.M., De Bruijn, I., Nybroe, O. and Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* **34**:1037–1062.

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., and Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil*. **321**: 341–361.

Rahman, M. F., Islam, M. R., Rahman, T., and Meah, M. B. (2010). Biochemical characterization of *Ralstonia solanacerum* causing bacterial wilt of brinjal in Bangladesh. *Progressive Agriculture*. **21**(1-2): 9-19.

Rahman, S., Ahmad, K., Ali, N., Idrees, M., & Ali, W. (2020). Isolation and characterization of *Agrobacterium tumefaciens* strains from malakander farm, University of Agriculture, Peshawar. *Pak. J. Zool.* **52**(5): 2019.

Ramanathan, A., Shanmugam, V., Raguchander, T. and Samiyappan, R. (2002). Induction of systemic resistance in ragi against blast disease caused by *Pseudomonas fluorescens*. *Ann. Plant Prot. Sci*. **10**:313-318.

Ramírez, V., Martínez, J., Bustillos-Cristales, M. D. R., Catañeda-Antonio, D., Munive, J. A., and Baez, A. (2022). *Bacillus cereus* MH778713 elicits tomato plant protection against Fusarium oxysporum. *J. Appl. Microbiol.* **132**(1). 470-482.

Rashid, M., Chowdhury, M. S. M., and Sultana, N. (2013). In-vitro screening of some chemicals and biocontrol agents against *Erwinia carotovora* subsp. *carotovora,* the causal agent of soft rot of potato (*Solanum tuberosum*). *The Agriculturists*. **11**(2): 1-9.

Rasool, L., Asghar, M., Jamil, A., and Rehman, S. U. (2015). Identification of *Azospirillum* species from wheat rhizosphere. *J. Animal Plant Sci*. **25**(4): 1081-86.

Rathore, P. (2014). Isolation, Biochemical Characterization and inoculation effect of *Azospirillum* on the growth of wheat. *Int. J. Sci. Res.* **3**(6), 626-628.

Rawat, R. and Tewari, L. (2011). Effect of abiotic stress on phosphate solubilisation by biocontrol fungus *Trichoderma* species. *Current Microbiology.* **62**: 1521-1526.

Rezzonico, F., Zala, M., Keel, C., Duffy, B., Moënne-Loccoz, Y. and Défago, G. (2007). Is the ability of biocontrol fluorescent pseudomonads to produce the antifungal metabolite 2, 4-diacetylphloroglucinol really synonymous with higher plant protection? *New Phytol.* **173**:861–872.

Robin, D.C. and Marchand, P.A. (2019). Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. *Pest. Manag. Sci.* 2019; 75:950–958. doi: 10.1002/ps.5199.

Rojas-Solís, D., Hernández-Pacheco, C. E., and Santoyo, G. (2016). Evaluation of *Bacillus* and *Pseudomonas* to colonize the rhizosphere and their effect on growth promotion in tomato (Physalis ixocarpa Brot. ex Horm.). *Revista Chapingo. Serie horticultura.* **22**(1): 45-58.

Roychowdhury, D., Paul, M., and Banerjee, S. K. (2015). Isolation identification and characterization of bacteria (*Rhizobium*) from chick pea (*Cicer arietinum*) and production of biofertilizer." *Eur J Biotechnol. Biosci.* **3**: 26.

Ruiu, L. (2018). Microbial Biopesticides in Agroecosystems. *Agronomy*. **8**: 235.

Sagar Aryal. (2022). Biochemical Test and Identification of *Pseudomonas aeruginosa* Microbiology info.com

Salar R.K., and Aneja K.R. (2007). Significance of thermophilic fungi in mushroom compost preparation: Effect on growth and yield of *Agaricus bisporus* (Lange) Sing. *J. Agric. Technol.* **3**:241–253.

Schippers, B., Bakker, A. W., & Bakker, P. A. (1987). Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annu. Rev. Phytopath.* **25**(1): 339-358.

Schreiter, S., Ding, G. C., Heuer, H., Neumann, G., Sandmann, M., Grosch, R. and Smalla, K. (2014). Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. *Frontiers in microbiology*. **5**: 144.

Shaikh, S.S., Wani, S.J. and Sayyed, R.Z. (2018). Impact of Interactions between Rhizosphere and Rhizobacteria: A Review. *J. Bacteriol. Mycol.* **5**: 1058.

Shanmugam, V., & Kanoujia, N. (2011). Biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycospersici* by plant growth-promoting rhizobacterial mixture. *Biol. Cont.* **57**(2): 85-93.

Sharma, D., and Singh, Y. (2019). Characterization of *Ralstonia solanacearum* isolates using biochemical, cultural, molecular methods and pathogenicity tests. *J. Pharmacognosy Phytochemistry*. **8**(4): 2884-2889.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D., et al. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Appl. Sci*. **1:** 1446.

Sharon, E., Chet, J. and Spiegel, Y. (2011). Biological Control of Plant-Parasitic Nematodes. **11**

Sindhu, S.S. and Dadarwal, K.R. (2001). Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. *Cicer* in chickpea. *Microbiol. Res*. **156**: 353–358.

Singh, B.K., Millard, P., Whiteley, A.S. and Murrell, J.C. (2004). Unravelling rhizosphere–microbial interactions: opportunities and limitations. *Trends Microbiol*. **12**(8): 386-393.

Singhal, N., Singh, N. S., Mohanty, S., Kumar, M., and Virdi, J. S. (2021). Rhizosphere *Lactobacillus plantarum (Lactiplantibacillus plantarum*) strains exhibit bile salt hydrolysis, hypocholestrolemic and probiotic capabilities in vitro. *Scientific Reports*. **11**(1): 1-9.

Someya, N., Nakajima, M., Watanabe, K., Hibi, T., & Akutsu, K. (2003). Influence of bacteria isolated from rice plants and rhizospheres on antibiotic production by the antagonistic bacterium Serratia marcescens strain B2. *J. General Plant Path.* **69**: 342-347.

Stark, C.H., Condron, L.M., O'Callaghan, M., Stewart, A. and Di, H.J. (2008). Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralization resulting from farm management history and organic matter amendments. *Soil Biol. Biochem*. **40**(6): 1352-1363.

Suhaida, S., Farid, M.A.R., Nellysa, N.A., and Shari Fuddin, S. (2022). Morphlogical Identification of *Trichoderma* species Isolated From Rhizosphere of Cocoa (*Theobroma cacao*). *Int. J. Sci. Environ. Technol.* **11**(1):23 – 33.

Tena, G. (2018). Recruiting microbial bodyguards. *Nat. Plants***. 4**: 857.

Tiwary, B. N., Prasad, B., Ghosh, A., Kumar, S., and Jain, R. K. (2007). Characterization of two novel biovar of Agrobacterium tumefaciens isolated from root nodules of Vicia faba. *Current microbiology*. **55:** 328-333.

Umar, W., Ayub, M.A., Rehman, M.Z., Ahmad, H.R., Farooqi, Z.U.R., Shahzad, A., Rehman, U., Mustafa, A., and Nadeem, M. . (2020). Nitrogen and phosphorus use efficiency in agroecosystems. Resources Use Efficiency in Agriculture. 213–257.

Usman, H. M., Farouq, A., Baki, A., Abdulkadir, N., and Mustapha, G. (2018). Production and characterization of orange pigment produced by Halophilic bacterium Salinococcus roseus isolated from Abattoir soil. *J. Microbiol. Exp*. **6**: 238-243.

Usta, C. (2013). Microorganisms in Biological Pest Control—A Review (Bacterial Toxin Application and Effect of Environmental Factors). In *Current Progress in Biological Research*. InTech Open: London, UK. **32**: 137–144

Vajna B., Szili D., Nagy A., Márialigeti K. (2012). An improved sequence-aided T-RFLP analysis of bacterial succession during oyster mushroom substrate preparation. *Microb. Ecol.* **64**: 702–713

Ventosa, A., Marquez, M. C., Kocur, M., and Tindall, B. J. (1993). Comparative study of “Micrococcus sp.” strains CCM 168 and CCM 1405 and members of the genus *Salinicoccus*. *Int. J. Syst. Bacteriol.* **43** (2), 245-248.

Wahyudi, A.T., Widyawati, A., Astuti, R.P. and Giyanto G. (2013). Screening of Pseudomonas sp. Isolated from Rhizosphere of Soybean Plant as Plant Growth

Promoter and Biocontrol Agent. *American Journal of Agricultural* and *Biological Science*. **6**:134-141.

Wahyudi, A.T., Widyawati, A., Astuti, R.P., Meryandini, A. and Nawangsih, A.A. (2011). Characterization of Bacillus sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. *Journal of Microbiology* and *Antimicrobials*. **3**:34-40.

Wang, X., Zhou, X., Cai, Z., Guo, L., Chen, X., Chen, X. and Wang, A. (2020). A biocontrol strain of Pseudomonas aeruginosa CQ-40 promote growth and control *Botrytis cinerea* in tomato. *Pathogens*. **10**(1): 22.

Wilhelm, S. (1973). Principles of biological control of soil-borne plant diseases. *Soil Biol. Biochem.* **5**(6): 729-737.

Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., and Lombardi, N. (2014). *Trichoderma*-based products and their widespread use in agriculture. *The Open Mycology Journal*. **8**(1):71-126

Wu, L., Wu, H.J., Qiao, J., Gao, X. and Borriss, R. (2015). Novel routes for improving biocontrol activity of Bacillus based bioinoculants. *Front. Microbiol*. **6**: 1395.

Yassin, M. T., Mostafa, A. A. F., Al-Askar, A. A., Sayed, S. R., and Rady, A. M. (2021). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, in vitro. *J. King Saud University-Sci.* **33**(3): 101-363.

Zamioudis, C. and Pieterse, C.M.J. (2012). Modulation of host immunity by beneficial microbes. *Mol. Plant Microbe Interact*. **25**(2): 139-150.

Zia-ul-Hussnain, S., Haque, M. I., Mughal, S. M., Shah, K. N., Irfan, A., Afghan, S., and Hafeez, F. Y. (2011). Isolation and biochemical characterizations of the bacteria (*Acidovorax avenae* subsp. *avenae*) associated with red stripe disease of sugarcane. *African J. Biotech.* **10**(37): 7191-7197.

Zolla, G., Badri, D.V., Bakker, M.G., Manter, D.K. and Vivanco, J.M. (2013). Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. *Appl. Soil Ecol.* **68**: 1-9.