Improving Soil Agriculture Properties by the Implication of Compost, Compost Tea, and Soil Microorganisms

BY

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By

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CERTIFICATE

This thesis submitted by **Ahsan Ullah** is accepted in its present form by the Department of Microbiology, Quaid-e-Azam University, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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DEDICATION

This work is dedicated to my late Father, beloved Mother, all my Family members, Teachers and Friends for their endless love, support, and encouragement throughout my studies.

DECLARATION

I am Ahsan Ullah certify that research work “Improving soil agriculture properties by the implication of compost, compost tea, and soil microorganisms” is my own work. The material and information contained in this thesis is not previously presented elsewhere for any other degree.

AHSAN ULLAH

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ACRONYMS/ABBREVIATIONS

m	=	meter	SIM	=	Sulphur Indole motility
ds	=	decisiemens	TSI	=	triple sugar iron
N	=	Nitrogen	MR	=	methyl red
P	=	Phosphorus	*Spp*	=	Specie
K	=	Potassium	BCAs	=	Biological control agents
BD	=	Bulk density	Mg	=	Milligram
OM	=	Organic Matter	ug	=	Microgram
EC	=	Electrical conductivity	MC	=	Moisture content
CM	=	compost	%	=	Percentage
CT	=	compost tea	PGP	=	Plant growth promoting
BC	=	Bacterial Consortia	PGPR	=	Plant growth promoting rhizobacteria
OM	=	Organic matter	OD	=	Optical density
gm	=	Gram	rpm	=	Round per minute
FO	=	*Fusarium oxysporum*	CFU	=	Colony forming unit
PDA	=	Potato dextrose agar	QAU	=	Quaid-i-Azam university-
TOC	=	Total organic carbon	C	=	Carbon
ACT		Aerated compost tea	ToMV	=	Tomato mosaic virus
NCT		Non aerated compost tea	GRAS		Generally, recognize as safe
FDA		Food and drug administration			

ABSTRACT

Soil fertility and productivity challenges are growing with every passing day in view of changing global climate and increasing human population. One of the most viable and valuable options is the application of sustainable biotechnological practices. Present study investigated the effects of compost, compost tea, and selected bacterial strains on soil properties and plant growth parameters in autoclaved and unautoclaved soils. Compost and compost tea were prepared using simple carbon source (molasses) following standard composting principles. Selected *Bacillus* strains were isolated from *Solanum lycopersicum* (tomato plant*)* rhizosphere soil and screened for antifungal activity against *Fusarium oxysporum, Aspergillus flavus*, and *Aspergillus niger*. The results showed that four *Bacillus* isolates S1, S2, S4, and S10 exhibited significant antagonistic activity and reduced *Fusarium oxysporum* growth by (41.9 ± 1%), (39.4 ± 1%), (42.1 ± 3%), and (46.6 ± 1%) respectively. *Bacillus* strain S4 caused an inhibition of *Fusarium oxysporum* (46.6% ± 1), *Aspergillus flavus* (62.40 ± 1%) and *Aspergillus niger* (66.6 ± 0%) in vitro experiments. In order to investigate plant growth promoting traits, bacteria were assessed for their abilities to solubilize phosphate and zinc along with nitrogen fixation, protease, catalase, oxidase production abilities. *Bacillus* strain S2 and S10 showed positive response toward phosphate and zinc solubilization. Soil organic matter in post-harvested soil was found to be 2.86 times higher as compared to pre-harvested one, while the elemental ratios were N (1.35 ± 0.19%), P (0.51 ± 0.07%) and K (0.38 ± 0%), respectively, after treatment with compost (CM) + compost tea (CT) + Bacterial consortia (BC). In both autoclaved and unautoclaved soils, the application of compost, compost tea, and bacterial consortia significantly influenced plant growth characteristics. In autoclaved soil, the addition of CM+CT+BC led to a remarkable plant height (77.66 ± 4.04 cm), fresh and dry weight (19.55 ± 3.05 g and 3.32 ± 0.97 g), primary root length (7.20 ± 1.80 cm) and chlorophyll content (43.63 ± 3.35) were achieved. In conclusion, this study highlights the potential benefits of compost, compost tea, and specific bacterial strains in improving soil quality and promoting plant growth leading to sustainable food production.

1. Introduction

The agriculture productivity has been challenged owing to climate change, deforestation, urbanization and soil and water pollution. Consequently, food security is becoming a possible implication to the aforementioned issues. It can be expected that millions of people worldwide could be affected in future (Schreinemachers *et al*., 2018; United Nations, 2020). Plant pathogens, primarily comprising microorganisms such as eukaryotic fungi, prokaryotic bacteria, protozoa and viruses, impose a serious threat to human health and economy (Book, 2009). Every year a significant loss caused by these pathogens during pre-harvest and post-harvest phases has been witnessed (Singh *et al*., 2017). Importantly, early every plant species appears to be susceptible for wide variety of plant pathogens.

Plant pathogenic fungi and bacteria often reside within their host plants and occasionally in the soil. These phytopathogens are highly persistent in their attacks, causing direct and indirect economic losses estimated at approximately 40 billion dollars worldwide (Pandith *et al*., 2022). Plant diseases result in an annual loss of 10-15% in the production of major crops, with fungal pathogens being responsible for 70-80% of these losses, severely impacting plant growth and yields (Peng *et al*., 2021). Notably, major food crops like tomatoes, rice, wheat, soybeans, and maize are frequently affected by fungal pathogens on a global scale (Almeida *et al*., 2019). The consequences of plant diseases extend beyond agricultural concerns, leading to a decline in species diversity, increased expenses for disease control measures, negative effects on human health, and food insecurity (Savary *et al*., 2019). Urbanization exacerbates the situation, with projections indicating an 80% reduction in arable land for food grain production in Africa and Asia (D'Amour *et al*., 2017). Considering that the world's population is expected to reach 10 billion by 2050, a 60% increase in agricultural production will be necessary to meet the growing demand (Fedoroff, 2015).

In order to enhance agricultural production, agrochemicals such as herbicides, pesticides, and fungicides are commonly employed (Carvalho, 2006). However, the use of chemical pesticides has resulted in soil and groundwater contamination, along with the emergence of pesticide-resistant pathogenic strains. Furthermore, pesticides have adverse effects on non-target organisms, including beneficial insects, pollinators, soil

microbes, and human health (Alizadeh *et al*., 2020). Soil fertility and biochemical processes are also impacted by various fungicides (Gikas *et al*., 2022).

Tomatoes (*Solanum lycopersicum*) hold a prominent global position as the second most important horticultural crop. China leads in tomato production, contributing to 31.0% of the world's total production and cultivating 20.6% of the global tomato-growing area. Tomato cultivation predominantly occurs in regions with arid or semi-arid Mediterranean climates, characterized by an average annual rainfall of less than 300 mm. However, tomato crop requires substantial water for their growth and development (Rodríguez-Ortega *et al*., 2019): (Ghosh *et al*., 2022). In the context of tomato farming, phytopathogens pose a substantial threat to both crop yield and quality, resulting in significant economic losses for farmers and affecting the global availability of this vital vegetable. Addressing this issue needs sustainable and eco-friendly strategies for phytopathogens control while optimizing tomato crop productivity. Tomatoes (*Solanum lycopersicum*) hold the highest value among fruit crops globally (Tieman *et al*., 2017) because of source essential vitamins, minerals, and antioxidants (Escobar Rodriguez *et al*., 2021).

Fungal pathogens represent a significant threat to tomato crops, causing various diseases with substantial economic consequences. Like, *Alternaria solani* is responsible for early blight, which manifests as circular lesions with concentric rings on leaves and stems, leading to premature defoliation and reduced fruit production. *Fusarium oxysporum* f. sp. lycopersici induces *Fusarium* wilt, a vascular disease that obstructs water uptake, resulting in wilting, yellowing, and stunted growth in tomato plants. Phytophthora infestans, the culprit behind late blight, can cause swift and widespread destruction of tomato foliage and fruit. Bacterial pathogens also pose a significant threat to tomatoes. *Ralstonia solanacearum*, responsible for bacterial wilt, is a notorious pathogen that infects the vascular system, leading to wilting and plant death. Viruses can induce a wide range of diseases in tomato plants, adversely affecting crop health and yield. *Tomato yellow leaf curl virus* (TYLCV) results in leaf yellowing and curling, stunted growth, and reduced fruit size and yield. Another notable viral pathogen is the *Tomato mosaic virus* (ToMV), causing mosaic patterns and yellow streaks on leaves, impacting photosynthesis and fruit development (Jones *et al*., 2014). Modern sustainable agriculture places a strong emphasis on the conservation of natural

resources and ensuring consumer safety (Lykogianni*et al*., 2021). Biological control methods have gained popularity in meeting these criteria for sustainable agricultural development due to their environmental friendliness, cost-effectiveness, low toxicity, positive effects on plant health, and minimal impact on soil flora (Parnell *et al*., 2016). Given the critical importance of managing plant pathogens and the diseases they cause to enhance crop productivity and quality, addressing the global food security challenge is imperative. This urgency arises as biodiversity loss and greenhouse gas emissions, currently at 60% and 25%, respectively, highlight the need for more sustainable agricultural practices. One such sustainable practice that receives much attention is the use of organic amendments such as compost.

Compost, commonly referred to as "black gold" in agriculture and horticulture, comes from the controlled decomposition of a variety of naturally occurring weeds (Jones *et al*., 2014). Composting is a traditional method that utilizes the decomposition of natural materials by microorganisms with bacteria, fungi, and other beneficial organisms. Provides a sustainable and environmentally friendly method around the process of recycling organic matter, and providing nutrient-rich material to increase soil fertility, improve soil structure, in plants can also support growth. Compost is a natural product, humus, and complex mixtures of microorganisms that act as reservoirs for nutrients and beneficial microorganisms, creating a vibrant and healthy soil ecosystem. Composting enriched soil in a slow release fashion with a variety of nutrients such as nitrogen, phosphorus, potassium and micronutrients. It happens, reducing the risk of nutrient imbalances and leaching, it also increases soil structure, improves aeration, drainage and root growth (Lykogianni*et al*., 2021). Compost increases soil cation exchange capacity (CEC) which helps in nutrient retention and plant consumption. Compost helps to enhance the diversity of microorganisms in the root zone, enhance nutrient cycling and enhance plant nutrient absorption, resulting in stronger root systems, increased yields, and soil overall health. Compost plays an important role in improving plant resistance by enhancing natural defense mechanisms. Beneficial microorganisms present in compost act as biocontrol agents against harmful plant pathogens. These microorganisms can directly combat pathogens or stimulate the plant's immune response, reducing disease incidence. Compost contains humic substances that act as signaling molecules, priming the plant's innate immune system upon pathogen attack.

Additionally, compost application enhances soil enzyme activities and nutrient transformations, contributing to sustainable and organic farming practices (Islam *et al*., 2018).

Compost tea, a liquid solution derived from compost, has emerged as a sustainable, biologically active plant growth promoter and an alternative to chemical fertilizers and pesticides (González- Hernández *et al*., 2022). Aerated compost tea contains high concentrations of beneficial microorganisms, such as bacteria, fungi, and protozoa, which facilitate nutrient solubilization, nitrogen fixation, and plant growth promotion. Its foliar application increases plant disease resistance and stimulates systemic acquired resistance in plants (Martínez *et al*., 2023). Compost tea can effectively reduce damping-off and combat soil borne plant diseases caused by fungal phytopathogens, making it a valuable tool in organic farming. Additionally, it contains plant growth regulators like auxins and cytokinins that influence plant development and stress tolerance. Foliar application of compost tea enhances nutrient absorption and translocation, contributing to overall plant health and vigor. While compost tea holds promise as an organic plant growth stimulant, its composition and brewing methods significantly affect its efficacy. It has shown positive effects on seed germination, early seedling growth, plant height, leaf area, and root length. In many agricultural settings, the utilization of biological controls involving beneficial microorganisms forms complex relationships with plants, influencing vital processes such as nutrient cycling, disease resistance, and overall plant health (Naidu et al., 2013) (Seddigh *et al*., 2018). These microorganisms play a pivotal role in breaking down organic matter into simpler forms through decomposition. One notable example is mycorrhizal fungi, which establish symbiotic relationships with plant roots, significantly enhancing nutrient uptake efficiency, particularly with phosphorus. Moreover, specific soil microorganisms serve as biocontrol agents, providing natural disease resistance for plants. Beneficial bacteria and fungi, including species like *Bacillus* and *Trichoderma*, can suppress soil-borne pathogens by producing antimicrobial compounds or competing for resources (Smith *et al*., 2012). These antagonistic interactions effectively reduce the incidence and severity of plant diseases, offering a sustainable and eco-friendly alternative to chemical pesticides.

Soil bacteria also play an important role in forming and maintaining soil structure. Like, fungal hyphae form stable aggregates in the soil, increasing soil porosity, aeration, and drainage. This in turn stimulates root growth and facilitates nutrient uptake into plants (Rillig *et al*., 2015). In addition, microbial activity contributes to the accumulation of organic matter in the soil, enriching humus. This humus further improves soil fertility and moisture retention (Raza *et al*., 2022). The combination of optimal soil systems and crop rotation is critical for sustainable agriculture and long-term food production. Given the increasing call for sustainable agricultural practices and the vital to reduce reliance on artificial and imported fertilizers and chemical compounds, there's a developing interest in exploring alternative approaches to enhance plant growth and soil properties. The concurrent application of compost, compost tea, and rhizospheric soil microorganisms holds significant promise in this regard. This study aims to uncover the synergistic effects of these three elements on various plant increase parameters and soil fitness signs. Through rigorous experimentation and analysis, the results of this research have the potential to shape strategies for fostering environmentally friendly and economically viable approaches that promote both productive yields and ecological harmony.

Aim and Objectives

The aim of the present study is "evaluate possible role of compost, compost tea, and soil microorganisms in improving agriculture productivity" and study specific objectives are,

Objectives

1. To prepare compost and composts tea, isolation and screening of PGP *Bacillus* strain having antifungal activity.
2. To conduct physicochemical analysis of compost, composts tea and soil
3. To assess the impact of compost, compost tea and selected bacterial strains on soil physicochemical properties and their effect on plant growth

2. Literature review

2.1. Food security

Ensuring food security means people have access to safe, nutritious and plenty of food all the time (FAOSTAT, 2022). Globally, millions of the humans suffering from hunger and according to estimates over 820 million population has been facing acute shortage of food because of inadequate access to safe and nutritious foods. This leads to immediate and long-term health issues, including conditions such as stroke, heart disease, diabetes and other physiological issues (IRRI, 2022). In order to limit food insecurity, increase in the global agricultural production by 70% by 2050 and by 100% in poor countries is required (FAOSTAT, 2022). Asia plays a vital role in the global food supply chain as the origin of a third of the world's under privileged population. Tomatoes, whether eaten raw or cooked, are especially useful for foods that are nutritious and maintain their nutritional value. Ironically, more than 80% of all tomato sales are processed products such as juices, soups, and ketchup (Collins *et al*., 2022). The diet of tomatoes and their derivatives is well known to provide numerous health benefits, of which their antioxidant properties are a major contributing factor (Kumar *et al*., 2012).

2.2. Global tomato production

Tomato (*Solanum lycopersicum*) is the 2nd most demanded vegetable after potato (Leite *et al*., 2018). It belongs to the *Solanaceae* family that include many important food crops (Knapp *et al*., 2016). Tomatoes formerly originated in the Andes region of South America. It was introduced to the Europe by the Spaniards in the 16th century, the cultivated species then spread to the Southeast Asia, Africa, and the Middle East and became widely distributed (BTEE, 2023) Tomatoes grow in tropical conditions throughout the growing season and requires a combination of hot and humid weather conditions (Chohan *et al*., 2017) (Worku *et al*., 2018). The tomato-based products industry is highly developed globally, and uses variety of cultivation techniques, processes and marketing strategies. Global production and consumption of tomatoes has grown rapidly because of many innovative products. Currently, about 170.75 million tons of fresh tomatoes are produced in more than 150 countries worldwide on 5.02 million hectares (Leite *et al*., 2018). Tomatoes are more profitable crops due to its

popularity and widespread consumption in the diet because of a good source of vitamins (A and C) and lycopene, an excellent antioxidant chemical compound (Maria *et al*., 2014). Globally, the leading countries for tomatoes production are China, the European Union, the United States and Turkey (FAOSTAT, 2019). Global tomato production is expected to grow continuously, including fresh and processed varieties. But some trends in the industry, such as the ratio of greenhouse to open field production can significantly change its productivity owing to the controlled growth rate, period shorter ripening period and higher yield per unit area (Gatahi *et al*., 2020). However, the real impact of this transformation yet remains elucidated. In addition, how these settings would reduce the application of pesticides and other agrochemicals to improve crop protection against pathogens need to be evaluated under varying growth conditions particularly considering new practices such as controlled farming, vertical farming etc.

2.3. Importance and utilization of tomato crop

Tomato crop has emerged as an increasingly popular staple vegetable over the past century. It is grown almost worldwide, whether in greenhouses, grid houses, or outdoor fields (Garrido and Luque-Romero, 2014). In addition to delicious taste, it offers many health benefits such as bone growth, cell division, immune regulation, maintenance of defense of ophthalmological system, respiratory system, urinary tract, and intestines. In addition, it helps to bones and teeth are retained, making them susceptible to metal absorption (Mozos *et al*., 2018). Another important nutritional benefit of tomatoes is the high content of lycopene, which is a powerful antioxidant that helps prevent various cancers (Viuda-Martos *et al*., 2018).

2.4. Important tomato crop diseases

Currently, tomato faces a huge challenge, with over 200 viruses and diseases identified, causing direct or indirect loss of tomato production (Singh *et al*., 2017) These diseases of cereal crops and vegetables with faults such as fungi, nematodes, viruses and viruses pose a serious threat and their impact is wider than just crop reduction; They can have adverse effects on the nutrient content of these crops, human health and the wider economy (Oliver *et al*., 2015).

2.4.1. Fungal diseases

Several severe diseases affect tomato plants due to fungal infection, resulting in 20% to 70% (Laurence *et al*., 2014). *Sclerotinia* rot has significant impact on tomato crop yield (Majumdar Purabi, 2021). Tomato rot, crown rot, and root rot diseases, all caused by *Fusarium* species, have been extensively studied (Laurence *et al*., 2014). Currently, this pest causes significant losses in this important vegetable crop, even under open field and greenhouse conditions, and remains a major restriction in tomato production (Singh *et al*., 2017).

2.4.2. Nematode diseases

Nematodes cause highly damaging and widespread tomato crop diseases (Zhou *et al*., 2016). Nematodes not only directly affect crops but also increase plant susceptibility (Ashraf and Khan 2010). It reduced tomato yield by up to 30–50% in China (Yang *et al*. 2011). However, effective prevention strategies are still being developed (Collange *et al*., 2011).

2.4.3. Bacterial diseases

Xanthomonas campestris bacteria is a common pathogen affecting tomato crop by causing leaf spot. It also severely damages the greenhouse and field environments, causing yield losses of 10% to 50% (Kallo 1991). (Sharma 2018) (Reddy *et al*. 2012). *Ralstonia solanacearum* pathogen cause more damage than any other pathogen affecting more than 200 plant families including tomatoes and inhibits their growth (Huang *et al*. 2013) *Clavibacter michiganensis* systematically causes wilt and root abscesses, while Blister-like spots can emerge on locally infected leaves, leading to significant economic losses in global tomato production. The virulence factor of *Clavibacter michiganensis* causing economic losses the development of rash compared to wilt, resulting in diseases occur in tomato plants (Chalupowicz *et al*. 2016).

2.4.4. Viral disease

Viral diseases affecting tomatoes include a variety of viral pathogens, *including tomato spotted wilt virus,* sometimes causing blight on tomato plants (Rossello *et al*. 1993) throughout the tropical and subtropical world. Another viral problem affecting locally

grown tomatoes is *tomato yellow leaf curl*, causing losses up to 100% in many areas in many areas no tomato yellow leaf curl represents a major obstacle to tomato production. The causative agents of this disease are the *Begomo virus* species, a group of twin viruses, collectively known as *tomato yellow leaf curl virus* and, the rapidly emerging *Pepino mosaic virus*, which is a major threat to tomato crops (Moriones and NavasCastle 2000).

2.5. *Fusarium*

The genus *Ascomycota*, which includes *Fusarium*, belongs to the group Hypocreles. *Fusarium* is assigned to the family Nectriaceae. There are more than 1,500 species in the genus *Fusarium*, with many species. These species include phytopathogens, pathogens that infect humans, and saprobes. Differentiating pathogenic from non-pathogenic bacteria based on morphology is challenging, and many species found in plants and animals produce mycotoxins (Bahadur, 2022). In agriculture, *Fusarium* remains one of the most important groups of phytopathogenic fungi due to its involvement in various diseases affecting many crops. According to the American Phytopathological Society (APS) *Fusarium* species infect 83 of 108 plant species affecting their productivity. A study to assess the scientific and economic importance of phytopathogenic fungi in molecular plant pathology showed that the mycotoxins, trichothecenes, and fumonisins of *Fusarium* species including the top ten pathogens *Fusarium oxysporum* and *Fusarium graminerum* have negative impact on animal productivity and human health. Individuals with compromised immune systems are particularly susceptible to *Fusarium* species (Summerell, 2019).

2.5.1. Genome of *Fusarium* species

Comparative genomics of *Fusarium* and other fungal genomes have shown that genomes can be divided into at least two parts: core and accessory genomes. These genomes differ in several characteristics: evolutionary speed, expression number and gene collection. Core genomes contain genes that regulate key metabolic processes where evolution occurs at a low level. These genes are highly conserved across species. The expression of the genes involved is much lower than the rest of the genome (Waalwijk *et al*., 2017).

2.5.2. Soil Seed Borne *Fusarium*

Fusarium species are capable of both parasitic and saprophytic behavior and are commonly soil and seed-borne. Among seed fungi, *Fusarium* species are of particular importance because they can affect both seed germination and agricultural production. Notably, *Fusarium oxysporum* and *Fusarium solani,* which cause fungal diseases, can cause crown and root rot. Blight caused by these fungi can cause crop losses, and these organisms can persist in the soil or subsoil despite the absence of the host (Blanco & Aveling, 2016).

2.5.3. Mechanism of *Fusarium* survival in the adverse environmental conditions

Soil-borne diseases exhibit slower spread than soil borne viruses, because they take more time to establish. This is why they rely on their ability to remain in the land. Soil-borne *Fusarium* begin infection in the rhizosphere and subsequently produce in the stem of living plants, giving them a distinct advantage over other plants in that environment. In plant roots they produce next-generation protection for sensitive crops in the future. This occurs when host plants die or senesce, causing vascular fungal hyphae lining the plant to leak into the thick-walled sclerenchyma and parenchyma tissues to form chlamydospores. When refuse from these plants is returned to the soil, lot of it will rot away. But chlamydospores of the pathogen in these soft tissues remain safe for a long time (Smith, 2007).

2.5.4. Methods to control diseases

The introduction of exotic plant pathogens into a pathogen free area can cause a more devastating epidemic than an existing pathogen. This phenomenon occurs because plant growth in environments where they are not exposed to pathogen have no chance of acquiring specific resistance genes. In addition, there are usually no microorganisms that can oppose or compete with a pathogen that enters a pathogen-free zone. Conversely, a foreign pathogen is in a situation where there is plenty of sensitive plant tissue available to feed freely and multiply without interference. To mitigate this risk, crop cultivation in area which has unfavorable environment for the pathogen, reducing the chances of pathogen attack. Governments have also introduced quarantine laws aimed at reducing pests that can be introduced through imports (Agrios, 2005; Karuppuchamy & Venugopal, 2016).

2.6. Cultural methods to control disease

Cultural methods of disease management reduce the pathogen levels or rate of disease development (Rodriguez *et al*., 2022).

2.6.1.1. Tillage

Tillage increases crop residue decomposition thereby reducing the availability of inoculums for many pathogens. Storage of crop residues by wind or water sprays also reduces pathogen dispersal because the residues do not remain on the soil surface but movement of soil and crop residue due to tillage results dramatically increase in the movement of inoculum of pathogen in soil (Ownley & Trigiano, 2016).

2.6.1.2. Crop rotation

Planting the same plants in the same area year after year increases the number of pathogens in the soil. Crop rotation helps prevent inoculation pathogens from rising to dangerous levels, as the pathogen cannot infect and feed on plants, so become weak and begin to die (Texas Plant Disease Handbook, 2022). On the other hand, rotation with a non-host crop will have a dramatic effect in the population of nematode specific to that crop resulting in non-target killing. *Fusarium oxysporum f.sp*. *vasinfectum* causative agent of wilt of cotton but failed to reduce the number of *Fusarium* spores in soil so that crop rotation alone would not eliminate the disease (Ownley & Trigiano, 2016).

2.6.1.3. Soil solarization

Soil solarization is a pre-planting method of plant management that uses solar energy to control soil microbial and weed seed populations. This method involves covering the flooded area with lightweight plastic to capture sunlight and raise the temperature. During hot summers, this treatment increases soil temperature to a level that reduces the spread of *Verticillium dahlia*, some *Fusarium* species, *Sclerotinia species*, *Agrobacterium tumefaciens*, *Streptomyces scabies*, other nematodes and weed seeds, but its application in agriculture is limited as it is a lengthy process and is exclusively rely on climatic conditions (Panth *et al*., 2020).

2.6.1.4. Resistant cultivars

Utilizing resistant crop species is a sustainable and economically effective technique. It is vital to find screening efforts to identify viable resistant varieties (Jing *et al*. In 2021). However, it's crucial to apprehend that the development of resistant varieties is a time-consuming process, regularly requiring 10-15 years to provide a cultivar that meets agronomic standards, even when a source of resistance is readily available, as stated in a 2016 information source. Furthermore, it is really worth noting that the lengthy-term efficacy of genetically encoded resistance is restricted. Over time, new fungal races can emerge which could overcome this resistance. This phenomenon, as emphasized by using Sain *et al*., in 2015, highlighted the development of maintaining durable resistance in crop types.

2.6.2. Physical management of seed borne fungi

Heat and hot water as a means of controlling severe seed diseases are physiological control measures, using temperatures of 50°C. Hot water treatment is outstanding as a highly effective method of controlling seed born fungi, including *Alternaria spp*., *Penicillium spp*. *Aspergillus flavus* and *Rhizopus spp*. Another method to control seed born fungi is radio frequency. It is frequency heat therapy. Radio frequency treatment offers a viable alternative and has shown efficacy in complete eradication of *Fusarium graminerum* from wheat seed while maintaining germination rate. The success of these physical methods necessarily depends on uniform heat distribution in seed lot and careful monitoring is required to ensure that no seeds are exposed temperature for a longer period, which can lead to loss of viability (Sharma *et al*., 2015).

2.6.3. Chemical control of plant diseases

When plants sense the presence of microorganisms, they activate defense mechanisms such as systemic resistance (SAR) to fight infection. Interestingly, plant exposure to abiotic objects, plants become aware of them and improving its protection and immunity. Abiotic stimulators such as probenazole (PBZ), acibenzolar-S-methyl (ASM), tiadinyl (TDL), and isothianil have been used to stimulate plant defenses and activate them. Plants recognize these abiotic stimuli as not their own products, increasing their immune response. Several types of fungicides are used to control seed

diseases and protect against seed and soil-borne pathogens during germination and establishment Fungicides applied to seeds promote yield survive and prevent diseases such as seed rot and seedling wilt. This method is effective in controlling fungi both internally and externally on seeds, as well as those living in soil or crop residues. Some common fungicides used to treat seeds are Captan, Thiram, Zineb, Mancozeb, Metalixal, Diazobene, Pentachloronitrobenzene (PCNB), and Chloroneb (Agrios and Amza 2018).

2.6.3.1. Negative effects of chemical control of plant diseases

Around the globe, approximately 4000 tons of chemical fungicides are employed, constituting about 17.5% of pesticide usage (Sharma *et al*., 2019). These fungicides can find their way into natural water sources through various pathways, including agricultural wastewater resulting from equipment washing and spraying, mishandling of empty containers leading to spillage, runoff, spray drift, leaching, and subsurface drainage (Schönenberger *et al*., 2022). Fungicides cover a diverse range of compounds with varying functional groups and mechanisms of action. Organic fungicides have been associated with both acute and chronic toxicity in aquatic and terrestrial organisms, raising concerns for public health. Studies on rats exposed to fungicides have revealed endocrine-disrupting effects, as well as biochemical, histopathological, and hematological impacts. The repeated application of fungicides with the same mode of action can lead to the development of resistance. World health organization (WHO) have characterized many fungicides as hazardous chemical and are banned in the European Union (Gikas et al., 2022). For instance, Methyl benzimidazole carbamates (MBCs) were among the first site-specific fungicides, and resistance has been documented in nearly 100 plant pathogenic species. Additionally, around thirty plant pathogens have developed resistance to azole fungicides, with some pathogens acquiring resistance after just two years of fungicide use (Corkley *et al*., 2021).

2.6.4. Biological control of plant disease

Biological control refers to the practice of inhibiting plant pathogens, enhancing plant immunity, or altering the environment through the use of beneficial microbes, compounds, or sustainable cropping systems to manage plant diseases. This approach offers several advantages over other disease management strategies. Biological control

agents (BCAs) primarily target specific groups of pathogens, resulting in fewer negative impacts on the ecosystem compared to fungicides. Additionally, many BCAs can establish themselves and persist in the environment without the need for continuous effort (Haddoudi *et al*., 2021; Köhl *et al*., 2019).

Microbes used as BCAs to protect plants from diseases through various mechanisms. They induce systematic resistance (ISR) in plants, making them immune to phytopathogens, and compete with pathogens for nutrients and space through indirect interactions. In direct interactions, biocontrol agents can kill phytopathogens by parasitizing them or by producing secondary metabolites with antimicrobial properties (antibiosis). Hyper-parasitism involves targeting pathogens by invading and damaging their mycelium, spores, and resting structures such as sclerotia. Antibiosis, based on the production of antimicrobial secondary metabolites, is a common method for killing phytopathogens. These antimicrobial compounds are applied either with or without living agents in biological control products (Köhl *et al*., 2019). Managing *Fusarium* phytopathogens once they have established themselves can be challenging (Bahadur, 2022). Given the adverse effects on the ecosystem, there is a growing need to reduce the use of chemical treatments, which are often less effective. Application of microorganisms like *fluorescen*t *Pseudomonas, Trichoderma viride*, *Bacillus subtilis*, and *Trichoderma harzianum* has been shown to be effective in controlling soil-borne phytopathogens responsible for root rot in various crop species (Shafique *et al*., 2016). Numerous organisms have been successfully employed as BCAs to control soil-borne phytopathogens. Common fungal and bacterial BCAs used in commercial applications include *Bacillus*, *Paecilomyces*, *Phlebiopsis*, *Serratia*, *Pseudomonas, Streptomyces*, *Rhizobium*, *Coniothyrrium*, *Gliocladium,* and *Trichoderma* (Mazzola & Freilich, 2017).

2.6.4.1. *Bacillus* as biological control agent

Bacillus belongs to the genus *Firmicutes*. These bacteria carry out their aerobic metabolism but can also alternatively switch to anaerobic metabolism (Fira *et al*., 2018). Several species in the genus *Bacillus* have antagonistic properties, *Bacillus* is more important than other bacterial species. *Bacillus* is used as a biological control (BCAs) are increasing speedily. *Bacillus* species are particularly interesting as

antimicrobial agents because of their ability to produce highly resistant enzymes. They multiply rapidly and are capable of producing antibiotics that effectively control a wide range of plant pathogens. Notably, *Bacillus subtilis* is listed in the US, it is recognized by the FDA as relatively safe (GRAS) and appropriate for use in the food industry. Furthermore, the volatile compounds produced by *B. subtilis* promote plant growth and stimulate defense mechanisms for the induction of induce systematic resistance (ISR). Enzymatic products from *B. subtilis* cells and endospores are also effective in controlling several fungal phytopathogens (Shafi *et al*., 2017). A large portion of the genome of the plant-associated *B. amyloliquefaciens* (FZB42) approximately 8% is used to synthesize a variety of antibiotics, with polyketides, bacteriocins, lipopeptides, siderophores, and antimicrobial peptides constructed as cyclic structures, as it is associated with fatty acid derivatives. Differences in fatty chain length and amino acid composition result in different types of isomerization. Lipopeptides can be divided into three families: surfactin, iturin, and fenzicin. The surfactin family includes different variants such as halobacillin, surfactin, lichenisin, and pumilacidin. These lipopeptides consist of seven amino acids attached to a beta-hydroxy fatty acid chain of 13 to 15 carbon atoms. Large numbers of surfactin family members can cause irreversible membranes in cell membranes, potentially leading to dissolution. They include members of the iturin family, such as mycosbatillin, iturin E, B, A, D, and bacillomycin. These lipopeptides are composed of seven amino acids bound to beta-amino fatty acid chains of varying chain length. Members of the iturin family can be ion-conducting membranes. The fengycin family includes lipopeptides such as fengycin, plippastatin, and several maltacin isomers. In this family, beta-hydroxy fatty acids with up to 18 carbon atoms are linked to ten amino acids. Fengsin lipopeptides interact with lipid bilayers, disrupting their structure and altering their permeability (Fira *et al*., 2018).

2.7. Compost

Compost is a mixture of decomposed organic matter, primarily derived from various sources comprises organic materials, such as grass clippings, dry leaves, and twigs. Food waste consists of items like fruits, vegetables, and kitchen scraps. Compost serves as a nutrient-rich soil amendment, enhancing soil structure and supplying essential nutrients to plants, thereby promoting healthy growth and vitality.

2.7.1. Composting

Composting is a biotransformation process that converts part of the organic waste into organic matter through the action of specific beneficial microorganisms, therefore the presence and activity of microorganisms is very important for successful composting. Composting requires the presence of a variety of microorganisms, including bacteria and fungi, that grow under mesophilic (medium temperature) or thermophilic (high temperature) conditions. The composition of these particles depends on biodegradable materials of naturally occurring compounds and mainly on their concentration in mixtures (Gogoi *et al*., 2015). To optimize the composting process, it is important to understand the role of beneficial microbial communities in the breakdown of hardy plant residues, especially cellulose, hemicellulose, and lignin, which account for about 40%, 20-30%, and 30% in plant residues, respectively. Cellulose is an important constituent of grass material, and its efficient degradation is an important component of composting brown rot fungi use varies mechanism for cellulose degradation (Martioniz *et al*., 2009).

2.7.2. Phases of composting process

The composting process consists of two primary phases. The first phase is marked by strong microbial activity, and the second phase involves the conversion of organic material into humic substances (Adani *et al*., 1999). Humification is the process of forming humic substances, is considered a key factor in enhancing quality of compost (Chen and Aviad, 1990; Chen *et al*., 1994).

2.7.2.1. Decomposition phase

The decomposition stage of composes includes all three stages: mesophilic (medium temperature), thermophilic (high temperature), and curing (cooling) phase, this stage involves the breakdown of simple and complex components by microorganisms. on the other hand, corresponds to the maturation phase of the compost represents how compost is converted into a mature, stable product, rich in humic substances to benefit soil health and structure (Azim *et al*., 2018).

i. Mesophile stage

During the mesophilic phase, the temperature of the compost pile gradually increases from the ambient temperature until it reaches a temperature of about 40°C. During this phase organic acid is produced which is the main cause of decreasing. These acids release from lipids and carbohydrates degraded by microorganism. Mesophilic microorganisms are successively replaced by thermophilic microorganisms' pH (Kaiser 1983; Mustin 1987; Tuomela 2000).

ii. Thermophile stage

The thermal phase is usually characterized by temperatures in the range of 50 to 60 °C. This phase contains many heat-tolerant and heat-loving fungi. During this phase, the pH rises as microorganisms break down proteins, releasing ammonia in the process. As the temperature exceeds 60 °C, the decomposition of organic matter begins to slow down, and above 70 °C, the which produced plays a role in the decomposition. After thermophilic stage the cooling phase starts in which the material returns to stabilize, mesophilic microorganisms begin to reestablish themselves (Azim *et al*., 2018).

iii. Curing or maturation stage

Maturation of compost occurs at ambient temperature and is dominated by mesophilic microorganisms, mainly bacteria and fungi (Makan *et al*. 2013). In maturation phase, the micro and macro fauna appear. Antibiotics are remarkably produced by the microorganisms. In this phase overall, heat releasing and weight loss of the composted materials remains low (Vobrkov *et al*. 2017).

2.7.3. Composting parameters

In existing literature, numerous composting parameters have been identified, including the carbon-nitrogen ratio (C/N), microbial activity, germination index, cation exchange capacity (CEC), humic substances content, compost concentration of water-soluble carbon (WSC), and dissolved organic matter. These factors serve as crucial indicators in evaluating compost quality, encompassing aspects such as nutrient balance, microbial efficiency, phytotoxicity potential, soil nutrient exchange, humic compound presence, accessibility of soluble carbon, and the dissolved organic content within the compost. There are newly developed monitoring systems that aim to provide more

accurate descriptions of biodegradation. It is important to note that no single parameter has been widely validated, therefore, a combination of tests is often necessary to accurately assess compost quality. Following are important composting parameters,

2.7.3.1. Carbon-nitrogen ratio

About 30 parts of cellulose for every nitrogen fraction in the decomposition process is utilized by microorganisms (Choi, 1999) The ratio of carbon to nitrogen in composting materials very important. (Mustin, 1987). Microorganisms use carbon as energy source and nitrogen is a main component of amino acids, proteins and also an important element of nucleic acid. Carbon used by the microbes fifteen to thirty times more than nitrogen. Duration of composting was found to increase with increasing initial C/N ratio. Trees made of wood, timber and other materials take a long time (approximately 18 months) to mature, as compared to household waste that matures in a relatively short period of time, usually about 7 months.

Carbon-N Ratio is one of the most important factors affecting the final product and properties of the composting process (Kumar *et al*., 2010) It was commonly found that C/N ratio which is optimal for different types of composting materials is between 25 and 35 (Choi, 1999). When the initial C/N ratio exceeds 35, the bacteria must go through several life cycles to oxidize the excess carbon, and in such cases aerobic due to the availability of nitrogen the rate of fermentation is limited but recent studies have shown that it can be used to produce effective compost with C/N ratios as low as 15 (Kumar *et al*.,2010). Materials rich in lignin and cellulose, such as tree stumps, roots and sawdust, have high C/N ratios due to the complexity of their carbon structure. These raw materials with high C/N ratios difficult to decomposed and takes more than 10 months for complete decomposition (Yulipriyanto, 2001).

2.7.3.2. Moisture content

Water is very important for all the microorganisms as, they rely on water to transport essential nutrients and energy products across the cells (Roman *et al*., 2015). Maintaining adequate moisture levels during composting can be very difficult, especially in open areas. In practice, this challenge is often addressed through

temperature monitoring, which provides valuable information about when to turn, hydrate, or aerate compost pile (Tiquia and Tam, 1998) Insufficient initial moisture content (less than 30%) results in rapid water loss of compost pile and result in alteration of biological process. (De Bertoldi *et al*., 1983). In contrast, higher moisture content (more than 80%) form anaerobic conditions in the compost. Consequently, determining the optimum water level is very important for the composting process (Yulipriyanto, 2001). Due to the combined effect of increased temperature and forcing of air, the moisture content decreases, causing vapor to disappear as the water evaporates

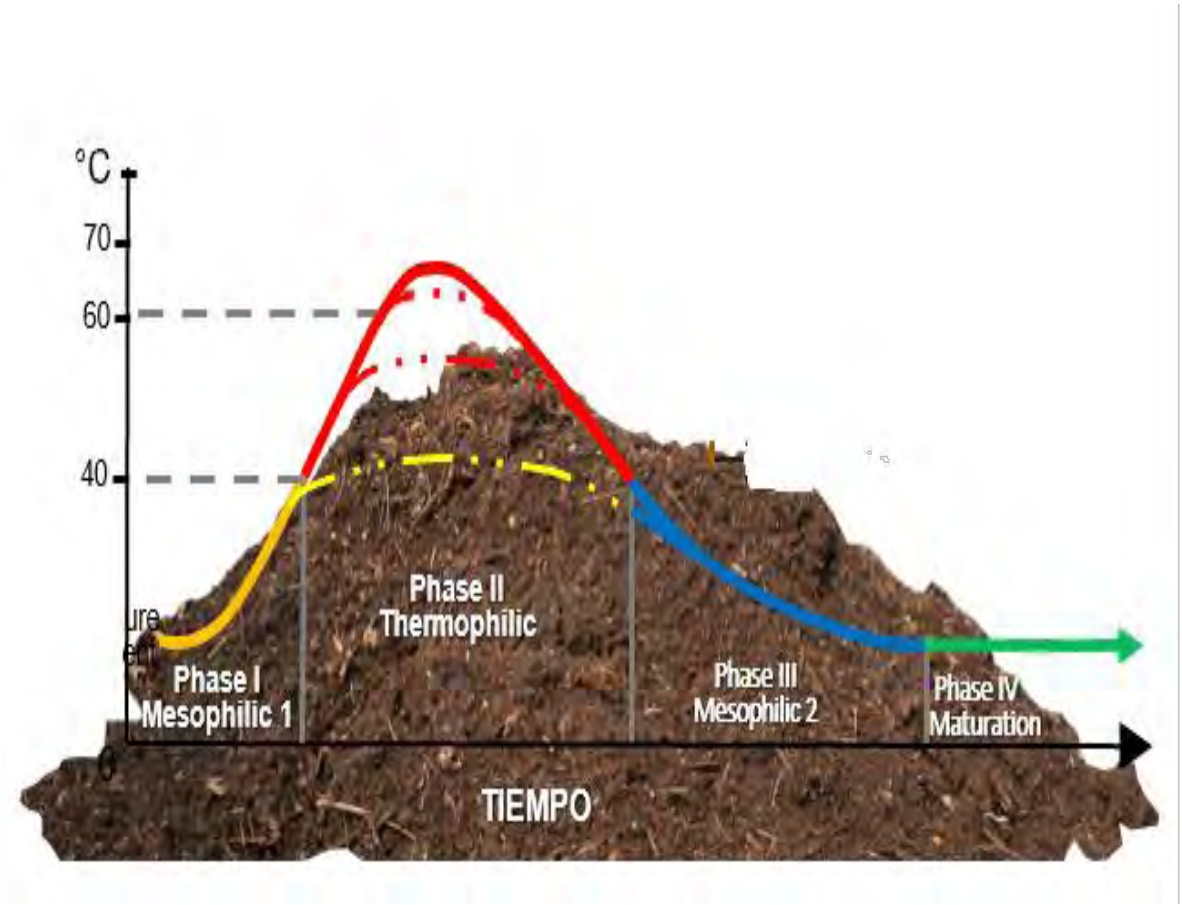


Figure 2.1 Different thermal phases of composting (https://opanatura.com/compost-and-composting/)

2.7.3.3. Microbial activity

Moisture content exerts a significant influence on microbial activity. In arid conditions, microbial activity reduces, while in waterlogged conditions, aerobic activity decreases

due to reduced air supply. The recommended optimal moisture content, as suggested by Epstein in 1997, falls within the range of 40% to 60% on a mass basis. Composting involves the development of various microorganisms, which progress in response to the temperature of the composting mass, marking distinct stages of the process, as highlighted by Keener *et al*. in 2000. Initially, bacteria dominate the composting process, with fungi being present throughout but becoming predominant when moisture levels drop below 35%, while they remain inactive at high temperatures.

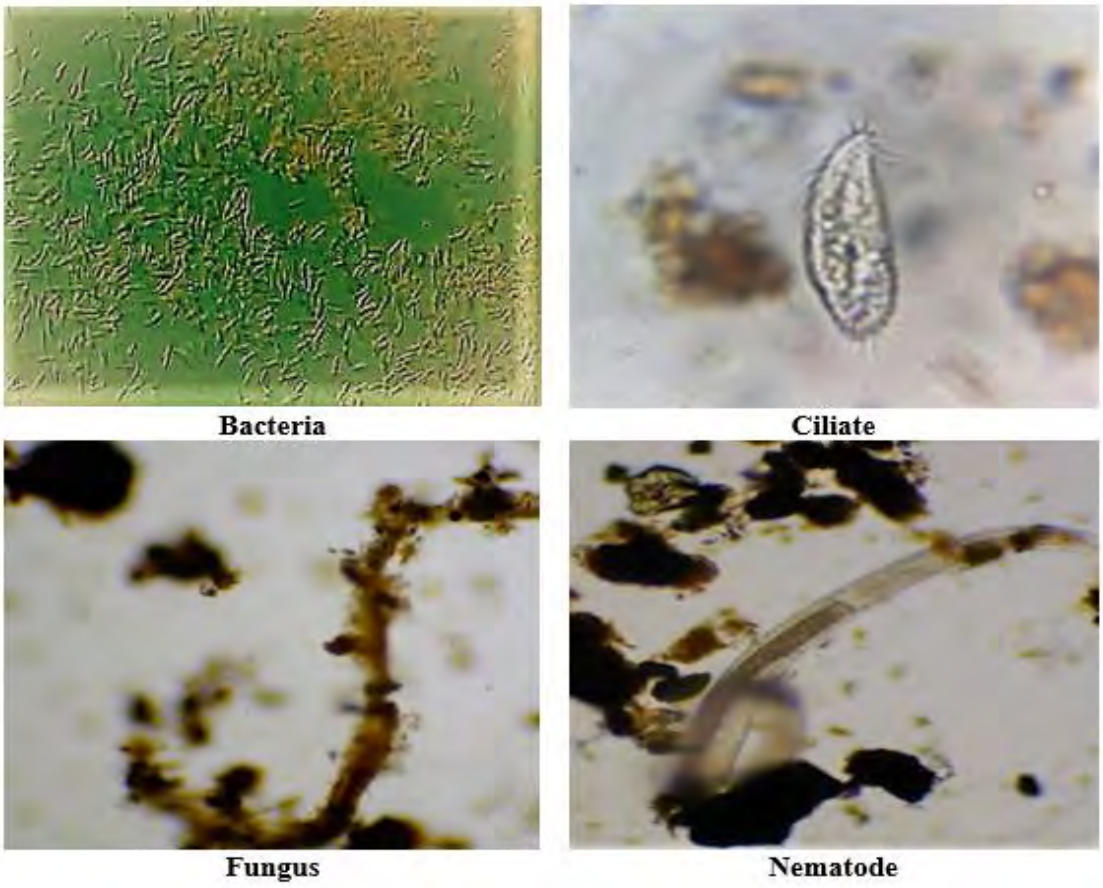


Figure 2.2: Microorganisms involved in composting process

2.7.3.4. Porosity and Bulk density

How much material is contained within a specific volume is called bulk density of compost. This density also influences the mechanical characteristics like strength, porosity, and compaction ease. Generally, dry bulk densities tend to fall within the range of 100-400 $kg.m^{-3}$, while wet bulk densities come in the range of 500- 900 $kg.m^{-3}$ as outlined by Agnew and Leonard in 2003. Higher bulk density values indicate increased mass but reduced porosity and air volume. Porosity allow air to aerate

compost pile to create aerobic conditions for microorganisms to degrade organic materials.

2.7.3.5. Oxygen content

In compost piles, the porosity (or pore space) must facilitate in aerobic conditions. To maintain optimal aerobic conditions within the compost pile, a minimum oxygen (O_2) content of 5% in the pore space is required, while anaerobic conditions occur when

there's less than 1% O_2, according to Mustin in 1987. Anaerobic compartments may also develop in less aerated sections of the compost pile, as indicated by Finstein *et al*. in 1999. In the initial phase of composting the oxygen demand increased 15 to 20% due to the increase in microbial number and decreases in curing and maturation phase. Oxygen is reliant on the porosity of the compost pile, so careful consideration must be given to factors like the size and shape of organic particles and the moisture content of the pile. Additionally, it's important to ensure that the concentration of carbon dioxide (CO_2) does not exceed 15%, as described by Mustin in 1987.

2.7.3.6. pH

A pH range of 6.7 to 9.0 is beneficial to encourage strong microbial activity during the composting process. The most favorable pH values fall within the range of 5.5 to 8.0, as suggested by De Bertoldi *et al*. in 1983 and Miller in 1992. It assumes significant importance in managing nitrogen losses through ammonia volatilization, which can be notably high when pH exceeds 7.5 as reported by De Bertoldi *et al*. in 1983, organic materials can undergo composting across a wide pH range, from 3 to 11, with the optimal values ranging between 5.5 and 8. Nearly neutral pH levels are ideal for the development of microorganisms, although fungi exhibit greater tolerance for pH deviations from neutrality compared to bacteria. In the initial phases, the pH value of composting decreases because of decomposition of organic materials which releases organic acids as described by McKinley and Vestal in 1985. Towards the end of the composting process, it is also possible for the pH to become acidic due to the release of H+ ions during nitrification, as noted by Fang and Wong in 1999.

2.7.3.7. Organic carbon (OC)

Organic carbon (OC) is the main component of organic waste. The total C content includes both total organic carbon and inorganic carbon in the form of carbonates and bicarbonates. Typically, total organic carbon makes up over 90% of the total carbon found in composts, as reported by Navarro *et al*. in 1993. In its raw state, green waste contains approximately 20 to 30% total organic carbon, as indicated by Riffaldi *et al*. in 1986 and Vallini *et al*. in 1993. Household waste comprises around 25 to 50% TOC, according to Avnimelech *et al*. in 1996, while sludge contains about 30 to 40% TOC, as observed by Diaz-Burgos *et al*. in 1993, Ayuso *et al*. in 1996, and Bernal *et al*. in 1998.

Throughout the composting process, the TOC content reduces as microorganisms break down organic substances essential for their metabolism, ultimately converting them into carbon dioxide (CO_2) through mineralization. As described by Beck-Friis *et al*. in 2003, methane emissions primarily occur during the thermophilic phase and typically account for less than 2% of the initial TOC in poorly ventilated compost piles.

2.7.3.8. Nitrogen

During the composting process, the organic nitrogen in the waste is primarily mineralized, forming ammonium (NH_4^+) and nitrate (NO_3-), especially when the nitrogen in the form of a portion of this mineral nitrogen is reabsorbed by microbes for their metabolic activity during composting, some combine with the organic matter in compost and part released in the form of inorganic N (nitrogen), as described in Larsen and McCartney 2000. As composting proceeds and reaches its final stage, the mineralization process becomes more pronounced, often increasing nitrate (NO_3^-) concentrations, a commonly observed phenomenon, as noted in 2001 by Sanchez-Monedero *et al*. As a result, an overall increase in total nitrogen content of matured compost occurred.

2.8. Compost tea

Compost teas (CTs) are produced by mixing of mature compost with tap water, usually in a ratio of 1:5 or 1:10 (volume/volume), followed by a specific incubation period (Finstein *et al*. in 1999). CT properties are influenced by various factors, such as

compost type, compost-to-water ratio and aeration, which together promote the growth of beneficial microorganisms. Furthermore, CTs retain soluble nutrients, valuable substances, and a variety of microorganisms (including bacteria, *actinomycetes*, filamentous fungi, *oomycetes*, and yeasts) that together contribute to disease prevention and plant growth (González-Hernández *et al*., 2023). Soluble nutrients, plant growth regulators, and humic acids are recognized as mediators for plant disease prevention (Keener *et al*., 2000). Compost tea usually contains several plant growths promoting

rhizobacteria (PGPR) that promote plant growth through various mechanisms, like nitrogen fixation, nutrient solubilization, growth hormone release, and enzyme production. These mechanisms of plant resistance also appear to be stimulated, enabling plants to withstand subsequent stress (González-Hernández *et al*., 2023).

2.8.1. Compost tea brewing methods

Compost tea can be produced using two brewing methods: aerated and non-aerated. The brewing or fermentation procedure involves soaking compost in water at a consistent temperature for a specified duration. This allows for the extraction of nutrients and microorganisms from the compost. Through this brewing process, microorganisms play a key role in converting insoluble nutrients into a form that is accessible. The soluble nutrients, in turn, foster the development of a diverse community of organisms within the compost tea

i. Non-aerated compost tea (NCT)

The traditional method involves a "passive" brewing process that requires no oxygen, resulting in Non-Aerated Compost Tea (NCT). It relies on solid sugar-free compost, under low oxygen conditions with periodic stirring of the extract. Although some recent publications refer to this process as anaerobic, it should be noted that since the reaction takes place in an open fermentation vessel, so the term "anaerobic" does not accurately describe this process. The average non-aerated compost tea brewing time is fourteen (14) days.

ii. Aerated compost tea (ACT)

A more modern method involves an "active" process using an aerator to introduce oxygen during the fermentation process and produces aerated compost tea (ACT). A

shorter brewing time period for aerated compost tea (ACT) ranging from 12 hours to three days. Nutritional additives and microbial-rich fermented products are often introduced during brewing to increase the beneficial microorganisms in compost tea.

Figure.2.3: Simple aerated compost tea Brewer (https://gardentherapy.ca/compost-tea-brewer/)

2.8.2. Factors influencing the compost tea process

The literature lacks extensive data directly comparing various processes for producing compost tea (Scheuerell 2006). Nevertheless, available data suggests that both aerated and non-aerated compost teas can exhibit batch-to-batch variations. These inconsistencies have been associated with several factors, including compost grade, compost-to-water ratio, brewing time, fermentation nutrients, microbial supplements, aeration, filtration, and dilution before application.

2.8.2.1. Compost grade

The compost consists of animal waste, soil agricultural plant material, biosolids, and food waste. Each of these materials has unique properties that play a role in the

characteristics of the resulting mature compost (Scheuerell 2002). Certain studies suggest that the microbial composition in compost is influenced by the type of feedstock. For instance, feedstocks abundant in carbon, such as dry leaves, sawdust, wood chips, and shredded newspaper, tend to yield compost with a higher fungal content. On the other hand, feedstocks rich in nitrogen, including hay, weeds, coffee grounds, herbaceous material, and manures, typically result in compost with a higher bacterial content. (Scheuerell *et al*.,2006). In addition, vermicompost with high nutrient content is often used as an ingredient in mass production of compost tea. Therefore, regardless of the method used, the compost selection should match the intended use. Mature compost must stable and be free from pathogens, while immature compost can be unstable and can harbor pathogens. Ensure that key indicators of compost stability are temperature fluctuations and carbon-nitrogen ratio (C:N) in the compost system. As the compost matures and hardens, the C:N ratio decreases (Noble *et al*.,2005).

2.8.2.2. Compost-to-water ratio

According to recent literature which indicate that the compost-to-water ratio (v/v), tends to vary depending on the production method. In the case of non-aerated compost tea, most studies commonly employ ratios ranging from 1:3 to 1:10, as observed by (Scheuerell *et al*. in 2002). However, for aerated compost tea, the specific ratio depends on the type of equipment used and is typically recommended by suppliers of compost tea equipment.

2.8.2.3. Brewing time

Regarding brewing time in the context of non-aerated compost tea (NCT) and its disease-suppression properties, multiple studies have consistently indicated that an ideal fermentation period for achieving effective disease control falls within the range of 8 to 16 days, as documented by Scheuerell in 2002. An extended brewing duration facilitates the enhanced extraction of nutrients from the compost and facilitates the accumulation of antibiotics. These antibiotics, in turn, activate natural plant defense

mechanisms, contributing to disease suppression, as suggested by Scheuerell in 2003.On the other hand, an alternative view point proposes that the optimal brewing period for compost tea is shorter, typically ranging from 18 to 24 hours. This time frame is believed to align with the maximum activity of microbial populations within the tea, as highlighted by Ingham in 2005.

2.8.2.4. Nutrient supplements

Substances like kelp, fish hydrolysate, molasses, and humic acid are introduced into the brewing process of compost tea to serve as catalysts or microbial starters, as documented by Naidu in 2010 and Scheuerell in 2002. These additives are utilized to facilitate the targeted enrichment of specific microorganisms. Furthermore, numerous compost tea equipment manufacturers offer pre-packaged nutrient blends with comparable compositions for added convenience. In both aerated compost tea (ACT) and non-aerated compost tea (NCT), these fermentation nutrients possess the capacity to either inhibit or stimulate the growth rates of various organisms, as indicated by Scheuerell in 2004. Recent research findings have highlighted that the addition of substances like molasses or other simple sugars in compost tea has the potential to encourage the proliferation of human pathogens such as *Salmonella* and *E. coli*, particularly when residual levels of these pathogens are present in the compost source, as noted by Ingram in 2007.

2.8.2.5. Microbial supplements

Compost contains a variety of microorganisms, especially bacteria and fungi, which play an important role in the decomposition of organic matter (Brinton *et al*., 2004). Bacteria can grow in both aerobic and anaerobic condition. Some of bacterial species isolated and observed in mature compost are, *Enterobacteria*, *Serratia*, *Nitrobacteria*, *Pseudomonas*, *Bacillus*, *Staphylococcus*, and *actinomycetes* various types. In parallel, fungal species such as *Trichoderma spp* (Brinton and Droffner, 1995). Some of these microorganisms are classified as “facultative anaerobes,” meaning they can thrive in low-oxygen environments, and even adapt to aerobic conditions. It is likely that these facultative anaerobes with compost that maturity is associated with disease-preventive properties Studies have shown that compost added to soil or soil-free growing materials effectively prevents various fungal root rot diseases (Hoitink *et al*., 1996). Primarily

ACT is associated with high levels of aerobic bacteria (Ingham 2005), whereas NCT usually refers to bacterial populations that are primarily facultative anaerobes (Weltzien 1991, Ingham 2005). Additional microbial formulations include fungi from the genus *Trichoderma* due to their ability to function as biocontrol agent since 1920s with successful results in maize crop (Harman and Hadar 1983) (Harman and Gary, 2011). *Trichoderma* species naturally live near plant roots, feeding on or parasitizing pathogenic fungi. In the absence of pathogenic fungi in the soil, the addition of *Trichoderma* may be of little or no value, these beneficial organisms will die-off without feeding on pathogens (Ingham 2005 and Sullivan 2004).

2.8.2.6. Aeration

The role of air or oxygen supply in the aerated compost tea (ACT) brewing process is believed to promote the growth and proliferation of various beneficial microorganisms extracted from compost, as shown by Ingham in 2005. Little or no oxygen in time can provide favorable conditions for growth, (Ingham 2005, Scheuerell *et al*., 2004). However, it is important to recognize that there is currently no scientific data to support the widespread claim that only low-oxygen conditions ideal for growth of most pathogens or aerobic conditions simply allow beneficial microorganisms to grow, according to Scheueller proposed in 2002. Early studies on anaerobic compost teas (NCTs) showed that transient low- oxygen brewing conditions can increase microbial activity as well as NCT diseases prevention (Scheuerell 2004). Also, disease prevention observed in microbial populations and laboratory tests with NCT sterilization, as reported by Scheueler *et al*., 2002. NCT and ACT brewing techniques comparison of aeration or not upon doing so, and with or without supplementary nutrients to control the fungus and Pythium wilting in cucumber shoots, the results did not show any significant association between microbial counts in continuously aerated compost tea and disease control.

However, the addition of nutrients to ACT during brewing consistently showed the best inhibition of Pythium damping off, suggesting that nutrients, rather than aeration, support microbial activity in ACT, as reported by Scheuerell in 2006. Furthermore, it is worth noting that the aeration during compost tea production process is usually materials. NCT odors have only been reported in cases where supplemental fertilizers

were introduced during fermentation, as observed by Scheuerell in 2006 and 2002. The need for aeration during composting tea remains is invisible, and note that aerated compost tea, or oxygenated tea is effectively anaerobic it occurs if not used immediately. Producers and users of ACT should consider the additional costs associated with brewing process.

2.8.3. Application of compost tea

i. Disease suppression

Biological interactions for control of plant pathogens are complicated by the dynamic nature of diseases caused by environmental pathogens. This interaction is believed to be mediated by the following mechanisms so that it occurs.

ii. Antibiosis

Some beneficial organisms have the ability to produce antibiotics or other compounds that exhibit toxicity against pathogens. For example, the bacterium *Pseudomonas fluorescens* produces hydrogen cyanide, which inhibits the growth of various bacteria. Other bacteria such as *Bacillus*, *Serratia* and fungal specie *Trichoderma* are able to effectively produce antimicrobial compounds from plant tissues, (Haas and ,2005).

iii. Competition

The growth medium contains beneficial microorganisms they often compete with pathogens or fungi for food sources (Hoitink 1993).

iv. Induced Resistance

Certain beneficial microorganisms living in plant roots or leaves have been found to induce plant resistance by activating genes that confer resistance to pathogens, as noted by Haas and Defago in 2005.

v. Parasitism

A few beneficial microorganisms have the ability to feed on specific pathogens. For example, *Trichoderma* species have been shown in studies to produce enzymes that break down the cell walls of some fungal root pathogens, as described by Handelsman in 1996. However, there are few control studies in aerated compost tea (ACT).,

especially when compared directly to non-aerated compost tea (NCT). (Conforti *et al*., 2002) conducted a field trial at Presidio Golf Course to evaluate the effectiveness of ACT in promoting turf growth and controlling common fungal disease (*Microdochium* Patch/*Fusarium* Patch) caused by *Microdochium nivale*. Compost prepared from vermicompost, wood chips, straw clippings, equivalent horse manure will be used, and horse bedding, on site. This compost is cured for at least four months before it is ready for tea. During brewing, fertilizers such as molasses, sea kelp, cane sugar, rock dust and yeast were added to the ACT. The resulting tea was applied to the green leaves at the rate of one gallon of compost tea per 1000 ft2 for twelve months. It was applied weekly during periods of high disease pressure and bi-weekly during periods of moderate or low disease pressure. Studies have shown that treated grasses appear to have significantly longer root lengths compared to untreated grasses. The field trial conducted in Ontario Canada (Hsiang 2007). The control of visible dollar spots caused by this fungus *Sclerotinia homoeocarpa* was observed. Fungicide testing was also conducted on neighboring plots, and the results were compared. Different levels of disease prevention were observed when ACT was used, ranging from 49% to 86%. The differences in suppression were due to the specific nutrient and nutrient sources used. The highest strain (86%) was observed when ACT was prepared using a mushroom solution ACT.

vi. Improve soil structure

Compost is a diverse size group of microorganisms, humic acids, and organic compounds such as carbon and nitrogen. These elements support soil health and promote healthy plant growth. While compost is not a fertilizer (compost enriches the soil, while fertilizer feed plants directly), high quality compost can increase soil moisture, density and enhance nutrients uptake by the plant. A review of the literature indicates that composted teas may retain some of the same benefits of compost to varying degrees. Compost tea can be made in shorter time period and can be applied directly to plant surfaces. However, it is important to note that the effects of compost tea do not last very long, and frequent applications are required to fill the plant or soil surface with nutrients and beneficial microorganisms These data are research based on by Brinton in 1995, Scheueller in 2002, and Ingham in 2005.

3. Material and Methods

3.1. Compost and composts tea preparation, isolation and screening of PGP *Bacillus* strain having antifungal activity

3.1.1. Site for compost pile

The composting process of organic waste materials was done at research field of faculty of Biological Sciences QAU, Islamabad located (33º44'56" N 73º08'7.6" E). The ground was cleared of weeds and other vegetations. The selected area was under partial sunlight and well drained to prevent waterlogged compost which creates anaerobic conditions and affects the composting process.

3.1.2. Compost pile preparation

The organic waste materials, which included dry leaves, grass clippings, trimmed bushes, and wood chips, straw, and small twigs were collected from gardens and research field of Biological Sciences faculty QAU, Islamabad. The area for the composting process was cleared of weeds and vegetation. Dry and green organic materials were continuously layered to create a compost pile with dimensions of 2 meters wide, 1-meter-high, and 3 meters long. Started with a 10-inch layer of brown, carbon-rich materials (dried leaves, straw, trimmed bushes, wood chips, straw, and small twigs). This layer acts as the foundation and helps provide aeration to the pile (Abdel-Haleem *et al*., 2022). Then added a 3-inch layer of green, nitrogen-rich materials like fruit and vegetable peels and fresh grass clippings. These materials provide nitrogen and other essential nutrients for the compost pile. Sprinkled water and soil between each organic layer to help introduce beneficial microorganisms and wet the pile to aid in the decomposition of the organic matter. The composting was performed in piles covered with a black plastic sheet to retain temperature and moisture. The aeration of the compost pile was performed by turning and mixing the pile on-site after every 15 days. The ratio of brown (carbon rich) and green (nitrogen rich) wastes was set at 25:1. A water content of about 55–60% was maintained throughout the composting process.

3.1.3. Aerated compost tea preparation

The solution of aerated compost tea was prepared of sieved mature compost in Applied and Environmental Microbiology Laboratory, QAU Islamabad by soaking 500 g of matured compost was taken in a compost tea bag and dipped into 10 liters of tap water for three days in a plastic Can (container) of 20 liters with continuous air provided by aquarium air pump. Three teaspoons of unsulfured molasses were added in water (plastic Can) for initiation of bacterial growth. (Ingham, 2005) and (Khan *et al*., 2011). Compost tea brewed for 3 days, and tea bag was shacked on daily basis. The prepared aerated compost tea was added and sprayed every 2 weeks 50 ml per pot (Abdel-Haleem *et al*., 2022) and Abou-El-Hassan, 2010).

3.1.4. Isolation and screening of PGP *Bacillus* strain from rhizosphere soil samples

Different rhizospheric soil samples of *Solanum lycopersicum* (tomato plants) were collected from tomato growing area of Urmar Payan (33°96'101"N 71°73'011"E), Chamkani (34º00'19.46" N 71º38'56.97"E), and Choha Gujar (33º59'57.17" N 71º37'48.51" E) Peshawar District, Pakistan. Closely adhered soil from plants roots were separated and kept in sterilized plastic bags. The samples were carried to the Applied and Environmental Microbiology Laboratory, QAU Islamabad, and then kept in a refrigerator at 4 ºC for further processing.

3.1.5. Soil enrichment

Enrichment was done in Minimal Salt Medium (MSM). One gram of rhizospheric soil was added into sterilized Erlenmeyer flasks containing MSM and incubated in a shaker incubator at 120 rpm, at 37℃ for approximately one week. After said time, serial dilution was accomplished for further processing. Table 3.1 shows the chemical composition of MSM. Rhizosphere soil of *Solanum lycopersicum (tomato plant)* was homogenized in 100 ml of distilled water in conical flask (250 ml) by continuous shaking for 10 minutes. One ml homogenized suspension of the rhizospheric soil was transferred separately in to 9 ml sterile distilled water to form 10^{-1} dilution and dilutions were made up to 10^{-7} (7 times tenfold dilution). 100 µl of each dilution from each sample was taken and spread in the plates. The plates were incubated at 32 – 37 ºC for 1-2 days.

Table 3.1: Chemical composition of MSM

Macronutrients	**Concentration (g/L)**
K_2HPO_4	10 gm
$NaH_2PO_4.2H_2O$	5 gm
$NaNO_3$	2 gm
$MgSO_4.7H_2O$	0.2 gm
$CaCl_2.2H_2O$	0.01 gm
$FeSO_4.7H_2O$	0.08 gm
Glucose	20 gm
Micronutrients	**Concentration (g/L)**
$MnSO_4.4H_2O$	0.80 mg
$CuSO_4.7H_2O$	1.20 mg
$ZnSO_4.\ 7H_2O$	1.40 mg
$CoCl_2.6H_2O$	1.20mg

3.1.6. Isolation of *Bacillus* species and compost isolates

For isolation of *Bacillus spp*. after enrichment of rhizosphere soil, serial dilution and spreading of serially diluted samples on plates containing Hi-Crome *Bacillus* Agar while for the isolation of bacterial isolates from compost same procedure was followed, however, spreading was done on nutrient agar plates. Both plates were incubated at 37℃ for 1-2 days.

3.1.7. Screening of PGP *Bacillus* species and compost isolates

A total of 24 strains, 20 from compost and 4 isolates of rhizospheric soil bacteria were screened for PGP tests including phosphate solubilization, zinc solubilization, nitrogen fixation, protease, catalase, cellulase assay and oxidase production.

3.1.8. Secondary screening of PGP *Bacillus* strains based on antifungal activity

All isolated rhizosphere soil bacterial strains (S1-S15) were screened for their antifungal potential. Out of 15 strains, 4 bacterial strains S1, S2, S4 and S10 showed strong antifungal potential and were selected for further characterization.

3.1.9. Antifungal activity of selected *Bacillus* strains

3.1.9.1. Point inoculation

A dual culture assay was applied to screen the antifungal activity of selected *Bacillus* strain and zone of inhibition was measured. Spore suspension of three isolated fungus *Fusarium oxysporum* (causative agent of seedling rot), genus *Aspergillus flavus* and genus *Aspergillus niger* was prepared in normal saline or distilled water in Eppendorf tubes. The isolated fungi inoculated in the form of lawn on the SDA medium and the *Bacillus* was point inoculated against the test fungi and incubated at 37℃ for examining after 24, 48, 72, 96 and 120 hours.

3.1.10. Percent growth inhibition

Using dual culture assay demonstrated by (Amna *et al*., 2020) with slight modification, 9 mm petri plates were taken containing SDA medium and the bacteria was inoculated by single line streak 3 cm apart from edge of plate. Plug of 5cm was inoculated towards the other edge 3 cm apart from the bacterial streak line. A plate with only fungal mycelial plug was used as control. The incubation period of five to seven day at 27±2℃ was fulfilled. Each treatment was subjected to replication. The formula of percent growth inhibition is given below.

Growth inhibition (%) = (radius of fungus cultured alone − radius of fungus cultured with bacteria) / radius of fungus cultured alone ×100.

3.1.11. Identification of selected *Bacillus* strains and compost isolates

3.1.11.1. Morphological characterization of *Bacillus* species

Selected bacterial strains S1, S2, S4 and S10 were streaked on LB agar plates and incubated at 30 - 32 ºC for 1 day. The bacterial colonies were observed for the colony

morphological traits including Gram's staining, color of colony, colony margins, colony shape, colony surface texture, and colony elevation.

3.1.11.2. Biochemical identification

i. Citrate utilization test

This test confirms the utilization of citrate as carbon source and ammonia as nitrogen source. The strain was streaked on the slant of Simmons citrate agar and incubation period of 24 hours at 37℃ was given, growth on the slant and colour change from green to blue show positive result.

ii. Indole test

Indole is the metabolic product of tryptophan amino acid. To confirm the presence of indole, the strain was grown in tryptone broth with an incubation period of 24 hours at 37℃. After one day, few drops of Kovac's reagent were poured slowly along the tube wall and change in colour from yellow to orange at the top was noticed.

iii. Triple Sugar Iron test

Freshly cultured bacterial colony was inoculated with the help of inoculating needle by stabbing from the center to the bottom of the test tube then streaked on the surface area of the Triple Sugar Iron (TSI) agar. The confirmation of fermentation of glucose, sucrose and lactose, production of hydrogen sulphide and acid was done after interpreting the result.

iv. Methyl Red

This test confirms the ability of bacteria to produce acid and maintaining its stability by fermenting glucose. *Bacillus* strain was inoculated in the methyl red broth and placed in incubator at 37℃ for 3 days. On the addition of methyl red reagent development of red colour is the indication of positive result showing acid production and yellow indicates no acid formation.

v. Catalase test

The test was used to detect the presence of catalase enzyme in selected bacterial strain. One-day old colony was placed on slide and mixed with 3% hydrogen peroxide solution. Existence of catalase test break the H_2O_2 into water and oxygen. Production of bubble was observed for the presence of catalase.

vi. Oxidase test

This test was used to confirm the existence of enzyme cytochrome-c oxidase in selected bacterial strain. One to two drops of oxidase reagent (tetra methyl para phenylenediamine) were poured on sterilized cotton swabs. The sterilized cotton swabs inoculated with bacterial culture and any change in colour was observed. Dark purple colour confirms the presence of cytochrome-C oxidase because it is oxidized into indole phenol product.

3.1.12. PGP activities of selected *Bacillus* strain and compost isolates

All bacterial strains were checked for various plant growth promoting characteristics using 24 hours old culture.

i. Protease production

For protease activity by bacterial isolates skim milk agar medium. The respective medium was prepared by adding (g/L) glucose,1 gm; peptone, 2gm; yeast extract, 5gm; K_2HPO_4, 1gm; $MgSO_4.7H_2O$, 0.2 gm; skimmed milk 5 gm; and agar, 15gm. After autoclaving the strains were spot inoculated in center of plates and placed in an incubator at 32 ºC for 2-3 days. Halo zone appearance surrounding the colony was indication of protease production (Nnolim *et al*., 2020).

ii. Cellulase assay

Cellulose Congo-red agar was employed to confirm the production of cellulase enzyme. One-day old bacterial colony was streaked on the plate containing Cellulose Congo Red Agar media. Discoloration of the media indicates the presence of cellulase after incubation period of 24 to 48 hours at 37℃. The pH of the media is 6.8 - 7.2 (Gupta *et al*., 2012).

iii. Phosphate solubilization assay

For the confirmation of solubilization of zinc oxide and phosphate, the bacteria were point inoculated on Pikovskaya's and zinc oxide individually, incubated at 27±2℃ for five to seven days. Halo zone around the colony indicates solubilization.

Table 3.2: Composition of Pikovskaya's Agar (Pikovskaya's Agar M520)

Ingredients	Concentration (g/L)
Dextrose	10
Yeast extract	0.5
Ferrous sulfate	0.0001
Ammonium sulfate	0.5
Potassium Chloride	0.2
Magnesium sulfate	0.1
Calcium phosphate	5
Magnesium sulfate	0.0001
Agar	15

iv. Zinc oxide solubilization assay

For the confirmation of solubilization of zinc oxide and phosphate, the bacteria were point inoculated on zinc oxide agar and incubated at 27±2℃ for five to seven days. Halo zone around the colony indicates solubilization.

Table 3.3: Composition of Zinc Solubilizing Agar (Di Simine *et al*., 1998)

Ingredients	Concentration (g/L)
Dextrose	10
Ammonium sulfate	1
Zinc oxide	1
Potassium Chloride	0.2
Magnesium sulfate, heptahydrate	0.2
DPHP	0.1
Agar	15

v. Nitrogen fixation assay

All bacterial isolates were screened for nitrogen fixation by inoculating in Jensen's media. After 2 days, bacterial growth at 32 °C indicated nitrogen fixing activity.

Table 3.4: Composition of Jensen's media (Di Simine *et al*., 1998)

Ingredients	**Concentration (g/L)**
K_2HPO_4	0.2
$MgSO_4.7H_2O$	0.2
KH_2PO_4	1.0
$FeSO_4 \cdot 7H_2O$	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.005
NaCl	0.2
Glucose	10
Agar	15

3.2. Physiochemical analysis

3.2.1. Physicochemical analysis of compost, compost tea and soil

The samples were carried to the Applied and Environmental Microbiology Laboratory, QAU Islamabad, and then kept in a refrigerator at 4 ºC for further processing. The compost tea Can (container) was shaken to dilute the solution and taken 3 samples of 50 ml into autoclaved falcons. The samples were stored at about 4°C in refrigerator for further processing. For lab analysis the soil sample was passed through a 2mm sieve and stored at 4°C for further process.

3.2.2. Analysis of moisture content and temperature of compost pile

Moisture content was analyzed using the standard oven-drying method by applying the formula.

$$\text{Moisture Content (MC\%)} = \frac{W-D}{W} \times 100$$

W=wet weight and D= dry weight (weight after drying sample)

A field thermometer was used to measure the compost pile temperature.

3.2.3. pH and electrical conductivity (EC)

The pH and EC were measure and estimated by using a digital pH and electrical conductivity meter after 1 hour shaking the prepared sample (1:10 w/v) (Teutscherova *et al*., 2018).

3.2.4. Bulk density of compost and soil

Bulk density of soil and compost were found by graduated cylinder technique. Taken 100 ml graduated cylinder added soil sample and compact the sample by dropping the graduated cylinder above 2–3inch height and then add soil sample up to a 50 ml mark used a spatula to level the top of sample in graduated cylinder added sample with the spatula the top of soil sample exactly levels with 50 ml mark. This was the bulk volume of sample. Weighted and recorded graduated cylinder + compact soil sample weight. Repeated the whole process for compost sample. Bulk density calculated by the formula.

Bulk density = D/ $B\ g/cm3 = Mm/Vh\ kg/m3$

Where: A= weight of graduated cylinder, B = bulk volume compacted soil, C = weight of cylinder + compacted sample, D = weight of sample (C - A).

3.2.5. Organic matter analysis of soil, compost and compost tea

Standard method recommended by Møller *et al*., 2000, was used, to examined soil and compost organic matter content by drying at 105 °C for 24 hours followed by weight loss analysis through ignition at 550 °C for 4 hours. The organic matter content of the compost tea was determined by Walkley-Black wet digestion. Total organic carbon (TOC): Organic carbon was determined based on 58% of total carbon released in organic matter (Nelson and Sommers, 1996) according to the following formula (TOC) = OM (58/100). Total soil nitrogen was determined by the micro Kjeldahl method described by (Jackson,1973). Also, C/N ratio: The ratio of total carbon to total nitrogen is calculated based on the following equation: C/N ratio = (% total carbon / % total nitrogen).

3.2.6. Analysis of microbial population of compost tea

The bacterial and fungal population of each aerated compost tea was analyzed directly, reported as colony forming unit CFU/ml by the aerated compost tea after 24 hours of fermentation. 1 ml samples of aerated compost tea were serially added to 7 test tubes containing 9 ml of autoclaved normal saline. 100 µl of diluted compost tea was serially spread evenly on five plates of nutrient agar (NA) and five plates of potato dextrose agar (PDA) while incubating at 37°C and 25°C for a minimum after 24 hours to 72 hours in PDA media were supplemented with molecular growth inhibition with Oxytetracycline antibiotic.

3.2.7. Microbial population of compost

One gram of grounded sample of compost was serially diluted into 7 test tubes containing sterile normal saline and labelled numbering wise from 1 to 7. One-gram grounded compost sample was taken into test tube 1 and mixed with pipette. From test tube 1, one ml was poured into 2nd test tube and mixed. The same procedure was performed till the last tube 7. Finally, 7 different serial dilutions were achieved. From

test tube 3, 4, 5, 6, 7, about 0.10 ml liquid was poured and spread on serial wise labelled plates containing nutrient agar for bacterial growth and five plates of Potato dextrose agar for fungal growth adding oxytetracycline antibiotic to PDA media to inhibit bacterial growth. The plates were incubated at 37℃ up to 2 days for bacterial and 27℃ for 7 days for fungal growth. Population densities were reported as colony forming unit (CFU)/g from each media.

3.3. Effect of compost, compost tea and selected Bacterial strain on plant growth parameters in greenhouse experiment.

A pot experiment was carried in green house at temperature range between 30-34 ℃. The sandy loam soil used in the current study was taken from research field Faculty of Biological Sciences QAU, Islamabad. The soil was air dried and passed through a 4 mm sieve for using in pot. The antagonistic *Bacillus* species were cultivated in nutrient broth and incubated at 37℃ in rotary shaker incubator at 150 rpm for 24hours. Broth was centrifuged and bacterial pellet was dissolved in autoclave distal water. The optical density (OD) of bacterial suspension was adjusted at 0.5 OD. soil was autoclaved and

placed in plastic pots of 12cm diameter. The sterilized tomato seeds were soaked in *Fusarium* fungal spore suspension and placed in shaker incubator at 27℃ for 36 hours at 150 rpm and then air dried in laminar hood. The infected seeds were soaked in *Bacillus* cells suspension for 2 hours and then sown in the pots.

3.3.1. Experimental design

The experimental design was carried out in pots. The pots were arranged in completely randomized design (CRD) and was followed to carry the experiment in triplicates and designed with positive and negative control and seven (7) treatments. There were two sets for experiment one for autoclave and one for non-autoclave soil. The experiment was carried for 45 days (Abdel-Haleem *et al*., 2022) (Limtong *et al*., 2020).

3.3.2. Experimental treatments

To inspect the efficacy of compost, compost tea, and bacterial consortia as a soil amendment and biocontrol agents for *Fusarium* wilt disease whole experiment was conducted in triplicates and designed with 9 treatments 1. Negative control (T-)

(no inoculum), 2. Positive control (T+) (with pathogen), 3. Compost (CM) 4. Compost + Compost tea (CM+CT), 5. Compost + Bacterial consortia (CM+BC) 6. Compost + Compost tea + Bacterial consortia (CM+CT+BC), 7. Compost tea (CT), 8. Compost tea+ Bacterial consortia (CM+BC), 9. Bacterial consortia (BC). 50% Compost was added as one dose during soil preparation, whereas the compost tea was added every 10 days 50 ml per pot (Sarker *et al*., 2012).

3.3.4. Measurement of plant growth parameters

After 45 days the plants were harvested, three plants were randomly chosen from each experimental treatment to determine plant fresh weight, dry weight, number of leaves, shoot length, root length, vigour index, chlorophyll content by using Minolta Chlorophyll Meter (Spad 501) of *Solanum lycopersicum* (tomato plants) were measured (Ahmad *et al*., 2018).

3.3.5. Statistical analysis

Statistical analysis was conducted by Microsoft Excel and Minitab® software to analyses the data and ANOVA was done. Means were compared by Turkey's multiple range test and means were considered significantly different by considering significance level of $p \leq 0.05$ (Limtong *et al*., 2020).

4. Results

4.1. Compost and Compost tea preparation from mature compost

The composting experiment was conducted using a combination of brown (carbon-rich) and green (nitrogen-rich) organic materials collected from the gardens and research field of the Faculty Biological Sciences at Quaid-i-Azam University, Islamabad. The compost pile, measuring 2 meters in width, 1 meter in height, and 3 meters in length, was carefully constructed following established composting principles. A complete mature compost and compost tea is shown in figure 4.3 (a) and (b).

Figure 4.1: Schematic process illustrating mature compost formation

Figure 4.2: In vitro synthesis of compost tea

a **b**

Figure 4.3: (a) Mature compost (b) Compost tea from a mature compost.

4.2. Isolation and screening of PGP *Bacillus* strain from rhizosphere soil samples having antifungal activity.

4.2.1. Primary screening of *Bacillus*

After serial dilution of soil samples collected from rhizosphere of agar tomato plant, bacterial strains were grown on HiCrome media. Growth of these bacterial strains is shown in figure 4.4

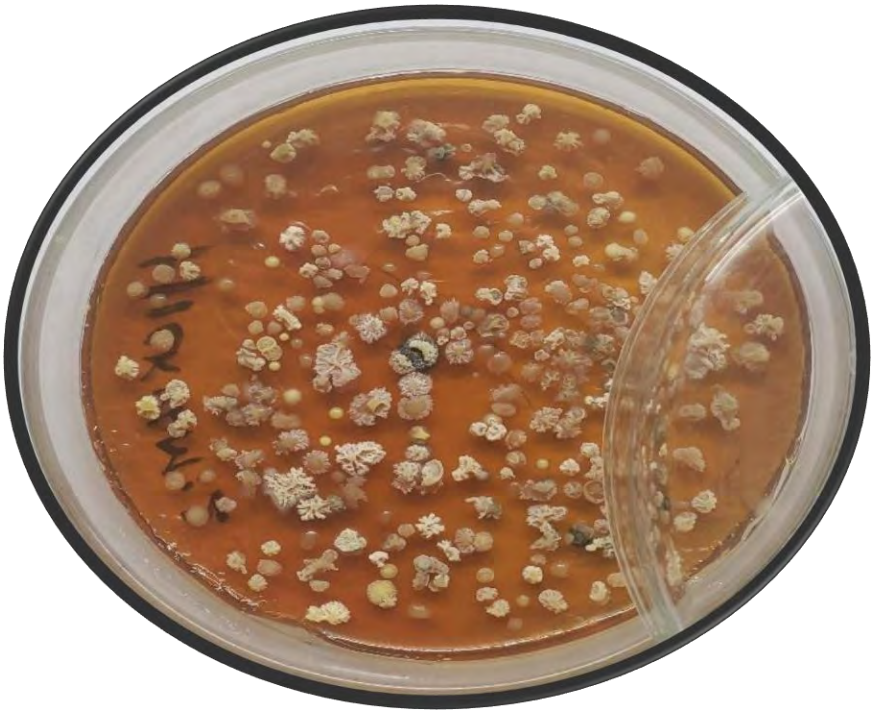


Figure 4.4 Growth of *Bacillus* stains on HiCrome media

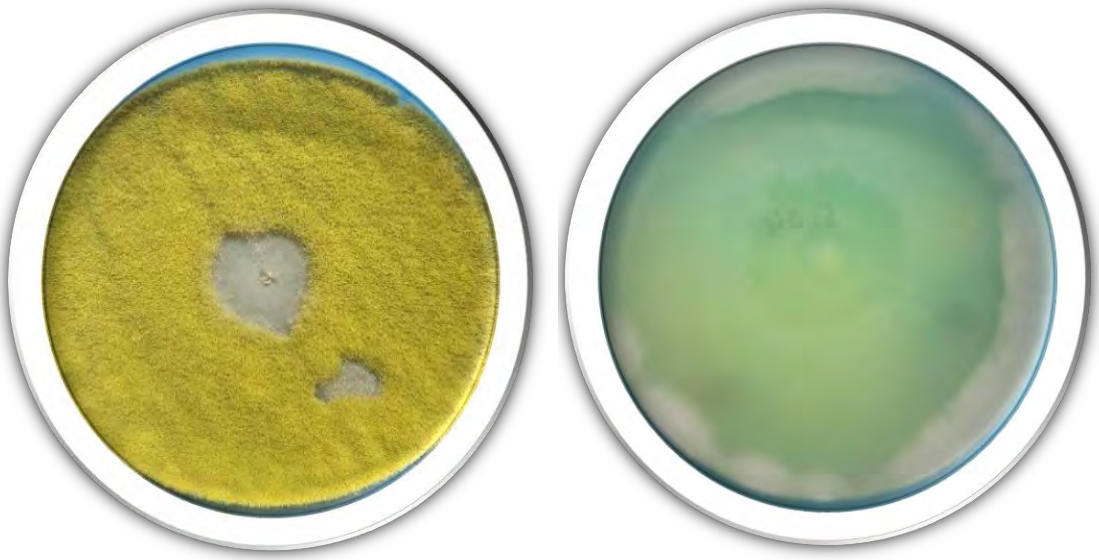
4.2.2. Secondary screening of selected *Bacillus* species based on antifungal activity

Out of fifteen selected bacterial strains, 4 strains were found with significant antifungal potential. The selected four *Bacillus* isolates were purified, preserved, and screened for antifungal activities against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*. *Bacillus* species were point inoculated on the SDA plates on which lawn of fungal spores' suspension was made. The plates were incubated at 27℃ for 5-7 days and zone of inhibition was measured in mm. The zone of inhibition produced by selected bacterial strains against *Aspergillus flavus*, *Fusarium oxysporum*, and *Aspergillus niger* species are shown in (Table 4.1). Maximum inhibition against

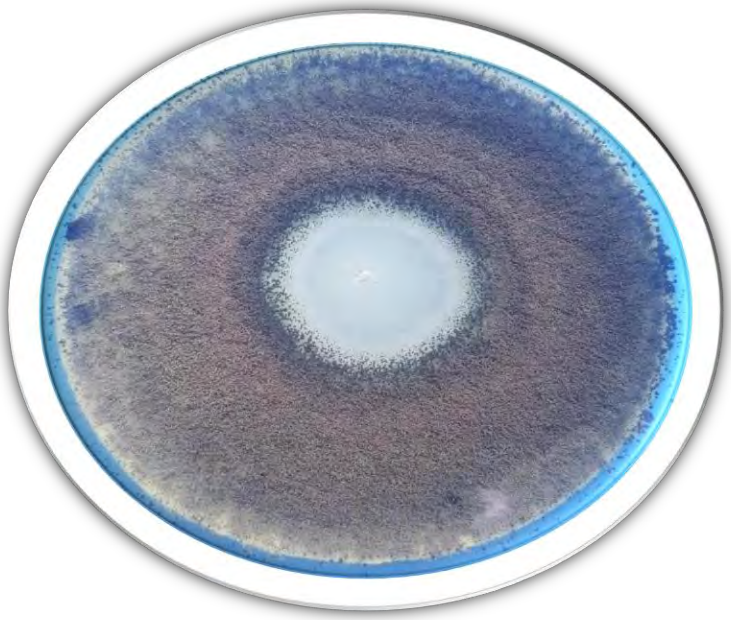
Fusarium oxysporum by S4 strain (71 ± 4.35 mm), S1 shown against *Aspergillus flavus* (55 ± 5.29 mm) while S10 shown against *Aspergillus niger* (46 ± 5.56 mm).

Table 4.1: Zone of inhibition by selected *Bacillus* species against *Fusarium oxysporum*, *Aspergillus flavus* and *Aspergillus niger*

Strain Code	**Zone of Inhibition (mm)**		
	Fusarium oxysporum	***Aspergillus flavus***	***Aspergillus niger***
S1	57 ± 5.0	55 ± 5.29	33 ± 4.58
S2	13 ± 4.0	45 ± 4.0	28 ± 5.0
S4	71 ± 4.35	10 ± 0.68	29 ± 2.64
S10	34 ± 3.0	34 ± 3.76	46 ± 5.56



A **B**



C

Figure 4.5: Zone inhibition of selected *Bacillus* strains against (**A**) *Aspergillus flavus* (**B**) *Fusarium oxysporum* (**C**) *Aspergillus niger*

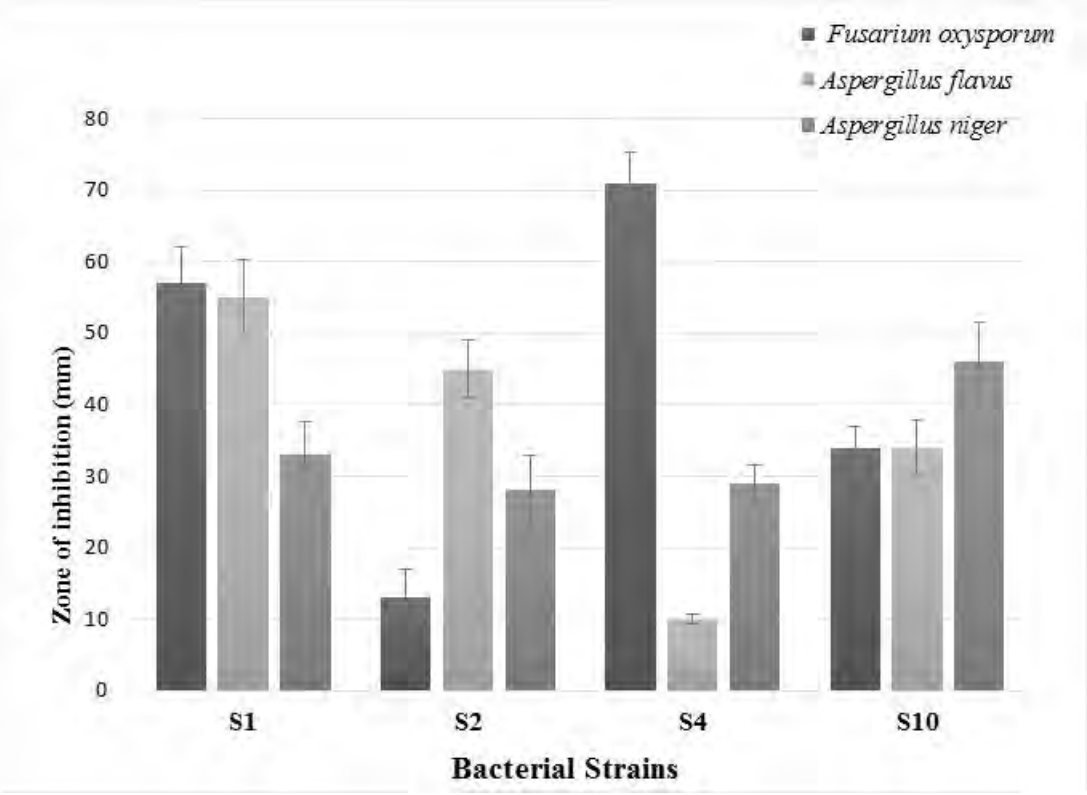


Figure 4.6: Zone of inhibition by selected *Bacillus* species against *Aspergillus flavus, Fusarium oxysporum*, and *Aspergillus niger*

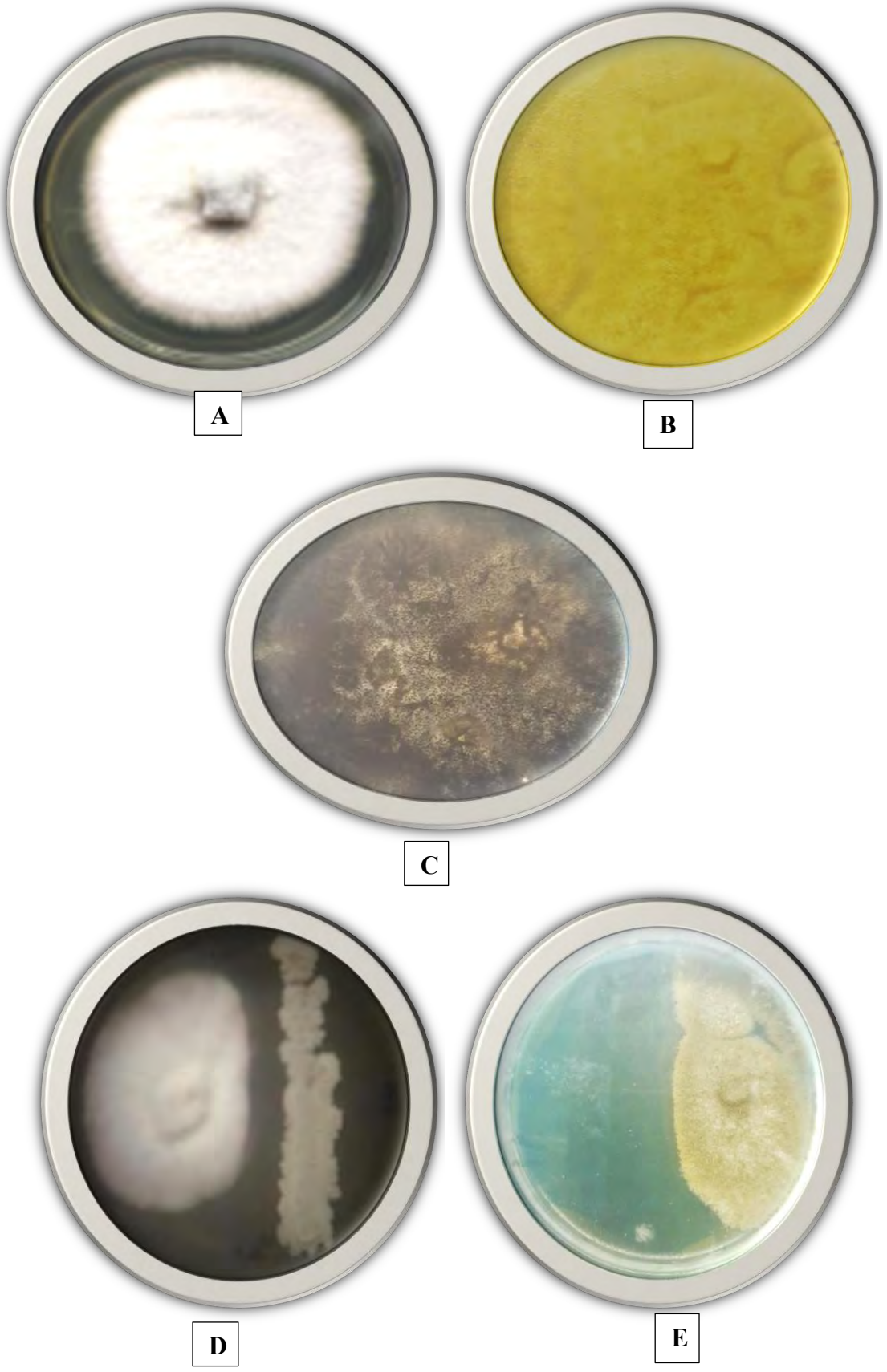
4.2.3. Percentage growth inhibition of fungi

Colony diameter of fungus inoculated with *Bacillus* strain was compared with the fungus inoculated alone on SDA after 5-7 days' incubation period at 27°C. The bacterial strains S1, S2, S4 and S10 reduced the *Fusarium oxysporum* specie growth by 41.9 ± 1%, 39.4 ± 1 %, 42.1 ± 3 % and 46.6 ± 1% respectively while in case of *Aspergillus flavus* and *Aspergillus niger* species, 57.77 ± 6 %, 46.44 ± 6%, 62.40 ± 1%, 30.62 ± 0%, and 51 ± 0%, 62.1 ± 0%, 66.6 ± 0%, 44.3 ± 1% respectively. Results of percentage growth inhibition of fungi by selected bacterial strains as shown in table 4.2 and figure 4.7.

Table 4.2: Percentage growth inhibition shown by selected *Bacillus* strains against *Fusarium oxysporum, Aspergillus flavus*, and *Aspergillus niger*

Strain Code	Inhibition assay of *Fusarium oxysporum*		Inhibition assay of *Aspergillus flavus*		Inhibition assay of *Aspergillus niger*	
	Mycelial growth (mm)	Mean % inhibition (±SD)	Mycelial growth (mm)	Mean % inhibition (±SD)	Mycelial growth (mm)	Mean % inhibition (±SD)
S1	43	41.9 ± 1	43	57.77 ± 6	35	51 ± 0
S2	56	39.4 ± 1	46	46.44 ± 6	25	62.1 ± 0
S4	53	42.1 ± 3	44	62.40 ± 1	21	66.6 ± 0
S10	45	46.6 ± 1	52	30.62 ± 0	41	44.3 ± 3

Each value represents a mean of three replicates while (±SD) indicate standard deviation


A
B
C
D
E

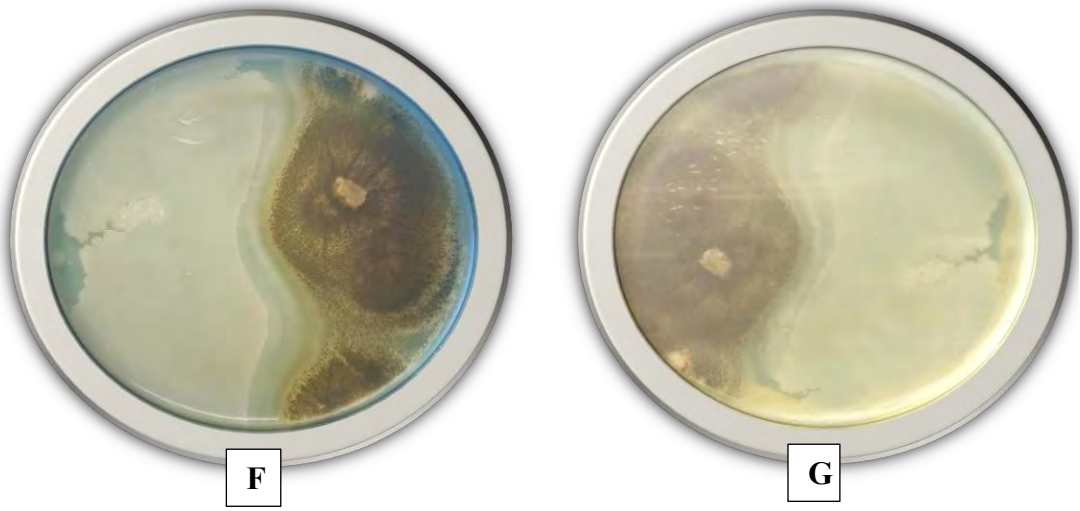


Figure 4.7: Percentage Growth inhibition of controls (**A**) *Aspergillus flavus* (**B**)*Fusarium oxysporum and* (**C**) *Aspergillus niger* shown by selected *Bacillus* Strains (**D**) S10 against *Aspergillus flavus (***E**) S1 against *Fusarium oxysporum (***F**) S2 against *Aspergillus niger (***G**) S4 against *Aspergillus niger*

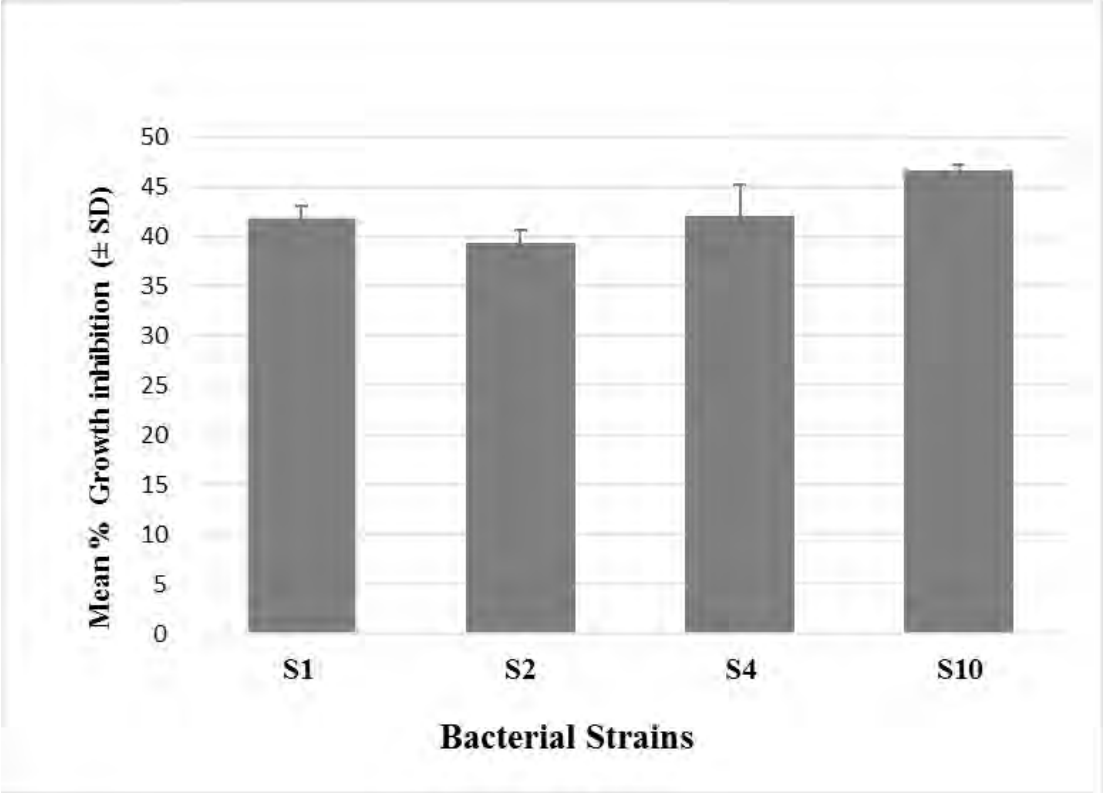


Figure 4.8: Percentage growth inhibition of *Fusarium oxysporum* by selected *Bacillus* strains

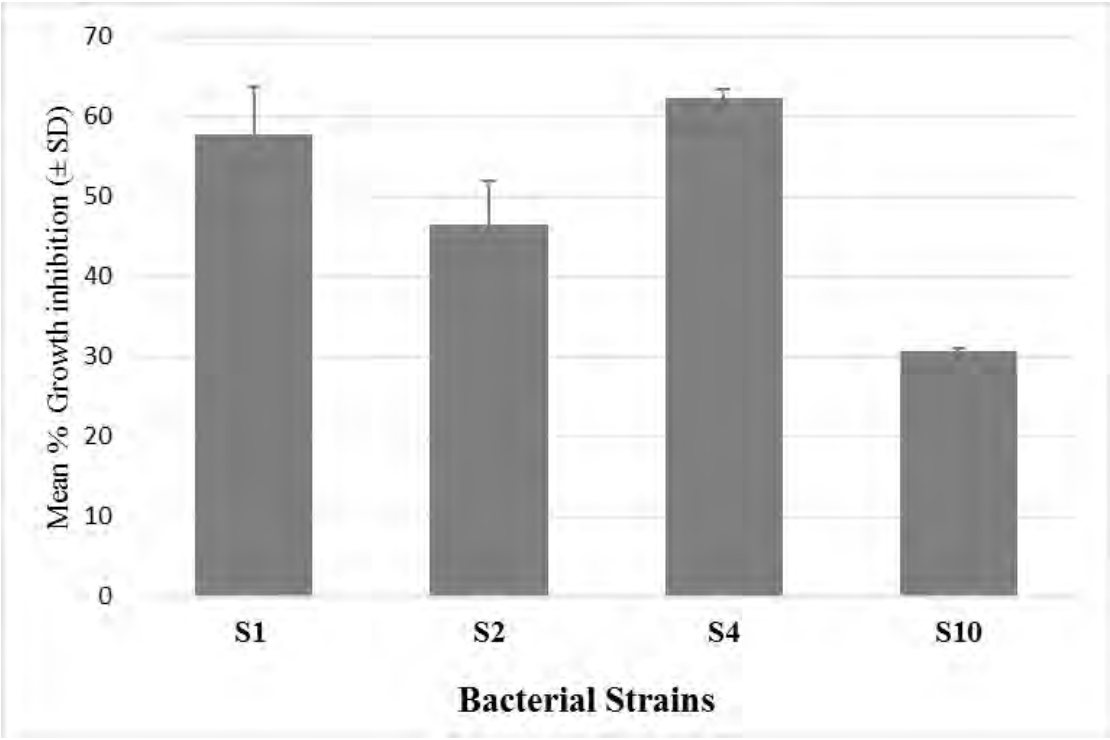


Figure 4.9: Percentage growth inhibition of *Aspergillus flavus* by selected *Bacillus* strains

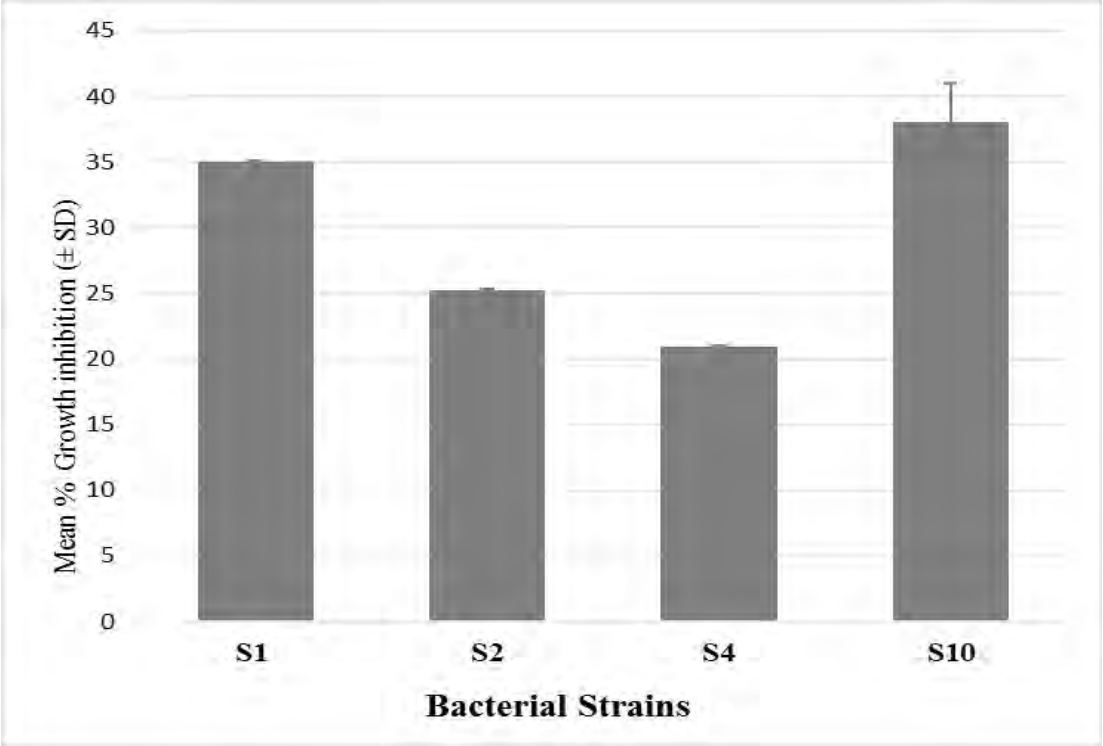


Figure 4.10: Percentage growth inhibition of *Aspergillus niger* by selected *Bacillus* strains

4.3. Identification of selected *Bacillus* strains and compost isolates

4.3.1. Morphological identification of selected *Bacillus* strains and compost isolates

All four rhizospheric soil and twenty compost isolated bacterial strains purified and characterized based on their Gram staining reaction, cell morphology, color, elevation, margin, and shape (Table 4.3). The color of bacterial colonies was whitish, some are off-white, while few were light yellow to light orange. These strains were also categorized by on the base of Gram staining and biochemical test screening. The overnight grown bacterial cultures were used for biochemical tests.

Table: 4.3: Morphological characterization of selected isolated strains

Strain Code	Gram staining	Shape	Colony	Form	Elevation	Margin
S1	+	Rod	Lime green	Punctiform	Umbonate	Undulate
S2	+	Rod	Off white	Irregular	Flat	Lobate
S4	+	Cocci	White	Punctiform	Pulvinate	Entire
S10	+	Rod	White	Circular	Convex	Curled
AH1	-	Rod	White	Rhizoidal	Flat	Filamentous
AH2	+	Cocci	Off white	Irregular	Umbonate	Lobate
AH3	+	Rod	White	Circular	Umbonate	Entire
AH4	+	Rod	White	Filamentous	Raised	Undulate
AH5	+	Rod	Off white	Circular	Convex	Lobate
AH6	+	Cocci	Orange	Circular	Flat	Lobate
AH7	+	Rod	Lime green	Regular	Flat	Curled
AH8	-	Rod	White	Irregular	Umbonate	Entire
AH9	-	Cocci	White	Filamentous	Raised	Undulate
AH10	+	Cocci	White	Circular	Convex	Filamentous
AH11	+	Rod	Off white	Circular	Umbonate	Entire
AH12	+	Cocci	Orange	Irregular	Flat	Lobate
AH13	-	Cocci	Yellow	Irregular	Flat	Lobate
AH14	+	Cocci	Off white	Circular	Raised	Entire
AH15	+	Rod	White	Rhizoidal	Pulvinate	Undulate
AH16	+	Rod	White	Filamentous	Flat	Lobate
AH17	-	Cocci	Off white	Irregular	Flat	Flat
AH18	+	Rod	White	Circular	Umbonate	Entire
AH19	-	Rod	Yellow	Circular	Convex	Undulate
AH20	+	Rod	Off white	Circular	Convex	Entire

3.2.2. Biochemical Identification of selected *Bacillus* strains and compost isolates.

Table 4.4: Triple Sugar Iron (TSI) test of selected *Bacillus* strains and compost isolates

Strains	Source	Slant	Butt	Gas	H_2S
		Acidic/Acidic	Alkaline/Acidic		
S1	Soil	No change	No change	-	+
S2	Soil	K	A	-	-
S4	Soil	K	A	+	-
S10	soil	K	A	-	+
AH1	Compost	No change	No change	-	-
AH2	Compost	No change	A	+	+
AH3	Compost	K	A	-	-
AH4	Compost	K	A	+	-
AH5	Compost	K	A	-	-
AH6	Compost	A	A	+	-
AH7	Compost	A	A	-	-
AH8	Compost	A	A	-	-
AH9	Compost	A	A	+	-
AH10	Compost	K	V	-	+
AH11	Compost	A	A	-	-
AH12	Compost	K	A	-	-
AH13	Compost	No change	No change	-	-
AH14	Compost	K	A	-	-
AH15	Compost	A	K	-	+
AH16	Compost	K	A	+	+
AH17	Compost	A	A	+	-
AH18	Compost	K	No change	-	-
AH19	Compost	K	A	-	+
AH20	Compost	No change	No change	-	-

(Key: Alkaline = K (Yellow color), Acidic = A (Red Color)

Table:4.5: Sulfur Indole Motility (SIM), Urease, Methyl Red (MR) Simmon Citrate (SC) Test

Strain Code	Source	SIM			Urease	MR	SC
		Sulfur	Indole	Motility			
S1	Soil	+	+	-	+	+	+
S2	Soil	-	-	-	+	-	-
S4	Soil	-	-	+	+	+	+
S10	Soil	+	-	+	+	-	-
AH1	Compost	-	-	+	+	+	+
AH2	Compost	-	-	-	+	+	-
AH3	Compost	-	-	-	+	-	-
AH4	Compost	-	-	+	+	+	+
AH5	Compost	-	-	-	+	+	+
AH6	Compost	-	-	+	+	-	-
AH7	Compost	+	-	-	+	-	+
AH8	Compost	-	+	-	+	+	+
AH9	Compost	-	-	+	+	-	+
AH10	Compost	+	-	+	+	-	-
AH11	Compost	-	-	+	+	-	+
AH12	Compost	-	-	+	+	-	+
AH13	Compost	-	-	-	+	+	-
AH14	Compost	-	-	-	+	+	-
AH15	Compost	-	-	+	+	+	-
AH16	Compost	-	-	-	+	+	-
AH17	Compost	-	-	-	+	+	+
AH18	Compost	+	-	-	+	+	+
AH19	Compost	+	-	-	+	+	+
AH20	Compost	+	-	-	+	+	+

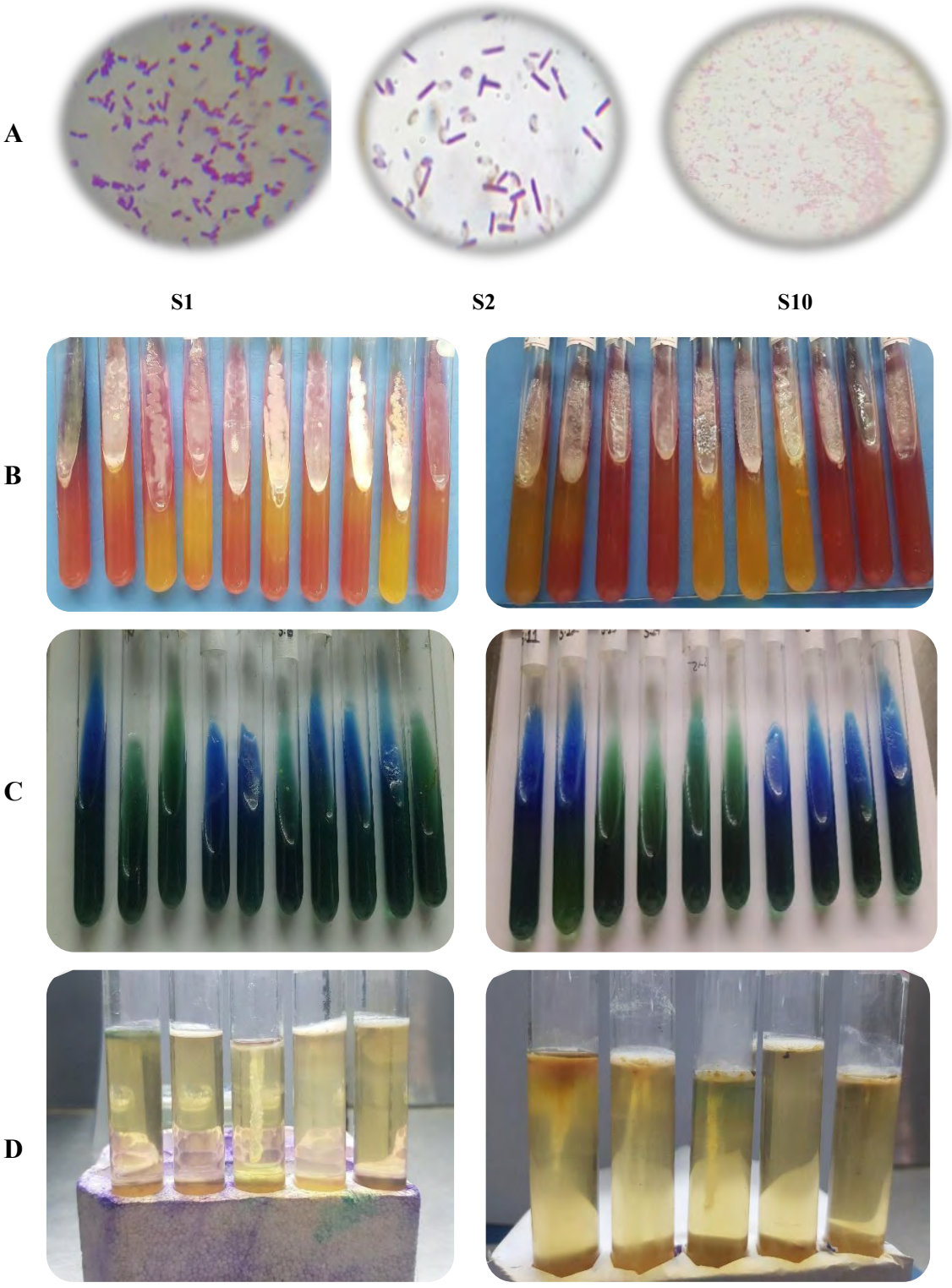


Figure 4.11: (**A**) Gram's staining (**B**)Triple Iron Sugar Test (**C**) Simmons Citrate Test (**D**) Sulfur IndoleMotility Test

4.3.3. Plant growth promoting activities of selected *Bacillus* strain and compost isolates

A total of twenty-four bacterial strains, 20 from compost and 4 isolates of rhizospheric soil bacteria were screened for plant growth promoting (PGP) tests including phosphate solubilization, zinc solubilization, nitrogen fixation, protease, catalase, cellulase assay and oxidase production.

4.3.3.1. Phosphate solubilization

The Pikovskaya's agar medium containing tricalcium phosphate as insoluble source of phosphorus was used for phosphate solubilization assay bacterial strain S2, S10, AH1, AH4, AH6, AH11, AH14, AH19 showed a positive result of phosphate solubilization (Table 4.6).

4.3.3.2. Zinc Solubilization

For zinc solubilization, AH3, AH8, AH10, AH11, AH16, Ah19, AH20 strains of compost while S1, S2 and S10 from rhizospheric soil isolates showed positive response by forming clear halo zone around the bacterial colony on respective zinc medium containing zinc oxide as insoluble source of zinc (Table 4.6).

4.3.3.3. Atmospheric nitrogen fixation ability

A total of 24 bacterial strains from two sources rhizospheric soil and compost. Nine compost isolated strains, AH1, AH4, AH8, AH13, AH14, AH17, AH18, AH19, AH20 while S2 and S10 from rhizospheric soil isolated strains showed positive results regarding atmospheric nitrogen fixation by showing growth on respective nitrogen free medium (DN medium) (Table 4.6).

4.3.3.4. Protease production

Skimmed milk agar medium was used to assess the protease activity of strains. Strain S1 and S10 rhizospheric sample isolates and AH1, AH3, AH11, AH18, AH19, AH20 of compost isolates showed positive response regarding protease activity (Table 4.6).

4.3.3.5. Catalase production

Out of total twenty-four bacterial strains of both samples (compost and rhizospheric soil) only three strains AH3, AH13 and AH17 showed no bubble formation while rest of the strains showed bubble formation upon addition of hydrogen peroxide to bacterial colony indicating the catalase producing ability of bacterial strain (Table 4.6).

2.3.3.6. Cellulose degradation

The antagonistic rhizospheric soil strains and compost isolates were point inoculated on cellulose Congo red agar and incubated at 37℃ for 24 hours. Bacterial strains showed clear zone around the bacterial colony in cellulose agar media amended with Congo red showed positive results for cellulase production. Strains S2, AH3, AH11, AH12, AH14 were positive while rest of strains showed negative results as there was no clear zone around the colonies of bacteria (Table 4.6).

2.3.3.7. Oxidase production

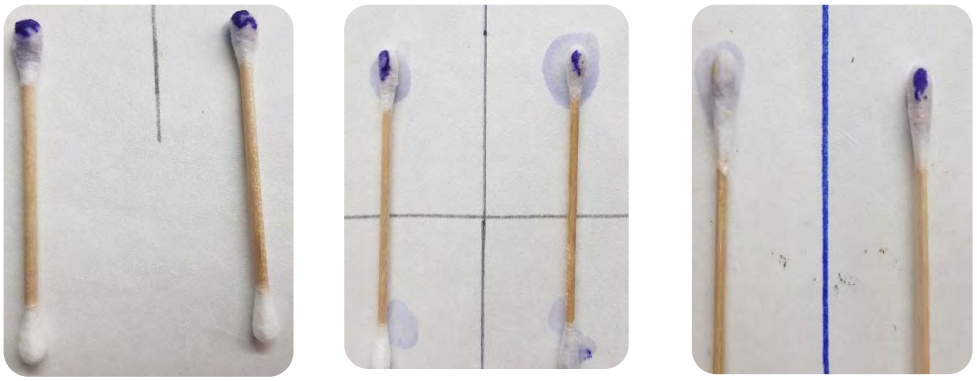
Out of 24 bacterial isolates only 2 strains AH9 and AH17 were oxidase negative while all other strains were oxidase positive two drops of oxidase reagent (tetramethyl para phenylenediamine) were poured on bacterial inoculated cotton swabs dark purple color confirms the presence of Cytochrome-C oxidase because it is oxidized into indole phenol product (Table 4.6)

Table 4.6: Qualitative characterization of strains regarding plant growth promoting activities.

StrainCode	Source	Phosphate solubilization	Zinc solubilization	Nitrogen Fixation	Catalase	Oxidase	Cellulose activity	Protease
S1	Soil	-	+	+	+	+	-	+
S2	Soil	+	+	-	+	+	+	-
S4	Soil	-	-	-	+	+	-	-
S10	Soil	+	+	+	+	+	-	+
AH1	Compost	+	-	+	+	+	-	+
AH2	Compost	-	-	-	+	+	-	-
AH3	Compost	-	+	-	-	+	+	+
AH4	Compost	+	-	+	+	+	-	-
AH5	Compost	-	-	-	+	+	-	-
AH6	Compost	+	-	-	+	+	-	-
AH7	Compost	-	-	-	+	+	-	-
AH8	Compost	-	+	+	+	+	-	-
AH9	Compost	-	-	-	+	-	-	-
AH10	Compost	-	+	-	+	+	-	-
AH11	Compost	+	+	-	+	+	+	+
AH12	Compost	-	-	-	+	+	+	-
AH13	Compost	-	-	+	-	+	-	-
AH14	Compost	+	-	-	+	+	+	-
AH15	Compost	-	-	-	+	+	-	-
AH16	Compost	-	+	-	+	+	-	-
AH17	Compost	-	-	-	-	-	-	-
AH18	Compost	-	-	-	+	+	-	+
AH19	Compost	+	+	-	+	+	-	+
AH20	Compost	-	+	+	+	+	-	+

(Key: (+): present, (-): absent; Values with bold show the best PGP activities of strains)

A

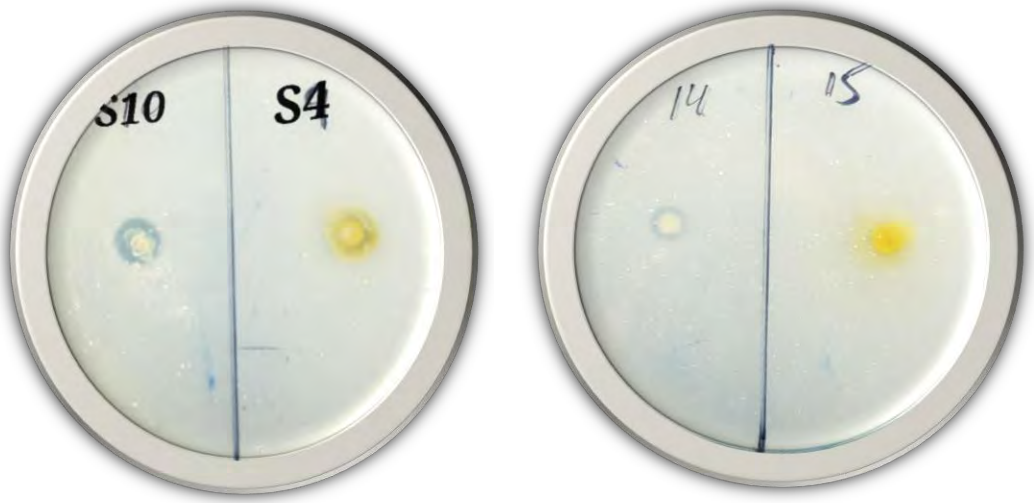


S1 **S2** **S4** **S10** **AH9** **AH10**

B

S1 **S2** **S4** **S10** **AH1** **AH2**

C



S10 **S4** **AH14** **AH15**

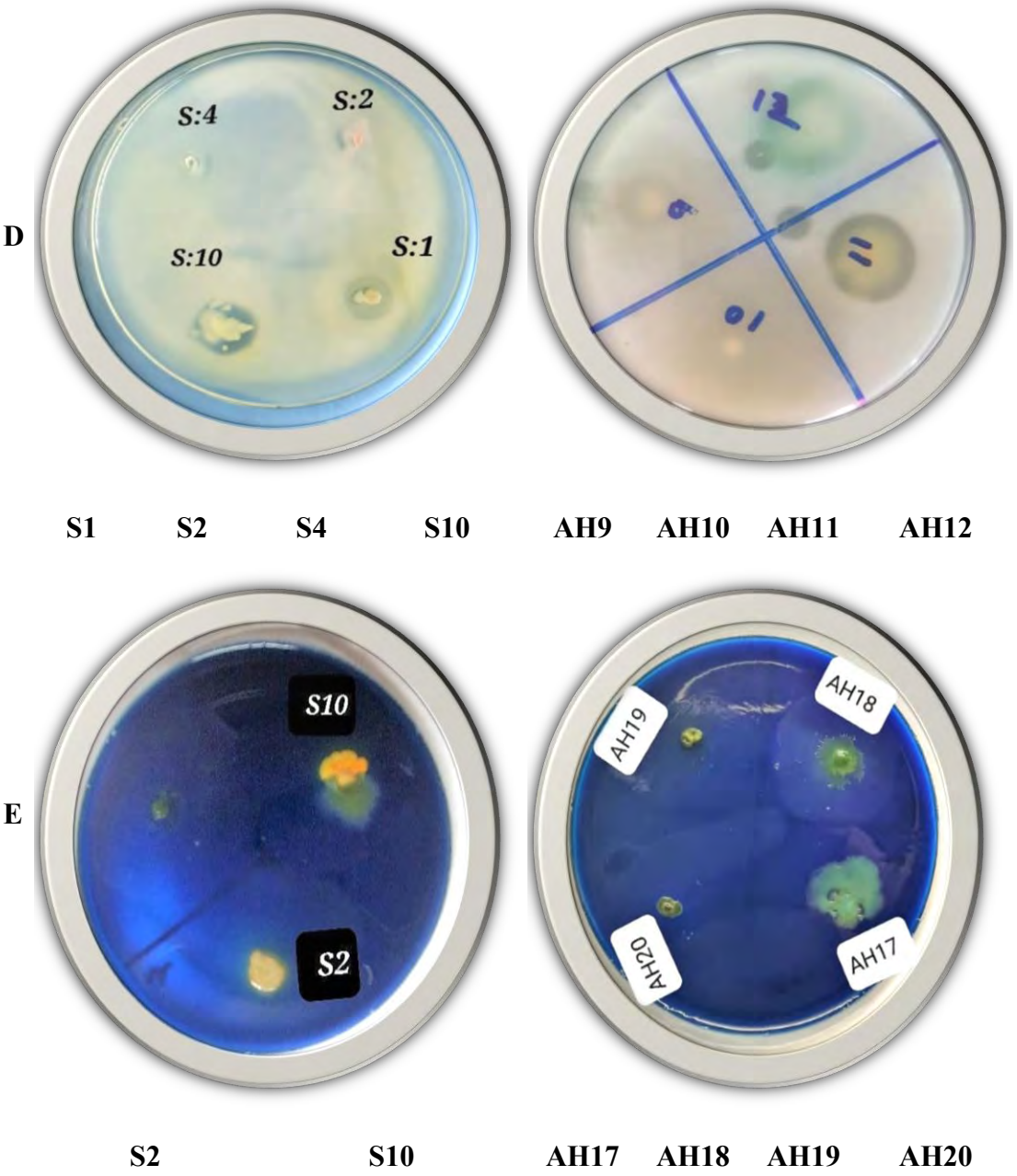


Figure 4.12: PGP Activities of selected *Bacillus s*pecies and compost isolates (**A**) Oxidase test (**B**)Catalase test (**C**) Phosphate production (**D**) Protease Production (**E**) Nitrogen Fixation Test

4.4. Physicochemical analysis of compost, compost tea and soil

Table 4.7: Physicochemical properties of compost and compost tea

Parameters	Compost	Compost tea
pH	8.21 ± 1.33	8.10 ± 0.66
EC	2.47 ± 0.29	2.81 ± 0.17
OM%	19.88 ± 0.96	5.23 ± 0.37
C/N ratio	20.73 ± 1.0	10.67 ± 0.56
Total N%	1.43 ± 0.09	0.61 ± 0.04
Total P%	1.12 ± 0.1	0.18 ± 0.07
Total K%	0.53 ± 0.03	0.09 ± 0

Table 4.8: Physiochemical properties of soil

Texture	pH	EC (ds/m	N%	P%	K%	OM %	BD g/cm^3
Sandy loam	7.83 ± 1	0.49 ± 0.12	0.19 ± 0.3	0.12 ± 01	0.5 ± 0.09	0.59 ± 0.12	1.37 ± 0.15

4.5. Physicochemical and biological analysis of compost pile

4.5.1. Temperature analysis

Through digital field thermometer compost pile temperature was monitored on daily basis up to 14 days from mid and both ends of the pile, and then on weekly basis temperature of compost pile was recorded. The highest temperature was reported on day thirteen 61.53℃ from day fourteen gradual decrease in temperature occurred (Table.4.9).

Table 4.9: Compost pile temperature analysis

Days/Week	Left side of pile temperature	Mid of pile temperature	Right side of pile temperature
1	29.73 ± 1.50	33.83 ± 1.45	29.76 ± 0.55
2	29.76 ± 1.00	33.46 ± .98	30.40 ± 0.80
3	31.30 ± 0.80	35.30 ± 0.79	31.60 ± 0.81
4	31.56 ± 0.90	36.63 ± 1.93	32.70 ± 0.91
5	33.46 ± 0.85	45.03 ± 1.42	35.33 ± 0.98
6	34.9 ± 0.8 8	46.33 ± 0.75	35.30 ± 1.90
7	36.83 ± 1.13	42.80 ± 4.17	33.23 ± 0.75
8	33.20 ± 1.68	48.13 ± 0.90	33.03 ± 1.45
9	35.90 ± 1.40	49.23 ± 1.04	37.96 ± 0.97
10	36.50 ± 0.75	58.23 ± 4.21	36.5 ± 0.88
11	36.23 ± 0.66	56.80 ± 3.53	33.86 ± 0.77
12	38.86 ± 0.83	55.56 ± 5.16	37.36 ± 1.01
13	37.70 ± 1.04	61.53 ± 2.31	39.26 ± 1.20
14	39.53 ± 0.75	61.23 ± 2.83	40.86 ± 0.35
3rd Week	38.90 ± 0.87	58.33 ± 2.25	37.96 ± 0.80
4th Week	38.76 ± 1.34	56.60 ± 2.35	37.96 ± 1.33
5th Week	37.83 ± 0.35	50.53 ± 1.10	38.36 ± 1.26
6th Week	37.26 ± 1.19	47.20 ± 1.24	36.56 ± 0.68
7th Week	35.80 ± 0.55	42.40 ± 0.70	35.06 ± 0.92
8th Week	36.16 ± 0.25	42.43 ± 0.65	35.83 ± 1.12
9thWeek	36.36 ± 0.90	38.93 ± 1.85	37.33 ± 0.90
10th Week	36.03 ± 1.17	37.93 ± 1.34	36.03 ± 0.51
11th Week	34.10 ± 0.43	38.93 ± 0.85	32.80 ± 1.11
12th Week	31.23 ± 1.30	38.80 ± 1.65	31.53 ± 1.62
13th Week	30.03 ± 1.25	39.06 ± 0.72	30.53± 2.21
14th Week	30.03 ± 0.70	37.13 ± 1.13	30.43 ± 0.75

Each value represents a mean of three replicates while (±SD) indicate standard deviation

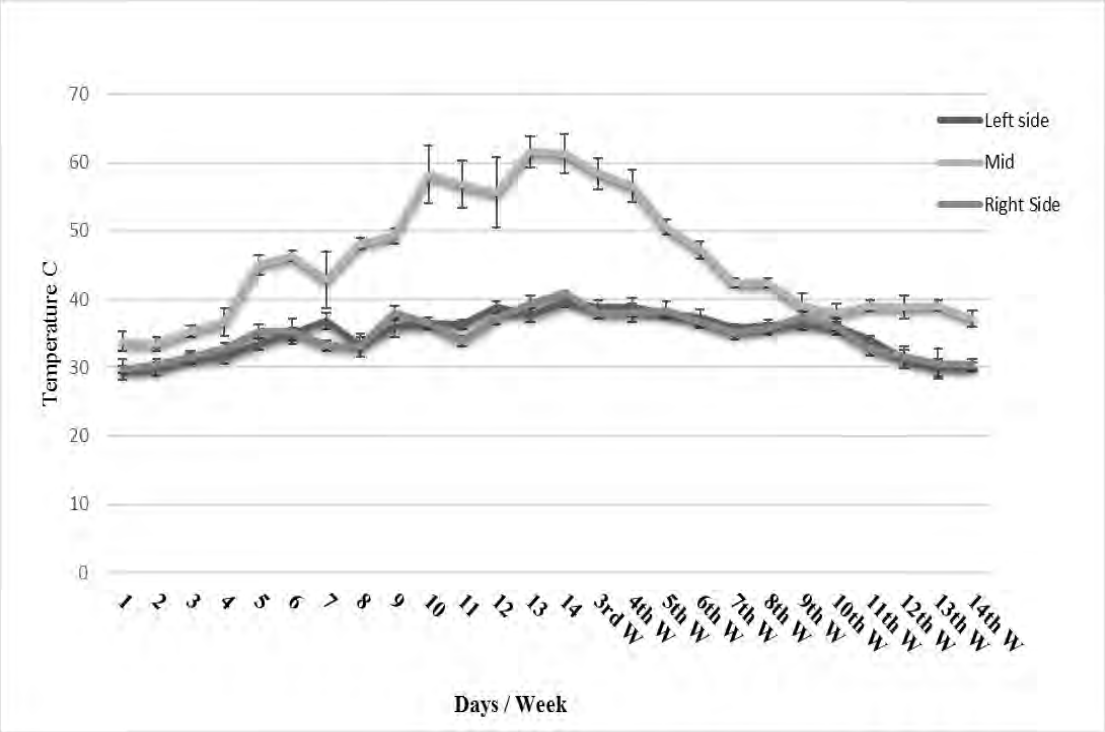


Figure 4.13: Temperature value of compost pile during composting process

4.5.2. pH analysis of compost during composting process

In initial phase of composting process gradual decrease was occurred in pH value up to 6th week of composting process and then increase in pH value reported (Table 4.10). The lower pH value lied between 4-6. The pH increased and stable around neutral at maturation phase of composting process. This stability of pH and temperature of composting process is the indication of maturity of compost.

4.5.3. Electrical conductivity analysis

Maximum EC value 9.4 ± 0.62 was observed in 2nd week then gradual decrease occurred in EC during composting process organic compound matter converts into more stable form due microbial activity. This transformation involves the breakdown of complex molecules including those containing salts as a result, the electrical conductivity decreases as these compounds broken down. The compost microorganisms utilize the available salts and decrease occurred in electrical conductivity value of the composting process shown in (Table 4.10).

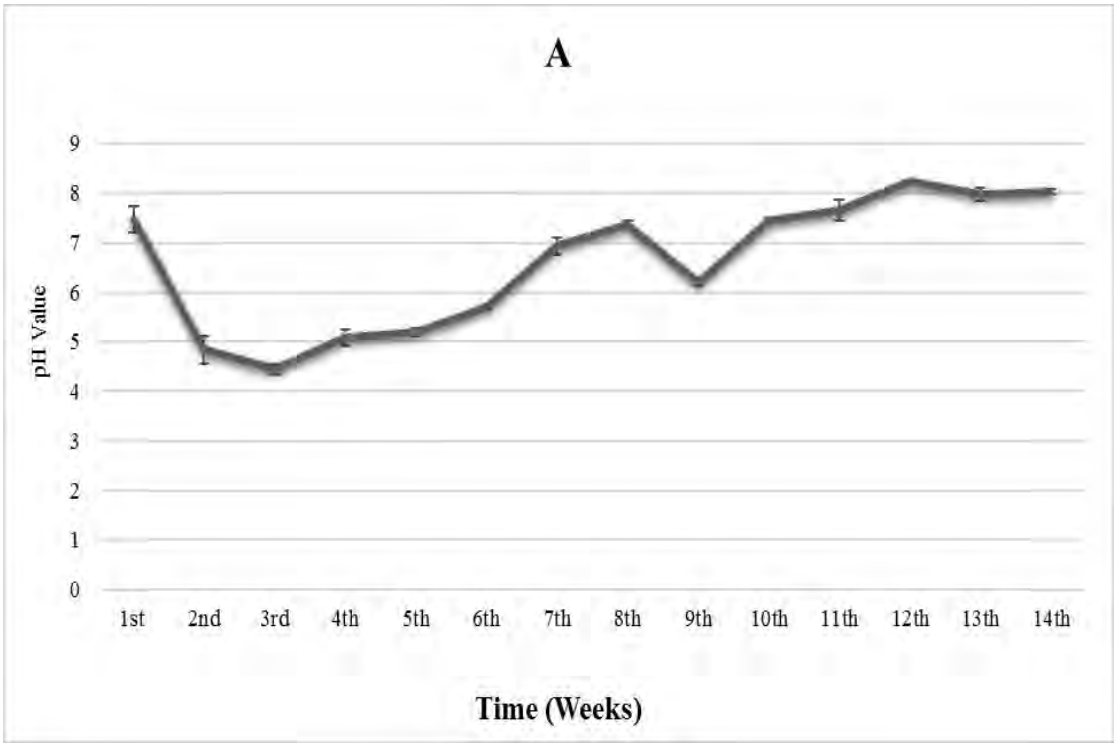
4.5.4. Moisture content analysis of compost pile during composting

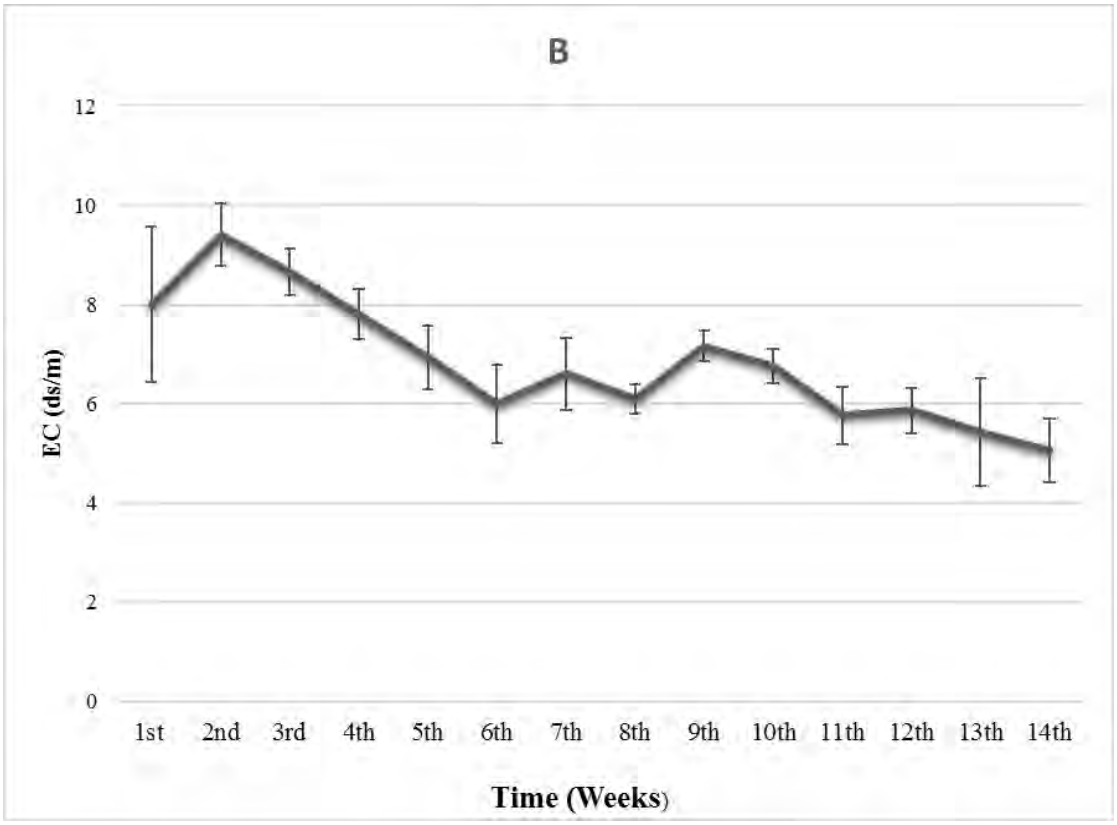
The moisture content of compost pile analyzed on weekly basis. The data in (Table 4.10) reveal notable trends in the moisture content of the compost pile over time. An initial moisture content of 61.6% in the 1st week. Followed by a decrease to 55.23 ± 2.70% in 2nd week. Then rising again to 59.25 3 ± 2.89, 61.333 ± 2.87, and 64.86 3 ± 3.28% in 3rd ,4th, and 5th week. The pattern continued throughout the study suggests that external factors like weather condition or management practices influenced moisture content of compost pile.

Table 4.10: Moisture content (MC), Electrical conductivity (EC), and pH profile of compost pile during composting process

Weeks	pH	EC (ds/m)	MC%
1st week	7.48 ± 0.26	8.0 ± 1.57	61.6 ± 2.46
2nd week	4.84 ± 0.27	9.4 ± 0.62	55.23 ± 2.70
3rd week	4.46 ± 0.10	8.66 ± 0.47	59.25 ± 2.89
4th week	5.08 ± 0.15	7.8 ± 0.5	61.33 ± 2.87
5th week	5.19 ± 0.08	6.93 ± 0.65	64.86 ± 3.28
6th week	5.69 ± 0.04	6.0 ± 0.79	42.93 ± 3.55
7th week	6.93 ± 0.16	6.6 ± 0.72	45.16 ± 6.11
8th week	7.36 ± 0.08	6.1 ± 0.30	45.8 ± 3.42
9th week	6.2 ± 0.09	7.16 ± 0.30	45.03 ± 3.71
10th week	7.45 ± 0.06	6.76± 0.35	58.23 ± 1.71
11th week	7.65 ± 020	5.76 ± 0.56	42.26 ± 1.40
12th week	8.23 ± 0.03	5.86 ± 0.45	36.8 ± 1.76
13th week	7.98 ± 0.12	5.43 1.07	31.06 ± 2.85
14th week	8.03 ± 0.04	5.06 ± 064	30.9 ± 1.40

Each value represents a mean of three replicates while (±SD) indicate standard deviation


A
pH Value
0
1
2
3
4
5
6
7
8
9
1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
11th
12th
13th
14th
Time (Weeks)


B
EC (ds/m)
0
2
4
6
8
10
12
1st
2nd
3rd
4th
5th
6th
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9th
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12th
13th
14th
Time (Weeks)

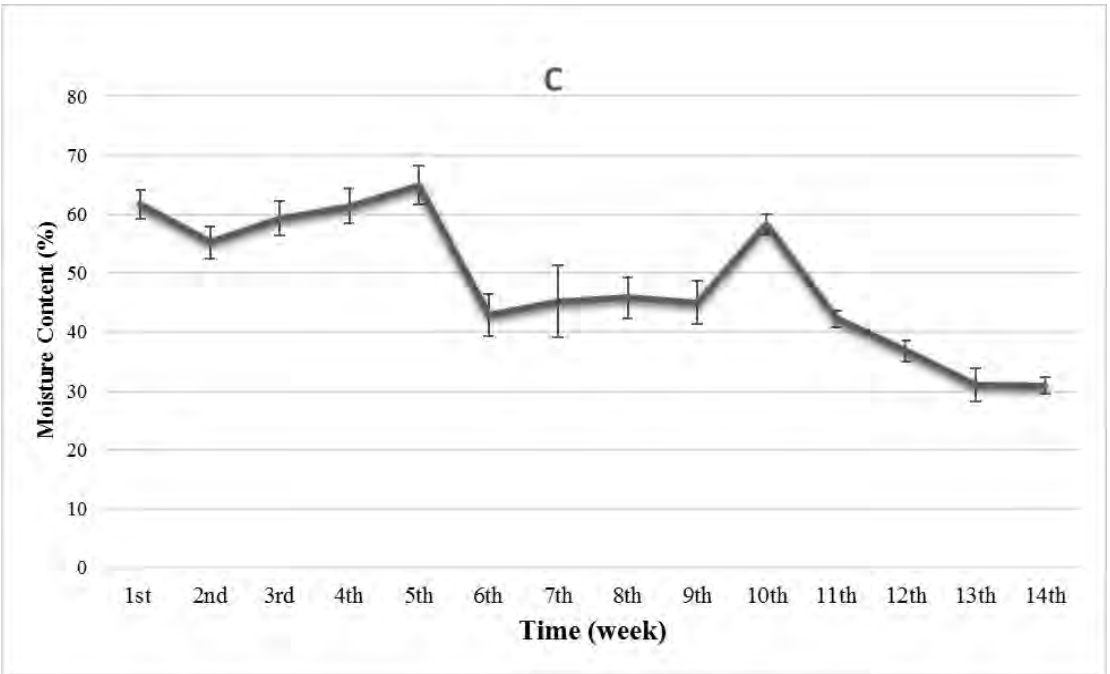


Figure.4.14: Analysis of (**A**) pH, (**B**) Electrical conductivity, and (**C**) Moisture content of compost pile during composting processing

4.6. Biological analysis of compost pile

4.6.1. Bacterial and fungal number during different phases of composting process

Figure (4.16) demonstrates the change in the number of bacteria during three different phases (mesophile, thermophile and curing stage) of organic composting of dry leaves were found to be positively correlated with temperature of compost pile. In mesophile, thermophile and in curing phase of composting, the bacterial counts were 64×10^7 cfu/g d.w, 28×10^7 cfu/g d.w and 46×10^7 cfu/g d.w (cfu = colony forming unit; dw. = dry weight) respectively. While Figure (4.17) shows the change in the pattern of the number of fungi during different phases of organic composting process. The experimental results revealed that in the mesophile, thermophile and curing phases of organic composting process the fungal counts were 32×10^4 cfu/g dw, 15×10^4 cfu/g dw, and 21×10^4 cfu/g dw respectively.

a. 46×10^{7} cfu/g **b.** 21×10^{4} cfu/g

Figure 4.15: (**a**) Bacterial count and (**b**) Fungal count during curing phase of composting

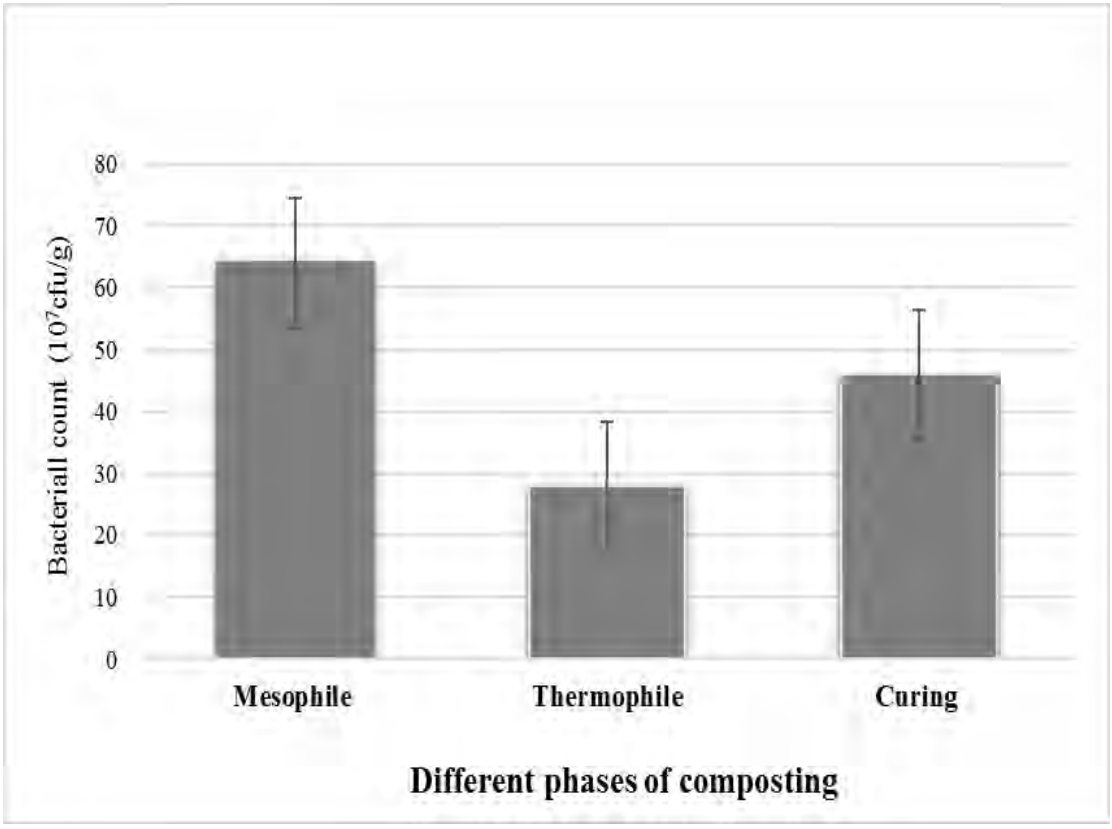


Figure 4.16: Bacterial count during different phases of composting

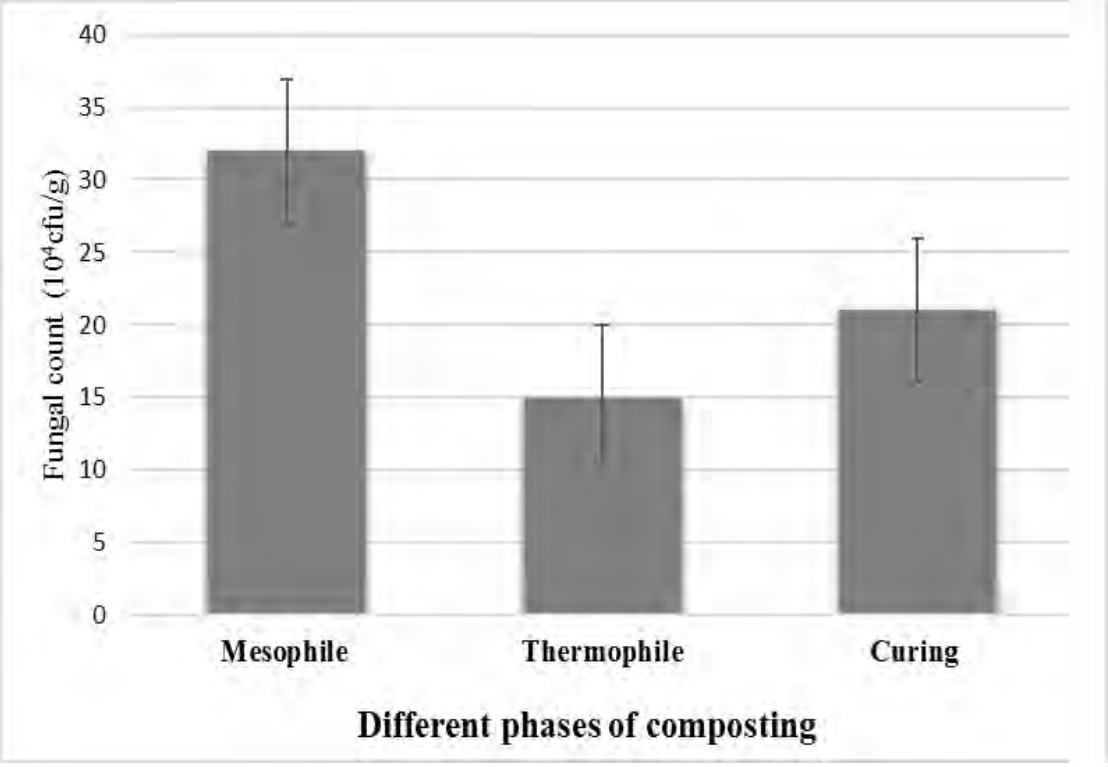


Figure 4.17: Fungal count during different phases of composting

4.7. Effect of compost, compost tea, and selected bacterial strains on soil physicochemical properties.

Impact of compost, compost tea, and rhizospheric soil bacterial consortia on soil properties. The results showed that positive significant changes in soil characteristics as compared with the soil before cultivation and control treatment, these changes are as follows:

4.7.1. Effect of different treatment on N, P, K percentage contents in soil after harvesting

Individual and combined application of organic fertilizers significantly increased the nutrient availability in soil (Table 4.11). The maximum nutrient content of N% (1.35 ± 0.19%), (1.15 ± 0.15%), and (1.12 ± 0.10%), were achieved in those treatments receiving CM+CT+BC, CM and CM+CT as compared to the soil (0.19 ± 0.03%) and both controls (0.15 ± 0.03%) and (0.73 ± 0.08%). On the other hand, P and K content significantly increased with applying compost and compost tea individually or combined as compared to the control treatment. The P and K contents in the soil were

increased (0.51 ± 0.07%), (0.39 ± 0.02%), (0.31 ± 0%) and (0.38 ± 0%), (0.34 ± 0.01%) and (0.30 ± 0.02%,) in pots treated with, CM+CT+BC, CM+BC, CM+CT, and CM+CT+BC, CM, CM+CT as compared with the soil and controls.

Table 4.11: Effect of different treatments on soil N, P, K % contents after harvesting

Treatments		N (%)	P (%)	K (%)
T+	Autoclaved soil + Pathogen	0.15 ± 0.03	0.16 ± 0.01	0.07 ± 0
T-	Autoclaved soil	0.73 ± 0.08	0.21 ± 0.01	0.13 ± 0.05
CM	Compost	1.15 ± 0.15	0.30 ± 0.05	0.34 ± 0.01
CM+CT	Compost + Compost tea	1.12 ± 0.10	0.28 ± 0.03	0.30 ± 0.02
CM+BC	Compost + Bacterial consortia	1.03 ± 0.09	0.39 ± 0.02	0.25 ± 0.03
CM+CT+BC	Compost + Compost tea + Bacterial consortia	1.35 ± 0.19	0.51 ± 0.07	0.38 ± 0
CT	Compost tea	0.95 ± 0.07	0.19 ± 0	0.19 ± 0.01
CT+BC	Compost Tea + Bacterial consortia	1.06 ± 0.12	0.31 ± 0	0.20 ± 0.02
BC	Bacterial consortia	0.41 ± 0.07	0.15 ± 0	0.16 ± 0

Each value represents a mean of three replicates while (±SD) indicate standard deviation

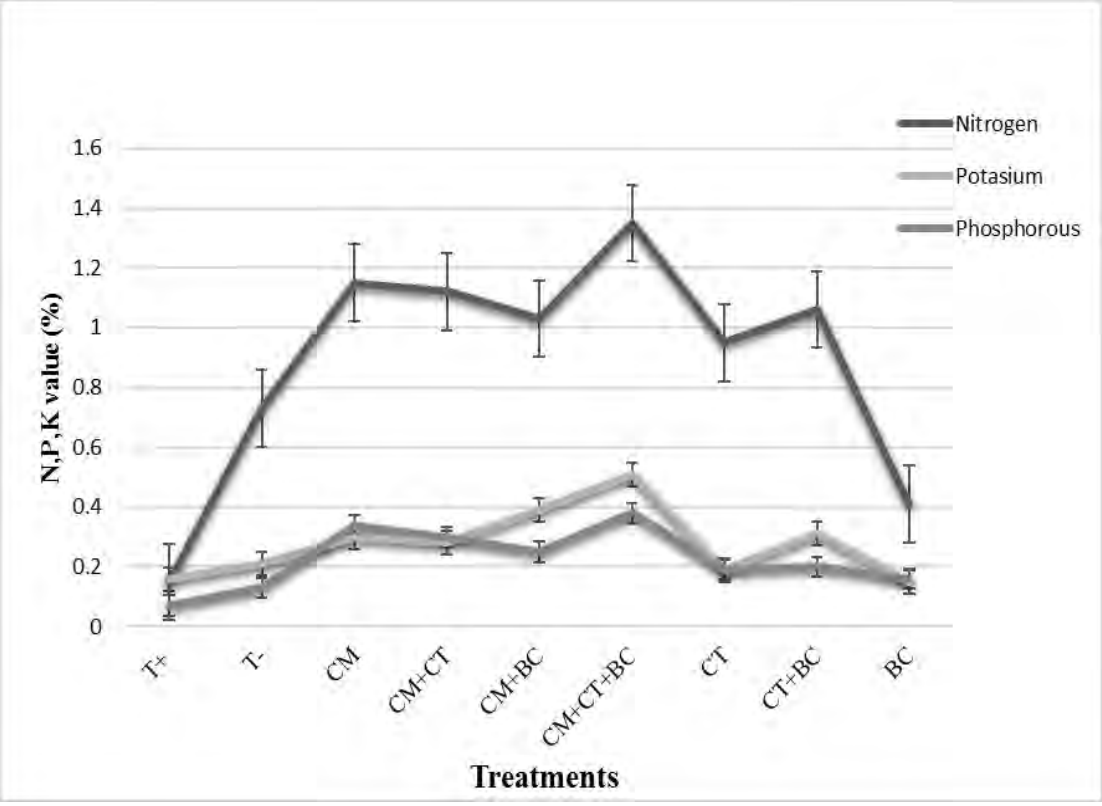


Figure 4.18: Effect of different treatments on soil N, P, K % contents after harvesting

4.7.2. Effect of different treatments on soil organic matter, pH, electrical conductivity and bulk density

4.7.2.1. Soil organic matter (OM)

The results showed that, in most cases, the individual and combined application of compost (organic fertilizer) significantly improved the soil organic matter content, in comparison with the control treatment (Table 4.12). The most pronounced increases after the harvesting the soil organic matter (3.45 ± 0.02, 2.95 ± 0.05, and 2.13 ± 0.04%) were obtained the tomato plants treated with CM+CT+BC, CM+CT, CM+BC as compared to control (0.42 ± 0.02% and 0.56 ± 0.01 %) and soil organic matter (0.59 ± 0.12%) respectively.

4.7.2.2. Soil pH

Table 4.12 results clearly show that, after harvesting, applying different treatments in current study, resulted in significant decreases in the soil pH compared to both control

treatments and the original soil pH. The soil pH decreases gradually (6.47 ± 0.02, 6.68 ± 0.10, 6.77, and 6.85 ± 0.13) in soil treated with CM, CM+CT, CM+BC, and CM+CT+BC respectively, compared to the original soil pH (7.83 ± 1) and control (8.17 ± 0.04 and 7.91 ± 0.03) The reduction in the soil pH after the end of experiment was more evident under compost applications alone or combined with compost tea and bacterial consortia compared to the sole application of compost tea or bacterial consortia.

4.7.2.3. Soil electrical conductivity

Electrical conductivity (EC) is considered as an indication of soil salinity. EC value of all treatments was increased except BC treatment which EC value was reported 0.85 ds/m which is low from control soil EC. While maximum EC value reported in treatment CM+CT+BC, CM+CT and CM+BC (2.15 ± 0.03, 1.95 1.95 ± 0.04, and 1.49 ± 0.02 ds/m) respectively, (table 4.12).

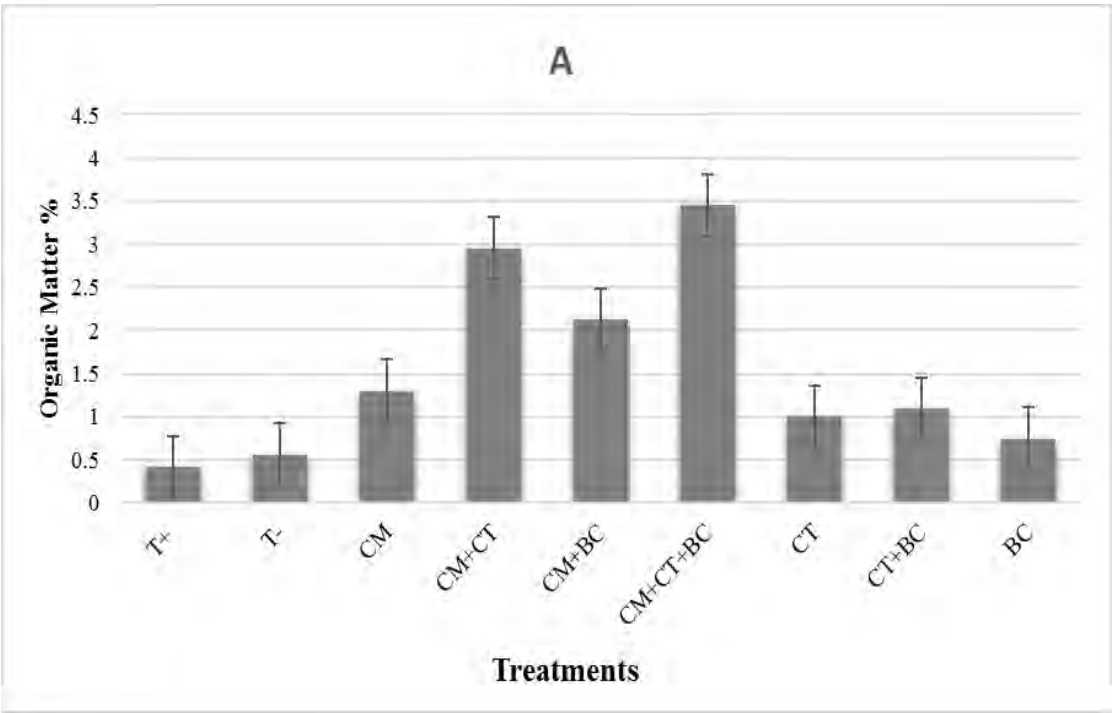
4.7.2.4. Soil bulk density

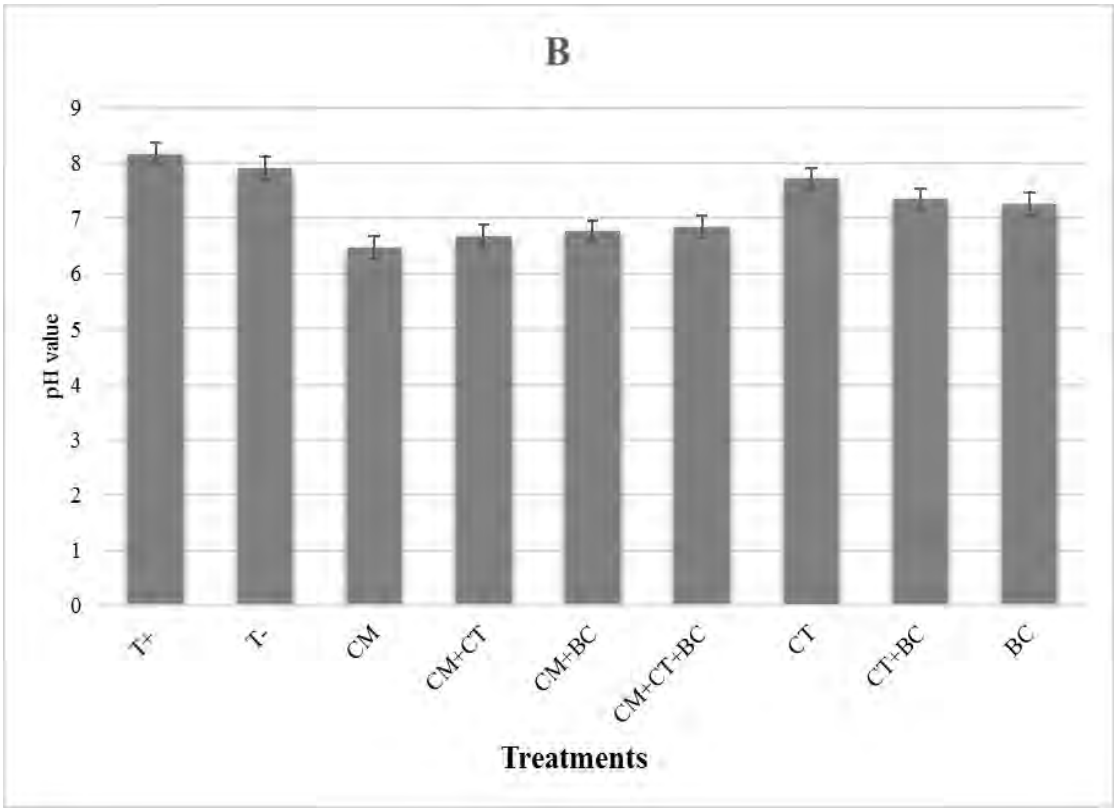
Soil bulk density is an indicator of soil compaction and soil health, the application of all the treatments under study led to decreases in the soil bulk density values compared to the control (Table 4.12). The low bulk density values were (1.12 ± 0.04, 1.14 ± 0.03, and 1.16 ± 0.02 g/cm^3) for, CM, CM+CT, and CM+BC treatments respectively, compared to the Soil bulk density (1.37 ± 0.15 g/cm^3) shown in (Table 4.8) and control. Various changes in soil bulk density were noted, and these changes were associated with different, application rates and type of organic amendments. Moreover, application of compost reduced the volume weight more than application of other treatments.

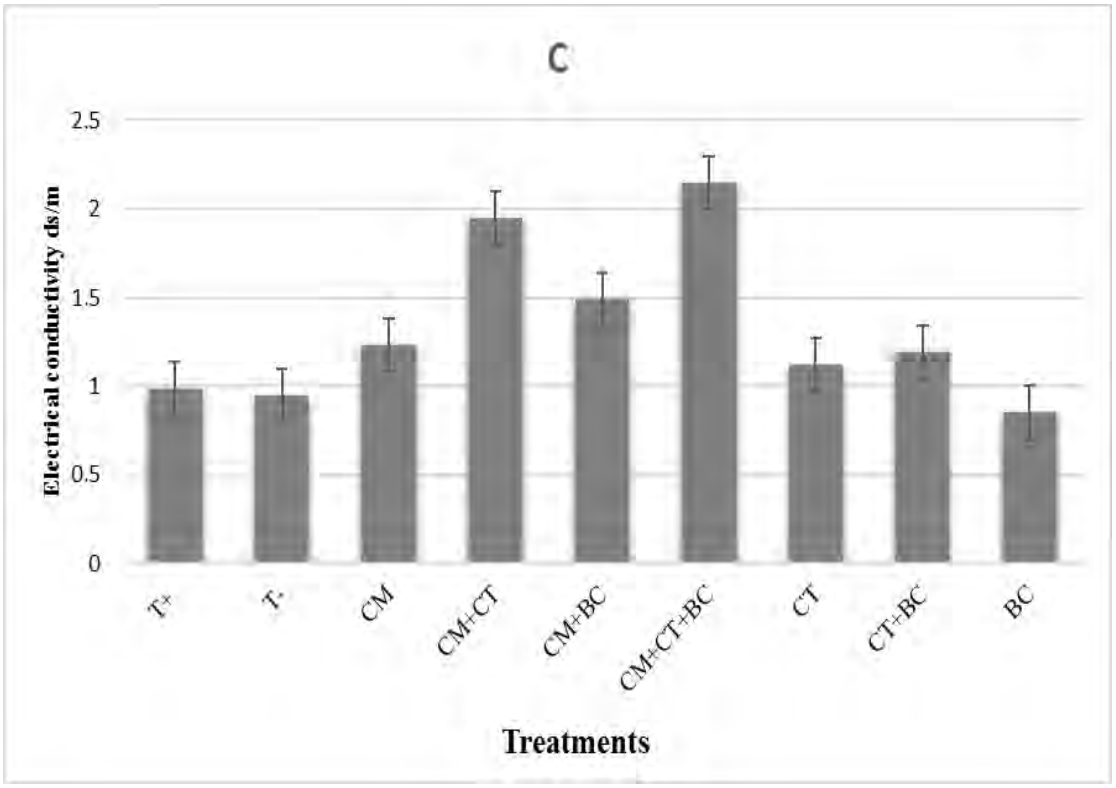
Table 4.12: Effects of compost, compost tea, and soil microorganisms on organic matter, pH, electrical conductivity, and bulk density of soil

Treatment	Organic Matter %	pH	EC ds/m	Bulk density
T+	0.42 ± 0.02	8.17 ± 0.04	0.99 ± 0.02	1.25 ± 0.03
T-	0.56 ± 0.01	7.91 ± 0.03	0.95 ± 0.03	1.26 ± 0.01
CM	1.30 ± 0.02	6.47 ± 0.02	1.23 ± 0.02	1.12 ± 0.04
CM+CT	2.95 ± 0.05	6.68 ± 0.10	1.95 ± 0.04	1.14 ± 0.03
CM+BC	2.13 ± 0.04	6.77 ± 0.10	1.49 ± 0.02	1.16 ± 0.02
CM+CT+BC	3.45 ± 0.02	6.85 ± 0.13	2.15 ± 0.03	1.19 ± 0.03
CT	1.0 ± 0.02	7.72 ± 0.08	1.12 ± 0.03	1.24 ± 0.03
CT+BC	1.09 ± 0.04	7.35 ± 0.45	1.19 ± 0.05	1.24 ± 0.06
BC	0.75 ± 0.04	7.26 ± 0.70	0.85 ± 0.01	1.22 ± 0.02

Each value represents a mean of three replicates while (±SD) indicate standard deviation




B
pH value
9
8
7
6
5
4
3
2
1
0
T+
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
Treatments


C
Electrical conductivity ds/m
2.5
2
1.5
1
0.5
0
T+
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
Treatments

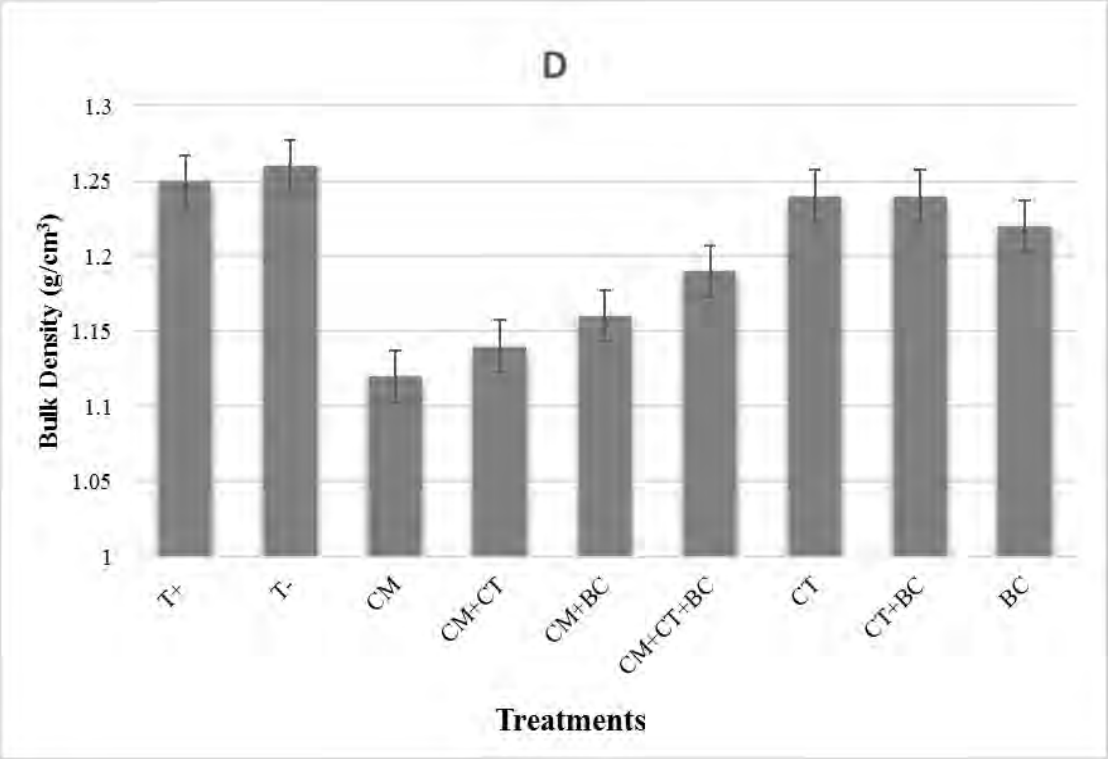


Figure 4.19: Effect of compost, compost tea and soil microorganisms on soil (**A**) Organic matter (**B**) pH value (**C**) Electrical conductivity and (**D**) Bulk

4.8. Impact of compost, compost tea, and soil microorganisms (bacterial consortia) on plant growth parameters in autoclaved and un-autoclaved soil

The organic fertilizers, compost, compost tea, and soil microorganisms (bacterial consortia) and their interactions had a significant effect on the plant height, number of leaves per plants, chlorophyll content, number of secondary shoots, primary and secondary root length, seed germination rate, plant fresh and dry weight and seedling vigour index ($P<0.05$) data presented in (Tables 4.13 and 4.14).

Figure 20: Pot experiment in autoclaved soil

Figure 21: Pot experiment in un-autoclaved soil

4.8.1. Impact of compost, compost tea and soil microorganisms on seed germination rate

In both autoclaved and un-autoclaved soil, treatment CM+CT+BC had the highest rate of seed germination (83.33%) in comparison with other treatments shown in (Table 4.13 and 4.14). But this rate was lower than that of the corresponding negative controls but higher than that of the positive controls.

4.8.2. Impact of compost, compost tea and soil microorganisms on plant chlorophyll content

Maximum SPAD units reading for chlorophyll content were obtained in plants treated with CM+CT+BC and CM+BC (43.63 ± 3.35, 38.96 ± 1.06) and (39.46 ± 0.56, 37.83 ± 4.80) in both autoclaved and un-autoclaved soil in comparison with their control treatments.

4.8.3. Impact of compost, compost tea, and soil microorganisms on plant height

The maximum plant height was achieved in those pots treated with a combination of CM+CT+BC (72.33. In autoclaved and unautoclaved soil, the addition of CM+CT+BC led to a remarkable (2.64, 1.38) and (1.85, 0.96) times increase in plant height compared to their positive and negative controls respectively (Table 4.13 and 4.14). The treatment CT was found to be less effective as compared to other treatment for plant height.

4.8.4. Impact of compost, compost tea and soil microorganisms on plant primary and secondary root length

The results in table (4.13 and 4.14) show maximum primary root length in treatment CM+CT+BC (7.20 ± 1.80 cm) followed by CM+BC (6.16 ± 1.44 cm) while highest secondary root length was observed in treatment CM+CT and CT+BC (13.66 ± 6.02 and 12.33 ± 4.61 cm) respectively in autoclaved soil higher than unautoclaved soil treatments. Notably treatment BC in unautoclaved soil is less effective in promoting both primary and secondary root lengths compared to other treatments.

4.8.5. Impact of compost, compost tea and soil microorganisms on number of leaves per plant

The highest number of leaves per plant was observed in plants treated with a mixture of CM+BC (40 ± 2.58), followed by a mixture of CM+CT+BC and CM+CT (35.33 ± 2.51 and 33.0 ± 5.19) in autoclaved soil, as compared to control treatments (9.66 ± 1.52 and 14.66 ± 1.52), respectively. The results in (Table 4.13 and 4.14) show that both controls of un-autoclaved soil have a greater number of leaves per plant in comparison with controls of autoclaved soil.

4.8.6. Impact of compost, compost tea and soil microorganisms on plant secondary shoots number

The highest number of secondary shoots (branches) per plant were reported in treatments CM+CT+BC (10 ± 1 and 7.66 ± 1.52) in both type of soil when compared to control treatments.

4.8.7. Impact of compost, compost tea and soil microorganisms on plant fresh and dry weight

Significantly plant fresh and dry weight were achieved in treatment CM+CT+BC and CM+BC (19.55 ± 3.05, 15.0 ± 3.77 gm) and (3.92 ± 0.97 and 2.86 ± 0.47 gm) in autoclaved soil while in (Table 4.14) unautoclaved soil treatments CM+CT+BC and CM+CT have maximum fresh and dry weight (16.53 ± 2.50, 14.60 ± 2.22 gm and 3.66 ± 0.57, 2.53 ± 0.56 gm) as compared to their control treatments.

4.8.8. Impact of compost, compost tea and soil microorganisms on seedling vigour index

Highest seedling vigour index was reported in treatment CM+CT+BC (7443.33 ±1355.0) and (7163.6771 ± 1321.0) in both autoclaved and un-autoclaved soil in comparison with respective positive and negative controls.

T- **T+** **CM** **CM+CT** **CM+BC** **CM+CT+BC** **CT** **CT+BC** **BC**

Figure 4.22: Impact of different treatments on plant growth parameters

Table:4.13: Impact of compost, compost tea and soil microorganisms on plant growth parameters in Autoclaved soil

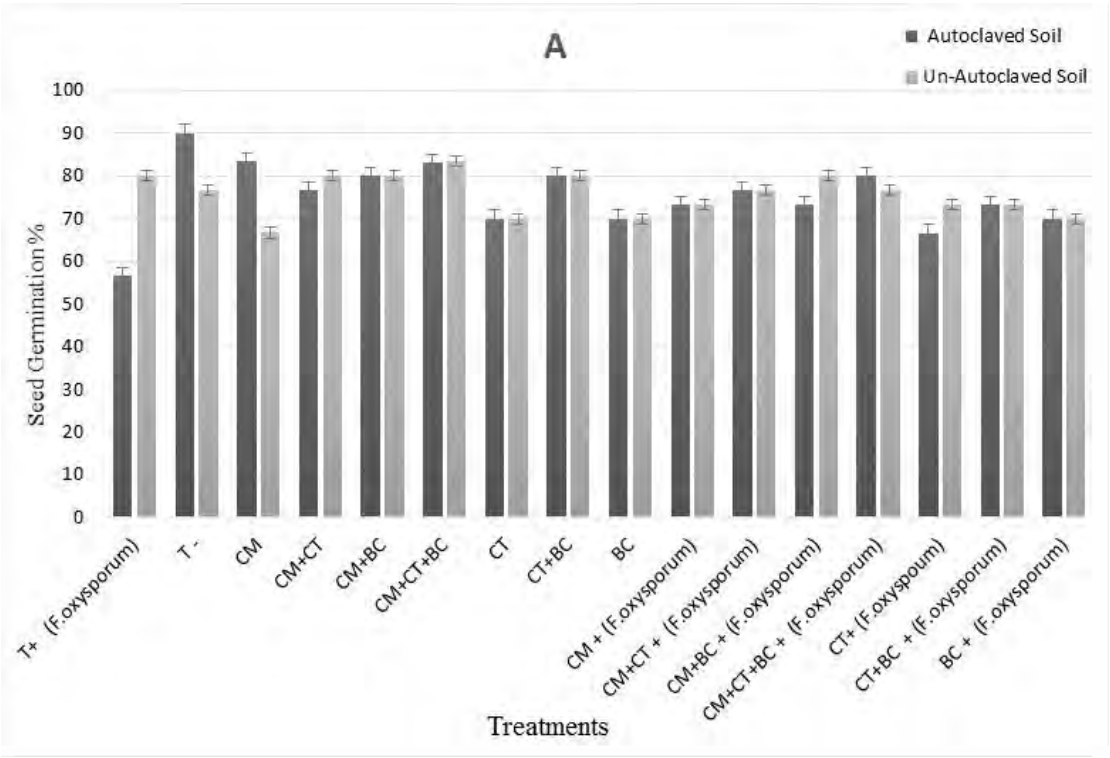
Treatments	Seed Germination %	Chlorophyll Content	Plant Height (cm)	Primary Root length (cm)	Secondary Root length (cm)	Number of leaves per plant	Secondary Shoot Number per plant	Fresh Weight (gm)	Dry Weight (gm)	Seedling Vigour index
Autoclaved soil without Pathogen										
T-	90.0 ± 10.0 a	35.9 ± 3.21 ab	32.50 ± 3.27 de	3.36 ± 0.56 bc	5.66 ± 0.57 ab	14.66 ± 1.52 de	4.33 ± 0.57 cd	2.33 ± 0.68 ef	0.71 ± 0.25 d	3460±720.83 cd
T+	56.66 ± 15.27 b	32.13 ± 5.35 a	21.30 ± 3.40 d	2.86 ± 0.60 b	4.0 ± 2.64 a	9.66 ± 1.52 c	2.66 ± 0.57 c	0.83 ± 0.25 f	0.32 ± 0.15 c	1404.33±246.70 e
CM	83.33 ± 15.54 a	36.40 ± 4.92 ab	63.86 ± 5.74 b	4.7 ± 0.36 abc	9.66 ± 2.30 ab	30.33 ± 4.72 abc	6.33 ± 2.30 bc	12.20 ± 1.86 bc	1.55 ± 0.18 bcd	6151.33±1198.98 ab
CM+CT	76.66 ± 5.77 a	35.96 ± 3.44 ab	65.9 ± 2.35 ab	5.33 ± 1.15 abc	13.66 ± 6.02 a	33.0 ± 5.19 abc	7.0 ± 1.0 abc	13.09 ± 1.66 bc	2.50 ± 0.50 b	6111.33±838.561 ab
CM+BC	80.0 ± 10.0 a	38.96 ± 1.06 ab	70.66 ± 2.51 ab	6.16 ± 1.44 ab	10.0 ± 3.0 A	40.0 ± 4.58 a	8.33 ± 1.15 ab	15.0 ± 3.77 ab	2.86 ± 0.47 bc	6450.0±765.44 ab
CM+CT+BC	83.33± 15.27 a	43.63 ± 3.35 a	77.66 ± 4.04 a	7.20 ± 1.80 a	11.66 ± 1.15 ab	35.33 ± 2.51 ab	10.0 ± 1.0 a	19.55 ± 3.05 a	3.92 ± 0.97 a	7443.33±1355.0 a
CT	70.0 ± 10.0 a	34.36 ± 4.75 ab	28.20 ± 2.88 de	5.20 ± 0.75 abc	5.33 ± 0.57 ab	23.33 ± 2.88 cd	6.66 ± 0.57 abc	5.0 ± 1.74 def	1.15 ± 0.23 cd	2369.33±564.07 cd
CT+BC	80.0 ± 17.32 a	36.40 ± 4.75 ab	45.33 ± 7.02 c	3.83 ± 0.76 bc	12.33 ± 4.61ab	31.33 ± 2.51 abc	7.33 ± 1.52 abc	8.63 ± 2.37 cd	1.46 ± 0.41 bcd	4580.0±1270.79 bc
BC	70.0 ± 17.32 a	35.36 ± 3.75 ab	36.33 ± 4.04 cd	4.4 ± 0.17 abc	8.66 ± 4.04 ab	29.33 ± 5.31bc	6.0 ± 1.0 bcd	7.53 ± 0.90 cde	1.13 ± 0.50 bcd	3100.0±556.776 cd
Autoclaved Soil with Pathogen										
CM	73.33 ± 15.27 ab	32.76 ± 1.25 a	58.33 ± 2.51 ab	4.3 ± 0.40 ab	9.30 ± 0.81 a	28.66 ± 4.0 a	5.33 ± 0.57 abc	12.20 ± 1.86 b	1.44 ± 0.16 b	4983±1221.64 abc
CM+CT	76.66 ± 5.77 ab	36.86 ± 3.27 a	59.86 ± 5.0 a	4.5 ± 1.15 ab	8.0 ± 2.0 a	30.66 ± 4.72 a	6.66 ± 1.52 ab	12.23 ± 1.60 b	1.43 ± 0.16 b	5204±693.31 ab
CM+BC	73.33 ± 5.77 ab	33.30 ± 4.30 a	62.80 ± 4.54 a	6.46 ± 0.98 a	9.50 ± 2.29 a	28.0 ± 2.64 a	7 ± 2.51 ab	11.85 ± 1.32 bc	1.37 ± 0.25 b	5287.33±163.46 ab
CM+CT+BC	80.0± 10.0 ab	38.13 ± 2.74 a	68.0 ± 6.0 a	5.56 ± 1.72 ab	9.26 ± 3.52 a	28.66 ± 2.51 a	8.66 ± 2.51 a	16.73 ± 1.91 a	2.36 ± 0.35 a	6235.33±1462.72 a
CT	66.66 ± 5.77 ab	37.20 ± 0.98 a	26.66 ± 2.51 d	4.36 ± 0.90 ab	6.83 ± 1.89 a	25.66 ± 6.02 a	8.0 ± 1.00 ab	2.26 ± 0.35 ef	0.88 ± 0.38 bc	2235±370.50 de
CT+BC	73.33 ± 5.77 ab	36.43 ± 3.04 a	45.23 ± 5.77 bc	3.90 ± 0.79 ab	9.60 ± 3.07 a	26.0 ± 3.0 a	6.0 ± 2.0 abc	8.13 ± 1.60 cd	1.37 ± 0.32 bc	4041.33±724.26 abcd
BC	70.0 ± 10.0 ab	34.26 ± 5.61 a	32.36 ± 3.39 cd	5.23 ± 0.75 ab	9.0 ± 2.64 a	22.66 ±2.08 ab	5.33 ± 0.57 abc	6.06 ± 1.85 de	0.56 ± 0.177 c	2856.67±132.85 cde

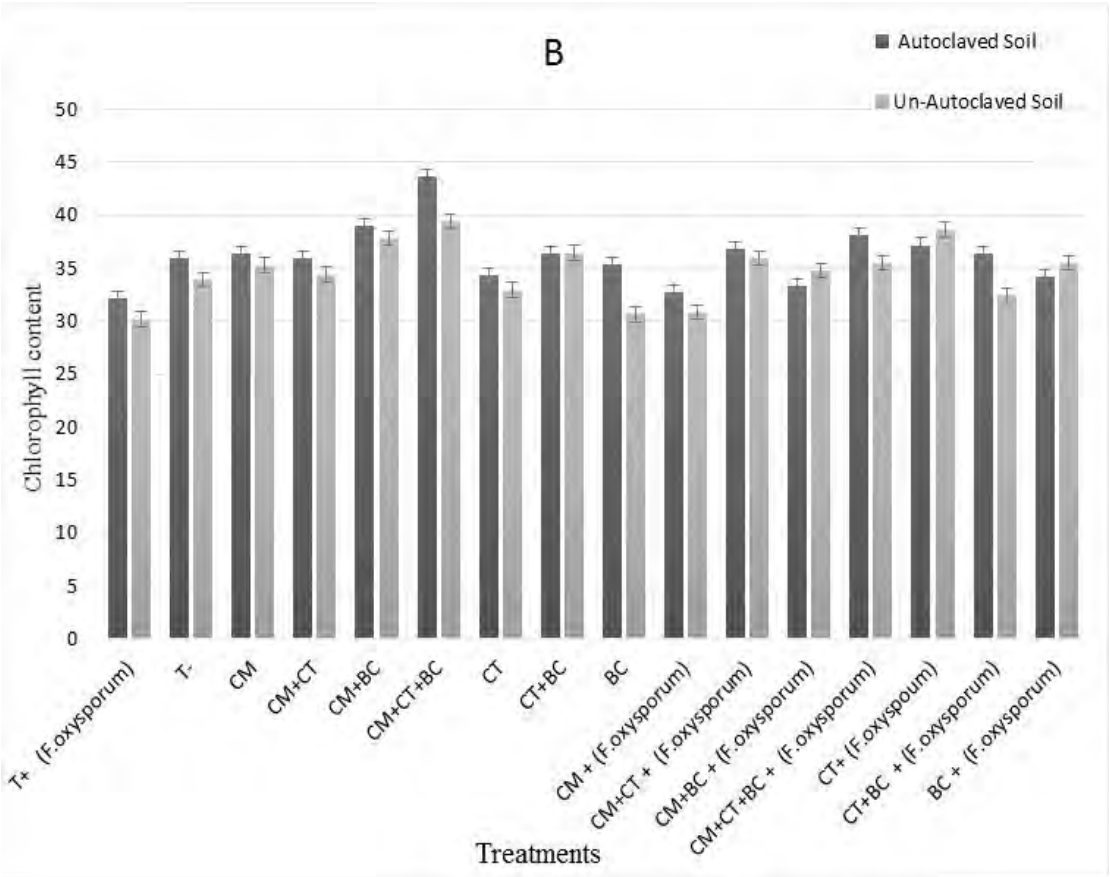
Each value represents mean while ± sign indicates standard deviation (SD). The values in the same column sharing not same lower-case letters differ significantly according to Tukey HSD all-pairwise comparisons test at alpha 0.05.

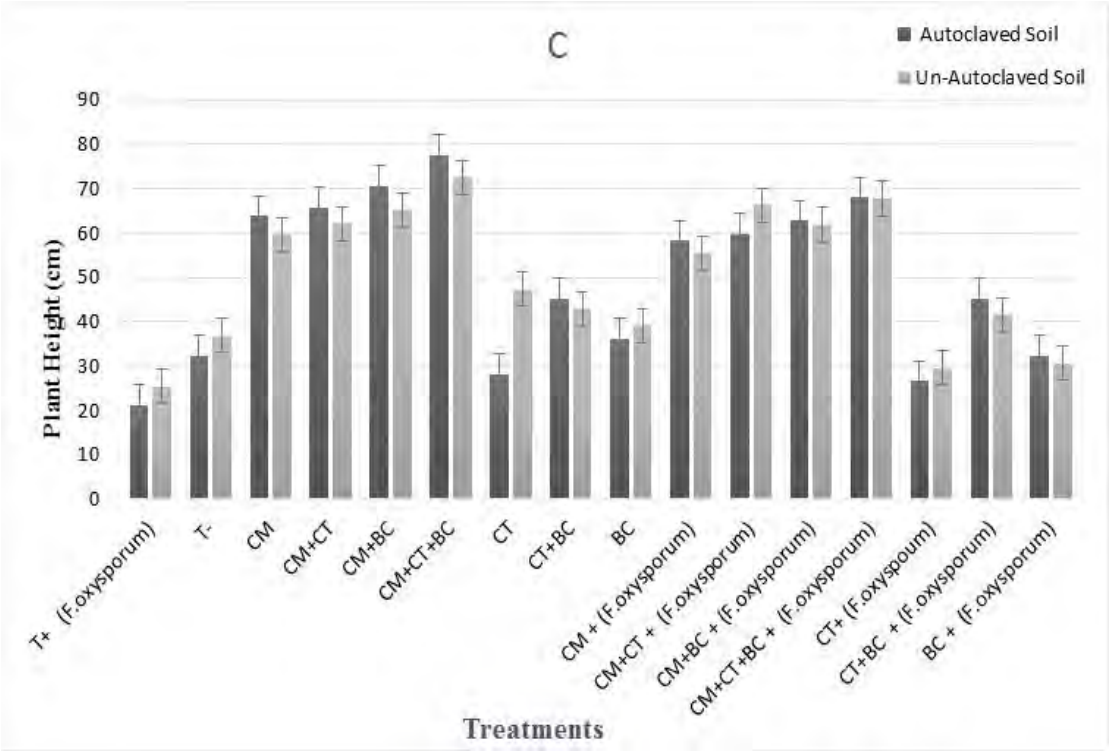
Table:4.14: Impact of compost, compost tea and soil microorganisms on plant growth parameters in Un-autoclaved soil

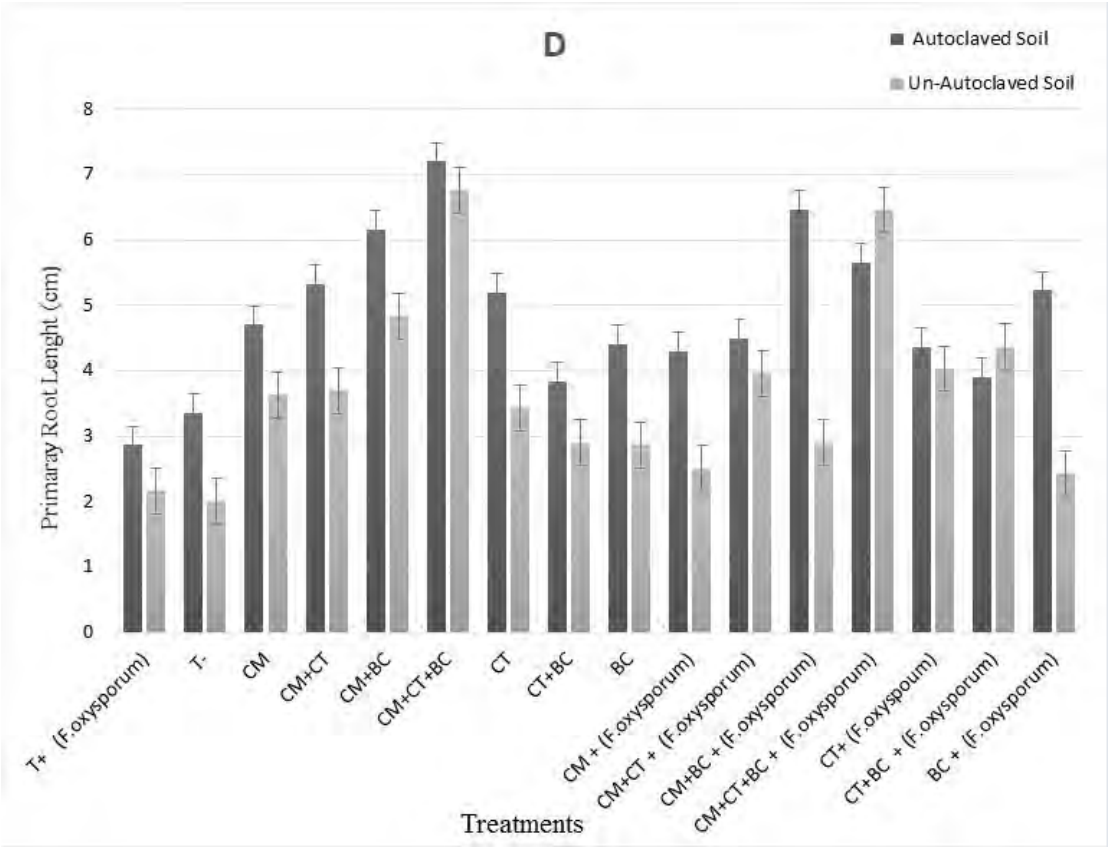
Treatments	Seed Germination %	Chlorophyll Content	Plant Height (cm)	Primary Root length (cm)	Secondary Root length (cm)	Number of leaves per plant	Secondary Shoot number per plant	Fresh Weight (gm)	Dry Weight (gm)	Seedling Vigour index
Un-Autoclaved Soil without Pathogen										
T-	76.66 ± 15.27 a	33.90 ± 1.50 ab	37.03 ± 3.67 de	2.0 ± 0.50 c	4.53 ± 070 d	17.0 ± 2.0 cd	5.0 ± 1.0 ab	2.56 ± 0.70 ef	0.86 ± 0.43 de	3726.67 ± 291.43 cd
T+	80.0 ± 10 a	30.2 ± 2.83 ab	25.46 ± 2.83 cd	2.16 ± 0.28 bc	5.30 ± 0.60 b	11.33 ± 2.51 bc	3.33 ± 0.57 a	1.50 ± 0.26 cd	0.39 ± 0.44 bc	2482.0 ± 553.33 bc
CM	66.66 ± 15.27a	35.30 ± 1.76 ab	59.70 ± 3.50 bc	3.63 ± 0.60 bc	8.56 ± 0.80 abcd	24.0 ± 3.60 bc	5.66 ± 2.08 ab	11.30 ± 2.36bcd	2.10 ± 0.45 bcd	4526.67 ± 336.14 bcd
CM+CT	80.0 ± 10 a	34.43 ± 1.30 ab	62.20 ± 5.46 ab	3.70 ± 0.45 bc	8.80 ± 1.20 abcd	31.73 ± 2.36 ab	7.0 ± 1.0 ab	14.60 ± 2.22 ab	2.53 ± 0.56 ab	5643.67 ± 416.59 abc
CM+BC	80.0 ± 10 a	37.83 ± 4.80 ab	65.33 ± 7.02 ab	4.83 ± 1.43 ab	9.76 ± 2.02 abc	29 ± 4.35 ab	5.0 ± 2.0 ab	12.36 ± 1.51 abc	2.25 ± 0.69 bc	6021.33 ± 395.75 ab
CM+CT+BC	83.33 ± 11.54 a	39.46 ± 0.56 a	72.66 ± 3.05 a	6.76 ± 1.38 a	12.90 ± 2.20 a	35.53 ± 5.31 a	7.66 ± 1.52 a	16.53 ± 2.50 a	3.66 ± 0.57 a	7163.67 ± 1321.20 a
CT	70.0 ± 10 a	32.96 ± 1.22 ab	47.43 ± 3.89 cd	3.43 ± 0.81 bc	7.0 ± 1.73 bcd	27.46 ± 4.84 abc	5.66 ± 1.15 ab	7.16 ± 0.95 de	0.82 ± 0.18 de	3786.0 ± 360.21 cd
CT+BC	80.0 ± 10 a	36.46 ± 2.98 ab	43.0 ± 4.58 d	2.90± 0.40 bc	11.33 ± 2.51 ab	27.0 ± 2.64 abc	5.0 ± 1.0 ab	8.40 ± 1.55 cd	1.35 ± 0.05 bcde	4320.0 ± 520.86 bcd
BC	70.0 ± 10 a	30.66 ± 4.04 b	39.23 ±3.91 d	2.86 ± 0.76 bc	8.06 ± 2.0 bcd	25.0 ± 4.58 abc	4.66 ± 1.52 ab	7.0 ± 1.32 de	1.0 ± 0.20 d cde	3328.0 ± 650.01 d
Un-Autoclaved Soil with Pathogen										
CM	73.33 ± 5.77 a	30.86 ± 2.80 b	55.6 ± 6.91 ab	2.50 ± 0.52 bc	8.86 ± 2.46 ab	27.33 ± 3.51 ab	5.0 ± 1.0 a	9.30 ± 2.91 ab	1.32 ± 0.08 bc	4723.33 ± 426.88 ab
CM+CT	76.66 ± 15.27 a	35.96 ± 3.52 ab	66.33 ± 4.16 a	3.96 ± 0.80 bc	9.33 ± 1.52 ab	28.66 ± 4.50 ab	6.33 ± 0.57 a	11.03 ± 1.28 a	1.90 ± 0.52 ab	5796.67 ± 1178.0 a
CM+BC	80.0 ± 10 a	34.83 ± 2.51 ab	61.9 ± 8.82 a	2.90 ± 1.13 bc	9.50 ± 2.17 ab	25.33 ± 5.13 ab	6.66 ± 1.52 a	11.63 ± 1.12 a	1.91 ± 0.25 ab	5747.0 ± 1211.0 a
CM+CT+BC	76.66 ± 5.77 a	35.56 ± 1.04 ab	67.83 ± 4.80 a	6.46 ± 0.76 a	9.80 ± 1.83 ab	31.0 ± 3.60 a	6.0 ± 1.73 a	11.74 ± 1.90 a	2.88 ± 0.97 a	5970.67 ± 815.46 a
CT	73.33 ± 11.54 a	38.63 ± 1.50 a	29.66 ± 8.14 cd	4.03 ± 0.50 bc	5.03 ± 0.45 ab	22.66 ± 4.93 abc	5.0 ± 20 a	6.46 ± 1.56 bc	0.95 ± 0.31 bc	2482.67 ± 143.18 c
CT+BC	73.33 ± 11.54 a	32.40 ± 1.85 ab	41.66 ± 3.05 bc	4.36 ± 1.30 ab	10.66 ± 4.30 a	28.66 ± 5.50 ab	4.0 ±1.0 a	8.73 ± 1.18 ab	1.32 ± 0.08 bc	3856.0 ± 913.68 abc
BC	70.0 ± 10 a	35.56 ± 1.70 ab	30.66 ± 4.61 cd	2.43 ± 0.47 bc	9.46 ± 2.24 ab	23.33 ± 5.03 abc	5.33 ± 0.57 a	5.76 ± 1.0 bcd	0.40 ± 0.25 bc	2768.0 ± 96.08 bc

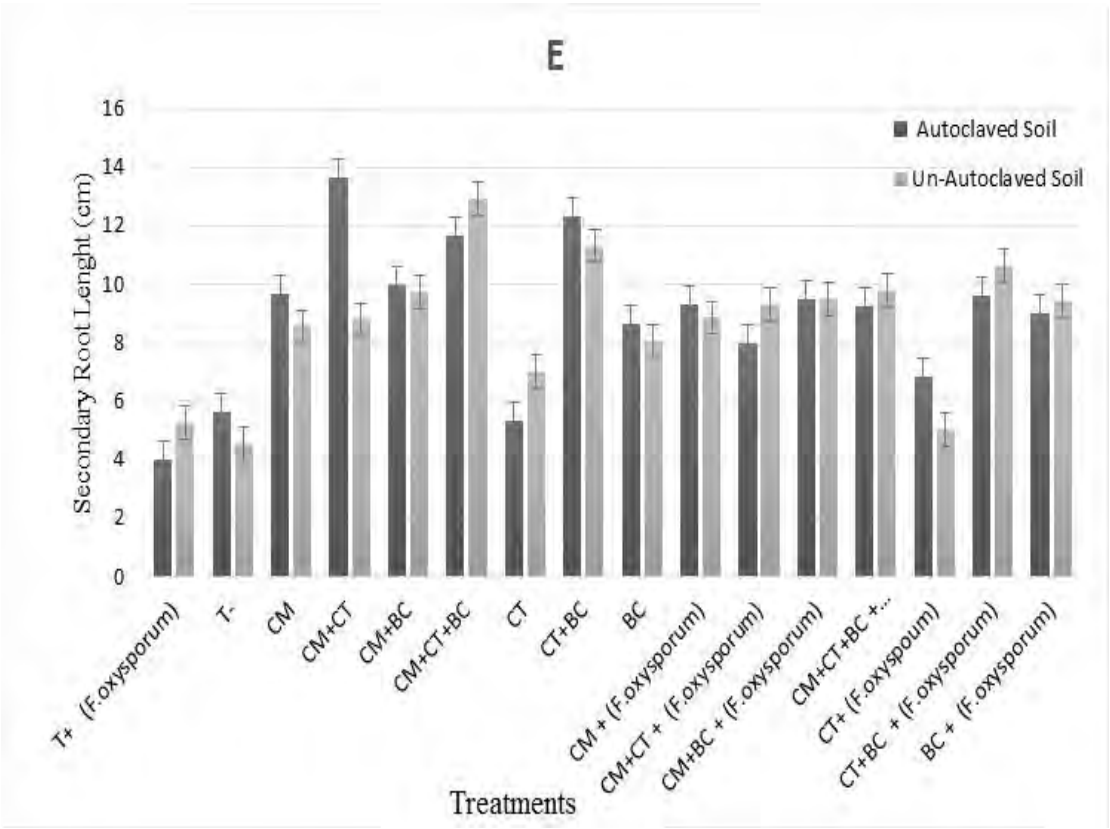
Each value represents mean while ± sign indicates standard deviation (SD). The values in the same column sharing not same lower-case letters differ significantly according to Tukey HSD all-pairwise comparisons test at alpha 0.05.

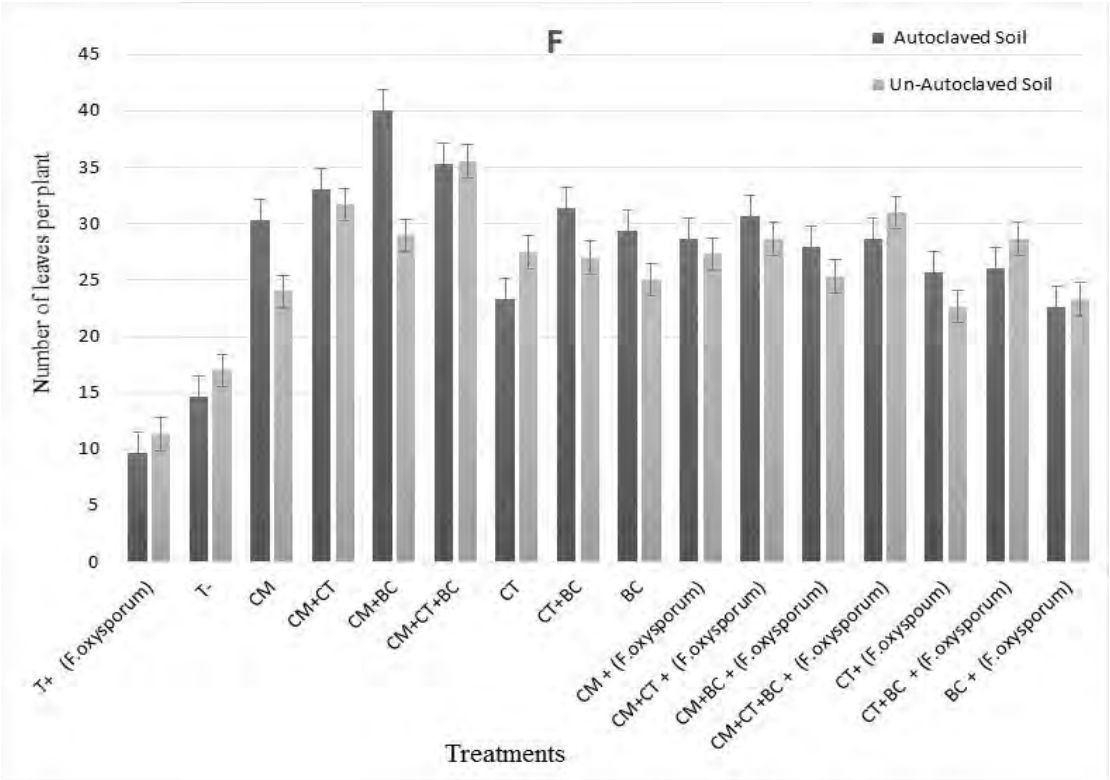

A
Autoclaved Soil
Un-Autoclaved Soil
Seed Germination %
100
90
80
70
60
50
40
30
20
10
0
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC + (F.oxysporum)
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments

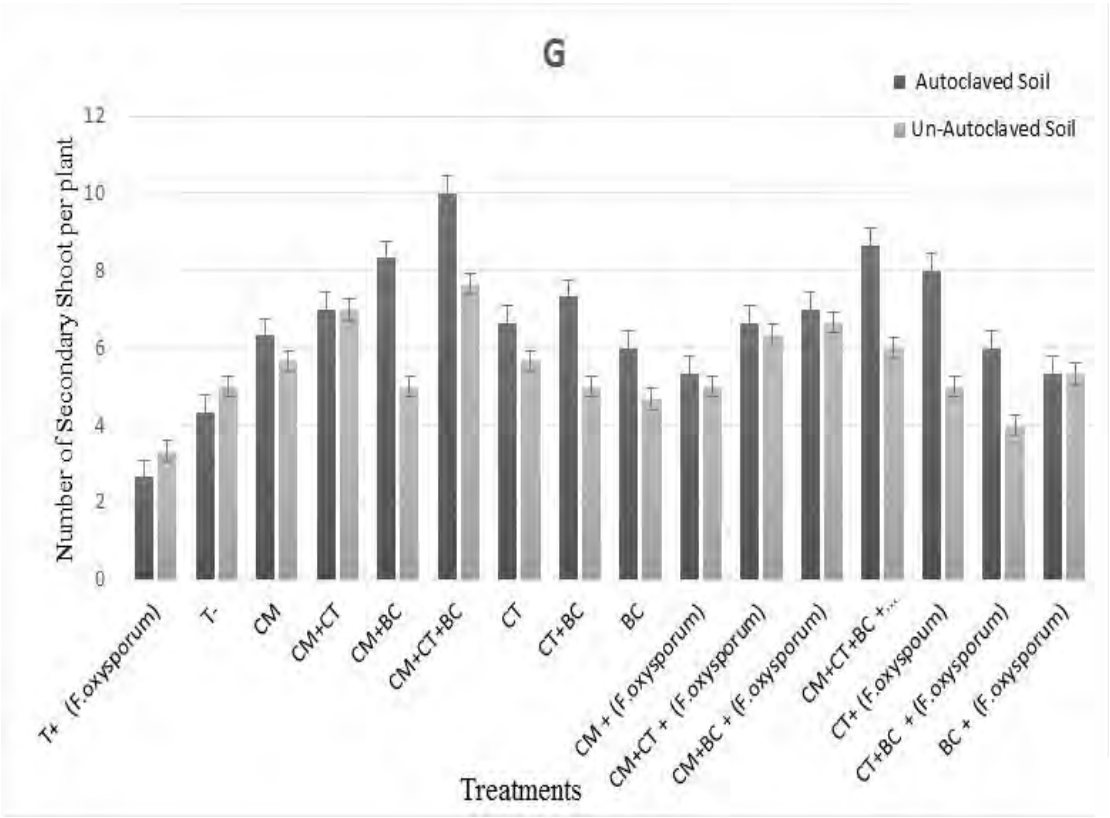

B
Autoclaved Soil
Un-Autoclaved Soil
Chlorophyll content
50
45
40
35
30
25
20
15
10
5
0
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC + (F.oxysporum)
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments

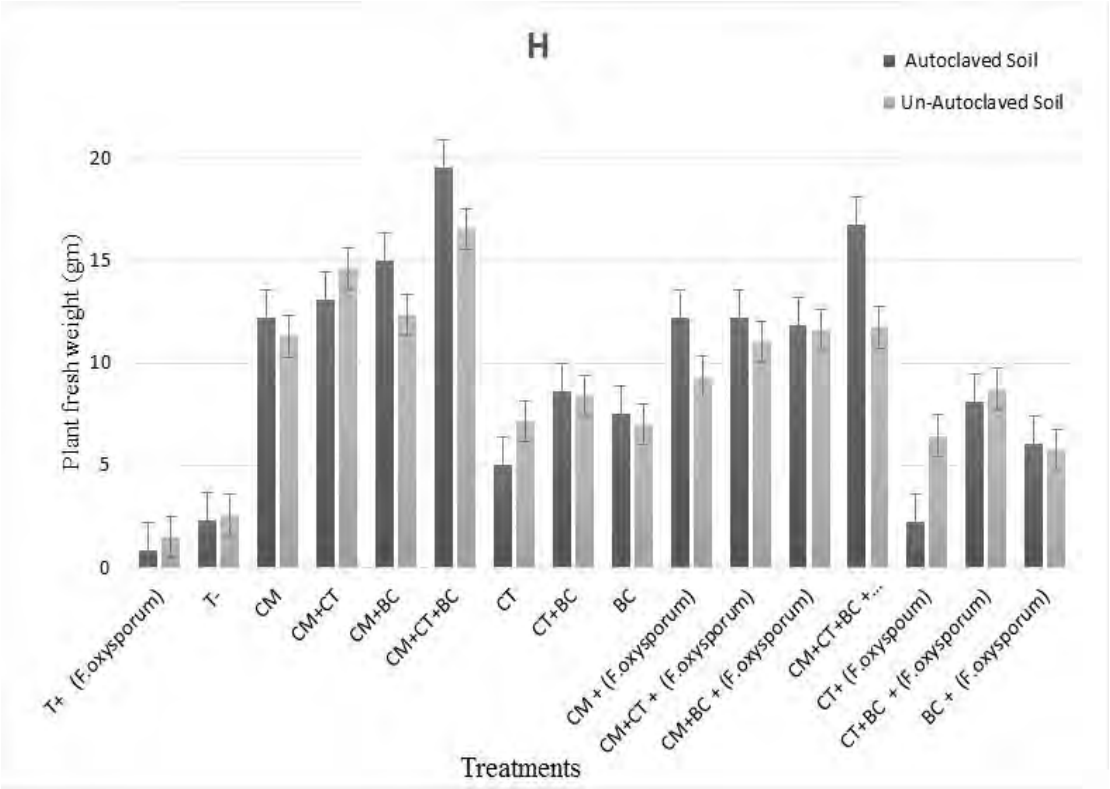

C
Autoclaved Soil
Un-Autoclaved Soil
Plant Height (cm)
0
10
20
30
40
50
60
70
80
90
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC + (F.oxysporum)
CT+ (F.oxyspoum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments


D
Autoclaved Soil
Un-Autoclaved Soil
Primaray Root Lenght (cm)
0
1
2
3
4
5
6
7
8
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC + (F.oxysporum)
CT+ (F.oxyspoum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments


E
Autoclaved Soil
Un-Autoclaved Soil
Secondary Root Lenght (cm)
0
2
4
6
8
10
12
14
16
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC +...
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments


F
Autoclaved Soil
Un-Autoclaved Soil
Number of leaves per plant
0
5
10
15
20
25
30
35
40
45
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC + (F.oxysporum)
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments


G
Autoclaved Soil
Un-Autoclaved Soil
Number of Secondary Shoot per plant
0
2
4
6
8
10
12
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC +...
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments


H
Autoclaved Soil
Un-Autoclaved Soil
Plant fresh weight (gm)
0
5
10
15
20
T+ (F.oxysporum)
T-
CM
CM+CT
CM+BC
CM+CT+BC
CT
CT+BC
BC
CM + (F.oxysporum)
CM+CT + (F.oxysporum)
CM+BC + (F.oxysporum)
CM+CT+BC +...
CT+ (F.oxysporum)
CT+BC + (F.oxysporum)
BC + (F.oxysporum)
Treatments

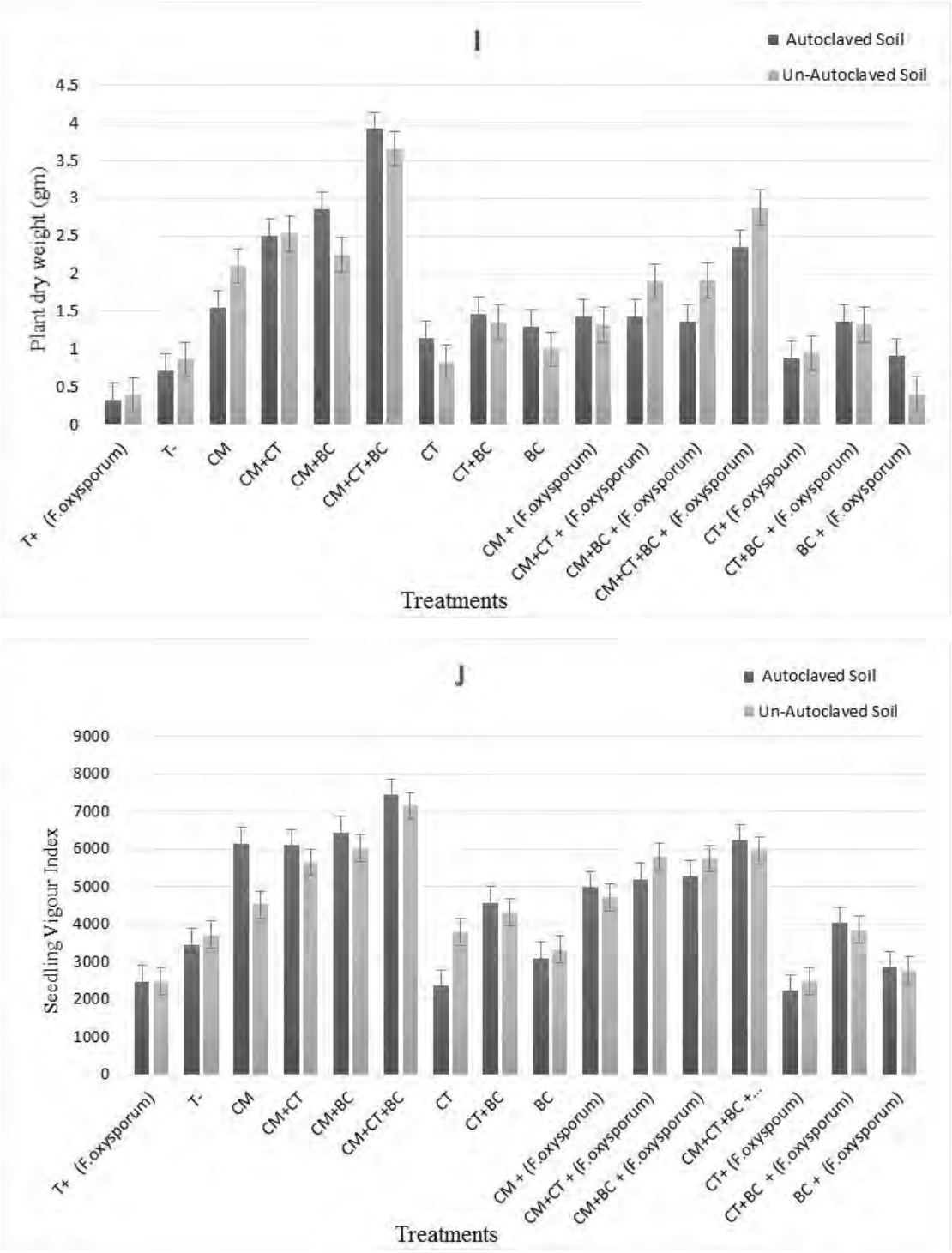


Figure 4.23: Impact of different treatments on plant growth parameters in autoclaved and un-autoclaved soil (**A**) Seed germination % (**B**) Chlorophyll content (**C**) Plant height (**D**) Primary root length (**E**) Secondary root length (**F**) Number of leaves per plant (**G**) Number of secondary shoots per plant (**H**) Plant fresh weight (**I**) Plant dry weight (**J**) Seedling vigour index

Discussion

Farming is essential for feeding a growing global population. However, traditional farming, which heavily depends on chemical fertilizers and pesticides, can harm the environment and human health. Recently, there's been more interest in sustainable farming methods that reduce the use of chemicals while improving soil quality and increasing crop yields. Compost is a valuable source of organic matter, essential nutrients, and beneficial microorganisms. When it is added to the soil, it works wonders by enhancing soil structure, increasing its ability to retain water, and holding onto nutrients. Scientific research has confirmed that regularly using compost can boost soil fertility and decrease the necessity for synthetic fertilizers. It provides shelter for beneficial soil microbes, improving the agricultural properties of the soil (Lehmann *et al*., 2015. The data presented in Table 4.11 demonstrates that incorporating compost into the soil improved soil health by increasing its levels of nitrogen (N), phosphorus (P), and potassium (K), organic matter content, and reducing soil bulk density. This indicates that composting is a practical approach to reduce the necessity for formers to add additional N.P.K fertilizers.

According to Weber *et al*., 2007, soil physical properties tend to improve shortly after adding compost. The findings from our current study indicate that, in most instances, using compost (organic fertilizers) either alone or in combination, substantially increased the amount of organic matter in the soil when compared to the control group (Table 4.12). The most significant increases in soil organic matter (OM) after harvesting were obtained 2.86, 2.36, and 1.54 times, the tomato plants treated with CM+CT+BC, CM+CT, and CM+BC, respectively. These notable improvements can likely be attributed to the stability of compost materials, which tend to have higher organic matter content compared to compost tea. These findings parallel with the research conducted by (Abdel-Haleem *et al*., 2022; Leifeld *et al*., 2002). The reductions in the soil pH were more evident under individual applications of compost or combined with compost tea and soil microorganism (bacteria). That reduction is depending on increased of organic matter content in compost compared to other treatments. As a result, oxidation of organic compounds, microorganism's activity increased, leading to higher production of CO_2 and other organic acids. These processes contributed to a reduction in soil pH. The soil pH decreases to 6.47 ± 0.02, 6.68 ± 0.10, 6.77 ± 0.10, and

6.85 ± 0.13 in soil treated with CM, CM+CT, CM+BC, and CM+CT+BC. These results are in accordance with those reported by (Abdel-Haleem *et al*., 2022). The soil's electrical conductivity (EC) increased when organic amendments were applied, whether alone or in combination. 2.15 ± 0.03 ds/m was reported in treatment CM+CT+BC. This can be explained by the presence of a high concentration of soluble salts in these organic amendments. These findings are consistent with the research conducted by (Forster *et al*.,2006) and Lakhdar (2009). Soil bulk density is an indicator of how compacted the soil is and indicates its overall health. When compost was applied, it resulted 22.32% reduction in soil bulk density compared to other treatments.

Compost tea is a liquid blend made from soaking compost. It includes beneficial microorganisms, organic matter and essential nutrients. When compost tea applied as a foliar spray or soil conditioner, compost tea provides the ability to enhance plant growth and to mitigate the incidence soil borne diseases, as mentioned in 2019. (Inbar *et al*., 2019). The analyzed compost tea, as detailed in Table 4.7, exhibited unique biochemical characteristics. It showed (5.23 ± 0.37%) organic matter, C/N ratio of (10.67 ± 0.56), and N.P.K content of (0.61 ± 0.04%), (0.18 ± 0.07%), and (0.09 ± 0%), respectively. The pH of the compost tea was recorded as 8.10 ± 0.66, with an electrical conductivity of 2.81 ± 0.17 ds/m. During the compost tea process, microorganisms from the compost were added to the water, where they multiplied. Compost tea has benefits in stimulating plant growth and controlling phytopathogens, as demonstrated (Scheuerell and Mahaffee 2002). To achieve this goal, the physicochemical properties of nutrients in compost tea, including its humic components including, enhance plant nutrition, exhibit direct toxicity to pathogens, and or systemic immunity may induce (Köhl *et al*., 2019). Furthermore, compost teas have been designed to act as biocontrol agents against beneficial pathogens and bacteria on leaf and root surfaces, (Diánez *et al*. 2006). The isolated strains from rhizosphere of tomato plants are found to solubilizers of inorganic phosphates via release of organic acids that result in the formation of halo zone around the bacterial colony (Karthika *et al*., 2020). Plant growth promoting rhizobacteria as phosphate solubilizers show an effective role in its solubilization and conversion to readily available form for plants for uptake and utilize for their growth promotion.

Biochemical tests were performed to analyse bacteria that showed positive response towards various compounds. That revealed the potential of these bacteria to utilize different sugars and substrates from surroundings in the rhizosphere while competing for resources in the rhizosphere. Among all isolated strains, it was noticed that *Bacillus* species are predominant and reported to be more tolerant to different environmental conditions as compared to other genera. (Nicholson *et al*., 2000). Isolated strains were found positive for plant growth promoting (PGP) traits including phosphate, protease, cellulase, zinc solubilization along with atmospheric nitrogen fixation. All these PGP traits have been documented extensively to boost plant growth. Atmospheric nitrogen fixing ability of bacterial strain is also an important characteristic of plant growth promotion. Out of four isolated rhizospheric *bacillus* strains two strains S1 and S10 were able to fix atmospheric nitrogen. It has been well documented that various *Bacillus spp*. have nifH gene with ability to produce nitrogenase (Alaylar *et al*., 2020). In another study by (Solanki *et al*., 2017) it has been reported that some of the *Bacillus* species. have the nifH gene and produce nitrogenase enzyme, which can fix atmospheric nitrogen (N_2) and provide it to plants to enhance plant growth and yield (Kuan *et al*., 2016). Besides plant growth promotion, pathogen invasion can be minimized by the synthesis of extracellular enzymes with capacity to degrade the fungal cell wall (Olanrewaju *et al*., 2019). All the studied bacterial strains were able to produce broad range of extracellular enzymes including protease, catalase, amylase, cellulase. All these enzymes have been reported to cause lyse of fungal cell wall (Simmons and Fry, 2017; Cheba and Zaghloul, 2020; Ullah *et al*., 2020).

Currently studied strains have broad antifungal activity which can inhibit the growth of *Aspergillus flavus*, *Fusarium oxysporum* and *Aspergillus niger*. The strains including. S1, S2, S4 and S10 showed significant antagonistic activity with a variable range of percentage inhibition against selected fungal strains. Results of current study revealed that isolated *Bacillus* strains S1, S2, S4 and S10 reduced the *Fusarium spp*. growth by (41.9 ± 1%), (39.4 ± 1%), (42.1 ± 3%) and (46.6 ± 1%) respectively while in case of *Aspergillus flavus* and *Aspergillus niger* species, (57.77 ± 6%), (46.44 ± 6%), (62.40 ± 1%), (30.62 ± 0%), and (51 ± 0%), (62.1 ± 0%), (66.6 ± 0%), (44.3 ± 3%) respectively. S4 strain caused 8.2% more inhibition as compared to previously reported *Bacillus subtilis* 30VD-1 against *Aspergillus flavus* (Djellel and Larous., 2018)

The findings of present study align with previous research indicating that the incorporation of compost as an organic amendment leads to a substantial reduction in soil bulk density (Abdel- Haleem *et al*., 2022) In present study the maximum nutrient content of N 1.35 ± 0.19%, 1.15 ± 0.15%, and 1.12 ± 0.10%), were achieved in those treatments receiving CM+CT+BC, CM and CM+CT as compared to the soil (0.19 ± 0.03 %). The maximum P and K contents 0.51 ± 0.07 % and 0.38 ± 0 % were achieved in treatment CM+CT+BC as compared to control and other treatments. Moreover, the application of compost and compost tea (organic fertilizers) led to notable and statistically significant alterations in various vegetative growth parameters. Specifically, the combination of CM+CT+BC treatments resulted in the most substantial increases in plant height, secondary shoot number, plant fresh weight, and plant dry weight. Conversely, the control treatment yielded the lowest values for these characteristics. In autoclaved and unautoclaved soil, the addition of CM+CT+BC led to a remarkable 2.64,1.38 and 1.85 and 0.96 times increase in plant height compared to the positive and negative control respectively (table 4.13 and 4.14). These findings highlight the efficacy of CM+CT+BC treatments in promoting the vegetative development of tomato plants.

Utilizing a combination of compost, compost tea, and soil microorganisms has been shown to enhance plant health, crop production, and nutritional quality, as documented in studies by Gamaley *et al*. (2001), Pant *et al*. (2009), and Pant *et al*. (2011). Compost tea, with its live microorganisms, holds the potential to enhance nutrient uptake and facilitate plant growth, as supported by research conducted by Scheuerell and Mahaffee (2002), Ingham (2005), and Hargreaves *et al*. (2008). Across all the treatments, there was a significant increase in the number of leaves per plant compared to the control. In autoclaved soil, CM+BC treatment resulted in the highest increase at a rate of 3.14 and 1.72 while in un-autoclaved soil, CM+CT+BC treatment led to a notable increase of 2.13 and 1.09 times. Furthermore, the variation in plant fresh and plant dry weight was significantly influenced by the organic fertilizer treatments (Table 4.13 and 4.14). The most substantial enhancements, with increases of 22.55 and 11.25 times for plant fresh and dry weights, respectively, were observed in autoclaved soil treated with CM+CT+BC. For instance, the relative chlorophyll content index (SPAD) reached its highest readings at 0.35 and 0.30 rate in both autoclaved and un-autoclaved soil, respectively, compared to their control treatments.

Results of the present study suggests that several variables were significantly affected by different treatments, making them valuable predictive indicators for optimal tomato plant growth and sustainable yields in the same agroecosystem. In this study, it was clear that the CM+CT+BC and CM+BC treatments consistently outperformed all others in terms of tomato growth parameter values, followed closely by the CM+CT treatment. This confirms the superiority of using a combination of compost, compost tea and bacterial consortia as the preferred treatment to maximize tomato growth parameters under tested conditions. These treatments showed continuous ability to deliver nutrients necessary for plants throughout their growth cycle express, and consequently enhance various important physiological mechanisms of action.

Conclusion

In the present study rhizospheric *Bacillus* strains were isolated, compost and compost tea were prepared and tested for plant growth promoting characteristics in different combinations. CM+CT+BC and CM+BC treatments were consistently more significant as compared to other treatments, in terms of growth parameters. This confirms the superiority of using a combination of compost, compost tea and bacterial consortia as the preferred treatment option to maximize plant growth and physicochemical properties of the soil. Soil analysis exhibited an increase of (1.35 ± 0.19%) for N, (0.51 ± 0.07%) for P and (0. 38 ± 0%) for K respectively. Soil organic matter (SOM) at post-harvesting stage was found to be 2.86 times greater as compared to pre-harvesting soil organic matter after treatment with CM+CT+BC. In autoclave soil, addition of CM+CT+BC led to a remarkable increased in plant height of 2.64, fresh and dry weight (22.55 and 11.25) and relative chlorophyll content index reached its highest readings of 0.35 times. Strain S1, S2, S4 and S10 reduced *Fusarium spp*. growth 41.9 ± 1%), (39.4 ± 1%), (42.1 ± 3%), and (46.6 ± 1%) respectively. *Bacillus* strain S4 caused an inhibition of *Fusarium oxysporum* (46.6% ± 1), *Aspergillus flavus* (62.40 ± 1%) and *Aspergillus niger* (66.6 ± 0%) in vitro experimentation. Overall, our study highlights the potential benefits of sustainable agricultural practices that can incorporate beneficial ecological and microbial changes to create healthy and productive soils. Further research and field trials are needed to validate and refine these promising materials for development of sustainable agriculture models.

Future Prospects

For exploring the future possibilities, researchers can continue to refine and expand the knowledge gained from this research, ultimately contributing to sustainable and non-destructive agricultural practices in the environment. Future prospects of the present study may include:

- Future research could be conducted for detailed insight into the microbial composition of composted soil, compost tea, and beneficial bacterial species.
- Examining long-term effects on soil microbial communities and their role in maintaining soil health provides valuable insights into sustainable agriculture.
- Further research may be exploring antifungal activity of the *Bacillus* genus may lead to the development of vaccines against fungal pathogens in agriculture and will greatly advance the understanding of these efficacy mechanisms and their potential to it will reduce the need for fungicides.
- Large-scale field trials to validate laboratory findings under real-world agricultural conditions will be an important next step. These trials can help determine the feasibility and effectiveness of commercial applications of compost, compost tea, and microbial treatment.

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