Assessing the Ecological Impacts of Biocontrol Agents on Soil Microbiota and Soil health in Goan Agricultural Soils

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DECLARATION BY STUDENT

I hereby declare that the data presented in this dissertation report entitled, **Assessing the Ecological Impacts of Biocontrol agents on Soil Health and Microbiota of Goan Agricultural soils** is based on the results of investigations carried out by me in the discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Ms. Snigdha Mayenkar and the same has not been submitted elsewhere for the award of a degreeor diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations /experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report —**Assessing the Ecological Impacts of Biocontrol agents on Soil Health and Microbiota of Goan Agricultural soils** I is a bonafide work carried out by Ms. Kaushiki Krishna Karnat under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

Agricultural practices worldwide are undergoing a transformative shift towards sustainable and environmentally friendly methods. One such approach is the use of biocontrol agents to manage pests and diseases in crops, minimizing reliance on synthetic chemicals. Goan agricultural soil is characterized by its unique ecological and agricultural dynamics. Understanding the impacts of biocontrol agents on soil microbiota and health is of paramount importance to sustainable agriculture. This study delves into the complex relationship between biocontrol agents and soil health, focusing on their effects on soil microbiota in Goan agricultural soil. By assessing the ecological impacts of these agents, we aim to contribute to the growing body of knowledge on sustainable agricultural practices. The findings from this research will not only enhance our understanding of soil microbiota dynamics but also inform strategies for optimizing the use of biocontrol agents in Goan agriculture. Through meticulous experimentation and analysis, this study endeavours to shed light on the complexities of the soil ecosystem and its responses to biocontrol agents. It is our hope that the insights gleaned from this research will pave the way for more sustainable and resilient agricultural practices in Goa and beyond.

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ABBREVIATIONS

FAO	Food and Agriculture Administration
UV	Ultraviolet
CWDE	Cell Wall Degrading Enzyme
NaCl	Sodium Chloride
HCl	Hydrochloric Acid
$BaCl_2$	Barium Chloride
FYM	Farm Yard Manure
Kg	Kilogram
cm	Centimeter
mg	Milligram
nm	Nanometer
°C	Degree Celsius
%	Percentage
λmax	Lambda max
PGPR	Plant Growth Promoting Rhizobacteria
rpm	Revolutions per Minute
CFU	Colony Forming Unit
TCO	Control soil from Taleigao
TPO	Taleigao soil treated with *Pseudomonas fluorescens*
TT	Taleigao soil treated with *Trichoderma viride*
TN	Taleigao soil treated with Neem (
TCH	Taleigao soil treated with
RCO	Control soil from Raia
RP	Raia soil treated with *Pseudomonas fluorescens*
RT	Raia soil treated with *Trichoderma viride*
RN	Raia soil treated with Neem (*Azadirachta indica*)
RCH	Taleigao soil treated with Carbendazim- 12% + Moncozeb – 63 %

ABSTRACT

Chemical pesticides and fertilizers are known to have harmful effects on environment and human health. Consequently, they have also been linked to a significant impact on the microbial composition of soil which in turn affects soil health and fertility. Therefore there is a need for greener alternative like biological control agents. This study assesses the influence of biocontrol agents *Pseudomonas fluorescens*, *Trichoderma viride*, Neem cake (*Azadirachta indica*) and a chemical pesticide (Carbendazim-12% + Moncozeb-63%) on agricultural soil in Goa. We examine soil physical, chemical, and biological parameters, along with pot trials with cowpea ―Alsando‖ plants. Results reveal that biocontrol agents enhance soil health, microbial diversity, and plant growth in comparision to chemical pesticides that exhibit detrimental effects on these parameters.

1. INTRODUCTION

1.1 BACKGROUND

Goa is a small, emerald state located on the west coast of the Indian peninsula. A vital aspect of the state's economy, agriculture is one of the dwindling occupations today,. The majority of people in coastal, urban, and rural areas make their living from the occupations such as mining, tourism, and fishing (Morakar et al., 2023). The State covers 3,702 square kilometres, of which 3,70,200 hectares, or around 35%, is used for agriculture. Cashew, coconut, and spices are a few of the key cash-rich crops grown in Goa (Oheraldo, 2022). Goa is home to special foods such as ―Alsane‖ (*Vigna unguiculata),* "Tambdi Baji‖ (*Amaranthus Cruentus*), "Bhendi" (*Abelmoschus esculentus),"*Mancurad‖ (*Mangifera indica*), and Khola chillies (*Capsicum frutescens*, some of which have earned Geographical Indication tags.

Types of land used for farming in Goa.

Khazan Land: This region is made up of low-lying lands around the estuaries. Monsoon paddy crops are grown on this site.

Ker Land: This is level terrain with an elevated water table that is slightly above sea level. Soils that are sandy to sandy loams are found here. In these locations, vegetables, legumes, and Rabi paddy crops are farmed.

Morod land: This type of terrain is either highland or terraced, ideal for single rain-fed rice crops or horticultural/plantation crops (ICAR- CCARI GOA)

Diseases affecting Goan crops and their management:

In many regions of the world, cowpeas (*Vigna unguiculata*) are consumed as a high-quality plant protein source (ICAR- CCARI GOA, 2024) Cowpeas are an essential dietary component for humans due to their high protein and carbohydrate quantities, relatively low-fat content and comparable amino acid pattern to cereal grains (Gupta, 2022). Cowpea is severely harmed economically by fungal disease. The most common diseases are cowpea mosaic, *Fusarium oxysporum* wilt, root rot, (ICAR- CCARI GOA). Chemical treatments such as methyl bromide fumigation are used to treat this condition. Applying organic manures such as FYM/poultry manure or utilizing biocontrol agents such as antagonistic *Pseudomonas fluorescens* treatment based on talc is found to be beneficial (ICAR- CCARI GOA, 2024).

The improper use of these chemical pesticides can have detrimental effects on both human health as well as on ecosystem due to its recalcitrant nature (Bose et al, 2022). In addition to their direct effects such as toxicity and altered substrate availability profile of the soil, all these factors indirectly cause a shift in the population dynamics of soil microflora (Prashar, 2016). Therefore there is a need for greener alternative like biological control agents.

The term "biological control agent" describes the use of naturally occurring or modified organisms to lessen the effects of unwanted organisms and promote beneficial insects, microbes, and crops. These agents produce antimicrobial compounds against pathogens, destroy and invade pathogen spores, as well as the pathogen's mycelium,

cell, and endospores. They also generate improved resistance against a pathogen, compete with it for space and nutrients. In order to manage plant diseases, these agents have become viable substitutes to combat many pest diseases as an alternative to chemical pesticides and fertilizers (Singh, 2020).

Fungi play a significant role in the biocontrol agent community due to their ability to detect hosts and their harmful effect on pests. They are efficient in managing pests that cause problems to both humans as well as plants (Dwivedi, 2021). Due to its ability to prevent the growth of certain phytopathogenic fungi, *Trichoderma sp*. is regarded as a promising biological control agent against these phytopathogenic fungi (Bastakoti et al., 2017).

The use of bacterial biocontrol agents has been widespread in reducing the frequency and intensity of a number of crop diseases that are significant to the agricultural sector (Narayanasamy, 2013). Numerous *Pseudomonas* strains have the ability to directly promote plant growth in the absence of pathogens by increasing the availability and uptake of mineral nutrients through solubilization of phosphate. Additionally, they can promote root growth by producing phytohormones or strengthening the plant's resistance to abiotic stress. They are good root colonizers and effective controllers of soil-borne diseases. By using induced systemic resistance in plants, some *Pseudomonas sp*. strains can also protect leaves from developing diseases (Lee et al., 2023).

Phytopesticides are natural pesticides derived from plants. they tend to have lower toxicity to non-target organisms and and degrade easily. Neem is environmentally friendly and biodegradable, provides all the macro and micronutrients needed to

nourish soil and plants, aids in the removal of denitrifying bacteria from the soil, is perfect for cash and food crops, boosts crop yields, lowers the need for fertilizer (KV et al., 2019). Excellent grade organic or natural manure that doesn't harm plants, soil, or other living things is also made from Neem cake.

Although many advantages of using biocontrol agents instead of chemical pesticides are known, there is not enough scientific data available on the effects of these biocontrol agents on the soil health. There is no known study as per our knowledge conducted on Goan agricultural soils. Hence, it is of paramount importance to understand the transient and long term effects of the frequently used biocontrol agents.

1.2 AIM AND OBJECTIVES

Aim:

Assessing the impacts of three different biocontrol agents on soil health and soil microbiome of Goan agricultural soil.

Objectives:

- To compare the effects of biocontrol agents and chemical pesticide on soil health via biochemical testing.
- To assess the ecological impacts of three biocontrol agents on the culturable soil microbiota of Goan agricultural soil.
- To study the effects of biocontrol agent on soil health via the growth of a local food crop cowpea "*Alsando*" (*Vigna unguiculata).*

1.3 RESEARCH HYPOTHESIS

Chemical pesticides have shown harmful effects on environment and human health. They have been linked to significant impacts on the microbial composition of soil which in turn affect soil health and fertility. Therefore there is a need for greener alternatives like biological control agents. It is hypothesized that biocontrol agents will have a net positive effect on the microbial community present in soil whereas chemical pesticide will have a net negative effect on growth of plant as well as microbial community in soil. Additionally, biocontrol agents will enhance the plant growth properties. The application of biocontrol agents in Goan agricultural soil will lead to improvements in soil health and soil microbiota, characterized by healthy microbial diversity, enhanced soil organic matter content, and improved nutrient availability, compared to untreated soil or soil treated with chemical pesticides.

1.4 SCOPE

The research will focus on agricultural soils in Goa, India, to assess the effects of biocontrol agents on soil health and microbiota. The study will evaluate the effects of *Pseudomonas fluorescens , Trichoderma viride,* and Neem (*Azadirachta indica*)) as biocontrol agents, and chemical pesticide (Carbendazim- 12% + Moncozeb – 63%) considering their prevalent use and potential impact on soils in Goa. The study will involve assessment of key indicators of soil health such as microbial diversity, soil pH, and nutrient availability of sulphate and phosphate. The study will employ a controlled experiment with treatments including biocontrol agents, chemical pesticides, and untreated controls to compare their effects on soil health and microbiota as well as study of plant health parameters. This study will provide insights into the effectiveness of biocontrol agents in improving soil health and microbiota in Goan agricultural soil, contributing to sustainable agriculture practices.

2.Literature review

Maintaining biodiversity and sustainable yields in agriculture depend heavily on managing soil health. For industrial agriculture, pesticides and fertilizers are an inevitable evil. Even while they are still vital tools for ensuring the security of the world's food supply, their negative effects cannot be ignored, especially in relation to the widespread emphasis on sustainable agriculture. In addition to the numerous well-documented negative consequences that chemical pesticides and fertilizers have on the environment and human health, they have also been linked to a significant impact on the microbial composition of soil. An essential part of agricultural ecosystems, soil microflora actively contributes to improved crop productivity and soil fertility in addition to being crucial to basic soil processes. In addition to having a significant effect on the physical characteristics of soil, microbial activity is also essential for the advancement of environmentally beneficial techniques such as phytopathogen biocontrol and bioremediation in agricultural soils. Thus, it is known that soil microorganisms are bioindicators of soil activity and health (Prashar, 2016).

The ability of the soil to support and sustain agricultural development while preserving environmental quality is referred to as soil health. An adequate but not excessive supply of nutrients, good structure, adequate depth for rooting and drainage, good internal drainage, low populations of plant disease and parasitic organisms, high populations of organisms that promote plant growth, low weed pressure, absence of chemicals that could harm the plant, resilience after a degradation episode defines high-quality soils. Innovatively combining several techniques that improve the soil's biological, chemical, and physical compatibility for crop production is the goal of management aimed at improving soil health (Magdoff, 2001).

An essential part of soil survey for determining the potentials and constraints of land use and management is soil texture, which is defined as the relative proportions of sand, silt, and clay particles in soils. Soil structure development, carbon sequestration, nutrient retention, water infiltration and storage, and other soil processes have all been recognized as being significantly influenced by soil texture (Xia et al, 2020) .

Soil moisture denotes the water content present in the soil, held within the pore space—the gaps between soil particles. It constitutes a significant aspect of soil concerning crop development and, along with soil pH, plays a key role in determining soil reactions, as well as heat and gaseous exchanges within the soil. In addition to meeting the water requirements of crops, the water present in the soil facilitates the absorption of nutrients by crops in the form of dissolved salts. The soil pH indicates the acidity or alkalinity present in the soil and is gauged on a scale from 0 to 14, with a pH below 7 being acidic and above 7 being alkaline. The optimal soil pH range that ensures broader nutrient availability for crops is between 5.5 and 7.5. Inaccurate soil pH levels may sometimes mimic signs of disease or pest infestation, potentially causing crop damage or unnecessary financial losses in the absence of a correct diagnosis. Therefore, a crucial element in effective crop nutrition management is the precise measurement of soil pH. Among the diverse methods available for determining litter decomposition, the teabag method stands out. This approach is easy, allowing farmers to easily implement it in the field without the need for technical expertise (FAO, 2020).

Plants primarily require nitrogen and phosphorus, with sulphur being the least critical. Plants absorb sulphur from the soil in the form of sulphates through their roots. The soil's sulphur requirements are adequately fulfilled through organic sulphur mineralization and atmospheric deposition. Although ion chromatographic methods are specific for sulphate estimation, they incur high initial costs. Alternatively, sulphur

content can be determined using spectrophotometric and turbidimetric methods. Among these methods, the spectrophotometric approach stands out as the most reliable, accurate, time-efficient, and effective means for assessing sulphur levels in soil and plant materials (Nair, 2020).

Phosphorous (P) is the primary component, along with nitrogen, that restricts agricultural yields in tropical soils, particularly in heavily worn, oxidic soils where the majority of the soil's phosphorous content is locked in oxides and clay minerals. Orthophosphates make up the accessible phosphorous in the soil solution, although microbial and organic phosphorous are other stocks that can quickly become available (Cardoso et al., 2013). Phosphorous is necessary for evaluating the quality of soil (Prashar, 2016).

The on-going use of chemical pesticides presents risks and challenges, highlighting potential hazards in soil fertility reduction. The accumulation of chemical residues in the soil poses dangers during crop rotation, potentially causing irreversible harm to the natural soil flora and fauna. Additionally, it can lead to the contamination of surface and groundwater, as well as water reservoirs, with the capacity to disrupt the natural ecosystem (Vanitha, 2022). The application of synthetic chemicals, while effective in inhibiting pathogens, adversely impacts beneficial microbes. Pesticide treatment hinders the formation of effective root nodules with nitrogen-fixing bacteria. In contrast, utilizing endophytic fungi as a biocontrol agent emerges as an environmentally friendly and cost-effective alternative compared to various other methods. Cultivating these eco-friendly fungi is straightforward, and their management is both easy and environmentally conscious (Yadav, 2020). Currently, there are no definitive solutions to counteract the adverse effects of chemical pesticides on soil

health. The only viable alternatives involve substituting or replacing them with biopesticides or biocontrol agents (Vanitha, 2022).

Biopesticides are becoming more popular due to their benefits for the environment, target specificity, efficacy, biodegradability, and suitability for use in integrated pest management (IPM) programs. Despite notable progress in their market penetration, biopesticides still constitute a comparatively little portion of pest management options. Global production of over 3000 tons annually is increasing at a rapid pace. Just 4.2% of India's overall pesticide market is made up of biopesticides. While the government has included biopesticides in a number of agricultural initiatives to encourage their usage, biopesticides face many challenges locally and are expected to grow at an astounding 10% annual rate (Chakraborty et al, 2023).

Fungal biofertilizers, known for their ability to solubilize phosphate, are frequently utilized as biological agents to enhance the growth and development of plants by improving the uptake of phosphorus. *Trichoderma* s*p.,* widely utilized in the production of biofertilizers, is acknowledged for its ability to improve crop nutrition, facilitate nutrient absorption, and enhance overall plant productivity (Yadav, 2020). *Pseudomonas sp*. possesses multiple mechanisms to inhibit plant diseases, including the secretion of antibacterial substances, competition with other bacteria for nutrients and space, and induction of ISR (Induced Systemic Resistance). Notably, the secondary metabolites produced by *Pseudomonas sp*. play a crucial role in the biological control of plant diseases. In non-pathogenic conditions, numerous *Pseudomonas* spp. can positively influence plant growth by improving the availability and uptake of mineral nutrients through the process of phosphate solubilization (Lee et al., 2023).The establishment of

biological control agents (BCA) is influenced by the soil's physicochemical properties (Leal, 2023).

Numerous horticultural mineral oils, botanicals, plant essential oils, and detergents are currently used for pest and disease control worldwide. Thousands of plants have been tried for this purpose. Neem has emerged as the best choice among the several disease-controlling plants and biopesticides. Neem is widely acknowledged as a natural substance with a variety of applications in industry, agriculture, medicine, and the environment. Worldwide research is currently being done on the benefits of Neem in agriculture. This amazing tree is used to make a wide variety of agricultural products. Products made from Neem include soil conditioners, fertilizers, manures, compost, insecticides, pesticides, and fumigants. Neem tree products are useful insect growth regulators (IGRs) that can also be used to control nematodes and fungus (Adusei, 2022).

Compared to other methods, biological control is less expensive and more affordable. Throughout the crop period, the crop is protected by biocontrol agents. Applying biocontrol agents makes both the environment and the application user safer. They leave minimal traces and proliferate readily in the soil. By promoting the healthy soil microflora, biocontrol agents not only prevent disease but also improve plant and root growth. Applying and handling biocontrol agents to the target is a relatively simple task. Biocontrol agents and biofertilizers can be mixed together. It is simple to produce them. It poses extremley low threat to humans (Sharma et al., 2013).

Biocontrol agents work by either directly parasitizing or infecting pests, competing with them for resources, or producing toxins that are harmful to the pests.

Competition: In order to multiply and endure in their native environments, microorganisms must contend for resources such as minerals, organic nutrients, and space. Both the phyllosphere and the rhizosphere have reported on this. It has been proposed that competition has a part in the luminous *Pseudomonas* strains biocontrol of *Fusarium* and *Pythium* species. Heterotrophic soil fungus relies on competition for substrates above anything else. The most significant competitive advantage belongs to the fungi that have the most propagules or the largest quantity of mycelia growth. The combination of physiological traits necessary for successful competitive colonization of dead organic substrates is known as competitive saprophytic ability (Sharma et al., 2013). Highly competitive microbes that use space and nutrition competition as a mechanism of action are promising alternatives for biological control (Köhl et al., 2019).

Antibiosis: Antibiosis is described as antagonism caused by lytic agents, enzymes, volatile chemicals, or other harmful substances, as well as by particular or non-specific metabolites of microbial origin. A key component of biological control is antibiosis. A condition known as antibiosis occurs when plant leftovers, soil microorganisms, underground plant sections, etc. produce metabolites. It happens when the antagonists' metabolic products inhibit or eradicate the pathogen.

Promotion of plant development: Biocontrol agents also generate growth hormones, such as gibberellins, auxins, and cytokinins. In addition to promoting plant development and suppressing harmful diseases, these hormones also raise yields. The research on the mechanism of growth promotion have shown that (Plant Growth Promoters) PGPR either directly or indirectly stimulates plant growth by producing plant growth regulators, siderophores, or antibiotics to protect plants from harmful

rhizosphere organisms or soil-borne pathogens. *Pseudomonas* species may promote plant development by mineralizing phosphates and generating compounds like gibberellins (Sharma et al., 2013). They are also known to improve the uptake of nutrients and water, or by generating substances like hormones that keep plants fit (Ayaz et al., 2021).

Hyperparasitism : The direct competitive relationship between two organisms in which one is obtaining nutrients from the other is known as parasitism. Hyperparasitism is the term used to describe a relationship where the host is also a parasite, such as a plant pathogen. The primary methods of parasitism involve the expulsion of CWDEs (Cell Wall Degrading Enzymes) sometimes when combined with the release of secondary metabolites in close proximity to the host cell, resulting in cell wall openings and subsequent cytoplasmic disarray (Köhl et al., 2019).

Most biopesticides fall under the category of basic or low-risk compounds, meaning that their risk factor to humans is low. A large number of them are utilized at levels similar to those observed in the environment. Low-risk materials break down quickly in comparison to chemical pesticides, which take a long time to decay and leave behind residue in food or the environment that could harm humans or other living things. The likelihood of microbial pesticides having a negative impact on human health is very low, and the fact that crystal protein needs an alkaline environment to transform into its active toxic form a state that is present in the guts of insects but absent from most mammalian systems may help to explain why they are not toxic to animals or humans. Moreover, the crystal protein binding site, which is assumed to be absent from human systems, is expressed by the targeted insect species (Ahmad et al., 2022).

Along with the merit there are few demerits of bio pesticides. Considering their rate of biodegradability, they have a very short shelf life. This affects production procedures, development expenses, and inconsistent field performance. Within the entire pest community, microbes make up a rather small percentage. Because of this, only a portion of the pest population is effectively controlled by these microbial bio pesticides. In addition, they work more slowly than chemical pesticides. Adverse weather conditions have an impact on the effectiveness of microbial insecticides. Desiccation, heat, UV rays, and other elements lessen the effect. Therefore, it is important that the delivery system be carefully designed. Moreover, they are less effective than conventiona pesticides and provide a minimal risk to diseases. The process of creating a biopesticide is quite costly in general (Essiedu et al., 2020)

3. Methodology

3.1 Soil sample collection

Agricultural soil was collected from the fields of Raia, Salcete, Goa (15.3166506°N,73.9998257°E) and Taleigao, Tiswadi, Goa (15.476648°N, 73.818062° E) in the month of September and October 2023.

3.2 Physical testing of soil physical parameters

3.2.1 Ribbon method for soil texture:

Handful of soil sample was placed on palm and water was added drop wise until it turned into modeling clay. The soil was rolled into a cigar shape with about 1/2-3/4 inch diameter and the cigar was gently pressed into a flat ribbon shape. As the ribbon developed, it was extended over a forefinger until it broke from its own weight. This procedure was followed for finding the texture of both the soil samples (FAO, 2020).

5.2.2 Soil moisture by gravimetric water content:

The wet weight of both Soil 1 and Soil 2 was measured (Wm). This soil sample was then dried in the oven at 100°C for one day and the dry weight was measured (Wd). The water content was calculated using the following formula (FAO, 2020).

Gravimetric water content = (Wm-Wd)/ x 100

Where

Wm = weight of moist soil (g)

Wd = weight of dried soil (g)

3.3 Field Experimental setup

500 g of soil was weighed and added in a total of 28 pots. 14 pots of Soil 1 (Taleigao soil) and 14 pots of soil 2 (Raia Soil). Out of 14 pots 3 pots were treated with *Pseudomonas fluorescens* (1.5 g in 25 mL of water) and 3 pots were treated with *Trichoderma viride* (1.5 g in 25 mL water), 3 pots with Neem cake (7.5g in 25mL of water) and 3 pots were treated with Carbendazim- 12% + Moncozeb – 63% (0.25g in 25mL water). 2 pots were kept as control for each soil. Soil was tested for different parameters for a period of 2 months.

3.4 Testing of soil chemical properties:

3.4.1 Soil pH using pH meter method:

The pH of soil sample of each treatment and control was calculated after every 15 days for a period of 2 months. 1g of soil sample was weighed and added to beaker containing 10 mL of distilled water. After mixing for few minutes the solution was then allowed to settle for 30 minutes. pH of the soil samples was measured by inserting the pH electrodes into the soil solution (FAO, 2020).

3.4.2 Sulphate test

1g of soil sample was weighed and added in 10 mL of distilled water. After vortexing, solution was filtered using Whatman filter paper. In 2.5 mL of filtrate, 0.5 mL of NaCl – HCl solution and 0.5 mL of Glycerol – ethanol solution and 0.075g of $BaCl_2$ was

added. After proper mixing absorbance was measured at λmax of 420 nm using distilled water as blank. The reagents were added according to following table.

3.4.2.1 Preparation of NaCl- HCl solution

It was prepared by dissolving 6 g of sodium chloride in minimum amount of distilled water in 100mL flask followed by addition of 0.5 mL of Analytical grade conc. HCl and diluting it up to the mark with distilled water.

3.4.3.1 Preparation of Glycerol- Ethanol solution

It was prepared by mixing 25 mL glycerol in 50 mL of ethanol (1:2).

3.4.4 Phosphate test

7 mL of Bray Extracting Solution was mixed with 1 g soil sample. Additionally, a tube containing only the Bray Solution was prepared as a blank. After vortexing vigorously 1 mL of this mixture was transferred to centrifuge tubes, and they were spun at 6,000 rpm for a period of 5 minutes. For further analysis, 0.50 mL of the supernatant, along with 2.0 mL of Reagent C, was dispensed into a colorimeter tube. This mixture was thoroughly mixed and allowed to stand for 30 minutes. Absorbance was measured and recorded at a wavelength of 882 nm.

3.4.4.1 Preparation of Bray's No. 1 solution

In deionised water 0.55 g Ammonium Fluoride A.R. (NH_4F) was dissolved and transferred to a 500mL volumetric flask. 1.25 mL concentrated hydrochloric acid was added and bulked to volume with deionised water.

3.4.4.2 Preparation of Reagent A

4.28 g of ammonium molybdate A.R. [$(NH_4)_6Mo_7O_{24}.4H_2O$] was dissolved in 50 mL of warm deionized water. Simultaneously, 0.098 g of potassium antimony tartrate A.R. (KSbO.C4H4O6) was dissolved separately in 37.5 mL of deionized water. 125 mL of deionized water was placed in a 500 mL volumetric flask, and 50 mL of concentrated sulphuric acid was slowly added with mixing. After cooling, the cooled molybdate and tartrate solutions were added, mixed thoroughly, and then topped up to volume with deionized water.

3.4.4 Preparation of Reagent C

0.265 g of L-Ascorbic Acid A.R. ($C_6H_8O_6$) was dissolved in deionized water and transferred to a 250 mL volumetric flask. Then, 35 mL of Reagent A was added, and the solution was bulked to volume with deionized water.

3.5 Testing of soil biological properties:

3.5.1 Viable plate count

3.5.1.1 Preparation of nutrient agar medium

Appropriate amount of nutrient agar powder was weighed according to the manufacturer's instructions and mixed with distilled water and then sterilized by autoclaving at 121°C for 20 minutes. After cooling, media was poured in petri plates and was allowed to solidify.

3.5.1.2 Serial dilution and viable count

1 g of soil was weighed and transferred to a test tube containing 10 mL of distilled autoclaved water. Each soil sample was then serially diluted in a series of tubes containing 9 mL of sterile autoclaved water. This procedure is repeated until the desired dilution is achieved. 0.1 mL of the dilution of 10-2 and 10-3 of each soil sample was spread evenly onto Nutrient agar media plates. Plates were then incubated at room temperature for 24 hours. The number of colonies was counted and their morphology was noted. Serial dilution and viable plate count was done after every 20 days. Viable count was calculated using the below given formula.

CFU/mL = (Number of colonies x Dilution factor) / volume plated in mL)

Where:

Number of colonies: the number of visible colonies on the agar plate.

Dilution factor: the factor by which the original sample was diluted.

Volume plated: the volume of the diluted sample plated on the agar plate

3.5.2 Assessing the presence of phosphate solubilizing microorganisms

3.5.2.1 Preparation of Pikovskaya media

Appropriate amount of nutrient agar powder was weighed according to the manufacturer's instructions and mixed with distilled water and then sterilized by autoclaving at 121°C for 20 minutes. After cooling, media was poured into petri plates and was allowed to solidify.

3.5.2.2 Serial dilution and spread plating

1 g of soil was weighed and transferred to a test tube containing 10 mL of distilled autoclaved water. Each soil sample was then serially diluted in a series of tubes containing 9 mL of sterile autoclaved water. This procedure is repeated until the desired dilution is achieved. 0.1 mL of the dilution of 10-2 and 10-3 of each soil sample was spread evenly onto Pikovskaya media plates. Plates were then incubated at room temperature for 24 hours. Plates were examined production of halo zones by phosphate solubilizing microorganisms.

3.5.3 Litter decomposition test

Tea bags were air dried and the initial dry weight was measured (Wi). These tea bags were then incubated in each of the soil samples. After two months of field incubation, the teabags were recovered. Organic material attached was removed and its weight was measured (W_f). Litter mass lost during the incubation period was calculated using the formula below (FAO, 2020).

Percentage of mass loss = (Wi-Wf)/Wf x 100

Where

Wi = initial teabag weight before field incubation (g)

Wf = final teabag weight after field incubation (g)

3.6 Pot trials with cowpea seeds

5.6.1 Seed sample Collection and surface sterilization

Cowpea seeds were collected from a local farmer in Margao, Goa. Seeds were sterilized by soaking in Sodium hypochlorite solution for 1 minute followed by washing in distilled water for 2-3 times.

5.6.2 Aseptic germination

Seeds were transferred to sterile petri plates containing cotton, aseptically in laminar air flow. Little amount of distilled water was added to soak the cotton. Water was sprinkled once a day under aseptic condition.

3.6.3 Experimental set – up

500 g of soil collected from Taleigao was weighed and added in total 14 pots. Out of 14 pots 3 pots were treated with *Pseudomonas fluorescens* (1.5 g in 25mL of water) and 3 pots were treated with *Trichoderma viride* (1.5 g in 25 mL water), 3 pots with Neem cake (7.5 g in 25mL of water) and 3 pots were treated with Carbendazim- 12% + Moncozeb – 63 % (0.25g in 25mL water). 2 pots were kept as control for each soil. 20 germinated seeds were potted in each pot. They were watered everyday with 40 mL of water and exposed to sunlight from 8:00 am to 1:00 pm.

3.7 Assessment of plant growth parameters:

5.7.1 Viability percentage/ Survival rate of plants

Viability percentage was found using the formula given below

Seed viability percentage = Number of plants grown/ Number of seeds potted x 100

3.7.2 Shoot length measurement:

After locating the shoot of interest from each pot, it was measured from the top of the shoot to its base, where it emerges from the soil, using a scale. Shoot length was measured after every 15 days.

3.7.3 Shoot dry weight measurement:

After carefully separating shoot sample from roots, it was weighed on a weighing balance and measurements were recorded in grams (W_1). Shoots were oven dried at 60-80 °C for 24 hours. Dried shoots were weighed and measurements were recorded (W_2). Shoot dry weight was calculated by using formula given below.

$$\textbf{Dry weight} = W_2 / W_1$$

Where,

W_1 is initial weight of fresh shoots

W_2 is the weight of dried shoots

3.7.4 Root length measurement:

With extreme caution to avoid damaging the roots, the plant was delicately taken from the soil.. Usually, the measurement began at the base of the stem, when the roots first appear, and continued to the tip of the longest root.

3.7.5 Root dry weight measurement:

Roots were taken carefully from the pot without any damage by carefully removing the dirt. It was then weighed on a weighing balance (W_1) and oven dried at 60-80 °C for 24

hours. Dried roots were then weighed (W_2) and measurements were recorded. Dry weight was calculated by the formula given below.

$$\textbf{Dry weight} = W_2 / W_1$$

Where,

W_1 is initial weight of fresh roots

W_2 is the weight of dried roots

4.Results and Discussion

4.1 Soil Sampling

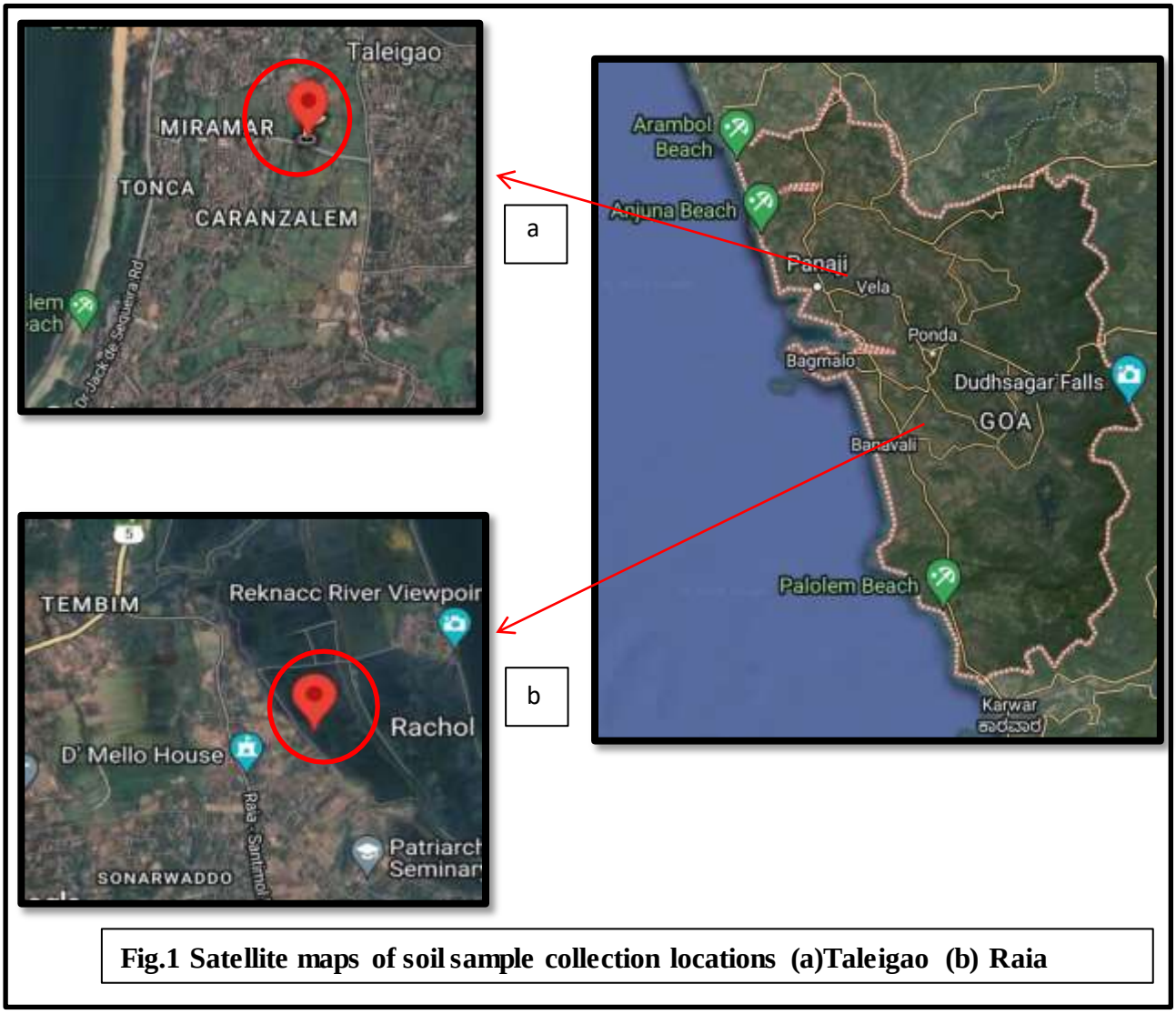


Fig.1 Satellite maps of soil sample collection locations (a)Taleigao (b) Raia

Fig 2: Soil sample collection

4.2 Soil physical properties:

4.2.1 Soil texture by ribbon method:

Soil 1 was of a typical sandy loam texture which is characterized by low water-holding capacity of the soil. Coarser soil particles are responsible for creating bigger holes that allows water to pass through it more easily. On the other hand Soil 2 had clayey loam texture which is characterized by higher water-holding capacity of the soil

Fig 3 : Soil 1

Fig 4 : Soil 2

4.2.2 Soil gravimetric content:

Table 1: Gravimetric content of soil 1 and soil 2

Sr No.	Soil type	M_T: weight of moist soil (g)	M_S: weight of dry soil (g)	M_w: M_T- M_S (g)	M_w/M_t x100 Gravimetric Content
1	Soil 1	30.6	25	5.6	18.30%
2	Soil 2	39	25	14.6	36.88%

The gravimetric content is calculated based on the weight of moist soil and the weight of dry soil. The results show that the Soil 2 has a higher gravimetric content of 36.88% compared to the Soil 1 which has a gravimetric content of 18.30%. Higher gravimetric content means, soil can hold more water which can be beneficial for plant growth. Differences in the soil gravimetric content can be explained by the differences in soil texture. Sandy loam texture of Soil 1 contributes to lower water holding capacity and clayey loam texture of Soil 2 is responsible for higher water holding capacity.

4.3 Soil chemical properties:

4.3.1 pH of soil

The pH of Raia soil and Taleigao soil treated with biocontrol, chemical pesticide and control was measured after every 10 days for period of 2 months. The results are presented in tabular form below.

Table 2: pH of Soil 1 from day 0 to day 40

Soil sample	Day 0	Day10	Day 20	Day 30	Day 40	Day 50
TCO	6.37	6.5	6.04	6.65	6.67	6.97
TP	6.37	6.94	7.53	5.53	6.51	7.65
TT	6.37	6.73	7.71	6.06	6.88	7.68
TN	6.37	6.18	6.52	4.99	5.94	7.47
TCH	6.37	6.43	6.45	5.51	5.95	7.26

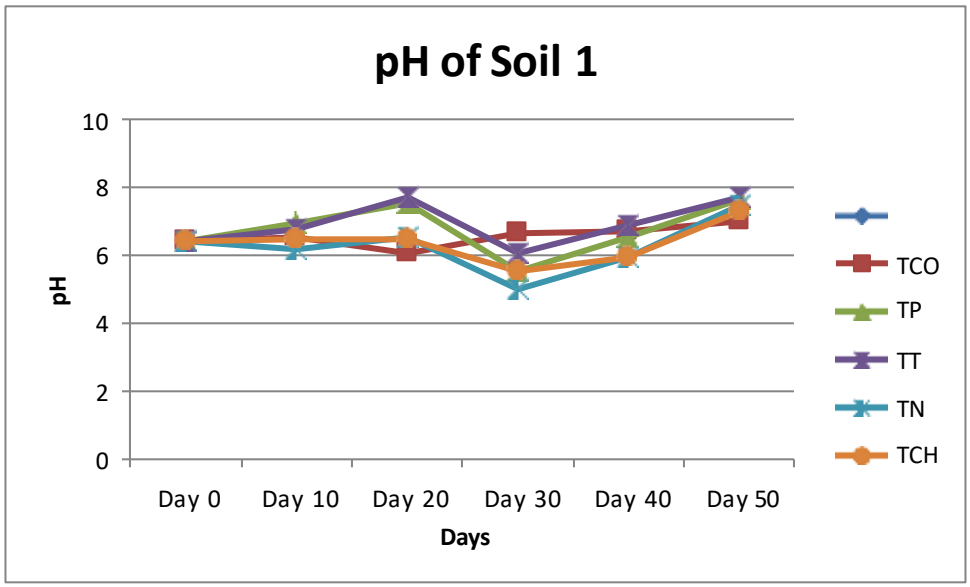


Fig.5 : Graph showing pH of soil 1

In Soil 1, the pH remained relatively stable at 6 across all treatments until day 10, after which it slightly increased in all treatments by day 20. However, by day 30, there was a slight decrease in pH across all treatments. By day 40 and 60, the pH was found to have increased again. Overall, the pH in Soil 1 exhibited a similar trend across all treatments throughout the experiment similar to another study (Thomas,1996). The reason for stable pH can be the mineral composition of sandy loam soil, which includes a mix of sand, silt, and clay particles, can influence pH stability certain minerals, like calcium carbonate, can act as buffers, helping to stabilize pH (McCauley et al, 2005). Another reason can be the presence of organic matter in sandy loam soil that contributes to pH stability (Fageria, 2012)

Table 3: pH of Soil 2 from day 0 to day 50

Soil sample	Day 0	Day10	Day 20	Day 30	Day 40	Day 50
RCO	6.2	6.03	5.98	6.2	6.29	5.95
RP	6.2	4.3	6.02	6.06	6.51	6.14
RT	6.2	6.04	6.27	6.46	6.88	6.54
RN	6.2	5.71	5.67	5.57	5.94	5.49
RCH	6.2	6.54	6.02	5.74	5.95	5.33

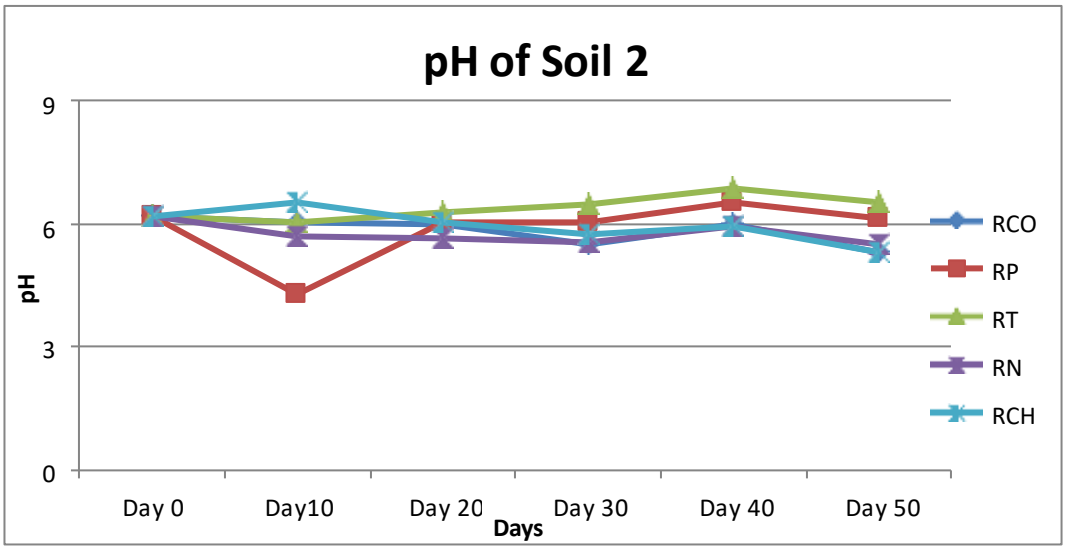


Fig.6: Graph showing pH of Soil 2

In Soil 2, the pH values on day 10 exhibited a consistent trend across all treatments, except for treatment TP, where a shift towards acidity was observed. However, by day 20, treatment TP showed an increase in pH. Subsequent observations on days 30, 40, and 50 revealed that the pH values across all treatments followed a similar trend.

Reason for low pH on day 20 in RP could be because *Pseudomonas* Are efficient in nutrient uptake, particularly nitrogen. This uptake of nutrients from the soil solution could lead to an increase in proton release, further lowering the pH of the soil as seen in another study (Israr et al, 2016). The introduction of *Pseudomonas sp*. to the soil may have influenced the overall microbial community composition and activity. Changes in the microbial community can affect nutrient cycling and organic matter decomposition, potentially leading to shifts in soil pH. Reason for stable pH across all the treatments could be because clayey loam soil often has a higher organic matter content, which can act as a buffer against pH changes as shown in study conducted by (Senesi, 2018). Organic matter can release acids or bases into the soil, helping to maintain a balanced pH Neem cake, contain compounds that can influence the soil microbial community and nutrient availability, potentially contributing to pH stability (Jatana et al, 2020).

4.3.2 Sulphate test

Sulphate test was carried out after every 15 days to find the concentration of sulphate in the soil treated with biocontrol agents, chemical pesticide and control over a period of 60 days.

The table below shows the absorbance measured at 420 nm on day 2, day 16 day 31 and day 46.

Table 5: Absorbance of Soil 2 for sulphate test measured after every 15 days

Soil sample	Day 2	Day 16	Day 31	Day 46
TCO	0.651	0.583	0.573	0.481
TP	0.462	0.258	0.161	0.102
TT	0.617	0.411	0.233	0.206
TN	0.480	0.142	0.145	0.120
TCH	0.539	0.337	0.237	0.217

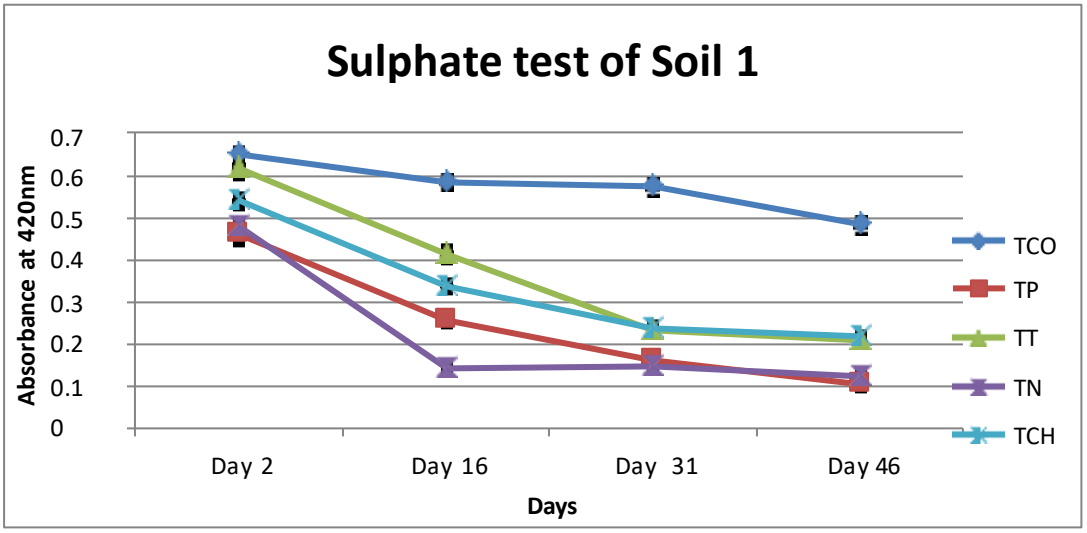


Fig 7 : Sulphate test for soil 1

In TCO absorbance values show a decreasing trend over time, suggesting a gradual decrease in sulphate levels in the soil. This could be due to factors such as sulphate uptake by plants or microbial activity. The absorbance values of TP show a sharp decrease from day 2 to day 16 and then remain relatively stable. This could indicate a rapid decrease in sulphate levels initially, followed by a more stable period.

TT show a decreasing trend over time, similar to TCO. This indicates a gradual decrease in sulphate levels in the soil. TN shows a slight decrease over time, indicating

a minor decrease in sulphate levels in the soil. While TCH show a decreasing trend similar to TCO and TT, suggesting a gradual decrease in sulphate levels in the soil.

Table 5: Absorbance of Soil 2 for sulphate test measured after every 15 days

Soil sample	Day 2	Day 16	day 32	Day 46
RCO	0.715	0.711	0.689	0.676
RP	0.559	0.799	0.839	0.595
RT	0.670	0.505	0.659	0.523
RN	0.619	0.521	0.780	0.339
RCH	0.875	0.722	0.724	0.236

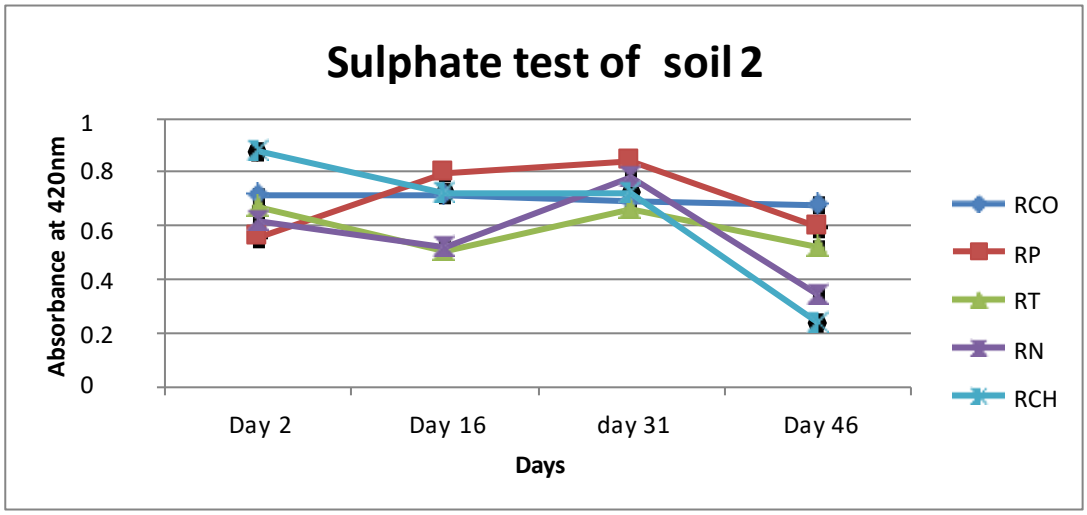


Fig 8: Sulphate test for Soil 2

In RCO the absorbance values fluctuate slightly over time, possibly indicating fluctuations in sulphate levels in the soil. The initial high value could be due to the presence of sulphate-releasing compounds in the treatment. In RP the absorbance values show an increasing trend until day 32, indicating a gradual increase in sulphate

levels. The decrease in absorbance on day 46 could be due to sulphate depletion. In RT The absorbance values show some fluctuations but remain relatively stable over time. This could indicate a consistent level of sulphate in the soil with minor variations. In RN The absorbance values initially increase and then decrease sharply. This pattern could be due to fluctuations in sulphate levels or other factors affecting the test results like soil texture similar to another study (Bloem, 2001). In RCH the absorbance values show a decreasing trend over time, indicating a decline in sulphate levels in the soil.

4.3.3 Phosphate test

Phosphate test was carried out after every 15 days to find the concentration of phosphorous in the soil treated with biocontrol agents, chemical pesticide and control over a period of 60 days. The table below shows the absorbance measured at 882 nm on day 3, day 17, day 32 and day 47 of treatment.

Table 6: Absorbance of Soil 1 for phosphate test measured after every 15 days

Soil sample	Day 3	Day 17	Day 32	Day 47
TCO	0.626	0.664	0.676	0.744
TP	0.436	0.628	0.458	0.748
TT	0.705	0.662	0.468	0.878
TN	0.635	0.681	0.397	0.746
TCH	0.568	0.572	0.537	0.898

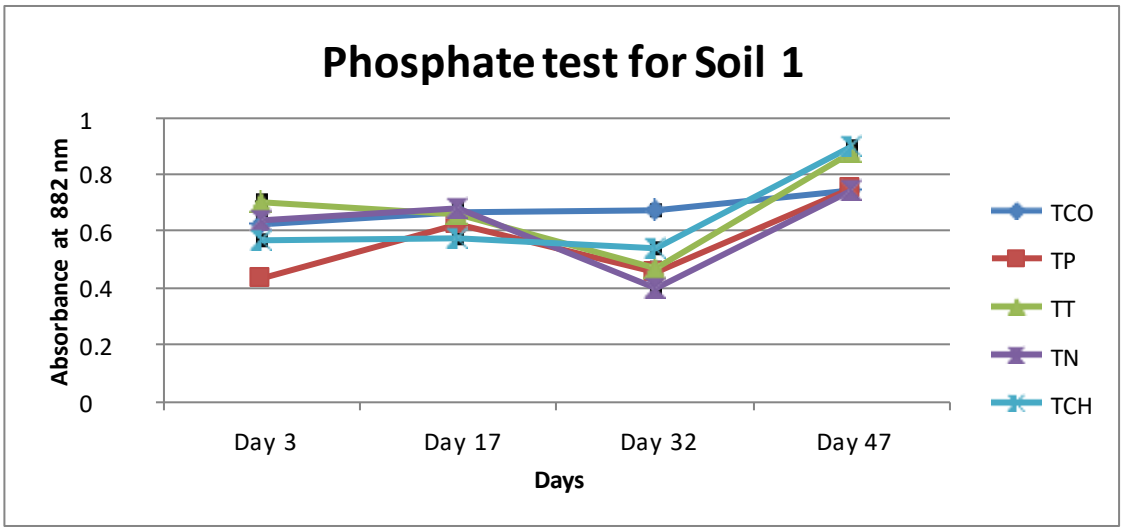


Fig 9: Phosphate test for Soil 1

In TCO, absorbance values show an increasing trend over time, suggesting an increase in phosphorus levels in the soil. This could be due to factors such as the gradual release of phosphorus from the treatment or changes in soil conditions that affect phosphorus availability (Penn et al, 2019). While in TP the absorbance values show some fluctuations but remain relatively stable over time. This could indicate a consistent level of phosphorus in the soil with some variations. Whereas in TT The absorbance values show fluctuations and a slight decrease over time, suggesting a potential decrease in phosphorus levels or changes in phosphorus availability in the soil. In the absorbance values show fluctuations but remain relatively stable over time. This could indicate a consistent level of phosphorus in the soil with minor variations. The absorbance values of TCH show fluctuations but remain relatively stable over time, similar to TP. This could indicate a consistent level of phosphorus in the soil with minor variations. Phosphorous test did not give the expected results in Soil 2 due to very low concentration of phosphorous. Additionally, clayey soils often have low organic matter content, which is a source of phosphorus (Mahajan et al, 2015).

4.4 Testing of soil biological properties:

4.4.1 Serial dilution and viable count

Fig 10: Colonies observed after Serial dilution and plating of Soil 1

Fig 11: Colonies observed after Serial dilution and plating of Soil 2

Table 7: Viable count of Soil 1 and Soil 2 on Day 1

Soil	Spread plate sample	CFU/mL
Soil 1	TCO	6×10^4
	TP	40×10^4
	TT	25.2×10^4
	TN	22.9×10^4
	TCH	No growth
Soil 2	RCO	12.4×10^4
	RP	7.1×10^4
	RT	No growth
	RN	9.2×10^4
	RCH	9.15×10^4

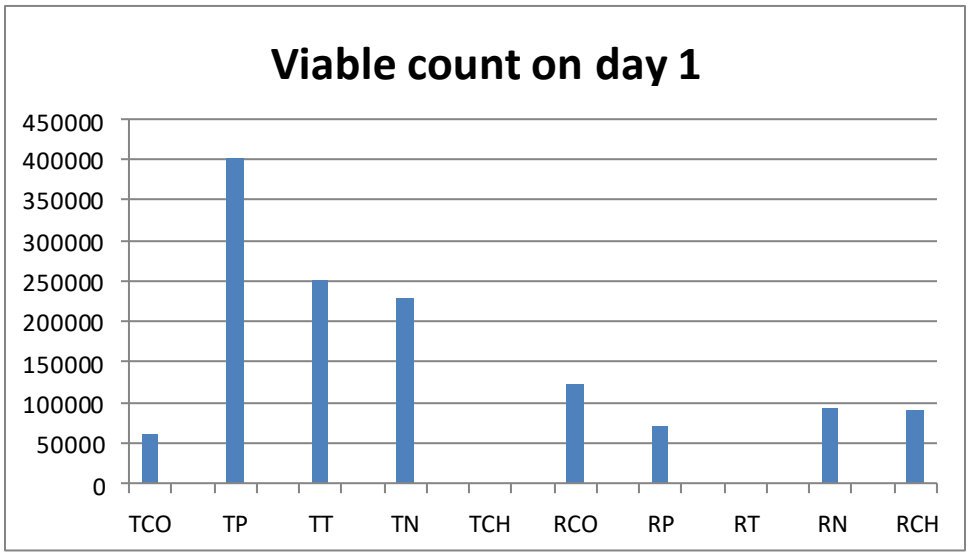


Fig 12: Viable count on day 1

In soil 1 control soil sample showed the lowest viable count of 6×10^4 CFU/mL on day 1 while TP had the highest viable count of 40×10^4 CFU/mL. TT and TN showed

almost similar viable count that is 25.2 x 10^4 CFU/mL and 22.9 x 10^4 CFU/mL while there was no growth seen in TCH.

In soil 2 control showed highest growth of 12.4 x 10^4 CFU/mL. RP showed lowest growth of 7.1 x 10^4 CFU/mL on the other hand RN and RCH showed similar growth of 9.2 x 10^4 CFU/mL and 9.15 x 10^4 CFU/mL, while RT showed no growth.

In Soil 1, the control sample initially had the lowest viable count, likely due to the absence of any treatment. TP showed the highest growth, indicating its effectiveness in promoting microbial activity. TT and TN had similar viable counts, suggesting comparable effects on microbial growth. The lack of growth in TCH indicates its strong inhibitory effect on microbial activity. In Soil 2, the control sample initially had the highest growth, possibly due to the natural microbial population in the soil. RP showed the lowest growth, indicating a suppressive effect. RN and RCH had similar growth, suggesting a similar impact on microbial activity. The lack of growth in RT indicates its ineffectiveness or inhibitory effect on microbial growth.

Table 8: Viable count of Soil 1 and Soil 2 on Day 20

Soil	Spread plate type	CFU/mL
Soil 1	TCO	18. x 10^4
	TP	26.4 x 10^4
	TT	22.7 x 10^4
	TN	22.6 x 10^4
	TCH	10 x 10^4
Soil 2	RCO	15.2 x 10^4
	RP	13 x 10^4
	RT	55.7 x 10^4

	RN	32.8 x 10^4
	RCH	13 x 10^4

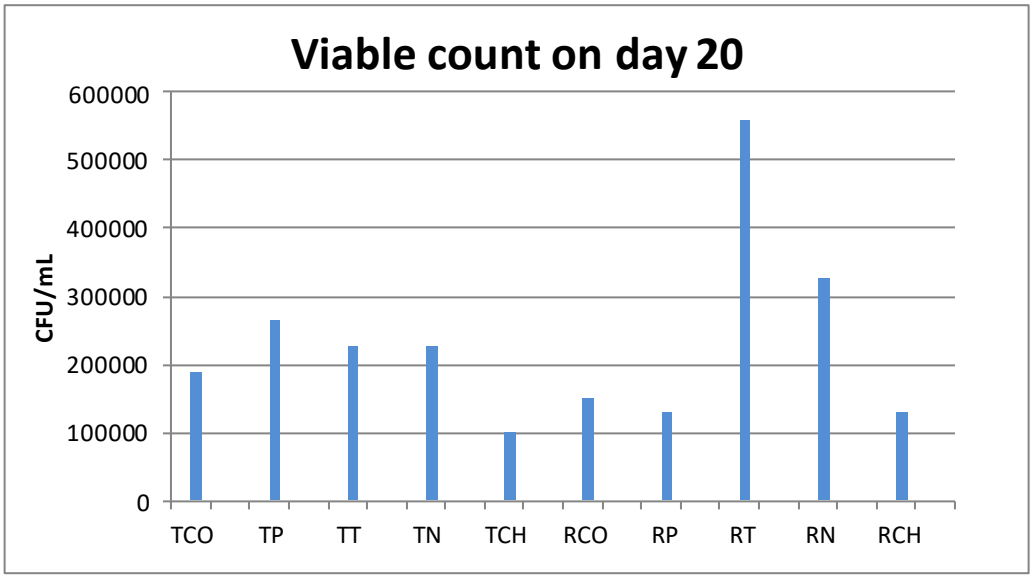


Fig 12: Viable count on day 20 days

In Soil 1, TP showed the highest growth of 26.4 x 10^4CFU/mL on day 20. While TT and TN showed similar viable count of 22.7 x 10^4CFU/mL and 22.6 x 10^4CFU/mL. TCO showed comparatively lower viable count of 18. x 10^4. On the other hand TCH showed the lowest viable count of 10 x 10^4CFU/mL.

In soil 2, RCO showed the viable count of 15.2 x 10^4CFU/mL. RT showed the highest viable count of 55.7 x 10^4CFU/mL. Viable count in RN was 32.8 x 10^4 CFU/mL. on the other hand viable count in RP and RCH was 13 x 10^4CFU/mL.

In Soil 1, the biocontrol agent TP showed the highest growth on day 20, indicating its effectiveness in promoting microbial activity in the soil. The treatments with *Trichoderma sp.* and Neem cake also showed similar viable counts, suggesting a moderate effect on microbial growth. TCO had a lower viable count compared to the

other treatments, indicating a less pronounced impact on soil microbiota. The chemical pesticide treatment (TCH) exhibited the lowest viable count, indicating a strong suppressive effect on microbial growth.

In Soil 2, the biocontrol agent RCO showed a moderate viable count, indicating a moderate impact on microbial growth. The treatment with *Trichoderma sp*. Showed the highest viable count, suggesting a strong stimulatory effect on microbial growth. RN had a viable count lower than RT but higher than RCO, indicating a moderate impact. RP and RCH had the lowest viable counts, indicating a strong suppressive effect on microbial growth, similar to the effect observed with TCH in Soil 1.

Table 9: Viable count of Soil 1 and Soil 2 on Day 40

soil	Spread plate type	CFU/mL
Soil 1	TCO	9.3×10^4
	TP	43×10^4
	TT	7.8×10^4
	TN	8.5×10^4
	TCH	No growth
Soil 2	RCO	15.9×10^4
	RP	19.6×10^4
	RT	30×10^4
	RN	1.5×10^4
	RCH	No growth

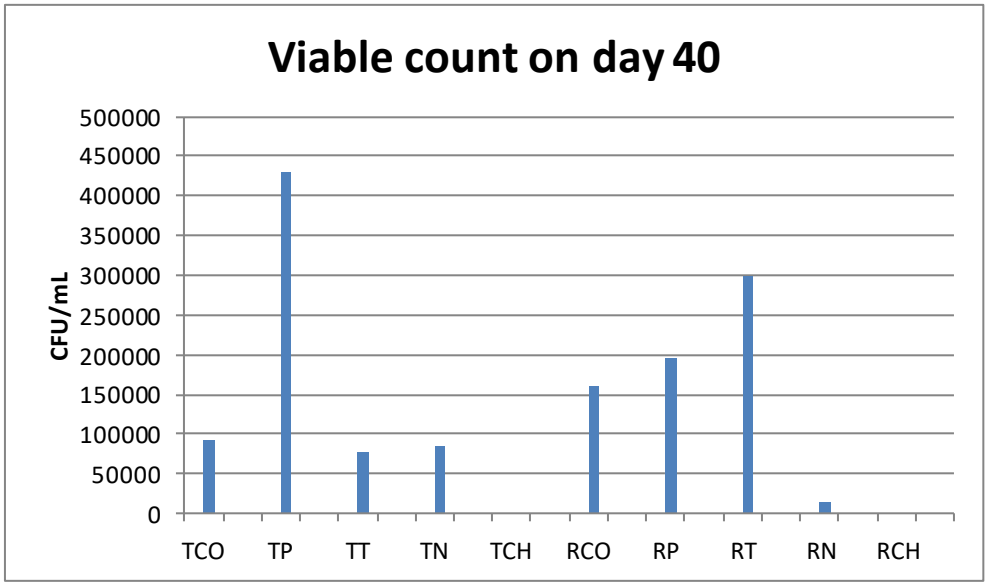


Fig 13: Viable count on day 40

On day 40, in Soil 1 TCO had a viable count of 9.3 x 10^4 CFU/mL while TP had significantly higher viable count of 43 x 10^4 CFU/mL. viable count in TT and TN was found to be 7.8 x 10^4 CFU/mL and 8.5 x 10^4 CFU/mL. However, there was no growth seen in TCH.

In Soil 2, RCO had a viable count of 15.9 x 10^4 CFU/mL while RP had significantly higher viable count of 19.6 x 10^4CFU/mL. On the other hand RT showed the highest viable count of 30 x 10^4 CFU/mL and RN had the least viable count of 1.5 x 10^4 CFU/mL. While no growth was seen in RCH.

The similar viable counts observed between the biocontrol agent-treated soil and the control sample in Soil 1, except for TP, could be due to the specific effects of the biocontrol agents used in the study. Biocontrol agents are typically used to control plant diseases by inhibiting the growth of pathogens. In this case, the biocontrol agents may have had minimal impact on the overall soil microbiota, resulting in viable counts similar to the control sample.

The lack of growth observed in the soil treated with chemical pesticide in both Soil 1 and Soil 2 is likely due to the toxic effects of the pesticide on soil microorganisms. Chemical pesticides are designed to kill or inhibit the growth of pests and pathogens, but they can also harm beneficial microorganisms in the soil. This can lead to a decrease in overall microbial activity and viable counts. In Soil 2, the higher viable counts observed in the soil treated with biocontrol agents compared to the control sample, except for Neem cake, could be because these agents may have promoted the growth of beneficial microorganisms in the soil.

Table 10: Viable count of Soil 1 and Soil 2 on Day 60

soil	Spread plate type	CFU/mL
Soil 1	TCO	150×10^4
	TP	173×10^4
	TT	70×10^4
	TN	48×10^4
	TCH	No growth
Soil 2	RCO	96×10^4
	RP	166×10^4
	RT	26×10^4
	RN	20×10^4
	RCH	No growth

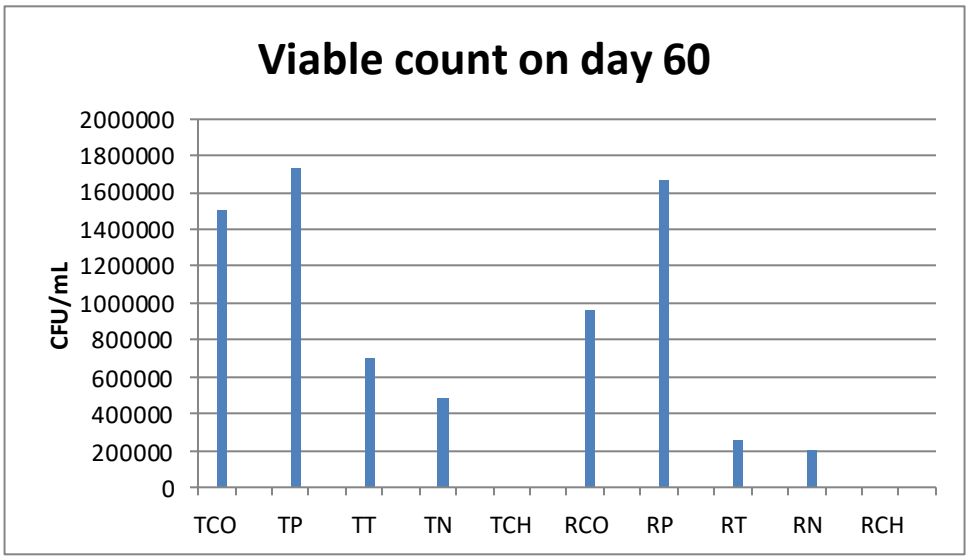


Fig 14 : Viable count on day 60

On day 60 in Soil 1 TCO had viable count of 150 x 10^4CFU/mL. TP had the highest viable count of 173 x 10^4 CFU/mL. TT had viable count of 70 x 10^4 CFU/mL. TN showed comparatively low viable count of 48 x 10^4CFU/mL. while there was no growth in TCH.

In Soil 2, RCO had viable count of 96 x 10^4 CFU/mL. while RP had the highest viable count of 166 x 10^4 CFU/mL. RT and RN had low viable count of 26 x 10^4CFU/mL and 20 x 10^4 CFU/mL. Whereas there was no growth in RCH.

These results suggest that the composition of the soil may have influenced the effectiveness of the treatments on soil microbiota. The presence of *Pseudomonas sp.* in the TP and RP treatments may have contributed to higher viable counts similar to study conducted by Aagot (2001), as *Pseudomonas sp.* is known for its ability to promote plant growth and enhance soil health. Conversely, the presence of chemical pesticides in the TCH and RCH treatments may have suppressed microbial growth, leading to no growth observed in these treatments similar to study conducted by Shahid (2022).

4.4.2 Estimate of culturable microbial diversity

4.4.2.1 Microbial diversity of Soil 1 on day 1

Table 11 : Colony characteristics and estimated number in TCO on day 1

TCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	60	50%
Circular, Entire, Flat, Opaque	39	32%
Irregular, Undulate, Flat, Opaque	4	3%
Filamentous, flat, opaque	2	2%
Irregular, undulate, flat smooth and shiny	13	11%
Punctiform, Entire, Flat, Opaque	2	2%

Table 12 : Colony characteristics and estimated number in TP on day 1

TP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire, Flat, Opaque	28	70%
Irregular, undulate, flat smooth and shiny	12	30%

Table 13 : Colony characteristics and estimated number in TT on day 1

TT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	16	46%
Circular, Entire, Flat, Opaque	12	36%
Irregular, undulate, flat, Dry	4	18%

Table 14 : Colony characteristics and estimated number in TN on day 1

TN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	10	53%
Circular, Entire, Flat, Opaque	20	26%
Irregular, undulate, flat, Dry	8	21%

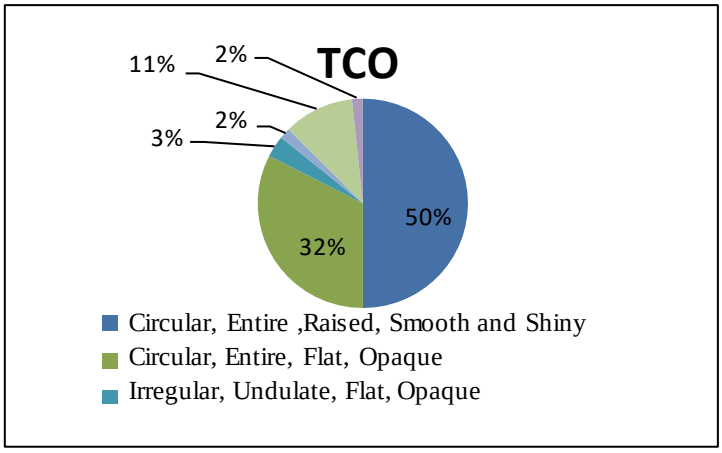


Fig 15: Microbial diversity in TCO on day1

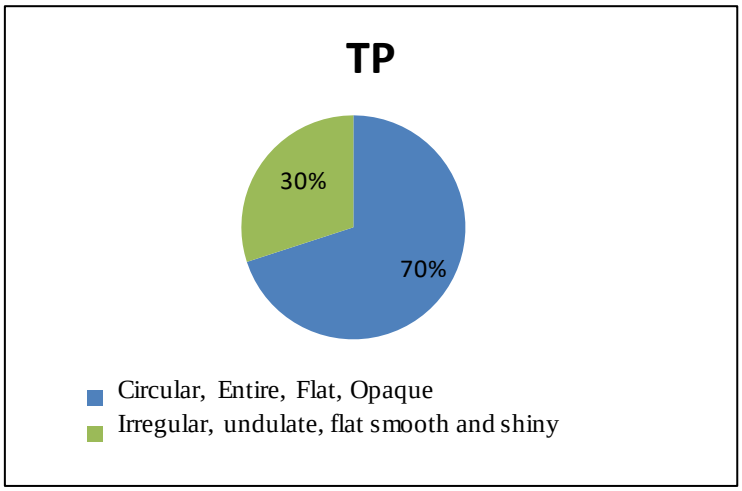


Fig 16: Microbial diversity in TP on day 1

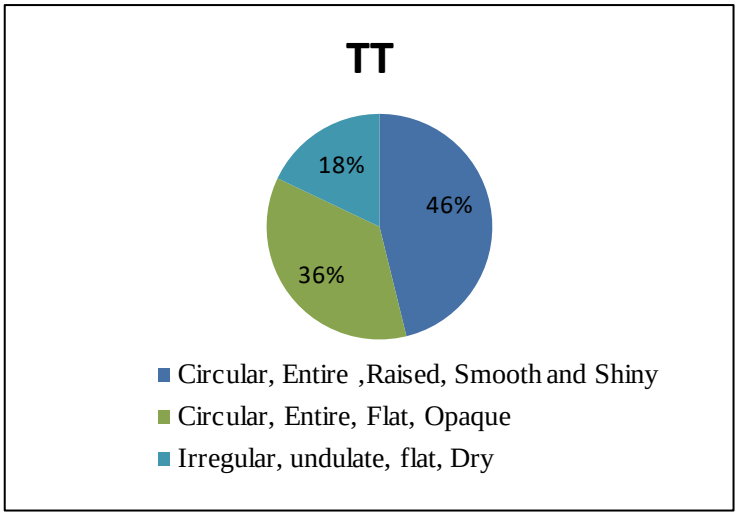


Fig 17 : Microbial diversity in TT on day 1

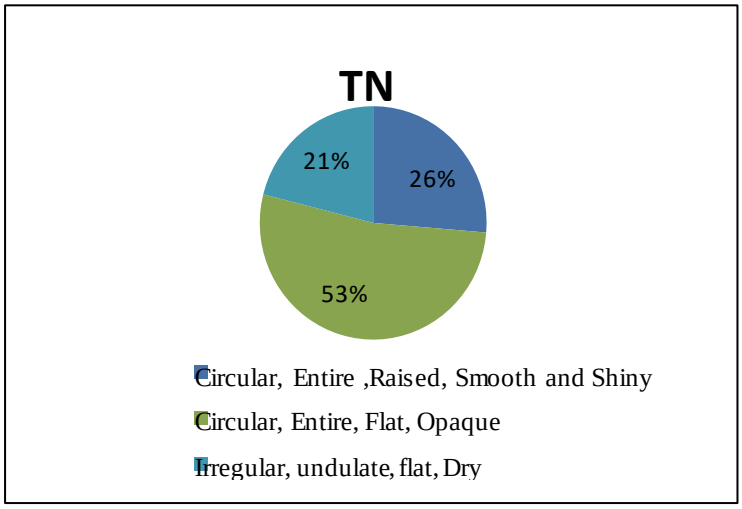


Fig 18: Microbial diversity in TN on day 1

4.4.2.2 Microbial diversity of Soil 1 on day 1

Table 15: Colony characteristics and estimated number in RCO on day 1

RCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular,entire,raised,smooth and shiny	2	11%
Circular, entire, flat, opaque	6	33%
Irregular, undulate, flat, dry	10	56%

Table 16: Colony characteristics and estimated number in RP on day 1

RP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, entire, raised,smooth and shiny	2	18%
Circular, entire, flat, opaque	3	27%
Irregular, undulate, flat, dry	6	55%

Table 17 : Colony characteristics and estimated number in RN on day 1

RN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire, Raised, Smooth and Shiny	10	67%
Irregular, Undulate, Flat, Dry	5	33%

Table 18 : Colony characteristics and estimated number in RCH on day 1

RCH		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	6	50%
Circular, Entire, Flat, Opaque	4	33%
Irregular, Undulate, Flat, Opaque	2	17%

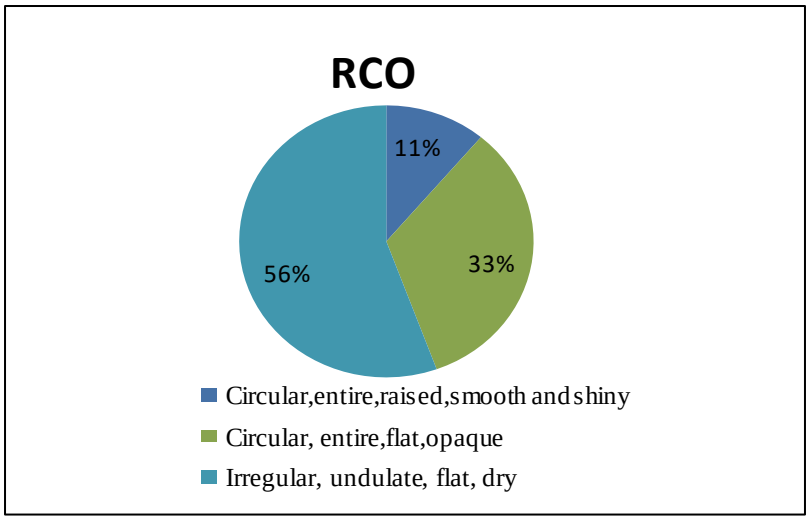


Fig 18: Microbial diversity in RCO on day 1

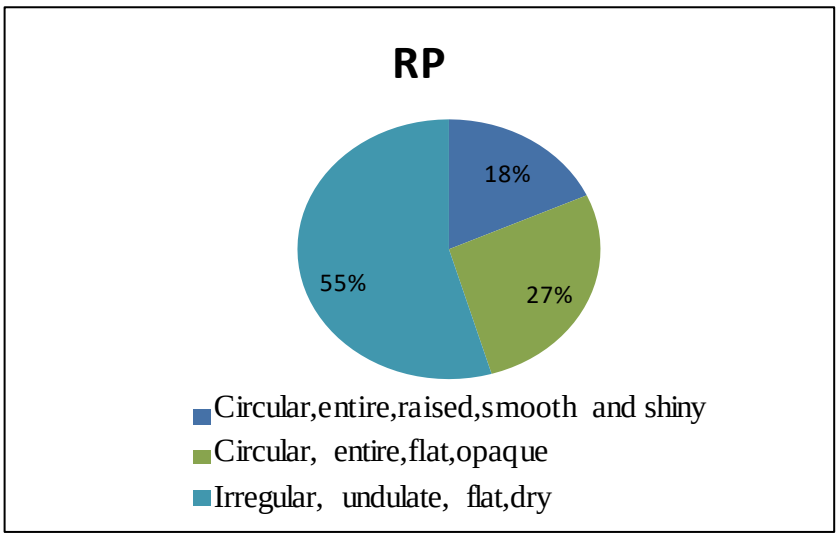


Fig 19: Microbial diversity in RP on day 1

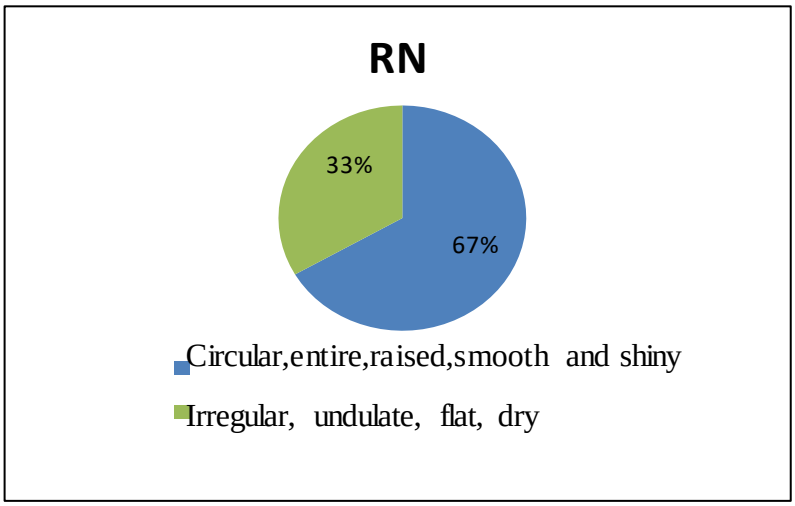


Fig 20: Microbial diversity in RN on day 1

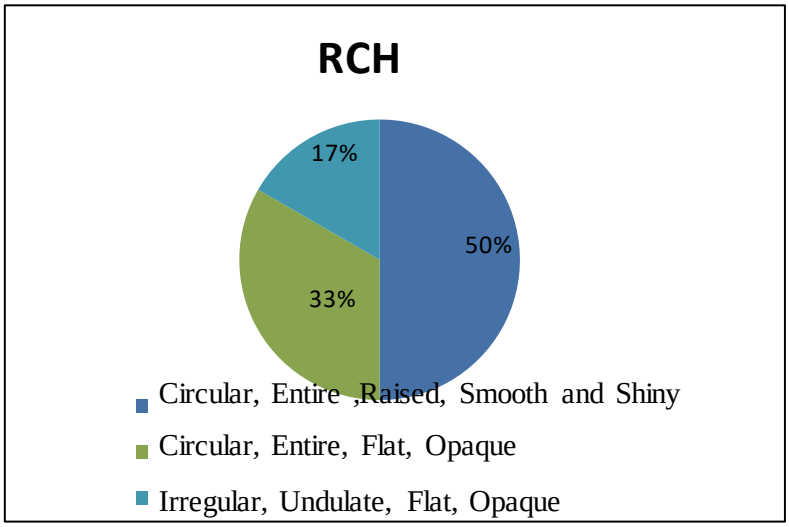


Fig 22: Microbial diversity in RCH on day 1

4.4.2.3 Microbial diversity of Soil 1 after 20 days

Table 19 : Colony characteristics and estimated number in TCO after 20 days

TC0		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	7	27%
Circular, Entire, Flat, Opaque	8	31%
Irregular, undulate, flat, Opaque	7	27%
Irregular, undulate, flat, Translucent	4	15%

Table 20 : Colony characteristics and estimated number in TP after 20 days

TP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	10	26%
Circular, Entire, Flat, Opaque	7	18%
Irregular, undulate, flat, Opaque	14	37%
Irregular, undulate, flat, Translucent	6	16%
Circular, Entire, Flat, Translucent	1	3%

Table 21 : Colony characteristics and estimated number in TT after 20 days

TT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	16	49%
Circular, Entire, Flat, Opaque	6	33%
Irregular, undulate, flat, Opaque	11	18%

Table 22 : Colony characteristics and estimated number in TN after 20 days

TN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	4	13%
Circular, Entire, Flat, smooth and shiny	25	84%
Irregular, undulate, flat, Smooth and shiny	1	3%

Table 23 : Colony characteristics and estimated number in TN after 20 day

TCH		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	8	35%
Circular, Entire, Flat, Opaque	9	39%
Irregular, undulate, flat, Opaque	6	26%

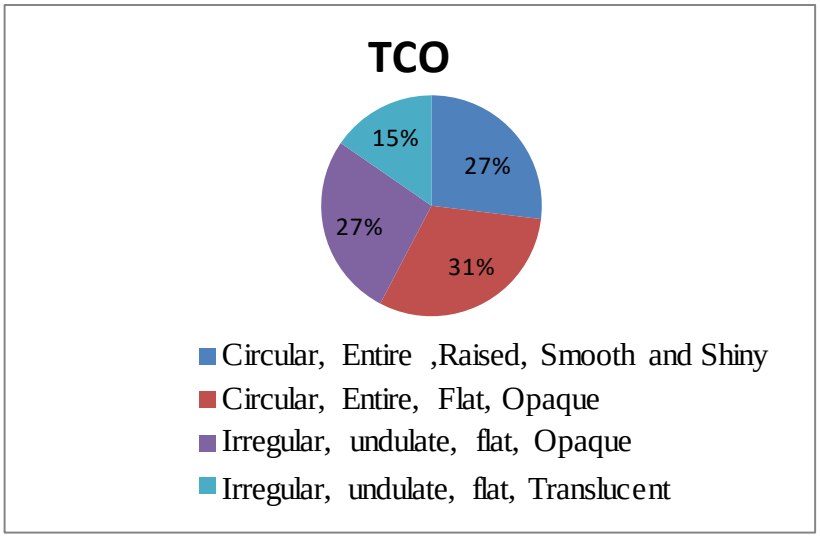


Fig 23 : Microbial diversity in TCO after 20 days

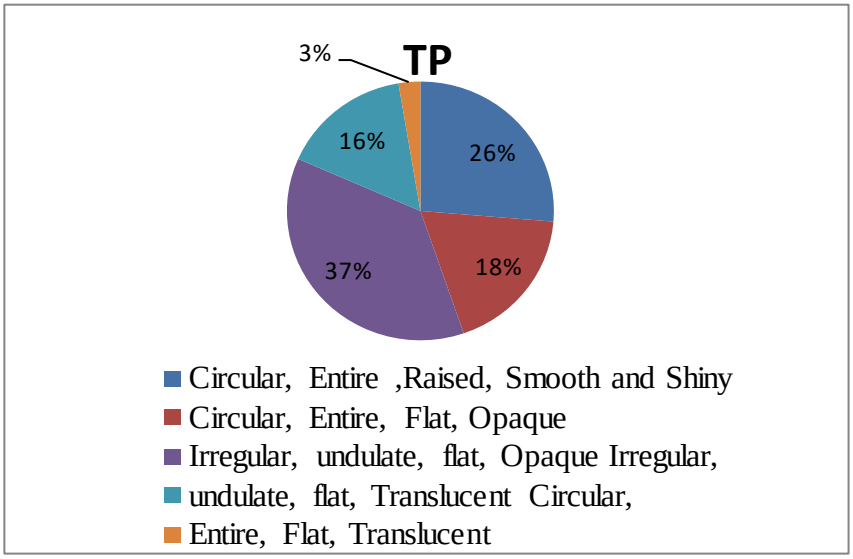


Fig 24 : Microbial diversity in TP after 20 days

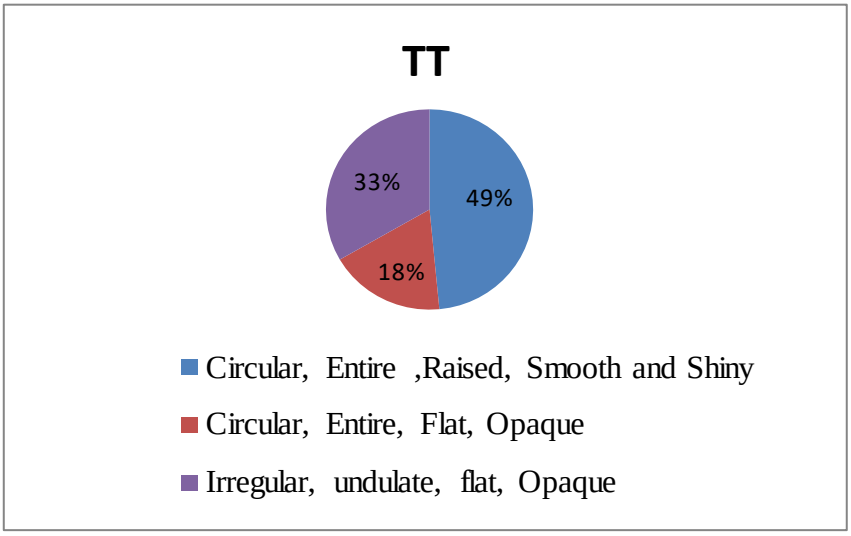


Fig 25 : Microbial diversity in TT after 20 days

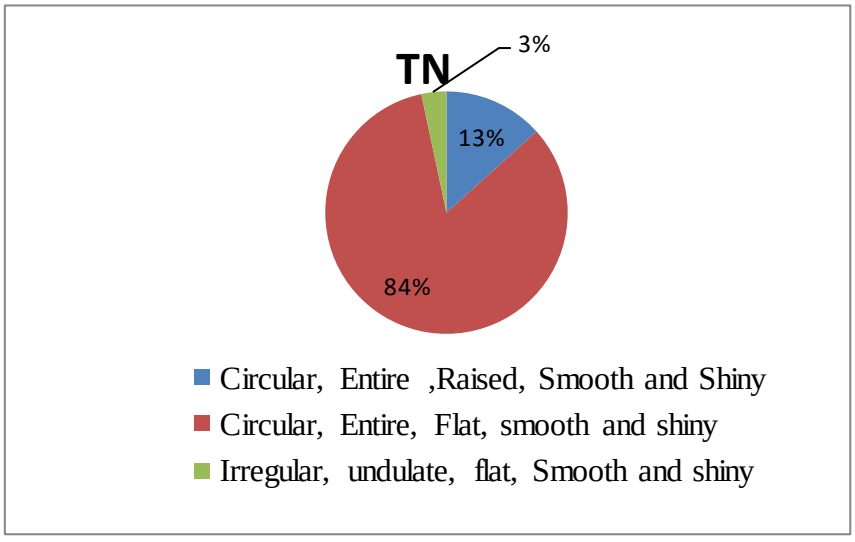


Fig 26 : Microbial diversity in TN after 20 days

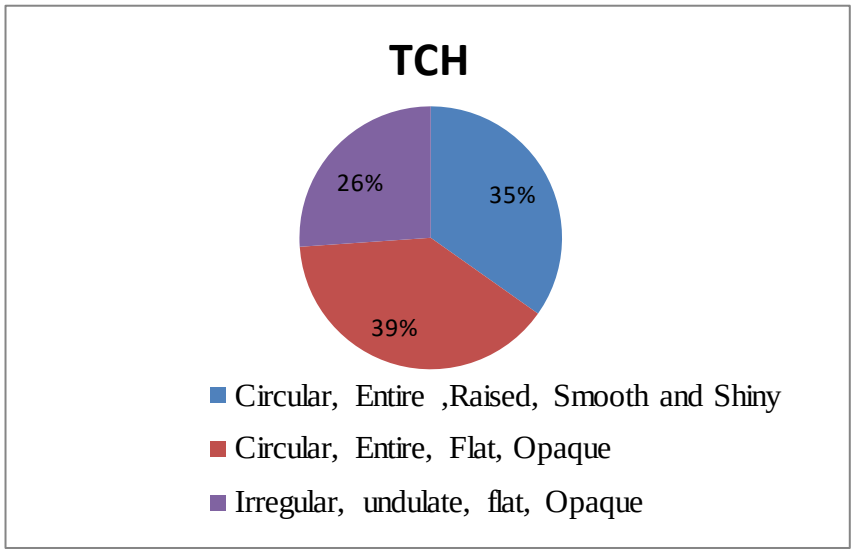


Fig 27 : Microbial diversity in TCH after 20 days

4.4.2.4 Microbial diversity of Soil 2 after 20 days

Table 24 : Colony characteristics and estimated number in TN after 20 days

RCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	1	6%
Irregular, undulate, flat, Opaque	5	31%
Circular, Entire, Flat, Smooth and Shiny	10	63%

Table 25 : Colony characteristics and estimated number in RP after 20 days

RP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	3	23%
Circular, Entire, Flat, Smooth and Shiny	6	46%
Irregular, undulate, flat, Opaque	4	31%

Table 26 : Colony characteristics and estimated number in RT after 20 days

RT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	19	22%
Circular, Entire, Flat, Smooth and Shiny	58	68%
Irregular, undulate, flat, Opaque	8	9%

Table 27 : Colony characteristics and estimated number in RN after 20 days

RN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	14	24%
Circular, Entire, Flat, Smooth and Shiny	16	27%
Irregular, undulate, flat, Smooth and Shiny	3	5%
Irregular, undulate, flat, Opaque	26	44%

Table 28 : Colony characteristics and estimated number in RCH after 20 days

RCH		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	6	9%
Circular, Entire, Flat, Smooth and Shiny	9	14%
Irregular, undulate, flat, Opaque	51	77%

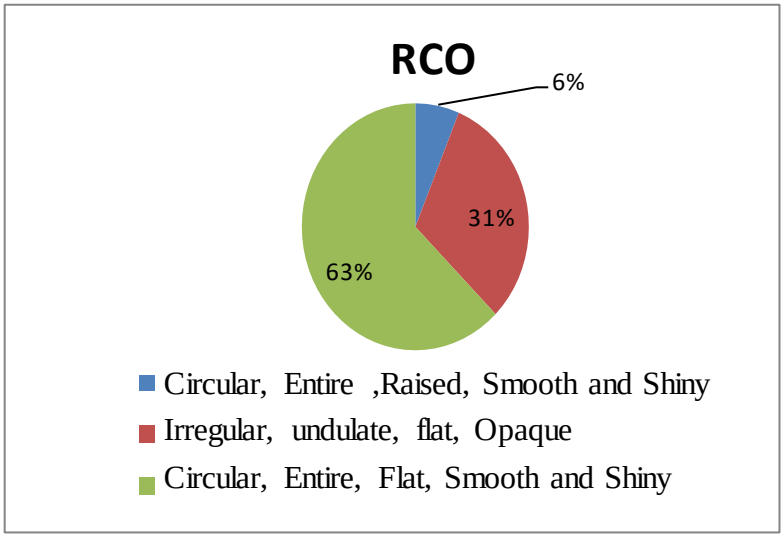


Fig 28 : Microbial diversity in RCO after 20 days

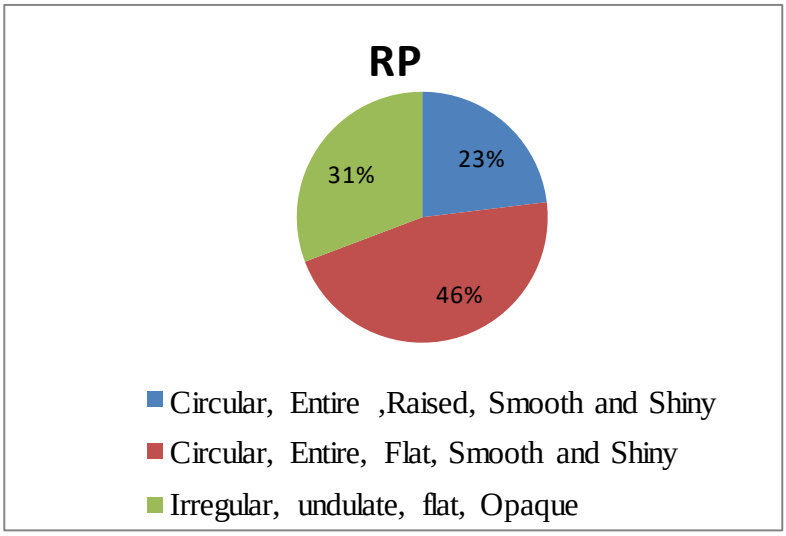


Fig 29 : Microbial diversity in RP after 20 days

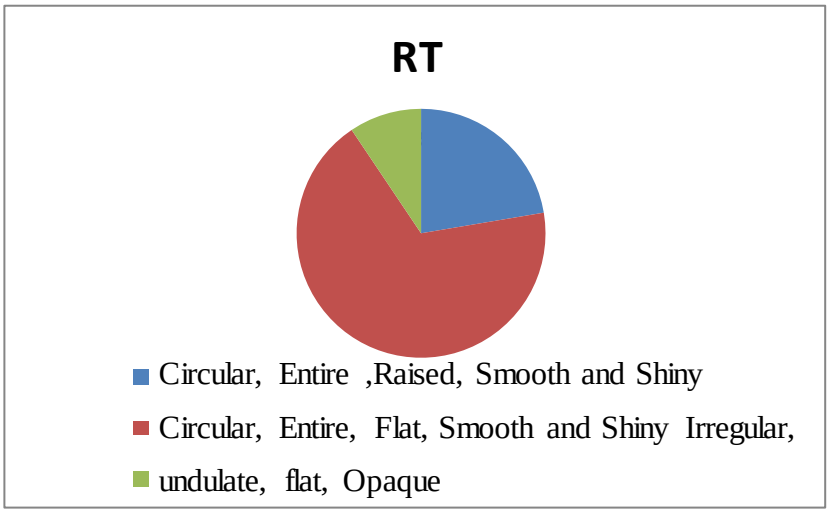


Fig 30 : Microbial diversity in RT after 20 days

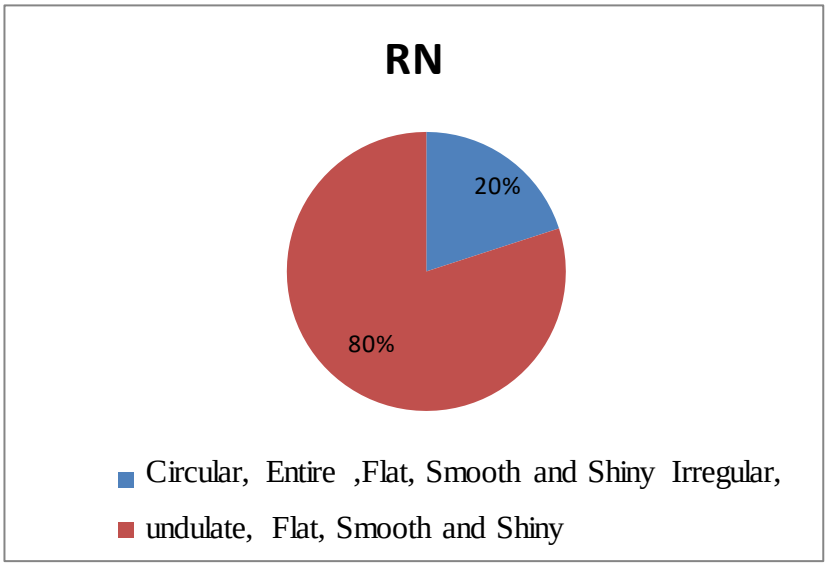


Fig 31 : Microbial diversity in RN after 20 day

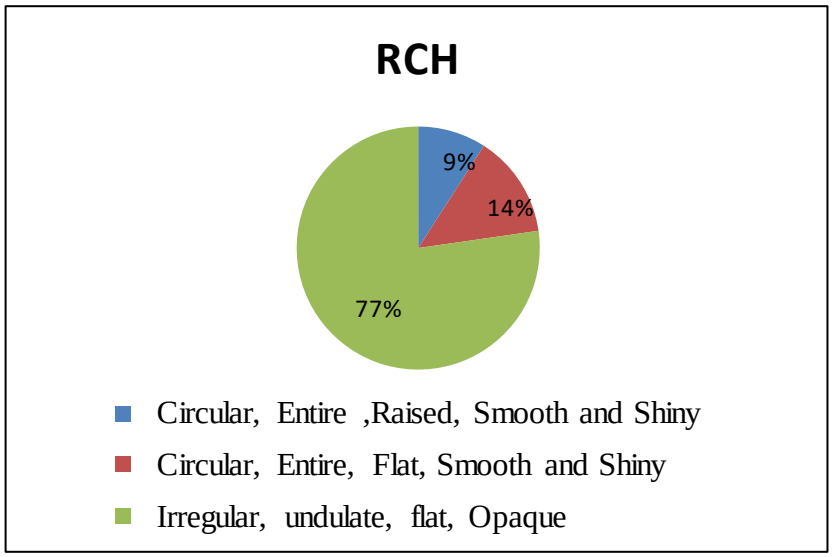


Fig 32 : Microbial diversity in RCH after 20 days

4.4.2.5 Microbial diversity of Soil 1 on day 40

Table 29 : Colony characteristics and estimated number in TCO after day 40

TCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	2	14%
Circular, Entire, Flat, Opaque	1	7%
Circular, Entire, Flat, Dry	1	7%
Irregular, undulate, flat, Opaque	7	50%
Irregular, undulate, flat, Dry	3	21%

Table 30 : Colony characteristics and estimated number in TP after day 40

TP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	3	7%
Circular, Entire, Flat, Opaque	5	12%
Irregular, undulate, flat, Opaque	35	81%

Table 31 : Colony characteristics and estimated number in TT after day 40

TT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	15	19%
Circular, Entire, Flat, Smooth and Shiny	10	13%
Irregular, undulate, flat, Opaque	24	31%
Irregular, undulate, flat, Smooth and Shiny	18	23%
Irregular, undulate, flat, Dry	11	14%

Table 32 : Colony characteristics and estimated number in TN after day 40

TN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	47	55%
Circular, Entire, Flat, Smooth and Shiny	10	12%
Circular, Entire, Flat, Dry	19	22%
Irregular, undulate, flat, Opaque	9	11%

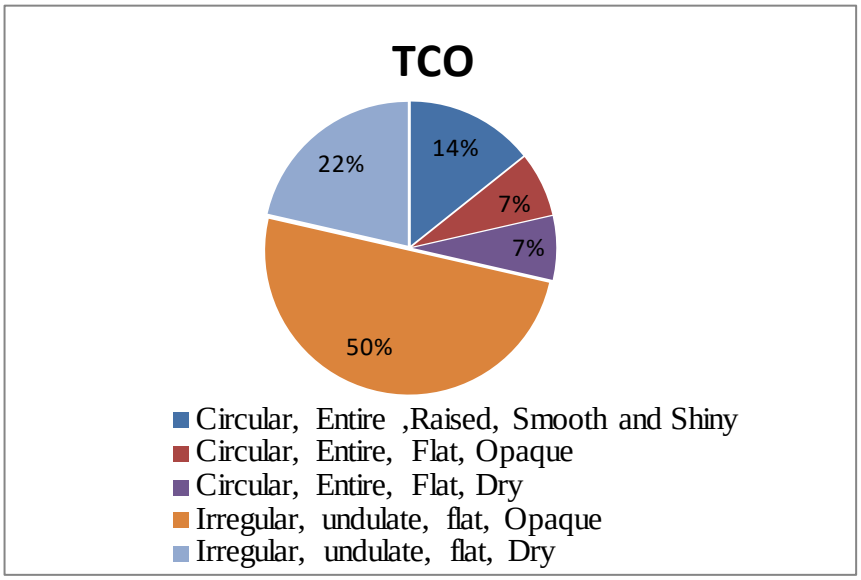


Fig 33 : Microbial diversity in TCO after 40 days

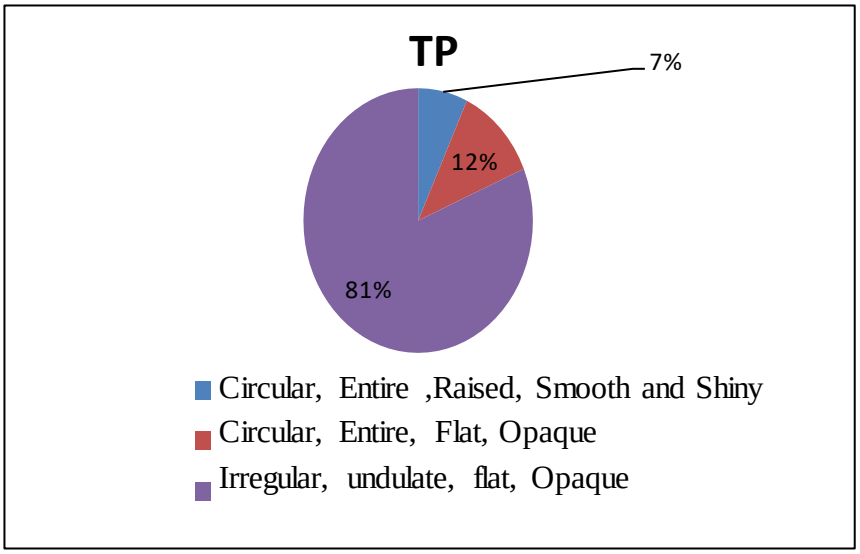


Fig 34 : Microbial diversity in TP after 40 days

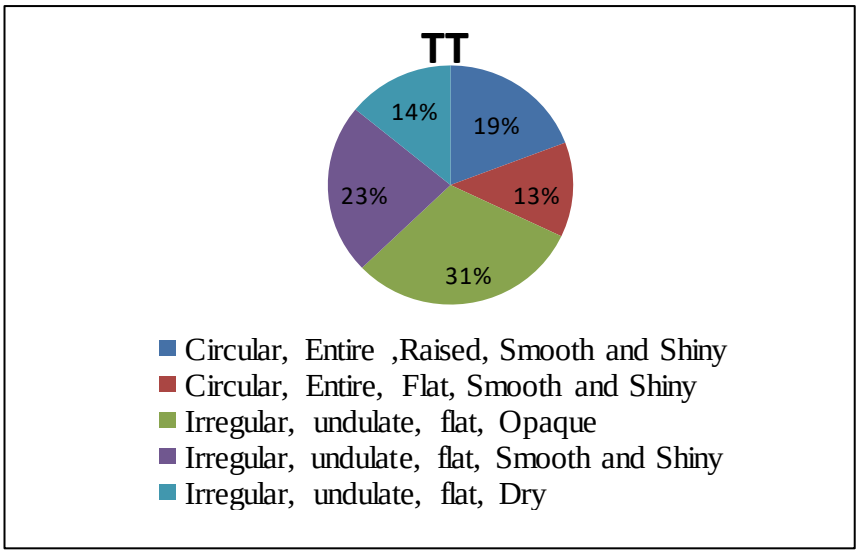


Fig 35 : Microbial diversity in TT after 40 days

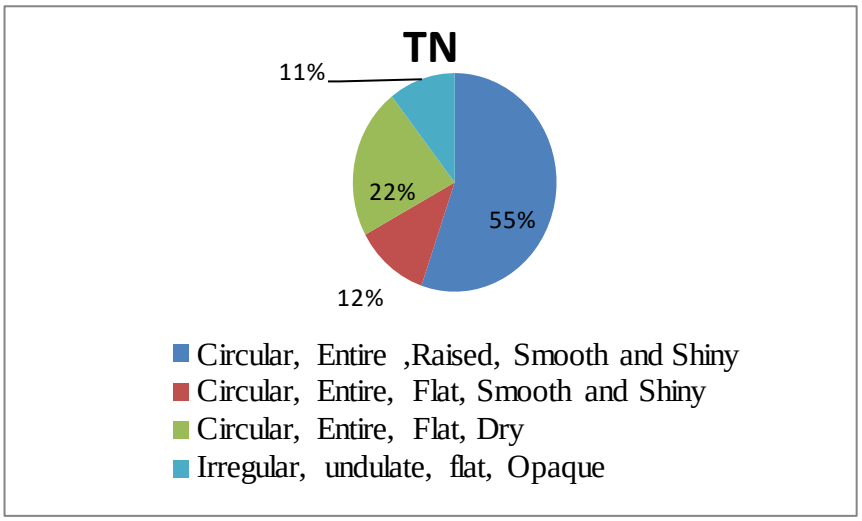


Fig 36 : Microbial diversity in TN after 40 days

4.4.2.6 Microbial diversity of Soil 2 on day 40

Table 33 : Colony characteristics and estimated number in RCO after day 40

RCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	5	20%
Circular, Entire, Flat, Smooth and Shiny	12	48%
Irregular, undulate, flat, Opaque	8	32%

Table 34: Colony characteristics and estimated number in RP after day 40

RP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	6	35%
Irregular, undulate, Flat, Smooth and Shiny	11	65%

Table 35: Colony characteristics and estimated number in RT after day 40

RT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	8	27%
Circular, Entire, Flat, Smooth and Shiny	12	40%
Irregular, undulate, flat, Opaque	10	33%

Table 36 : Colony characteristics and estimated number in RN after day 40

RN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Flat, Smooth and Shiny	3	20%
Irregular, undulate, Flat, Smooth and Shiny	12	80%

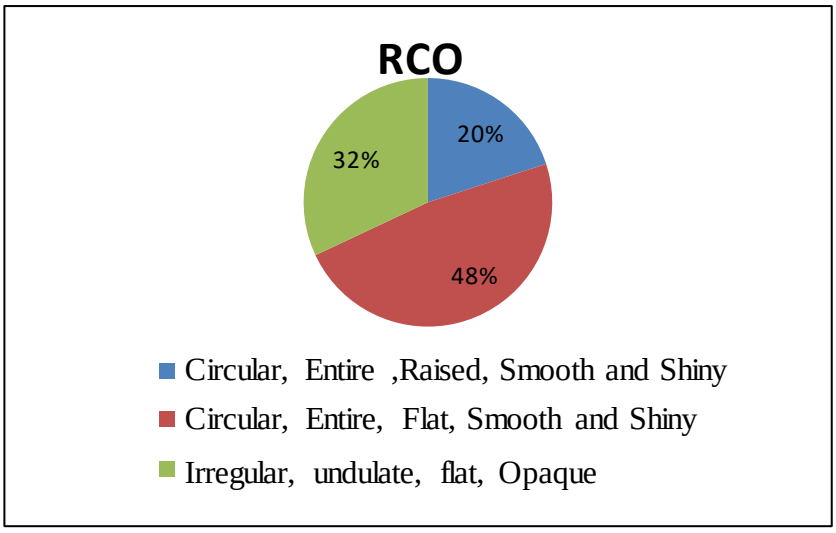


Fig 37 : Microbial diversity in RCO after 40 days

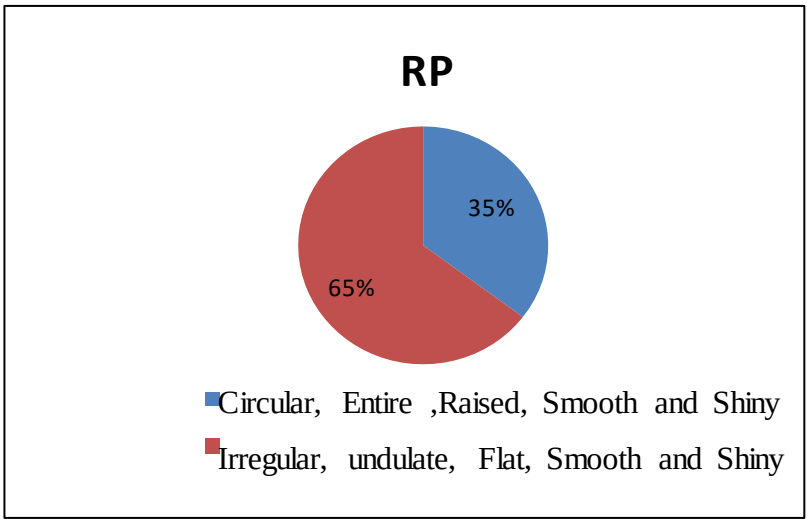


Fig 38 Microbial diversity in RP after 40 days

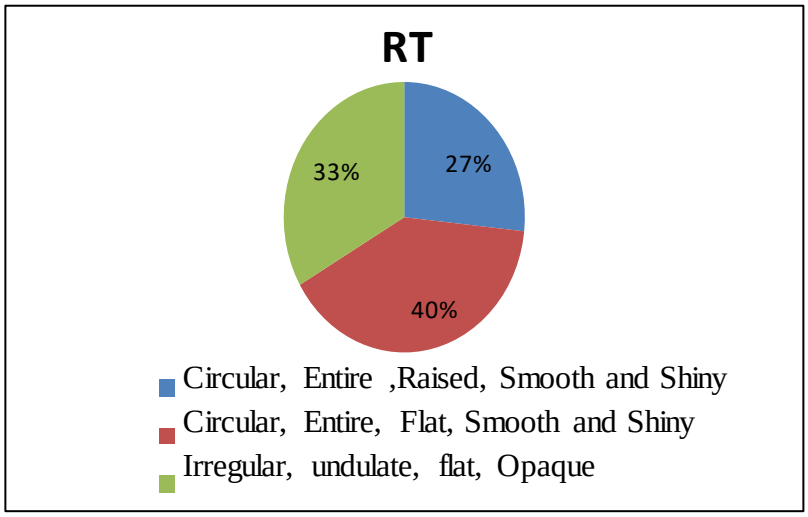


Fig 39: Microbial diversity in RT after 40 days

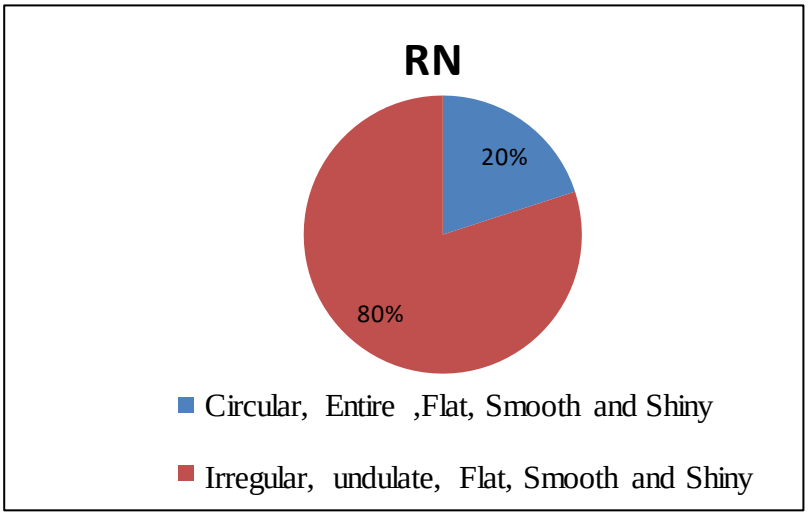


Fig 40: Microbial diversity in RN after 40 days

4.4.2.7 Microbial diversity of Soil 1 on day 60

Table 37 : Colony characteristics and estimated number in TCO after day 60

TCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	14	9%
Circular, Entire, Flat, Opaque	50	33%
Irregular, undulate, flat, Opaque	49	33%
Irregular, undulate, flat, Dry	37	25%

Table 38 : Colony characteristics and estimated number in TP after day 60

TP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	16	9%
Circular, Entire, Flat, Opaque	32	19%
Irregular, undulate, flat, Dry	35	20%
Circular, entire, flat, Translucent	90	52%

Table 39 : Colony characteristics and estimated number in TT after day 60

TT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	3	4%
Circular, Entire, Flat, Opaque	9	13%
Irregular, undulate, flat, Dry	18	26%
Circular, entire, flat, Translucent	40	57%

Table 40 : Colony characteristics and estimated number in TN after day 60

TN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	1	2%
Circular, Entire, Raised, Opaque	12	25%
Circular, entire, flat, Dry	16	33%
Irregular, undulate, flat, Dry	19	40%

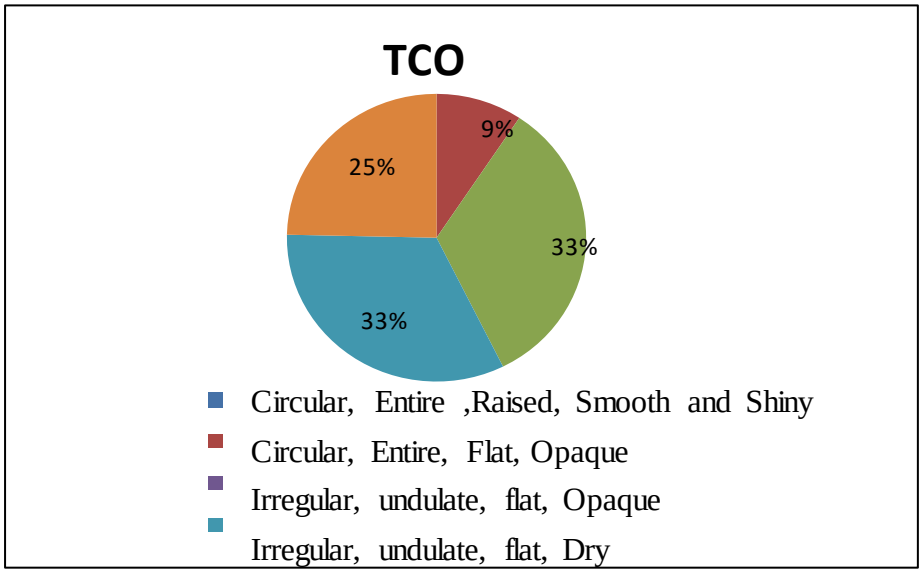


Fig 41 : Microbial diversity in TCO after 60 days

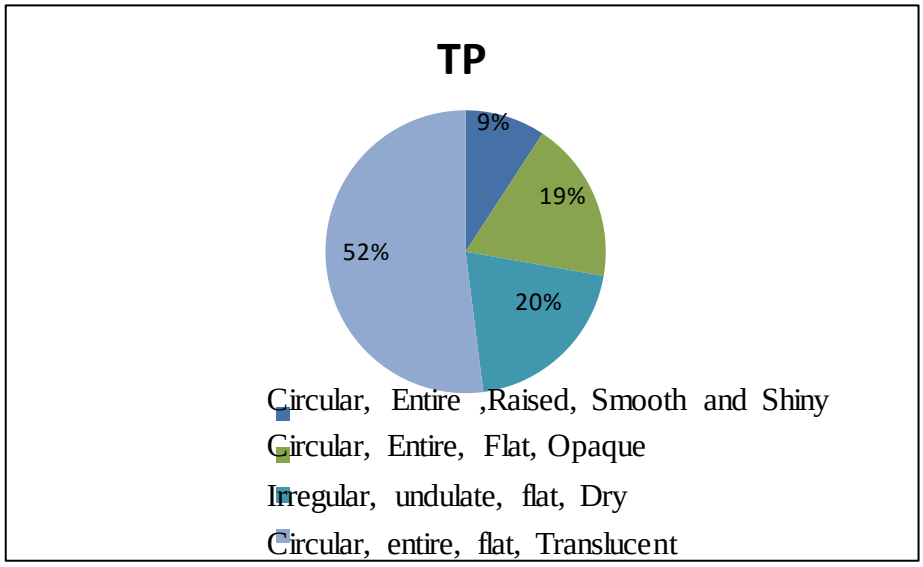


Fig 42 : Microbial diversity in SMTP after 60 days

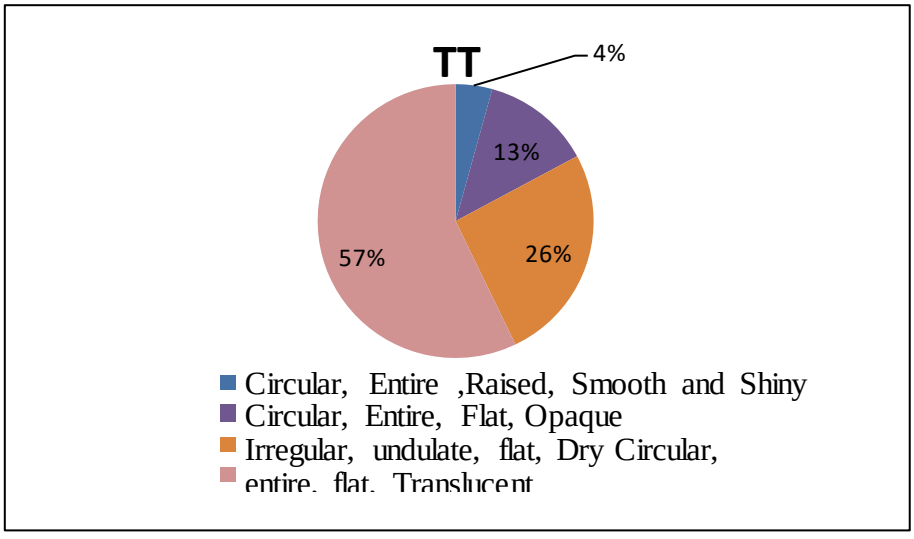


Fig 43 : Microbial diversity in TT after 60 days

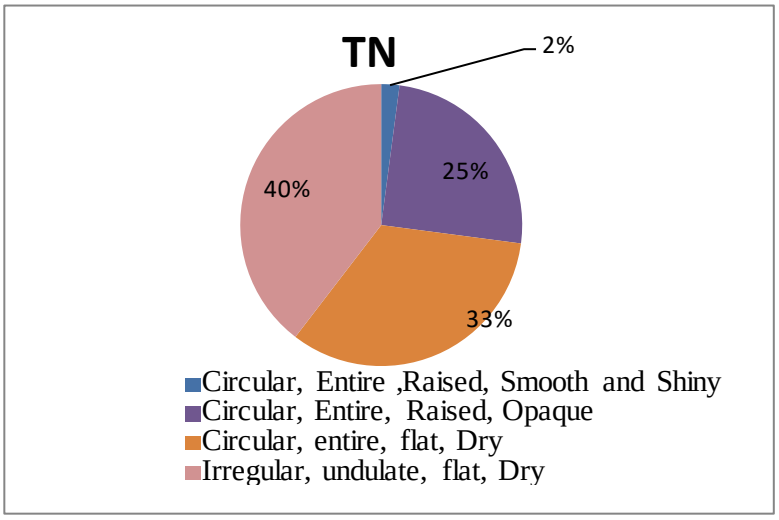


Fig 44: Microbial diversity in TN after 60 days

4.3.2.8 Microbial diversity of Soil 2 on day 60

Table 41 : Colony characteristics and estimated number in RCO after day 60

RCO		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	2	2%
Circular, Entire, Flat, Translucent	64	67%
Irregular, undulate, flat, Translucent	30	31%

Table 42 : Colony characteristics and estimated number in RP after day 60

RP		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	2	1%
Circular, Entire, Flat, Translucent	104	63%
Irregular, undulate, flat, Translucent	60	36%

Table 43 : Colony characteristics and number in RT after day 60

RT		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	5	25%
Circular, Entire, Flat, Translucent	15	75%

Table 44 : Colony characteristics and number in RN after day 60

RN		
Colony characteristics	**Number of colonies**	**Percentage**
Circular, Entire ,Raised, Smooth and Shiny	112	87%
Irregular, undulate Flat, Dry	16	37%

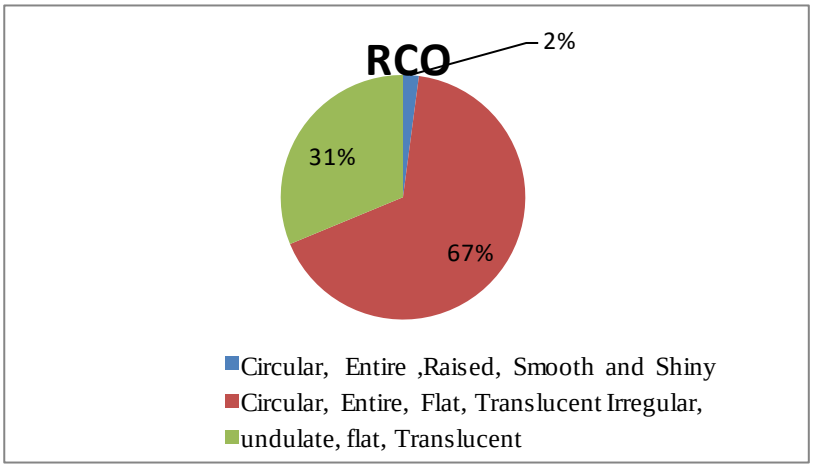


Fig 45 : Microbial diversity in RCO after 60 days

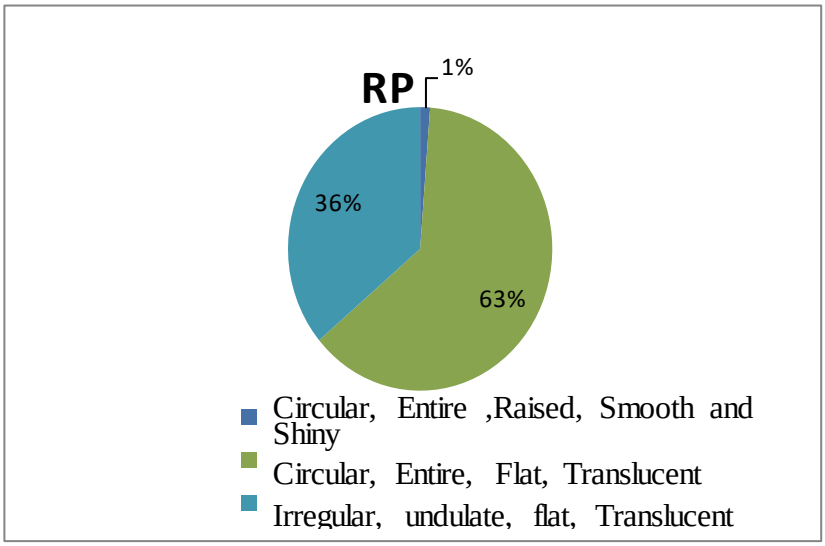


Fig 46 : Microbial diversity in RP after 60 days

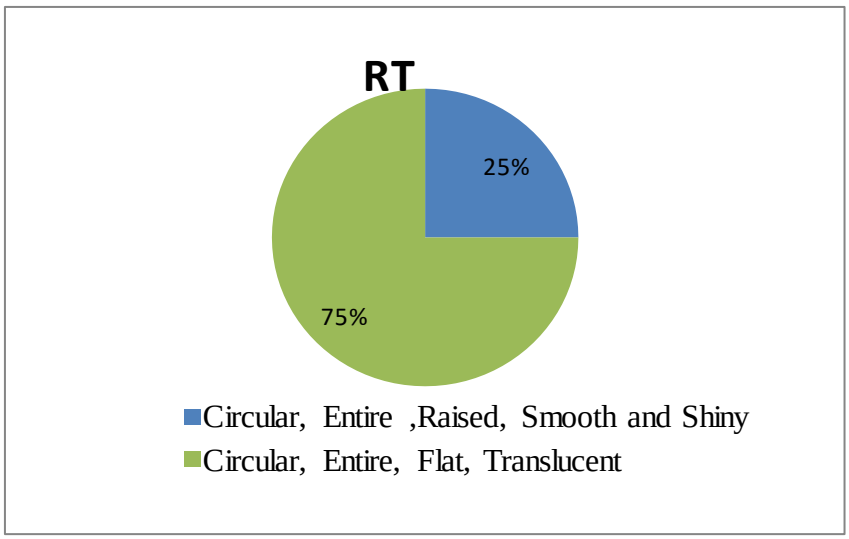


Fig 47 : Microbial diversity in RT after 60 days

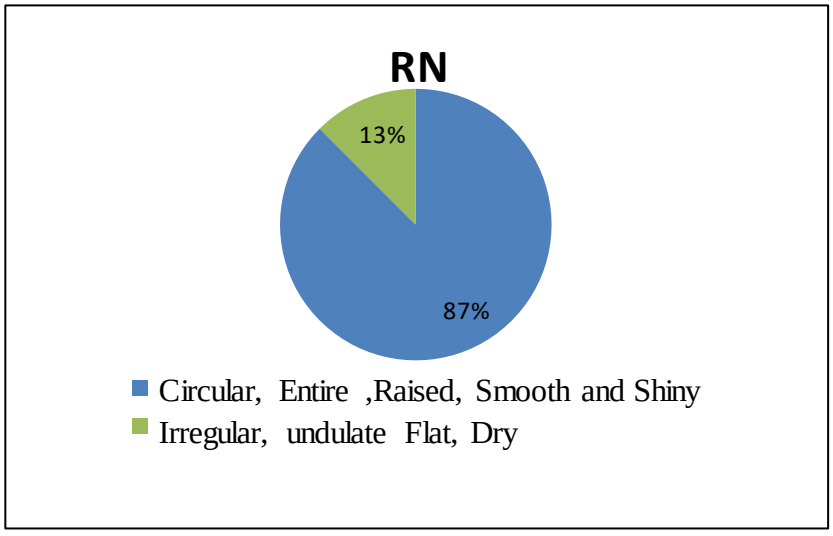


Fig 48 : Microbial diversity in RN after 60 days

6 morphologically distinct colonies were observed in soil 1 on day 1. While TP had 2 different types of colonies. 3 in TT and TN . TCH showed no growth. This means that the application *of Pseudomonas sp., Trichoderma sp.* and Neem cake must have caused some changes in the soil that may have inhibited the growth of certain microbes on day 1. After 20 days of treatment TP had 5 distinct colonies this indicates that *Pseudomonas sp*. must have promoted the growth of other microbes. TT, TN and TCH showed 3 different colonies even after 20 days. However, after 40 days of application TP showed 3 different types of colonies this indicates that *Pseudomonas sp*. must have inhibited the growth of other microbes in the soil. TT showed 5 and TN showed 4 distinct colonies. These results suggest that TP, TT, and TN promoted the growth of microbes after 40 days, indicating a shift towards the original soil microbial composition. After 60 days 4 distinct colonies were seen in all the treatments except TCH indicating that soil came back to its original microbial composition after 60 days however there was complete loss of microbial diversity in TCH.

3 morphologically distinct colonies were observed in soil 2 on day 1. While in RP 3 different types of colonies were observed indicating that *Pseudomonas sp*.did not have any effect on microbial diversity on day 1. In RT no growth was observed which suggests that *Trichoderma sp.* had a negative effect on soil microbial diversity. RN on the other hand had 2 distinct colonies indicating that it inhibited the growth of certain microbes in the soil. RCH had 3 distinct colonies indicating that there was no effect on the microbial community. After 20 days RP, RT and RCH had 3 distinct type of colonies. RN had 4 distinct type of colonies this suggests that Neem cake promoted the growth of microbes in the soil. After 40 days, RP and RN showed reduction in the microbial diversity. However, RT showed 4 distinct colonies indicating that

Trichoderma sp. must have promoted the growth of microbes. No growth was observed in RCH. After 60 days, soil returned back to its original composition.

4.4.3 Assessing the presence of phosphate solubilizing microorganisms

TCO

TP

Fig 49: Clearance zone seen in TCO and TP plates after 8 days

RCO

RP

RT

Fig 50: Clearance zones in plates plated with soil 2 after 8 days of treatment

RP

RT

Fig: 51 Clearance Zones seen on plates after plating RP and RT after 23 days

Table 43: Number of phosphate solubilizing bacteria on day 8

Soil type	Soil sample	Number of colonies with Halo zone	Dilution factor
Soil 1	TCO	6	10^{-3}
	TP	1	10^{-3}
	TT	0	NA
	TN	0	NA
	TCH	0	NA
Soil 2	RCO	11	10^{-2}
	RP	12	10^{-3}
	RT	14	10^{-3}
	RN	0	NA
	RCH	0	NA

On day 8 in soil 1, control had 6 halo zones produced by phosphate solubilizing colonies at a dilution 10^{-3} .TP had 1 colony with halo zone at 10^{-3} dilution. No colonies producing halo zones were observed in other treatments.

In soil 2, 11 halo zones were observed in RCO at 10^{-2} dilution while 12 halo zones were observed around the colonies in RP at 10^{-3} dilution. 14 halo zones were observed in RT at 10^{-3} dilutions. No halo zones were seen in other treatments. Soil 1 which is a sandy loam soil typically has good drainage and aeration properties, which can affect microbial activity and colonization. The presence of 6 halo zones in the control

suggests that some native soil microbes or conditions might promote phosphate solubilisation. *Trichoderma sp*. known for its biocontrol and plant growth-promoting properties, likely had a limited effect in this soil, it had only 1 colony with a halo zone. Soil 2 which is a clayey loam soil has a higher clay content, which affects nutrient availability and microbial activity. The higher number of halo zones in the control (RCO) suggests a more favourable environment for phosphate solubilisation. *Pseudomonas sp*. is known for its ability to solubilize phosphates and produce organic acids. The presence of 12 halo zones indicates its effectiveness in solubilizing phosphates in this soil type, even at a lower dilution. *Trichoderma sp*. also known for its phosphate solubilisation ability showed the highest number of halo zones among all treatments similar to another study (Lee et al., 2023). Neem cake and chemical pesticides may have exerted phytotoxic effects on the phosphate-solubilizing microbes, inhibiting their growth and activity similar to another study (Esitken, 2011).

Table 44 : Number of phosphate solubilizing colonies on day 23

Soil type	Soil sample	Number of colonies with Halo zone	Dilution factor
Soil 1	TCO	0	NA
	TP	0	NA
	TT	13	10^{-3}
	TN	0	NA
	TCH	0	NA
Soil 2	RCO	0	NA
	RP	7	10-2
	RT	0	NA
	RN	0	NA
	RCH	0	NA

In Soil 1, on day 23, the TT exhibited 13 halo zones at a 10^{-3} dilution, indicating significant phosphate solubilisation activity. This suggests that *Trichoderma sp.* was able to effectively solubilize phosphates in the sandy loam soil. No halo zones were observed in any other treatment, indicating that only *Trichodermas sp.* was actively solubilizing phosphates at this stage.

In Soil 2, on day 23, the RP had 7 halo zones at a 10^{-2} dilution, indicating moderate phosphate solubilisation activity. No other treatment exhibited halo zones, indicating that only *Pseudomonas sp.* was actively solubilizing phosphates in the clayey loam soil at this stage.

After day 23, no halo zones were observed in any of the treatments in both soils. This may be because to the phosphate-solubilizing microbes may have depleted the available phosphates in the soil, leading to a decrease in activity. Other microbial species in the soil may have outcompeted the phosphate-solubilizing microbes for nutrients or space, reducing their activity.

4.4.4 Litter decomposition test

Fig 50: Tea bags buried in Soil 1 after 60

Table 45: Rate of litter decomposition in Soil 1

Soil sample	Litter Decomposition %
TCO	13.51%
TP	6.56%
TT	9.45%
TN	31.29%
TCH	10.73%

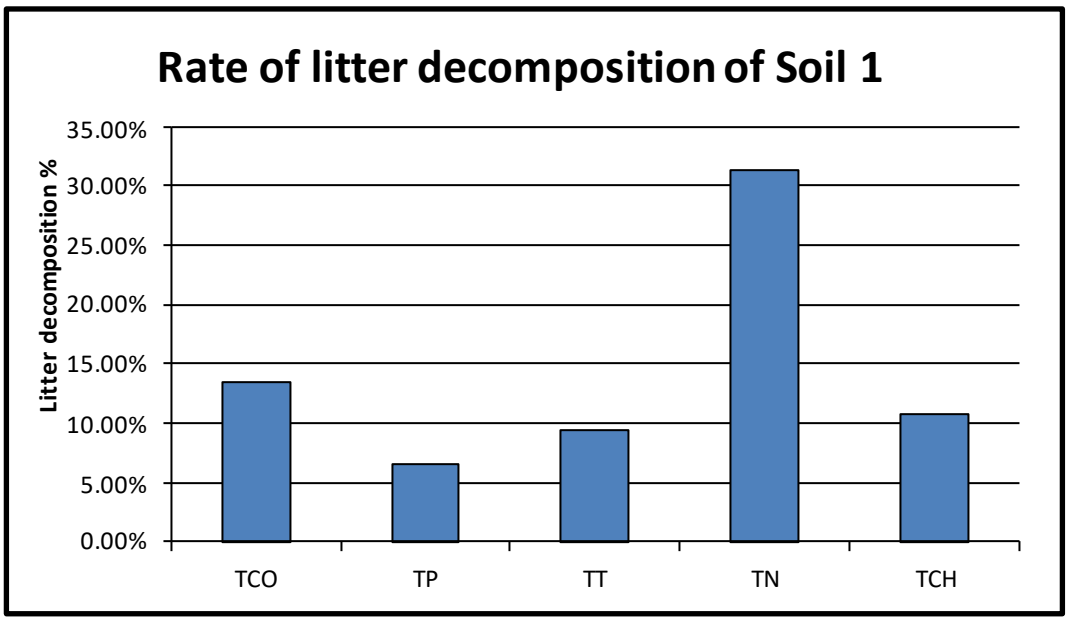


Fig 51: Rate of litter decomposition in Soil 1

In Soil 1, after 60 days, TCO exhibited a decomposition rate of 13.51%, TP showed a relatively low decomposition rate of 6.56%, TT had a decomposition rate of 9.54% slightly higher than TP, TN demonstrated the highest decomposition rate of 31.29%similar to another study (Kumar et al., 2005) and TCH had a decomposition rate of 10.73%.

Sandy loam soil typically has lower organic matter content compared to other soil types. The addition of Neem products, which are rich in organic matter, may significantly increase the substrate available for microbial decomposition, leading to higher decomposition rate (Agyarko et al, 2006). In Soil 1 there may be other microbial species competing for resources, which could limit the ability of *Pseudomonas sp*. to effectively decompose organic matter as a result it showed low rate of decomposition.

Control TP TT TN TCH

Fig 52 : Tea bags buried in Soil 2 after 60 days

Table 46: Rate of litter decomposition in Soil 2

Soil sample	Litter Decomposition %
RCO	27.70%
RP	33.42%
RT	22.12%
RN	18.62%
RCH	28.10%

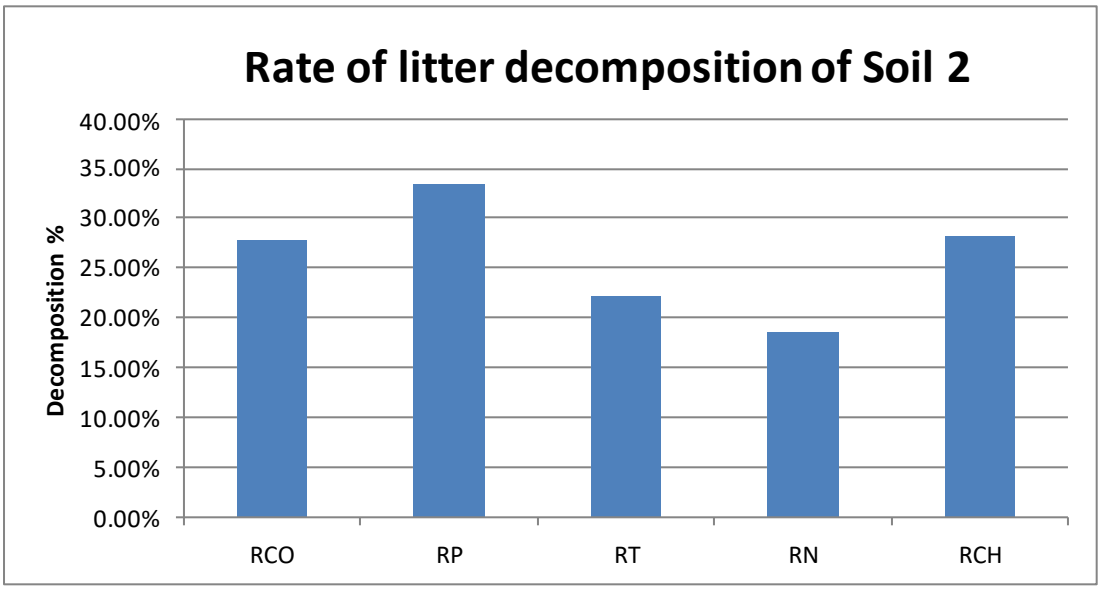


Fig 53: Rate of litter decomposition in Soil 2

In Soil 2, after 60 days, RCO exhibited a decomposition rate of 27.70%, RP had the highest decomposition rate of 33.42%, RT showed a decomposition rate of 22.12%, TN had the lowest decomposition rate of 18.62%, and RCH had a decomposition rate almost similar to the control at 28.10%.

Pseudomonas sp.are known for their ability to enhance soil microbial activity. They can stimulate the decomposition of organic matter by producing enzymes and organic acids that break down complex organic compounds into simpler forms that are more readily available to other microorganisms (Wallenstein, 2011) this explains the high decomposition rate of RP. Clayey soil has unique physical and chemical properties that can influence microbial activity and decomposition rates. These properties may interact with the effects of chemical pesticides, resulting in similar decomposition rates between RCO and RCH.

Low decomposition rate of TN in soil 2 could be because neem cake can have physical effects on soil structure, such as increasing soil aggregation. These physical changes can affect the accessibility of organic matter to decomposers, potentially slowing down

decomposition rates. Some Neem compounds can persist in the soil for an extended period, especially under certain environmental conditions. This prolonged presence of Neem cake compounds can continue to inhibit microbial activity and decomposition over time

4.5 Pot trials with cowpea seeds

Fig 54: Pots potted with cowpea seeds in soil 1

Control TP TT TN TCH

Fig 55: Plant growth on day 15

Control TP TT TN TCH

Fig 56: Plant growth on day 30

Fig 57: Plant growth on day 45

4.5.1 Viability percentage

Table 47 : Viability percentage of Cowpea

Sample	Viability percentage
TCO	35%
TP	38.33%
TT	31.66%
TN	38.33%
TCH	21.66%

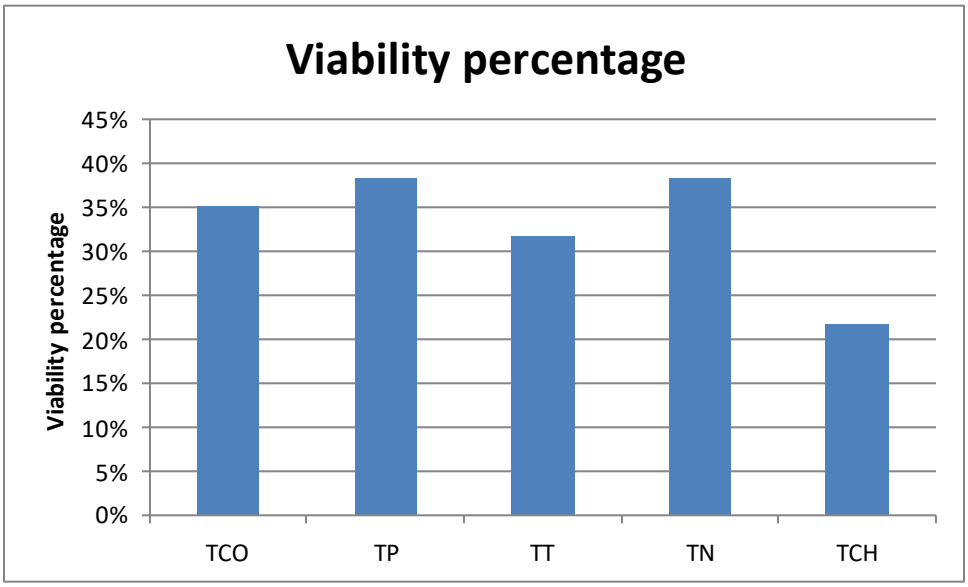


Fig 58 : Viability percentage of Cowpea

TCO had viability percentage of 35%.TP resulted in a viability percentage of 38.33%. *Pseudomonas sp.* is known to promote plant growth and protect plants from diseases. The higher viability compared to the control could indicate a beneficial effect of *Pseudomonas sp.* on the soil.

TT resulted in a viability percentage of 31.66%. *Trichoderma* species are also known to promote plant growth and suppress plant pathogens. The lower viability compared to the control and *Pseudomonas sp*. treatments could suggest that *Trichoderma sp.* was less effective. TN resulted in a viability percentage of 38.33%, similar to Pseudomonas. Neem is known for its pesticidal properties and effects on soil health. The similar viability to *Pseudomonas* could indicate a comparable effectiveness. TCH Chemical pesticide treatment resulted in a viability percentage of 21.66%, the lowest among all treatments. Chemical pesticides are generally effective at controlling pests but can have negative impacts on soil health and beneficial microorganisms similar to another study (Shahid, 2022), which could explain the lower viability compared to the other treatments.

4.5.2 Shoot length measurement

Table 48: Shoot length of plants grown in Soil 1

Soil sample	Day 15	Day 30	Day 45
TCO	15	17.5	18
TP	14.3	16	17
TT	12.6	15.5	16
TN	15	19	22
TCH	12.3	13	15.5

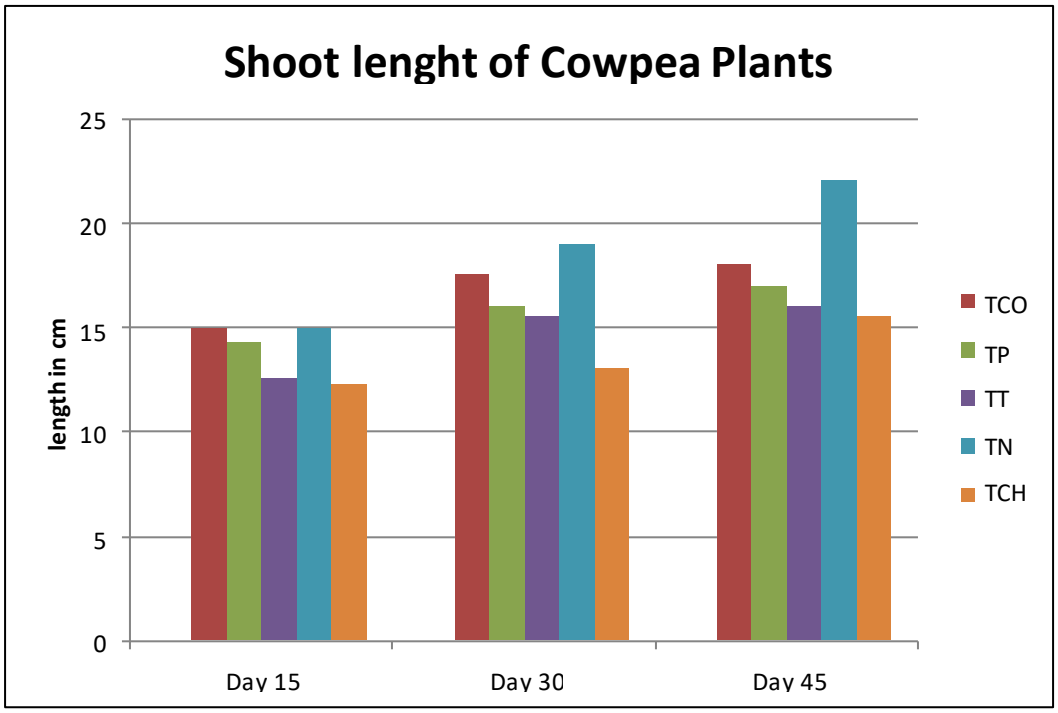


Fig 59 : Shoot length of plants grown in Soil 1

On day 15, the shoot length of plants grown in TCO was comparable to plants grown in TN, while plants grown in TP exhibited greater shoot length than those in TT. However, plants in TCH showed the lowest shoot length among all treatments.

By day 30 and 45, plants in TN displayed the greatest shoot length compared to all other treatments similar to another study (Doshi et al., 2020). Plants in TCO exhibited slightly shorter shoot length than those in TN but longer than both TP and TT, while plants in TCH consistently showed the shortest shoot length across all treatments similar to another study (Khan et al., 2020).

These results demonstrate that the use of chemical pesticides has a negative impact on cowpea plants while use of biocontrol agents may had a slightly less negative impact. Overall, these results suggest that the use of biocontrol may be a more sustainable alternative to chemical pesticides. Neem products have been reported to improve soil health by increasing microbial activity and organic matter content (Mweetwa et al, 2016). Improved soil health can lead to better nutrient uptake and overall plant growth. Reason why plants grown in soil treated with *Pseudomonas sp.* and *Trichoddrma sp.* did not grow that well compared to soil without any treatment could be because *Pseudomonas sp.* and *Trichoderma sp.* are beneficial microbes that can enhance plant growth through various mechanisms, such as nutrient cycling, disease suppression, and hormone production.

4.4.3 Shoot dry weight measurement:

Table 49 :Shoot dry weight of plants grown in Soil 1

Soil sample	Day 15	Day 30	Day 45
TCO	0.3	0.48	0.66
TP	0.26	0.46	0.56
TT	0.5	0.52	0.52
TN	0.59	0.7	1.24
TCH	0.25	0.42	0.4

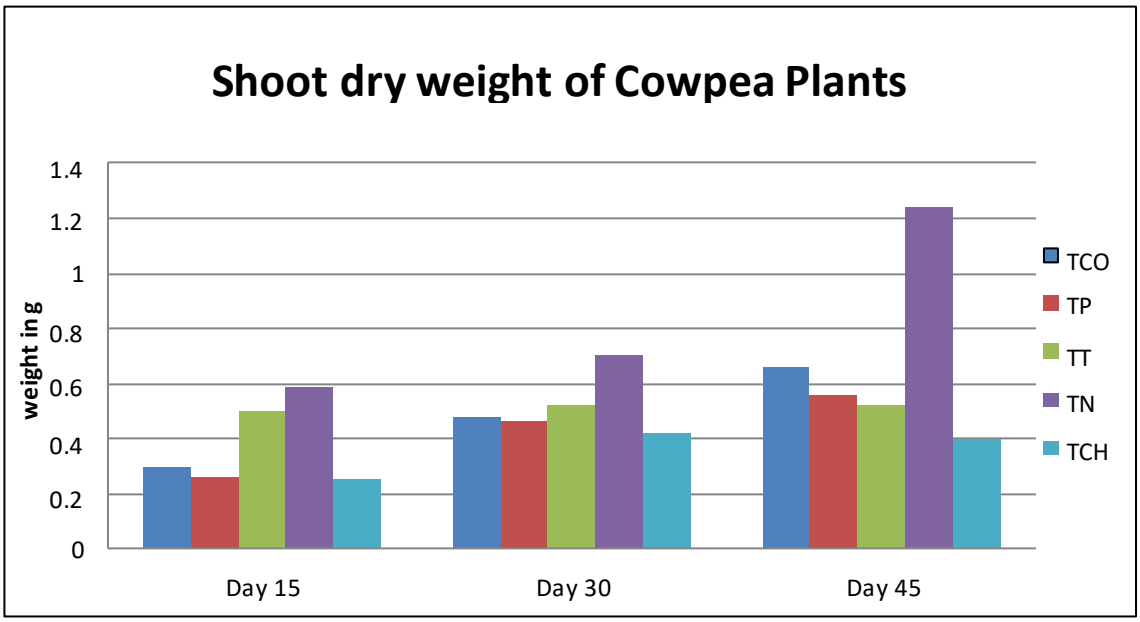


Fig 60: Shoot dry weight of plants grown in Soil 1

On day 15, the shoot dry weight of cowpea plants grown in TCO was slightly higher than the dry weight of plants grown in TP, which was similar to those grown in TCH. The shoot dry weight of plants in TN was the highest compared to all other treatments.

Dry weight of plants grown in TT was lower than TN but higher than all other treatments. On day 30 the shoot dry weight of plant grown in TCO was little higher that TP but lower than TT. Plants grown in TN showed highest shoot dry weight while plants grown TCH had the lowest shoot dry weight. On day 45, the shoot dry weight of plants grown in TCO was higher than those grown in TP, TT, and TCH. Plants grown in TN exhibited the highest shoot dry weight, while plants in TCH had the lowest shoot dry weight.

Neem products, such as Neem cake, are known to be rich in nutrients like nitrogen, which are essential for plant growth (Lokanadhan et al, 2012). The nutrients released from Neem products may have been more readily available to the plants, leading to increased shoot dry weight.

4.5.4 Root length measurement:

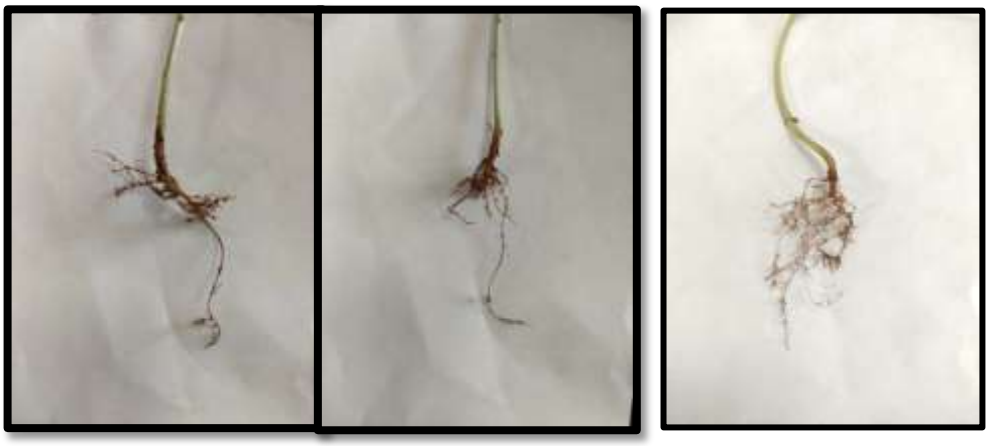


Fig 61: Roots of cowpea plants grown in Soil 1

Table 50 :Root length of plants grown in Soil 1

Soil sample	Day 15	Day 30	Day 45
TCO	6.00	7.60	8.20
TP	6.20	7.50	8.00
TT	5.80	6.50	7.00
TN	6.00	8.20	8.50
TCH	5.00	6.00	7.00

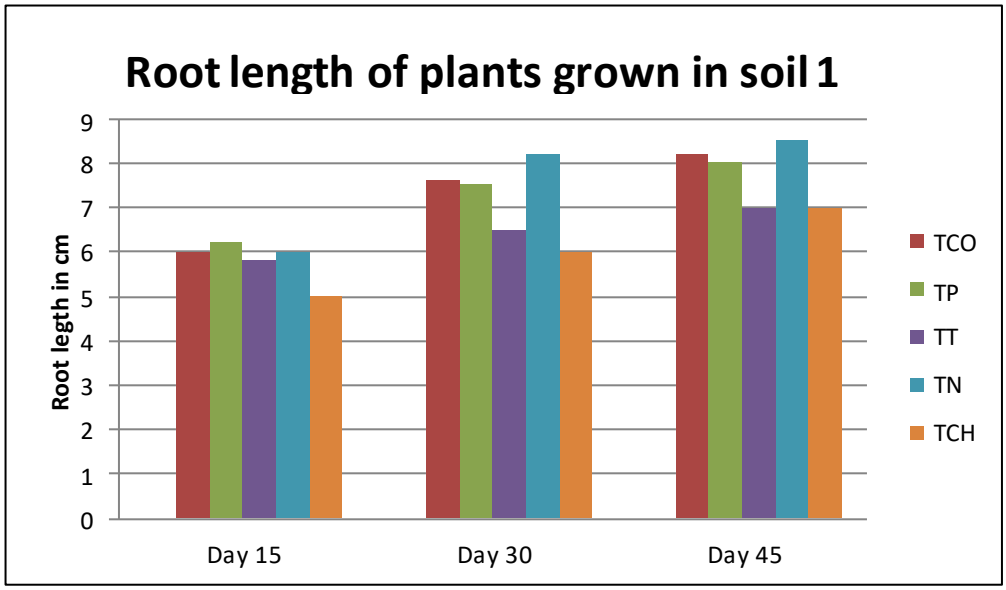


Fig 62 : Root length of plants grown in Soil 1

On day 15, the root lengths of plants in all treatment groups were comparable, except for the treatment group exposed to TCH where the root length differed significantly. On day 30 and day 45 the root lengths of plants in the TCO and TP treatment groups were similar. The root length in the TN treatment group was the highest, while the TT treatment group had slightly longer roots than the TCH group, which exhibited the lowest root length measurements similar to another study (Khan et al., 2020).

The differences in root lengths among the treatment could be due to the varying effects of the treatments on root growth. The TN treatment, which includes Neem,cake may have promoted root growth due to the presence of bioactive compounds known to enhance plant growth and development. The TCO and TP treatments, which did not contain Neem cake might have influenced root growth differently, resulting in comparable lengths. The TT treatment, although containing *Trichoderma sp*. showed slightly better root growth compared to TCH, which may indicate a differential impact of *Trichoderma sp*. compared to chemical pesticide on root development.

4.5.5 Root dry weight measurement:

Table 51: Root dry weight of plants grown in Soil 1

Soil parameter	**Day 15 (g)**	**Day 30 (g)**	**Day 45 (g)**
TCO	0.08	0.09	0.14
TP	0.07	0.13	0.17
TT	0.07	0.08	0.07
TN	0.15	0.24	0.32
TCH	0.01	0.03	0.03

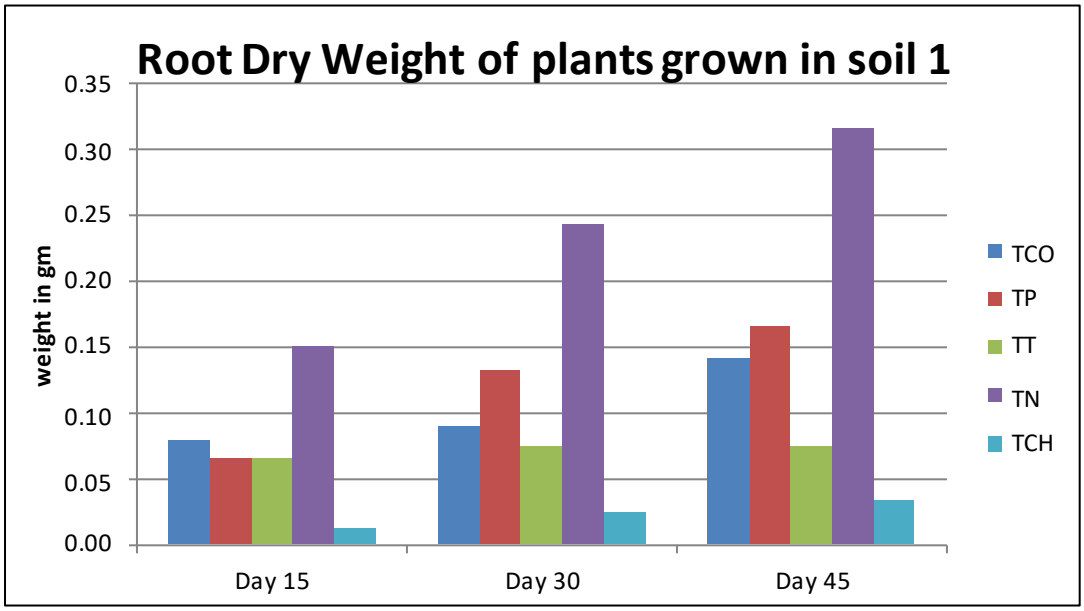
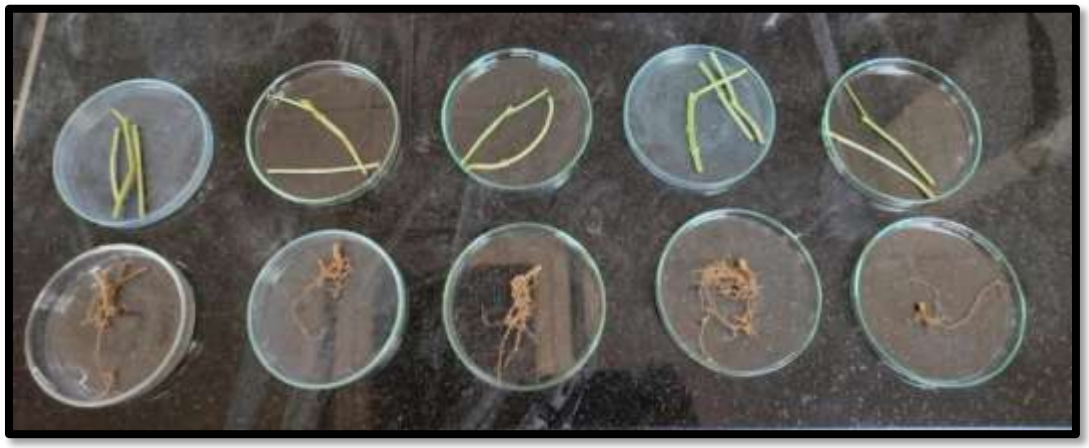


Fig 63 : Root dry weight of plants grown in Soil 1

Results show that root dry weight of plants grown in TCO,TP and TT was comparable on day 15. While weight of roots in TN was found to be higher than all the treatments. TCH on the other hand showed the lowest weight. On both day 30 and day 45, the root weights of plants in the TP treatment group were higher than those in the TCO and TT groups. The TN treatment consistently resulted in the highest root weights, while the TCH treatment consistently showed the lowest root weights across all time points.

The higher root dry weight in the TN treatment group compared to all other treatments suggests that the Neem cake may have stimulated root growth more effectively than the other treatments. *Pseudomonas sp.* is known for its plant growth-promoting properties, which could have contributed to increased root biomass in the TP treatment. Consistently low root weights in the TCH treatment group across all time points may indicate that the combination of Carbendazim and Moncozeb had a negative impact on root growth compared to the other treatment these results were similar to study conducted by Khan et al. in 2020.



Control TP TT TN TCH

Fig 64: Petri plates containing roots and shoots of plants grown in soil 1 with various treatments before calculating dry weight

CONCLUSION

This study aimed to evaluate the impact of biocontrol agents on soil health and microbiota of Goan agricultural soils. According to study findings, in both Taleigao Soil and Raia soil, biocontrol agents *Pseudomonas fluorescens* , *Trichoderma viride,* and Neem cake showed significant benefits compared to the chemical pesticide (Carbendazim-12% + Moncozeb-63%). Among them, *Pseudomonas sp.* exhibited the most favorable results, promoting both microbial growth and plant development in both the soils Although microbial diversity study suggests that they were less diverse but it had the highest soil recovery time compared to other treatments. *Pseudomonas sp.* is known for its plant growth-promoting properties and its ability to enhance soil health, which likely contributed to its quick recovery. Additionally, the presence of a higher number of phosphate-solubilizing bacteria in the soil treated with *Pseudomonas sp.* suggests a positive influence on growth of phosphate-solubilizing bacteria and in turn of the cowpea plant in Taleigao soil. *Pseudomonas sp.* are known to stimulate the decomposition of organic matter by producing enzymes and organic acids this explains its high decomposition rate in Soil 2 compared to other treatments. In terms of soil growth, the Taleigao soil treated with neem cake showed the best results.Neem products, like neem cake, are recognized for their richness in nutrients such as nitrogen, crucial for plant development. The nutrients released from neem products might have been more readily accessible to the plants, thereby enhancing their growth.

To conclude, using biocontrol agents rather than chemical pesticides is more beneficial to the environment and a sustainable choice. Encouraging the application of biocontrol agents in the Goan agriculture is essential to help farmers increase their yield and thus boost the economy of the state.

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APPENDIX – I

Media used

1. Nutrient Agar

Components	(Gram/ Litre)
Peptone	5
Meat extract	5
Sodium Chloride	3
Agar	15
Distilled Water	1000 mL
pH	7.4 ± 0.2

2. Pikovskaya Agar

Components	Grams/ Litre
Yeast extract	0.5
Dextrose	10
Calcium Phosphate	5.
Ammonium Sulphate	0.5
Potassium Chloride	0.2
Magnesium Sulphate	0.1
Manganese Sulphate	0.0001
Ferrous Sulphate	0.0001
Agar	15
Distilled Water	1000mL
pH	7 ± 0.2

APPENDIX – II

Reagent preparation

1) Preparation of NaCl- HCl solution

It was prepared by dissolving 6 g of sodium chloride in minimum amount of distilled water in 100mL flask followed by addition of 0.5 mL of Analytical grade conc. HCl and diluting it up to the mark with distilled water.

2) Preparation of Glycerol- Ethanol solution

It was prepared by mixing 25 mL glycerol in 50 mL of ethanol (1:2).

3) Preparation of Bray's No. 1 solution

In deionised water 0.55 g Ammonium Fluoride A.R. (NH_4F) was dissolved and transferred to a 500mL volumetric flask. 1.25 mL concentrated hydrochloric acid was added and bulked to volume with deionised water.

4) Preparation of Reagent A

4.28 g of ammonium molybdate anhydrous was dissolved in 50 mL of warm deionized water. Simultaneously, 0.098 g of potassium antimony tartrate anhydrous was dissolved separately in 37.5 mL of deionized water. 125 mL of deionized water was placed in a 500 mL volumetric flask, and 50 mL of concentrated sulphuric acid was slowly added with mixing. After cooling, the cooled molybdate and tartrate solutions were added, mixed thoroughly, and then topped up to volume with deionized water.

5) 3.4.4 Preparation of Reagent C

0.265 g of L-Ascorbic Acid A.R. ($C_6H_8O_6$) was dissolved in deionized water and transferred to a 250 mL volumetric flask. Then, 35 mL of Reagent A was added, and the solution was bulked to volume with deionized water.