Studies on Soil Microbial Diversity in Mokokchung District, Nagaland

by
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A thesis submitted to the Department of Botany, Nagaland University, Lumami, Nagaland in partial fulfilment for the requirement of Degree of Doctor of Philosophy in Botany

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DEDICATION

This thesis is dedicated to my supervisor **Professor Talijungla**

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on Soil Microbial Diversity in Mokokchung District, Nagaland**" which is being submitted by Ms. **Asangla Kichu, bearing registration No.778/2017,** with effect from **29/07/2016**, in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany is an original research work carried out by the candidate under my supervision and guidance. The matter embodied in this thesis has not been submitted anywhere for any other degree.

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DECLARATION

I, Ms. **Asangla Kichu**, bearing Ph.D. registration number 778/2017, with effect from 29/07/2016, hereby declare that, the thesis entitled **"Studies on Soil Microbial Diversity in Mokokchung District, Nagaland"** being submitted to Nagaland University, Lumami, for the degree of Doctor of Philosophy in Botany is an original and independent research work carried out by me under the supervision of Prof. Talijungla, Department of Botany, Nagaland University, Lumami.

This thesis has not previously been submitted for award of any other degree or diploma to any university or other tertiary institutions. My declaration/thesis is hereby forwarded by my supervisor and head of the department.

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CHAPTER 1

Introduction

1.1 Soil and its importance

The soil is the foundation of every terrestrial ecosystem, and it plays a crucial role in the major global biogeochemical cycles of carbon, nutrients, and water (Smith et al. 2015). Soil has a wide range of chemical and physical characteristics. Numerous different soil types are created by the interaction of natural processes like leaching, weathering, and microbial activity. In addition to acting as a structural support for the plant, soil provides the vital nutrients, water, oxygen, and root support that plants require to grow and flourish (Powlson et al. 2001). It also serves as a buffer to safeguard delicate plant from drastic temperature changes (Onwuka and Mang, 2018). Healthy soil is a living, dynamic ecosystem that is teeming with small and large organisms that convert dead and decaying matter, among other essential functions. Fertile soil contains primary plant nutrients like nitrogen, phosphorus and potassium along with minor nutrients that aid in the growth of agricultural and horticultural crops (Kumar and Singh, 2016). Soil is a ubiquitous medium for growing all types of crops and as a result, soil is crucial to human societies because we rely on it to produce food and other resources (Tilman et al. 2002). Efforts to stop soil degradation must become a top priority in our globalized world to maintain and increase food production. The world's population is estimated to reach between 8 to 10 billion in the next 50 years (Bongaarts, 2009; Lutz et al. 2001) with food demand expected to rise by a two-fold increase (Alexandratos, 1999). If poor management of the soil resources keeps reducing the fertility of the soil and the amount of productive arable land (Pimentel et al., 1995), then we will lost a crucial component of sustainable

agriculture (Tilma, 1999). The state of the soil has a significant impact on the environmental sustainability of forestry, horticulture, and agriculture. Additionally, the production of wholesome food, which affects human health, is directly related to soil health (Frac et al., 2018).

1.2 Soil microbial diversity

Soil supports a wide range of microbial diversity comprising of a variety of microorganisms. The diverse ranges of microorganisms that are present in the soil interact with one another and with other organisms that make up the soil biota. The aboveground plants may be impacted favourably or unfavourably by these interactions. Without a doubt, the soil microorganisms engagement in the cycling of nutrients serves as their primary purpose. However, they play a variety of tasks that go beyond just this, from the breakdown of organic matter to having a direct or indirect impact on plant performance (McGrath et al., 2001). According to Torsvik et al. (2002), one gram of soil may contain up to 10 billion microorganisms, with thousands of different species many of which are still unidentified. At various levels of biological organization, complexity and variability are described by microbial diversity. It includes the genetic diversity found within taxa (species), as well as the quantity (richness), proportionality (evenness), and distribution of taxa and functional groups within communities (Zak et al. 1994). A variety of microorganisms are involved in crucial soil functions, and there has been an increase in interest in the significance of microbial diversity in soil habitats. This is because maintaining soil health and quality largely depends on the type and number of microorganisms and their

activity in the soil. It is noted that microbial population can have detrimental impacts on plant development, growth, and yield or even cause plant death. Additionally, because some microorganisms can react to changes in soil quality more quickly than soil carbon or available nutrients, they can serve as early warning indicators of those changes. (Kennedy and Papendick, 1995). Studies on microbial diversity are crucial for comprehending the function of microbes in soil as it plays essential role in both agroecosystems and natural ecosystems (Lecomte et al., 2011). According to Pandey et al. (2011), microbial activities have an impact on the ecosystem structure and function in the soil, which in turn affects plant growth. The soil microorganism regulates the breakdown of organic matter and controls the release of mineral nutrients into the soil which is crucial for the functioning of the ecosystem.

According to Wardle (1992), the soil microbial community's temporal dynamics are probably crucial in determining the mineralization and, consequently, the availability of nutrients for plant productivity. Among the soil microorganisms, bacteria and fungi are the active decomposers of organic matter, preserving soil health and facilitating the availability of nutrients in the soil, which are crucial for plant growth and productivity. With numbers as high as $1.5x10^{10}$ per gram of soil, bacteria are one of the most numerous groups of the soil microflora. Fungi, on the other hand, are a significant group of the microflora and are essential for humans, plants, and animals (Torvsvik et al., 1990). In addition to being active nutrient cyclers, soil bacteria and fungi also function as biofertilizers and bioremediation because they are crucial to soil processes like biochemical

transformation and mineralization (Magnet et al., 2013), which have a significant impact on the structure and functioning of an ecosystem and are thus essential to many ecological services (Ramkumar et al., 2017). Fungi and bacteria are an important group of microbes to study because they have enormous value in agriculture, food, and industries as a source of numerous enzymes, antibiotics, and medicines (Abed et al., 2017) that makes them a valuable group of microbe to study. Soil phosphorus is an important limiting micronutrient in the soil and soil microbial diversity regulates its availability in the soil. According to studies by Sharma et al. (2013), Kanse et al. (2015), and Yu et al. (2022), soil microbial activity and population increased substantially with the availability of organic phosphorus in the soil, and as a result helped in converting the organic phosphorus into available form for plants use. Phosphate-solubilizing soil fungi like *Aspergillus*, *Penicillium*, and *Tricoderma* showed dominancy, which accelerated the mineralization of phosphorus in the soil. Elias (2016) reported that soil fungal species such as *Aspergillus, Penicillium and Fusarium* significantly increased available P in their study. In order to increase agricultural output, phosphate-solubilizing bacteria from the genera *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Kluyvera*, *Streptomyces*, *Pantoea*, and *Pseudomonas* has also been extensively studied (Kang et al., 2014, Alori et al., 2017). The microbial flora can vary depending on native soils (Shi et al., 2002; Gleason et al., 2004). While certain fungi are found throughout soil, others are confined to particular areas. The amount and kind of organic matter in the soil, as well as other soil and climate factors, surface vegetation, and soil texture, all have an

impact on where these organisms are found (Marschner et al., 2003). Sharma et al., 2015 studied the fungal diversity from twelve vegetational zones of Arunachal Pradesh, India and reported the dominance of the forest soil by fungal genera *Oidiodendron*, *Acremonium*, *Cladosporium*, *Humicola*, *Aspergillus*, *Penicillium* and genera under *Blastomyces*, *Cercospora*, *Metarrhizium* and *Rhizomucor* were found to be restricted to particular soil type, altitude and physicochemical paroperties. In relation to vegetation and abiotic soil variables, such as season, soil microbial communities fluctuate. *Penicillium* and *Mortierella* species are prevalent in temperate to high latitudes, which show that certain fungal species are features of particular types of flora. At low latitudes, *Aspergillus* species are prevalent in grasslands and deserts. Grassland soils tend to have *Fusarium* and *Periconia*, whereas forest soils typically have *Mortierella*, *Mucor*, and *Penicillium* which reveals that the soil fungal community undoubtedly exhibits tremendous diversity. Durovade et al. (2008) in their study of soil fungi in various locations in Ilorin, isolated 34 common fungi, with *Aspergillus*, *Penicillium*, and *Fusarium* predominating other fungal genera. They also found that the physicochemical characteristics of the soil samples had an impact on the distribution and population of fungi.

There is a growing interest regarding the significance of microbial diversity in soil habitats since, a wide variety of microorganisms are involved in crucial soil functions. It is thought that the degree of microorganism diversity in soil is essential for maintaining soil health and quality (Meliani et al., 2012). The ecological niche and nutritional habits of soil microorganisms are very diverse,

and human intervention can change the type and quantity of the microbial diversity (Atlas, 1984). Moreover, the quantity and activity of soil's microorganisms control the quality of the nutrients that plants can access. Soil microbial diversity is a key component for the preservation of the soil health and other related processes. According to Waksman (1927), the relative abundances of bacteria, fungi, and other microorganisms provide a sensitive indicator of soil fertility as well as the soil's chemical make-up. High microbial diversity improves soil ecosystem functions and resistance to disturbances like alterations in land use, changing seasons, and fertilizer use. On the other hand, a decline in diversity could result in loss of ecosystem services, which would reduce the stability of the system. To ensure the long-term provision of ecosystem services, soil microorganism diversity is therefore necessary (Madegwa and Uchida, 2021). Apart from the importance of soil mycoflora in agriculture, fungi are valuable medically due to their ability to synthesize antibiotics and in food sector. The *Penicillium* spp., *Saccharomyces cerevisiae* and *Aspergillus niger* are some of the most common and economically significant members of the microfungi (Meklin et al., 2004; Portnoy et al., 2004).

According to Seth et al. (2016), only 5-10% of the estimated 1.5 million fungal species in the world have been formally characterized. Of the 1.5 million fungal species, 1/3 is found in India, demonstrating the necessity of identifying the understudied native fungal communities (Chandrashekar *et al.*, 2014). Although physiological, biochemical, and molecular methods are important for identifying fungi and bacteria, morphological properties based on culture and

microscopical characteristics are still widely used methods, making them crucial for identifying fungi and bacteria (Chandini and Rajeshwari, 2017). Fungal and bacterial species are generally characterised morphologically based on the characteristics of their colonies and microscopical examinations, such as colony characteristics, size, colour variations, exudates secretions, mycelia arrangements, spore orientations, etc., photography along with descriptions of the isolated soil fungi are crucial for identifying the soil fungi (Senanayake et al., 2020) with biochemical test, all of which provide visible information on the investigated fungal and bacterial species (Germain and Summerbell, 1996; Schmit and Lodge, 2004). In addition to molecular techniques that aid in the extraction of bacterial and fungal DNA for identification up to the species level (Mushimiyimana et al., 2016). Molecular techniques have made it easier to determine the microbial flora (Nannipieri et al., 2017) and the diversity of the *r*RNA gene is a helpful tool for researching the taxonomy and phylogenetic classification of bacteria and fungi (Lim et al., 2005).

1.3 Factors affecting soil microbial diversity

Given the significance of microbial diversity in soil, numerous researchers have examined the soil microbial population and found that elements like temperature and acidity have a significant impact on the distribution of bacterial and fungal species (Warcup, 1951). According to Jacoby et al. (2017), physicochemical and ecological interactions in the soil have a significant impact on the activities of soil microorganisms, which are largely responsible for the transformation of organic matter and nutrients as well as other soil processes.

There are significant external factors such as moisture, *p*H, temperature, available nutrients, organic and inorganic fertilizers, organic matter content, type of vegetation, plowing, and season that affect the structure and function of microbial community and activity in soil (Griffin, 1972; Grayston et al., 2001; Jamir and Ajungla, 2018). Chemical components like acids, micro- and macro-elements, and clay elements give soil microorganisms the nutrition they need to grow, function, and survive in a variety of ecological niches.

The structure, spatial distribution, and activity of the microbial population which are potential important markers of soil health and quality can be directly influenced by the physicochemical properties of soils (Schnurer et al., 1985). According to Kaiser et al. (1992), the amount and quality of soil organic matter, soil texture, soil *p*H, and other characteristics of soil all affect the size and activity of microbial populations. Tiwari et al. (1987) found significant differences in the distribution of microbial populations, their activities, and biochemical transformations with various moisture regimes. They concluded that changes in soil moisture status affect a variety of parameters, including the population and activity of microbes. Low soil moisture contents are known to be intolerable to many soil microorganisms (Paul and Clark, 1989). According to Tiwari et al. (1989), soil moisture has a significant impact on the microbial population, its activity, and the interactions between various parameters. The dynamics of soil microbial communities are influenced by the temperature and moisture regimes of the soil, which are influenced by the different climates at different locations (Wardle and Parkinson, 1990). According to Liu et al. (2000), soil temperature,

moisture content, and the availability of substrate are the main variables that affect the growth and population density of soil microbes. Gray et al. (2011) found that the variation in soil temperature and moisture affects the soil microbial population. Chandini (2020) found that soil moisture and temperature was one of the most important factors that affected the soil fungal population in their study in Mattavara forest, Karnataka, India and among the fungal genera *Penicillium* and *Aspergillu*are were dominant. Therefore, an optimum soil moisture and temperature is of paramount importance for the multiplication and activity of the soil microbes.

There are other factors that influence soil microbial activity such as soil texture (Hamarashid et al., 2010). Pandey and Palni (1997) found that the number of species and populations of microfungi were highest in the rhizospheric soil and the microbial population gradually decreased as soil depth increased due to decrease in organic matter content. The influence of soil *p*H on the soil microbial communities depends on a number of factors including the study location, type of vegetation, and soil type. As a result, soil *p*H is also a crucial factor that regulates microbial communities for thriving in the soil (Matthies et al., 1997; Pietri et al., 2008). Several workers such as Melaini et al. (2012), Hemkemeyer (2018), and Seaton et al. (2020) has reported that soil with a higher clay and silt content is more preferable for microbial growth because of their greater capacity to maintain more soil organic matter and nitrogen than in sandy soil. Soil *p*H affects the microbial activity of the soil (Ali et al., 2021). Goswami et al. (2017) in their study on tea garden soil in India, discovered a significant relationship between

soil *p*H and microbial diversity, and Rousk et al. (2010) found that the amount and distribution of soil bacteria and fungi changed as soil *p*H varied.

The composition of the soil microflora, its biomass, and its activity are significantly influenced by the soil characteristics, particularly the soil nutrient status (Arunachalam et al. 1999). For any soil ecosystem to remain sustainable, soil microbes are essential. They are well known for their part in the cycling of nutrients and general capacity to serve as a relatively labile source of nutrient elements in the soil (Duxbury et al., 1989; Singh et al., 1989). According to Klose et al. (2004), variations in the quantity and quality of the forest litter produced affected the microbial and biochemical properties in their study. Sharma et al. (2014) found a positive relationship of SOC with soil microbial population in tea garden in India. Additionally, other workers also reported in their studies that, soil organic matter, moisture and available nutrient content have a positive impact in increasing the multiplication and activity of the soil fungal population (Lyngdoh and Karmakar, 2018; Chandini, 2020). According to Saplalrinliana et al. (2016) in their study in jhum land in Northeast India, found that fire activity results in rapid decomposition of soil organic matter in jhum land and does not provide enough time to incorporate it into the soil, as a result reduces the organic carbon content and available nutrients after burning the jhum fields which has a negative impact on the soil microbial population. However a long jhum fallow length or more than 10 years is found to play a significant role in regenerating the depleted soil fertility status and increase in microbial diversity (Temjen et al. 2021). Other researchers have also reported that jhum fallow length plays important role in the

regeneration of soil fertility and increased microbial activity (Arunachalam and Pandey, 2003; Majumder et al., 2010).

Another important factor that influences the microbial diversity is the season. Díaz-Raviña et al. (1995) investigated the microbial population in four pinewoods in Spain, and discovered that seasonal variations, soil type, and the interaction of the season and soil type had a significant impact on the soil microbial population and recorded higher microbial population in spring season and lower in winter season probably as a result of moisture in the summer and cold temperatures in the winter. While other workers has reported the occurrence of higher soil microbial population in summer and spring season which was associated with the favourable temperature and mositure with higher soil nutrients that influenced the multiplication of the soil microbial population (Bhattacharyya and Jha, 2010; Kaushal and Singh, 2013) which also shows the specificity of soil microbes towards particular season, geographical location and vegetation type (Mishra and Sharma, 1977; Karaoglu and Ulker, 2006). Lyngoh and Karmakar (2018) in their study in microbial population under forest, agricultural and horticultural crop land in Meghalaya, India, found that the different climatic conditions in various seasons of the year altered the soil dynamics, which resulted in variation in the microbial population in the different land uses. Seaonal effect on diversity of soil fungal diversity in other parts of India has also been reported by Ashok et al. (2015), Kavitha et al. (2020), and Pandey et al., (2022).

Land-use for different cultivation practices, have been reported to have an impact on the soil microbial community. Anthropogenic activities and the use of

pesticides can negatively affect soil microbial diversity. There is a need for reliable and correct mechanisms of studying soil microorganisms under different land use to address how changes in microbial community structure can influence ecosystem as microorganism plays a crucial role in the cycling of nutrients (Kirk et al., 2004). According to Shannon et al. (2002), when an ecosystem is stressed or disturbed, microbial communities react by changing the amount of energetic compounds needed to support their populations. Soil microbial population can be altered by human intervention. The demand for more agricultural land due to the increasing human population has led to deforestation and pressure on agricultural land for more productivity in a short period which causes adverse effects on the soil microbial population. Studying the affect of different cultivation management practices along with their vegetation cover with seasonal differences under various land use type can help increase our knowledge in understanding the linkage between the soil and soil microbes and their role in plant productivity which will allow better soil nutrient management decisions (Frac et al., 2018) as soil microbes maintain a healthy soil structure which is one of the most important characteristics of soil ecosystem (Swer et al., 2011). Meliani et al. (2012) investigated at how different soil types and soil depth affected the diversity of soil microbes and discovered that rhizospheric soil at a depth of 0–5 cm recorded a higher microbial population than soil at a depth of 5–10 cm with *Triticum* sp. as the dominant fungal species and *Pseudomonas* sp. and *Bacillus* sp. as the dominant bacterial species. Additionally they found that the different cropping and soil management practices primarily affected the bacterial population rather

than the fungal population. Kennedy and Smith (1995), in their study between prairie and cultivated soils discovered that different management techniques resulted in variations in the soil microbial communities. Joshi et al. (2019) found that the amount of soil organic matter deposition in jhum cultivation was impacted by the use of fire, which in turn had an effect on the soil microbial communities. Panda et al. (2017) and Miah et al. (2014) discovered *Bacillus* as the most prevalent bacterial genus under jhum land in India, demonstrating the ability of the Bacillus genera to survive the heat as during the event of burning slashed vegetation in jhum fields, there is an increase in soil temperature that can changes the soil microbial community and furthermore they added the use of fire had a negative impact on the soil microbial community (Kendawang et al., 2004). Jalali et al. (2016) studied the distribution of bacterial community between cultivated soil (orchards and farm) and uncultivated soil (shrubland) in Iran, and reported that the structural diversity of bacterial community was influenced by physicochemical properties and the management practice under the different land use type. Gömöryová et al. (2020) studied the impact of different land use (forest, grassland, and cropland) in Europe and discovered that the soil organic matter was primarily affected by the various land use type, which directly influenced the soil microbial population. Onwona-Kwakye et al. (2020) in their study found that use of chemicals and disturbances caused in the soil resulted in a lower soil bacterial population. The bacterial community and physicochemical characteristics in scrublands were drastically altered by agriculture over time, and these changes may have an adverse effect on the natural soil ecosystem (Ding et

al., 2013). Wani et al. (2018) in their study on soil microbial diversity and activity under five different land use (Forestry, Horticulture, Agriculture, Agri-Horti, and Pasture) found that soil under forest recorded higher microbial number and diversity due to the presence of higher soil organic carbon than the other land use types. In a natural forest, agricultural, and degraded habitat, research was done to investigate the changing microbial population and the health of the soil. The results demonstrated that the natural forest recorded the highest soil microbial diversity followed by agricultural land and degraded land had the lowest soil microbial diversity indicating that the conversion of forest ecosystems to agricultural soils accelerated soil health deterioration (Anggrainy et al., 2020). Several other workers reported that agricultural activities caused disturbance in the soil in shifting cultivated land and other agricultural land that lead to a decrease in the soil organic matter content, which directly affects the soil microbial population (Kendawang et al., 2004; Bossio et al., 2005; Miah et al, 2014; Hosen et al., 2016; Szoboszlay et al., 2017). Pandey et al. (2011) studied the effect of fire in shifting cultivated land in Arunachal Pradesh, India and reported that only heat resistant bacterial species will remain after the fire event in shifting cultivation and the heat-intolerant ones are eliminated. They found bacterial genera under *Bacillus* and *Pseudomonas* to survive the fire event. Majunder et al., 2010 found that the plant residues after harvest in a shifting cultivated land can be a good source of adding nutrient in the soil and furthermore found that fungal and bacterial population varied depending upon the litter nutrient concentration in the soil. Banerjee et al., (2017) isolated beneficial

bacterial genera under *Bacilllus* and *Curtobacterium* and fungal genera under *Penicillium* and *Metarhizium*, native to shifting cultivated land in Northeast, India, to restore the soil fertility status of shifting cultivated land. According to Li et al. (2016), the structure and composition of the soil bacterial population in tea orchards in China were strongly impacted by the length of continuous cropping age. *Penicillium* and *Trichoderma* species predominated the tea soil in the Indian Himalayan region as a result of interactions between the plants, the microbial populations, and the environmental parameters (Pandey et al., 2001). Some common fungal genera under *Aspergillus, Penicillium, Tricoderma, Mucor, Cladosporium, Fusarium* were isolated by Temsurenla and Ajungla (2018) and Jamir et al., (2022a) from tea garden and from shifting cultivated land by Temjen et al. (2021) in Nagaland, India which was attributed to the antagonistic nature of these bacterial and fungal genera and their greater ability to adapt better to seasonal fluctuations and variations in agricultural practices. Whereas some bacterial and fungal species was found to be confined to a particular season and site expressing high sensitivity to seasonal changes and different land use that can result from the interaction of plant and microbes, agricultural systems, and environmental factors which exerts a selective pressure on the soil microbial community (Pandey et al., 1997; Temsurenla, 2021). According to Renla and T. (2017) in their study in tea garden under Nagaland, India found that the number and type of the microbial population was influenced by the amount of the soil organic matter, seasonal fluctuations and geographical locations.

1.4 Soil enzyme

Seasonal factors, such as temperature, have an impact on the amount of microbial and enzymatic activity in the soil (Batra et al., 1997). According to Dick (1994), soil enzymes may serve as an early indicator of the health and quality of the soil and the level of soil enzyme activity is extremely sensitive to changes in soil moisture. The level of soil enzyme activity can be extremely sensitive to changes in soil moisture (Chen, 2003). Furthermore, seasonal fluctuations and sample collection period can affect soil enzyme activity and higher enzyme activity was found to be directly related with microbial activity (Gracia et al., 2002; Baum et al., 2003). Extracellular soil enzymes, which are required for the breakdown of insoluble soil nutrients and contribute significantly to soil nutrient cycling, are influenced by the microbial flora of the soil. As a result, the soil microbial flora serves as a useful indicator of the organic matter turnover in the soil. (Arunachalam, 2003). Studies of soil enzyme activity provide information on the biochemical processes occurring in the soil as soil enzyme catalyze a number of reactions during the decomposition of organic matter in the soil that are crucial for soil life processes and act as an early indicator of management changes in agriculture.

Soil phosphatase enzyme

Measuring phosphatase activity gives an idea of how much phosphate enzyme may be present in the soil. Seasonal variation appears to influence the soil phosphatase activity by a variety of elements, including aeration, soil moisture, temperature, *p*H, vegetation, nutritional status, organic matter concentration, and microbiota (Neal, 1990; Tabatabai, 1994). The subsequent rise in phosphate

activity may be related to an increase in the number of bacteria which regulates the transformation of organic and inorganic phosphorus molecules in the soil. The phosphatase activity may have a big impact on how much native soil organic P molecules are available to plants as phosphorus (Nannipieri, 1994; Dotaniya, 2012). According to Skujins (1976) and Pankhurst et al. (1995), phosphatase activity can be used to estimate the overall level of microbial activity in the soil. Phosphatase is thought to play a crucial role in the phosphorus cycle, respectively, in soil ecosystems (Margalef et al., 2017). Šarapatka, (2003). investigated the measurement of phosphatase activity in soils and discovered that using the ideal *p*H for enzyme activity measurements gives a value of the enzyme's maximum potential activity under natural conditions. The conversion of forests for various land uses and agricultural activities are thought to have a significant impact on soil enzyme activity, which is one of the crucial soil characteristics that determine soil health (Salam et al., 1998) and has led to significant changes in soil fertility-related quality, and biological disturbances (Liu et al., 2018).

1.5 Soil microbial diversity in Nagaland and Northeast India

Since time immemorial, the Northeastern region of India has fascinated and inspired naturalists and scientists. Its diverse and rich flora and fauna attract biologists from all over the world. It offers numerous diverse microclimates and ecological niches to higher plants as well as to microorganisms. The Northeastern region has been identified as a hotspot for biodiversity, so the scientific community must also pay attention to its microbial diversity. Therefore, given the diverse biodiversity of Northeastern India, including Nagaland state, extensive

exploration of the soil fungal and bacterial diversity is essential. Nagaland is a hilly State in the Northeast India. It has a total geographical area of 16,579 sq km. It is bordered by Manipur in the South, Myanmar in the East, Assam in the West, Arunachal Pradesh and a portion of Assam in the North. It is located between 93°20′ to 95°15′ E longitude and 25°60′ to 27°40′ N latitude. The climate in the state ranges from subtropical to temperate and experiences summer rain from the South West Monsoon and winter rain from the North East Monsoon, with an average annual rainfall of 2000–2500 mm. The temperature ranges from 16 to 31°C in summer, while in winter it can drop down upto 4°C. Mokokchung district under Nagaland state, has a hilly topography and several mountain ranges, narrow valleys, and a few plains, due to which agriculture can only be practised on manageable slopes (Jamir et al., 2015). As a result, agriculture is primarily subsistence-based and only partially satisfies human needs. The Mokokchung district is home to many villages, and its residents rely heavily on the environment for survival. The favourable climatic condition in the area results in luxuriant growth of forest (Bendangtemjen and Pongener, 2017). Jhum cultivation has been practiced for a very long time and is deeply embedded in the Ao-Naga culture. However, with an increase human population and dependence on jhum cultivation for crop production in the area has lead to reduction in jhum fallow cycle, deforestation which results in depletion of soil health and disappearance of natural habitats and bio-resources (Kuotsuo et al., 2014; Lemtor et al., 2022; Sarkar, 2022). Another new practice in the area is tea cultivation (Debojit 2017; Benla and Sharma, 2019), and proper cultivation practice should be used because

monoculture is linked to soil sickness such as increased soil acidity, decreased soil fertility, and overuse or improper application of chemicals like herbicides and pesticides that can lead to a loss of soil biodiversity (Temsurenla and Ajungla, 2017). Agriculture that uses a monoculture system has the potential to alter certain soil properties, including species richness, microbial activity, and community structure. The dynamics of the microbial community in soil have been shown to be considerably impacted by changes in land use patterns (Gupta et al., 2022).

As a result, the unexplored areas under jhum cultivation, tea cultivation and forest, in the district provides a unique habitat for isolating and studying the diversity of soil fungi and bacteria. Knowing the type and quantity of these populations can help one understand the state of the soil health and can also shed light on how microbial communities change in agricultural and forest soil. The significance of microbial communities for the long-term survival of terrestrial ecosystems in the area is poorly understood. Therefore, research on microbial diversity is necessary to explore not only the new fungal and bacterial diversity but also to learn about their relationship with seasonal variations and land use. A stable taxonomic diversity of soil microbes in a region's chosen ecological niche (jhum land, tea garden, reserve forest) will help with better land use planning and management, soil health and quality, and the chance to further investigate the potential of native soil microbes. These areas were chosen for study in consideration of their ecological and economic significance, such as jhum cultivation, as the majority of those living in hilly areas rely on this system of

cultivation for their subsistence and tea is a very popular beverage on the global market and a healthy component of our diet. Due to the lack of information in the area, it is necessary to study the microbial diversity because it may be useful to both current and future microbiologists who are interested in the microbial diversity of this area.

1.6 Scope of the study

Microorganisms in the soil are crucial for maintaining soil fertility and quality, and soil is crucial in defining sustainable land management. The soil nutrient cycle, bioavailability, and environmental biotransformation are influenced by soil microorganisms. However, a number of variables, including disturbances brought on by anthropogenic activities, seasonal variations, and geographic location, can affect the quantity and variety of the soil microbial community. Soil microorganisms are a living part of the soil environment that responds quickly to anthropogenic factors. In general, soil microbe activity and diversity are regarded as sensitive indicators of the condition of a particular environment. It is vital to research how cultivation methods, seasonal variations, and geographic locations affect the diversity of the soil microbial community, physiochemical properties, and soil enzymatic activity in a given area.

The current study was conducted in the Mokokchung district of Nagaland under different land uses based on the significance of the soil microflora. It is important to research the soil micro flora because its diversity is related to the health of the soil and above-ground plants. A proper understanding of a region's

microbial diversity will aid in the development and establishment of sustainable land use management strategies.

1.7 Aims and objectives of the present study

The main objective of the present study titled "Studies on Soil Microbial Diversity in Mokokchung District, Nagaland" is to explore the seasonal fluctuations in soil physicochemical properties, soil microbial flora, number and types of the native soil microbial flora and the soil enzyme activity in different land use *viz*. cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest under the Mokokchung district of Nagaland. The present study was proposed with following objectives-

1) To determine the soil properties such as *p*H, moisture, temperature, texture and organic carbon content.
2) To estimate the concentrations of available nutrients such as nitrogen, phosphorus and potassium.
3) To enumerate the soil microbial population.
4) To determine the soil phosphatase enzyme activity.
5) To evaluate the seasonal variations in soil physicochemical properties, the soil microbial flora and soil enzyme activity at different land use.
6) To observe the relationship between soil physicochemical properties, the soil microbial flora and soil enzyme activity.

The outline of the thesis is presented in Table 1.1.

Chapter 1	**Introduction**
Chapter 2	**Study on Soil Physicochemical properties**
Chapter 3	**Study on Soil Microbial Diversity**
Chapter 4	**Study on Soil Enzyme Activity**
Chapter 5	**Summary and Conclusion**
References	

Figure 1.1. Outline of the thesis

CHAPTER 2

Study on soil physicochemical properties

2.1 Introduction

Use of land for agricultural purpose with different cultivation practices effects soil physico-chemical properties (Tumayro and Tesgaye, 2021). The variations in the physico-chemical properties are found to be influenced by seasonal fluctuations, geographical locations, environmental factors and the different management activities involved in the study area (Negasa, 2020). The need to produce more food in a short period of time with the increase in population has put pressure on the land. This causes dilution of the forest cover for firewood and cultivation for crop production that deteriorates the soil heath and degradation of the environment (Girma, 2020). In Northeast, tea plant is an important cash crop and jhum cultivation is a predominant practice in most part of the state (Renla and Ajungla, 2017; Kumar et al., 2017). Most districts in Nagaland are giving due importance to tea cultivation including Mokokchung district (Temsurenla and Ajungla, 2017) and jhum cultivation is a major cultivation practice in the districts of Nagaland (Bhan, 2009). Cultivation practice involved in tea cultivation and jhum cultivation are known to negatively affect the soil physicochemical properties. Monoculture of tea crop in soil causes acidification, reduction in available nutrients due to the high requirement of nutrients for the development of tea leaves and use of chemical fertilizers (Arafat et al., 2020; Chien et al., 2019). In Nagaland, jhum cultivation has been reported as the major cause of soil fertility, biological degradation due to a decrease in jhum fallow period of >10 years, use of fire for burning slashed vegetation that causes environmental problems, poor fallow management, continuous slashing and cultivation over the same land due to short-cycle of jhum has negatively affected the soil properties and soil health (Karthik

et al., 2009; Saha et al., 2012). Forest soil acts as a vital role in conserving the terrestrial ecosystem and maintaining the health of the environment, as forest soil have better soil physicochemical characteristics which should be further expanded and protected from agricultural disturbances (Mizra et al., 2020). Therefore, it is necessary to estimate and characterise the status of the physical and chemical properties of both agricultural and forest soil of a region for appropriate management of land use and sustainable use of the soils which can be achieved only by having proper understanding and knowledge of physical-chemical properties of soils. The present study was conducted to comprehend the variations of soil physico-chemical properties over different seasons at selected agricultural land and forest namely cultivated jhum land under Khensa village, abandoned jhum land under

Mekuli village, tea garden under Longmisa village and Minkong reserve forest under Chuhchuyimpang village, under Mokokchung district, Nagaland.

Description of study location

Nagaland is a hilly state situated on the north eastern part of India with a total geographical area of 16579 km^2 and has a hilly topography. It lies between 25º 6′N to 27º 4′N latitude and 93º 20′E to 95º 15′E longitude. Nagaland state is bounded by Assam in the North and West, Myanmar and Arunachal Pradesh in the North and Manipur in the south. Mokokchung is a district located in the north-west of Nagaland between 25° 56′ N and 27° 40′N latitude and between 93° 53′E and 94° 53′E longitude. It has a geographical area of 1615 km^2 and is mostly hilly in topography and few plain lands. Mokokchung district has a mild to warm climate throughout the

year. Four different land use soil systems from Mokokchung district, Nagaland was selected for the present study. The four different study sites selected was under cultivated jhum land (CJL) located at Khensa village, abandoned jhum land (AJL) at Mekuli village, tea garden (TG) at Longmisa village and Mingkong reserve forest (MRF) at Chuchuyimpang village, Mokokchung district, Nagaland. The geographical location, study map, and photographs of the four study sites are provided in Table 2.1, Figure 2.1 and Figure 2.2. The climate of Mokokchung district is characterized by winter (December-February), spring (March-May), summer (June-August), Autumn (September-November). Geographical coordinates of the study sites were determined by a global positioning system (Garmin, 12H) and the map of the study area was generated using ArcGIS Desktop 10.8

In cultivated jhum land, rice (*Orzya* spp) is the main crop cultivated along with other crops such as chilly (*Capsicum frutescens*), cucumber (*Cucumis sativus*), *Solanum nigrum*, tomato (*Lycopersicum esculentum*), pumkin (*Cucurbita* sp), yam (*Colocasia esculenta*), ginger (*Zingeber officinale*), maize (*Zea mays*), Tulsi (*Occimum sanctum*), sponge gourd (*Luffa* sp), Yardlong bean (*Vignia unguiculata*), chives (*Allium tuberosum*), green onion (*Allium cepa*), velvet bean (*Mucuna* sp). Weeds like *Ageratum conyzoides*, *Artemisia nilagirica*, *Artemisia vulgaris*, *Eupatorium adenophorum*, *Drymaria cordala*, *Biden pilosa*, *Chlromolaena odorat*, *Gynura crepidioides* were some dominant species. In abandoned jhum land, some of the tress such as *Ablizza chinensis*, *Schima wallichi*, *Albizia chinensis*, *Macaranga denticulate* and weeds like *Mikania cordata* were dominant. In Minkong reserve forest, trees such as *Schima wallichi*, *Terminalia myriocarpa*, *Albizia chinensis*, *Alnus*

nepalensis, *Anthocephalus cadamna*, *Macaranga* sp, *Trema orientalis* sp, *Saurauia panuana*, *Prunus phaeosticta*, *Archidendron clypearia* and weeds like *Mikania cordata* were dominant. In tea garden, shade trees present in the study were mostly *Parkia roxburghii* along with some other plants such as *Alnus nepalensis*, *Albizia chinensis*, *Emblica officinalis*, *Psidium guajava*, *Prunus persica*, *Pyrus* sp, and *Mangifera indica*.

In the present study, cultivated jhum land at Khensa village was cultivated after 7 years of fallow period. The forest is slashed in December and burnt in the month of February. The soil was prepared for cultivation in February itself and sowing of crops was done in March followed by regular weeding until the major crop (rice) was harvested in September. After one year of cultivation, the land was abandoned to undergo the jhum fallow rotation period for the next 7 years. *Ageratum conyzoides* and *Eupatorium* sp dominated the first year of the jhum fallow period. The abandoned jhum land in Mekuli village was a 10 year old jhum fallow which was left to naturally build up the soil fertility and regenerate the vegetation from the previous cultivation. Minkong reserve forest is a Protected Forest under the Department of Forest, Nagaland and has an area of 275.32 hectares. The tea garden was 10 year old and tea leaves were plucked at regular intervals during the present study starting from March, May, July, September and light pruning of tea plant was done in December which was kept to decomposed on the surface of the soil. No chemical fertilizer were used in the soil, herbicide (Oxyfluorfen) was used thrice in a year.

Table 2.1: Study sites with geographical coordinates

Study site	Geographical coordinates	Altitude (masl)
Cultivated jhum land (CJL)	N 26 ˚20’54.4 ″ E 94 ˚29’58.5″	1027
Abandoned jhum land (AJL)	N 26˚21′05.3″ E 094˚28′16.6″	1043
Tea garden (TG)	N 26˚21′0″ E94˚33′36″	1182
Mingkong rerserve forest (MRF)	N 26˚21′36″ E94˚33′0″	1313

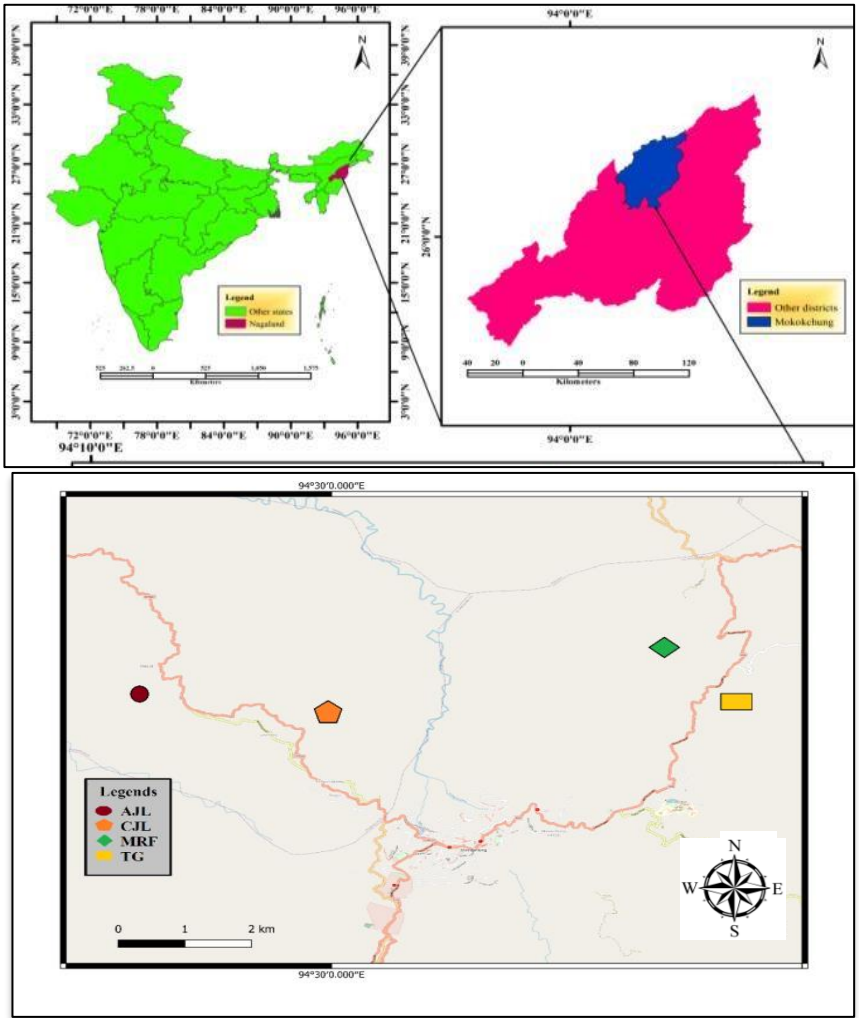

Figure 2.1. Study map depicting an outline map of India, Nagaland state and sampling sites under Mokokchung district

2.2 Materials and methods

The details of materials and methods used for the sample collection and laboratory experiments carried out during the course of this research work are discussed in this section.

2.2.1 Soil sampling

Soil samples were collected seasonally (winter, spring, summer and autumn) from all the four study sites for duration of two years i.e., December 2016- November 2018. The samples were collected at a soil depth of 0-15cm from five different locations at each of the study sites and mixed nicely to make a composite mixture. Plant materials and visible debris and were removed from the composite soil mixture**.** Collections were done under sterile conditions and the soil samples were transferred to the laboratory in sterile sealing bags. Within 24 hours of collection, estimation of different parameters such as soil moisture content, soil pH, culture and isolation of soil fungi and bacteria were carried out. The soil samples were stored at a temperature of 4° C and -20°C for enzymatic studies and molecular studies respectively, some portion of soil samples were air-dried and sieved through a 2mm mesh sieve.

Figure 2.2. Images of the study sites, A: Cultivated jhum land, Khensa village; B: Abandoned jhum land, Mekuli village; C: Tea garden, Longmisa village; D: Mingkong reserve forest, Chuchuyimpang village

2.2.2 Soil physicochemical analysis

Physicochemical analysis of all the collected soil samples was carried out in triplicates by following standard procedures and published methods. Analysis was carried out for soil texture (sand %, silt % and clay %), moisture content, soil temperature (ºC), soil *p*H, SOC, available N, available P and available K.

2.2.2.1 Soil texture

The texture of the soil was determined by hydrometer method and soil textural classification was done as proposed by USDA (Weil and Brady, 2016). 15 gm of soil sample (<2 mm) was weighed and taken in a beaker to which 92.7 ml of 0.5% Hexametaphosphate was added, shaken on a reciprocating shaker for 16 hour. The mixture was sieved through standard 0.5 mm mesh and 0.053 mm mesh sieve for separating sand particles and other particles solution. During the sand particle separation, the solution and particles sit (silt + clay) passing the sieve were collected. The silt and clay solution was stirred thoroughly to suspend all particles and left undisturbed for around 90 minutes at room temperature after which the suspended particle (clay) was decanted and the settled particles (silt) was oven dried at 105°C. The sand %, silt % and clay % were calculated by the formula given below:

$$\text{Sand \%} = \frac{Oven\ dry\ sand\ mass}{Original\ sample\ mass} \text{ X } 100\%$$

$$\text{Silt \%} = \frac{Oven\ silt\ mass}{Original\ sample\ mass} X100\%$$

Clay % = 100- (sand % - silt %)

2.2.2.2 Soil moisture content

10g of fresh soil sample was weighed into glass petriplates using an analytical balance (Wensar, PGB 200) and oven-dried at 105 ºC for 24 hours. The oven-dried sample was reweighed. Moisture content was calculated by the formula given below:

$$\text{Moisture content (\%)} = \frac{Fresh\ weight - Dry\ weight}{Dry\ weight} \text{ X } 100$$

2.2.2.3 Soil temperature

Soil temperature was recorded at the time of soil sampling using a soil thermometer (MAXTECH, DT-9) in all the four study sites. The *p*H of any solution is the negative logarithm of the hydrogen ion activity that measures the alkalinity or acidity of any solution.

2.2.2.4 Soil *p*H

For determining soil *p*H, 5g of soil sample were used to prepare a saturated solution (1:2.5:: soil:water) and soil *p*H reading was recorded from an electronic digital *p*H meter (Systronics microcontroller-based *p*H meter system, 361).

2.2.2.5 Soil organic carbon

Soil organic carbon was determined by following Walkley and Black, 1934. 1g of each soil sample in triplicates were weighed and taken in a 250ml glass jar. 10ml 0f 0.5M $K_2Cr_2O_7$ and 0.25g of Ag_2SO_4 was added into the glass jar after that 20ml of conc. H_2SO_4 was added into the mixture for oxidizing total organic matter and swirled gently. The mixture was allowed to stand for 30 minutes. 200ml of

distilled water and 10ml of ortho phosphoric acid (H_3PO_4) was added for diluting the reaction mixture followed by adding 1ml of diphenylamine indicator by after which the mixture turned to blue-violet in colour. Unreacted $K_2Cr_2O_7$ was titrated back by taking 10ml of the standard ferrous ammonium sulphate (FAS) and the end point was recorded when the colour changed from blue-violet to brilliant green. The blank was run without soil samples in triplicates. The percentage organic carbon of all the soil samples were calculated by the following formula:

$$\%\text{Organic carbon} = \frac{10\,(S-T)X\ 0.003}{S}\ \text{X}\ \frac{100}{\text{weight of the soil sample}}$$

Where,

S= ml of FAS used in the blank

T= ml of FAS used in the soil sample

2.2.2.6 Available nutrients

Available nitrogen

Available N was determined through N distillation apparatus (Kelpus, DISTYL EM VA) as given by Motsara and Roy (2008). Triplicates of 5g of air-dried soil sample was weighed and added to the digestion tube and loaded in distillation unit through the slider mechanism in the apparatus. In the digestion tube, 25 ml of 0.32 % KMNO4 and 25 ml of 2.5 % NaOH were added. Distillation was done for 9 minutes. Prior to distillation, 25 ml of 2.5 % boric acid and an indicator was mixed in a 250 ml conical flask and put at the end of the receiver of the distillation unit for

collecting the liberated ammonia. The green coloured collected distillate was titrated against 0.02N H2SO4 and the end point was taken when the colour changed from green to the original pinkish colour. Blank was run without soil sample. The amount of available N content in the soil sample was calculated as:

Available N (kg/ha) =

$$\frac{14X\ (Normality\ of\ acid)X\ (Titrant\ value\ of\ the\ sample)X\ 2{,}24\ X\ 1000000}{Weight\ of\ the\ soil\ sample\ X\ 1000}$$

Available phosphorus

Available P was determined by Bray and Kurtz method as given by Motsara and Roy (2008). This method is specially used for acidic soil. 5g of air-dried soil sample in triplicates was taken into a 250 ml conical flask and added 50 ml of Bray's P-1. The mixture was shaken for 5 minutes on a reciprocating shaker and Whatman filter paper No.42 was used to filter to get a clear filtrate. From the clear filtrate, 5 ml was taken into a 50 ml conical flask to which 5 ml of molybdate reagent followed by 10 ml of distilled water, 1ml of working SnCl2.2H2O solution and then diluted up to 25 ml with distilled water. Blank was also prepared without the soil.

For standard P, stock P solution was made by dissolving 0.2195g of oven-dried KH2PO4 in 1 litre of distilled water and from this 10 ml of the stock solution was taken and diluted up to 500 ml. This was used as intermediate standard phosphorus from which 0, 2.5, 5.0, 7.5, 10 and 12.5 µg/ml P were taken in a series of 25 ml conical flasks. 5 ml of Bray's solution No.1 was added and standards were prepared in the same way as used in the soil sample.

After 10 minutes, the intensity of blue colour formed in standards and soil sample extracts were determined using spectrophotometer against the blank at 660 nm (ELICO, SL 210 UV VIS spectrophotometer). The amount of available P content in the soil sample was calculated using the following formula:

$$\text{Available P} = \frac{A}{1{,}000{,}000} \text{ X } \frac{Volume\ of\ the\ extract}{Volume\ of\ extract\ taken\ for\ estimation} \text{ X } \frac{2{,}000{,}000}{Weight\ of\ the\ soil}$$

Where, A = content of P observed in the sample, as read from the standard curve

Available potassium

Available K was determined by following Toth and Prince method as given by Motsara and Roy (2008) through flame photometry after extraction of the soil with 1N neutral ammonium acetate.

5 g of air-dried soil sample in triplicates was weighed and taken in a 50 ml conical flask. 25 ml of ammonium acetate extractant was added to the conical flask with the soil and shaken for 5 minutes on a reciprocating shaker and the mixture was filtered by using Whatman filter paper No.42 to collect a clear filtrate. Blank was used as 1N ammonium acetate without soil samples.

For standard K solution, stock K solution of 1000 µg/ml was made by dissolving 1.908 g of oven-dried potassium chloride (KCl) in 1000 ml of 1N ammonium acetate solution. Working K standard solutions were made by transferring 0, 2, 4, 6, 8 and 10 ml of the stock solution into a series of 100 ml glass jar and diluted up to the marks with 1N ammonium acetate solution

Available K content in the filtrate was determined photometrically by using a digital flame photometer (Systronics, 130) set under K filter. The flame photometer was adjusted to 0 with distilled water and to 100 with 10 ppm K solution followed by reading the standards and samples. The amount of available K content in the soil sample was calculated by the given formula:

$$\text{Available K(kg/ha)} = \frac{A}{1{,}000{,}000} \text{ X Volume of the extract X } \frac{2{,}000{,}000}{Weight\ of\ the\ soil}$$

2.3 Statistical analysis

The statistical analysis was done to determine any significant difference among the soil parameters that was analysed during the experimental process. Statistical analysis for all the collected data were done by SPSS version 16 software package. Each test was carried out in triplicate and mean values with ± standard error of the triplicates for all the study sites were processed. One-way Analysis of Variance (ANOVA) was used to ascertain statistically significant differences in each of the soil physicochemical parameters between the sampling seasons ($p \leq 0.05$). If the ANOVA results were significant, Tukey's HSD *post hoc* tests were performed. Two way Analysis of Variance (ANOVA) was used to determine the effects of site and season on the soil physicochemical parameters in the four study sites. Pearson correlation analysis was done for test the relationship among the soil physicochemical parameters. The level of significance referred to in the results is $P < 0.05$.

2.4 Results

Seasonal variations in soil physicochemical properties in all the four study sites were observed throughout the present study period during (2016-2018). The average values of the physicochemical parameters of different seasons from all the study sites (CJL, AJL, TG and MRF) are shown here in tables and figures.

2.4.1 Soil texture

The soil texture in cultivated jhum land, Khensa village belonged to the sandy clay loam to sandy laom of the textural classification triangle (Figure 2.3a). In the first year, the average content of sand, silt and clay in the soil ranged from 49.49-59.27%, 24.34-25.34% and 16.37-25.17% respectively while in second year, the sand, clay and silt content in the soil ranged from 55.23-59.88%, 23.34-28.34% and 13.17-21.43% respectively (Table 2.2). In the first year, the highest and lowest content of sand was recorded during summer and winter season, silt during winter and summer season, and clay during winter and summer respectively whereas in the second year, highest and lowest content of sand was recorded during summer and spring, silt during autumn and spring, clay during spring and summer respectively.

Table 2.2. Soil texture of cultivated jhum land during 2016-2018

Soil properties	2016-17				2017-18			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Sand	49.49 ±0.57^{c}	57.10 ±0.95^{ab}	59.27 ±0.37^{a}	55.14 ±1.19^{b}	58.13 ±0.09^{b}	55.23 ±0.07^{d}	59.88 ±0.14^{a}	56.49 ±0.28^{c}
Silt	25.34 ±0.08^{a}	25.30 ±0.11^{a}	24.36 ±0.18^{b}	24.38 ±0.55^{b}	27.31 ±0.05^{b}	23.34 ±0.11^{c}	26.95 ±0.42^{b}	28.34 ±0.19^{a}
clay	25.17 ±0.82^{a}	17.60 ±1.03^{c}	16.37 ±0.56^{c}	20.48 ±0.17^{b}	14.56 ±0.92^{b}	21.43 ±0.10^{a}	13.17 ±0.17^{c}	15.17 ±0.07^{b}

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters (^{abc}and^{d}) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

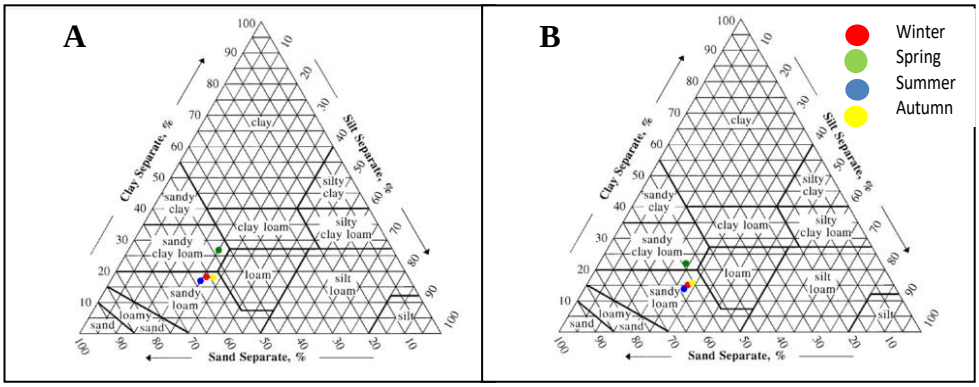

Figure 2.3a. Soil textural classification triangle of cultivated jhum land, A: 2016-17 and B: 2017-18

The soil texture in abandoned jhum land, Mekuli village belonged to the sandy clay loam of the textural classification triangle (Figure 2.3b). In the first year, the average content of sand, silt and clay in the soil ranged from 55.40-50.97%, 21.54-24% and 23.06-25.03% respectively while in the second year the sand, clay and silt content in the soil ranged 51-58.88%, 19.61-22%, 21-27% respectively (Table 2.3). In both year, highest content of these soil properties was recorded during sand (summer), silt and clay (spring) whereas, the lowest content of these soil properties in

first and second year was observed during sand (winter, spring), silt (summer, spring) and clay (summer)

Table 2.3. Soil texture of abandoned jhum land during 2016-2018

Soil properties	2016-17				2017-18			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Sand	53.30 ±0.40 [c]	50.97 ±0.06 [d]	55.40 ±0.03 [a]	54.00 ±0.49 [b]	53.13 ±0.05 [c]	51.00 ±0.48 [d]	58.88 ±0.07 [a]	55.39 ±0.35 [b]
Silt	22.63 ±0.22 [b]	24.00 ±0.08 [a]	21.54 ±0.05 [c]	22.40 ±0.18 [b]	21.47 ±0.06 [b]	22.00 ±0.12 [a]	20.12 ±0.08 [c]	19.61 ±0.08 [d]
clay	24.07 ±0.03 [b]	25.03 ±0.27 [a]	23.06 ±0.11 [d]	23.60 ±0.31 [c]	25.40 ±0.03 [b]	27.00 ±0.24 [a]	21.00 ±0.09 [d]	25.00 ±0.27 [c]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc test*

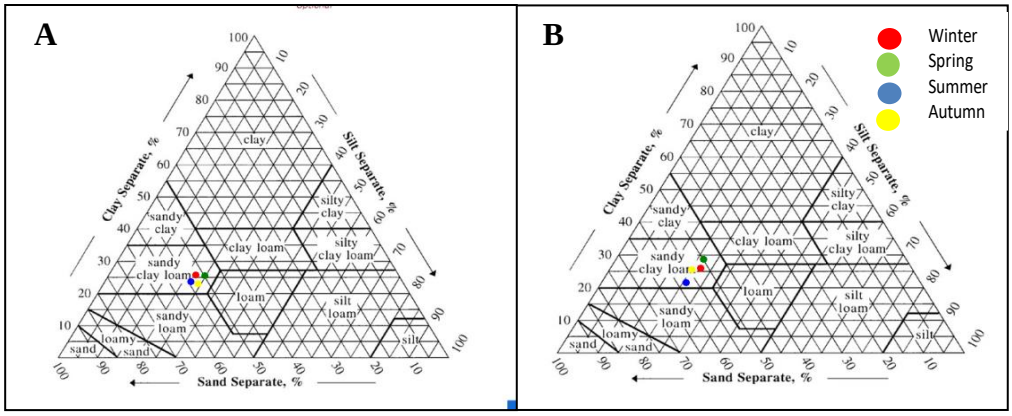

Figure 2.3b. Soil textural classification triangle of abandoned jhum land, A: 2016-17 and B: 2017-18

The soil texture in tea garden, Longmisa village belonged to the sandy clay loam of the textural classification triangle (Figure 2.3c). In the first year, the average content of sand, silt and clay in the soil ranged from 51.13-56.73%, 20.67-24.60% and 21.33-24.27% respectively while, in the second year the sand, clay and silt content in the soil ranged from 51.53-59.87%, 20.13-21.67% and 20-27% respectively (Table 2.4). In both the years, highest content of these soil properties was

recorded during sand (summer), silt and clay (spring) while the lowest content of these soil properties in the first was observed in sand (spring), silt (summer) and clay (autumn) whereas in the second year the lowest content of these soil properties was observed in sand (spring), silt and clay (summer).

Table 2.4. Soil texture of tea garden during 2016-2018

Soil properties	2016-17				2017-18			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Sand	54.00 ±0.36 ^{c}	51.13 ±0.95 ^{d}	56.73 ±0.11 ^{a}	55.14 ±0.04 ^{b}	53.93 ±0.09 ^{c}	51.53 ±0.60 ^{d}	59.87 ±0.63 ^{a}	55.60 ±0.18 ^{b}
Silt	22.00 ±0.18 ^{c}	24.60 ±0.13 ^{a}	21.27 ±0.19 ^{c}	23.53 ±0.69 ^{b}	20.67 ±0.18 ^{b}	21.47 ±0.12 ^{a}	20.13 ±0.07 ^{c}	20.40 ±0.08 ^{bc}
clay	24.03 ±0.01 ^{a}	24.27 ±0.17 ^{a}	22.00 ±0.07 ^{b}	21.33 ±0.12 ^{c}	25.40 ±0.09 ^{b}	27.00 ±0.36 ^{a}	20.00 ±0.11 ^{d}	24.00 ±0.11 ^{c}

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters (^{abc}and^{d}) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

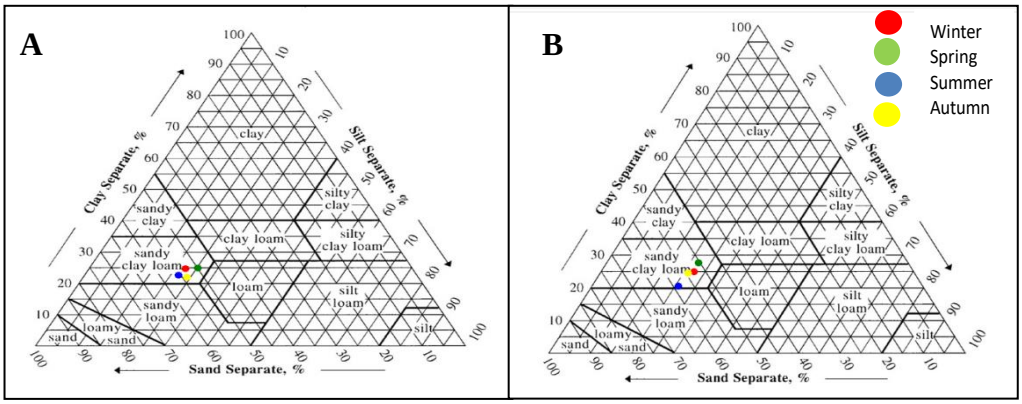

Figure 2.3c. Soil textural classification triangle of tea garden, A: 2016-17 and B: 2017-18

The soil texture in Mingkong reserve forest, Chuchuyimpang village belonged to the laom of the textural classification triangle (Figure 2.3d). In the first year, the average content of sand, silt and clay in the soil ranged from 42.78-44.24%, 29.78-31.22% and 25.98-26.13% respectively the second year the sand, clay and silt content in the soil ranged from 43.12-46%, 28.21-3.94% and 25.79-26.07% respectively. In

both the year, highest content of these soil properties was recorded in sand (summer), silt (spring) and clay (autumn) whereas the lowest content of these soil properties was recorded in sand (spring), silt and clay (summer)

Table 2.5. Soil texture of mingkong reserve forest during 2016-18

Soil properties	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
Sand	43.00 ±0.05 [b]	42.78 ±0.09 [b]	44.24 ±0.10 [a]	44.00 ±0.05 [a]	43.23 ±0.25 [c]	43.12 ±0.24 [c]	46.00 ±0.23 [a]	45.46 ±0.05 [b]
Silt	31.00 ±0.03 [a]	31.22 ±0.20 [a]	29.88 ±0.04 [b]	29.87 ±0.03 [b]	30.94 ±0.10 [a]	30.86 ±0.26 [a]	28.21 ±0.03 [c]	28.47 ±0.04 [b]
clay	26.00 ±0.02 [b]	26.00 ±0.08 [b]	25.88 ±0.03 [c]	26.13 ±0.04 [a]	26.03 ±0.54 [a]	26.02 ±0.50 [a]	25.79 ±0.57 [b]	26.07 ±0.04 [a]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

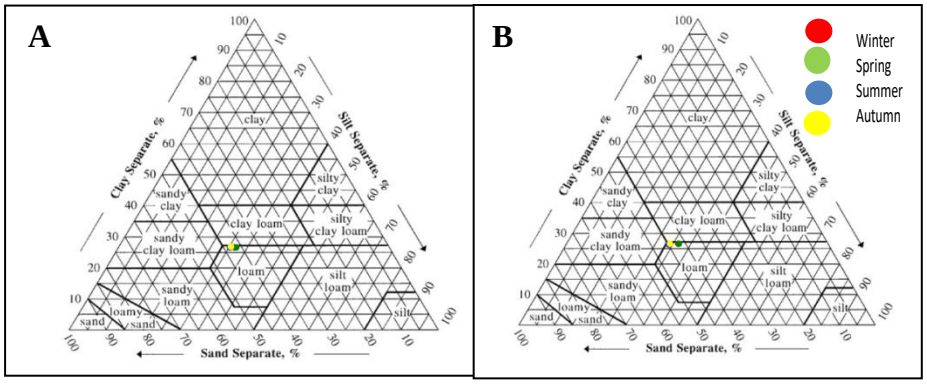

Figure 2.3d. Soil textural classification triangle of Mingkong reserve forest, A: 2016-17 and B: 2017-18

2.4.2. Soil *p*H

The mean values in *p*H of the collected soil samples from all the four study sites are shown in Table 2.6. The mean *p*H values in CJL at Khensa village in the first year ranged from 4.36-5.38 and in the second year, it ranged from 4.40-4.65. In both years, the highest soil *p*H was recorded in summer season and the lowest soil *p*H was

recorded in winter season. The soil *p*H value at cultivated jhum land was strongly acidic.

The mean *p*H values of the collected soil samples in AJL at Mekuli village in the first year ranged from 5.08-5.91 and in the second year, it ranged from 5.42-6.14. In both years, the highest soil *p*H was recorded in summer season respectively and the lowest soil *p*H was recorded in winter season in both the year. The soil *p*H value at AJL was moderately acidic.

The mean *p*H values of the collected soil samples in tea garden at Longmisa village in the first year ranged from 4.51-5.74 and in the second year, it ranged from 4.41-5.62. In both years, the highest soil *p*H was recorded in autumn season and the lowest soil *p*H in both the years were recorded in winter season. The soil *p*H value at TG was strongly acidic.

The mean values in *p*H of the collected soil samples in MRF at Chuchuyimpang village in the first year ranged from 5.61-6.42 and in the second year, it ranged from 5.73-6.35. In both the years, the highest and lowest soil *p*H was recorded during summer and winter season respectively. The soil *p*H value at MRF was moderately acidic.

Table 2.6. Soil *p*H of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	5.31 ±0.26 [a]	5.38 ±0.02 [a]	5.15 ±0.04 [a]	4.36 ±0.01 [b]	4.40 ±0.01 [d]	4.49 ±0.01 [c]	4.65 ±0.03 [a]	4.57 ±0.04 [b]
AJL	5.08 ±0.08 [d]	5.32 ±0.04 [c]	5.91 ±0.10 [a]	5.64 ±0.08 [b]	5.42 ±0.08 [b]	5.68 ±0.17 [b]	5.95 ±0.07 [a]	6.14 ±0.04 [a]
TG	4.51 ±0.44 [c]	5.02 ±0.01 [bc]	5.42 ±0.08 [ab]	5.74 ±0.15 [a]	4.41 ±0.53 [b]	5.05 ±0.03 [ab]	5.57 ±0.07 [a]	5.62 ±0.07 [a]
MRF	5.61 ±0.11 [c]	5.89 ±0.10 [bc]	6.42 ±0.11 [a]	6.13 ±0.13 [b]	5.73 ±0.14 [b]	5.83 ±0.07 [b]	6.35 ±0.15 [a]	6.15 ±0.03 [a]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

2.4.3 Soil moisture

The mean values of soil moisture of the collected soil samples from all the four study sites are shown in Table 2.7. The soil moisture content in CJL at Khensa village ranged from 36.69-47.16% in the first year while, in the second year, it ranged from 29.63-43.24%. In AJL at Mekuli village, it ranged from 40.22-66.59% in the first year while the second year, it ranged from 41.09-59.61%. In TG at Longmisa village it ranged from 34.20-50.74% in the first year while in the second year, it ranged from 32.66-47.85%. In MRF at Chuchuyimpang village it ranged from 52.81-76.61% in the first year while in the second year, it ranged from 49.62-82.91%. In both first and second year, the highest and lowest moisture content was recorded during summer and winter season respectively in all the four study sites.

Table 2.7. Soil moisture content of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	36.69 ±0.03 [d]	37.03 ±0.02[c]	47.16 ±0.03 [a]	38.82 ±0.21 [b]	29.63 ±0.20 [d]	36.59 ±0.57c	43.24 ±0.95 [a]	41.62 ±0.35 [b]
AJL	40.22 ±0.02 [d]	46.55 ±0.04 [c]	66.59 ±0.03 [a]	54.58 ±0.07 [b]	41.09 ±0.03 [d]	55.88 ±0.20 [b]	59.61 ±0.30 [a]	53.09 ±0.03[c]
TG	34.20 ±0.14 [c]	46.33 ±1.10 [b]	50.74 ±0.22 [a]	47.50 ±0.14 [b]	32.66 ±0.16 [d]	40.84 ±0.08 [c]	47.85 ±0.07 [a]	45.82 ±0.09 [b]
MRF	52.81 ±0.13 [d]	60.08 ±0.34 [c]	76.61 ±0.17 [a]	64.17 ±0.27 [b]	49.62 ±0.19 [d]	58.53 ±0.18 [c]	82.91 ±0.07 [a]	80.91 ±1.00 [b]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

2.4.4 Soil temperature

The mean values of soil temperature of the collected soil samples from all the four study sites are shown in Table 2.8. Mean values of soil temperature recorded in CJL at Khensa village ranged from 21.03-27.03 ºC in the first year while in the second year it varied from 21.67-28.04 ºC. Mean values of soil temperature in AJL at Mekuli village ranged from 18.83-24.13 ºC in the first year while in the second year it varied from 15.04-23.40 ºC. Mean values of soil temperature in tea garden at Longmisa village ranged from 21.43-29.32 ºC in the first year while in the second year it varied from 20.52-29.05 ºC. Mean values of soil temperature in MRF at Chuchuyimpang village ranged from 15.36-22.09 ºC in the first year while in the second year it varied from 12.60-21.03 ºC. In all the four study sites, among the seasons, the highest and the lowest soil temperature were recorded in summer and winter season in both the years.

Table 2.8. Soil temperature (ºC) of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	21.03 ±0.14 [c]	24.20 ±0.90 [b]	27.03 ±0.04 [a]	26.15 ±0.57 [a]	21.67 ±0.22 [c]	24.04 ±0.03 [b]	28.04 ±0.04 [a]	27.30 ±0.96 [ab]
AJL	18.83 ±0.25 [c]	21.13 ±0.91 [b]	24.13 ±0.05 [a]	23.08 ±0.02 [a]	15.04 ±0.04 [d]	20.04 ±0.04 [c]	23.40 ±0.23 [a]	21.80 ±0.46 [b]
TG	21.43 ±0.51 [c]	26.57 ±0.61 [b]	29.32 ±0.44 [a]	28.67 ±0.89 [a]	20.52 ±0.03 [d]	26.33 ±0.03 [c]	29.05 ±0.04 [a]	27.40 ±0.26 [b]
MRF	15.36 ±0.15 [c]	20.08 ±0.34 [b]	22.09 ±0.05 [a]	20.06 ±0.32 [b]	12.60 ±0.06 [c]	18.06 ±0.23 [b]	21.03 ±0.22 [a]	20.73 ±0.18 [a]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

2.4.5 Soil organic carbon

The mean values of soil temperature of the collected soil samples from all the four study sites are shown in Table 2.9. In CJL at Khensa village, the average value

of SOC content, in the first year ranged from 1.12-2.22%. While in the second year, it ranged from 0.98-1.19%. In first year, the highest and the lowest SOC content was recorded during the spring and autumn season respectively whereas in the second year, the highest and lowest value of SOC was recorded in spring and winter season respectively.

In AJL at Mekuli village, the average value of SOC content, in the first year ranged from 2.02-2.53%. While in the second year, it ranged from 2.03-2.68%. In both first year and second year, the highest SOC content was recorded during spring season while the lowest SOC content was recorded in winter season.

In tea garden at Longmisa village, the average value of SOC content, in the first year ranged from 1.38-1.72%. While in the second year, it ranged from 1.29-1.68%. In both years, the highest SOC content was recorded in spring season while the lowest SOC content was recorded in winter season.

In MRF at Chuchuyimpang village, the average value of SOC content, in the first year ranged from 2.31-2.69%. While in the second year, it ranged from 2.21-2.75%. In both years, the highest SOC content was recorded in spring season while the lowest SOC content was recorded in winter season.

Table 2.9. Soil organic carbon (%) of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	1.91 ±0.01 [b]	2.22 ±0.10 [a]	1.73 ±0.02 [c]	1.12 ±0.09 [d]	0.98 ±0.02 [b]	1.19 ±0.01 [a]	1.04 ±0.03 [b]	1.12 ±0.03 [a]
AJL	2.02 ±0.04 [b]	2.53 ±0.18 [a]	2.25 ±0.18 [ab]	2.31 ±0.13 [ab]	2.03 ±0.05 [c]	2.68 ±0.10 [a]	2.35 ±0.06 [b]	2.47 ±0.03 [b]
TG	1.38 ±0.01 [d]	1.72 ±0.01 [a]	1.55 ±0.03 [b]	1.49 ±0.02 [c]	1.29 ±0.03 [d]	1.68 ±0.01 [a]	1.53 ±0.02 [b]	1.44 ±0.20 [c]
MRF	2.31 ±0.07 [b]	2.69 ±0.32 [a]	2.51 ±0.04 [ab]	2.62 ±0.04 [ab]	2.21 ±0.11 [b]	2.75 ±0.11 [a]	2.48 ±0.12 [ab]	2.50 ±0.12 [ab]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters (^{abc}and^{d}) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

2.4.6 Soil available nutrients

Available nitrogen

The mean values of soil available N of the collected soil samples from all the four study sites are shown in Table 2.10. In CJL at Khensa village, the average value of available N content, in the first year ranged from 122.08-319.82 kg/ha while in the second year, it ranged from 232.90-271.50 kg/ha. In first year, the highest and the lowest available N content was recorded during the winter and autumn season respectively whereas in the second year, the highest and lowest value of available N content was recorded in spring and summer season respectively.

In AJL at Mekuli village, the average value of available N content, in the first year ranged from 372.44-492.39 kg/ha while in the second year, it ranged from 356.26-514.57 kg/ha. In both first year and second year, the highest available N content was recorded in the spring while the lowest available N content was recorded in winter season.

In tea garden at Longmisa village, the average value of available N content, in the first year ranged from 252.41-401.21 kg/ha while in the second year, it ranged from 256.41-459.30 kg/ha. In both years, the highest and the lowest available N content was recorded in the spring and winter season respectively.

In MRF at Chuchuyimpang village, the average value of available N content, in the first year ranged from 493.48-581.53 kg/ha while in the second year, it ranged

from 465.84-598.54 kg/ha. In both first and second year, the highest available N content was recorded in the spring season while the lowest available N content was recorded in the winter season.

According to the Methods Manual of Soil Testing in India (2011), available N is catergorized as very low (< 140 kg/ha), low (< 141-280 kg/ha), medium low (281 - 420 kg/ha), medium (421 - 560 kg/ha), high (561-700 kg/ha) and very high (>700 kg/ha). Following this manual, the available N in cultivated jhum land was very low-low, in abandoned jhum land it was medium low-medium, in tea garden it was low to medium and in Mingkong reserve forest it was medium to high.

Table 2.10. Soil available N (kg/ha) of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	319.82 ±0.31 [a]	266.51 ±0.25 [b]	236.74 ±0.07 [c]	122.08 ±0.06 [d]	257.61 ±0.15 [c]	271.50 ±0.15 [a]	232.90 ±0.01 [d]	263.55 ±0.26 [b]
AJL	372.44 ±0.277 [d]	492.39 ±0.36 [a]	402.79 ±1.18 [c]	448.88 ±0.10 [b]	356.26 ±0.80 [d]	514.57 ±0.51 [a]	366.59 ±0.55 [c]	446.43 ±0.24 [b]
TG	252.41 ±0.27 [d]	401.21 ±0.19 [a]	398.60 ±0.50 [b]	348.60 ±0.05 [c]	256.41 ±0.33 [d]	459.30 ±0.74 [a]	381.56 ±0.16 [c]	454.26 ±0.09 [b]
MRF	493.48 ±0.17 [d]	581.53 ±0.15 [a]	552.53 ±0.15 [c]	569.51 ±0.24 [b]	465.84 ±0.10 [d]	598.54 ±0.19 [a]	536.66 ±0.21 [c]	553.76 ±0.16 [b]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey *post hoc* test

Soil available phosphorus

The mean values of soil available P of the collected soil samples from all the four study sites are shown in Table 2.11. In CJL at Khensa village, the average value of available P content, in the first year ranged from 14.65-34.65 kg/ha while in the second year, it ranged from 17.45-29.25 kg/ha. In first year, the highest and the lowest P content was recorded in the spring and autumn season respectively whereas

in the second year, the highest and lowest value of P was recorded in spring and summer.

In AJL at Mekuli village, the average value of available P content, in the first year ranged from 30.10-48.81 kg/ha while in the second year, it ranged from 42.69-59.78 kg/ha. In both first and second year, the highest value of available P content was recorded in the spring season while and the lowest value of available P content was recorded in the summer season.

In tea garden at Longmisa village, the average value of available P content, in the first year ranged from 20.29-45.47 kg/ha while in the second year, it ranged from 38.87-52.92 kg/ha. In the first year, the highest value of available P content was recorded in the spring season while the lowest value of available P content was recorded in the winter season. In the second year, the highest value of available P content was recorded in the spring season while the lowest value of available P content was recorded in the summer season.

In MRF at Chuchuyimpang village, the average value of available P content, in the first year ranged from 42.14-53.89 kg/ha while in the second year, it ranged from 42.28-55.66 kg/ha. In both first and second year, the highest value of available P content was recorded in the spring season while the lowest value of available P content was recorded in the summer season.

According to the Methods Manual of Soil Testing in India (2011), available P is catergorized as very low (<16 kg/ha), low (16-32 kg/ha), medium low (32-48 kg/ha), medium (48-64 kg/ha), high (64-80 kg/ha) and very high (>80 kg/ha).

Following this manual, the available P in cultivated jhum land was very low-medium low, in abandoned jhum land it was low-medium, in tea garden it was low to medium and in Mingkong reserve forest it was medium low to medium.

Table 2.11. Soil available P (kg/ha) of CJL, AJL, TG and MRF during 2016- 2018

Sites	2016-17				2017-18			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
CJL	30.03 ±0.01 [b]	34.65 ±0.32 [a]	21.45 ±0.04 [c]	14.65 ±0.32 [d]	24.67 ±0.10 [c]	29.25 ±0.03 [a]	17.45 ±0.12 [d]	26.44 ±1.00 [b]
AJL	33.83 ±0.05 [c]	48.81 ±0.16 [a]	30.10 ±0.02 [d]	38.44 ±0.32 [b]	46.09 ±0.05 [c]	59.78 ±0.13 [a]	42.69 ±0.68 [d]	54.91 ±0.06 [b]
TG	20.29 ±0.07 [d]	45.47 ±0.12 [a]	35.26 ±0.14 [b]	27.70 ±0.19 [c]	42.56 ±0.10 [c]	52.92 ±0.22 [a]	38.87 ±0.18 [d]	48.20 ±0.1 [b]
MRF	48.82 ±0.14 [c]	53.89 ±0.09 [a]	42.14 ±0.07 [d]	50.42 ±0.08 [b]	48.69 ±0.20 [c]	55.66 ±0.17 [a]	42.28 ±0.20 [d]	52.64 ±0.24 [b]

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters ([abc]and[d]) in the same row in each year indicate significant differences between season (p<.05) after a Tukey post hoc test

Soil available potassium

The mean values of soil available K of the collected soil samples from all the four study sites are shown in Table 2.12. In CJL at Khensa village, the average value of available K content, in the first year ranged from 91.18-130.67 kg/ha while in the second year, it ranged from 90.76-117.56 kg/ha. In both years, the highest and the lowest value of available K content was recorded in the spring and summer season respectively

In AJL at Mekuli village, the average value of available K content, in the first year ranged from 132.57-202.29 kg/ha while in the second year, it ranged from 130.87-208.54 kg/ha. In both first and second year, the highest value of available K content was recorded in the spring season while the lowest value of available K content was recorded in the summer season.

In tea garden at Longmisa village, the average value of available K content, in the first year ranged from 98.68-143.96 kg/ha while in the second year, it ranged from 94.29-149.11 kg/ha. In both years, the highest value of available K content was recorded in autumn season while the lowest value of available K content was recorded in the winter season

In MRF at Chuchuyimpang village, the average value of available K content, in the first year ranged from 150.99-270.30 kg/ha while in the second year, it ranged from 152.08-271.71 kg/ha. In both years, the highest value of available K content was recorded in the spring season while the lowest value of available K content was recorded in the winter season.

According to the Methods Manual of Soil Testing in India (2011), available K is catergorized as very low (<120 kg/ha), low (121-180 kg/ha), medium low (181-240 kg/ha), medium (241-300 kg/ha), high (301-360 kg/ha) and very high (> 360 kg/ha). Following this manual, the available K in cultivated jhum land was very low-low, in abandoned jhum land it was low-medium low, in tea garden it was very low to low and in Mingkong reserve forest it was low to medium.

Table 2.12. Soil available K (kg/ha) of CJL, AJL, TG and MRF during 2016- 2018

	2016-17				2017-18			
Sites	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	110.18 ±0.05 ^{c}	130.67 ±0.30 ^{a}	91.18 ±0.98 ^{d}	100.08 ±0.54 ^{b}	102.32 ±0.20 ^{c}	117.56 ±0.24 ^{a}	90.76 ±1.00 ^{d}	112.41 ±0.23 ^{b}
AJL	172.62 ±0.16 ^{c}	202.29 ±0.07 ^{a}	132.57 ±0.52 ^{d}	192.55 ±0.12 ^{b}	168.97 ±0.12 ^{c}	208.54 ±0.11 ^{a}	130.87 ±0.14 ^{d}	184.25 ±0.62 ^{b}
TG	98.68 ±0.03 ^{d}	141.65 ±0.08 ^{b}	135.23 ±0.12 ^{c}	143.96 ±0.03 ^{a}	94.29 ±0.13 ^{d}	121.01 ±0.78 ^{b}	119.36 ±0.20 ^{c}	149.11 ±0.09 ^{a}
MRF	208.98 ±0.11 ^{c}	270.30 ±0.11 ^{a}	150.99 ±0.18 ^{d}	266.58 ±0.28 ^{b}	211.86 ±0.04 ^{c}	271.71 ±0.10 ^{a}	152.08 ±0.09 ^{d}	246.05 ±0.04 ^{b}

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Mean ±standard error mean
Different letters (^{abc}and^{d}) in the same row in each year indicate significant differences between season (p<.05)

after a Tukey *post hoc* test

2.5 Statistical Analysis

Statistically significant differences in each of the soil physicochemical properties between the sampling seasons was analyzed by one-way analysis of variance (ANOVA) at $p<0.01$ and $p<0.05$ significant levels. If the ANOVA results were significant, Tukey's HSD post hoc test was performed to identify the mean significant differences in all the soil parameters and shown by different letters (a>b>c>d). One-way ANOVA test results showed that there were mean significant difference in all the soil physicochemical properties between the sampling seasons ($p\leq.05$ and $p\leq.01$). Tukey's *post hoc* test of significance for mean differences between the sampling seasons (winter-spring, winter-summer, winter-autumn, spring-summer, spring-autumn, summer-autumn) showed statistically significant differences in all the soil parameters in all the four study sites in both the years (Table 2.3). Two-way ANOVA test between site and sampling season also revealed statistically significant effect of their interaction ($p\leq.01$) in all the soil parameters (Table 2.4).

Table 2.13. One-way ANOVA of soil physicochemical parameters of CJL, AJL, TG and MRF during 2016-2018

Para meters	CJL				AJL				TG				MRF			
	2016-17		2017-18		2016-17		2017-18		2016-17		2017-18		2016-17		2017-18	
	F(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)
***p*H**	37.38	.001	65.13	.001	64.28	.001	28.47	.001	15.06	.001	13.11	.002	28.80	.001	20.93	.001
Moist.(%)	6.28	.001	320.12	.001	2.11	.001	5.80	.001	481.65	.001	1.24	.001	5.10	.001	3.07	.001
Temp.(%)	80.83	.001	101.15	.001	73.39	.001	598.31	.001	94.57	.001	2.64	.001	399.45	.001	1.32	.001
SOC (%)	144.26	.001	28.62	.001	6.48	.016	52.81	.001	192.39	.001	171.43	.001	5.18	.028	10.99	.003
AN(kg/ha)	5.08	.001	3.10	.001	2.08	.001	5.23	.001	1.59	.001	1.56	.001	1.39	.001	3.17	.001
AP(kg/ha)	4.60	.001	290.02	.001	6.16	.001	1.54	.001	1.89	.001	4.31	.001	7.53	.001	2.39	.001
AK(kg/ha)	6.08	.001	1.46	.001	3.60	.001	2.99	.001	2.44	.001	9.08	.001	2.84	.001	1.50	.001
Sand (%)	76.10	.001	445.22	.001	256.80	.001	6.19	.001	428.15	.001	188.30	.001	13.52	.002	1.86	.001
Silt (%)	10.40	.004	253.98	.001	39.15	.001	1.65	.001	48.48	.001	70.70	.001	116.76	.001	5.98	.001
Clay (%)	99.33	.001	182.19	.001	106.65	.001	904.10	.001	546.97	.001	660.48	.001	31.28	.001	73.85	.001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table 2.14. Two-way ANOVA of soil physicochemical parameters of CJL, AJL, TG and MRFduring 2016-2018

Parameters	Source	2016-17		2017-18	
		***F*(3,8)**	***p*(value)**	***F*(3,8)**	***p*(value)**
***p*H**	Site	95.80	.001	225.86	.001
	Season	31.21	.001	47.77	.001
	SitesxSeason	4.18	.001	5.45	.001
Moist.(%)	Site	1.21	.001	1.32	.001
	Season	1.26	.001	5.78	.001
	SitesxSeason	457.38	.001	609.99	.001
Temp.(%)	Site	484.67	.001	2.07	.001
	Season	400.21	.001	1.69	.001
	SitesxSeason	6.45	.001	11.61	.001
SOC (%)	Site	207.37	.001	1.20	.001
	Season	40.78	.001	86.65	.001
	SitesxSeason	13.97	.001	4.82	.001
AN(kg/ha)	Site	1.48	.001	1.19	.001
	Season	9.50	.001	2.76	.001
	SitesxSeason	8.24	.001	3.90	.001
AP(kg/ha)	Site	4.28	.001	1.63	.001
	Season	1.81	.001	3.98	.001
	SitesxSeason	4.18	.001	80.97	.001
AK(kg/ha)	Site	3.00	.001	2.32	.001
	Season	9.67	.001	5.81	.001
	SitesxSeason	1.40	.001	1.16	.001
Sand (%)	Site	1.61	.001	6.87	.001
	Season	171.41	.001	1.27	.001
	SitesxSeason	65.07	.001	90.68	.001
Silt (%)	Site	2.02	.001	1.27	.001
	Season	104.20	.001	179.30	.001
	SitesxSeason	16.79	.001	403.83	.001
Clay (%)	Site	626.47	.001	3.39	.001
	Season	149.02	.001	815.56	.001
	SitesxSeason	97.20	.001	135.59	.001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Correlation analysis was employed to assess mean difference and the association between soil variables. Correlations among the soil properties were determined by Pearson's correlation coefficient. Pearson correlation analysis was done using the software SPSS 16.0 for test of significance. Unless otherwise stated, the level of significance referred to in the results is $p \leq .05$ and $p \leq .01$. Pearson correlation coefficient was done to measure the relationship between the means of the physicochemical parameters. The correlation coefficient between the physicochemical properties of all the four study sites from 2016-2018 are presented below (Table 2.15 – Table 2.22).

In CJL at Khensa village, the results showed both positive and negative significant correlation ($p \leq .05$ and $p \leq .01$) among the soil physicochemical properties in both first (Table 2.15) and second (Table 2.16) year. In the first year, the results showed a positive significant correlation between temperature and moisture (r=.75**, p=.005), between SOC and *p*H (r=.92**; p=.001) and a significant negative correlation between available K and moisture (=-.73**; p=.006). Available N showed a significant positive correlation with *p*H (r=.91**; p=.001), SOC (r=.85**; p=.001) and a significant negative correlation temperature (=-.69*; p=.013). There was a significant positive correlation of available P with *p*H (r=.87**; p=.001), SOC (r=.96**; p=.001), and available N (r=.85**; p=.001) whereas a significant negative correlation with temperature (r=-.64*; p=.026). Sand showed a significant positive correlation with temperature (r=.84**; p=.001), and moisture (r=.67*; p=.016), silt showed a significant positive correlation with *p*H (r=.60*; p=.040), SOC (r=.65*; p=.022), available N (r=.70**; p=.012), available P (r=.82**; p=.001), and a

significant negative correlation with moisture (r=-.66*; *p*=.019) and temperature (r=-.78*; *p*=.003), clay was significantly and negatively correlated with moisture (r=-.63*; *p*=.029), temperature (r=-.80**; *p*=.002), and sand (r=-.98**; *p*=.001),

In the second year, moisture was significantly and positively correlated with *p*H (r=.96**; *p*=.001), temperature showed a positive significant correlation with *p*H (r=.89**; *p*=.001) and moisture (r=.96**; *p*=.001). Available N showed a negative significant correlation with *p*H (r=-.61*; *p*=.035). Available P showed a positive significant correlation with SOC (r=.59*; *p*=.041) and available N (r=.99**; *p*=.001) whereas a negative significant correlation with *p*H (r=-.59*; *p*=.042). Available K showed a positive significant correlation with SOC (r=.73**; *p*=.007), available N (r=.97**; *p*=.001) and available P (r=.97**; *p*=.001). Sand was negatively correlated with SOC (r=-78**; *p*=.003), available N (r=-.96**; *p*=.001), available P (r=-.96**; *p*=.001) and available K (r=-.99**; *p*=.001). Silt showed a significant negative correlation with SOC (r=-.58**; *p*=.049) and clay (r=-.87**; *p*=.001). Clay showed a positive significant correlation with SOC (r=.78**; *p*=.003), available N (r=.76*; *p*=.004), available P (r=.81**; *p*=.002), K (r=.69*; *p*=.001) whereas it showed a significant negative correlation with sand (r=-.86**; *p*=.001).

In AJL at Mekuli village, the results showed both positive and negative significant correlation ($p<.01$ and $p<.05$) among the soil physicochemical properties in both first (Table 2.17) and second (Table 2.18) year. In the first year, moisture showed a positive significant correlation with *p*H (r=.97**; *p*=.001), temperature showed a positive significant correlation with *p*H (r=.94**; *p*=.001) and moisture

(r=.94**; *p*=.001), available N showed positive significant correlation with SOC (r=.81**; *p*=.002), available P showed positive significant correlation with SOC (r=.66**; *p*=.019) and available N (r=.89**; *p*=.001). Available K was positively correlated with available N (r=.68*; *p*=.014) and available P (r=.86**; *p*=.001) whereas negatively correlated with moisture (r=-.66*; *p*=.020). Sand was positively correlated with *p*H (r=.68**; *p*=.015), moisture (r=.71**; *p*=.009) whereas negatively correlated with available N (r=-.64*; *p*=024), available P (r=-.92**; *p*=.001) and available K (r=-.82**; *p*=.001). Silt was positively correlated with available N (r=.67*; *p*=.018), available P (r=.91**; *p*=.001), available K (r=.82**; *p*=.001) whereas negatively correlated with *p*H (r=-.60*; *p*=.038), moisture (r=-.67*; *p*=.018) and sand (r=-.96**; *p*=.001). Clay showed a positive correlation with available N (r=.58*; *p*=.049), available P (r=.88**; *p*=.001), available K (r=.78**; *p*=.001) and silt (r=.95**; *p*=.001) whereas a negative correlation with *p*H (r=-.70*; *p*=.011), moisture (r=-.74**; *p*=.006), temperature (r=-.61*; *p*=.037) and sand (r=-.98**; *p*=.001)

In the second year, a positive significant correlation was observed between moisture and *p*H (r=.66*; *p*=.021), temperature showed a positive significant correlation with *p*H (r=.83**; *p*=.001) and moisture (r=.94**; *p*=.001), SOC showed a positive significant correlation with moisture (r=.72**; *p*=.008) and temperature (r=.59*; *p*=.043), available N was positively correlated with SOC (r=.88**; *p*=.001), available P was positively correlated with SOC (r=.75**; *p*=.005) and available N (r=.96**; *p*=.001), available K showed positive significant correlation with available N (r=.85**; *p*=.001), available P (r=.95**; *p*=.001) and sand was negatively

correlated with available N (r=-.61*; *p*=.037) available P (r=-.72**; *p*=.009) and available K (r=-.89**; *p*=.001) and positively correlated with temperature (r=.63*; *p*=.028), silt was negatively correlated with *p*H (r=-.83**; *p*=.001), temperature (r=-.63**; *p*=.028), sand (r=-.81**; *p*=.001), clay showed a positive correlation with available N (r=.66*; *p*=.018), available P (r=.81**; *p*=.002), available K (r=.96**; *p*=.001) silt (r=.63*; *p*=.029), whereas a negative correlation with sand (r=-.96**; *p*=.001).

In TG at Longmisa village, the results showed both positive and negative significant correlation (*p*<.01 and *p*<.05) among the soil physicochemical properties in both first (Table 2.19) and second (Table 2.20) year. In the first year, moisture showed a positive significant correlation with *p*H (r=.78**; *p*=.003), temperature showed a positive significant correlation with *p*H (r=.82**; *p*=.001) and moisture (r=.97**; *p*=.001), a positive significant correlation was observed between SOC and moisture (r=.61*; *p*=.037), available N was positively correlated with moisture (r=.92**; *p*=.001), temperature (r=.84**; *p*=.001) and SOC (r=.85**; *p*=.001), available P showed a positive correlation with moisture (r=.65**; *p*=.022), SOC (r=.99**; *p*=.001) and available N (r=-.89**; *p*=.001), available K showed a positive correlation with *p*H (r=.77**; 003), moisture (r=.91**; *p*=.001), temperature (r=.89**; *p*=.001), SOC (r=.71*; *p*=.010), available N (r=.88**; *p*=.001) and available P (r=.71**; *p*=.010), silt was positively correlated with SOC (r=.60*; *p*=.041) whereas it showed a negative correlation with sand (r=-.79**; *p*=.002) and clay was negatively correlated with *p*H (r=-.81**; *p*=.001), moisture (r=-.60*; *p*=.038), temperature (r=-.73**; *p*=.007) and sand (r=-.79**; *p*=.002).

In the second year, moisture showed a positive significant correlation with *p*H (r=.90**; *p*=.001), temperature showed significant positive correlation with *p*H (r=.86**; *p*=.001), moisture (r=.98**; *p*=.001), SOC showed a positive significant correlation with temperature (r=.68**; *p*=.015), available N showed significant positive correlation with *p*H (r=.68*; *p*=.015), moisture (r=.71**; *p*=.010), temperature (r=.78**; *p*=.003), SOC (r=.78**; *p*=.003), available P showed a positive significant correlation with available N (r=.65*; *p*=.023). Available K showed a positive correlation with *p*H (r=.78**; *p*=.003), moisture (r=.76**; *p*=.004), temperature (r=.73**; *p*=.007) and available N (r=.85**; *p*=.001). Sand showed a positive significant correlation with moisture (r=.63*; *p*=.029) whereas a negative correlation with available P (r=-.81**; *p*=.001). Silt and sand was negatively and significantly correlated (r=-.89**; *p*=.001). Clay showed a positive correlation with available P (r=.80**; *p*=.002) and silt (r=.86**; *p*=.001) whereas it showed a negative correlation with moisture (r=-.66**; *p*=.021001) and sand (r=-.99**; *p*=.).

In MRF at Chuchuyimpang village, the results showed both positive and negative significant correlation ($p<.01$ and $p<.05$) among the soil physicochemical properties in both first year (Table 2.21) and second year (Table 2.22). In the first year, moisture was positively correlated with *p*H (r=.94**; *p*=.001), temperature was positively correlated with *p*H (r=.86**; *p*=.001) and moisture (r=.90**; *p*=.001), available N was positively correlated with temperature (r=.80**; *p*=.002) and SOC (r=.74**; *p*=.006), available P showed negative significant correlation with *p*H (r=-.60*; *p*=.040), moisture (r=-.70; *p*=.011), a positive significant correlation was found between available K and available P (r=.95**; *p*=.001). Sand showed positive

significant correlation with *p*H (r=.83**; *p*=.001) and moisture (r=.75**; *p*=.005) whereas a negative significant correlation with available P (r=-.67*; *p*=.018). There was a significant positive correlation between silt and available P (r=.63*; *p*=.018) whereas a negative significant correlation between silt and *p*H (r=-.80**; *p*=.001), moisture (r=-.75**; *p*=.003) and sand (r=-.90**; *p*=.001).

In the second year, there was significant positive correlation between moisture and *p*H (r=.89**; *p*=.001), temperature showed positive significant correlation with *p*H (r=.82**; *p*=.001) and moisture (r=.92**; *p*=.001), available N showed positive significant correlation with temperature (r=.67*; *p*=.018) and SOC (r=.89**; *p*=.001), available K showed positive significant correlation with available P (r=.99**; *p*=.001), sand showed a positive significant correlation with *p*H (r=.92**; *p*=.001), moisture (r=.95**; *p*=.001), temperature (r=.80**; *p*=.002) whereas it showed a negative significant correlation with available P (r=-.59**; *p*=.045) and available K (r=-.59**; *p*=.042). Silt showed negative significant correlation with *p*H (r=-.91**; *p*=.001), moisture (r=-.97**; *p*=.001), temperature (r=-.83**; *p*=.001) and sand (r=0.99**; *p*=.001). Clay showed a positive significant correlation with available P (r=.83**; *p*=.001), available K (r=.84**; *p*=.001) whereas it was negatively correlated with *p*H (r=-.58**; *p*=.047).

Table 2.15. Correlation between soil physicochemical properties at CJL during 2016-17

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1.00									
Moist. (%)	-.04	1.00								
Temp.(℃)	-.45	.75**	1.00							
SOC(%)	.92**	-.19	-.46	1.00						
AN (Kg/ha)	.91**	-.18	-.69*	.85**	1.00					
AP (Kg/ha)	.87**	-.44	-.64*	.96**	.85**	1.00				
AK (Kg/ha)	-.17	-.73**	-.15	.09	-.26	.26	1.00			
Sand(%)	-.08	.67*	.84**	-.03	-.39	-.21	-.02	1.00		
Silt(%)	.60*	-.66*	-.78**	.65*	.70*	.82**	.33	-.43	1.00	
Clay(%)	-.002	-.63*	-.80**	-.09	.31	.10	-.03	-.98**	.39	1.00

CJL- Cultivated jhum land

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.16. Correlation between soil physicochemical properties at CJL during 2017-18

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1.00									
Moist. (%)	.96**	1.00								
Temp.(℃)	.89**	.96**	1.00							
SOC(%)	.16	.35	.49	1.00						
AN (Kg/ha)	-.61*	-.42	-.30	.57	1.00					
AP (Kg/ha)	-.59*	-.41	-.30	.60*	.99**	1.00				
AK (Kg/ha)	-.41	-.20	-.06	.73**	.97**	.97**	1.00			
Sand(%)	.39	.18	.05	-.78**	-.96**	-.96**	-.99**	1.00		
Silt(%)	.22	.17	-.02	-.58*	-.40	-.43	-.44	.52	1.00	
Clay(%)	-.34	-.20	-.01	.78**	.76**	.79**	.81**	-.85**	-.87**	1.00

CJL- Cultivated jhum land

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.17. Correlation between soil physicochemical properties at AJL during 2016-17

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1.00									
Moist. (%)	.97**	1.00								
Temp.(℃)	.94**	.94**	1.00							
SOC(%)	.23	.16	.31	1.00						
AN (Kg/ha)	.07	-.01	.24	.81**	1.00					
AP(Kg/ha)	-.38	-.45	-.22	.66*	.89**	1.00				
AK (Kg/ha)	.55	-.66*	-.40	.40	.68*	.86**	1.00			
Sand(%)	.68*	.71**	.56	-.46	-.64*	-.92**	-.82**	1.00		
Silt(%)	-.60*	-.67*	-.50	.55	.67*	.91**	.82**	-.96**	1.00	
Clay(%)	-.70*	-.74**	-.61*	.45	.58*	.88**	.78**	-.98**	.95**	1.00

AJL-Abandoned jhum land

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.18. Correlation between soil physicochemical properties at AJL during 2017-18

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1.00									
Moist. (%)	.66*	1.00								
Temp.(℃)	.83**	.94**	1.00							
SOC(%)	.45	.72**	.59*	1.00						
AN (Kg/ha)	.19	.38	.23	.88**	1.00					
AP (Kg/ha)	.12	.15	.04	.75**	.96**	1.00				
AK (Kg/ha)	-.16	-.16	-.29	.53	.85**	.94**	1.00			
Sand(%)	.57	.44	.63*	-.22	-.61*	-.72**	-.89**	1.00		
Silt(%)	-.83**	-.34	-.63*	.04	.30	.31	.51	-.81**	1.00	
Clay(%)	-.39	-.43	-.55	.27	.66*	.81**	.96**	-.96**	.63*	1.00

AJL-Abandoned jhum land

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.19. Correlation between soil physicochemical properties at TG during 2016-17

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1									
Moist. (%)	.78**	1								
Temp.(℃)	.82**	.97**	1							
SOC(%)	.19	.61*	.48	1						
AN (Kg/ha)	.57	.92**	.84**	.85**	1					
AP (Kg/ha)	.22	.65*	.51	.99**	.89**	1				
AK (Kg/ha)	.77**	.91**	.89**	.71*	.88**	.71*	1			
Sand(%)	.44	.29	.38	-.53	-.03	-.46	-.02	1		
Silt(%)	.09	.12	.09	.60*	.30	.51	.49	-.79**	1	
Clay(%)	-.81**	-.60*	-.73**	.22	-.27	.19	-.49	-.79**	.27	1

TG- Tea garden

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.20. Correlation between soil physicochemical properties at TG during 2017-18

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1									
Moist. (%)	.90**	1								
Temp.(℃)	.86**	.98**	1							
SOC(%)	.39	.52	.68*	1						
AN (Kg/ha)	.68*	.71**	.78**	.78**						
AP (Kg/ha)	-.002	-.08	.05	.52	.6*	1				
AK (Kg/ha)	.78**	.76**	.73**	.36	.85**	.40	1			
Sand(%)	.52	.63*	.51	-.15	-.10	-.81**	.16	1		
Silt(%)	-.35	-.43	-.26	.49	.25	.80**	-.17	-.89**	1	
Clay(%)	-.54	-.66*	-.55	.08	.07	.80**	-.15	-.99**	.86**	1

TG- Tea garden

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Table 2.21. Correlation between soil physicochemical properties at MRF during 2016-17

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1									
Moist. (%)	.94**	1								
Temp.(℃)	.86**	.90**	1							
SOC(%)	.17	.06	.39	1						
AN(Kg/ha)	.52	.46	.80**	.74**	1					
AP(Kg/ha)	-.60*	-.70*	-.32	.52	.30	1				
AK (Kg/ha)	-.41	-.55	-.16	.57	.45	.95**	1			
Sand(%)	.83**	.75**	.55	-.15	.19	-.67*	-.44	1		
Silt(%)	-.80**	-.75**	-.57	.06	-.23	.63*	.36	-.90**	1	
Clay(%)	-.34	-.46	-.25	.19	.18	.64*	.80**	-.17	-.02	1

MRF- Minkong reserve forest
Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 2.22. Correlation between soil physicochemical properties at MRF during 2017-18

Parameters	*p*H	Moist. (%)	Temp. (℃)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	Sand (%)	Silt (%)	Clay (%)
pH	1									
Moist. (%)	.89**	1								
Temp.(℃)	.82**	.92**	1							
SOC(%)	.19	.23	.53	1						
AN (Kg/ha)	.22	.36	.67*	.89**	1					
AP(Kg/ha)	-.55	-.31	-.13	.45	.51	1				
AK (Kg/ha)	-.56	-.32	-.15	.44	.49	.99**	1			
Sand(%)	.92**	.95**	.80**	-.01	.09	-.59*	-.59*	1		
Silt(%)	-.91**	-.97**	-.83**	-.04	-.15	.51	.52	-.99**	1	
Clay(%)	-.58*	-.38	-.40	-.02	.011	.83**	.84**	-.55	.49	1

MRF- Minkong reserve forest
Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

2.6 Discussions

Some selected soil physicochemical properties including *p*H, moisture, temperature, SOC, available nutrients and soil texture under different land use types are discussed under this section.

2.6.1 Variation in soil texture

Determination of soil texture is important as it can indicate the quality of the soil (Shukla, 2006). The level of intensity involved in cultivation (Assefa et al., 2020) and parent material influences the variations in soil textural fractions of sand, silt and clay between different land uses (Oguike and Mbagwu, 2009). Two-way ANOVA result (Table 2.14), showed a significant difference due to interaction effect between site and season in the distribution of soil textural fractions such as sand, silt and clay was observed between the study sites (p<01). The soil texture was classified as sandy clay loam and sandy loam in cultivated jhum land, sandy clay loam in abandoned jhum land and tea garden while loam in Minkong reserve forest (Figure 2.3a-2.3d) according to USDA (2004) classification. The soil textural class of each study site remained unchanged throughout the study period in each of the respective study sites. The loam soil textural class in Minkong reserve forest shows that its soil has a good soil structure to support plant growth as loam soil is highly porous to air, water and plant roots that results in better distribution and retention of soil nutrients (Fentie et al., 2020). The sandy clay loam-sandy laom soil textural class in cultivated jhum land, abandoned jhum land and tea garden. The present result is comparable with Mishra and Francaviglia (2021), where the author also reported the same soil textural class in their study in other districts under Nagaland. This shows the homogeneity of the parent soil material and uniformity of the soil forming processes however activities such as weathering, soil erosion and deposition alters the composition of soil texture (Moges et al., 2013)

From one-way ANOVA analysis, the results shown in table 2.13, showed a significant effect of sampling season ($p<.05$) on sand, silt and clay content in all the four study sites in both the study years. Even though there was a significant differences in the distribution pattern of sand, silt and clay over the season, sand was recorded to be higher during summer season throughout the study period. Correlation results showed that sand was significantly correlated ($p<.05$ and $p<.01$) with soil moisture and temperature and their relationship was strong and positive (Table 2.15-2.22). Therefore, the reason for the higher sand percentage during summer may be because of the increased moisture due to higher rainfall and a higher temperature during the summer season in the present study. Silt and clay content showed an irregular pattern over the season throughout the study period. Although there was variation in the distribution of the soil fractions between the study sites, sand percentage remained the highest among the three soil particles (Table 2.2-2.5). The total mean value of sand percentage in both first and second year of the study period was comparatively higher in cultivated jhum land (55.25 and 57.43%) and tea garden (53.42 and 54.60%) as compared to abandoned jhum land (54.25 and 55.23%) and Minkong reserve forest (43.51 and 44.45%) respectively. Manjunatha and Singh (2020) and Moges et al., (2013) has reported that interruption in cultivated soil which is caused by cultivation practices such as soil tillage, clearing, burning and regular removal of vegetation can intensify soil erosion and allow the finer soil particles like silt and clay to be washed away by rain and thereby an increase in sand percentage in cultivated soil. This could be the possilbe reason in the present finding as well, because in the cultivated jhum land and tea garden, agricultural activities such as

slashing and burning of vegetation, harvesting and removal of vegetation and soil tillage was practiced whereas in the other two study sites i.e, abandoned jhum land Minkong reserve forest, the soil and the vegetation cover were not disturbed by any such agricultural practices as no cultivation was done. However, contradicting result to the present finding has also been reported by Chemeda et al. (2017) where the authors indicated a higher sand fraction in the soil surface of forest land in comparison with cultivated land. These variations in the sand, silt and clay content may be due to different soil inherent parent material that leads to variation in soil fractions between the study sites and also due to different soil management activities (Tufa et al., 2013). The silt and clay fractions were higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land, indicating the soil under the forest was better in terms of nutrient storage and availability for plants (Asseffa et al., 2020; Rawat et al. 2021)

Significant positive and negative correlations of soil textural particles (sand, silt, clay) with other soil properties were established in the present study ($p<.05$ and $p<.01$). There was a negative correlation of sand with available N, available P, available K and SOC and a positive correlation with soil *p*H, temperature and moisture, a significant positive and negative correlation was found between silt and *p*H, SOC, available N, available P, available K, sand and clay, clay showed a significant positive correlation with SOC, available N, available P, available K and a and significant negative correlation with *p*H, temperature and moisture, silt and sand in the present study (Table 2.15-2.22). The results showed that higher sand percentage in the soil decreases the availability of nutrients which may be due to

anthropogenic activities and higher rainfall with increased temperature during summer season in the present study can intensify soil erosion therefore resulting in a higher sand fraction and reduced silt and clay fractions.

2.6.2 Variation in soil *p*H

Significant effect of season and site interaction ($p<.01$) was reported on soil *p*H during the study period (Table 2.14). The maximum mean value of soil *p*H varied from 4.36-5.36, 4.51-6.02, 4.51-6.02 and 5.61-6.42 in cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest (Table 2.6). The *p*H values of soil in the four study sites ranged from acidic to slightly acidic. The acidic nature of soil in other districts of Mokokchung and Nagaland has also been reported by Amenla et al., (2010) and Mishra and Francaviglia (2021) where the authors has reported that it may be because of the high rainfall in Northeast region that intensifies leaching of basic cations from soil colloids or maybe due to slow decomposition of the organic matter that lead to constant release of low molecular acids which may lead to the acidic nature of these soils.

Seasonal variation in soil *p*H was found to be significant ($p<.05$) in all the four study sites (Table 2.13) and higher soil *p*H was recorded during autumn and summer season and lower during winter season (Table 2.6) which can be due to the fluctuations in moisture and temperature during seasonal changes, also a significant positive correlation ($p<.05$) was established between soil *p*H with temperature and moisture in the present study. In comparison between the four study sites, soil of cultivated jhum land were more acidic followed by tea garden, abandoned jhum land

and Minkong reserve forest (Table 2.6). The lower soil *p*H value in the cultivated jhum land and tea garden may be because of the management practices as Girma (2020) also reported that lower soil *p*H value in cultivated land may be due to the removal of basic cations during vegetation harvesting or removal, runoff due to soil erosion and leaching and high rainfall. However, Yimer et al. (2007) reported a contrasting result to the present finding that a higher soil *p*H was found in cultivated soil than the *p*H of forest and grassland as a result of the regular cation uptake and removal of cations around the tree roots in the forest. Eventhough the values of soil *p*H in Minkong reserve forest and abandoned jhum land was higher than the two cultivated land the soil *p*H value of Minkong reserve forest and abandoned jhum land were moderately to slightly acidic which can be due to the continuous uptake of basic cations by tree roots, canopy leachates, decay products of plant litter (Muche et al., 2015; Semy et al., 2022) and an increase in soil *p*H in jhum fallow is reported by Manjunantha and Singh (2020) which may be due to the continuous deposition of plant organic matter with increasing fallow age that leads to successive enhancement with bases. Variations in soil *p*H values were observed between the study sites however the soil in all the four study sites remained strongly acidic to moderately acidic throughout the study period.

In the cultivated jhum land, soil *p*H was found to be higher after slashing and burning of vegetation and decreased with subsequent cultivation (Table 2.6). The present result is in agreement with the findings by Tawnenga et al. (1997) and Wapongnungsang et al. (2021a) that slashing and burning of vegetation in jhum land increases soil *p*H followed by a consequent decrease in the *p*H value during

cultivation which maybe because of the incorporation of cations released after burning and release of humic acid due to destruction of organic matter. The soil *p*H in tea soil was slightly acidic to acidic in the present study. Similar reports of acidic nature in tea cultivated soil have been reported by Chien et al. (2019) which is a common phenomenon due to the release of protons during the uptake of NH_4-N by the tea plant and nitrification in soil with excessive use of N fertilizers, however no N fertilizers were used in the present study. Therefore, the acidic soil of the tea garden in the current study may be mainly due to the addition of organic debris from pruning of tea bushes that leads to an increase in acidity of tea soil, similar results has been reported by other workers such as Temsurenla and Ajungla (2017) in tea garden under Nagaland, and Gogoi et al. (2016) in tea garden under Assam. Soil *p*H is an important factor that influences the solubility and availability of nutrients in the soil. Kebebew et al. (2022) and Mishra et al. (2021) reported that soil in forest should be slightly acidic for proper nutrient supply and according to USEPA (2008) the tea plant grows best in acidic soil between an ideal optimum soil *p*H of 4.0-6.0. Altering the optimum soil *p*H requirement can have a negative impact on soil nutrient availability which adversely affects crop productivity and sustainability of agricultural systems (Guo et al., 2010). Significant positive correlation ($p<.05$) was also observed between soil *p*H and SOC, available K and available N which indicates that soil *p*H influences the SOC, available K and available N content in the present soil whereas it was it was significantly ($p<.05$) negatively correlated with soil available P, silt and clay during the study period (Table 2.15-2.22). Therefore,

maintaining an optimum *p*H in any agricultural soil is one of the most important conditions for better growth and function of plants.

2.6.3 Variation in soil moisture

Zhang and Li (2022) has reported that soil moisture is important for regulation and function of physical processes in the soil and land use is considered as one of the key factor in the variations that occurs in moisture and temperature in the soil coupled with other various factors such as precipitation, amount of water used up by plants, air temperature, runoffs, type of vegetation, weather and topography. D'Odorico et al. (2007) have reported that soil moisture is an environmental variable that combines the effects of climate, soil, and vegetation on the dynamics of water-stressed ecosystems and in contrast to abiotic factors such as soil texture and rainfall regime, the influence of vegetation composition and structure on soil moisture variability is poorly understood. Rawat et al. (2021) have mentioned that soil moisture influences microbial activity that influences organic carbon content in the soil. It is important to maintain an optimum moisture content in soil so that plants can readily uptake water because variation in soil moisture content can alter vegetation development, availability of soil nutrients, formation of forest canopy, diversity of tree species and crop productivity.

Significant seasonal variation ($p<.05$) in soil moisture was recorded (Table 2.13) and soil moisture content was recorded to be highest during summer season and lowest during winter season throughout the study period in all the four study sites. The present result concur with the higher moisture content in the soil during summer

season because of maximum rainfall received during this season (July) and lower soil moisture content due to the decrease in rainfall during the dry winter season (January) at the Mokokchung district. Similarly, Temsurenla (2021) also reported that soil moisture level tends to rise with an increase in rainfall during summer season and tends to go down with a decrease in rainfall during dry winter season in Mokokchung district and variations in soil moisture content over the season may be regulated by soil temperature, evapotranspiration process, runoffs, and uptake of water by plants. This was further ascertained by the significantly positive correlation ($p<.01$) between soil moisture and soil temperature in all the four study sites in both the study year (Table 2.15-2.22).

Significant effect of site interaction ($p < .01$) with the sampling season was also reported for soil moisture (Table 2.14) in the present study. Between the study sites moisture content in soil was recorded to be higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land. This can be attributed to the dense vegetation over the fallow period, thick canopy in the forest and higher silt and clay content representing increase water retention capacity (Assefa et al., 2020). Simliarly, Fentie et al. (2020) reported forest soil have higher moisture content than a cultivated land, eucalyptus plantation and a grazing land in their study, Temjen et al. (2020) reported that fallow agricultural land have higher soil moisture content compared with a newly cultivated land. Soil moisture is associated with vegetation type (El Masri, 2017) and the lower soil moisture content in the cultivated jhum land and tea garden may be because of the high rate of precipitation and utilization of water by plants for transpiration, regular removal of vegetation, use of

fire for burning slashed vegetation in jhum land, soil tillage which accelerates soil erosion and water runoff are intensified (Arunachalam, 2003; Wapongnungsang et al., 2021b).

2.6.4 Variation in soil temperature

Soil temperature is an important feature that affects the biological events taking place in soil and directs physical and chemical processes. Various factor affect soil temperature such as vegetation cover, climatic conditions and soil topography (Yan et al., 2018; Ni et al., 2019). Soil temperature varies seasonally and daily due to changes in radiant energy and energy changes that occur at the soil surface as well as being heavily influenced by ambient temperature (Gash and Shutterworth, 1991; Wang et al., 2018) and influences plant processes, soil microbial diversity, mineralization process of organic matter and is closely linked to crop growth (Sabri et al., 2018).

Significant seasonal variation ($p<.05$) in soil temperature was established in the present study (Table 2.13). Soil temperature was recorded to be highest during summer season and lowest during winter season throughout the study period. Interaction effect between study site and season for soil temperature was also significant ($p<.01$) in the present study (Table 2.14) and the mean value of soil temperature between the study sites were found to be higher in cultivated jhum land followed by tea garden, abandoned jhum land and Minkong reserve forest. The present result could be attributed to the higher solar radiation during summer season and decreased solar radiation during winter season reaching the surface of the soil

(Lozano-Parra et al., 2018). Increase in temperature may be attributed to the direct exposure of the surface soil to sunlight in absence of dense vegetation at cultivated jhum land and tea garden whereas the lower soil temperature in the Minkong reserve forest and abandoned jhum land can because the solar radiation is relatively intercepted by the thick canopy before reaching the surface of the soil during the radiation absorption of soil that leads to lower soil temperature (Facelli et al.,1999) as variation in vegetation cover can sensibly alter soil temperature responses (Song et al., 2013). Chen and Dirmeyer (2020) have reported that land use and land management is associated with altering the features of local land surface which affects the atmosphere and climate due to land-atmosphere interaction through surface fluxes. Thus, the atmospheric response to changes in land use or land management can also be linked to temperature changes.

2.6.4 Variation in soil organic carbon

The different land use was found to have significant effect on the SOC content. The mean SOC value was under different study sites followed the order Minkong reserve forest>abandoned jhum land>tea garden>cultivated jhum land (Table 2.9). The interaction effect of site and season (p=.01) on SOC during the study period (Table 2.14). The higher SOC content in Minkong reserve forest soil is attributed to the large acculumation of organic matter added to the soil surface in the form of leaf litter that remains undisturbed in the forest soil in the absence of anthropogenic activities. This is in accordance with Puket and Drinkwater (2001) who reported that minimal distrurbance in forest soil leads to more stable soil

aggregates that contribute to higher retention of the organic matter input under the soil of trees in the forest. Saplalrinliana et al. (2016) observed that jhum fallow length is related to the amount of litter accumulation in the fallow soil, that a longer jhum fallow length accumulates higher organic matter as more plant residues are abundantly available which are returned to the soil and are left to decompose in the jhum fallow land.

Lower SOC content in cultivated jhum land and tea garden is due the destruction of the soil surface while uprooting of vegetation, tillage and use of fire in jhum cultivation. Girma (2020) has reported that organic matter input is lower in soil under cultivation land use due to the disturbances in the soil that accelerates the oxidation of organic matter from the soil that includes annual soil tillage that provides less physical protection for SOC retention in the soil. Several other workers (Sarkar et al., 2015; Arunachalam, 2003) has reported that the use of fire in jhum cultivation changes the SOC content in the soil as burning of the slashed vegetation affects the soil temperature by increasing it and accelerating the litter decomposition in the surface soil, exposing the mineral soil and intensify loss of nutrients through soil erosion and water runoff. Arafat et al. (2020) reported that continuous use of land for cultivation of single crop like tea crop for a long period of time results in loss of organic matter because of management practices, removal of crops and water erosion that reduces the organic matter input. Hence, the SOC content was higher in Minkong reserve forest and abandoned jhum as compared with tea garden and cultivated jhum land. In cultivated jhum land the SOC content was found to decrease with the subsequent cropping period, and in tea garden the SOC content also decreased in the

second year of study but the value of SOC decreased was negligible between the study years whereas in Minkong reserve forest and abandoned jhum land the SOC content was found to moderately increase (Table 2.9). This study shows that different land use types have a significant (p<.01) effect on the SOC (Table 2.14). The SOC content in cultivated jhum land during first year was found to be lowest after the harvesting period (autumn season) and it was left to fallow after 1-year of cropping. In the second year, the SOC was found to be lower than the first year however it was found to slightly increase with the following fallow period (Table 2.9) with the addition of new organic matter from the remains of vegetation and dead plants after harvest in the previous cropping period. This result concurs with the findings by Kendawang et al. (2004). The accumulation of soil organic matter is intensified by longer fallow length, therefore a higher SOC content in abandoned jhum land which was undergoing its 10 years of fallow period during the first year of study period. Similar result is reported by Saplalrinliana et al. (2016) where SOC was significantly higher in jhum land with longer fallow length than a shorter jhum fallow length and the importance of fallow length in litter accumulation for soil nutrient regeneration. The present findings show that SOC content was affected most by the jhum cultivation as compared to the other study sites.

All the study sites showed a significant seasonal variation (p<.05) in SOC content (Table 2.13). In both year, the SOC content was found to be highest during spring season in all the study sites and the lowest SOC content was found during winter season in all the study sites except for cultivated jhum land where it was observed to be lowest during autumn season in the first year. Babur and Dindaroglu

(2020) have reported that the temperature and moisture are the most important climatic factor that influences the microbial activity and SOC in the soil. Similarly, in the present study SOC showed a positive correlation in all the study sites with temperature and moisture however the correlation was significant ($p<.05$) with temperature and moisture in abandoned jhum land, tea garden and Minkong reserve forest and not significant in cultivated jhum land (Table 2.15-2.22). This result indicates that temperature and moisture are important for SOC mineralization process in the soil. It is likely that the quality and quantity of litter input coupled with microbial activity determines the amount of SOC content.

In the present study, the higher SOC during spring season in the study sites may be because of higher microbial activity and higher deposition of organic matter in the soil due to the existing favourable condition like moisture and temperature during these seasons. Whereas in winter the soil microbial activity, temperature and moisture is lower which reduces the organic matter decomposition in soil resulting in a lower organic matter content. Similar result has been discussed by other workers (Rosa and Debska, 2017; Temsurenla et al., 2021). In addition, the reason for a higher SOC content during the spring season in cultivated jhum land in the first year could be due to burning of the slashed vegetation which lead to a slight increase in the SOC content because burning increases soil temperature that accelerated the decomposition of organic matter. However, the SOC was not significantly different from the SOC content before burning was carried out (winter season). This may be because the organic matter present in the soil was rapidly decomposed during the process of burning resulting in reduced incorporation of litter materials into the soil of the burnt

jhum fields. The SOC content further decreased with subsequent cropping which may be due to the agricultural activities involved in jhum cultivation that made the soil more susceptible to erosion and lowest SOC during autumn season maybe because a considerable amount of organic matter is lost during crop harvest which correlated with the present study as harvesting was done during autumn season in the current jhum field. These findings are in line with (Tian et al., 2005; Zodinpuii et al., 2016). Acccording to Wuest (2014), SOC can experience significant fluctuations in seasonal patterns over the course of a year, that vary depending on soil management and changes in SOC content are caused by seasonal plant residue, root, and exudate input or decomposition. SOC is crucial for regulating nutrients in soil which is influenced by several factors. This is in agreement with the significant correlation of SOC with other properties in the soil. SOC showed significant positive correlation ($p<.05$) with available N, available P, available K and silt content in all the four study sites (Table 2.15-22) which indicates that SOC is essential for regulating the availability of nutrients in the soil. Waqas et al. (2020) has reported through their findings that SOC and soil nutrients should be optimized together through management strategies for a sustainable crop productivity which shows that SOC and available nutrients are interrelated and dependent on each other. The present study shows that proper management of SOC is important for sustaining soil productivity, crop security and protection from land degradation.

2.6.4 Soil available nutrients (N, P, K)

In order to preserve soil health and agricultural sustainability, it is necessary to examine how agricultural practices related to various soil management activities affect the environment, water, and soil as well as agricultural productivity (Eivazi et al., 2003). A successful crop production depends on the presence of enough plant nutrients since soil fertility directly influences plant development and productivity, and quantification of soil macro- and micronutrients is critical for determining soil health. One of the most important macronutrients in soil for plant growth and production is soil NPK (Pandey, 2018). In the present study, soil available N, available P, and available K were examined in all four study sites, and the influence of different soil management practices on the soil nutrients. To check whether there is a significant relationship between the soil available N, P, and K and the other soil properties a correlation was established to assist in the study. The results showed a positive correlation of soil available N, available P, and available K with other soil properties and significant ($p<.05$) positive correlation was established among available N, available P, and available K, and also between the available N, available P, and available K with moisture, temperature, silt, clay and SOC (Table 2.15-2.22). The mean value of available N (Table 2.10), available P (Table 2.11) and available K (Table 2.12) under different study sites followed the order Minkong reserve forest>abandoned jhum land>tea garden>cultivated jhum land throughout the study period.

2.6.4.1 Variation in soil available N

Soil N is the most limiting nutrient in the soil for plants and is available for plant uptake in inorganic forms as ammonium (NH_4^+) and nitrate (NO_3^-). Maintaining optimum N in the soil is crucial as excess N is harmful to plants and insufficient N can effect plant growth and reduce plant productivity (Broadbent, 1986; Bhuyan et al., 2014). In comparison to the other three study sites, available N was higher in Minkong reserve forest. This difference can be attributed to the higher organic content brought about by the addition of more plant litter, which increases the number of soil microbes and aids in the decomposition and release of nitrogen in the soil. This result is consistent with several workers such as Kumar et al. (2013) who reported that forest soil has more nitrogen content than other agricultural soil because it receives more dead organic matter, has beneficial microbes present, and has a higher mineralization rate. Karam et al. (2013) found relationship between the quantity of organic matter and the accessibility of nitrogen in forest soil. Additionally, Rawat et al. (2021) mentioned that soil texture can also influence the availability of the nitrogen in the soil and a loam to sandy loam soil texture is favourable for higher available N content. Whereas the cultivated jhum land, abandoned jhum land, and tea garden all had lower levels of organic matter and microbial diversity than Minkong reserve forest. This could be the cause of the lower levels of available nitrogen in these three study sites when compared to the Minkong reserve forest. It is also reported by other workers that extensive cultivation speeds up decomposition and oxidation process that lowers nitrogen content in soil (Tumayro and Tesgaye, 2021) which can be attributed to the lower available N content in the

present finding in cultivated jhum land and tea garden than the abandoned jhum land and Minkong reserve forest. Two-way ANOVA study between the interaction effect of site and season on available N was found to significantly vary (p<.01) during the study period. Significant positive correlation (p<.05) of available N was established with SOC indicating that with an increase in organic matter there an increase in available N, and a Significant positive correlation (p<.05) of available N with clay shows greater accumulation of available N with increased clay percentage in the soil. There was also a significant positive correlation (p<.05) of available N with available P and available K in all the study sites throughout the study period (Table 2.15-2.22).

Seasonal variations on soil available N content in the study sites showed an irregular pattern of fluctuation over the season during the study period. Available N was found to be highest during spring season in all the study sites except in cultivated jhum land it was highest during winter season, whereas lowest available N was found during summer season in tea garden, during autumn season in cultivated land, during winter season in abandoned jhum land and Minkong reserve forest. In the cultivated jhum land, higher available N recorded during winter season in the first year can be attributed to the large amount of soil organic matter accumulated in the soil surface during the fallow period of 7 years in the present study. The soil available N was higher before burning (winter season) and lower after burning which can be due to the lost of soil organic nitrogen during the burning as oxidized N gases and N_2 through thermal oxidation. This finding is consistent with several workers such as Raison (1979), Døckersmith et al. (1999), Eldiabani et al. (2018), who has reported that with an increase in temperature, the volatilization of ammonia (NH_3) and nitric

acid (HNO_3) is intensified and therefore use of fire for burning in jhum fields causes rapid volatilization and oxidation resulting in reduced avialable N in soil. The lower available N during autumn season in the first year can be due to the lower organic matter during autumn due to crop harvesting which concurs with Wibowo and Kasno (2021) who has mentioned that the soil capacity to hold onto nitrogen will increase and decrease with its organic matter concentration and suggested that by preserving the soil organic matter composition, the availability of nitrogen can be maintained in the soil. With the subsequent cropping phases, the available N content declined and was recorded to be lower in the second year than the first year. After one year of cropping, the present jhum land was abandoned for nutrient regeneration in the soil because the soil lost a significant amount of available N during the cropping year due to cultivation and agricultural practices like tillage, regular weed removal and burning of slashed vegetation which causes the changes in soil properties, increases soil erosion, and slashing and burning of vegetation exposes the soil surface to direct sunlight, rain, wind and also kills soil microbes present which are essential for mineralization process in the soil. The higher available N in the present study sites during spring season may have resulted due to the soil temperature and moisture which were more suitable than the winter and summer seasons for the mineralization of N. According to Mirza and Patil (2020), soil temperature and moisture are important factors for the mineralization of available N thereby increasing the accessibility of available N in the soil. Soil available N showed positive correlation with moisture and temperature and the positive correlation was found significant ($p<.05$) with moisture and temperature in the tea garden and in Minkong reserve

forest, available N showed positive significant correlation (p<.05) with temperature. The variations of soil available N content due to seasonal fluctuations was also found to be significant (p<.05) by one-way ANOVA analysis in all the study sites (Table 2.13).

Environmental factors and agricultural practices can cause a decrease in the amount of nitrogen that is available in the soil and the loss of nitrogen from the soil can be accelerated by soil erosion, leaching and vegetation loss (Tian et al., 2005). In cultivated jhum land, the higher available N during spring season can be due to higher organic carbon due to the addition of litter by burning. This concurs with Wibowo and Kasno (2021) where the authors have mentioned that the soil capacity to hold onto nitrogen will increase with its organic carbon concentration and by preserving the soil organic matter composition, the availability of nitrogen can be maintained in the soil. Correlation between available N and SOC established a positive significant relationship (r=-.85*, p=.001) in the present study. With the subsequent cropping phases, the available N content declined and was recorded lower in the second year as compared to the first year. After cultivating for one year, the present jhum land was abandoned for nutrient regeneration in the soil because the soil losses a significant amount of available N during the cropping year due to cultivation and agricultural practices like tillage, regular weed removal and burning of slashed vegetation which causes the changes in soil textural composition, increases soil erosion, and slashing and burning of vegetation exposes the soil surface to direct sunlight, rain, wind and also kills soil microbes present which are essential for mineralization process in the soil. During summer season there is increased rainfall in the study area which

intensifies soil erosion that might have caused some amount of available N in the soil to be lost through water runoff aided by the sloping topography of the study site, through leaching or percolation and rapid uptake of available N by the crops might have lowered the content of available N in the soil. Yu-song et al. (2016) have also reported results similar with the current finding. In the present tea garden soil, the higher level of available N content during spring season may be attributed to increased SOC which was found to be significantly and positively correlated (r=-.85*, *p*=.001; r=-.78*, *p*=.003) due to addition of litter in the soil surface from dead decayed pruned tea leaves and branches and lower available N content during summer season can be due to soil erosion with heavy rainfall and rapid absorption of soil available N by the vigorously growing tea plants during this season. The present finding is consistent with the results reported by Yuan et al. (2013) where the author has also found similar reasons for the variation in available N content due to seasonal fluctuations in tea garden soil. Tian et al. (2005) has highlighted in their work that available N is highly sensitive to changes on the soil surface therefore vegetation loss, tillage and use of fire for the burning of vegetation can greatly change the N content in the soil as such activities can aggravate the soil. In the abandoned jhum land and Minkong reserve forest, the available nitrogen was found to be higher in the spring season that can be attributed to the favourable soil temperature and moisture, which might have accelerated the soil mineralization process after a cold and dry winter season. Mirza and Patil (2020) has also mentioned that soil nutrients are typically accessible during spring and late-spring when the soil temperature and moisture are suitable for mineralization. Winter recorded the lowest available nitrogen which

could be due to the low moisture and temperature during this season as low moisture and temperature are associated with slower mineralization rates (Hajiboland, 2017; Ray and Mukhopadhyay, 2012).

2.6.4.2 Variation in soil available phosphorous

The variations of soil available P content due to seasonal fluctuations was found to be significant ($p<.01$) by one-way ANOVA analysis in all the study sites (Table 2.14). In the present study, available P was higher during spring season and was found to be least during summer season in Minkong reserve forest and abandoned jhum land. This can be due to the organic matter accumulated during the winter season as availability of phosphorus rise with the addition of organic matter in the soil. Therefore with a moderate rise in temperature after a cold and dry winter and with the help of active soil microbial decomposers, the mineralization of organic matter is increased resulting in a release of higher available P into the soil. The lower soil available P during summer season may be due to the loss of the available P through water runoff due to heavy rainfall received during summer in the study area and uptake of available P by the roots of the actively growing plants in the forest and abondoned land during summer. The present finding is in line with several workers such as Weaver and Forcella (1979), Omer et al. (2018), who also reported similar results. The spring season recorded the highest soil available P in the tea garden which may be related to the accumulation of leaf litter in the soil that increases the organic matter content because each time tea plants are pruned, significant amount of leaf litter is returned to the soil in the tea garden (Arafat et al., 2020; Yuan et al.,

2013) resulting in more accumulation of available P in the soil. The amount of the available P in the soil decreased during the summer season due to the quick uptake by the tea crop and the heavy rains that accelerated the loss of the nutrients from the soil through surface runoff. This result is consistent with Baruah et al. (2012) who found that while higher rainfall during the wet season speeds up the mineralization of organic P to available P, it also causes higher losses through leaching and runoffs in tea garden belts of Golaghat district, Assam, India. In the cultivated jhum land, the available P was higher in the spring season in the first year, this may have been due to the addition of ash after burning slashed vegetation in the first year, and in the second year, the higher available P during the autumn season may have been due to the accumulation of higher organic matter from the leftover plant debris after harvest in the first year as well as due to the leaf litters of the plants that grew in the soil that remained undisturbed after the land was abandoned whereas uptake of available P by plant and loss through surface runoff may be the causes of the lower available P during the summer. The current result can be further ascertained by the positive significant correlation found between available P and SOC (r=.96**, *p*=.001) in the first year and (r=.60*, *p*=.04) in the second year under cultivated jhum land. The present result concurs with the finding of other workers such as Kumar et al. (2017) in jhum field of Arunachal Pradesh, India and Tulaphitak et al. (1985) in shifting cultivated land, Thailand.

According to the results obtained from the current study, the amount of available P content in Minkong reserve forest ranged from 42.24-53.89 $kgha^{-1}$ in the first year and 42.28-55.66 $kgha^{-1}$ (Table 2.11) in the second year, which shows that

the available P belong to the medium low- medium category and in abandoned jhum land the amount of available P ranged from 20.29-45.47 kgha^{-1} in the first year and 38.87-52.92 kgha^{-1} (Table 2.11) in the second year, which shows that the available P belong to the low- medium low category. From the present result, it shows that available P increased from first year to second year in both the study during the study period. This can be attributed to the long fallow period in abandoned jhum land and conservation and protection of the natural environment of the reserve forest from anthropogenic activities which was continuously supplemented with plant litter and dense vegetation for a very long time, making the soil richer in nutrients and possibly the rapid rates of P cycling in the soil surface through decomposition and mineralization of more rich P helped maintain greater concentrations of available P in these two study sites. Positive correlation was observed between available P and SOC however it was significant only in abandoned jhum land (r=.66*, *p*=..02; r=.75**, *p*=.01). This is in accordance with Zhao et al., (2018) research on the effects of different land cover changes on various P fractions, and Manjunatha and Singh (2020), who studied on jhum fields in the West Garo Hills District of Meghalaya, and has reported in their study that a longer jhum fallow period and a minimal disturbance in the forest soil leads to greater accumulation of available P.

In the present cultivated jhum land, the available P ranged from 14.65-34.65 kgha^{-1} in the first year and 17.45-29.25 kgha^{-1} (Table 2.11) in the second year which shows that the available P belong to the low-medium low category. In the cultivated jhum land, during the first year, the available P was highest after burning the slashed vegetation. This may be because of the addition of P through ash that increased the

available P but it decreased afterward with the following cropping period. Available P was found to be lower in the second year than the first year, which is an indication that the agricultural practice involved in jhum cultivation in the present Mokokchung district resulted in a decline in soil organic matter. The amount of organic matter content in the soil regulates the increase and decrease of available P, similarly in the present study the available P showed positive significant correlation with SOC in both the years (r=-.96**, *p*=.001; r=-.60*, *p*=.04). In the second year, the land was abandoned for a natural regeneration of the soil nutrients, with only the crop residue left after harvest that provides minimum protection of the soil from runoff and erosion and later on succession by weeds that further decreases the available P in the soil. The present finding is in line with several workers such as Wapongnungsang (2018), Mishra et al., (2017) and Tripathi and Barik, (2003) who reported similar observation in their study on the effects of shifting cultivation in Northeast India. In the present tea garden, the available P ranged from 20.29-45.47 $kgha^{-1}$ in the first year and 38.87-52.92 $kgha^{-1}$ (Table 2.11) in the second year which shows that the available P belong to the low-medium category. The tea garden soil also showed a decrease in the availability of P from first to second year of cultivation during the study period. The available P in the present tea garden was influenced by the type of the inherent parent material and also due to the acidic nature of tea garden which may increase the fixation of the P that leads to a decrease in soil available P. This finding is in conformity with Temsurenla (2021) who also reported similar level of available P in tea garden soil of Mokokchung district, Nagaland, and found that several factors including soil *p*H, type of parent soil material, altitude, moisture and

temperature affected the availability of the P in the tea garden of the district. Similarly, in the present tea garden, the soil available P showed significant positive correlation with SOC (r=.98**, p=.001), available N (r=.90**, *p*=.001; r=.65*, *p*=.02), moisture (r=.65*, p=.02) shown in table 2.15-2.22.

Two-way ANOVA study between the interaction effect of site and season on available N was found to significantly vary ($p<.01$) during the study period. The available P content was higher in the Minkong reserve forest and abandoned jhum land than the cultivated jhum land and tea garden which can be because a considerable amount of available phosphorus can be lost through soil erosion intensified by crop removal in cultivated areas (Rawat et al., 2021). However the soil of Minkong reserve forest and abandoned jhum land was not disturbed because no agriculture was practiced at these two study sites which may have resulted in a higher retention of the soil's accessible P. Available phosphorous was recorded between 42.14-55.66 $kgha^{-1}$ in Minkong reserve forest, 30.10-59.78 $kgha^{-1}$ in abandoned jhum land, 14.65-34.65 $kgha^{-1}$ in cultivated jhum land and 20.29-52.92 $kgha^{-1}$ in tea garden (Table 2.11) which reveals that cultivated jhum land and tea garden has much less accessible phosphorus than Minkong reserve forest and abandoned jhum land which can be attributed to the soil texture, and higher soil *p*H of the abandoned jhum land and Minkong reserve forest. This result is consistent with several workers such as Rawat et al. (2021), Bueis et al. (2019), and Hipps et al. (2005).

2.6.4.3 Variation in soil available potassium

Avaialbility of K in the soil is influenced by several factors such as the inherent parent material, soil *p*H, moisture, temperature and geographical location (Temsurenla and Ajungla, 2017). Potassium is one of the most important macronutrient along with nitrogen and phosphorus for plant growth and is required for almost all the biochemical and physiological processes for plant development (Wang et al., 2013). Potassium is known to be one of the limiting growth factor for tea plant (Huang et al., 2022) as 90-98% of the total potassium in the soil is unavailable to plants (Sindhu et al., 2014) and its deficiency can result in reduced production in the number and size of leaves (Pettigrew, 2008). It is important to maintain an optimum level of potassium along with the other macronutrients because an unbalanced level of nutrients in the soil can cause fixation of the nutrients that can lead to a reduction in available form of K to plants (Xu et al., 2020). The available K was found to be highest during spring season except in tea garden where it was recorded highest in autumn season whereas it was recorded to be lowest during summer season in the Minkong reserve forest, cultivated jhum land and abandoned jhum land and during winter season in the tea garden. In the current study, it was observed that the available K was influenced by seasonal fluctuation and other soil properties. Significant correlation ($p<.05$) between available K with the soil properties such as soil *p*H, soil texture, SOC, available N, available P, moisture and temperature was observed in the present study (Table 2.15-2.22). Seasonal variations on soil available K content in the study sites showed an irregular pattern of fluctuation over the season between the study sites. The variations of soil available K

content due to seasonal fluctuations was found to be significant (p<.01) by one-way ANOVA analysis in all the study sites (Table 2.14) and further, a two-way ANOVA study between the interaction effect of site and season on available K was found to significantly vary (p<.01) during the study period. The higher content of available K during spring and autumn season might be due to the ashes added into the soil by burning of the slashed vegetation during the winter season in the cultivated jhum land that lead to an increase of available K in the spring season (Yeboah et al., 2022; Mishra and Francaviglia, 2021), spring and autumn season recorded the highest SOC in the present study sites which can be the reason for the increased available K during these seasons (Chase and Singh, 2014). There was a positive correlation between SOC and available K and it was significant in second year (r=.73**, p=.01) in cultivated jhum land. Lowest available K during winter may be due to the harvesting of the crops that result in reduced basic cations (Kebebew et al., 2021) as pruning of tea leaves and branches was carried out during December-February (winter season) in the present tea garden. Additionally, Zhu et al. (2012), has mentioned that the soil losses a considerable amount of soil organic matter input during crop harvesting which can result in reduced availability of K in the soil and Weaver and Forcella (1979), who has reported that lower microbial activity could lead to a decrease in soil available K. During winter season there is reduced cation exchange in the soil due to low *p*H which causes a decrease in the binding capacity of K+ ions to the negatively charged clay complex (Temsurenla, 2021; Rawal et al., 2021) and during summer season the available k can be reduced through leaching by increased rainfall (Ralte 2017).

The mean value of available K was higher in Minkong reserve forest (150.99-271.71 $kgha^{-1}$) followed by abandoned jhum land (130.57-208.54 $kgha^{-1}$), tea garden (94.29-149.11 $kgha^{-1}$) and lowest in cultivated jhum land (90.76-117.56 $kgha^{-1}$) which is shown in Table 2.12. Average value of available K in the present study showed that the available K ranged from very low-low in cultivated jhum land, low-medium low in abandoned jhum land, very low-low in tea garden and low-medium in Minkong reserve forest. This result is in line with Singh and Munth (2013) who has reported that the average content of available K in soil of Nagaland is low to medium. This can be due to increased competition for cation exchange capacity sites because in acidic soil there is high concentration of H^+, Al^{3+}, Fe^{3+} that results in low availability of K in the soil (Rawal et al., 2021) and in the present study, the soil was acidic in nature. Additionally, the lowest soil available K recorded in cultivated jhum land can be due to leaching, lower clay percentage, regular tillage that depleted the available K, higher uptake of available K by the growing crops which were not replaced because of low soil organic matter in the cultivated jhum land unlike in Minkong reserve forest and abandoned jhum land that was replaced by the litter fall from trees that remained undisturbed by any agricultural practice thereby increasing the accumulation of soil organic mattter. Similar result has been reported by Tumayro and Tesgaye (2021). The higher soil available K content in the Minkong reserve forest and abandoned jhum land than the tea garden and cultivated jhum land can be attributed to the higher soil organic matter during the present study. As reported by Girma (2020), that agricultural practices involved in cultivation like tillage, weed removal and harvesting of crops with little input of organic matter into the soil

disturbs the distribution of available K and enhances in the depletion of available K from the soil. This could be the reason for the lower available K content in the tea garden and cultivated jhum land. Correlation established a significant positive relationship with silt, clay, SOC, available N, available P, moisture, temperature and a significant negative correlation with sand during the present study (Table 2.15-2.22). Several other workers have reported that the available K is mostly influenced by the inherent parent materials, proportion of clay percentage in the soil, contents of exchangeable bases, amount of soil organic matter in different study sites such as in tea cultivated soil (Temsurenla and Ajungla, 2017; Chien et al., 2019; Gogoi et al., 2016), in fallow jhum land (Nielsen and Calderón, 2011; Kozak and Pudelko, 2021), in forest land (Gemeda and Datt, 2018) and in jhum cultivated land (Bimantara et al., 2022; Tian et al., 2005; Juo and Manu, 1996) and also by the use of K fertilizers (Yu-song et al., 2016). However in the present study, K fertilizers were not used in any of the study sites and can be another reason for the low available K in the present study. According to Kumar et al., (2013), a higher accessibility of available K in the forest soil indicates that the soil nutrient content is undisturbed which leads to higher accumulation of available K for the uptake by trees and plants and a lower value of available K in the cultivated area can be because of leaching and soil erosion.

2.7 Conclusion

These results in the present study shows that the different land use type for agricultural purpose affected the soil physicochemical properties. The soil under the Minkong reserve forest showed the SOC, available nutrients, silt, clay, moisture and

*p*H invariably the highest value of all the soil parameters whereas cultivated jhum land, showed the lowest value of all these soil parameters. The present study revealed that the greater accumulation of the plant litter with minimial anthropogenic disturbances in the Minkong reserve forest was the possible reason for the current observations as compared to cultivated jhum land where agricultural practices such as use of fire for burning slashed vegetation, tillage and crop harvesting intensified soil erosion coupled with high rainfall in the Mokokchung district was the major reason for the reduction in SOC and available nutrients. The soil in abandoned jhum land showed lower values in term sof available nutrients than Minkong reserve forest but higher than the tea garden and cultivated jhum land. This indicates that the jhum fallow length plays an important role in regaining and keeping up the soil health in shifting cultivated land in Mokokchung district. The results shows that a fallow period of 10 years can accumulate a substantial amount of soil nutrients that can support plant growth and productivity however the soil under abandoned jhum land could be managed better with different management strategies so as to further enhance the soil fertility and to make jhum cultivation sustainable in the study site as depletion of soil nutrient with continuous cultivation and soil disturbtion after every fallow rotation can cause adverse affect on the soil heath. The cultivated jhum land which was slashed and burned after 7-years of fallow period in the present study, showed lower soil nutrient than the 10-years of abandoned jhum land. The SOC and available nutrients was significantly ($p<.05$) reduced by a great amount during the cropping period which is a matter of concern in jhum cultivation in the present study. The current cultivated jhum land was abandoned after one year of cropping. The jhum

fallow land after abandonment is left to naturally regenerate its soil fertility for a period of time before it is cleared for cultivation. Therefore, from the present investigation, during the jhum abandonment period in the present study site, scientific intervention is needed and other alternate soil management strategies in relation to jhum fallow ages should be adopted for a healthy and sustainable soil management. Several workers such as Devi and Choudhury (2013), Ramakrishnan (1984), Arunachalam (2003), Mishra and Francaviglia (2021), Kuotsuo et al. (2014), Tripathi and Barik (2003), Bhan (2009) and Rathore et al. (2010), have recommended alternate management strategies in the jhum fallow land such as agroforesty, minimize burning by slashing and mulching of the vegetation, interplanting of leguminous crops which could enhance restoration of the degraded jhum land. The results observed from the present tea garden shows that the soil under the tea garden was very low-medium in available nutrients (N,P,K) that can affect the growth and productivity of tea plant. Production of high quality tea leaves require a large amount of nutrients which can be supplemented through organic manure, plant residues and chemical fertilizers. In the present study, no chemical fertilizers or organic manures were used except for the plant residues as a source of organic matter which might have resulted in the low-medium content of available nutrients in the soil. Even though the soil pH was found to be favourable for the growth of tea crop, continuous monoculture such as tea crop is known to cause soil acidification in the long term and a decline in available nutrients (N,P,K) due to leaching. Workers like Li et al. (2016) have reported that the use of plant residues as a source of organic matter can still cause soil acidification in the lower layers of the tea cultivated soil and have

suggested no-till farming and lesser plant density in the tea cultivated areas to reduce the leaching of soil nutrients and soil acidification. The Minkong reserve forest under Mokokchung district, is protected by the Nagaland state government, and the soil under the forest was more suitable in terms of organic matter content, nutrient status and soil texture to support plant growth than the other study sites. Therefore, the available soil resources should be further maintained and strategies should be adopted to protect the associated biological diversity from agricultural expansion in the present studied area. Therefore, it can be concluded that the different agricultural practices and its management activities can affect the soil properties and the essential nutrients.

The present study can be used as baseline information for assessing the soil quality and for the better management of the soil in cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest in the Mokokchung district of Nagaland. Further detailed studies are recommended to better understand the effect of agricultural practice on soil physicochemical properties along with the plant response and productivity and nutrient requirement by the plants. Further researches in jhum land with different fallow period, tea garden of different age and other natural forest with varying altitudes in the district should be studied to compare and examine its effect on soil physicochemical properties for a sustainable land management.

CHAPTER 3

Study on Soil Microbial Diversity

3.1. Introduction

Soil acts as a reservoir for a diverse range of microorganisms. Soil microorganisms, especially bacteria and fungi play vital role in ecosystem functioning because of their ability in regulating biogeochemical cycle (Ali et al., 2021; Jacoby et al., 2017). The variation in diversity and distribution of soil bacteria and fungi occurs between and within the region which are affected by several factors such as geographical location and type of vegetation (Kaushal and Singh, 2013), variation in soil physicochemical properties (Díaz-Raviña et al., 1995), seasonal fluctuations linked with changes in climatic factors (Pandey et al., 2022) and different land-use (Szoboszlay et al., 2017). It is extremely important to have knowledge about the type and number of soil bacteria and fungi that exist in any terrestrial ecosystem as microbial activities is linked with the soil fertility status, plant growth and productivity. They act as useful indicator in the turnover of soil organic matter in the soil ecosystem and thereby contribute appreciably to soil nutrient cycling (Arunachalam, 2003; Gömöryová et al., 2020). However, the ever-increasing human population has put a pressure on land for increasing agricultural productivity which has resulted in deforestation coupled with different agricultural practices that consequently affects on soil microbial population (Joshi et al., 2019; Ding et al., 2013; Dhull et al., 2005). Therefore, preserving the ecosystem integrity is prerequisite for a sustainable ecosystem functioning (Ullah et al., 2019).

There are about 1.5 million fungal species distributed worldwide, out of which only 5% has been characterised so far (Sharma et al., 2015) and according to Ingham (2009), 1 gm of soil contain 100 million to 1 billion of bacteria. Therefore,

there is a need to explore the unexplored bacterial and fungal diversity and record the identified bacterial and fungal species. The isolation of soil fungal and bacterial species in different culture media and characterization through morphological, microscopical and biochemical studies is the oldest and commonly used method for the identification of soil fungi and bacteria (Thathana et al., 2017; Jamir and Ajungla, 2018) along with molecular methods which helps in extraction of the fungal and bacterial DNA for its identification up to species level (Mushimiyimana et al., 2016). In Nagaland, jhum cultivation is prevalent and tea cultivation is emerging as an important economic crop that involves different agricultural practice and information on the soil microbial population is scanty in these areas (Bhan 2009; Konwar, 2017; Temsurenla and Ajungla, 2017). Therefore, it provides a unique and excellent medium to study the soil microbial diversity for the present work. To understand the variation in soil bacterial and fungal communities in agricultural and forest land, it is necessary to enumerate the soil microbial population and analyse the factors that affects the type and number of the soil microbial communities (Mishra and Francaviglia, 2021). This will provide an opportunity for documenting both the explored and unexplored native soil bacterial and fungal population which can be useful information for proper management of soil health (Jamir et al., 2022a). In the present study, soil bacterial and fungal population was enumerated in culture media for colony forming unit (CFU) by serial dilution. Identification of soil bacteria was done through phenotypic, biochemical test and 16S *r*RNA sequencing method whereas soil fungal population was identified through morphological and microscopical studies. The current work was undertaken to study the variation in

soil bacterial and fungal population over different season and selected study sites namely cultivated jhum land under Khensa village, abandoned jhum land under Mekuli village, tea garden under Longmisa village and Minkong reserve forest under Chuhchuyimpang village, in Mokokchung district, Nagaland.

3.2. Materials and methods

3.2.1. Enumeration of soil fungi and bacteria

For enumeration of fungi and bacteria, serial dilution method was used (Johnson and Curl, 1972). Isolation of fungi was carried out in Rose bengal agar (M842), Czapex dox agar (M075), Malt extract agar (M137), Potato dextrose agar (M095) and for bacteria, McConkey agar (M082), Nutrient agar (M001), nutrient broth (M002), Jensen's agar (M710), and Simmons citrate agar (M099) were used. All the culture media was purchased from Himedia.

3.2.2. Sterilization and preparation of agar medium

Ahead of preparing the culture media, all the required glasswares and necessary equipments were washed, dried and autoclaved for 30 minutes at 121 ºC. For preparing both fungal and bacterial culture media, instruction provided by the manufacturer (Himedia) was followed. Media were boiled to melt the agar and autoclaved at 121 ºC for 15 minutes. The culture plates were prepared in horizontal laminar air flow system for fungi and bacteria and in each of the fungal culture media before pouring into culture plates 30 µg/ml streptomycin sulphate (for inhibiting bacterial growth) was added. The culture plates with the media were all treated with UV radiation in the laminar air flow for 5 minutes before inoculation.

3.2.3. Serial dilution

One gram of soil sample was taken into a test tube containing 10 ml of sterilized distilled water to give 10^{-1} dilution. For an even distribution, the test tube was closed with the test tube cap and shaken for few minutes. From this, 10 ml was pipette out to another test tube containing 9 ml of sterilized distilled water to make 10^{-2} dilution. Likewise, a series of dilution was made serially up to 10^{-6} (Waksman 1922).

3.2.4. Estimation of fungal population

For fungal isolation, 100 µl of 10^{-3} and 10^{-4} dilution was used for inoculating culture plates with Rose Bengal Agar (RBA) and Potato Dextose Agar (PDA) medium in triplicates, spread evenly with the help of a spreader and plates were sealed with parafilm. Culture plates were inoculated in triplicates for each sample and all the plates were incubated at 25 ± 2° C for 5-7 days upside down in incubator. All the colonies observed were further sub-cultured and pure cultures were maintained in four different culture media for each fungal species (RBA, PDA, MEA and CDA) and incubated at 25 ± 2° C for 5-7 days in an incubator.

Estimation of Colony forming unit (CFU) of fungi was estimated by counting the number of fungal colonies. An average of the triplicates of each culture media was noted and combined. The CFU of fungi per gram soil was calculated by following the formula (Johnson and Case, 2007):

$$CFUg^{-1} = \frac{\text{Number of fungal colonies X Dilution Factor}}{\text{Volume of inoculum(ml)}}$$

3.2.5. Identification of fungi

All the pure fungal isolates were identified based on their morphological (colony colour, margin, shape, structure and size, presence and absence of exudates) and microscopical structures (shape of conidiophore, mycelium, arrangement of conidia on conidiophore) along with the help of following standard identification manuals and available literature (Afzal *et al*., 2013; Asan, 2004; Bamett 1965; Carmen and Sciortino, 2017; Domsch, 1980; Duss and Laane, 1984; Frisvad and Samson, 2004; St Germain and Summerbell, 1996; Gilman, 1957; Gilman, 2001; Giraldo et al., 2015; Hauser, 2006; Ho *et al*., 2004; Jayasiri et al., 2019; Klich, 2002; Leslie and Summerbell, 2006; Nelson et al., 1983; Raper and Thom, 1949; Rifai, 1969; Samson and Pitt, 2000; Samson et al., 2004; Samson et al., 2014; Siddiquee, 2017; Pitt and Hocking, 2009; Thathana *et al.*, 2017, Thilagam, 2018; Wagner *et al*., 2013, Watanabe, 1937; Watanabe, 2002; Webster and Weber, 2007). Isolated fungi were transferred onto a clean glass slide with a flame sterilized needle and stained with lactophenol cotton blue. The same needle was used to tease the fungi for a thin spreading smear in the lactophenol cotton blue slide. Coverslip was placed gently from one edge of the stain. Microscopic examination including mycelium, hyphae shape, conidial development, conidial shape, conidiophore dimension, size and shape of metulae, philiade, chlamydospores spores and other special fungal structure were observed using cotton blue stain.

3.2.6. Preparation of microscopic slides

In a clean and dried glass slide, a drop of lacto phenol cotton blue was taken and a small amount of mycelia from the pure culture plate was picked with the help

of a sterilised needle and smeared on the glass slide. The slide was studied under a microscope (Moitic, BA210LED) at different magnifications (10x, 40x and 100x) and microscopic images were taken at a bar scale of 10µm and features such as shape, length and size of conidiophore and conidia, metulae, arrangements of spores, philiade were observed and examined.

3.2.7. Estimation of bacterial population

Isolation of bacteria was carried out by inoculating 100 µl of 10^{-5} and 10^{-6} serial dilution on four different culture media (Nutrient agar, Simmons citrate agar, Jensen's agar and Mc conkey) in triplicates. It was spread evenly on the surface of the culture media in the petri plates and sealed with parafilm. All the inoculated culture plates were incubated for 48 hours at 28°C±2. From the colonies observed each possible species of the soil microbe was taken with a sterile culture loop and streaked and re-streaked onto freshly prepared NA for purification followed by incubation for 24 h at 28°C±2. Each of the purified isolates were inoculated on nutrient broth for extracting bacterial DNA and transferred in to a shaking incubator at 250 rpm at 28°C±2 for 48 hours. For maintaining the pure culture isolates glycerol stock (20%) was prepared and stored at -20°C for further analysis.

Estimation of Colony forming unit (CFU) of bacteria was done by counting the number of bacterial colonies. An average of the triplicates of each culture media was noted and combined. The CFU of fungi per gram soil was calculated as (Johnson and Case, 2007):

$$CFUg^{-1} = \frac{\text{Number of bacterial colonies X Dilution Factor}}{\text{Volume of inoculum(ml)}}$$

3.2.8. Identification of bacteria

Identification of all the pure cultured bacteria was carried out based on phenotypic characterization such as colony morphology, gram staining, citrate utilization test, sugar fermentation test, catalase test, Methyl red test and Voges Prokauer test (Cappuccino and Sherman, 2002; Godkar, 1999). By following standard protocol, the results were examined (Garrity et al., 2005; Holt et al., 1994; Ludwig et al., 2009) and by using ABIS online software version 12, a laboratory tool for identification of bacteria based on the morphological and biochemical characters. Furtehrmore, molecular characterization was carried out by 16S *r*RNA sequencing technique.

3.2.8.1 Phenotypic characterization

Colony morphology

The pure culture isolates were studied for colony morphology such as colour, shape, margin and texture.

Gram staining

Gram staining was done for the all the pure isolates by following standard procedure. A smear of each of the bacterial cells was made on sterilized glass slide and fixed by gently sliding the glass slide over a flame. Then, it was flooded with crystal violet solution for one minute and smear was washed carefully with distilled water and mordant Gram's iodine was used. After this, the smear was decolorized with 95% ethyl alcohol and again rinsed with distilled water. For counterstaining, safranin was used for 60-80 sec and washed with distilled water. Slides were then

examined under microscope at 100x magnification. Gram-positive bacteria appeared crystal violet in colour whereas gram negative bacteria appeared to be pink in colour.

Catalase test

The presence of enzyme catalase in the bacterial isolates that breakdown hydrogen peroxide (H_2O_2) into oxygen and water was performed through catalase test. Fresh pure cultured bacterial colonies (16-18 hours) from the nutrient agar plate was taken and smeared on an autoclaved glass slides. 2-3 drops of 3% H_2O_2 was added and mixed with the help of a loop. The presence of the catalase enzyme was evident in the bacterial isolate by the formation of oxygen bubbles

Oxidase test

Oxidase test is done to confirm the presence of the enzyme cytochrome oxidase in bacterial isolates that catalyses the oxidation of reduced cytochrome by oxygen. Fresh cultured bacteria of 16-18 hours were used for the test. Oxidase disc soaked in sterilized water was transferred to a sterilized glass slide. The bacterial isolate was confirmed oxidase positive if it changed to purple colour within 5-10 seconds.

Citrate utilization test

This test is done to test an organism ability to utilize sodium citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole of the nitrogen. Bacteria that can grow on the medium can produce an enzyme called citrate permease which can convert citrate to pyruvate. The pyruvate enters the organism's metabolic cycle of the production of

energy and when bacteria metabolize the citrate, ammonium salts gets breakdown to ammonia that leads to an increase in alkalinity that results in the change of the bromothymol blue indicator in the medium from green to blue colour above *p*H 7.6.

The simmon-citrate medium was prepared and autoclaved and poured into glass vials. The bacterial isolate was streaked on to the autoclaved citrate medium. Incubation was done aerobically at at 37 ºC for 48 - 72 hours at 37 ºC. Colour change of the medium was observed from deep green to a prussian blue colour. Uninoculated vials were maintained as control.

Starch hydrolysis test

Starch hydrolysis was done to test the ability of the bacterial isolate in secreting extracellular enzyme that can breakdown large nutrient sources like starch into glucose sub-units outside the cell membrane so that it can be absorbed inside the cell. Fresh bacteria cultures (16-18 hours) were used for inoculating the agar medium in the culture plate and then incubated for 24-48 hours. After incubation, the surface of each of the culture plates with the bacterial isolate was flooded with gram iodine solution. Results were recorded positive for starch hydrolysis if a transparent clear zone appeared around the bacterial colony whereas lack of transparent clear zone indicated negative for the starch hydrolysis by the bacterial isolate.

Methyl Red -Voges Proskauer test

Methyl Red -Voges Proskauer test was carried out to determine the ability of the microorganisms to ferment glucose. Methyl red (MR) test identifies bacterial ability to produce stable acid end products by glucose fermentation and also

differentiates two major type of facultative enteric bacteria based on the production of the acid. The Voges Proskauer (VP) test shows the capability of the organism to produce acetyl methyl carbinol from fermentation of glucose and it differentiates two major types of facultative anaerobic enteric bacteria based on production of neutral products.

For the MR test, 10 ml each of MR-VP broth (glucose phosphate broth) was used for inoculating bacterial isolates and incubated at 37 ºC for 48 hours. After which, 5-6 drops of methyl red indicator was added to the bacterial broth. If the colour of the broth change from yellow to red it was identified as positive isolate whereas methyl red negative bacteria and control tube was identified by no change in the yellow colour of culture broth.

For the VP test, 10 ml of MR-VP broth (glucose phosphate broth) was inoculated with the bacterial isolates and incubated at 37 ºC for 48 hours. Afterwards, 5-6 drops of VP reagent I (α-napthol) and 2-3 drops of VP reagent II (40% KOH) were added to the bacterial broth. The test tube was shaken for 2-3 minutes and allowed to settle around 10-15 minutes. Change in colour to pink was observed in positive isolates whereas no colour change were identified as VP negative bacteria along with the control tube.

Triple sugar iron test

The triple sugar iron (TSI) test was done to test the ability of microorganism to utilize a certain carbohydrate such as lactose, sucrose and glucose and to produce hydrogen sulfide. Bacterial isolates were transferred and streaked in autoclaved TSI agar in gals vials for testing fermentation reaction, gas and H_2S production.

Incubation was carried out at 37 ºC for 48 - 72 hours. The following results were recorded:

i) Glucose fermentation was identified by an alkaline slant (red) and acid butt (yellow), with or without breaks in agar butt in the bacterial isolates.

ii) Lactose and or sucrose fermentation were identified by an acid slant (yellow) and acid butt (yellow), with or without breaks in agar butt in the bacterial isolates.

iii) An alkaline slant (red) and alkaline butt (red), with no change in agar butt were confirmed by no fermentation in the bacterial isolates

iv) iv) Gas and H_2S production was determined by cracking and blackening of the medium respectively in the tubes.

3.2.8.2. Molecular characterization of bacterial isolates by 16s *r*RNA gene

Reagents and chemicals used are as follows:

1x Tris-EDTA/ 1x TE buffer, pH 8.0

1.21 g of tris base and 0.292 g of EDTA was dissolved in 500 ml distilled water. The pH was adjusted with concentrated HCl and final volume of buffer was made to 1000 ml with sterilized distilled water, autoclaved for 15 minutes at 121℃.

10% SDS

10 g of SDS dissolved in 100 ml of sterilized distilled water

0.5 M EDTA, pH 8.0

186.12g EDTA.Na2 dissolved in 800ml sterilized distilled water and 50 ml of NaOH added, mixed and the final volume was made to 1000 ml with sterilized distilled water followed by autoclaving for 15 minutes at 121℃

Proteinase K (20 mg/ml)

To 1 ml of 50mM Tris-Cl (pH 8.0) and 1ml of a solution containing 1.5 mM calcium acetate, 20 mg of Proteinase K was added and dissolved and stored at -20 ºC

C:I (24:1)

For 500 ml, 20 ml of isoamyl alcohol was mixed with 480 ml of chloroform and stored at 4℃.

RNase (10 mg/ml)

10 mg of RNase was taken and made to dissolve in 1ml of 0.01 M sodium acetate (pH 5.2). It was heated for 15 minutes at 100 ℃ followed by cooling of the solution to room temperature. Afterward, 0.1 volume of 1 M Tris-Cl (pH 7.4) was added and stored at -20 ℃.

3M Sodium acetate, pH 5.2

In 70 ml of sterilized distilled water, 24.6 g of sodium acetate (anhydrous) was dissolved, glacial acetic was used to maintain acid pH and sterilized distilled water was used to make a final volume upto 100 ml.

70% ethanol

70 ml of ethanol mixed with 30 ml of distilled water to make 100ml of 70% ethanol

Running buffer (Stock 50x TAE), pH 8.0

242 g of Tris base, 57.1 ml glacial acetic acid and 100 ml 0.5 M EDTA (pH-8.0) and the final volume brought to 1000ml with sterilized distilled water from which 1X TAE (working buffer) was prepared

Ethidium bromide

10 mg of ethidium bromide (EtBr) powder was dissolved in containing 1 ml of sterilized distilled water and kept in a refrigerator

Extraction of bacterial genomic DNA

Genomic DNA extraction was done by following Sambrook and Russell (2001) and Wilson (2001) with slight modifications from bacterial cultures grown in nutrient broth within 48 hours at 28°C±2. 2 ml of the cultured broth was centrifuged at 12,000 rpm at 4℃ for 5 minutes and bacterial cells (pellets) were collected by discarding the supernatant. This was followed by adding 1X TE buffer (485µl), 10% SDS (30 µl) and 30 mg/ml proteinase K (3µl) for re-suspending the pellets and incubated for an hour at 60℃ in a water bath. After 1 hour of incubation, chloroform: isoamyl alcohol (C:I) in the ratio 24:1 was added and mixed by inverting the centrifuge tube for 5 minutes before centrifuging at 12,000 rpm for 10 minutes. The upper acqueous layer was pipetted out carefully and added 1µl of RNase solution and incubated for 10 minutes at 35℃. Addition of C:I to the and centrifugation at 12000 rpm for 10 minutes was repeated 3M sodium acetate (10 µl) added and the centrifuge tube was filled with ice cold isopropanol. The tube was kept at -20℃ for 5

-10 minutes following centrifugation at 12, 000 rpm for 10 minutes, discarding the supernatant, filling the tube with 70% ethanol, centrifuging again at 12, 000 rpm for 5 minutes and discarding the supernatant. Finally, the centrifuge tubes were dried by putting it upside down inside laminar airflow after which 1X TE (50 µl) was added to dissolve the bacterial DNA. Extracted DNA was checked by electrophoresis for the quality and estimation of quantity of the DNA approximately by using 1%

16S *r*RNA PCR amplification

PCR amplification was carried out in a Thermal Cycler (ThermoFisher, 5020). PCR amplification of the bacterial isolates was performed by bacterial 16S rRNA gene universal primer, 9ñ20F (5'-CGCGGGATCCGAGTTTGATCCTGGCTC-3') and 1492R (5′- GGCCGTCGACACGGATACCTTGTTACGACTT- 3′). The volume of each reagent used for the reaction mix is provided below:

Ingredient	**Quantity (µl)**	**Final concentration**
5X PCR buffer	12	1X
25mM MgCl2	3.6	1.5mM
10mM dNTPs	1.5	250 mM
10µM forward primer	1.5	0.25 µM
10µM reverse primer	1.5	0.25 µM
DNA	6	-
5 units (U) *Taq* DNA polymerase	0.6	0.05 U
sterile purified water	33.3	-

PCR reaction volume	60	-

PCR was carried out by the following program: denaturation at 94 ℃ for 10 min; then denaturation at 94℃ for 1 minute for 35 cycles, annealing at 55ºC for 45 seconds and extension at 72ºC for 1 min, finally, conclusion at 72ºC for 10 minutes. PCR products were checked by electrophoresis on 1.2% agarose gels after staining with ethidium bromide and visualized through a UV transilluminator and photographed through Gel Doc system (BIOSTEP, UST-20M-BE).

Purification and sequencing of amplified products of 16S *r*RNA gene was done using the commercial sequencing service provided by Bangalore (Barcode BioSciences Pvt. Ltd., Bangalore, India). Bioedit software was used to trim and remove ambiguous sequences and checked in NCBI GenBank by using BLASTN tool to compare with other sequences for identification of the species. Only the highest matching sequences were downloaded. The sequences were deposited to the GenBank database and accession numbers were obtained (www.ncbi.nlm.nih.gov).

3.2.9. Diversity analysis

Diversity indices sum up the species richness and abundance and they are essential as a primary approach towards estimation of the diversity of species. To evaluate the microbial diversity, Relative abundance, Simpson index of diversity (1-D) and Shannon-Weiner diversity index (H') were used for quantitative measure of the different species of in the study sites. Relative abundance is the propotion of a species in an ecosystem or sample of a community. The value of Shannon-Weiner diversity index (H') range from 0 to 5, and a higher Shannon-Weiner diversity index

(H') value indicates higher species diversity. In Simpson index of diversity (1-D), the value ranges from 0-1 and bigger the diversity index value, the greater is the species richness and relative abundance.

The diversity and dominance of the soil fungi and bacteria were calculated by the following formulas:

1. Shannon-Wiener index, calculated by the equation **(**Shannon and Weaver, 1949**)**

$$H' = -\sum_{i=1}^{S} (pi \ln pi)$$

Where, S = the total number of species

Ln= Natural Log

Pi = frequency of the ith species

2. The Simpson's index of diversity (1-D), calculated by the equation (Simpson, 1949)

$$1 - \mathrm{D} = 1 - \sum_{\mathrm{i}=1}^{\mathrm{S}} \left(\frac{\mathrm{ni}(\mathrm{ni}-1)}{\mathrm{N}(\mathrm{N}-1)}\right)$$

Where, ni = the number of individuals of species I which are counted

N = the total number of all individuals counted

3. Relative abundance which is the distibution of a species in an ecosystem or sample of a community was also calculated by the following formula as

Relative abundance (RA) was expressed as

$$RA = \frac{\text{Number of individuals of the same species}}{\text{Total number of individuals for all species}} \times 100$$

3.2.10. Statistical analysis

The significant seasonal variation for soil fungal population CFU^{-1} and bacterial population CFU^{-1} was done by one-way ANOVA and if the results were significant Tukey's *post hoc* test was carried out. Two-way ANOVA analysis was done to determine the effect of the season and study sites on the soil fungal and bacterial population. The significant difference was based with a probability of $p< 0.05$ and $p< 0.01$. Pearson's correlation was done to determine the relationship between the soil fungal and bacterial population with physicochemical parameters. The significant difference was based with a probability of $p< 0.05$ and $p< 0.01$. All the statistical analysis was done by using the software SPSS 16.0 for test of significance.

3.3. Results

3.3.1. Seasonal and site variation in colony forming Units ($x10^7$ $CFUg^{-1}$) of bacteria

In all the four study sites, seasonal variations in the bacterial population from the collected soil samples were observed. The mean value of the soil bacterial counts, recorded in different sampling season in cultivated jhum land at Khensa village, abandoned jhum land at Mekuli village, in tea garden at Longmisa village and in Minkong reserve forest at Chuchuyimpang village during 2016-2017 is shown in figure 3.1 and during 2017-2018 is shown in figure 3.2.

In cultivated jhum land, the soil bacterial counts ranged from 44-73 ($x10^7$ $CFUg^{-1}$) in the first year and from 41-71 ($x10^7$ $CFUg^{-1}$) during second year. In the first year, the soil bacterial counts was found higher during the summer season (73±1.73) followed by spring season (62±2.00), winter season (50±2.65) and autumn season (44±2.00) while in the second year, the soil bacterial counts was found higher during the spring season (71±1.73) followed by autumn season (62±1.00), summer season (53±1.00) and winter season (41±1.73).

In abandoned jhum land, the soil bacterial counts ranged from 62-106 ($x10^7$ $CFUg^{-1}$) in the first year and from 67-119 ($x10^7$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil bacterial counts was found higher during the summer season (106±2.65; 119±2.00) followed by autumn season (94±2.00; 89±2.00), spring season (75±2.00; 78±3.00) and winter season (62±2.00; 67±2.65).

In tea garden, the soil bacterial counts ranged from 52-98 ($x10^7$ $CFUg^{-1}$) in the first year and from 49-88 ($x10^7$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil bacterial counts was found higher during the spring season (98±1.00; 88±1.73) followed by summer season (85±1.73; 74±1.70), winter season (72±2.65; 57±1.73) and autumn season (52±2.65; 49±1.73).

In Minkong reserve forest, the soil bacterial counts ranged from 66-140 ($x10^7$ $CFUg^{-1}$) in the first year and from 70-136 ($x10^7$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil bacterial counts was found higher during the spring season (140±3.6; 136±1.73) followed by summer season (104±1.73; 101±1.7), autumn season (74±1.73; 84±2.65) and winter season (66±1.73; 70±2.65).

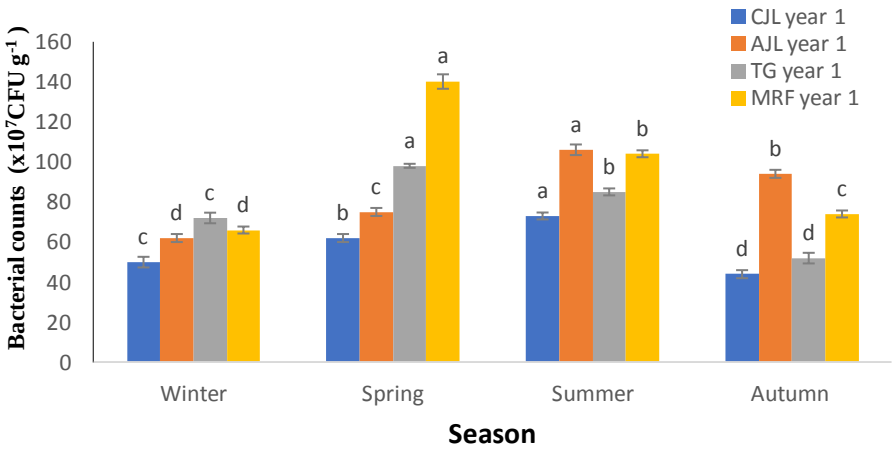

Figure 3.1. Bacterial counts (x10^7 $CFUg^{-1}$) among different sampling seasons during 2016-17 at cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean. Different letters ^{abcd} in each study site indicates the significant differences at p<0.05 as measured by Tukey's *post hoc* test.

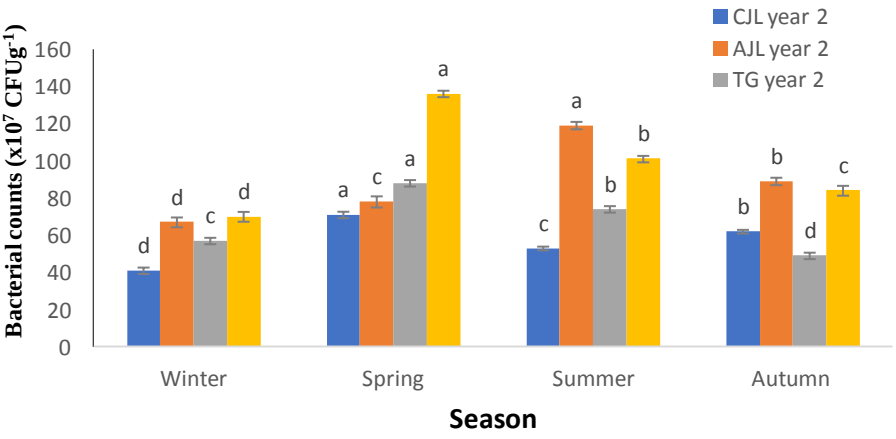

Figure 3.2. Bacterial counts (x10^7 $CFUg^{-1}$) among different sampling seasons during 2017-18 at cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean Different letters ^{abcd} in each study site indicates the significant differences at p<0.05 as measured by Tukey's *post hoc* test.

3.3.2. Seasonal and site variation in colony forming Units (x10^6 $CFUg^{-1}$) of fungi

The mean value of the soil fungal counts was recorded in different sampling season in cultivated jhum land at Khensa village, abandoned jhum land at Mekuli village, in tea garden at Longmisa village and in Minkong reserve forest at Chuchuyimpang village during 2016-2017 is shown in figure 3.3 and during 2017-2018 is shown in figure 3.4.

In cultivated jhum land, the soil fungal counts ranged from 26-59 ($x10^6$ $CFUg^{-1}$) in the first year and from 30-47 ($x10^6$ $CFUg^{-1}$) during second year. In the first year, the soil fungal counts was found higher during the summer season (26±2.00) followed by spring season (48±1.70), winter season (30±1.73) and autumn season (26±2.00) while in the second year, the soil fungal counts was found higher during the spring season (47±2.65) followed by autumn season (43±1.73), summer season (38±1.70) and winter season (30±2.00).

In abandoned jhum land, the soil fungal counts ranged from 71-54 ($x10^6$ $CFUg^{-1}$) in the first year and from 72-53 ($x10^6$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil fungal counts was found higher during the summer season (90±1.73; 94±1.70) followed by spring season (71±1.70; 72±2.65), autumn season (62±2.65; 62±2.65) and winter season (54±1.73; 53±1.73).

In tea garden, the soil fungal counts ranged from 39-71 ($x10^6$ $CFUg^{-1}$) in the first year and from 36-58 ($x10^6$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil fungal counts was found higher during the spring season (71±1.70; 58±1.15) followed by summer season (58±4.36; 55±2.00), autumn season (45±4.58; 43±2.65) and winter season (39±1.00; 36±1.53).

In Minkong reserve forest, the soil fungal counts ranged from 51-94 ($x10^6$ $CFUg^{-1}$) in the first year and from 64-96 ($x10^6$ $CFUg^{-1}$) during second year. In both the first year and second year, the soil fungal counts was found higher during the spring season (94±1.70; 96±2.65) followed by summer season (80±2.00; 92±2.6), autumn season (64±3.61; 76±2.65) and winter season (51±1.73; 64±4.00).

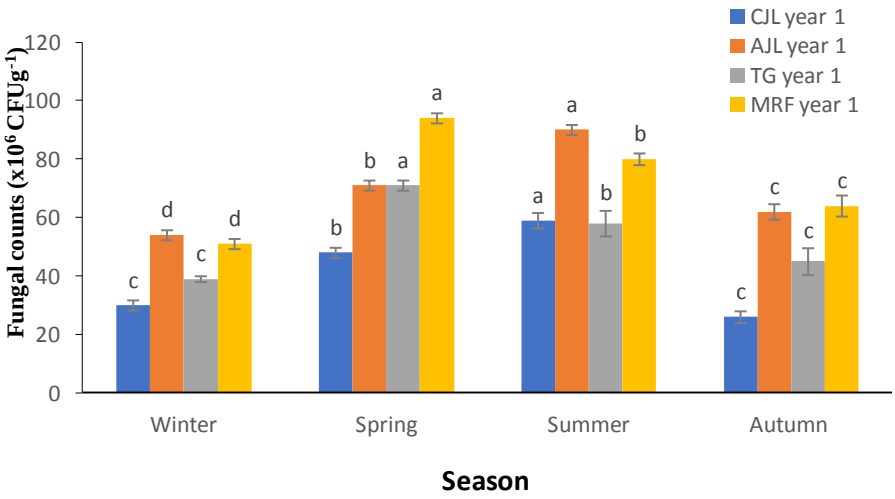

Figure 3.3. Fungal counts ($x10^7$ $CFUg^{-1}$) among different sampling seasons during 2016-17 at cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean Different letters ^{abcd} in each study site indicates the significant differences at $p<0.05$ as measured by Tukey's *post hoc* test.

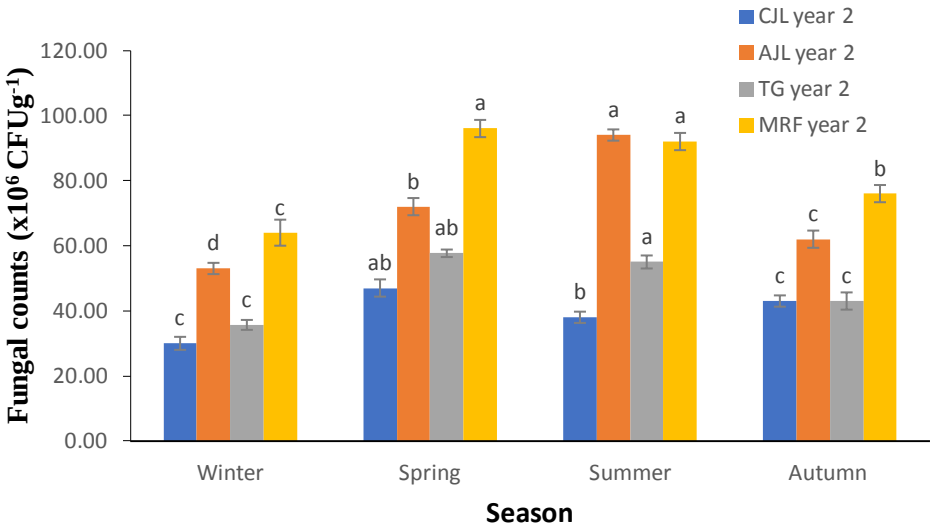

Figure 3.4. Fungal counts (x10^7 $CFUg^{-1}$) among different sampling seasons during 2017-18 at cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean Different letters ^{abcd} in each study site indicates the significant differences at $p<0.05$ as measured by Tukey's *post hoc* test.

3.3.3. Statistical analysis

Results from one-way ANOVA showed that variations in the bacterial and fungal counts were significantly ($p<0.5$) affected by seasonal fluctuations in all the study sites (Table 3.1) and a Tukey's *post hoc* test revealed significant difference in the bacterial and fungal counts between the season within each study site. From the results of two-way ANOVA, it was observed that the interaction effect of site and season significantly ($p<0.5$) affected the variation in the bacterial and fungal counts (Table 3.2).

Table 3.1. One-way ANOVA of bacterial and fungal counts during 2016-18 in CJL, AJL, TG and MRF

Parameters	CJL		AJL		TG		MRF	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)	F(3,8) p(value)
Bacterial counts	110.83 .001	246.38 .001	241.84 .001	250.46 .001	256.61 .001	304.67 .001	615.27 .001	486.55 .001
Fungal counts	169.12 .001	37.88 .001	179.69 .001	186.55 .001	55.34 .001	87.12 .001	183.09 .001	70.92 .001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table 3.2. Two-way ANOVA of bacterial and fungal count during 2016-18

Parameters	Source	2016-17		2017-18	
		***F*(3,8)**	***p*(value)**	***F*(3,8)**	***p*(value)**
Bacterial counts	Site	659.88	.001	1.07	.001
	Season	687.42	.001	727.42	.001
	SitesxSeason	199.35	.001	202.714	.001
Fungal counts	Site	413.88	.001	835.34	.001
	Season	410.76	.001	276.57	.001
	SitesxSeason	28.36	.001	32.27	.001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

3.3.4. Diversity indices

The diversity indices of soil bacterial species in all the four study sites were recorded (Table 3.3). Shannon-Weiner diversity index (H') and Simpon's index of diversity (1-D) was recorded highest in Minkong reserve forest (H'=2.29 and 1-D =0.92; H'=2.46 and 1-D =0.94) followed by abandoned jhum land (H'=1.69 and 1-D =0.86; H'=2.09 and 1-D =0.91), tea garden (H'=1.64 and 1-D =0.88; H'=1.64 and 1-D =0.85) and cultivated jhum land (H'=1.61 and 1-D =0.83; H'=1.28 and 1-D =0.70) in both first and second year repectively.The relative abundance of each soil bacterial species in the respective study site showed variation (Table 3.5). Throughout the study period, *Bacillus* sp. and *Bacillus subtilis* showed dominance over the other bacterial species across the four study sites in both the study year. The percentage contribution under *Bacillus* sp. and *Bacillus subtilis* in Minkong reserve forest showed the same percentage contribution by both the bacterial species with 0.21% each in the first year and in the second year, *Bacillus* sp. and *Bacillus subtilis* were dominant with 0.18% each in the second year. In abandoned jhum land, the highest percentage contribution was also recorded under *Bacillus* sp. and *Bacillus subtilis* with 0.31% each in the first year and 0.20% each in the second year. In tea garden, the highest percent contribution was recorded under *Bacillus subtilis* (0.36%) in the first year and in the second year under both *Bacillus* sp. and *Bacillus subtilis* with 0.23% each. In cultivated jhum land, the highest percent contribution was recorded under *Bacillus* sp. and *Bacillus subtilis* with 0.27% each in the first year whereas in the second year, *Bacillus subtilis* showed highest dominance with a percent contribution of 0.40%.

The diversity indices of soil fungal species in all the four study sites were recorded (Table 3.4). Shannon-Weiner diversity index (H') and Simpon's index of diversity (1-D) was recorded highest in Minkong reserve forest (H'=4.34and 1-D =0.97; H'=4.41 and 1-D=0.97) followed by abandoned jhum land (H'=4.14 and 1-D =0.94; H'=4.20 and 1-D =0.95), tea garden (H'=4.02 and 1-D =0.91; H'=4.03 and 1-D =0.90) and cultivated jhum land (H'=3.93 and 1-D =0.90; H'=3.79 and 1-D =0.54) in both first and second year repectively. The relative abundance of the individual bacterial species in the respective study site showed variation (Table 3.6). Each individual soil fungal species contributed a different percentage in all the study sites. Throughout the study period, no soil fungal species had the same position in the hierarchical order of dominance across the four study sites. In the Minkong reserve forest, the highest percent contribution was recorded under *Penicilllium chrysogenum* (5.94%) in the first year and by *Aspergillus fumigates* (5.18%) in the second year. In abandoned jhum land, the highest percent contribution was recorded under *Aspergillus niger* (6.14%) in the first year and by *Penicilllium chrysogenum* (4.98%) in the second year. In tea garden, the highest percent contribution was recorded under *Aspergillus flavus* (5.0%) in the first year and by *Aspergillus niger* (4.55%) in the second year. In cultivated jhum land, the highest percent contribution was recorded under *Penicilllium chrysogenum* (6.75%) in the first year and by *Aspergillus niger* (6.96%) in the second year.

Table 3.3. Diversity indices of soil bacteria from CJL, AJL, TG and MRF during 2016-18

Diversity indices	2016-17				2017-18			
	CJL	**AJL**	**TG**	**MRF**	**CJL**	**AJL**	**TG**	**MRF**
Shannon-Wiener index (H')	1.61	1.69	1.64	2.29	1.28	2.09	1.64	2.46
Simpson's index (1-D)	0.83	0.86	0.88	0.92	0.70	0.91	0.85	0.94

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table 3.4. Diversity indices of soil fungi from CJL, AJL, TG and MRF during 2016-18

Diversity indices	2016-17				2017-18			
	CJL	**AJL**	**TG**	**MRF**	**CJL**	**AJL**	**TG**	**MRF**
Shannon-Wiener index (H')	3.93	4.14	4.02	4.34	3.79	4.20	4.03	4.41
Simpson's index (1-D)	0.90	0.94	0.91	0.97	0.54	0.95	0.90	0.97

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table3.5. Realtive abundance (%) of soil bacteria from CJL, AJL, TG and MRF during 2016-18

Bacterial species	CJL		AJL		TG		MRF	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
Acinetobacter seiferti	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Bacillus aerophilus	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Bacillus amyloliquefaciens	0.00	0.00	0.18	0.00	0.00	0.10	0.00	0.00
Bacillus atltitudinis	0.00	0.00	0.09	0.00	0.00	0.05	0.00	0.00
Bacillus cereus	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Bacillus halotolerans	0.13	0.00	0.00	0.00	0.00	0.10	0.00	0.05
Bacillus licheniformis	0.00	0.00	0.00	0.05	0.00	0.00	0.08	0.05
Bacillus pumilus	0.13	0.15	0.09	0.05	0.20	0.10	0.08	0.05
Bacillus safensis	0.13	0.08	0.09	0.05	0.10	0.10	0.15	0.09
***Bacillus* sp**	0.27	0.31	0.18	0.21	0.30	0.20	0.23	0.18
Bacillus	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.05

stercoris								
Bacillus subtilis	0.27	0.31	0.36	0.21	0.40	0.20	0.23	0.18
Bacillus tequilensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus velezensis	0.00	0.08	0.00	0.05	0.00	0.10	0.00	0.05
Bacillus zhangzhouensis	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Chryseobacterium takakiae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
***Lysinibacillus* sp**	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00
Microbacterium imperial	0.00	0.00	0.00	0.00	0.00	0.05	0.15	0.00
***Microbacterium* sp**	0.07	0.08	0.00	0.00	0.00	0.00	0.00	0.05
Ralstonia picketti	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table3.6. Realtive abundance (%) of soil fungi from CJL, AJL, TG and MRF during 2016-18

	CJL		**AJL**		**TG**		**MRF**	
Fungal Name	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
Absidia cylindrospora	1.23	1.90	1.44	1.42	1.67	1.52	0.70	1.52
Absidia glaucas	0.61	1.27	1.08	0.36	0.83	0.51	1.05	1.52
***Absidia* sp1**	0.00	0.00	0.36	0.00	0.00	0.00	0.70	0.91
***Acremonium* sp1**	1.23	1.27	1.08	0.36	0.83	0.51	0.70	1.22
***Acremonium* sp2**	0.00	0.00	0.72	0.00	0.00	0.00	0.70	1.22
Alternaria alternata	0.61	0.63	0.72	0.71	1.67	1.01	0.70	1.22
Alternaria sp	0.00	0.63	1.44	0.36	0.00	0.51	0.70	0.91
***Arthtrographis* sp**	0.00	0.00	1.44	1.42	0.00	0.00	0.00	0.00
Apophysomyces elegans	0.00	0.00	1.44	1.42	0.00	0.00	0.00	0.00
Aspergillus flavus	3.68	5.70	5.42	3.91	5.00	4.04	3.15	1.83
Aspergillus fumigatus	3.68	3.80	3.25	2.49	2.50	3.03	3.50	5.18
Aspergillus heteromorphus	0.61	1.90	2.17	2.14	0.83	1.01	2.10	2.44
Aspergillus niger	6.13	6.96	6.14	4.63	4.17	4.55	2.80	3.66
Aspergillus ochraceus	1.84	3.16	1.44	1.78	2.50	3.03	1.40	0.00
Aspergillus oculeatus	0.61	0.00	0.36	0.71	0.00	0.00	1.40	1.83
Aspergillus oryzae	0.61	2.53	0.00	0.00	2.50	1.01	1.05	0.91
Aspergillus terreus	2.45	1.27	2.53	1.78	0.00	0.00	1.75	1.83
Aspergillus versicolor	1.84	1.90	1.81	1.78	0.00	0.00	1.40	1.22
***Aspergillus* sp1**	0.00	0.00	1.08	0.71	1.67	1.01	1.40	0.91
***Aspergillus* sp2**	0.00	0.00	1.08	0.36	2.50	1.01	1.40	0.61
***Athrinium* sp**	0.00	0.00	0.36	0.71	2.50	0.00	1.05	0.61
***Botrytis* sp**	0.00	0.00	0.36	1.07	0.00	0.00	1.05	1.22
Chaetomium globosum	2.45	1.90	1.81	1.78	0.83	2.02	1.40	0.91
***Chaetomium* sp1**	0.61	1.27	1.44	1.42	2.50	0.51	1.40	0.91

***Chaetomium* sp2**	0.00	1.27	1.44	1.78	0.00	3.03	1.40	0.61
***chrysosporium* sp1**	0.00	0.63	1.08	0.00	1.67	0.00	1.05	0.91
***chrysosporium* sp2**	0.61	0.00	0.36	1.78	0.00	0.00	0.70	0.30
***Cladophialophora* sp**	1.23	0.00	1.08	1.42	0.00	0.51	0.35	0.30
Cladosporium cladosporioides	1.23	2.53	1.08	1.78	1.67	2.53	2.10	0.91
Cladosporium oxysporum	1.23	0.63	0.72	0.00	0.83	0.00	0.70	1.22
Cladosporium herbarium	1.23	0.00	0.36	0.00	1.67	0.00	0.00	0.91
***Cladosporium* sp**	1.23	0.00	0.36	0.00	0.83	1.01	0.00	0.30
***clonostachys* sp**	1.84	2.53	0.00	0.00	0.00	0.00	0.00	0.00
***Colletotrichum* sp**	0.00	0.63	1.08	0.36	0.00	1.52	0.35	0.91
Cunninghamella echinulata	0.00	0.00	0.00	0.00	0.00	0.00	0.35	1.22
***Emmonsia* sp**	0.00	0.00	0.36	0.00	0.00	0.00	1.05	0.91
***Epicoccum* sp**	0.00	0.00	0.00	0.00	1.67	2.02	0.00	0.00
Exophiala	1.84	2.53	0.00	0.00	0.00	0.00	0.00	0.00
Fusarium oxysporium	0.61	1.27	0.72	1.42	0.83	1.01	0.00	1.52
Fusarium solani	0.61	0.63	0.00	1.42	1.67	0.51	0.00	1.52
***Fusaruim* sp1**	0.61	0.00	0.00	0.71	0.00	0.51	0.00	0.61
***Fusarium* sp2**	0.00	0.00	0.00	0.00	0.00	1.01	0.70	0.61
***Fusaruim* sp3**	0.00	0.00	0.00	0.00	0.00	1.01	0.70	1.22
Geotrichum candidum	2.45	2.53	1.44	1.07	1.67	2.53	1.40	1.83
***Geotrichum* sp1**	0.00	0.00	0.72	1.42	0.00	0.00	0.70	1.52
***Geotrichum* sp2**	0.00	0.00	0.00	0.00	0.00	0.00	0.35	1.22
***Gliocladium* sp**	1.23	0.63	0.36	0.36	0.00	0.51	0.00	0.91
Humicola sp	2.45	2.53	0.00	0.00	0.00	0.00	0.00	0.00
***Madurella* sp**	0.00	1.27	0.36	0.00	0.83	0.00	1.40	0.61
***Microascus* sp**	0.00	0.00	0.36	0.00	0.83	0.00	1.05	0.61
Mortierella	2.45	0.00	0.72	1.78	0.83	0.00	1.40	0.61
Mucor circinelloides	3.07	2.53	2.89	1.78	2.50	2.53	3.50	3.35
Mucor heimalis	2.45	0.63	1.44	1.07	2.50	2.53	1.40	0.61
Mucor plumbeus	0.00	2.53	1.44	1.07	0.83	2.53	1.75	0.91
Mucor racemosus	1.23	1.27	0.72	1.07	2.50	2.53	2.10	1.22
Mucor sp	1.84	0.00	0.00	1.07	0.00	0.00	0.70	0.91
***Neoscytalidium* sp**	0.00	0.00	0.00	0.00	2.50	2.02	0.00	0.00
Nigrospora osmanthi	0.00	0.00	0.00	0.00	1.67	2.53	0.00	0.00
***Paecomyciles* sp1**	0.00	0.00	0.72	0.71	0.00	1.01	0.00	0.61
***Paecomyciles* sp2**	2.45	1.27	0.00	1.42	0.00	1.01	0.70	0.91
Penicillium chrysogenum	6.75	6.33	3.97	4.98	3.33	1.52	5.94	1.83
Penicillium citrinum	3.68	1.27	3.25	2.85	2.50	1.01	1.40	3.96
Penicillium commune	3.07	2.53	0.72	1.78	0.83	1.01	1.40	1.22
Penicillium harzanium	2.45	1.90	1.44	1.42	1.67	1.01	0.70	1.22
Penicillium italicum	2.45	2.53	1.81	1.42	2.50	1.01	1.05	0.30
Penicillium janthinellum	1.84	2.53	0.72	1.07	2.50	3.03	0.00	0.00
Penicillium koningii	1.84	2.53	2.53	1.42	1.67	2.02	1.05	0.61

Penicillium palitans	0.00	0.00	0.00	1.07	0.00	0.00	1.05	0.30
Penicillium waksmani	1.23	1.90	2.53	1.07	0.83	0.00	1.05	0.91
***Penicillium* sp1**	0.00	0.00	0.00	0.00	0.83	0.00	0.70	0.30
***Penicillium* sp2**	0.00	0.00	0.00	0.00	1.67	0.00	0.70	0.61
***Penicillium* sp3**	0.61	0.63	0.00	0.00	1.67	0.00	1.05	0.61
***Periconia* sp1**	0.00	0.00	0.00	0.71	0.00	1.01	0.70	0.61
***Periconia* sp2**	0.00	0.00	0.00	0.71	0.83	1.01	0.70	0.61
***Pestaliotopsis* sp**	0.61	0.63	0.72	0.71	1.67	0.51	1.05	0.61
Phaeoacremonium	0.61	0.00	1.08	1.42	0.00	1.01	1.05	0.61
Phoma glomerata	1.23	2.53	2.17	2.14	0.83	1.52	0.35	0.61
***Phoma* sp1**	1.23	0.00	1.44	1.42	1.67	0.51	0.70	0.61
***Phoma* sp2**	0.00	0.00	1.08	1.07	1.67	0.51	0.35	0.61
***Phoma* sp 3**	0.00	0.00	0.00	0.00	0.00	0.00	1.05	0.61
Phomopsis amananthicola	0.00	0.00	0.00	0.00	0.00	0.00	1.05	1.22
Rhizopus microspores	0.00	0.00	0.00	0.71	0.00	0.00	0.70	0.30
Rhizopus stolonifer	0.61	0.00	0.72	0.36	0.83	0.51	0.70	0.61
***Scedosporium* sp 1**	0.00	0.00	2.17	0.71	0.00	0.00	0.70	0.30
***Scedosporium* sp 2**	0.61	0.00	1.44	0.71	0.00	0.00	0.70	0.61
***Sclerotiana* sp**	0.00	0.00	0.00	1.07	0.83	0.00	0.70	0.30
scopulariopsis brumtptii	0.00	0.00	0.00	0.00	0.00	0.00	1.40	1.22
Scytalidium dimidiatum	0.00	0.00	0.00	0.00	0.00	0.00	1.05	0.61
***Scytalidium* sp**	0.61	0.63	0.00	0.36	0.00	0.00	0.35	0.30
***Taloromyces* sp**	0.61	0.63	0.00	1.42	0.00	2.53	1.05	0.30
***Trichophyton* sp1**	0.00	0.00	1.44	0.71	1.67	1.01	0.00	0.30
***Trichopyton* sp2**	0.61	0.00	1.44	1.07	1.67	0.51	0.00	0.30
Tricoderma hamatum	1.84	1.90	1.81	2.49	0.83	2.53	2.10	0.61
Tricoderma harzianum	3.68	3.16	2.53	2.85	1.67	3.54	3.85	4.27
Tricoderma longibrachiatum	1.84	0.00	1.44	2.85	1.67	3.03	1.75	0.30
Tricoderma viride	0.61	3.16	3.61	2.14	1.67	2.53	1.05	3.05
***Tricoderma* sp1**	0.00	0.00	0.00	0.00	0.83	2.53	1.40	1.83
***Tricoderma* sp2**	0.00	0.00	0.00	0.00	0.83	2.53	1.05	0.61
***Ulocladium* sp**	0.61	0.00	0.00	1.07	0.00	0.00	0.35	0.61
***Verticillium* sp1**	0.61	1.27	0.00	0.36	0.00	1.01	0.00	3.05
***Verticillium* sp2**	0.00	0.00	1.08	1.07	0.83	1.52	0.70	0.00

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Perason's correlation coefficient identified both positive and negative correlations between the physicochemical properties with bacterial counts in all the study sites (Table 3.7). Bacterial counts showed both negative correlation and positive correlation the physicochemical properties. Bacterial counts were positively correlated with SOC, available N, available P, *p*H, moisture, and temperature throughout the study in all the study sites whereas the relationship between the bacterial counts with available K, sand, silt and clay did not show a uniform relationship in the present study.

In cultivated jhum land, during the first year, bacterial counts showed positive significant correlation with soil moisture (r=.72**; *p*=.009), whereas in the second year, bacterial counts showed positive significant correlation with temperature (r=.61*; *p*=.035), SOC (r=.95**; *p*=.001), available K (r=.68*; *p*=.014), clay (r=.74**; *p*=.006), and negative significant corealtion with sand (r=.72*; *p*=.008).

In abandoned jhum land, during the first year, bacterial counts showed positive significant correlation with soil *p*H (r=.97**; *p*=.001), moisture (r=.98**; *p*=.001), temperature (r=.96**; *p*=.001) whereas in the second year positively correlated with soil *p*H (r=.64*; *p*=.64*), moisture (r=.79**; *p*=.002), temperature (r=.86**; *p*=.001) and negatively correlated with available K (r=-.71**; *p*=.009).

In tea garden, during the first year, bacterial counts showed positive significant correlation with SOC (r=.71**; *p*=.009), available P (r=.75**; *p*=.005), clay (r=.64*; *p*=.027) whereas in the second year, the bacterial counts were positively correlated with SOC (r=.82**; *p*=.001) and silt (r=.58*; *p*=.046).

In Minkong reserve forest, during the first year, bacterial counts showed positive significant correlation with SOC (r=.63*; *p*=.027) and available N (r=.68*; *p*=.015) and during the second year, bacterial counts showed positive significant correlation with SOC (r=.82**; *p*=.001) and available N (r=.87**; *p*=.001).

Table 3.7. Correlation between bacterial counts and soil physicochemical properties at CJL, AJL, TG and MRF during 2016-18

	CJL		**AJL**		**TG**		**MRF**	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
Parameters	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**	**Bacterial counts**
pH	.57	.28	.97**	.64*	-.37	.02	.20	.001
Moist. (%)	.72**	.47	.98**	.79**	.15	.12	.26	-.01
Temp. (℃)	.43	.61*	.96**	.86**	-.02	.30	.54	.37
SOC (%)	.50	.95**	.22	.20	.71**	.82**	.63*	.82**
AN (Kg/ha)	.32	.50	.09	.25	.48	.32	.68*	.87**
AP (Kg/ha)	.26	.52	-.37	.45	.75**	.28	.27	.39
AK (Kg/ha)	-.10	.68*	.52	-.71**	.08	.21	.17	.37
Sand (%)	.68	-.72*	.67	.88	-.47	-.21	-.28	-.20
Silt(%)	-.13	-.59	.64	.64	.13	.58*	.32	.17
Clay (%)	-.71	.74	-.71	-.88	.64*	.13	-.29	-.16

CJL-Cultvated jhum land, A.984JL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

Perason's correlation coefficient identified both positive and negative correlations between the physicochemical properties with fungal counts (Table 3.8). Fungal counts showed negative correlation with sand while positive correlation with SOC, available N, available P and available K throughout the study in all the study sites whereas the relationship between the bacterial counts with soil *p*H, moisture, temperature, silt and clay did not show a uniform relationship in the present study.

In cultivated jhum land, during the first year, bacterial counts showed positive significant correlation with moisture (r=.71*; *p*=.010) whereas, in the second year, bacterial counts showed positive significant correlation with temperature (r=.64*; *p*=.025), SOC (r=.91**; *p*=.001), available K (r=.62*; *p*=.033), clay (r=.67*; *p*=.016) and negatively correlated with sand (r=-.66*; *p*=.020),

In abandoned jhum land, during the first year, bacterial counts showed positive significant correlation with *p*H (r=.78**; *p*=.003), moisture (r=.85**; *p*=.001), temperature (r=.74**; *p*=.006), and negatively correlated with available K (r=-.64*; *p*=.025) whereas, in the second year, bacterial counts showed positive significant correlation with moisture (r=.86**; *p*=.001), temperature (r=.78**; *p*=.003) and negatively correlated with clay (r=-.72*; *p*=.008).

In tea garden, during the first year, bacterial counts showed positive significant correlation with SOC (r=.95**; *p*=.001), available N (r=.84**; *p*=.001), available P (r=.97**; *p*=.001), available K (r=.61*; *p*=.035) whereas the second year, bacterial counts showed positive significant correlation with moisture (r=.59*; *p*=.043), temperature (r=.74**; *p*=.006), SOC (r=.94**; *p*=.001) and available N (r=.65*; *p*=.022).

In Minkong reserve forest, during the first year, bacterial counts showed positive significant correlation with temperature (r=.71**; *p*=.009), SOC (r=.61*; *p*=.035), and available N (r=.79**; *p*=.002) whereas the second year, bacterial counts showed positive significant correlation with temperature (r=.63*; *p*=.028), SOC (r=.73**; *p*=.007), and available N (r=.82**; *p*=.001).

Table 3.8. Correlation between fungal counts and soil physicochemical properties at CJL, AJL, TG and MRF during 2016-18

	CJL		AJL		TG		MRF	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
Parameters	**Fungal counts**	**Fungal counts**	**Fungal counts**	**Fungal counts**	**Fungal counts**	**Fungal counts**	**Fungal counts**	**Fungal counts**
pH	.52	.36	.78*	.38	.12	.42	.40	.32
Moist. (%)	.71*	.54	.85**	.86**	.58	.59*	.44	.32
Temp.(℃)	.49	.64*	.74**	.78**	.45	.74**	.71**	.63*
SOC(%)	.48	.91**	.33	.36	.95**	.94**	.61*	.73**
AN(Kg/ha)	.26	.43	.12	.06	.84**	.65*	.79**	.82**
AP(Kg/ha)	.25	.46	.22	.31	.97**	.25	.16	.04
AK(Kg/ha)	-.06	.62*	-.64*	-.56	.61*	.25	.13	.02
Sand(%)	.75	-.66*	-.39	.64	-.46	.09	-.04	.19
Silt(%)	-.17	-.52	-.37	-.26	.42	.28	.11	-.20
Clay(%)	-.79	.67*	-.40	-.72*	.26	-.17	-.19	-.50

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium

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

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

3.3.5. Identification of bacterial isolate

Phenotypic characterization

In the present study a total of 133 bacterial isolates from cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest were studied for morphological characteristics such as colony morphology such as shape, texture, margin texture, colour (Table 3.9), gram characters and some selected biochemical test (Table 3.10). Phenotypic characterizations of some bacterial isolate are shown in figure 3.5. The results showed gram-positive and gram-negative bacteria isolates that varied from rod to cocci shape in their morphology. Biochemical test such as citrate utilization test, sugar fermentation test, catalase test (Table 3.10) showed both negative and positive results indicating the capability of the bacterial isolates to excrete enzymes. Some images of the biochemical test are shown in figure 3.6.

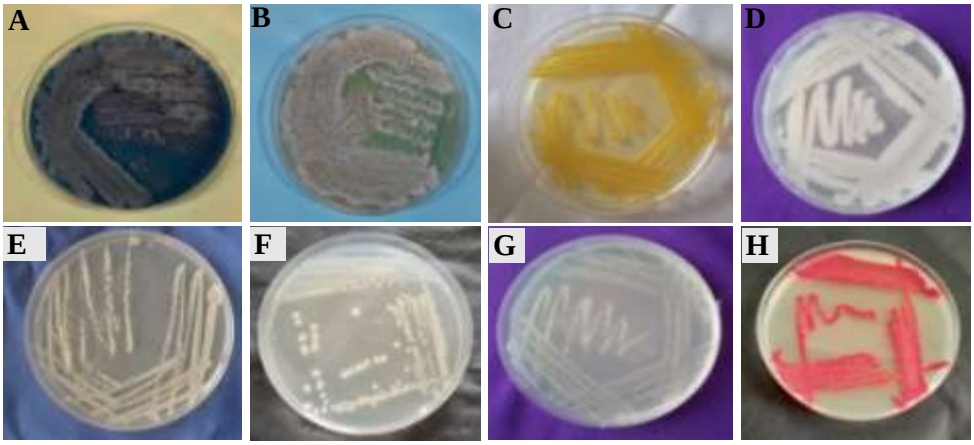

Figure 3.5. Pure culture plates of some bacterial isolate from the study sites. A-AK12, B-AK122, C-AK179, D-AK58, E-AK192, F-AK47, G-AK137, H-AK43

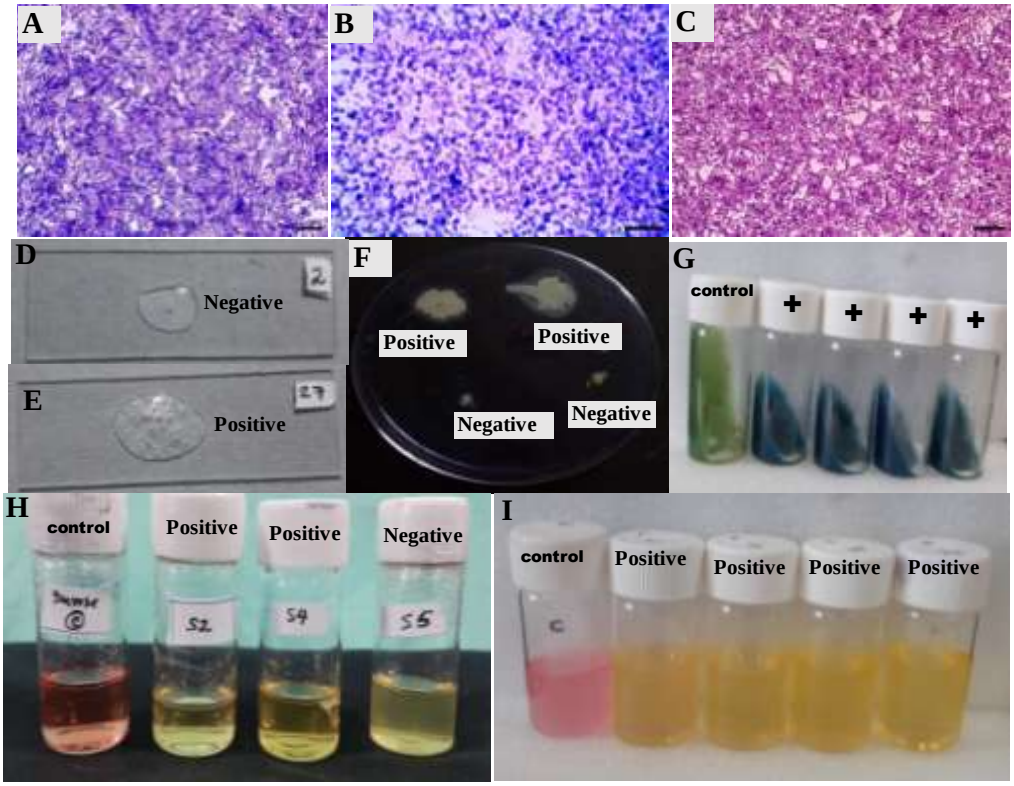

Figure 3.6. Characterization of bacterial isolates by gram characters and biochemical test. A-Gram positive, rod-shaped, B-Gram negative, rod-shaped, C-Gram positive,cocci shape, D-Catalase test, E-Starch hydrolysis test, F-Citrate utilization test, G-H-Sugar fermentation test of surcose, and lactose

Table 3.9. Culture plate morphology of the isolated bacterial colonies

Bacterial strain	Shape	Margin	Texture	Colour
AK5	Circular	Undulate	Dry	Yellow
AK13	Circular	Entire	Mucoid	Yellow
AK39	Circular	Entire	Dry	Cream
AK44	Irregular	Undulate	Mucoid	Cream
AK45	Irregular	Lobate	Mucoid	White
AK46	Circular	Fimbriate	Mucoid	Cream
AK47	Circular	Entire	Dry	Cream
AK56	Irregular	Entire	Moist	White
AK58	Circular	Entire	Moist	Yellow
AK61	Irregular	Undulate	Moist	Cream
AK65	Irregular	Lobate	Mucoid	White
AK93	Irregular	Entire	Moist	White
AK105	Circular	Undulate	Botyrous	Cream
AK136	Irregular	Entire	Mucoid	Yellowish cream
AK137	Circular	Entire	Botyrous	Orange
AK146	Irregular	Entire	Dry	Cream
AK158	Irregular	Entire	Moist	Cream
AK159	Circular	Entire	Dry	White
AK171	Irregular	Undulate	Dry	Cream
AK178	Circular	Undulate	Moist	Cream
AK179	Circular	Entire	Moist	Red
AK186	Circular	Fimbriate	Mucoid	White
AK187	Circular	Entire	Moist	Cream
AK206	Irregular	Entire	Dry	Cream
AK207	Irregular	Entire	Moist	Cream
AK11	Irregular	Undulate	Dry	White
AK17	Wrinkled	Crenate	Dry	White
AK110	Circular	Circular	Moist	Yellow

AK27	Circular	Undulate	Moist	Cream
AK28	Irregular	Entire	Dry	Cream
AK30	Irregular	Entire	Moist	Yellow
AK41	Circular	Undulate	Butyrous	White
AK48	Circular	Entire	Moist	Cream
AK63	Irregular	Entire	Dry	Pinkish white
AK67	Irregular	Entire	Moist	Cream
AK87	Circular	Entire	Dry	White
AK91	Irregular	Fimbriate	Dry	Yellowish white
AK95	Irregular	Undulate	Botyrous	Whire
AK96	Irregular	Fimbriate	Mucoid	White
AK97	Circular	Entire	Moist	Cream
AK100	Circular	Entire	Dry	Cream
AK109	Circular	Entire	Dry	White
AK116	Circular	Entire	Mucoid	White
AK118	Irregular	Undulate	Moist	White
AK122	Circular	Undulate	Moist	Pinkish white
AK123	Irregular	Fimbriate	Mucoid	Yellowish white
AK127	Circular	Entire	Moist	Cream
AK128	Circular	Undulate	Butyrous	Cream
AK129	Circular	Undulate	Dry	Cream
AK130	Wrinkled	Crenate	Moist	Pinkish white
AK131	Circular	Entire	Dry	White
AK142	Irregular	Lobate	Mucoid	Pinkish white
AK147	Circular	Undulate	Butyrous	Cream
AK149	Circular	Entire	Moist	Cream
AK150	Irregular	Entire	Dry	Cream
AK156	Irregular	Entire	Moist	Cream
AK164	Circular	Entire	Moist	Cream
AK170	Circular	Entire	Mucoid	Cream
AK177	Circular	Undulate	Dry	Cream

AK180	Irregular	Lobate	Mucoid	White
AK181	Circular	Entire	Dry	white
AK185	Circular	Entire	Butyrous	Yellow
AK189	Irregular	Crenate	Mucoid	Cream
AK 4	Irregular	Undulate	Moist	Orange
AK6	Circular	Undulate	Moist	Cream
AK7	Irregular	Entire	Dry	Cream
AK14	Irregular	Fimbriate	Mucoid	White
AK16	Irregular	Lobate	Mucoid	white
AK49	Circular	Entire	Moist	Cream
AK25	Irregular	Undulate	Dry	Cream
AK31	Circular	Entire	Mucoid	White
AK33	Irregular	Crenate	Moist	Cream
AK53	Circular	Entire	Mucoid	white
AK55	Circular	Entire	Mucoid	White
AK59	Irregular	Undulate	Mucoid	Cream
AK66	Irregular	Lobate	Mucoid	White
AK70	Circular	Entire	Moist	Cream
AK88	Circular	Entire	Mucoid	Yellow
AK102	Circular	Undulate	Dry	Pinkish white
AK103	Circular	Undulate	Butyrous	Cream
AK107	Circular	Entire	Dry	white
AK132	Circular	Lobate	Mucoid	Cream
AK133	Circular	Undulate	Butyrous	Yellowish white
AK134	Circular	Entire	Dry	Yellow
AK135	Wrinkled	Fimbriate	Dry	White
AK139	Circular	Entire	Moist	Cream
AK155	Irregular	Lobate	Mucoid	White
AK157	Wrinkled	Fimbriate	Dry	Yellow
AK173	Circular	Undulate	Butyrous	Cream
AK203	Irregular	Undulate	Butyrous	Yellow

AK204	Irregular	Fimbriate	Mucoid	White
AK12	Wrinkled	Undulate	Moist	Cream
AK40	Wrinkled	Fimbriate	Moist	Cream
AK18	Irregular	Entire	Mucoid	White
AK20	Circular	Undulate	Moist	Cream
AK21	Circular	Entire	Dry	White
AK29	Circular	Entire	Moist	Cream
AK36	Circular	Entire	Moist	Cream
AK42	Irregular	Lobate	Mucoid	White
AK43	Circular	Entire	Dry	white
AK52	Circular	Entire	Butyrous	Yellow
AK64	Irregular	Crenate	Mucoid	Cream
AK92	Irregular	Undulate	Moist	Orange
AK104	Circular	Undulate	Moist	Cream
AK106	Irregular	Entire	Dry	Cream
AK108	Irregular	Entire	Moist	Yellow
AK120	Circular	Undulate	Dry	White
AK148	Wrinkled	Crenate	Moist	Pinkish white
AK152	Circular	Entire	Dry	Grayish white
AK154	Circular	Entire	Moist	Cream
AK161	Circular	Entire	Dry	Yellow
AK162	Wrinkled	Fimbriate	Dry	White
AK163	Circular	Entire	Moist	Cream
AK165	Irregular	Lobate	Mucoid	White
AK166	Circular	Entire	Moist	Pinkish white
AK167	Irregular	Entire	Moist	Cream
AK175	Circular	Entire	Mucoid	Cream
AK182	Irregular	Undulate	Moist	Cream
AK183	Irregular	Undulate	Dry	White
AK191	Irregular	Undulate	Moist	Orange
AK192	Circular	Entire	Dry	Grayish white

AK193	Circular	Undulate	Dry	Cream
AK195	Wrinkled	Fimbriate	Moist	Cream
AK201	Irregular	Entire	Mucoid	White
AK202	Circular	Entire	Dry	Cream
AK208	Circular	Undulate	Dry	Cream
AK209	Circular	Fimbriate	Dry	Yellow
AK22	Circular	Lobate	Mucoid	Cream
AK26	Circular	Entire	Mucoid	White
AK23	Irregular	Crenate	Moist	Cream
AK71	Circular	Entire	Mucoid	white
AK168	Circular	Entire	Mucoid	White
AK169	Circular	Entire	Mucoid	Yellowish white

Table 3.10. Characterization of bacterial isolates by gram characters and biochemical test

Bacterial strain	Gram character	Shape	MR	VP	Citrate utiliztion	Catalase	oxidase	Starch	Sugar fermentation test					Presumptive identification
									Dextrose	Glucose	Lactose	Fructose	Maltose	
AK17	+	R	-	+	-	-	+	+	+	+	+	+	+	*Bacillus* sp.
AK110	+	R	+	-	-	+	+	-	-	-	+	+	+	*Bacillus* sp.
AK13	+	R	-	+	+	+	-	+	+	+	+	+	-	*Bacillus* sp.
AK44	+	R	-	+	+	+	-	+	+	+	+	+	+	*Bacillus* sp.
AK61	+	R	-	+	+	+	-	-	-	-	+	+	+	*Bacillus* sp.
AK39	+	R	-	-	+	+	-	+	+	+	+	-	+	*Bacillus* sp.
AK58	+	R	-	+	+	-	+	+	-	+	-	+	+	*Bacillus* sp.
AK136	+	R	+	-	+	+	-	+	-	+	+	+	+	*Bacillus* sp.
AK45	+	R	+	-	+	-	+	+	+	+	+	-	+	*Bacillus* sp.
AK65	+	R	+	+	+	+	-	+	+	+	-	+	+	*Bacillus* sp.
AK93	+	R	-	+	-	-	+	+	+	+	+	-	+	*Bacillus* sp.
AK105	+	R	+	+	+	+	-	+	+	-	-	+	+	*Bacillus* sp.
AK137	+	R	-	+	-	-	+	-	+	-	+	+	-	*Bacillus* sp.
AK158	+	R	+	+	+	+	-	+	+	+	+	+	+	*Bacillus* sp.
AK207	+	R	+	+	-	+	-	-	+	+	-	-	+	*Bacillus* sp.
AK5	+	R	+	+	+	-	-	+	+	-	+	+	-	*Bacillus subtilis*
AK46	+	R	-	+	-	+	+	-	-	+	-	+	+	*Bacillus subtilis*
AK47	+	R	-	-	+	-	-	+	+	-	+	+	-	*Bacillus subtilis*
AK56	+	R	+	-	-	+	+	-	+	+	+	+	+	*Bacillus subtilis*

AK146	+	R	+	+	+	+	-	+	+	+	-	+	+	*Bacillus* sp.
AK159	+	R	+	-	+	+	-	+	+	+	+	+	+	*Bacillus* sp.
AK171	+	R	-	+	-	+	-	-	+	+	-	+	+	*Bacillus subtilis*
AK178	+	R	+	+	-	-	+	+	+	+	+	+	+	*Bacillus subtilis*
AK179	+	R	+	+	-	-	+	+	+	+	+	+	+	*Bacillus subtilis*
AK186	+	R	+	-	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
AK187	+	R	+	+	-	-	+	-	+	+	+	+	+	*Bacillus subtilis*
AK206	+	R	-	+	+	-	-	+	+	+	+	+	+	*Bacillus subtilis*
AK11	+	R	+	+	-	+	+	-	+	+	-	+	+	*Bacillus subtilis*
AK181	+	R	-	-	+	+	-	+	+	-	+	+	+	*Bacillus* sp.
AK118	+	R	+	+	+	+	-	+	+	-	+	+	-	*Bacillus* sp.
AK91	+	R	-	+	-	-	+	-	-	+	+	-	+	*Bacillus* sp.
AK109	+	R	+	+	+	+	-	+	+	-	+	+	-	*Bacillus* sp.
AK63	+	R	-	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
AK67	+	R	-	+	+	+	-	+	+	+	-	+	+	*Bacillus subtilis*
AK149	+	R	-	+	+	+	-	+	+	-	+	+	+	*Bacillus* sp.
AK116	+	R	-	+	+	+	-	+	-	-	+	-	+	*Bacillus* sp.
AK142	+	R	+	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
AK185	+	R	-	+	-	+	-	-	+	-	+	-	+	*Bacillus subtilis*
AK48	+	R	+	-	-	-	+	+	+	+	+	+	+	*Bacillus* sp
AK30	+	R	+	+	-	-	+	+	+	-	+	+	+	*Bacillus* sp.
AK87	+	R	+	-	+	+	-	+	+	+	+	+	+	*Bacillus* sp.
AK95	+	R	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* sp.
AK97	+	R	+	+	+	+	-	+	+	-	-	+	+	*Bacillus* sp.

AK130	+	R	+	+	+	+	-	-	-	+	+	-	+	*Bacillus* sp.
AK150	+	R	-	+	-	-	+	+	+	+	-	+	+	*Bacillus* sp.
AK164	+	R	-	+	-	-	+	+	+	+	+	+	+	*Bacillus* sp
AK189	+	R	-	+	-	+	+	+	+	-	+	-	-	*acillus* sp.
AK96	+	R	-	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
AK100	+	R	-	+	+	+	-	+	+	-	+	-	+	*Bacillus subtilis*
AK122	+	R	+	-	+	-	+	-	+	+	+	+	+	*Bacillus subtilis*
AK123	+	R	+	+	+	+	-	-	+	+	-	+	+	*Bacillus subtilis*
AK129	+	R	+	-	+	-	+	-	+	+	+	-	+	*Bacillus subtilis*
AK131	+	R	-	+	+	+	-	+	+	-	+	+	+	*Bacillus subtilis*
AK147	+	R	-	-	-	-	+	+	+	+	-	-	+	*Bacillus subtilis*
AK170	+	R	-	+	+	+	-	+	+	-	-	+	+	*Bacillus subtilis*
AK177	+	R	-	+	+	+	-	+	+	+	-	-	+	*Bacillus subtilis*
AK180	+	R	+	+	+	+	-	+	+	+	-	+	-	*Bacillus subtilis*
AK127	+	R	-	+	+	+	-	-	+	-	+	+	+	*Bacillus subtilis*
AK128	+	R	-	+	+	-	+	+	+	+	+	-	+	*Bacillus subtilis*
AK156	+	R	-	+	-	-	+	+	+	+	-	+	-	*Bacillus subtilis*
AK41	+	R	-	+	+	+	+	-	+	+	-	+	+	*Bacillus subtilis*
AK27	+	R	+	-	-	-	+	-	+	+	-	+	+	*Microbacterium* sp.
AK28	+	R	+	-	-	+	+	+	+	+	+	-	+	*Microbacterium* sp.
AK55	+	R	-	+	+	+	-	+	+	-	-	+	+	*Bacillus subtilis*
AK59	+	R	+	-	+	+	-	+	+	-	+	-	+	*Bacillus subtilis*
AK157	+	R	+	-	+	+	-	-	+	+	-	-	+	*Bacillus subtilis*
AK 4	+	R	-	-	+	+	-	-	+	-	+	+	-	*Bacillus subtilis*

AK134	+	R	-	+	+	-	+	+	-	+	+	-	+	*Bacillus subtilis*
AK53	+	R	-	+	+	+	-	-	+	-	+	-	+	*Bacillus pumilus*
AK66	+	R	-	+	+	+	-	-	+	+	-	+	-	*Bacillus pumilus*
AK88	+	R	-	+	+	+	-	-	+	-	-	+	+	*Bacillus safensis*
AK107	+	R	+	-	+	+	+	+	-	+	+	+	-	*Bacillus safensis*
AK135	+	R	+	-	+	+	-	+	+	+	-	+	+	*Bacillus safensis*
AK139	+	R	-	+	+	+	-	+	-	+	-	+	-	*Bacillus safensis*
AK16	+	R	-	+	+	-	+	+	+	-	+	-	+	*Bacillus* sp
AK6	+	R	+	-	+	+	-	+	-	+	-	+	+	*Bacillus* sp.
AK31	+	R	+	-	+	+	-	-	+	-	+	+	-	*Bacillus* sp.
AK33	+	R	+	+	+	-	+	+	+	+	+	+	+	*Bacillus* sp.
AK103	+	R	+	-	-	+	+	-	-	+	-	-	+	*Bacillus* sp.
AK155	+	R	-	+	+	+	+	+	+	+	+	-	+	*Bacillus* sp.
AK7	+	R	-	+	+	+	-	+	+	+	-	-	+	*Bacillus subtilis*
AK14	+	R	-	+	+	+	-	+	+	-	-	+	-	*Bacillus subtilis*
AK25	+	R	-	+	+	+	-	+	-	+	+	+	-	*Bacillus subtilis*
AK70	+	R	-	+	+	+	-	-	+	-	-	+	+	*Bacillus subtilis*
AK102	+	R	-	+	-	-	+	+	-	+	+	+	+	*Bacillus subtilis*
AK133	+	R	-	+	+	+	-	-	+	+	+	+	+	*Bacillus subtili*
AK173	+	R	-	+	+	+	-	-	-	+	-	+	+	*Bacillus subtilis*
AK203	+	R	+	+	+	+	-	+	+	-	+	+	+	*Bacillus subtilis*
AK204	+	R	-	+	+	+	-	-	+	+	-	+	+	*Bacillus subtilis*
AK132	+	R	+	-	+	-	+	+	+	-	+	+-	+	*Bacillus* sp.

AK49	+	R	+	-	+	-	-	+	+	+	-	-	+	*Bacillus* sp.
AK12	+	R	-	+	+	+	-	-	+	+	+	+	+	*Bacillus* sp.
AK40	+	R	-	+	+	-	-	-	+	-	+	-	+	*Ralstonia* sp.
AK168	+	R/C	-	+	+	+	+	+	-	-	+	-	+	*Acinetobacter* sp.
AK169	+	R/C	-	+	-	-	+	+	+	+	-	-	+	*Bacillus* sp.
AK43	+	R	+	-	+	+	+	+	+	-	-	+	-	*Bacillus* sp.
AK163	-	R	+	-	-	-	-	+	+	-	+	+	+	*Bacillus* sp.
AK182	+	R	-	+	+	+	-	-	+	+	-	-	+	*Bacillus cereus*
AK183	+	R	-	+	-	+	-	+	+	+	+	+	+	*Bacillus cereus*
AK175	+	R	-	+	+	-	-	+	+	-	-	+	+	*Bacillus* sp.
AK195	+	R	-	+	-	+	+	-	+	+	-	-	+	*Bacillus* sp.
AK36	+	R	-	+	+	-	-	-	-	+	-	+	-	*Bacillus* sp.
AK167	+	R	-	+	-	+	-	-	-	+	-	+	+	*Bacillus* sp.
AK42	+	R	+	-	+	+	-	+	-	-	-	+	+	*Bacillus* sp.
AK92	+	R	-	+	+	+	-	--	+	+	-	+	-	*Bacillus* sp.
AK106	+	R	+	-	-	+	-	+	-	+	+	-	+	*Bacillus* sp.
AK18	+	R	-	-	-	-	+	+	+	-	+	-	+	*Bacillus* sp.
AK108	+	R	-	+	-	-	+	-	-	+	-	+	+	*Bacillus* sp.
AK161	+	R	-	-	+	+	-	-	+	-	+	-	+	*Bacillus* sp.
AK165	+	R	+	-	-	-	+	+	+	-	-	+	+	*Bacillus* sp.
AK166	+	R	+	+	+	-	+	+	+	+	+	-	-	*Bacillus* sp.
AK191	+	R	-	+	-	+	-	+	-	-	+	-	+	*Bacillus* sp.
AK208	+	R	+	-	+	+		-	+	-	+	-	+	*Bacillus* sp.

AK209	+	R	+	-	+	+	-	-	-	-	-	+	+	*Bacillus* sp.
AK20	+	R	-	+	-	-	-	-	+	-	-	+	-	*Bacillus* sp.
AK120	+	R	-	+	-	-	-	+	+	-	-	+	+	*Bacillus* sp.
AK148	+	R	-	+	-	-	-	+	+	+	-	-	-	*Bacillus* sp.
AK21	+	R	-	+	+	+	-	+	+	-	+	-	+	*Bacillus* sp.
AK29	+	R	+	-	+	+	-	-	+	-	-	+	+	*Bacillus* sp.
AK52	+	R	-	+	+	+	-	+	-	+	-	+	-	*Bacillus subtilis*
AK104	+	R	-	-	-	+	+	-	+	+	+	+	+	*Bacillus subtilis*
AK152	+	R	-	-	+	+	+	-	-	+	-	+	-	*Bacillus* sp.
AK154	+	R	-	-	+	+	+	+	+	+	+	+	+	*Bacillus subtilis*
AK192	+	R	-	-	+	+	+	+	+	-	+		+	*Bacillus subtilis*
AK193	+	R	-	+	-	+	+	+	+	+	+	+	+	*Bacillus subtilis*
AK202	+	R	+	+	-	+	+	-	-	+	+	-	+	*Bacillus* sp.
AK201	+	R	-	+	+	+	+	-	+	+	+	+	+	*Bacillus* sp.
AK64	+	R	-	+	-	-	+	+	+	+	+	+	+	*Bacillus* sp.
AK162	+	R	-	+	+	+	-	+	-	+	-	-	+	*Bacillus* sp.
AK26	-	R	-	+	-	+	_	+	+	+	+	+	+	*Chryseobacterium* sp.
AK22	-	R	-	-	+	+	-	+	+	-	+	-	+	*Chryseobacterium* sp.
AK71	+	R	-	+	+	+	+	-	+	+	-	+	+	*Lysinibacillus* sp.
AK23	+	R	-	+	-	-	+	+	+	+	+	+	-	*Bacillus* sp.

MR-Methyl red, VP- Voges-proskauer, R- Rod shape, R/C-Rod, slightly cocci shape

16s *r*RNA characterization of bacterial isolates

Bacteria DNA extracted from all the isolates were amplified by universal primer pairs and the sequenced bacterial isolates was BLAST search in the NCBI GenBank database to check the similarity of the aligned sequence of each of the bacterial isolates. The different band in agarose gel (1.2%) is shown in figure 3.7. The bacterial isolate submitted in NCBI GenBank is shown (Table 3.11) with the GenBank accession number. All the isolated and identified bacterial isolates have been stored in the Nagaland University, Lumami, India under Microbiology laboratory. In the present study, 133 bacterial isolates was identified, out of which 133 isolates, 99 isolates were identified upto species level whereas 34 isolates were identified till the generic level. The 133 isolates were identified under six (6) genera out of which *Bacillus* was dominant with isolates, *Acinetobacter* with 2 isolates, *Chryseobacterium* with 2 isolates, *Lysinibacillus* with 1 isolates, *Microbacterium* with 5 isolates, and *Ralstonia* with 1 isolate.

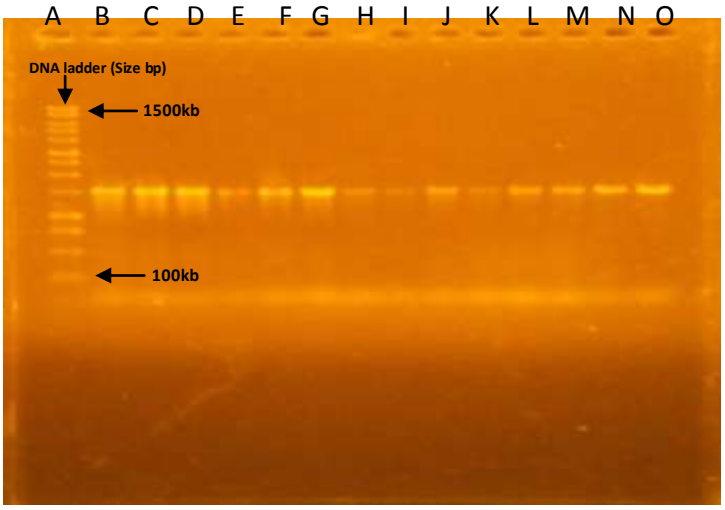

Figure 3.7. Agarose gel electrophoresis. Agarose gel, 1.2% (w/v) with PCR amplified products of bacterial isolates. The agarose gel electrophoresis shows DNA ladder (Lane A), negative control (Lane O) and the remaing lane (Lane B-M) denotes amplified 16S *r*RNA gene of some bacterial isolates

Table 3.11. 16s *r*RNA based identification of bacteria from CJL, AJL, TG and MRF with NCBI GenBAnk accession number

Representative strain	Organism	Percent similarity	Query cover	NCBI GeneBank accession number
AK5	*Bacillus subtilis*	100	99.50	ON140796
AK13	*Bacillus pumilus*	99	98.65	ON150922
AK39	*Bacillus safensis*	96	97.91	ON152310
AK44	*Bacillus pumilus*	97	100	0N152313
AK45	*Bacillus* sp.	99	92.20	ON171227
AK46	*Bacillus subtilis*	97	99.85	ON152383
AK47	*Bacillus subtilis*	100	96.24	ON152386
AK56	*Bacillus subtilis*	97	97.43	ON152399
AK58	*Bacillus safensis*	94	99.78	ON152402
AK61	*Bacillus pumilus*	99	92.03	ON152406
AK65	*Bacillus* sp.	99	97.94	ON152410
AK93	*Bacillus* sp.	100	98.55	ON152421
AK105	*Bacillus* sp.	99	99.05	ON155839
AK136	*Bacillus safensis*	100	98.86	ON155917
AK137	*Bacillus* sp.	100	98.43	ON155920
AK146	*Bacillus subtilis*	100	95.81	ON155993
AK158	*Bacillus* sp.	100	99.41	ON155998
AK159	*Bacillus Subtilis*	99	96.83	ON156005
AK171	*Bacillus Subtilis*	100	87.29	ON156006
AK178	*Bacillus Subtilis*	100	97.91	ON156033
AK179	*Bacillus Subtilis*	99	96.31	ON156015
AK186	*Bacillus Subtilis*	100	98.52	ON156400
AK187	*Bacillus Subtilis*	99	97.33	ON156449
AK206	*Bacillus Subtilis*	99	96.15	ON156439
AK207	*Bacillus* sp.	99	97.17	ON156455
AK11	*Microbacterium* sp	99	98.81	ON150859
AK17	*Bacillus halotolerans*	99	99.32	ON158773
AK110	*Bacillus halotolerans*	97	99.74	ON171376

AK27	*Microbacterium imperiale*	100	100	ON166492
AK28	*Microbacterium* sp.	99	99.77	ON159960
AK30	*Bacillus* sp.	98	98.25	ON141506
AK41	*Bacillus pumilus*	97	96.67	ON166551
AK48	*Bacillus* sp	95	97.71	ON166503
AK63	*Bacillus pumilus*	94	91.30	ON166708
AK67	*Bacillus pumilus*	96	99.35	ON166657
AK87	*Bacillus* sp.	99	97.48	ON166709
AK91	*Bacillus halotolerans*	99	99.23	ON166768
AK95	*Bacillus* sp.	97	99.13	ON166784
AK96	*Bacillus subtilis*	98	99.87	ON166812
AK97	*Bacillus* sp.	98	99.86	ON166813
AK100	*Bacillus subtilis*	99	99.71	ON166825
AK109	*Bacillus halotolerans*	99	99.03	ON166826
AK116	*Bacillus safensis*	100	99.86	ON170419
AK118	*Bacillus amyloliquefaciens*	100	99.79	ON170420
AK122	*Bacillus subtilis*	98	99.68	ON171202
AK123	*Bacillus subtilis*	99	97.90	ON171203
AK127	*Bacillus velezensis*	97	98.20	ON171204
AK128	*Bacillus velezensis*	97	99.36	ON171215
AK129	*Bacillus subtilis*	99	95.65	ON171216
AK130	*Bacillus* sp.	97	99.57	ON171220
AK131	*Bacillus subtilis*	99.03	99	ON171219
AK142	*Bacillus safensis*	97	99.36	ON171225
AK147	*Bacillus subtilis*	99	99.75	ON171229
AK149	*Bacillus pumilus*	98	99.86	ON171231
AK150	*Bacillus* sp.	99	99.37	ON171234
AK156	*Bacillus velezensis*	99	99.54	ON171335
AK164	*Bacillus* sp.	99	98.49	ON171318
AK170	*Bacillus subtilis*	96	99.84	ON171367
AK177	*Bacillus subtilis*	100	100	ON171369
AK180	*Bacillus*	93	95.79	ON171372

	amyloliquefaciens			
AK181	*Bacillus altitudinus*	97	99.53	ON171373
AK185	*Bacillus safensis*	99	97.23	ON166704
AK189	*Bacillus* sp.	99	97.08	ON166676
AK 4	*Bacillus licheniformis*	100	99.79	ON140985
AK6	*Bacillus* sp.	99	99.29	ON158766
AK7	*Bacillus subtilis*	99	97.86	ON158768
AK14	*Bacillus subtilis*	98	100	ON158771
AK16	*Bacillus* sp	99	99.42	ON158774
AK49	*Bacillus zhangzhouensis*	96	97.93	ON152390
AK25	*Bacillus subtilis*	99	99.23	ON158775
AK31	*Bacillus* sp.	94	94.17	ON158776
AK33	*Bacillus* sp.	90	98.36	ON158777
AK53	*Bacillus pumilus*	100	93.54	ON158778
AK55	*Bacillus allitudinis*	91	98.63	ON171374
AK59	*Bacillus amyloliquefacien*	97	99.14	ON158781
AK66	*Bacillus pumilus*	96.36	99	ON158788
AK70	*Bacillus subtilis*	99	97.85	ON158787
AK88	*Bacillus safensis*	100	99.84	ON158790
AK102	*Bacillus subtilis*	99	98.74	ON158789
AK103	*Bacillus* sp.	99	99.41	ON075548
AK107	*Bacillus safensis*	99	98.14	ON158791
AK132	*Bacillus tequilensis*	99	98.15	ON158792
AK133	*Bacillus subtilis*	98	99.29	ON158799
AK134	*Bacillus pumilis*	100	99.43	ON158801
AK135	*Bacillus safensis*	99	97.56	ON158802
AK139	*Bacillus safensis*	99	97.97	ON158803
AK155	*Bacillus* sp.	99	99.14	ON158804
AK157	*Bacillus amyloliquefacien*	100	98.40	ON158806
AK173	*Bacillus subtilis*	100	100	ON158805
AK203	*Bacillus subtilis*	100	98.08	ON158807

AK204	*Bacillus subtilis*	98	99.35	ON158808
AK12	*Microbacterium imperiale*	99	99.02	ON158772
AK40	*Ralstonia picketti*	92	99.27	ON158779
AK18	*Bacillus* sp.	99	99.23	ON159204
AK20	*Bacillus stercoris*	99	99.51	ON159203
AK21	*Bacillus subtilis*	99	99	ON159205
AK29	*Bacillus subtilis*	99	99.69	ON159273
AK36	*Bacillus pumilus*	97	99.77	ON159260
AK42	*Bacillus safensis*	96	99.62	ON159274
AK43	*Bacillus aerophilus*	96	99.48	ON159276
AK52	*Bacillus subtilis*	99	99.12	ON159275
AK64	*Bacillus zhangzhouensis*	98	100	ON159279
AK92	*Bacillus safensis*	99	95.16	ON159280
AK104	*Bacillus subtilis*	99	99.87	ON159556
AK106	*Bacillus safensis*	100	99.61	ON159559
AK108	*Bacillus* sp.	99	97.29	ON075533
AK120	*Bacillus stercoris*	99	99.68	ON159594
AK148	*Bacillus stercoris*	100	100	ON171409
AK152	*Bacillus subtilis*	100	96.29	ON159597
AK154	*Bacillus subtilis*	99	98.23	ON159596
AK161	*Bacillus* sp.	100	100	ON159599
AK162	*Bacillus zhangzhouensis*	99	98.75	ON159598
AK163	*Bacillus aerophilus*	99	99.47	ON159601
AK165	*Bacillus* sp.	100	97.73	ON159602
AK166	*Bacillus* sp.	99	99.30	ON159607
AK167	*Bacillus pumilus*	99	98.11	ON159603
AK175	*Bacillus licheniformis*	100	95.14	ON159612
AK182	*Bacillus cereus*	99	98.65	ON159611
AK183	*Bacillus cereus*	98	99.13	ON159610
AK191	*Bacillus* sp.	100	96.86	ON159616
AK192	*Bacillus subtilis*	100	99.36	ON159617

AK193	*Bacillus subtilis*	99	98.15	ON159619
AK195	*Bacillus licheniformis*	97	93.41	ON171412
AK201	*Bacillus velezensis*	95	99.32	ON159621
AK202	*Bacillus velenzensis*	100	94.56	ON159620
AK208	*Bacillus* sp.	97	94.32	ON159706
AK209	*Bacillus* sp.	99	97.07	ON159707
AK22	*Chryseobacterium takakiae*	99	99.58	ON159206
AK26	*Chryseibacterium takakiae*	99	99.71	ON159259
AK23	*Microbacterium* sp.	99	98.48	ON159258
AK71	*Lysinibacillus* sp.	92	100	ON159277
AK168	*Acinetobacter seifertii*	100	98.58	ON159608
AK169	*Acinetobacter seifertii*	100	98.98	ON159609

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

NCBI database at (https://blast.ncbi.nlm.-nih.gov) was used to compare 16S *r*RNA gene sequences of the present isolates with other 16S *rRNA* sequences (Altschul et al., 1990). Multiple sequences were aligned using the Clustal W algorithm against corresponding nucleotide sequences (Thompson et al., 1994). This would display the closest matches to respective known species. Sequence analysis and Phylogenetic tree construction were then conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software verison 11. Partial 16S *r*RNA sequences of all the 133 isolates were compared with other 16S *r*RNA sequences of valid microbial strains published in NCBI database at (https://blast.ncbi.nlm.-nih.gov). A comprehensive analysis of the 16S *r*RNA of *Bacillus*, *Acinetobacter*, *Chryseobacterium*, *Lysinibacillus*, *Microbacterium*, and *Ralstonia* gene tree was

conducted in order to clarify the genetic distance between the present 16S *r*RNA gene sequence of the isolates of *Acinetobacter*, *Chryseobacterium*, *Lysinibacillus*, *Microbacterium*, and *Ralstonia* (Table 3.11). The nucleotide sequence of *Rhodopirellula caenicola* (LR133894.1) was downloaded from NCBI GenBank database and used as an outgroup. The evolutionary distance for 16S *r*RNA gene sequence of the isolates under *Acinetobacter*, *Chryseobacterium*, *Lysinibacillus*, *Microbacterium*, *Ralstonia* were performed with *Rhodopirellula caenicola* (outgroup) and the phylogenetic tree was generated using neighbor-joining method (Kumura, 1980). The analyses were performed using the Kimura-2-parameter model with 1000 bootstrap replications to represent the evolutionary history of taxa analyzed and are in units of the number of base substituitions per site (Figure 3.8).

The phylogenetic tree generated from the 16S *r*RNA gene sequence of the isolates using neighbor-joining method is shown in Figure 3.8. The evolutionary distances were computed by using the Kimura-2-parameter method (Kimura, 1980) and the tree generated from neighbor-joining method showed two clades and three main sub-clades. The bacterial isolates were present in various clusters in the phylogenetic tree. Bacterial species belonging to genera *Bacillus*, *Lysinibacillus*, and *Microbacterium* forms a single monophyletic clade but on separate branches with a bootstrap value of 69% which may infer that they share more common ancestry and is again divided into three sub-clades. However, genera *Bacillus* and *Lysinibacillus* formed a clade together with a bootstrap value of 89% separated from the genus *Microbacterium* which may be because *Bacillus* and *Lysinibacillus* belong to the same order Bacillales under class Bacillaceae. *Bacillus lichineformis* AK4

(ON140985), *Bacillus tequilensis* AK132 (ON158792), *Bacillus stercoris* AK20 (ON159203), *Bacillus subtilis* AK5 (ON140796), *Bacillus halotolerans* AK17 (ON158773), *Bacillus amyloliquefaciens* AK157 (ON158806), *Bacillus velezensis* AK202 (ON159620) belonged to the same cluster with a bootstrap value of 97%, *Bacillus safensis* AK136 (ON155917), *Bacillus altitudinis* AK181 (ON171373), *Bacillus aerophilus* AK163 (ON159601), *Bacillus zhangzhouensis* AK162 (ON159598), *Bacillus* sp. AK93 (ON152421) and *Bacillus pumilus* AK53 (ON158778) formed another cluster with a bootstrap value of 81% while *Bacillus cereus* AK182 (ON159611) and *Lysinibacillus* sp. AK71 (ON166492) formed a distinct cluster with bootstrap value of 52% and 89% respectively, and *Microbacterium imperial* AK27 (ON166492) and *Microbacterium* sp. AK28 (ON159960) formed a cluster with a boothstrap value of 99% and was placed close to *Lysinibaciilus* sp AK71 (0N159277). *Chryseobacterium talakiae* AK22 (0N159206) and *Acinetobacter seifertii* AK169 (0N159609) formed a cluster with a boothstrap value of 69% and *Ralstonia pickettii* AK40 (ON158779) was present on a separate branch close to the outgroup *Rhodopirellula caenicola* (LR133894.1) with 67% boothstrap value.

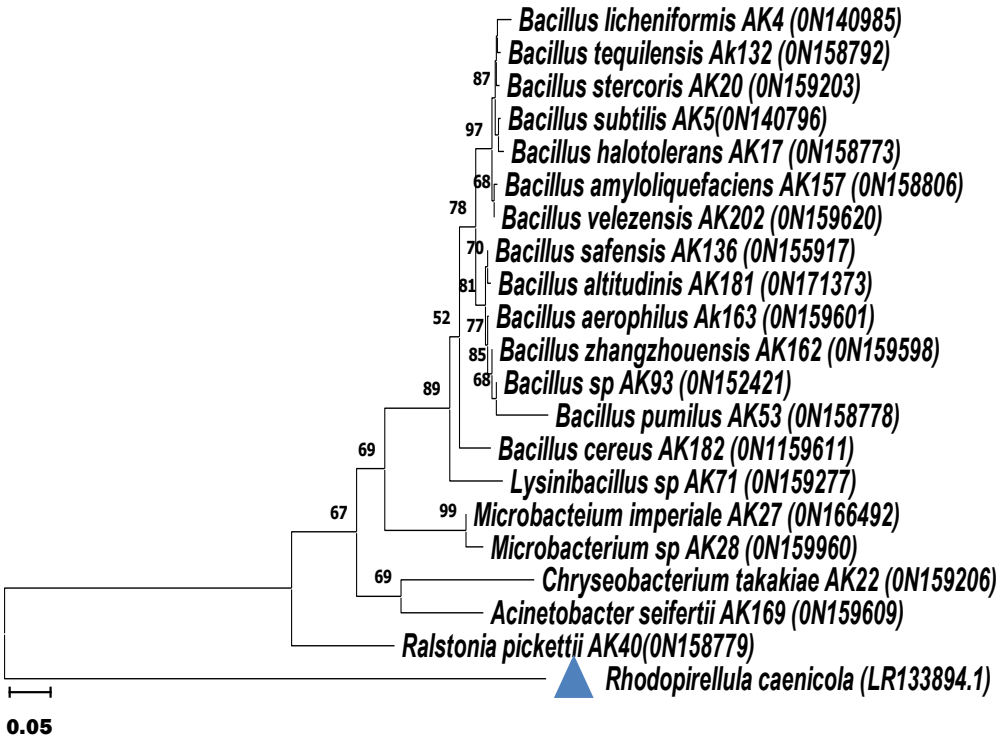

Figure 3.8. Phylogenetic tree generated on the basis of 16S *r*RNA gene sequences using Neighbor Joining Method. Genetic distances were computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values greater than 50% (expressed as percentage of 1000 replications) are given above the branch points. Enteries from this work is represented as generic name and GenBank accession number (in parentheses). Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

The phylogenetic tree generated from the 16S *r*RNA gene sequence from the isolates of bacterial species under *Acinetobacter*, *Bacillus*, *Lysininbacillus*, *Microbacterium*, *Ralstonia* with other nucleotide sequences from closely related *Acinetobacter* species, *Bacillus* species, *Lysininbacillus* species, *Microbacterium* species, and *Ralstonia* species is shown from Figure 3.9-3.13. The evolutionary distances were computed by using the Kimura-2-parameter method (Kimura, 1980) and *Rhodopirellula caenicola* (LR133894.1) was used as an outgroup in all the

following phylogenetic tree generated. The analyses were performed using the Kimura-2-parameter model with 1000 bootstrap replications to represent the evolutionary history.

Figure 3.9 shows the phylogenetic tree from the 16S *r*RNA gene sequence of the bacterial species under *Acinetobacter* with other nucleotide sequences from closely related *Acinetobacter* species. The bacterial isolates are present in distinct clusters with different bootstrap values from 35-88%. *Acinetobacter seifertii* AK169 *(ON159609)* was placed closely to with the reference strain *Acinetobacter seifertii* (ON159609.1) forming a clade together with a bootstrap value of 88% which again forms a monophyletic clade with *Acinetobacter seifertii* AK168 (ON159608) with a bootstrap value of 71 %. When the isolates AK168 and AK169 were examined using BLASTn, the sequences showed highly aligned (100%).

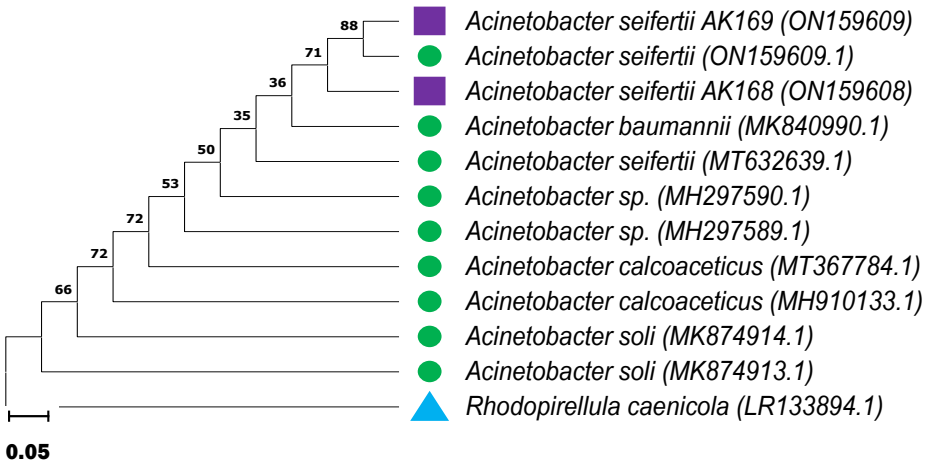

Figure 3.9. Phylogenetic tree of *Acinetobacter* species (AK168 and AK169) on the basis of 16S rRNA gene sequences, and showing the relationship with other closely related *Acinetobacter* species. Genetic distance was computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values (expressed as percentage of 1000 replications) are given above the branch. Enteries against (■) represents isolates from this work and entries agianst (●) are generic name and GenBank accession number (in parentheses) from public databases. Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

Figure 3.10 shows the phylogenetic tree from the 16S *r*RNA gene sequence of bacterial species under *Chryseobacterium* with other nucleotide sequences from closely related *Chryseobacterium* species. The bacterial isolates are present in two clades and three main sub-clades. *Chryseobacterium takakiae* AK22 (ON159206) was placed closely with the reference strain with a bootstrap value of 50% forming a clade together and showing close relation with the reference strain, *Chryseobacterium takakiae* (MF767440.1), which again forms a monophyletic clade with *Chryseobacterium takakiae* AK26 (ON159259) with a bootstrap value of 82%. When the isolates AK22 and AK26 were examined using BLASTn, the sequences showed highly aligned (99%).

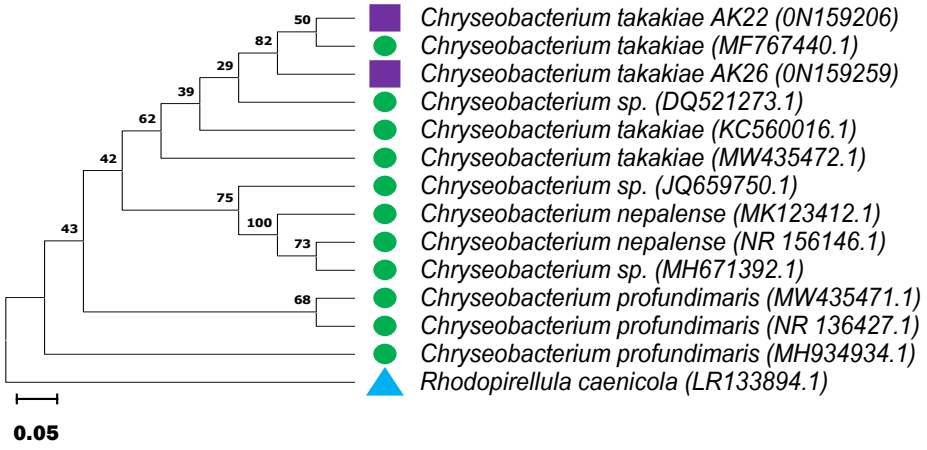

Figure 3.10. Phylogenetic tree of *Chryseobacterium* species (AK22 and AK26) on the basis of 16S r*RNA* gene sequences, and showing the relationship with other closely related *Chryseobacterium* species. Genetic distance was computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values (expressed as percentage of 1000 replications) are given above the branch. Enteries against (■) represents isolates from this work and entries agianst (●) are generic name and GenBank accession number (in parentheses) from public databases. Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

Figure 3.11 shows the phylogenetic tree from the 16S *r*RNA gene sequence of the bacterial species under *Microbacterium* with other nucleotide sequences from closely related *Microbacterium* species. The bacterial isolates are present in distinct clusters with different bootstrap values from 50-79%. *Microbacterium imperiale* AK28 (ON159960), *Microbacterium imperiale* AK23 (ON159258), *Microbacterium* sp. AK27 (ON166492), *Microbacterium* sp. AK12 (ON158772), was placed close to each other with bootstrap values of 50-66%. *Microbacterium* sp. AK12 was close to the outgroup *Rhodopirellula caenicola* (LR133894.1) *and Microbacterium imperiale* AK28 was close to the reference strain *Microbacterium* sp. (ON150859.1) with a bootstrap value of 52%. *Microbacterium* sp. AK11 (ON150859) was placed in another clade close to the reference strain *Microbacterium imperiale* (KF254682.1) with a boothstrap value of 36%. When the isolates AK11, AK12, AK23, AK27 and AK28 were examined using BLASTn, the sequences showed highly aligned (99-100%).

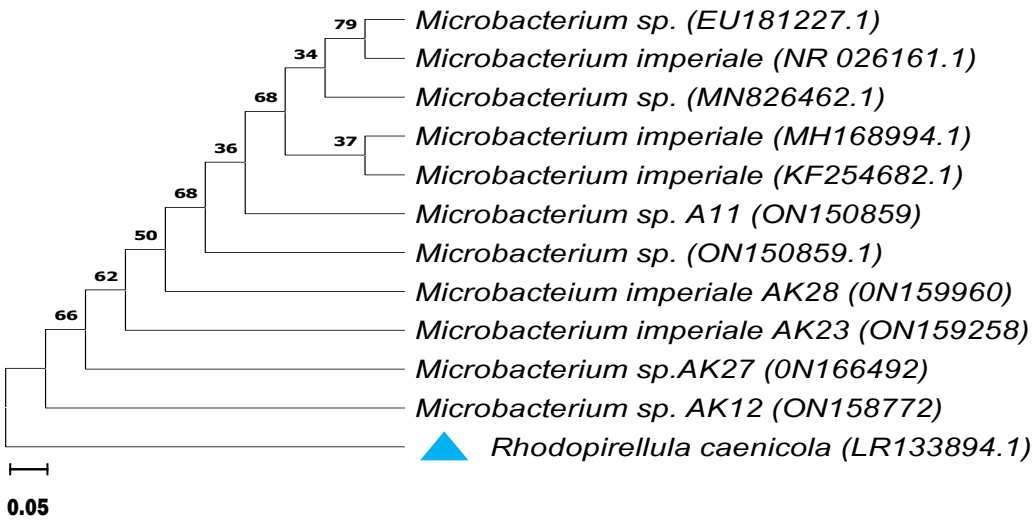

Figure 3.11. Phylogenetic tree of *Microbacterium* species (AK168 and AK169) on the basis of 16S r*RNA* gene sequences, and showing the relationship with other closely related *Microbacterium* species. Genetic distance was computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values (expressed as percentage of 1000 replications) are given above the branch. Enteries against (■) represents isolates from this work and entries agianst (●) are generic name and GenBank accession number (in parentheses) from public databases. Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

Figure 3.12 shows the phylogenetic tree from the 16S *r*RNA gene sequence of the bacterial species under *Ralstonia* with other nucleotide sequences from closely related *Ralstonia* species. The bacterial isolates are present in distinct clusters with different bootstrap values from 45-77%. *Ralstonia pickettii* AK40 (ON158779) was placed close to *Ralstonia* sp. (KJ6546841.1) with a boothstrap value of 69%. When the isolate was examined using BLASTn, the sequence showed highly aligned (92%).

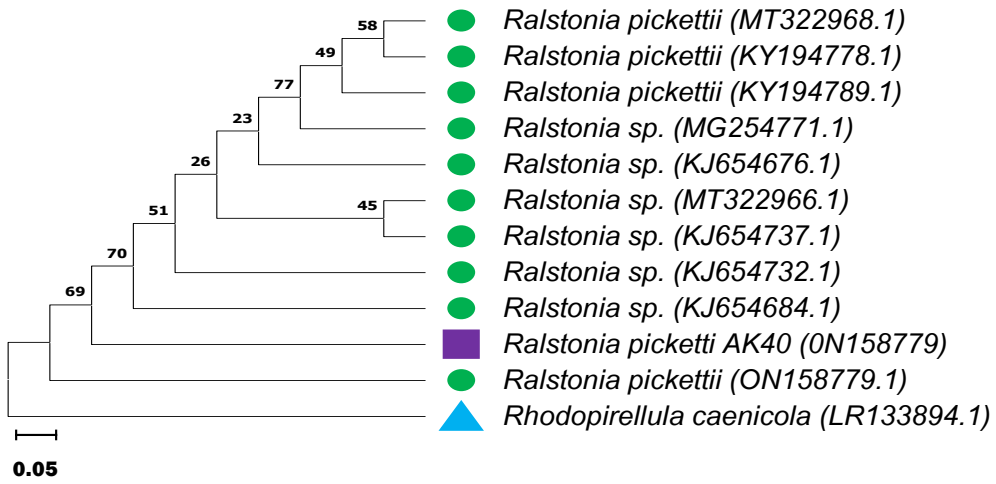

Figure 3.12. Phylogenetic tree of *Ralstonia* species (AK40) on the basis of 16S r*RNA* gene sequences, and showing the relationship with other closely related *Ralstonia* species. Genetic distance was computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values (expressed as percentage of 1000 replications) are given above the branch. Enteries against (■) represents isolates from this work and entries agianst (●) are generic name and GenBank accession number (in parentheses) from public databases. Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

Figure 3.13 shows the phylogenetic tree from the 16S *r*RNA gene sequence of *Bacillus species* with 124 isolates and *Lysinibacillus* species with 1 species from the present study. The phylogenetic tree showed 14 large clades and 40 main sub-clades. The bacterial isolates were present in various clusters in the phylogenetic tree. *Lysinibacillus* sp. AK71 (ON159277) formed a monophyletic group with *Bacillus cereus* AK183 (ON159610) and *Bacillus* sp. AK191 (ON159616). *Lysinibacillus* sp. AK71 was placed close to *Bacillus cereus* AK182 (ON1559661). *Bacillus areophilus* AK163 (ON159601), *Bacillus subtilis* AK187 (ON156449), *Bacillus amyloliquefaciens* AK118 (ON170420), *Bacillus pumilus* AK167 (ON159603), *Bacillus pumilus* AK134 (ON158801), *Bacillus zhangzhouensis* AK49

(ON152390), *Bacillus pumilus* AK53 (ON158878), *Bacillus* sp. AK108 (ON175533), *Bacillus subtilis* AK100 (ON166825), *Bacillus subtilis* AK177 (ON171369), *Bacillus subtilis* AK25 (ON158775), *Bacillus* sp. AK30 (ON141506), *Bacillus* sp. AK189 (ON166676), *Bacillus velezensis* AK127 (ON171204), *Bacillus subtilis* AK178 (ON156033), *Bacillus subtilis* AK123 (ON171203), *Bacillus subtilis* AK204 (ON158808), *Bacillus licheniformis* AK4 (ON140985), *Bacillus subtilis* AK171 (ON156006), *Bacillus velezensis* AK201 (ON159621), and *Bacillus altitudinis* AK181 (ON171373) all formed separate distinct braches with a bootstrap value of 52-100%. All the *Bacillus* strains were very distinct from each other as they had highly variable bootstrap values and formed various sub-clades. *Bacillus altitudinis* AK181 was present close to the outgroup *Rhodopirellula caenicola* (LR133894.1)**.** All these strains from the present study showed that we have been able to isolate high culture diversity, reflected by their high genetic variability and biochemical diversity.

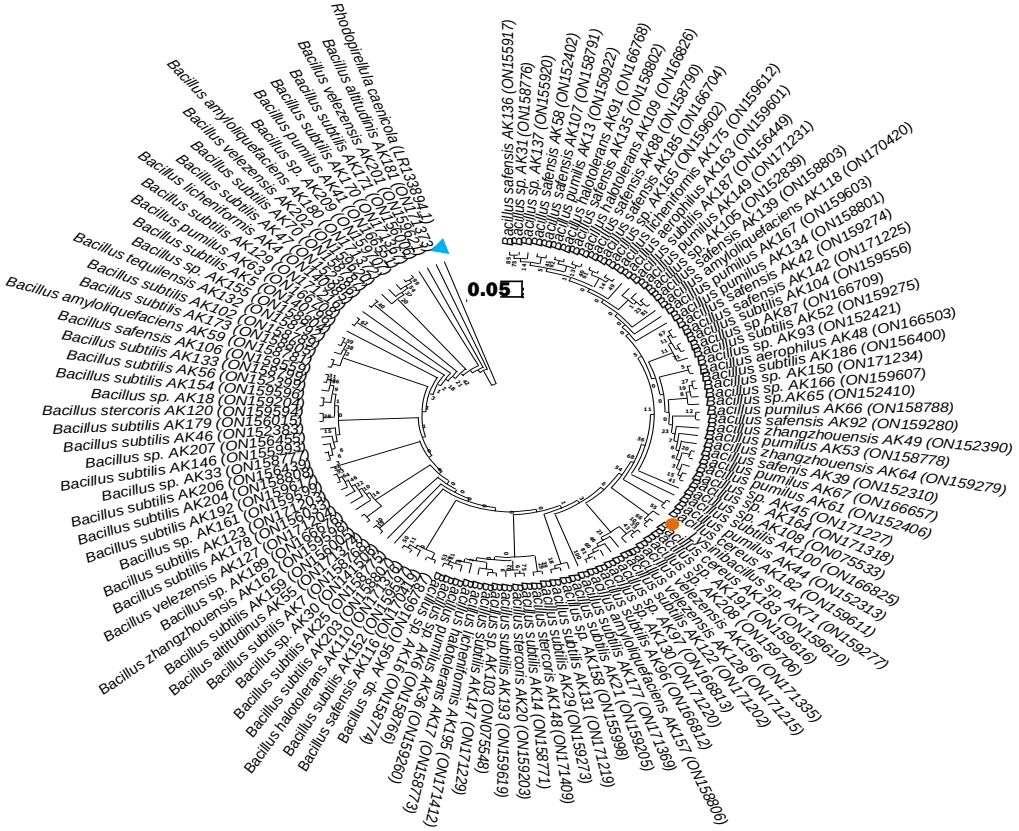

Figure 3.13. Phylogenetic tree of *Bacillus* and *Lysinibacillus* species on the basis of 16S rRNA gene sequences using Neighbor Joining Method. Genetic distance was computed by using Kimura's model. Bar=0.05 substitution per site. Bootstrap values (expressed as percentage of 1000 replications) are given above the branch. Enteries against (●) represents *Lysinibacillus* sp. AK71 (ON159277). Enteries against (▲) represents *Rhodopirellula caenicola* (LR133894.1) used as an outgroup from public database GenBank

3.3.6. Identification of fungal isolates

Soil fungal isolates were identified through morphological and microscopical characterization with the help of taxonomic keys and following relevant literatures (Afzal *et al*., 2013; Asan, 2004; Bamett 1965; Carmen and Sciortino, 2017; Domsch, 1980; Duss and Laane, 1984; Frisvad and Samson, 2004; Germain and

Summerbell, 1996; Gilman, 1957; Gilman, 2001; Giraldo et al., 2015; Hauser, 2006; Ho *et al*., 2004; Jayasiri et al., 2019; Klich, 2002; Leslie and Summerbell, 2006; Nelson et al., 1983; Raper and Thom, 1949; Rifai, 1969; Samson and Pitt, 2000; Samson et al., 2004; Samson et al., 2014; Samson, 1974; Siddiquee, 2017; St Pitt and Hocking, 2009; Thathana *et al.*, 2017, Thilagam, 2018; Wagner *et al*., 2013, Watanabe, 1937; Watanabe, 2002; Webster and Weber, 2007). The maximum fungal isolate recorded was under the genera *Penicillium*, *Aspergillus*, *Tricoderma*, *Mucor* and *Fusarium* with 12, 11, 6, 5 and 5 species respectively. Some of the identified fungal isolates are shown in figure 3.14-3.57.

***Absidia cylindrospora* Hagem. (1908)**

Colonies reached 2.2-3.4 cm, 2.3-3.5 cm, 3.1-4.1 cm and 2.5-3.1 cm in diameters on RBA, PDA, MEA and CDA within 5 days respectively. Colonies on top at first white in all the media plates becoming grayish brown with age, fast growing showing a radial growth and on the bottom, colony colour were yellow, brown and colourless. Zygospores covered with stellate projections, finger-like appendages arising from suspensors. Sporangiophores arising singly or branched, 2-3 from the same place of the stolon bearing apophysate sporangia and with a septum, upto 200µm long and 1.8-2.5 µm wide. Sporangia 10-12 µm in diameter, pyriform, smooth, columella present. Cylindrical spores, 2.1-2.4x3.5-4.1 µm with rounded ends.

***Absidia glauca* Hagem. (1908)**

Colonies reached 2.3-3.2 cm, 2.3-3.4 cm, 3.1-3.3 cm and 2.4-3.1 cm in diameters on RBA, PDA, MEA and CDA in 5 days respectively. Colonies initially

white in all the media plates which turned brownish olive in RBA, bluish green in PDA, grayish brown in CDA and on bottom all the media plates were colourless and fast growing. Sporangiophores erect, 2-3 arising from the same place on stolons, 260-300 µm long, 4.3-6 µm wide, with a septum and rhizoids present. Sporangia pyriform, 22.2-48.3µm in diameter with columellae above apophysis, smooth with pointed spine at top.

***Absidia* sp.1 Tiegh. (1878)**

Colonies reached 1.1-1.6 cm, 1.5-2.2 cm, 1.6-2.7 cm and 1.1-1.5 cm in diameters on RBA, PDA, MEA and CDA in 5 days respectively. Colonies on top were brownish grey, fluffy and bottom white in all the media plates. Sporangiospores 3.2-2.2 µm long, zygospores covered with stellate projections to 4.1 µm long, finger-like appendages arising from suspensors.

***Acremonium* sp.1 Link (1809)**

Colonies reached 1.3-1.9 cm, 2.3-2.8 cm, 3.1-3.3 cm and 2.5-2.9 cm in diameters on RBA, PDA, MEA and CDA in 7 days respectively. Colonies on the top of the media plates were white and slightly raised in the centre and the colony color in the bottom of these plates were light brown (MEA) and colourless (RBA, PDA and CDA). Septate, hyaline and branched hyphae with phialides. Phialides septate, hyaline, 15-38 µm long and 1.2-3.1 µm wide with basal septum tapered towars the apex with conidial masses. Conidia solitary, 1-celled, elongate ellipsoidal, cylindrical to oval, 2.9-3.4 x 1.5-2.4 µm.

***Acremonium* sp.2 Link (1809)**

Colony diameters on RBA, PDA, MEA and CDA in 5 days were 1.2- 1.4 cm, 1.1-1.4 cm, 1.4-2 cm and 1-1.9 cm respectively. Colonies on the top of the media plates appeared pinkish white with a pungent odour, cottony, flat and felty. Colony colour varied on the bottom of these plates from salmon to colourless. Branched, septate and hyaline hyphae with phialides. Phialides erect, hyaline, unbranched, septate, 1.3-2.2 µm wide and 21-40 µm long with a basal septum, tapered towards the apex with terminal conidial masses. Conidia 1-celled, hyaline, solitary, cylindrical to ellipsoidal, 3.1-4.9 µm x 1.8-2.2 µm.

***Alternaria alternate* (Fr.) Keissl. (1912)**

Colony diameters on RBA, PDA, MEA and CDA in 7 days were 2.2-3.4cm, 2.1-3.6 cm, 2.2-3.5cm and 2.1-3.1 cm respectively. Colonies on top initially were grayish-white which rapidly turned into olive color and fluffy. Bottom of the media plates were black. Conidiophore septate, straight, 23-46 µm long. Conidia golden brown branched in chains, smooth-walled, obclavate, tapered like a beak, two to six transverse septa, 15-34 x 6-15 µm.

***Alternaria* sp. Nees**

Colony diameters on RBA, PDA, MEA and CDA in 5 days were 2.6-3.4cm, 2.5-3.9 cm, 2.2-3.7cm and 2.1-3.1 cm respectively. Colony color on top appeared as grayish-white at first, rapidly turned olive, velvety and bottom of the media plates dark brown to black color. Septate hyphae, conidiophores light golden brown in color, septate, curved, 2.2-4.1 µm wide. Conidia solitary, golden brown, short

conical beak with germ tubes, two to seven transverse septa with one or two longitudinal septa, 3.5-10 x 10-20 µm.

***Arthtrographis* sp. G. Cochet ex. Sigler & J.W. Carmich. (1976)**

Colony diameters on RBA, PDA, MEA and CDA in 7 days were 1.7-2.1cm, 1.8-3.9 cm, 1.5-2.9cm and 2.1-3.2 cm respectively. Colonies initially smooth and yeast like later become velvety. White on top and white to tan on bottom of the media plates. Hyphae septate and rough in whorls and stucked together, branched conidiophores with anthroconidia. Anthroconidia boxcar shaped, 2-3.2 x 3-6.5 µm.

***Apophysomyces elegans* P.C.Misra, K.J.Srivast. & Lata (1979)**

Colonies fast growing reaching 4.2-4.9 cm, 4.2-4.7 cm, 4.2-5 cm and 4-4.1 cm in diameter within 5 days on RBA, PDA, MEA and CDA respectively. Colony colour on the top of these media plates were creamy white, raised, cottony, irregular and on the bottom, colony colour were pale brown and yellow. Hyphae septate, hyaline, branched, 3-5 µm and irregular. Foot cells present at the base of sporangiophore. Sporangia multispored, pyriform, 14-50 µm wide. Columellae present and apophyses funnel- shaped. Sporangiophore erect, unbranched, tapered towards the apex, ellipsoidal, 2.3-3.8µm wide and 100-350µm long.

***Aspergillus flavus* Link. (1809)**

Colonies were 3.9-5.1 cm, 3.5-4 cm, 3.6- 4cm and 2.2-3 cm in diameter within 5 days on RBA, PDA, MEA and CDA respectively. Colony color on top at first yellow-green later becoming dark green and bottom light brown, pale yellow and colourless. Conidiophores hyaline, rough walled bearing vesicle. Vesicles

globose to subglobose. Conidial heads radiating, biseriate and uniseriate. Phialides bearing conidia measuring 3-4.4 µm in diameter, smooth and aseptate.

***Aspergillus fumigatus* Fresenius. (1863)**

Colony slow growing, reaching 0.8-1.8 cm, 0.6-1.6 cm, 0.9-1.3 cm and 0.8-1.2 cm in RBA, PDA, MEA and CDA respectively in 5 days. Colony colour on top greenish-brown with exudate and white margins, velvety, bottom of these media plates were pale brown in color. Conoidiophores unbranched and sub-hyaline, straight to flexuous, smooth and aseptate, 2.4-3µm wide. Vesicles globose to sub-globose, conidial heads sub-spherical, phialides borne directly on the vesicle and uniseriate. Conidial head erect, compact and columnar. Conidia globose, echinulate and 1.5-2µm in diameter.

***Aspergillus heteromorphous* Batista and H. Maia (1957)**

Colony diameters on RBA, PDA, MEA and CDA were 2.9- 3.7 cm, 3.2-4.1 cm, 3.9-4.7 cm and 3.2-4.4 cm respectively. Colony colours on the top of the media plates were brownish black (RBA, PDA, MEA) and black (CDA). Bottom colour of the media plates were pale brown to brown. Colonies were granular with white floccose mycelium. Hyphae aseptate and biserrate. Conidiophores smooth, 400-790 µm long and 10-12µm wide. Vesicles globose, 33-47µm diameter. Phialides 5.2 -7.2 µm long and 2-3 µm wide. Conidia globose, rough, 3-4 µm diameter.

***Aspergillus niger* van Tieghem (1867)**

Colony diameters on RBA, PDA, MEA and CDA were 1.8- 2.6 cm, 2.2-3.1 cm, 2.5-3.7 cm and 3.2-4.1 cm respectively in 5 days. Colonies black on top surrounded by white margin. Bottom colorless in RBA, brown in PDA, cream in

MEA and CDA. Conidiophores sub-hyaline and pale brown, erect, smooth, unbranched, aseptate and 7.6-9 µm wide and 300-500 µm long. Phialides 7-8 µm long and 3-4 µm wide. Conidial heads biseriate, brown black to black with globular, aseptate unbranched chain of conidia. Conidia mostly measured 3-3.4 µm in diameter and appeared brownish, globose and rough.

***Aspergillus ochraceus* Wilhelm (1877)**

Colony diameters in 7 days on RBA, PDA, MEA and CDA were 3.2- 3.8cm, 3.1-3.9cm, 3.1-4cm, 2.4-3cm respectively. Colony colours on the top were brownish yellow (RBA, PDA and MEA) and light yellow in CDA, colonies were powdery, convoluted, flat, floccose-felty. On the bottom colony colours were colourless (RBA), pale brown (PDA) cream (MEA and CDA). Septate and hyaline hyphae. Conidiophores branched, erect, 4-7 µm wide, 250-400 µm long. Conidia 3-5 µm in diameter, ellipsoidal and hyaline.

***Aspergillus oculeatus* Iizuka (1953)**

Colony diameters on RBA, PDA, MEA and CDA were 3- 3.7cm, 3-3.4cm, 3.1-3.9cm, 2.4-2.9cm respectively in 7 days. Colony colours were black on the top of the media plates and on the bottom wrinkled and colony colours were colourless (RBA), brown (PDA) cream (MEA and CDA). The colonies were floccose and slightly raised in the centre. Conidiophores 7-14 µm wide, 300-500 µm long, thick-walled and hyaline. Phialides 7-12µm long and 2-3 µm wide, phialides covered the globose vesicles, vesicles 20-30 µm in diameter, dull yellow to light brown. Conidia 2-3 µm in diameter, rough and globose to subglobose.

***Aspergillus oryzae* Cohn (1884)**

Colony diameters in 7 days on RBA, PDA, MEA and CDA were 3.2- 4 cm, 3-4.7 cm, 4.2-5 cm, 2.7.4-3.7 cm respectively. Colonies on top appeared as light brownish green in colour. Conidiophores rough, 450-800 µm long and 12-15 µm wide. Conidia slightly roughened, 5-6 µm in diameter, 1-3 nucleate.

***Aspergillus terreus* Thom. (1918)**

Colony diameter 2-2.9 cm, 2.1-2.8 cm, 2.2-3.1 cm, 1.6-2.1 cm on RBA, PDA, MEA and CDA respectively in 5 days. Colonies on top of these media plates were bright yellow in colour surrounded by white colour and the bottom of the plates were colourless (RBA) tom pale yellow (PDA, MEA and CDA). Conidiophores smooth walled, hyaline, 100-250 µm long with hemispherical vesicles, metulae present. Conidial heads columnar, conidia slightly ellipsoidal, smooth-walled, 1.5-2.4 µm diameter.

***Aspergillus versicolor* (Vuillemin) Tiraboschi (1908)**

Colonies on RBA, PDA, MEA and CDA varied from 1.7-1.9 cm, 1.3-2.2 cm, 1.3-2.2 cm and 1.5-2 cm. Colony on top were brownish green with dark brown coloured exudates on RBA, PDA and MEA plates. Colonies surrounded with white colour mycelium, floccose, velvety and sulcate. Bottom color of these media plates were colourless (RBA), pale yellow (PDA and CDA), deep brown (MEA) and wrinkled. Conidiophores hyaline, smooth-walled, closely interwoven mycelium and 210-700µm long, sub-globose, biseriate. Phialides 5-8µm long and 2-2.5 µm wide. Vesicles 9-15 µm diameter and pyriform. Conidia ellipsoidal, rough, hyaline and 2-3.6 µm diameter.

***Aspergillus* sp.1 Micheli (1729)**

Colony diameters on RBA, PDA, MEA and CDA were 2.9- 3.7 cm, 2.5-3.2 cm, 3.1-3.9 cm and 2.1-2.7cm respectively in 7 days. Colony colours on top of these media plates were greenish-green (RBA and MEA) and dark green (PDA and CDA). Bottom light to dark brown in colour. Colonies were velvety, with off white mycelium in RBA and MEA. Conidiophores long, aseptate, hyaline, smooth-walled, 3-5 µm wide, 300- 800 µm long. Phialides 5-6 µm long, conidia smooth and globose, 2-3 µm diameter.

***Aspergillus* sp.2 Micheli (1729)**

Colony diameters on RBA, PDA, MEA and CDA were 2.9- 3.7 cm, 2.5-3.2 cm, 3.1-3.9 cm and 2.1-2.7 cm respectively in 7 days. Colony colours on top yellowish green with white mycelial margins, velvety and bottom of these media plates were colorless(RBA), pale yellow (PDA and MEA) and cream (CDA). Conidiophores hyaline, erect, aseptate, smooth-walled, 120-240 µm long, 3-5 µm wide. Phialides 2.2-3 µm wide and 3-4 µm long. Vesicles 10-30 µm diameter, globose. Conidia 2-3 µm in diameter and globose.

***Arthrinium* sp. Kunze (1817)**

Colony diameters in 5 days on RBA, PDA, MEA and CDA were 3.1- 4 cm, 3.2-4.2 cm, 3.2-4.5 cm and 1.5-2.2 cm respectively. Colony colours on the top were white, cottony, slightly raised in the centre, spreading, circular and aerial mycelium. Bottom colour of the plates were colourless (RBA), pale yellow (PDA and MEA) and cream (CDA). Hyphae hyaline, septate and branched, 1.5-4 µm diameter. Conidiophores reduced to conidiogenous cells, clustered in aggregation on hyphae,

doliiform to ampulliform, sympodial or polyblastic, 5-9.7 µm long and 3-6 µm wide. Conidia brown to dark brown, globose, smooth, central scar, pale equatorial slit, ellipsoidal, 8.1-12.6 µm x 6.1-10.6 µm.

***Botrytis* sp. P. Micheli ex Pers. (1794)**

Colony diameters in 5 days on RBA, PDA, MEA and CDA were 3.3- 4.2 cm, 3-4.7 cm, 3-4.5 cm and 1.3-2.5 cm respectively. Colony colours on the top were creamy white in RBA, MEA and CDA and greyish-white in PDA, colonies floccose to effuse, raised in the center, cottony and irregular. Bottom colour of these plates were colourless in (RBA), light brown (PDA and MEA) and cream (CDA). Mycelium septate, branched and filamentous. Conidiophore branched, erect, septate, brown, raised directly from mycelium, 5.5-8 µm wide. Conidia pale brown, hyaline, globose to ellipsoidal, smooth walled, 1-celled, 6-8 µm wide and 9-11 µm long.

***Chaetomium globosum* Kunze (1817)**

Colony diameters in 7 days on RBA, PDA, MEA and CDA were 3- 4 cm, 3.2-4.2 cm, 3.8-4.8 cm and 1.9-2.5 cm respectively. The colony colour on the top of these media plates were white to grey (RBA), greyish-yellow (PDA, MEA and CDA), cottony, granulated, floccose, and lobate to entire. Bottom colour of the colonies were pale yellow to dark colour. Ascomata dark brown, globose to subglobose, 170-250µm wide and 200-350µm long, lateral hair dark brown radiating in all directions. Terminal hairs dark olive-brown, 3.3-4.5 µm wide. Asci hyaline and clavate. Ascospores ellipsoidal, apiculate ends and 6-8 µm wide

***Chaetomium* sp.1 Kinze (1817)**

Colony diameters measured 2.3-3 cm, 2.1-2.6 cm, 2-2.7cm and 1.7-2.5 cm in 7 days on RBA, PDA, MEA and CDA respectively. The top of the colonies were grayish-white (RBA), and yellowish-brown (PDA, MEA and CDA), floccose, cottony, irregular, entire and granulated. Bottom color of these media plates appeared as colourless (RBA and MEA), pale yellow (PDA), light brown (CDA). Ascomata globose and dark brown, 200-250 µm wide and 220-350 µm long. Ascomatal wall radiating in all directions, 180-250 µm wide and 200-350 µm long. Terminal hairs in a dense tuft, lateral hair wavy and dichotomously branched. Ascospores sub-globose to ellipsoidal, brown, 7-10 µm wide.

***Chaetomium* sp.2 Kinze (1817)**

Colony diameters measured 3.1- 4.2 cm, 3.3-4.5 cm, 3.1-4 cm and 2.5-3 cm in 7 days on RBA, PDA, MEA and CDA respectively. Colony on the top of the media plates were white (RBA), whitish olivaceous grey (PDA), greyish-white (MEA and CDA), cottony, floccose and entire colonies. Bottom colour of the colonies were colourless to pale yellow. Ascomata globose to sub-globose. Peridium dark rough, brown and 200-300 µm wide and 250-390 µm long. Ascomatal wall radiating in all directions. Terminal hairs branched, undulate and 3-5 µm wide. Asci hyaline, clavate and fasciculate. Ascospores sub-globose, irregular and 6.5- 9 µm wide.

***Chrysosporium* sp.1 Corda (1833)**

Colony diameters measured 3- 4.1 cm, 3.2-4.7 cm, 3.2-4.1 cm and 1.5-2.6 cm in 7 days on RBA, PDA, MEA and CDA respectively. Colony colours on the top

of these media plates were white, floccose, slightly raised in the center, circular and entire whereas, the colours on the bottom of these plates varied from colourless (RBA and CDA), yellowish-brown (PDA) and brown (MEA). Hyphae hyaline, straight to racquet, branched, 1-3µm wide. Conidiophores with short chains of 2-4 alternate anthroconidia, 37-50 µm long. Anthroconidia cuneiform, pyriform to clavate, with a broad truncate base, smooth, 2-5 x 2-4 µm. Conidia hyaline, aleuriosporus globose to clavate and pyriform, basal scars, 1-celled, intercalary as well as lateral, terminal, 3-5 x 1.5-2.5 µm.

***Chrysosporium* sp.2 Corda (1833)**

Colony slow growing, colony diameters in 7 days were 0.6-1.2 cm, 0.2-1.5 cm, 0.6-1.5 cm and 0.5-1 cm on RBA, PDA, MEA and CDA respectively. Colonies on the top of the media plates appeared as olive to brown, flat, dense and with white margins. On the bottom of the media plates the colony colour were black. Conidia arising directly from the hyphae with 1-3 conidia forming ampulliform swellings, subglobose to pyriform, thick-walled, hyaline, 9-10 x 6.5-9 µm.

***Cladophialophora* sp. Borelli (1980)**

Colony diameters in 5 days were 1.6-3.2 cm, 1.2-3.5 cm, 1.6-2.5 cm and 1.5-2.2 cm on RBA, PDA, MEA and CDA respectively. Colonies on the top of the media plates appeared as black with a velvety surface. On the bottom of the media plates the colony colour were pale yellow to brown. Hyphae hyaline and septate, conidiophores short with chains of conidia producing sterigmata, 2-5 µm long, conidia lemon shaped to round, 1-2.5 x 1.5x3 µm.

***Cladosporium cladosporiodes* (Frescen.) G.A. de Vries, (1952)**

Colonies grow fast but the growth was poor in CDA, the colony diameters in 5 days were 1.5-3.2 cm, 1.6-3.5 cm, 1.5-3.5 cm and 0.5-1 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top of these media plates were first whitish-grey becoming olivaceous brown, velvety, floccose, slightly raised, round and entire to scalloped. On the bootom of these media plates the colony colour appeared as black. Hyphae septate and branched. Condiophore straight, short, branched, solitary with 3 to 4 cylindrical ramoconidia, 300-350 µm long and 3.5-6.5 µm wide. Conidia numerous, branched, one-celled, ellipsoidal to lemon shaped, smooth walled, 3-5 µm long and 2-2.5 µm wide.

***Cladosporium herbarum* (Pers.) Link (1816)**

Colonies diameters in 5 days were 4- 5 cm, 3-4.5 cm, 3-4 cm and 3-3.5 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top of these media plates were initially whitish-grey then changed to olivaceous green (RBA and PDA), pale olivaceous grey (MEA) and grey (CDA), floccose to velvety, slightly wrinkled, folded and wrinkled in the centre, radially furrowed and irregular. Bottom colour of the colonies were olivaceous-black. Hyphae septate and branched and 2-3.7 µm wide. Conidiophore erect, sepatae, branched, cylindrical, terminal and intercalary swelling, 200-250µm long. Ramoconidia septate, cylindrical and ampulliform. Conidia ovoid to ellipsoidal with rounded ends, golden brown, 1 to 2-celled, 3- 5.5 x 4.5-8.5 µm.

***Cladosporium oxysporum* Berk. & M. A. Curtis (1868)**

Colonies diameters in 5 days were 1.5- 3 cm, 1.8-3.5 cm, 1-3cm and 1-2.8 cm on RBA, PDA, MEA and CDA respectively. Colonies on the top of the media

plates were grey, floccose, slightly raised, irreugular, undulate to entire whereas the bottom color of the colonies were colourless (RBA) and leaden grey (PDA, MEA and CDA). Hyphae septate and branched, 2.2-5.8 µm wide. Conidiophore long, cylindrical to filiform, unbranched with intercalary swelling, 2-4 x 230-250 µm. Conidia ovoid to cylindrical, greyish brown, 1-celled.

***Cladosporium* sp. Link (1816)**

Colonies diameters in 5 days were 1.2- 3.3 cm, 1.5-3.5 cm, 1.4-3.6 cm and 1.2-2.9 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top of these media plates varied from bright green to olivaceous grey (RBA, PDA and MEA), dark green (CDA), colonies round, slightly raised to raised, floccose-felty and scalloped to entire. The colours on the bottom of these plates varied from black (RBA, MEA and CDA) to colourless (PDA). Hyphae septate and hyaline. Conidiophores erect, branched, solitary with 3 to 4 cylindrical ramoconidia, long and 4.2-7.5 x300-410 µm. Conidia in chains, hyaline, limoniform and ovoid, 1 to 2-celled, sympodial, 1.5-2.5 x 4-5.2 µm.

***Clonostachys* sp.**

Colonies diameters in 7 days were 2.2- 3.4 cm, 2.5-3.5 cm, 2.4-4 cm and 0.6-1.9 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top of all these media plates were white and on the reverse of these plates the colours were colourless (RBA) and pale yellow (PDA, MEA and CDA). Colonies were cottony, raised, felty and entire. Hyphae hyaline and 1.5-5 µm wide. Conidiophores primary and secondary, erect, hyaline and branched. Primary conidiophore 2-3 x 100-300 µm, verticillium-like branches and secondary conidiophore with 10.5-14.5 µm long

phialides, Penicillium-type, solitary or aggregated, hyaline and divergent phialides. Chlamydospores 7.1-18 µm in diameter, intercalary to terminal. Conidia hyaline, 1-celled, globose to ellipsoidal, 3-3.5 x 2.2-5 µm.

***Colletotrichum* sp. Corda (1831)**

Colonies diameters in 5 days were 3.2- 4.8 cm, 3.3-4.6 cm, 3.8-5 cm and 3.2-4.2 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top were white and bottom creamy yellow. Colonies were floccose, cottony, smooth and entire. Conidiophores septate and conidiogenous cells hyaline. Appresoria entire and obovate. Conidia aseptate, hyaline, fusiform to slightly cylindrical with an obtuse end, 1-septa and 5-6 X 14-17 µm.

***Cunninghamella echinulata* Thaxt. ExBlakeslee. (1905)**

Colonies diameters in 5 days were 3.1-4.5 cm, 4.2-5.1 cm, 4-5 cm and 3.2-4.3 cm on RBA, PDA, MEA and CDA respectively. Colony colours on the top of all media plates were white and bottom color of the media plates showed colourless (RBA, PDA and MEA) and cream (CDA). Colonies floccose, cottomy, raised and entire. Hyphae aseptate and branched. Sporangiophores branched, erect, vesicles at the terminal end and braches in the apical region, 3.5-8 µm wide. Terminal vesicles 30- 50 µm in diameter, vesicles on branches 14-30µm in diameter, hyaline, covered with sporangioles and pyriform. Spores hyaline to brownish, globose, 1-celled, 9-13µm in diameter.

***Emmonsia* sp.**

Colonies diameters in 7 days were 3.3-4.2 cm, 3.8-4.5 cm, 4.3-5 cm and 0.2-0.8 cm on RBA, PDA, MEA and CDA respectively. Growth was poor in CDA.

Colony colours on the top on media plates were white with tan, velvety, raised, irregular with brown spots in the centre. Bottom color of the colonies were colourless (RBA) and brown (PDA, MEA and CDA). Hyphae septate, hyaline to bamboo- like with darkening at septa. Conidiophore branched. Conidia in chains, hyaline and 2.5-5 µm in diameter. Adiaspores 23-52 µm in diameter.

***Epicoccum* sp. Link. (1809)**

Colony fast growing reaching a diameter of 2.5-3.6 cm, 2.4-3.7 cm, 2.8-4 cm and 2.1-3 cm in 5 days on RBA, PDA, MEA and CDA respectively. Colony on top yellowish, felty, irregular and bottom colourless to pale yellow. Hyphae septate, hyaline, conidia globose to pyriform with a funnel-shaped base and broad attachment scar, septate, dark brown and 15-25 µm diameter.

***Exophiala* sp.**

Colonies diameters in 5 days were 2.1-3.1 cm, 2.2-3.4 cm, 2.4-3.9 cm and 1.3-1.8 cm on RBA, PDA, MEA and CDA respectively. Top colony colour on these media plates were brown (RBA), olivaceous black and black (PDA, MEA and CDA) and bottom colour black. Colonies were velvety, floccose, smooth, velvety, slightly raised, radially sulcate. Hyphae septate and pale brown. Conidiophores short. Conidiogenous cells flask-shaped and terminal. Conidia subhyaline, 1-celled, cylindrical to slightly ellipsoidal. 3.5–4.8 x 4.3-8.8 µm

***Fusarium oxysporum* Schlecht. emend. Snyder & Hansen (1940)**

Colonies fast growing reaching diameters in 5 days were 3.1-4.5 cm, 3.2-4.8 cm, 3.5.1 cm and 3.1-3.8 cm on RBA, PDA, MEA and CDA respectively. Colony colours on top white (RBA) and peach with orange center (PDA, MEA, CDA),

bottom colourless (RBA), dull brown (PDA, MEA and CDA). Colonies floccose becoming felted, aerial mycelium, slightly raised, irregular and undulate to entire. Mycelium hyaline, septate and sporulating. Conidiophore septate, hyaline and branched with abundant micro-conidia. Microconidia aseptate, ellipsoidal to cylindrical, curved, on short monophialides, 2.3-3.7 x 5-12 µm. Macroconidia fusiform, hyaline, curved, pointed at both ends, 3-5 septate, foot-shaped basal cells, 3-4.6 x 24-45 µm. Chlamydospores abundant and solitary.

***Fusarium solani* (Mart.) Sacc. (1881)**

Colonies fast growing reaching 3.1-3.8 cm, 3.2-4.8 cm, 3.5-5 cm and 2.3-3.2 cm in diameter on RBA, PDA, MEA and CDA respectively within 5 days. Colony colours on the top pinkish-white and the bottom colourless (RBA and MEA) and brown (PDA and CDA). Colonies cottony, floccose, little aerial mycelium, irregular to regular and entire. Hyphae hyaline, septate and branched. Microconidia abundant, hyaline, oval, 0-1 septa, ellipsoid, conidiogenous cell monophialidic, 2-4 x 8-15 µm. Macroconidia hyaline, curved, smooth, apical cell short and blunt, tapering ends, 3-5 septa, 4.5-7.1 x 45-73.4µm. Chlamydospore abundant, hyaline, globose, intercalary to terminal and 6-8 µm in diameter.

***Fusarium* sp.1 Link (1809)**

Colonies in diameter reaching 3.1-4.2 cm, 3.3-4.8 cm, 3.5-4.5 cm and 2.5-3.1 cm on RBA, PDA, MEA and CDA in 5 days respectively. Colony colours on top of these media plates were creamy white (RBA and MEA), white to purple (PDA) and brownish grey (CDA). Bottom colour of the colonies were colourless (RBA), and brown (PDA, MEA and CDA). Colonies cottony, floccose, raised in the center,

irregular and undulate to entire. Conidiophore septate, hyaline and branched. Macroconidia hyaline, curved apical cell, to slightly curved, straight poorly developed basal cells, 3.2-4.4 x 25.1-45.4 µm. Microconidia oval to bean-shaped, 0 septa, produced on monophialides and polyphialides, 2-3 x 25-45 µm. Chlamydospore absent.

***Fusarium* sp.2 Link (1809)**

Colonies slow growing, colony diameter in 5 days were 1-1.9 cm, 1.2-2 cm, 1.3-2.2 cm and 0.6-1.5 on RBA, PDA, MEA and CDA respectively. Colony on top white covered with an orange slime, floccose mycelium and bottom yellowish-brown due to diffusing pigment. Conidia slender, sickle-shaped, curved, 1-septate with basal cell hardly pedicellate, 14-35 x 2.5-3.2 µm. Chlamydospore absent. Macroconidia 1-3 septate, 15-55 x 2,5-3.5 µm.

***Fusarium* sp.3 Link (1809)**

Colonies fast growing, colony diameter in 5 days were 2.8-3.8 cm, 3.2-4 cm, 3.1-4.2 cm and 2.6-3.1 on RBA, PDA, MEA and CDA respectively. Colony on top white pink colour with abundant aerial mycelium, floccose, round, entire and smooth. Bottom colony colour was pale yellow to yellow. Conidiophore branched, phialides with numerous sympodial proliferations bearing one micro-conidium. Micro-conidia fusiform or elongate, 8.5-11.7 x 2.5-3.6 µm. Macro-conidia 3-5 septate, slightly curved, 28.6-36.5 x 2.5-4.2 µm. Chylamydospores intercalary, rough, 5.7-15.2 µm diameter.

***Geotrichum candidum* Link. (1809)**

Colonies in diameter reaching 2.6-3.5 cm, 3.3-4.5 cm, 3.1-4.3 cm and 1.3-2.7 cm on RBA, PDA, MEA and CDA in 5 days respectively. Colony colour on top were creamy white and bottom colourless (RBA), brown (PDA and MEA), cream (CDA). Colonies appeared yeast-like, spreading, soft, raised in the center, irregular with a fruity odour. Mycelium hyaline, septate and sporulating. Hyphae septate, hyaline and branched, 3.2-5 µm wide. Anthroconidia rectangular, thick wall, 1-celled, 3-10 x 2-5 µm. Chalamydospores subglobose, solitary and 4-6 µm in diameter. Conidia in chains, 1- celled and cylindrical, arthosporous, smooth, 5-15 x 3-6 µm.

***Geotrichum* sp.1 Link (1809)**

Colonies in diameter reaching 2.3-3.2 cm, 2.4-3.5 cm, 2.1-3.3 cm and 1.3-2.5 cm on RBA, PDA, MEA and CDA in 5 days respectively. Colony colours on top and bottom on these media plates were white and pale yellow. Colonies appeared yeast-like, dry, growth non-aerial, slightly granular, regular and entire. Mycelium septate, hyaline and sporulating. Hyphae septate, hyaline and branched, 3.6-4.7 µm wide. Conidia in chains, 1- celled, arthosporous, cylindrical with blunt ends, smooth, and 3.8-12 x 3.3-5 µm. Chalamydospores subglobose, solitary and 4.5-5.7 µm in diameter.

***Geotrichum* sp.2 Link (1809)**

Colonies slow growing with a diameter of 1-2.4 cm, 1.2-2.5 cm, 1.4-2.9 cm and 0.6-1.8 in 7 days on RBA, PDA, MEA and CDA respectively. Colonies on top white, yeast-like texture, soft, flat and round. Bottom colour colourless (RBA), pale

yellow (PDA and MEA) and cream (CDA). Hyphae dichotomously branched, 6.3-10.7 µm wide. Conidia in chains, erect, thick-walled, 6-14 x 4.2-6.5 µm.

***Gliocladium* sp. Corda (1840)**

Colony diameter on RBA, PDA, MEA and CDA in 5 days were 2.4-3.1, 2.2-3.4, 2.1-3.5 and 1.6-2.8 respectively. Colony on top olive-green, spreading, floccose, cottony, raised, entire and bottom colourless to yellow. Conidiophore erect, verticliium-like, smooth walled, 105-125 µm long. Conidia elongate, 3.5-7.5 x 3.4-4.3 µm. Phialides on whorls of 3-4.

***Humicola* sp. Traaen (1914)**

Colony diameter in 5 days were 1.8-2.1 cm, 2-2.4 cm, 2-2.8 cm and 1.2-1.9 cm on RBA, PDA, MEA and CDA respectively. Colonies on top grayish white, cottony, aerial mycelium, irregular and bottom grayish black. Hyphal cells and aleuriconidia plurinucleate, 5-7 nucleate. Aleuriconidia dark brown, 10.2-16.4 µm diameter. Phialoconidia uninucleate, obovoid, 3-3.5 x 1.5-2 µm.

***Madurella* sp Brumpt (1905)**

Colony diameter in 5 days were 2.5-3.2 cm, 2.4-3.5 cm, 2.9-3.6 cm and 1.8-2.6 cm on RBA, PDA, MEA and CDA respectively. Colony top yellowish-brown (RBA), drak grey (PDA, MEA and CDA) and bottom dark brown (RBA), yellow (PDA, MEA and CDA).

Mycelia septate and toruloid, hyphae septate, condensed, rounded with chlamydoconidia. Chlamydoconidia intercalary with finger-like projections.

***Microascus* sp. Zukal.**

Colony diameter in RBA, PDA, MEA and CDA in 5 days were 3.1-4 cm, 3.4-4.1, 3.2-4.5 m and 3-4.2 cm respectively. Colony colour on the top of these media plates were brown (RBA), grayish-white (PDA, MEA and CDA) and bottom black. Colonies velvety with aerial mycelium, granular, radially culcate.

***Mortierella* sp Coem. (1863)**

Colonies in diameter reaching 3.3-3.6 cm, 3.4-4 cm, 3.1-4.7 cm and 3-3.8 cm on RBA, PDA, MEA and CDA in 5 days respectively. Colony on top produced a concentric pattern of growth and milky white, cottony, have a thin spreading mycelium. On the bottom, colony colour cream. Conidiophores unbranched, less than 100 µm in length, aseptate, slightly widened below sporangium. Sporangia globose, smooth, 9-11µm in diameter, small chlamydospores abundantly present, thick walled, irregular in shape, 9-10 µm in diameter. Conidia globose, 9-11 cm in diameter.

***Mucor* circinelloides Tiegh., 1875 Tiegh. (1875)**

Colonies in diameter reaching 2.3-3.2 cm, 3.1-4 cm, 3-3.8 cm and 2.6-3.5 cm on RBA, PDA, MEA and CDA in 5 days respectively. Colonies first white turning whitish-grey (RBA and CDA) and brownish-grey (PDA and MEA). Bottom colourless (RBA and CDA) and brown (PDA and MEA). Colony fast growing, cottony, floccose, turf thick, raised and irregular. Sporangiophores were branched sympodially, hyaline terminated by sporangium, erect, slightly curved, 2.5-5 x 20-24 µm. Columellae present. Sporangiospores were globose, echinulate, columellate on dehiscence and 20-23.5 µm in diameter. Columellae hyaline, ellipsoidal, with a

collar. Chlamydospores subglobose, 12-22 µm wide. Spores subglobose, 1-celled and 3.3-4.4 x 2.4-3.5 µm.

***Mucor heimalis* Wehmer (1903)**

Colony diameters on RBA, PDA, MEA and CDA varied from 2.4-3.1 cm, 2.5-3.5 cm, 2.3-3.6 cm and 3-4 cm in 5 days. Colony colours on top of these media plates varied from grey (RBA), grayish yellow (PDA), deep grayish-brown (MEA) and grayish-white (CDA). Bottom color were colourless to pale yellow to brown. Colonies cottony, floccose, aerial mycelium and smooth. Sporangiophores 10-13 µm wide, erect, branched sympodially. Sporangia globose, columellate on dehiscence, 52-68.7 x 61.3-80 µm. Columellae globose to subglobose, truncate base, with a collar, 15-30 µm wide. Chlamydospores globose, 9-16 µm wide. Spores subglobose to ellipsoidal, 1-celled and 2.4-4 µm

***Mucor plumbeus* Bonord. (1864)**

Colony diameter 1.3-3 cm, 1.4-2 cm, 2.1-3 cm and 1.2-2.1 sm. Colony colour on top of the media plates were dark grey (RBA and CDA) and olive-grey (PDA and MEA). Bottom color of the media plates were colourless (RBA), brown (PDA and MEA) and black (CDA). Colonies thick tufts, cottony, aerial mycelium, raised, soft, entire, smooth and round. Sporangiophores branched both sympodially and monopodially, constricted towards sporangium, 16-20.5 µm wide. Sporangia hyaline, columellae pyriform, obovoid, ellipsoidal to cylindrical, brown and 62-70 x 28-43 µm. Sporangiospores globose to ellipsoidal or irregularly shaped, 5.6-8.2 µm in diameter, yellowish brown. Chlamydospores absent.

***Mucor racemosus* Bull. (1791)**

Colony diameters on RBA, PDA, MEA and CDA varied from 2.2-3.2 cm, 2.1-3.2cm, 2.3-3.2 cm and 2.1-3.1 cm in 5 days. Colonies on top olivaceous-grey (RBA), smoke-grey (PDA and MEA) and grayish-white (CDA), fast growing, aerial mycelium, cottony, round and bottom colourless. Sporangiophores branched sympodially and monopodially, 14-17 µm wide. Numerous chlamydospores present in the sporangiophores, barrel-shaped to subglobose, 20-25µm in diameter. Sporangia 80-90 µm in diameter. Columellae ovoid, oval, or cylindrical-oval, 55-64 x 37-40 µm.

***Mucor* sp. Frescen.**

Colony diameters on RBA, PDA, MEA and CDA were 3.5- 4.1 cm, 3.2-4 cm, 3.2-4.7 cm and 2.4-3.7cm respectively in 5 days. Colony colours on top of these media plates varied from yellowish-grey (RBA), pale brown (PDA and MEA), brownish-grey (CDA) and pale yellow in bottom. Colonies appeared cottony, turf thick, floccose, slightly raised in the center, irregular. Hyphae septate. Sporangiophores hyaline, erect, globose to subglobose, slightly curved terminated by sporangium, sympodial branching, 3.5-5 x 5-11.4 µm. Sporangia globose, erect, columellate on dehiscence, echinulate, 30-63 µm wide. Columellae hyaline, sub-globose, with a collar. Chlamydospores 6-20 µm wide. Spores ellipsoidal, 1-celled and 5.7-9.8 µm.

***Neoscytalidium* sp. (Penz.) Crous & Slippers (2006)**

Colony growish rapidly filling entire plate within 5 days. Colony diameter were 3.2-4.2, 3.1-4.7, 3.2-5, 2.9-3.6. Colonies on top appeared as grayish brown

(RBA) and brown (PDA, MEA and CDA). Colony woolly, raised, aerial mycelium, entire and round. Hyphae branched, septate and hyaline, Anthroconidia with flattened ends, consecutive with no space, 4.8-5.2 x 3.2-12 µm.

***Nigrospora osmanthi* Zimm.**

Colony diameters on RBA, PDA, MEA and CDA were 2.5-3.4 cm, 3.5-4.8 cm, 3.3-4.4 cm and 2.4-3.4 cm in 5 days. Colony colour on top were initially white but turned grayish-white (RBA and PDA) and brownish-grey (MEA and CDA) whereas the bottom color of the colonies were colourless, pale yellow to brown. Colonies floccose, flat, flocosse, sparse aerial mycelia, irregular to regular and fimbriate. Mycelium smooth, hyaline, septate, micronematous, branched, hyphae 2.4-3.7 µm diameter. Conidiophores reduced to conidiogenous cells, monoblastic, solitary, discrete, ampulliform, 5.2-11.8 x 4.5-9 µm wide. Conidia black, solitary, aseptate, shiny, smooth and globose to sub- globose, 12.5-17 µm in diameter.

***Paecilomyces* sp.1 Samson (1974)**

Colony diameters on RBA, PDA, MEA and CDA reached 2.4 -3.3 cm, 2.5-3.1 and 2.2- 3.2 cm in 5 days. Colony colours on the top of these media plates were pale pink, flat and entire whereas the bottom colour of these plates were colourless (RBA) and yellowish (PDA, MEA and CDA). Hyphae septate and hyaline. Conidiophores loosely branched and 2.1-2.8 µm wide. Phialides solitary to pairs, swollen at the base and tapered towards the apex. Conidia ellipsoidal to ovoid, hyaline, 1-celled, slightly apiculate at one end, phialosporous, 1-1.5 x 3-4 µm.

***Paecilomyces* sp.2 Samson (1974)**

Colony diameters were 2.3- 3.4cm, 2.3-3.1cm, 2.5-3.6cm and 2.4-3.3cm in 7 days on RBA, PDA, MEA and CDA. Colony colours on the top of these media plates were yellowish-white, velvety, flat and entire whereas on the bottom, colony colours were brown. Conidiophores erect, hyaline, loosely branched, 2-2.7 µm wide. Hyphae hyaline and septate. Phialides solitary to pairs. swollen at the base and tapered towards the apex. Conidia subglobose to ovate, terminal, 1-celled, phialosporous and 3.5 x 4.6 µm.

***Penicillium chrysogenum* Thom (1910)**

Colony diameters on RBA, PDA, MEA and CDA reached 2.1 – 3.8 cm, 2.5-3.9 cm, 2.9-4 cm and 2.5-3.1 cm respectively in 5 days. Colony colour the top were grass green with pale to bright yellow exudates, velvety, entire, raised with a fruity smell and bottom vinaceous. Conidiophores 2-3-stage branched at rather wide angles. Conidia smooth-walled, ellipsoidal, globose to sub-globose and 3-3.8 x 2.5-3.2 µm.

***Penicillium citrinum* Thom, C. 1910**

Colony diameters on RBA, PDA, MEA and CDA reached 2.1 - 2.6 cm, 2.3-2.9 cm, 2-2.6 cm and 2.1-3 cm respectively in 5 days. Colony colour the top were dark green, powdery, round and bottom were wrinkled and colourless (RBA), yellowish-orange (PDA and MEA), cream (CDA). Conidiophores long, raised from subsurface hyphae, 100–300 µm long. Hyphae monoverticilate to terverticilate, smooth-walled stipes, metulae cylindrical. Phialides ampulliform and 7–9.5 x 2-2.5µm. Conidia globose to sub- globose and 2.2-3.5 µm.

***Penicillium commune* Charles Thom (1910)**

Colony diameters on RBA, PDA, MEA and CDA reached 2.2-3.5 cm, 2.4-3.3 cm, 3.2-3.8cm, 2.1-3.1cm in 7 days. Colony colour on the top of these media plates bluish-green with white mycelium margins, smooth, velvety to floccose, sulcate to slightly sulcate and bottom colourless (RBA), yellow (PDA), brown (MEA), reddish brown (CDA). Hyphae smooth-walled stipes, terverticillate and 190-380 µm long. Conidiophores raised from the subsurface, 120-280 µm long. Metulae cylindrical and 12-16.5 µm wide. Phialides ellipsoidal to globose, ampulliform, 7.3- 9.6 µm long. Conidia ellipsoidal to globose and 2.6-3.5 µm wide.

***Penicillium harzianum* Link.**

Colony on top of the media plates were brownish grey and bottom light yellow to brown. Colonies with aerial mycelium, sulcate and soft. Conidiophore arises from hyphae, short, 9-19 µm long, monoverticilliate, branched, with small phialides. Conidia in short chains, globose, conspicuously roughened, 2-2.6 µm diameter.

***Penicillium italicum* Wehmer, (1894)**

Colony diameters on RBA, PDA, MEA and CDA reached 1.2-1.7 cm, 2.2-3.6 cm, 2.4-3.7 cm and 0.5-1.5 cm in 5 days. Colony colour on the top and bottom of these media plates were grey and colourless (RBA), deep green and yellow (PDA), grayish-blue and pale brown (MEA) and grey and grey (CDA). Colonies were floccose, velvety, radially sulcate with white edge and transparent exudates. Conidiophores raised from the subsurface hyphae, smooth-walled stipe,

terverticillate, 110-330 µm long. Metulae cylindrical, 13-15 µm. Philiades cylindrical, 8-10 x 2-5 µm. Conidia ellipsoidal and 3.3-4 µm wide.

***Penicillium janthinellum* Link.**

Colony diameter were 2.3-3.6 cm, 2.8-3.8 cm, 3.1-3.7 cm and 2.1-3.2 cm in 5 days. Colony top pale grey to greenish grey whereas bottom pale yellow to brown. Colony spreading broadly, woolly, aerial mycelium, round and entire. Conidiophore branched and smooth-walled. Conidia simply ellipsoidal with apiculate ends, rough-walled, 3-3.2 µm long.

***Penicillium palitans* Westling, R. 1911**

Colony diameters on RBA, PDA, MEA and CDA reached 1-3.3 cm, 0.5-3.5 cm, 1-3.1 cm and 0.7-2.7 cm in 5 days. Colony colour on the top light green however and bottom pale brown and yellow. Colonies flat, velvety and radially sulcate. Conidiophores raised from the subsurface hyphae, rough-walled stipe, terverticillate, 245-370 µm long. Metulae cylindrical, 10.4-13.8 µm. Philiade 9.3-10.6 x 2- 3.1 µm. Conidia globose, 2.4- 4.1 µm in diameter.

***Penicillium koningii* Link.**

Colony diameters were 2.1-2.5 cm, 1.6-2.2 cm, 1.9-2.5 cm and 0.8-1.7 cm on RBA, PDA, MEA and CDA respectively in 5 days. Colony dull blue-green, velvety to floccose, irregular and bottom colourless to slightly greenish. Conidiophores branched, 50-100 µm long. Conidia forming loose columns, smooth-walled, ellipsoidal to subglobose, 1.7-2.3 µm long. Conidia in chains, globose to sub-globose, 2.1-3.5 µm wide.

***Penicillium waksmanii* Link.**

Colony diameters on RBA, PDA, MEA and CDA were 2-2.5 cm, 2-3 cm, 1.2-2.7 cm and 1-2 cm in 5 days. Colony colour on top dull green and bottom colour colourless (RBA), yellow (PDA and MEA) and cream (CZA). Colonies appeared with transparent and yellow exudates, velvety, radially sulcate, pale yellow margin around the colonies. Conidiophore biverticillate borne on the hyphae, smooth-walled, 210-440 µm long stipes. Metulae cylindrical, 4.4-5.8 µm long, ampuliform. Phialids 6.1-8.4 µm long. Conidia globose and 2.2-3 µm diameter.

***Penicillium* sp.1 Link (1809)**

Colony diameters on RBA, PDA, MEA and CDA were 2.3-3 cm, 2.5-3.2 cm, 2.2-3.3 cm, and 2-3.1 cm respectively in 5 days. Colony colours on top creamish-yellow (RBA and MEA), yellowish with white margins around the colonies (PDA, CDA) and bottom colony colour were colourless (RBA), yellow (PDA), brown (MEA and cream (CDA). Colonies were slightly velvety, granular, round to undulate and wrinkled. Hyphae septate and hyaline. Conidiophore hyaline, erect, unbranched, septate, monoverticillate. Metulae absent. Phialides ampulliform, hyaline, 2.8-4.1 x 9-11.7 3.2-4.5 µm. Conidia 1-ceeled, hyaline, globose, 3.1-3.8 µm.

***Penicillium* sp.2 Link (1809)**

Colony diameters in 7 days on RBA, PDA, MEA and CDA were 2- 3.7 cm, 2.2-3.6 cm, 2.1-3.8 cm and 2-2.3 cm. Colony on top appeared white at first and turned bluish-green and bottom colourless (RBA) and cream (PDA, MEA, and CDA). Colonies were velvety with brown exudates on MEA and CDA plates, entire, round and raised, wrinkled, undulate. Hyphae septate, hyaline, smooth-walled.

Conidiophores hyaline, erect, raised from the surface, stipes smooth, simple, terverticilate. Metulae cylindrical and phialides ampulliform. Conidia 1-celled in short chains, globose, 3-3.5 µm wide.

***Penicillium* sp.3 Link (1809)**

Colony diameters on RBA, PDA, MEA and CDA reached 2.5-3.2 cm, 2.1-3.5 cm, 2.6-3.2 cm and 1.1-2.8 cm in 7 days. Colony colours on the top of these media plates were blue-green to grayish and bottom colourless (RBA), cream (PDA and CDA) and brown (MEA). Colonies velvety to powdery, flat on margins and raised in the center of the colony, wrinkled and irregular. Hyphae hayaline and septate. Conidiophores arises from the hyphae, 150-700 µm, stipes smooth –walled, terverticilate. Metulae 3-4, cylindrical, 7.3-13 µm x 3-4 µm. Philiades ampulliform. Conidia globose , 2.5-3 µm wide.

***Periconia* sp.1 Tode Ex Fries (1791)**

Colony diameters on RBA, PDA, MEA and CDA reached 3.1-3.9 cm, 3.3-4.4 cm, 2.8-3.6 cm and 1.1-2.1 cm respectively in 7 days. Colony colours on the top of these media plates were pinkish-grey (RBA, PDA and MEA) and white (CDA). The bottom of these plates colourless (RBA), yellowish-brown (PDA), brown (MEA), black center surrounded with off-white margins (CDA). Colonies were effuse, woolly, crateriform (RBA and MEA), raised (PDA and CDA) undulate. Hyphae septate, hyaline and branched. Conidiophore dark brown, micronematous and macronematous, slightly bent, verruculose, solitary to small groups with globose spore heads at the apex, 4.2-5.6 x 342-417 µm long. Conidiogenous cells

monoblastic. Stipe verruculose. Conidia aseptate, globose, brown, blastosporous, 1-celled, catenate and 8.4-14.6 µm in diameter.

***Periconia* sp.2 Tode Ex Fries (1791)**

Colonies fast growing, reaching a diameter of 2.4-3.5, 2.8-3.8, 2.5-4.2, 2.2-3.6 in 5 days. Colonies on top of the media plates were dark brown, compactly cottony, aerial mycelia, smooth and spreading. Bottom dark and wrinkled. Conidiophores dark brown, branched and 6.3-11.2 x 320-400 µm. Conidia coarsely echinulate with spines, 18-30 µm diameter, 2-7 long, verruculose cells 5-8 µm diameter.

***Pestalotiopsis* sp**

Colonies on RBA, PDA, MEA and CDA were 3.5-4.1 cm, 3.7- 5.1 cm, 3-4.7 cm and 2.3-2.7 cm in 5 days. Colony growth in CDA was slow, colony on top varied with greyish brown (RBA), grey (PDA), greyish-white (MEA), white (CDA) and bottom of these plates showed colourless (RBA), black with off-white margins around the colonies. Colonies were slightly dense, fluffy, slightly raised aerial mycelium on the central surface, smooth and entire.

Phaeocremonium sp.

Colonies on RBA, PDA, MEA and CDA were 3.2-4.3 cm, 3.3- 4.1 cm, 3.2-4.3 cm and 2.1-2.8 cm in 7 days on top are initially cream later turning olive-gray and dark brown, mycelium velvety or suede, aerial mycelia, and smooth. Reverse black. Conidiophores reduced to discrete conidiogenous cells. Hyphae are septate, hyaline, appearing in circular whorls with phialides radiating outward. Phialides (2-3x12-30 µm), long, brown with brown basal septation, tapered at the tips.

Conidiophore branched, conidia hyaline, oval shaped occuring in chains, 4 21-28 x 5-7.5 µm. Basal cells hyaline, thin-walled, smooth, brown, 3 median cells, 3.5-5.7 µm long. Apical cells hyaline, unbranched, thin-walled, subcylindrical, 2-3 appendages and 4-6 µm long.

***Phoma glomerata* (Corda) Wollenw and Hochaptel. (1936)**

Colony diameters on RBA, PDA, MEA and CDA reached 2.3-3 cm, 2.1-2.6 cm, 1.4-2.7 cm and 1.1-2.7 cm in 5 days. Colony colour on the top of these media plates were creamish-white, flat, soft, round, entire, wrinkled (PDA) whereas on the bottom colony colour were colourless (RBA and MEA), pink with white margins (PDA and CDA). Pycnidia mostly regularly globose. Conidia ellipsoidal, 6-6.2 x 3-3.2 µm. Dictyochlamydospores commonly arising in unbranched/branched chains of 2-20, 18-20 x 12-30 µm.

***Phoma* sp.1 Saccardo (1880)**

Colony diameters on RBA, PDA, MEA and CDA reached 0.4- 1 cm, 0.2-1.2 cm, 0.5-1 cm and 0.4-1.3 cm in 5 days. Colony colour on the top of these media plates were yellow, circular, punctiform, whereas on the bottom of these plates colony colour varied from colourless (RBA and MEA), pale yellow (PDA), bright yellow (CDA). Hyphae septate and hyaline. Pycnidia sub-globose to pyriform, 90-150 µm wide. Conidia aseptate, hyaline, ellipsoid to ovoid, 1-celled and 3.6-6.8 x 2-2.6 µm.

***Phoma* sp.2 Saccardo (1880)**

Colony diameters on RBA, PDA, MEA and CDA reached 1.3-2.3 cm, 1.1-2.6 cm, 1.2-2.8 cm and 1.5-2.9 cm in 5 days. Colony colour on the top of these

media plates were white, concentric, irregular, raised in the center, wrinkled whereas on the bottom colony colour were colourless (RBA), pale yellow (PDA, MEA and CDA). Hyphae septate and hyaline. Pycnidia irregular, sub-globose to pyriform, 75-140 µm wide. Conidia aseptate and hyaline, ellipsoid to ovoid, 1-celled, 3.2-6.4 x 1.3-2.6 µm. Chlamydospores globose.

***Phoma* sp.3 Saccardo (1880)**

Colonies fast growing, colony diameter in 5 days on RBA, PDA, MEA and CDA were 3.2-4 cm, 3-4.3 cm, 3.4-4.2 cm, 3.1-4.1 cm respectively. Colonies on top appeared Creamish-white (RBA and CDA), brownish-white (PDA), blueish-white (CDA) and bottom colourless (RBA), brown (PDA and MEA), cream (CDA). Colonies flat, granular, round, circular, concentric rings (MEA), wrinkled (RBA, PDA and CDA) and powdery and velvety. Pycnidia abundantly produced in the center of the colony, conidia ellipsoidal to cylindrical, 3-6.3 x 1-2.8 µm.

***Phomopsis amaranthicola* Sacc & Roum. (1884)**

Colony diameters on RBA, PDA, MEA and CDA were 3.4- 4.2 cm, 3.2-4.5 cm, 3.1-4.7 cm and 3-3.9 cm in 7 days. Colony colour on the top of these media plates were white whereas on the bottom colony colours were colourless (RBA) and brown (PDA, MEA and CDA). Colonies were woolly, flat margin and raised in the center, turf thick, wavy, with prominent concentric rings and irregular. Conidiophore septate, hyaline and branched. Conidiomata cylindrical. Alpha conidia aseptate, hyaline, ellipsoidal,1-celled, 2.1-5.2 µm. Beta conidia slightly curved, filiform, hyaline and 2-11.5 µm.

***Rhizopus microspores* Tiegh. (1875)**

Colonies diameters on RBA, PDA, MEA and CDA were 1.7-3 cm, 2.4-4.7 cm, 2.8-4.8 cm and 3.5-4.7 cm respectively in 5 days. Colony colour on the top were brown (RBA, PDA and MEA), grey (CDA), and bottom of these media plates varied from colorless (RBA and PDA), dull brown (MEA and CDA), colonies were floccose, cottony, aerial mycelium, and entire. Hyphae hyaline and branched. Sporangia globose to sub-globose, columellate on dehiscence, 80.5-100 µm in diameter. Sporangiophores solitary to groups, unbranched, erect, connected by stolons, 7.6- 15.7 µm wide. Apophyses noticeable. Stolons hyaline, 3.5-7 µm wide. Rhizoids subhyaline. Columellae globose to obovoid, 24-65 µm wide. Chlamydospores globose, in short chains, 7-17 µm wide. Conidia cylindrical, striated and 3.4-4.3 x 5.2-6.5 µm.

***Rhizopus stolonifer* Vuillemin (1902)**

Colony diameters on RBA, PDA, MEA and CDA in 5 days were 3.3-4.5 cm, 4.2-5 cm, 4.1-5.3 cm and 3.4-4.8 cm. Colonies on top at first white becoming grey (RBA and MEA), yellowish-brown (PDA), off-white (CDA). Bottom color of the colonies were colourless (RBA), brownish black (PDA), dull brown (MEA and CDA). Colonies were cottony, raised, floccose and irregular. Hyphae hyaline, branched, irregular, 4.4-12.6 µm. Sporangia globose to sub-globose, erect, echinulate, columellate on dehiscence and 98.4-165 µm wide. Columellae hyaline, globose to sub-globose, hemispheric and 45-78 µm wide. Sporangiophores hyaline, septate, globose to ellipsoidal, unbranched, connected by stolons, striated and 7.5-16.5 µm wide. Sporangiospores sub-globose to oblong, striated and 3.5-5 µm wide. Rhizoids septate, hyaline, branched. Stolons hyaline and septate.

***Sarocladium* sp.1 (Sawada) W. Gams & D. Hawksw. (1976)**

Colonies diameters on RBA, PDA, MEA and CDA were 1-2.2 cm, 2-2.7 cm, 1.6-2.8 cm and 1-1.5 cm in 7 days. Colony growth in CDA was poor. Colonies on the top of these media plates were initially white which turned to greyish and the bottom of these plates showed colourless (RBA and MEA), black (PDA and CDA), concentric, raised, velvety, wrinkled, entire and irregular. Hyphae septate, hyaline, branched and 1.2-1.6 µm wide. Conidiophores branched, erect, hyaline and slightly bent. Phialides hyaline, tapered toward apex, conidial mass slimy, 1.3-1.7 x 1.5-2.5 µm. Conidia hyaline to sub-hyaline, oval to ellipsoidal, 1-celled, grouped in slimy heads and 2.6-3.1 x 1-2.2 µm.

***Scedosporium* sp.1**

Colonies diameters on RBA, PDA, MEA and CDA ranged from 1.2-2.2 cm, 1.6-2.9 cm, 3.3-5.2 cm and 3.2-5.2 cm in 5 days. Colony colour on the top of these media plates were off-white (RBA and PDA), greyish-white (MEA and CDA), cottony, forming mycelia tufts as the colony grows, circular, raised, entire and the bottom color of these colonies were colorless (RBA), brown surrounded by cream colour margin (PDA), smoky brown (MEA), yellow (CDA). Hyphae septate and hyaline. Conidiogenous cells cylindrical, flask shape to elongated neck. Conidia produced laterally in hyphae, 1-celled, solitary or in small groups at the apex of the neck, ovoid and clavate, smooth, 3 µm wide.

***Scedosporium* sp.2**

Colonies 1.2-2.5 cm, 1.4-2.9cm, 1.8-3.1 and 1.1-1.8 cm in diameter within 5 days on RBA, PDA, MEA and CDA. Colony on top appeared as woolly to cottony,

round, smooth, initially white then produced aerial hyphae that later become grey-brown and bottom colourless (RBA), yellow (PDA and CDA) and black (MEA). Hyphae septate and hyaline, 2-3.5 µm in diameter. Conidia produced in small groups on long, simple, branched conidiophores. Conidia single-celled, pale brown, broad to egg-shaped, 4-9 x 6-10 µm, rounded on the far end like ballons and base truncate.

***Sclerotinia* sp. Fuckel (1870)**

Colonies on RBA, PDA, MEA and CDA were 2.5-3.2 cm, 2.6-3.5 cm, 2.8-3.6 cm and 2.1-2.8 cm in 5 days. Colonies on the top of these media plates were initially off- white and bottom color brown. Sclerotia with yellow and white drops, cushion-shaped, dark brown, thick-walled. Micro-conidia abundant on flask-shaped phialides. Hyphae septate, hyaline, multinucleate, 2.4-4.6 µm in wide.

***Scopulariopsis brumtptii* Bainier (1907)**

Colonies in diameter 0.6-1.2cm, 0.8-1.4 cm, 1-1.5 cm and 0.4-1 cm in 5 days. Colonies on top of the media plates were initially white becoming grey with a central floccose tuft, woolly, rounded and bottom smoke grey to fuseous-black. Mycelial hyphae hyaline, annellophores solitary, conidiogenous cells ampulliform, 4-9.5 x 2.2-3.5 µm. Conidia ovoid, truncate at base, dark brown, smooth-walled, in chains, 4.2-5 x 3.1-4.3 µm.

***Scytalidium dimidiatum* Pesante.**

Colony diameters on RBA, PDA, MEA and CDA reached 3.1- 4 cm, 3.2-4.1 cm, 3-4 cm, 2.2-3.6 cm in 7 days. Colony on top of these media plates appeared as greenish-grey (RBA), pale grey (PDA and CDA), grey (MEA) and bottom yellow (RBA, PDA, CDA), dark brown (MEA). Colonies flat, entire, radially sulcate and

lanose. Hyphae hyaline to subhyaline, septate, 2.2-4 µm wide. Fertile hyphae produced from the main hyphae. Conidiophores not present. Arthroconidia aseptate, hyaline, 2.3-7 x 1.4-2.5 µm, square to short-cylindrical. Vegetative hyphae present producing irregularly shaped cells in chains and globose. Conidiogenous cells hyaline.

***Taloromyces* sp. C.R.Benj. (1955)**

Colonies diameters on RBA, PDA, MEA and CDA were 1.1- 1.6 cm, 1.3-1.5 cm, 0.6-1.2 cm and 0.4-1.7 cm in 5 days. Colony colours on top appeared as varied from dark green (RBA). lime green (PDA) to bluish green (MEA and CDA). Colonies surrounded by a yellow margin (RBA and PDA) and by a white margin (MEA and CDA), On the bottom of these plates colony colour were colourless (RBA), brownish (PDA and MEA) and yellow (CDA). Colonies raised, curled margin, sulcate, irregular and velvety. Conidiophores raised from substratum, hyaline, biverticillate. Metulae 3-6, divergent, 10.4-12.5 x 2-3 µm. Phialides 4-6, acerose. 9.7-12.4 x 1.6-2.6 µm. Conidia hyaline, ellipsoidal, 1.4-3 x 2.3-3.7 µm, stipe septate and smooth.

***Trichophyton* sp.1 (Castell.) Sabour. (1911)**

Colony diameters on RBA, PDA, MEA and CDA were 2.2- 3 cm, 2.1-3.2cm, 2.5-3.4 cm, and 2.1-3 cm in 5 days. Colony colour on top of these media plates varied from creamy-white (RBA and MEA) to white (RBA and CDA), bottom color were colourless (RBA) and pale yellow (PDA, MEA and CDA). Colonies appeared soft, filamentous, cottony, flat, round with a red pigment on MEA plate. Hyphae septate, hyaline, spiral, macroconidia produced laterally and directly on the hyphae.

Macroconidia cigar or club-shaped, multicellular, 4.3-7.5 x 8.1- 47.3 µm. Microconidia clavate to pyriform, 1-celled, 1.7-3.3 x 2.1-4.5 µm.

***Trichophyton* sp.2 (Castell.) Sabour. (1911)**

Colony diameters on RBA, PDA, MEA and CDA were 2.4-3.1 cm, 2.8-3.3 cm, 2.7-3.5cm, and 2.5-3.1 cm in 5 days. Colony on top of these media plates appeared white (RBA) to yellowish (PDA, MEA and CDA) and the bottom of these plates were colourless (RBA), pale yellow (PDA and CDA) and yellow (MEA). Colonies were cottony, irregular, raised, irregular, wrinkled and entire. Hyphae septate and hyaline. Macroconidia cigar or club-shaped, multicellular, 3.4-6.5 x 7.5-40.5 µm, microconidia clavate to pyriform, 1-celled, 1.1-2.3 x 2.5-3.5 µm.

***Tricoderma hamatum* (Bonord.) Bainier 1906**

Colony diameters on RBA, PDA, MEA and CDA in 5 days were 3.4-5 cm, 3.5-5 cm, 3.2-5.2 cm and 3.3-4.3 cm respectively. Colonies on top were first white beoming yellowish-green (RBA and MEA), bluish green (PDA) and green (CDA). Colony colour on the bottom of these media plates were colourless to brown. Colonies flat to slightly raised in the center, entire, woolly and compact tufts Hyphae septate, hyaline and branched. Conidiophore short, erect, hyaline, branched with 3-5 phialides on each branch, undulate to hamate. Phialides septate, hyaline, tapered towards the apex, flask-shaped, densely clustered, 2.3-3.5 x 4.5-8.4 µm. Conidia oblong to ellipsoidal, 1- celled and 2-3 x 3-4 µm. Chlamydospore sub-globose, terminal and intercalary, 6.8-10.5 µm diameter.

***Trichoderma harzianum* Rifai (1969)**

Colonies fast growing reaching a diameter of 4.1-5 cm, 4.3-5.3 cm, 4.2-5.5 cm and 3.8- 4.8 cm on RBA, PDA, MEA and CDA respectively in 5 days. Colonies on the top of these media plates were whitish green (RBA and MEA) to yellowish green (PDA and CDA) and bottom colourless to pale yellow. Colonies appeared as powdery, floccose, with concentric rings, raised and entire. Hyphae septate and hyaline. Conidiophores hyaline, bearing right angled branches to the tip, erect, 60.5-103 µm long. Phialides 2-3 in each branch, flask shaped and appeared in pairs, 3-6.5 µm long. Conidia short, 2.6µm in diameter, globose, smooth and 1-celled. Conidia short, globose, smooth and 1-celled, 2.6 µm in diameter. Chlamydospore sub-globose, 6.6-8.1 µm in diameter.

Tricoderma longibrachiatum* Rifai (1969*)

Colony diameters on RBA, PDA, MEA and CDA in 7 days were 3.4- 4.6 cm, 4.3-5 cm, 4-5.3 cm and 3.2- 4.1 cm respectively. Colony colours on the top of these media plates at first appeared as white turning greenish-yellow and bottom colourless (RBA) to greenish-yellow (PDA, MEA and CDA). Colonies flat, cottony, undulate, compact tufts with white pustules and green to yellowish conidia on the colony surface. Hyphae septate, hyaline and branched. Conidiophore with long main axis and short side branches, hyaline and erect. Phialides slender, hyaline, slightly bent at the apex, alternate, solitary, lageniform and 2-3.6 x 6.8-9 µm. Conidia obovoid to ellipsoidal, 1-celled, hyaline, 2-4 µm. Chlamydospore globose, terminal and intercalary

***Tricoderma viride* Pers. (1974)**

Colonies on RBA, PDA, MEA and CDA were 3.1-4.3 cm, 3.3-4.6 cm, 3.6-5 cm and 3.4-5.2 cm respectively within 5 days. Colony on top of these media plates appeared as white becoming yellowish- white and light-green and bottom color colourless (RBA) to pale yellow (PDA and CDA) to brown (MEA). Colonies were slightly raised to flat, floccose, undulate, powdery with a yellowish ring, white pustules and green conidia appered on the surface of the colonies. Hyphae branched, septate and hyaline. Conidiophore branched, right angled, erect, hyaline with 2-3 phialides in opposite pairs, solitary or in whorls, cylindrical to board in the middle, ampulliform, flask-shaped, 2-3.5 x 6.8-11 µm. Conidia globose to obovoid, 1-celled, hyaline, 2.8-4.3 x 3.2-4 µm. Chlamydospore globose to sub-globose, intercalary and 5.3-6.9 µm in diameter.

***Tricoderma* sp.1 Pers. (1801)**

Colony growth on MEA and CDA was poor. Colony diameters on RBA and PDA were 2.8-3.6 cm and 3-4.2 cm respectively in 5 days. Colony colour on the top at first white turning green and bottom colourless in RBA and brown in PDA. Colonies were floccose, dense, flat and entire. Hyphae septate and hyaline. Conidiophore erect, hyaline, sparingly branched with short side branches, 64-100 µm long. Phialides solitary to 2-5 in each branch in whorls, 2.3-3.5 x 6.2- 7.8 µm. Conidia hyaline, oval to ellipsoidal, 1-celled, 1.5-3 x 3-4 µm. Chlamydospore globose, 7.5-10 µm in diameter.

***Tricoderma* sp.2 Pers. (1801)**

Colony diameters on RBA, PDA, MEA and CDA plates reached 3-4.2 cm, 3.6-4.7vcm, 4-5.2 cm and 3.8-4.3 cm respectively in 5 days. Colonies on top were first white later becoming green (RBA), bluish-green (PDA and MEA), greenish-yellow (CDA) whereas bottom colour of these plates appeared as colourless (RBA and MEA) and pale yellow (PDA and CDA). White pustules present on the surface of the colonies, compact tufts, cottony, irregular, flat and undulate. Hyphae branched, septate and hyaline. Conidiophore with short right angled side branches, and long main axis, hyaline and erect. Phialides slender, solitary, slightly bent at the apex, 1.1-2.5 x 4.1-5.5 µm. Conidia hyaline, 1-celled, ellipsoidal. Chlamydospore sub-globose, terminal to intercalary and 2.1-3.1 x 3.5-4.1µm.

***Ulocladium* sp. Preuss (1851)**

Colony diameters on RBA, PDA, MEA and CDA were 2.8-3.5 cm, 3.1-4.1 cm, 2.7-3.9 cm and 0.5-1.6 cm in 7 days. Colony colour on the top were dark to whitish-grey (PDA, MEA and CDA) and bottom colourless (RBA), brown (PDA) and black (MEA and CDA) with a pale-yellow margin around the colonies. Colonies cottony, woolly, umbonate and entire. Hyphae septate and hyaline, Conidiophores smooth, septate, sub-hyaline, 3.1-4.5 µm wide. Conidiogenous cells solitary to fascicles of a few hyphae. Conidia dark brown, solitary, 1-2 septa, septa transverse and longitudinal, obovoid to ellipsoidal, slightly verrucose, muriform, constricted at or near septa, 28.2-35 x 17.6-20 µm,

***Verticillium* sp1 Nees(1816)**

Colonies in diameter were 2.2-3.1 cm, 2-3.3 cm, 2.1-3.5 cm and 2.5-3.2 cm. Colonies on top of the media plates were white, ciliate, entire, round, flat to slightly raised, and bottom colourless (RBA) and light brown (PDA, MEA and CDA). Conidiophores erect, verticillately branched, bearing whorls of slender flask-shaped. Phialides produced singly or in whorls on aerial hyphae, slender, tapering towards the tip and 21-25.2 µm long. Conidia hyaline forming slimy heads, ellipsoidal with apiculate base, 3-4 x 1.5-1.8 µm. Dictyochlamydospores abundantly produced in the aerial mycelium on 9-12 µm long stalks.

***Verticillium* sp.2 Nees(1816)**

Colonies in diameter were 1.8-2.5 cm, 2-2.8 cm, 2.1-3 cm and 1.5-2.7 cm. Colonies on top of the media plates were white, entire, ciliate, irregular, flat, spreading the entire plate, and bottom colourless (RBA) and pale yellow (PDA, MEA and CDA). Conidiophores erect, hyaline with several whorls of 3-4 phialides . Phialides subulate, 16-35 x 1-2.5 µm. Conidia ellipsoidal to short cylindrical, hyaline, 1-celled, septate, 2.3-7.2 x 1.2-3.1 µm.

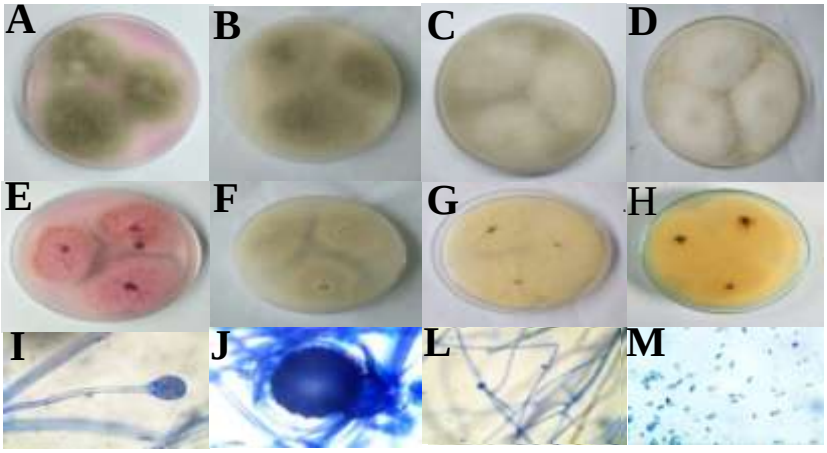

Figure 3.14. *Absidia cylindrosopora*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: sporongiophores under 40x, J-K: zygospore with finger-like projection under 40x and 100x, L:spores under 40x, scale bar = 10 µm

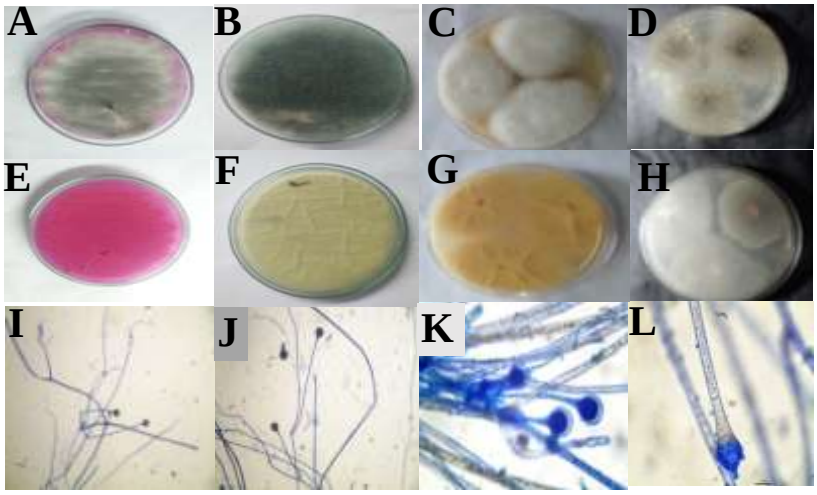

Figure 3.15. *Absidia gluaca*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: sporangiophores with sporangium under 10x, J: sporangium with intact sporangiospores and columellae under 40x, K: deliquesced sporangia, L: sporangiophores and rhizoids (arrow), scale bar = 10 µm

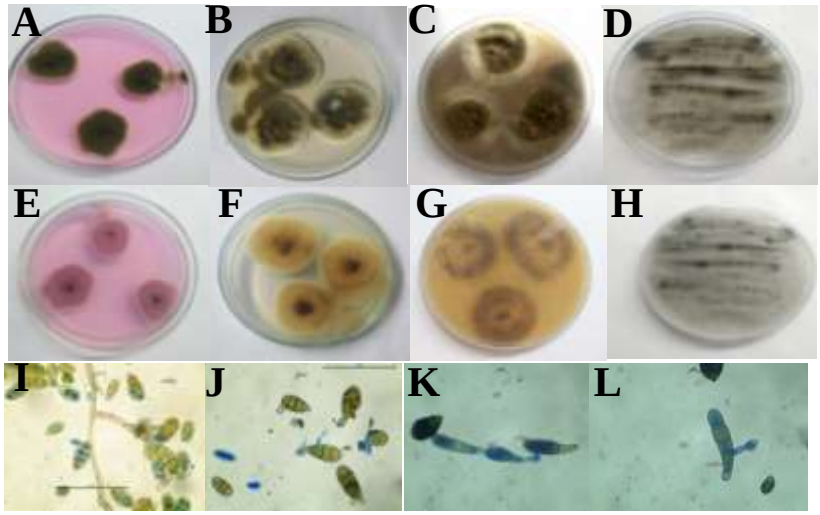

Figure 3.16. *Alternaria* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: conidiophores with conidia under 40x, J: different size and length conidia under 40x, K: conidia in chains, L: conidia with germ tube, scale bar = 10 µm

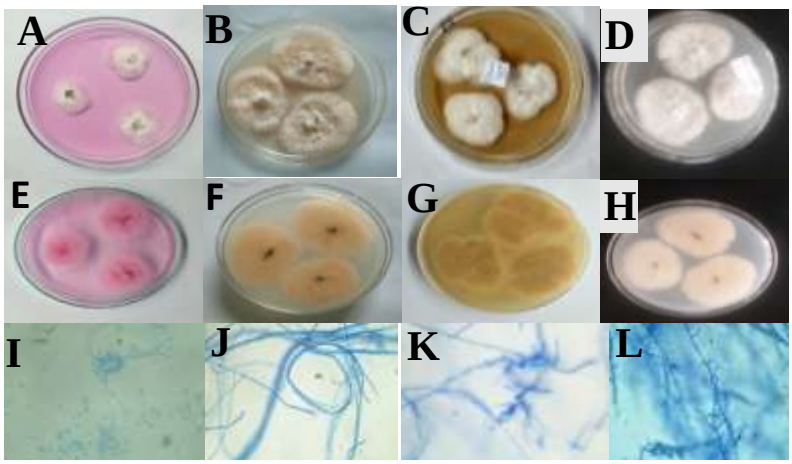

Figure 3.17. *Arthtrographis* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: hyphae in whorls under 40x, K: branching conidiophores with anthroconidia under 40x, L: anthroconidia in chains under 40x, scale bar = 10 µm

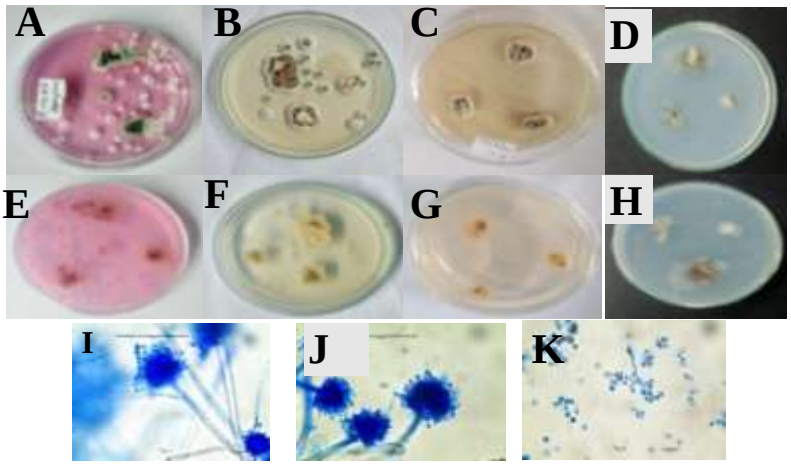

Figure 3.18. *Aspergillus fumigatus,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: conidia head under 100x, K: conidia under 10x, scale bar =

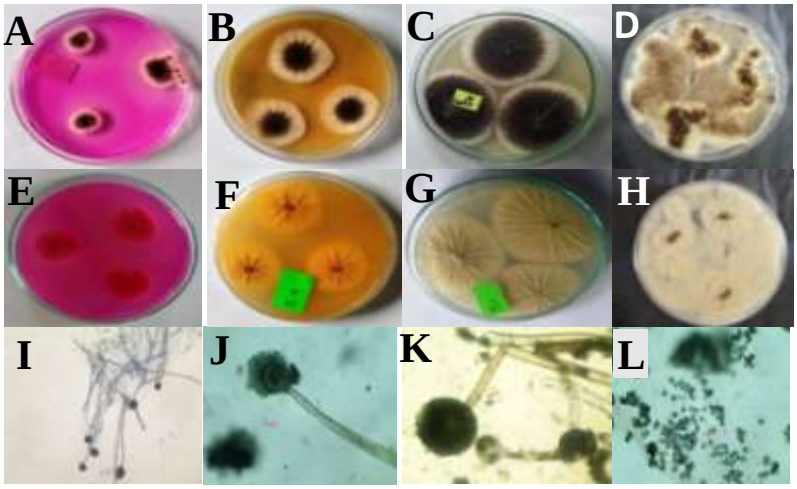

Figure 3.19. *Aspergillus niger,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: conidiophores with conidia under 10x, J-K: conidial heads under 40x and 100x, L: conidia under 40x, scale bar = 10

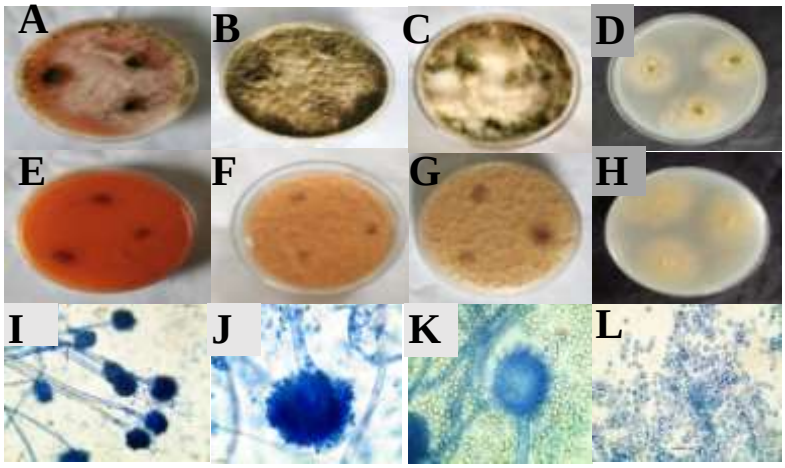

Figure 3.20. *Aspergillus flavus*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: conidiophores with conidial head under 40x, J-K: conidial head under 40x, L: conidia under 40x, scale bar = 10 µm

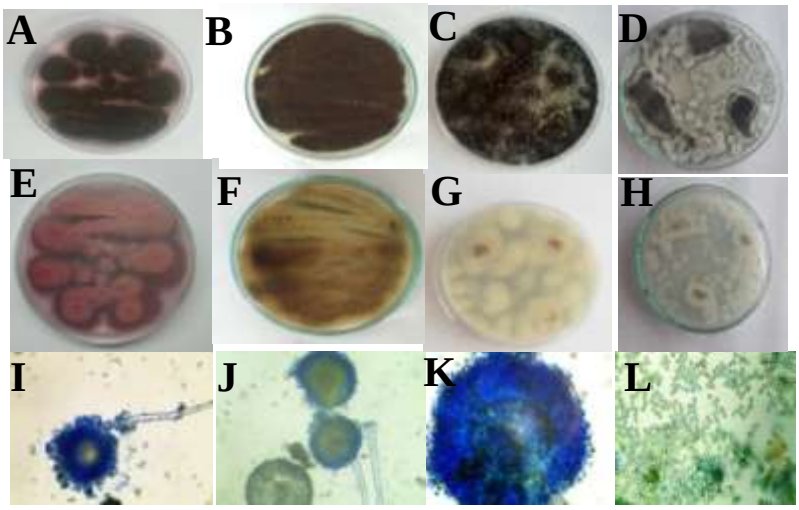

Figure 3.21. *Aspergillus oculeatus*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-K: conidial head under 40x and 100x, K: conidia under 40x, scale bar = 10 µm

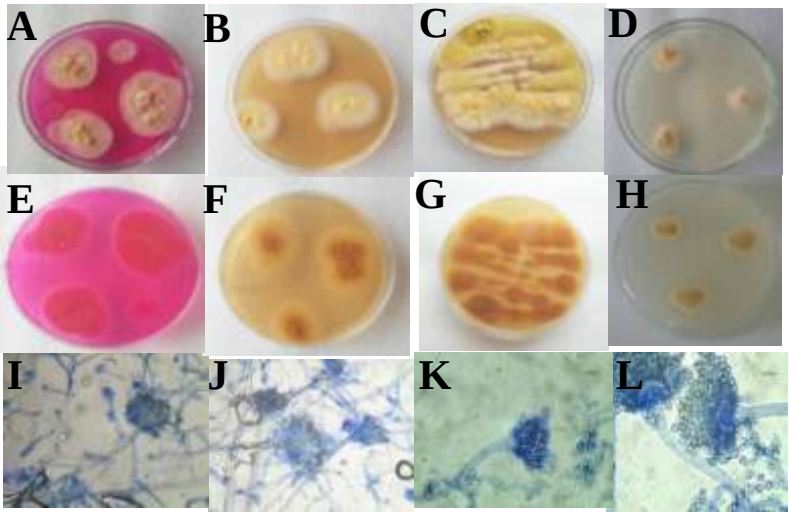

Figure 3.22. *Aspergillus terreus*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: conidiophores with conidial head under 40x, K-L: conidial head with conidia under 40x and 100x, scale bar = 10 µm

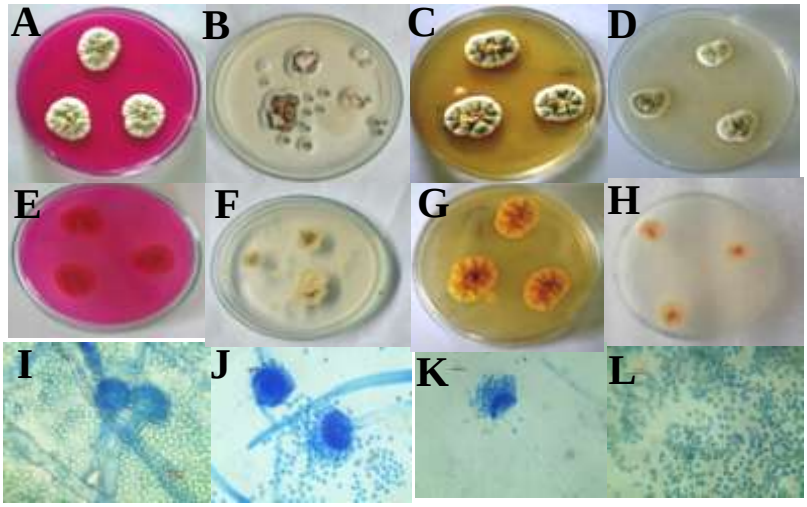

Figure 3.23. *Aspergillus versicolor*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-K: conidiophores and conidial head under 40x, L: conidia under 40x, scale bar = 10 µm

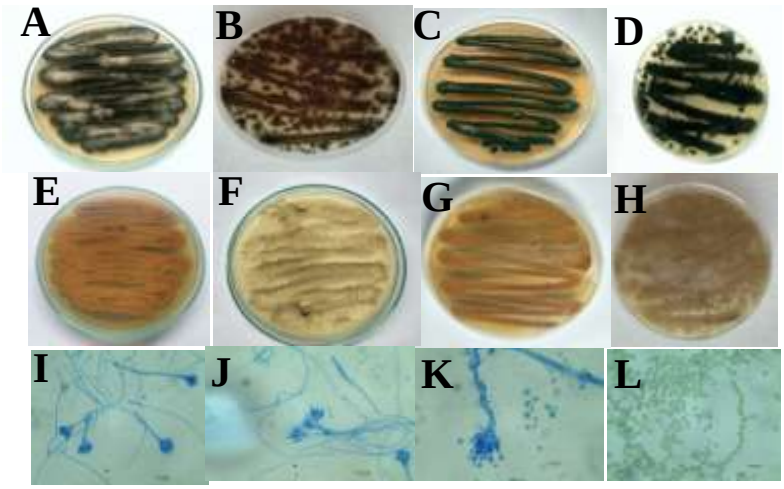

Figure 3.24. *Aspergillus* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: conidiophores and conidial heads under 40x, K: conidial haeds with phialides and conidia under 40x, L: conidia under 40x, scale bar = 10 µm

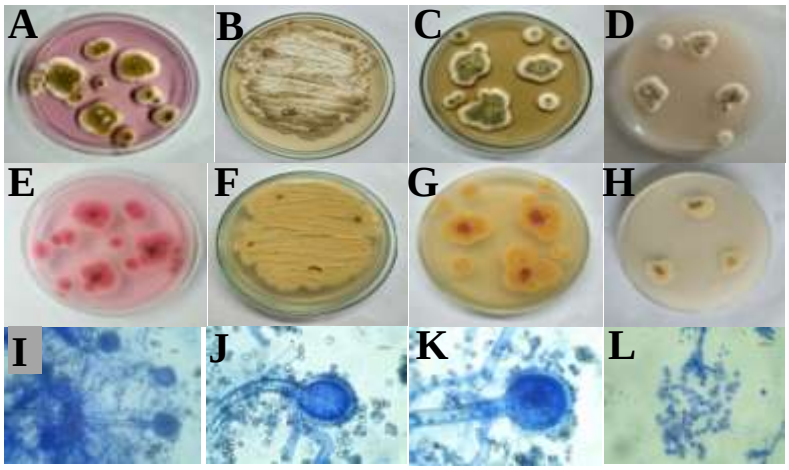

Figure 3.25. *Aspergillus* sp.2, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: conidiophores under 40x, J-K:conidial heads with phialides under 40x, L: conidia under 40x, scale bar = 10 µm

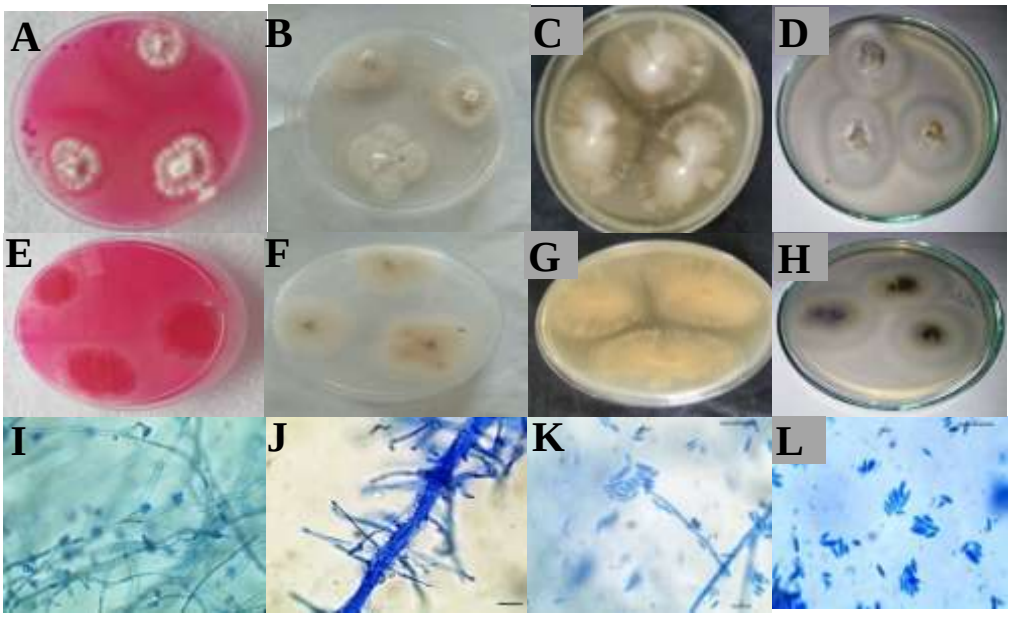

Figure 3.26. *Acremonium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: hyphal bundle with conidiophores and conidia heads under 40x, K: Phialides with conidial clusters L: conidia under 40x, L:spores under 100x, scale bar = 10 µm

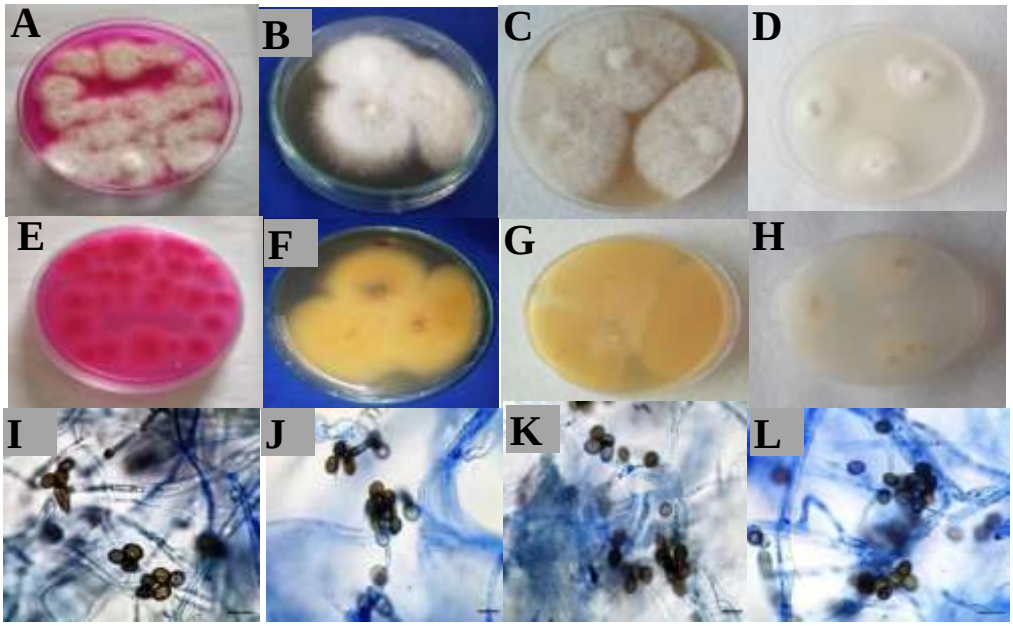

Figure 3.27. *Athrinium* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-L: dark brown conidia under 40x scale bar = 10 µm

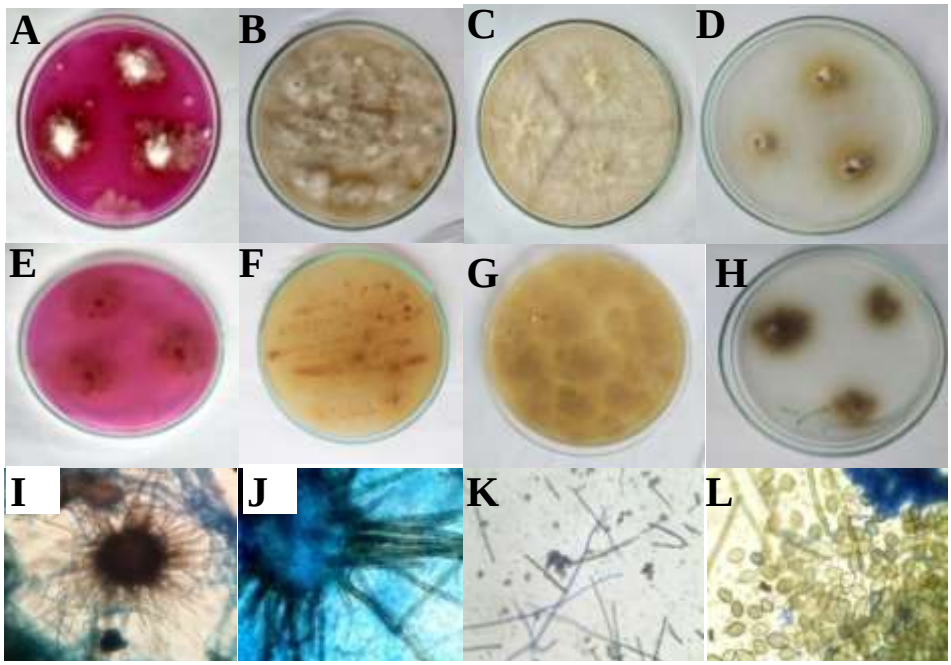

Figure 3.28. *Chaetomium globosum*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: perithecium with irregular hairs under 40x, J: perithecium under 100x, K-L:ascospores under 40x and 100x, scale bar = 10 µm

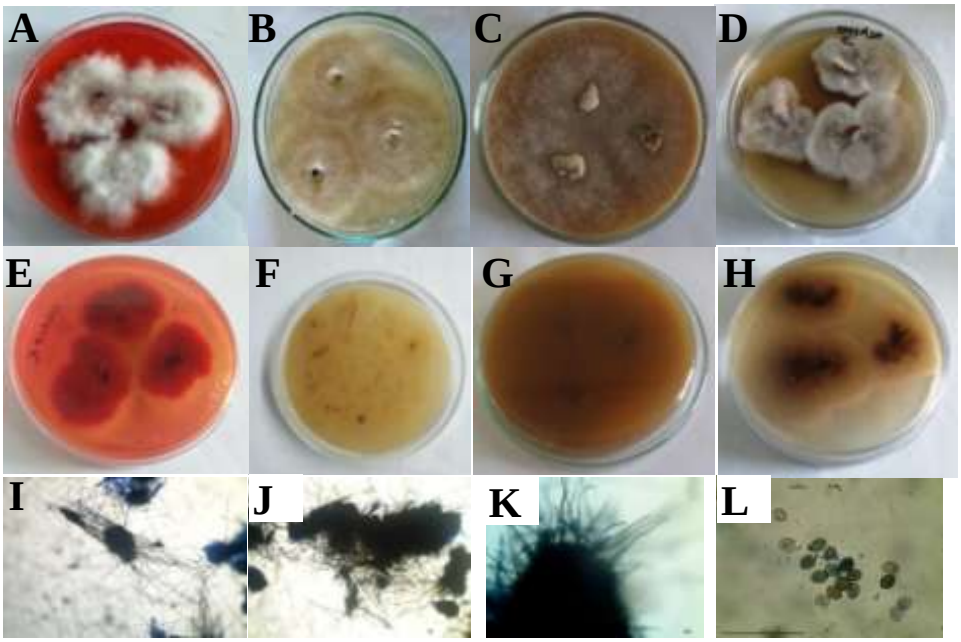

Figure 3.29. *Chaetomium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: perithecia with dichotomously branched hairs under 10x, K: perithecial hair under 40x, L:ascospores under 40x, scale bar = 10 µm

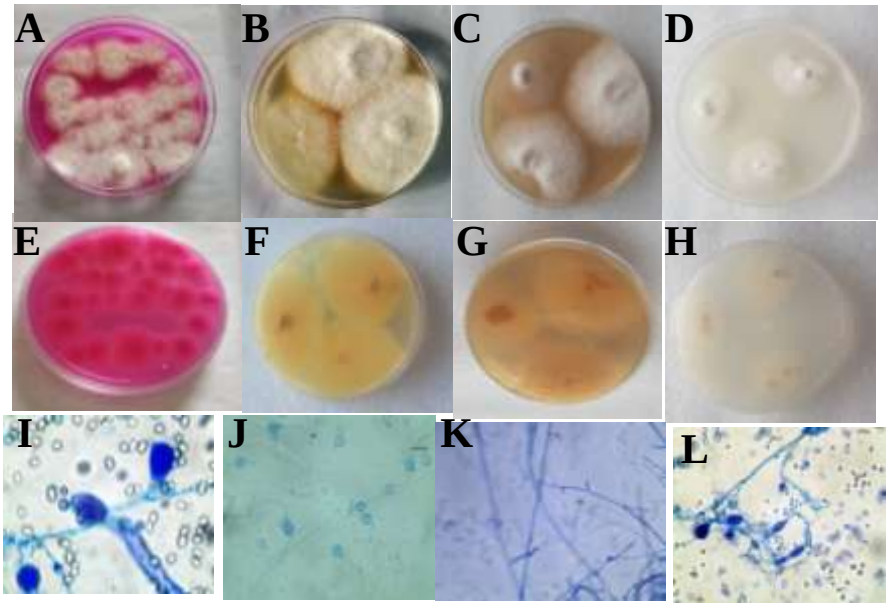

Figure 3.30. *Chrysosporium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: Hyphae under 10x, J: conidia on hyphae under 40x, K: alternating arthroconidia under 40x and 100x, L:spores under 40x, scale bar = 10 µm

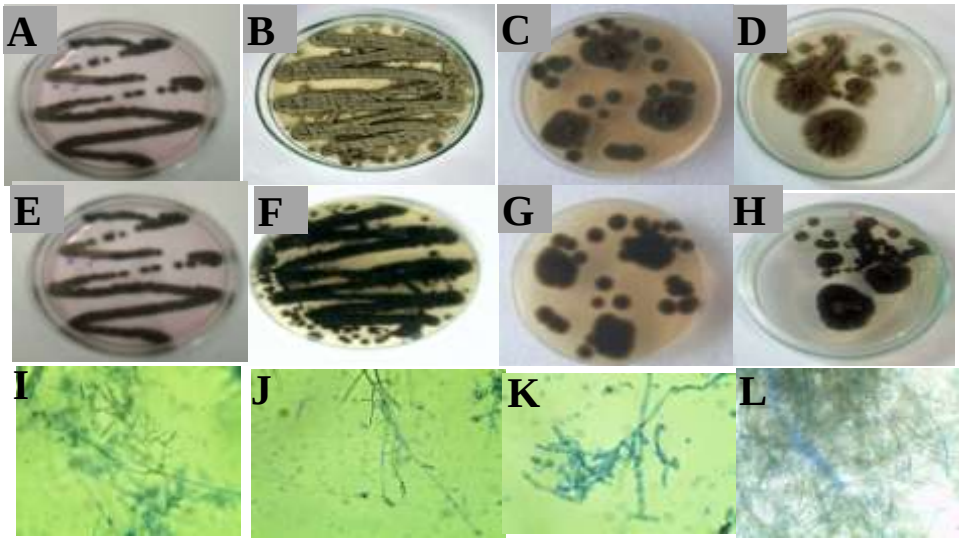

Figure 3.31. *Cladophialophora* sp. A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: short conidiophores with chains of conidia under 40x, K-L: long chains of conidia under 100x, scale bar = 10 µm

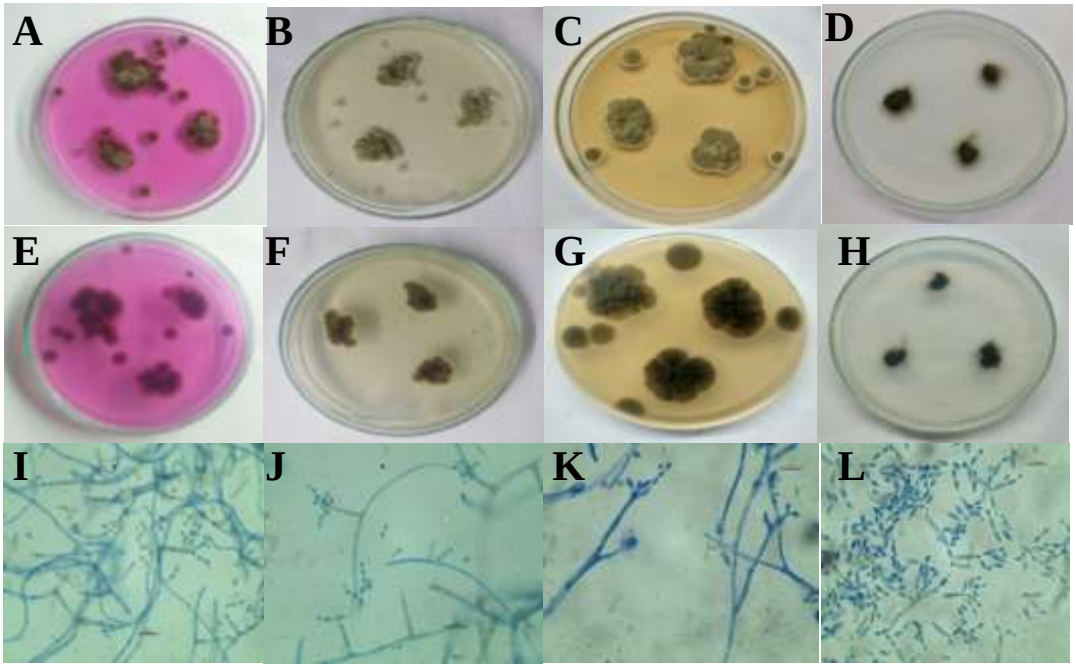

Figure 3.32. *Cladosporium cladosporioides*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: Hyphae under 40x, J-K: short conidiophores with conidia under 40x, L: conidia and ramoconidia under 40x, scale bar = 10 µm

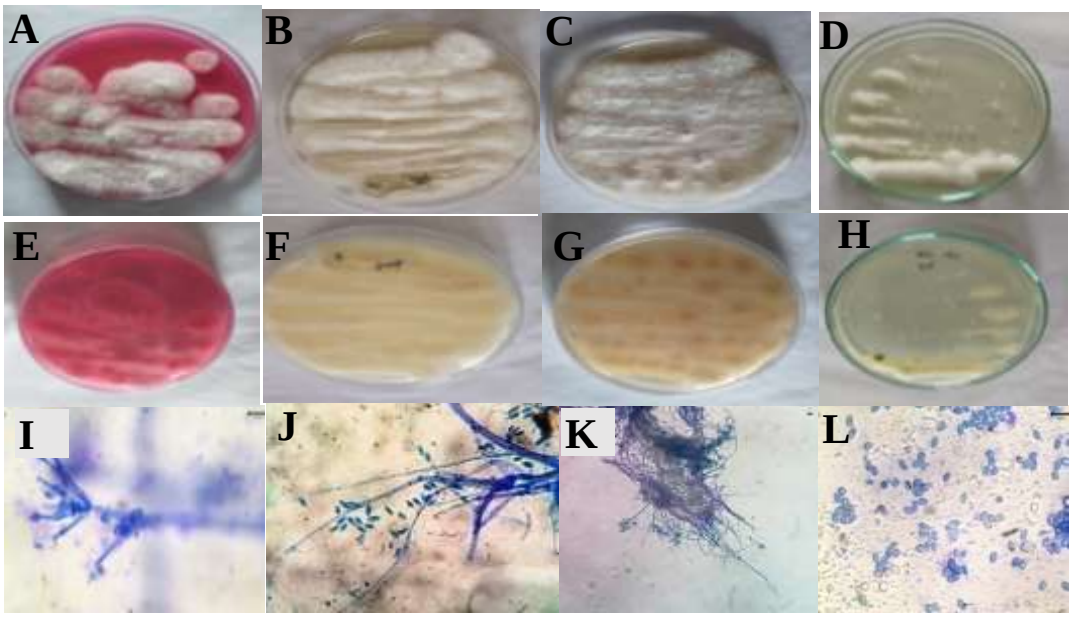

Figure 3.33. *Clonostachys* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: hyphae under 10x, J: conidiophores under 40x, K-L: conidia under 40x, scale bar = 10 µm

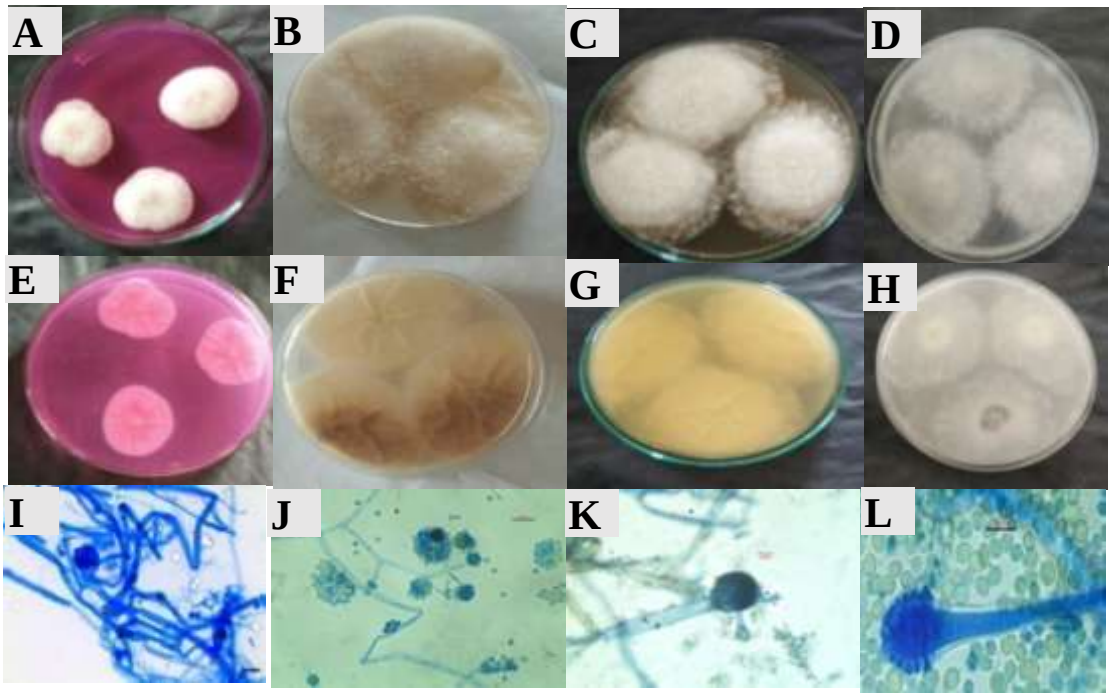

Figure 3.34. *Cunninghamella echinulata*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: sporangiophores with round and pitted sporangiola under 40x, K: swollen vesicles under 40x, L: sporangiola under 100x, scale bar = 10 µm

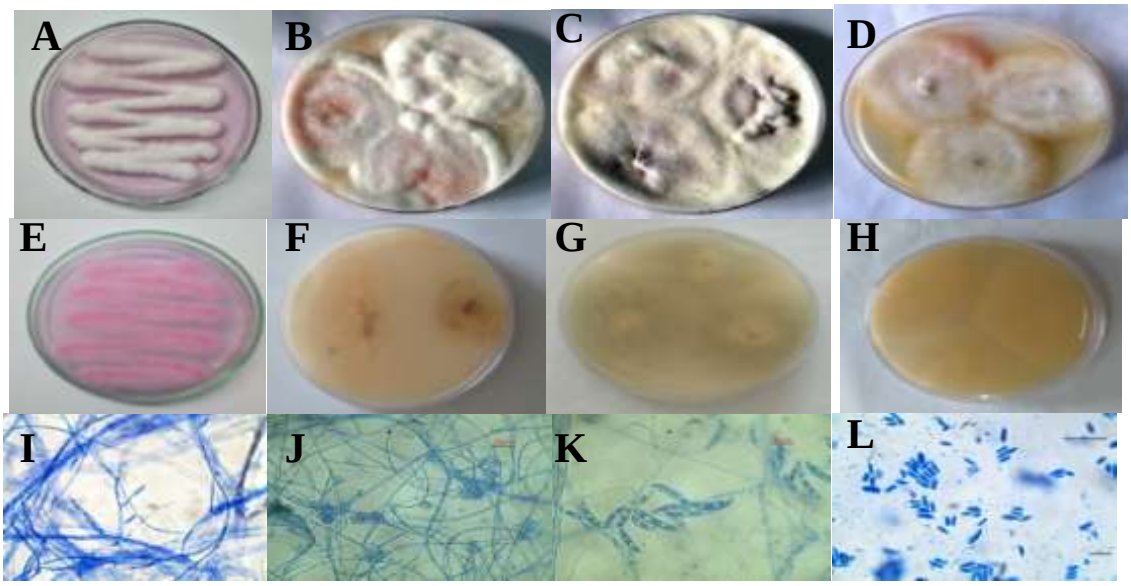

Figure 3.35. *Fusarium solani*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: hyphae under 40x, K: Macroconidia under 40x, L: microconidia under 40x, scale bar = 10 µm

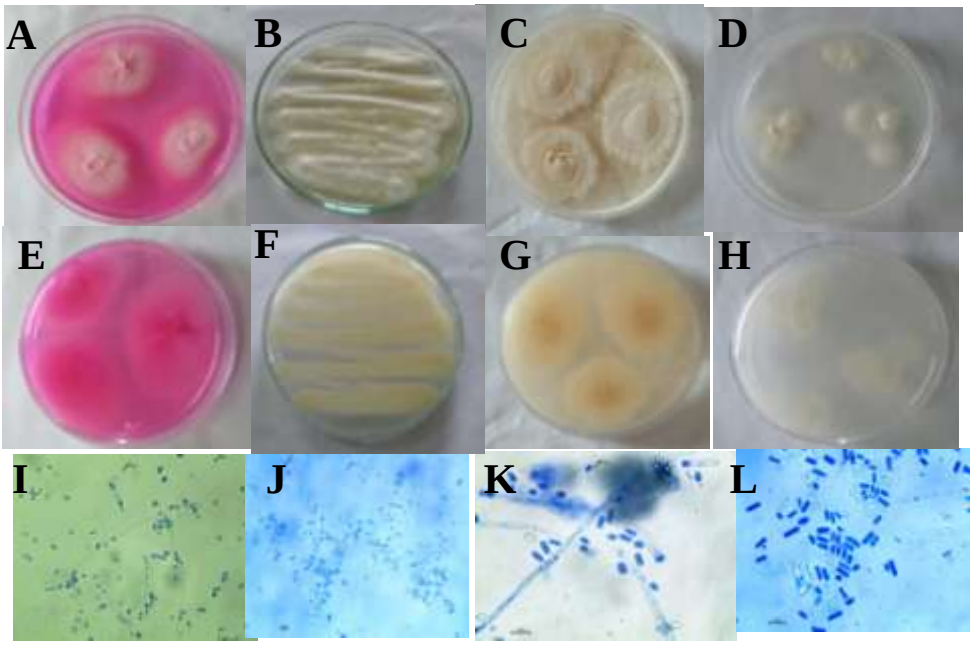

Figure 3.36. *Geotrichum candidum,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: hyphae and anthroconidia under 10x and 40x, K-L: anthroconidia under 40x and 100x, scale bar = 10 µm

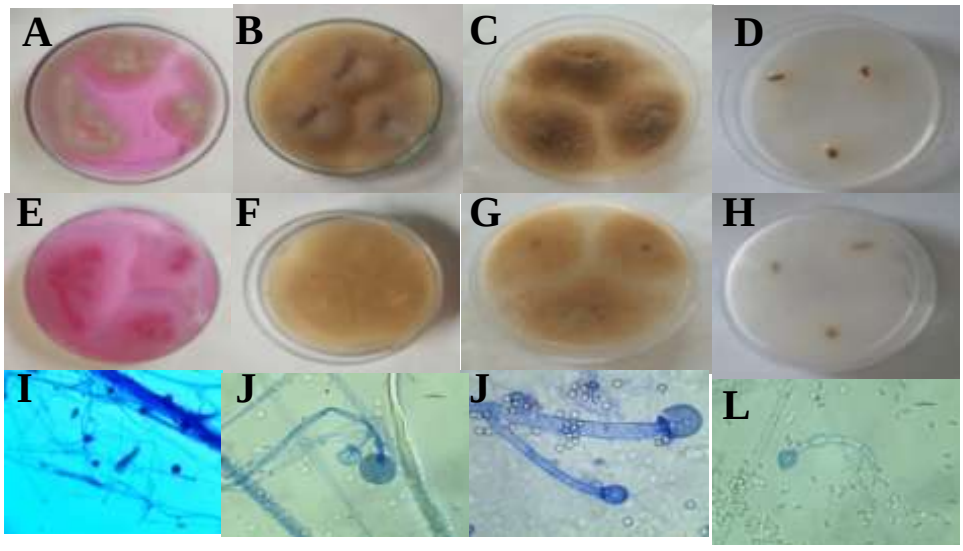

Figure 3.37. *Mucor circinelloides,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: sporangiophores with sporangium under 10x and 40x, K: sporangium with columellae and sporangiospores under 40x, L: chlamydospores under 40x, scale bar = 10 µm

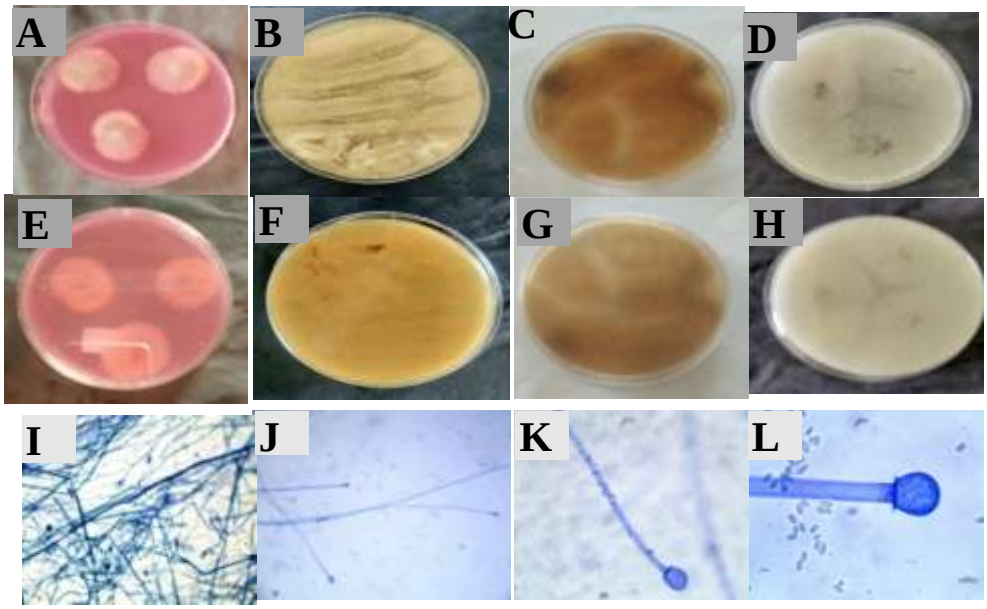

Figure 3.38. *Mucor heimalis,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: sporangiophores with sporangium under 10x, K-L: sporangium with columellae and sporangiospores under 40x, scale bar = 10 µm

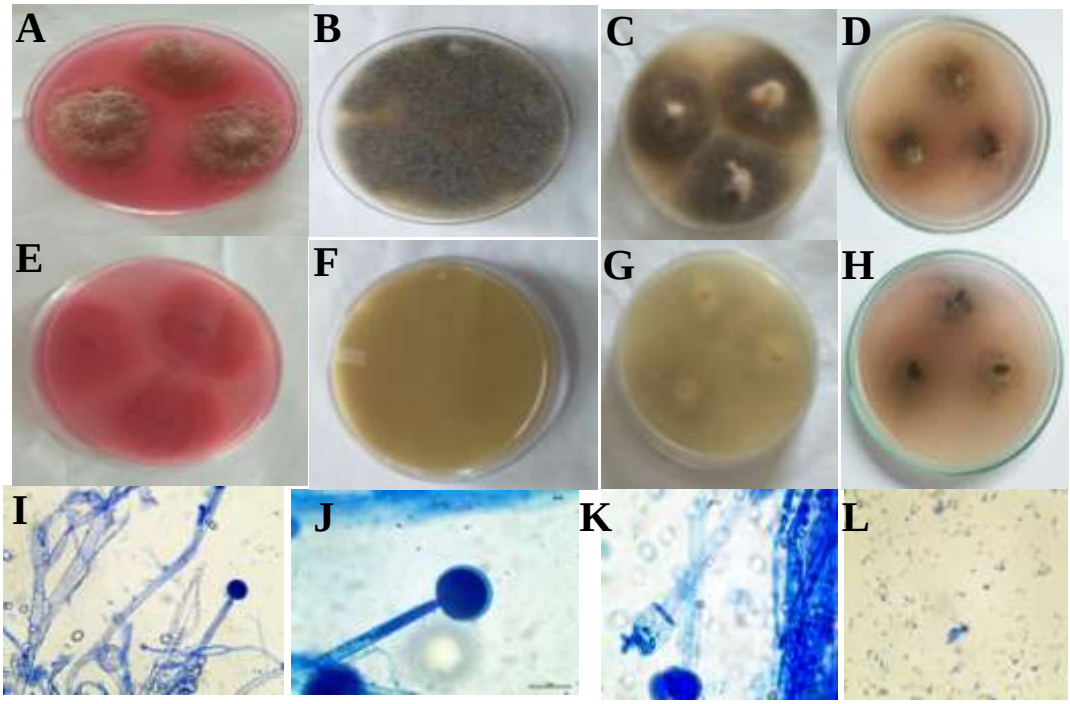

Figure 3.39. *Mucor plumbeus,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: sporangiophores with sporangium under 10x, J-K: sporangium under 40x, L: sporangiospores under 40x, scale bar = 10 µm

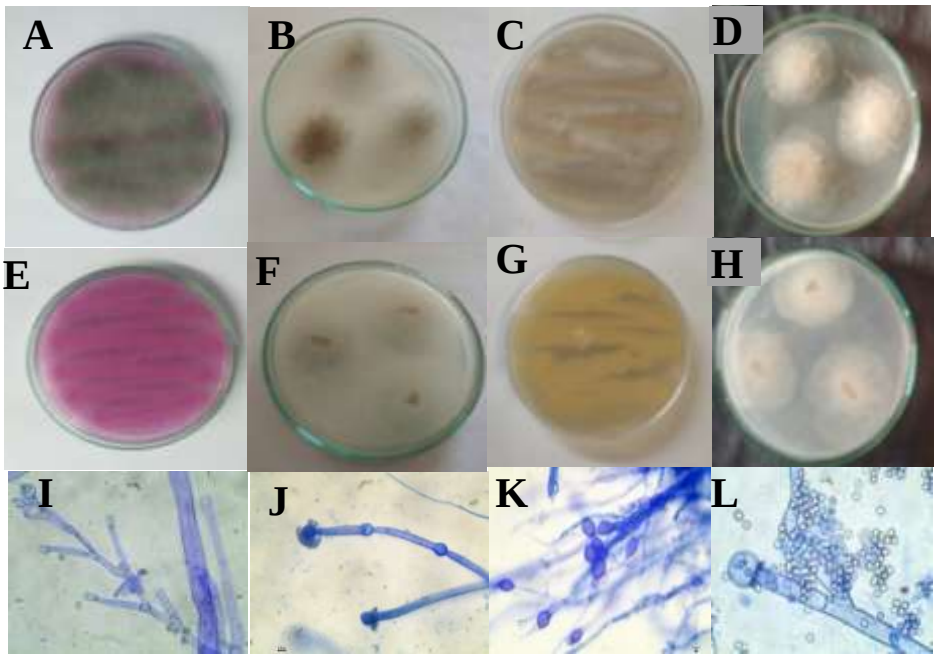

Figure 3.40. *Mucor racemosus,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: branching sporangiophores with sporangium under 40x, J: chlamydospores formation in the sporangiophores (arrow), K: chlamydospores under 40x, L: sporangium and sporangiospores under 40x, scale bar = 10 µm

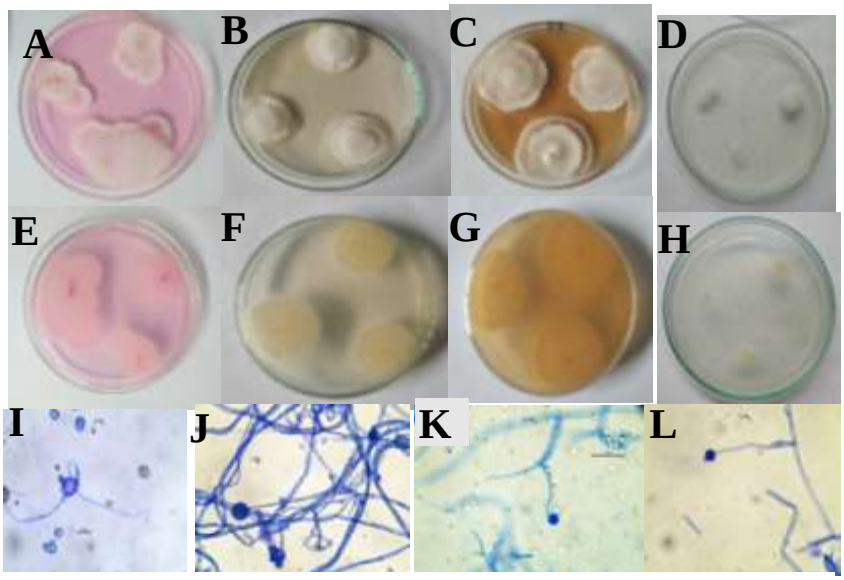

Figure 3.41. *Mortierella* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: Hyphae under 10x, J-K: conidiophores with sporangium under 40x, L: chlymadospore under 100x, scale bar = 10 µm

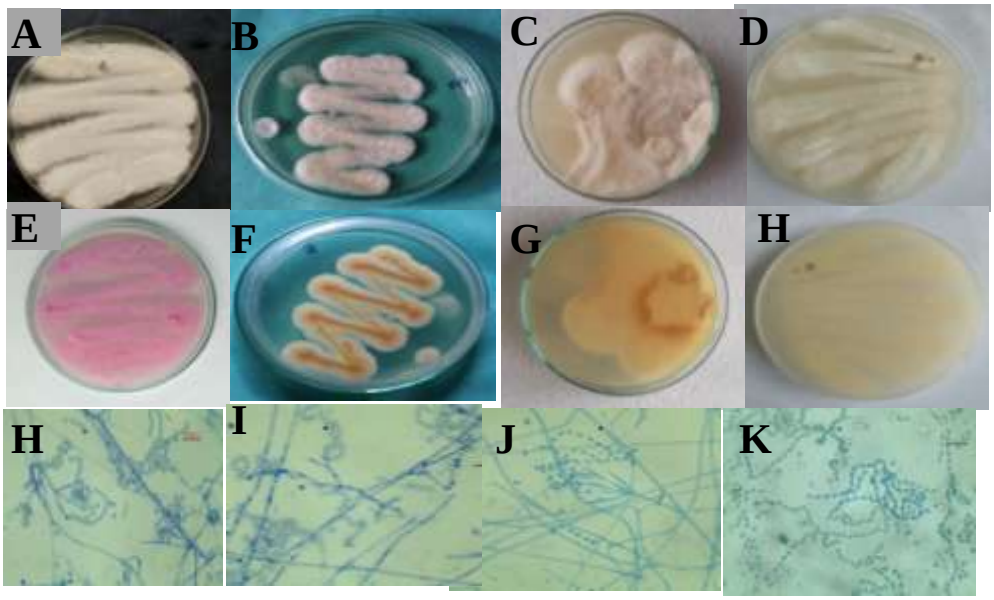

Figure 3.42. *Paecomyciles* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: short conidiophores with philiades under 40x, J: philiades with swollen base (arrow) under 40x, K-L: long chains of conidia under 40x, scale bar = 10 µm

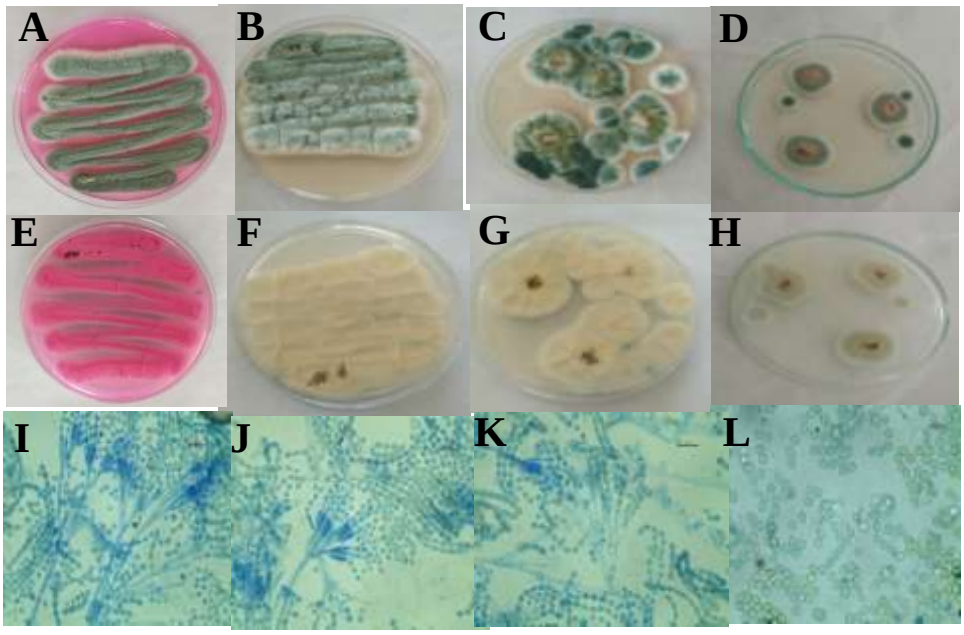

Figure 3.43. *Penicillium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-K: conidiophores with conidia under 40x, L: conidia under 40x, scale bar = 10 µm

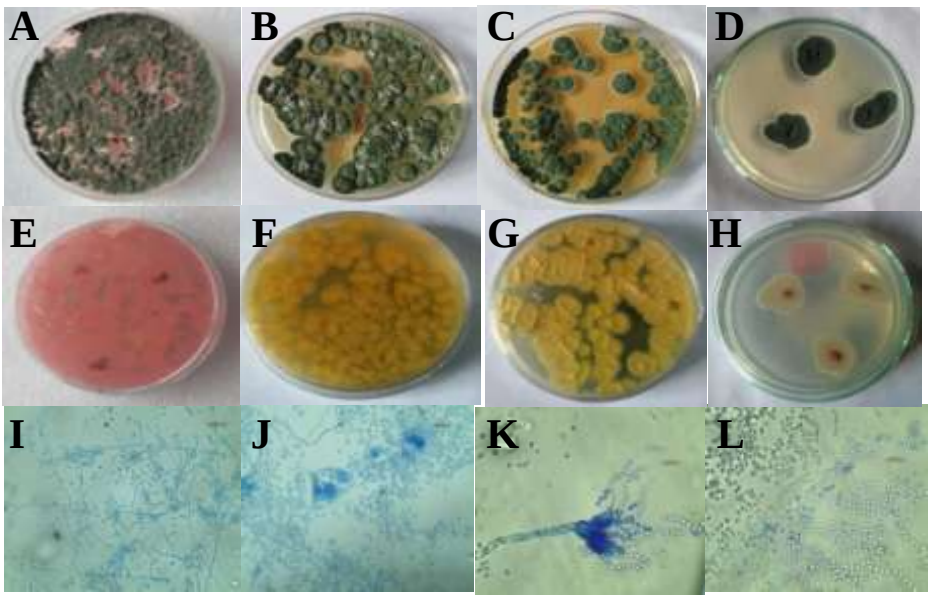

Figure 3.44. *Penicillium citrinium*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: conidiophores under 40x, J-K: philiades with long chains of conidia under 40x and 100x. L: conidia under 40x. scale bar = 10 um

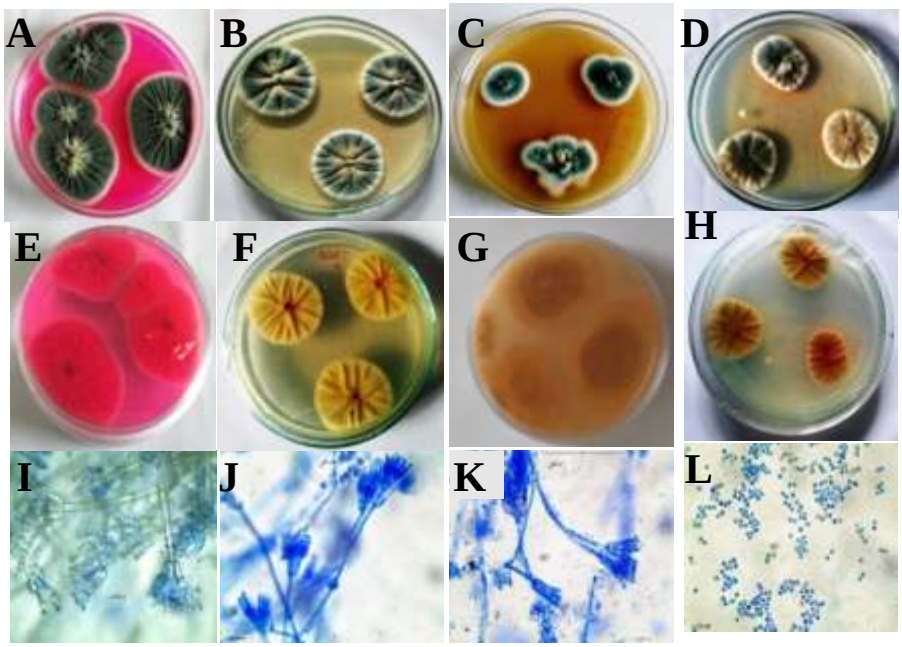

Figure 3.45. *Penicillium commune*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-K: conidiophores with conidia under 40x, L: conidia under 40x, scale bar = 10 µm

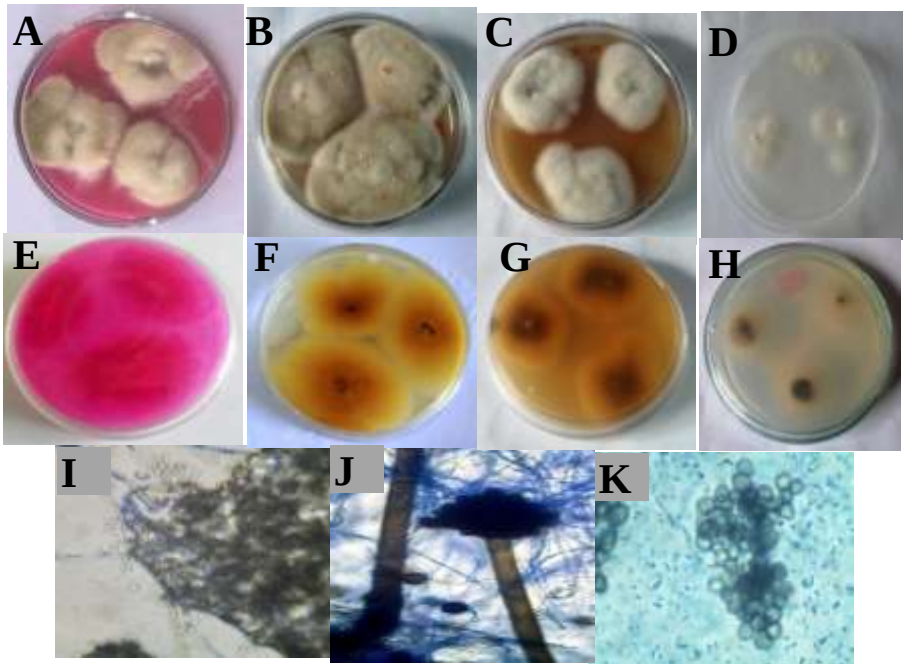

Figure 3.46. *Periconia* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: hyphae under 10x J: conidiophores with conidia under 40x and 100x, K: conidia under 100x. scale bar = 10 um

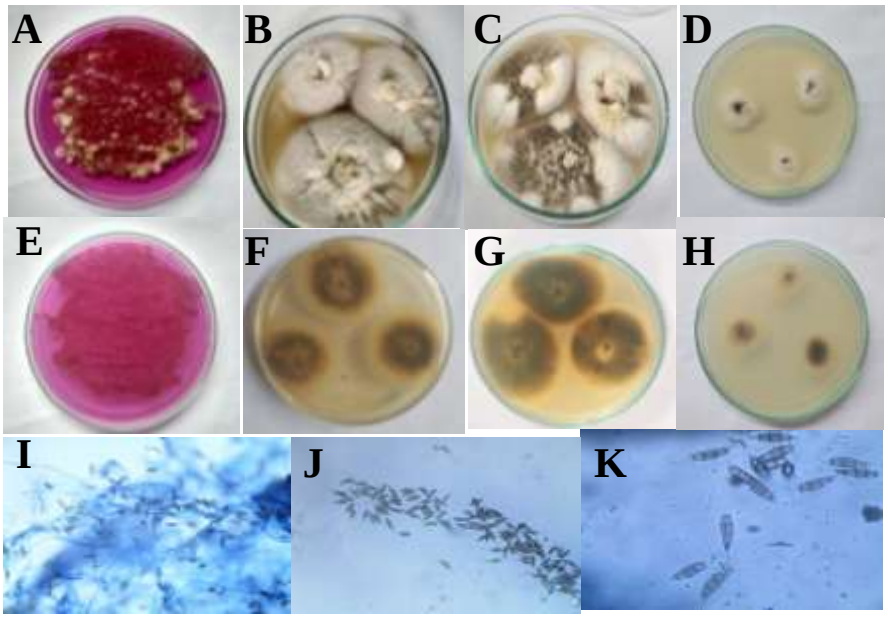

Figure 3.47. *Pestalioptopsis* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: Hyphae under 10x, J-K: conidia with basal and apical appendages under 10x and 40x, scale bar = 10 µm

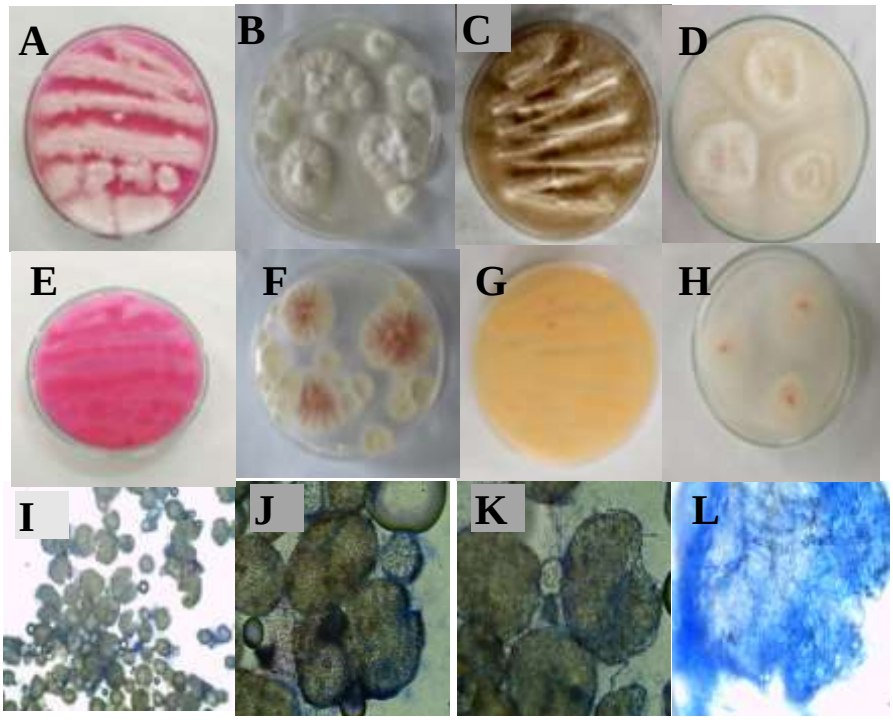

Figure 3.48. *Phoma* glomerata, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: pycnidia under 10x, J-K: pycnidia under 100x, L; chlamydospores under 100x, scale bar = 10 µm

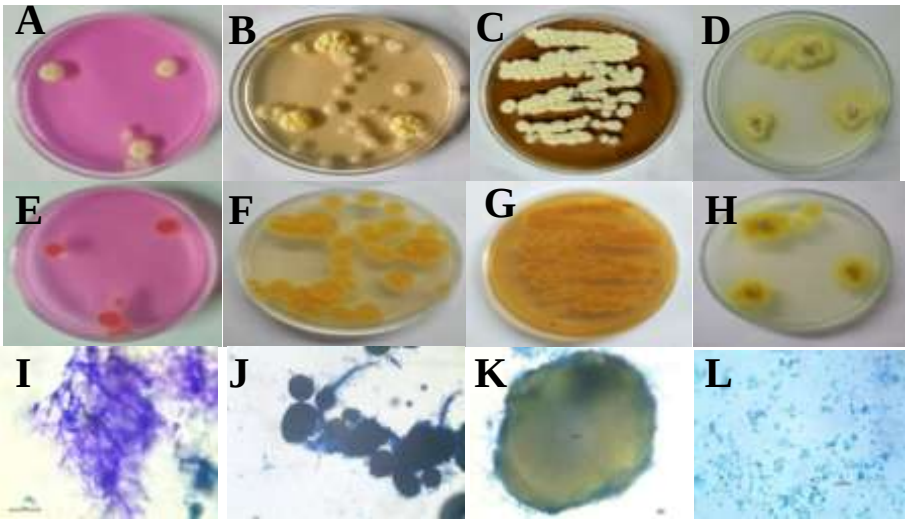

Figure 3.49. *Phoma* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: hyphae under 10x, J-K: pycnidia under 40x and 100x, L; conidia under 40x, scale bar = 10 µm

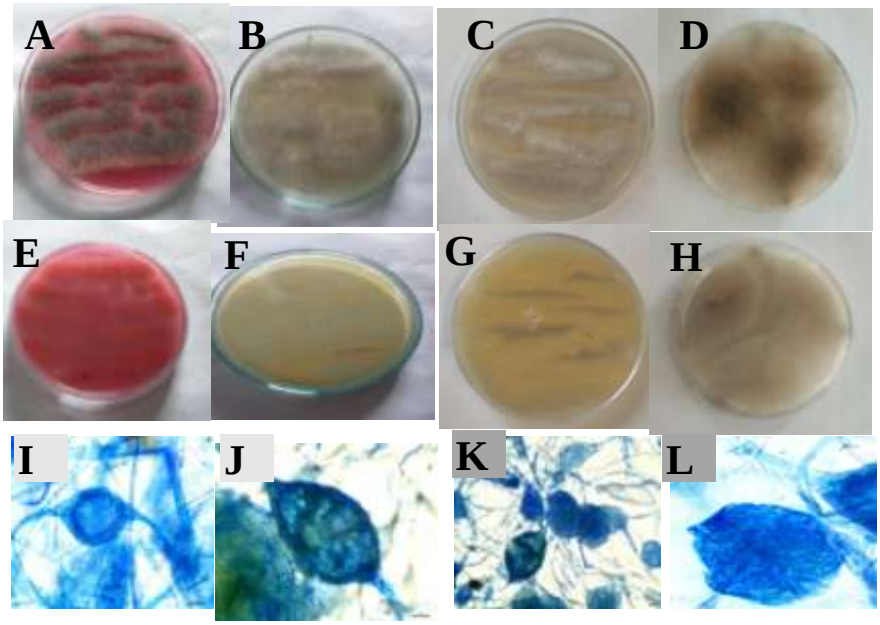

Figure 3.50. *Phoma* sp.2, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-K: pycnidia under 40x, L; chlamydospores under 100x, scale bar = 10 µm

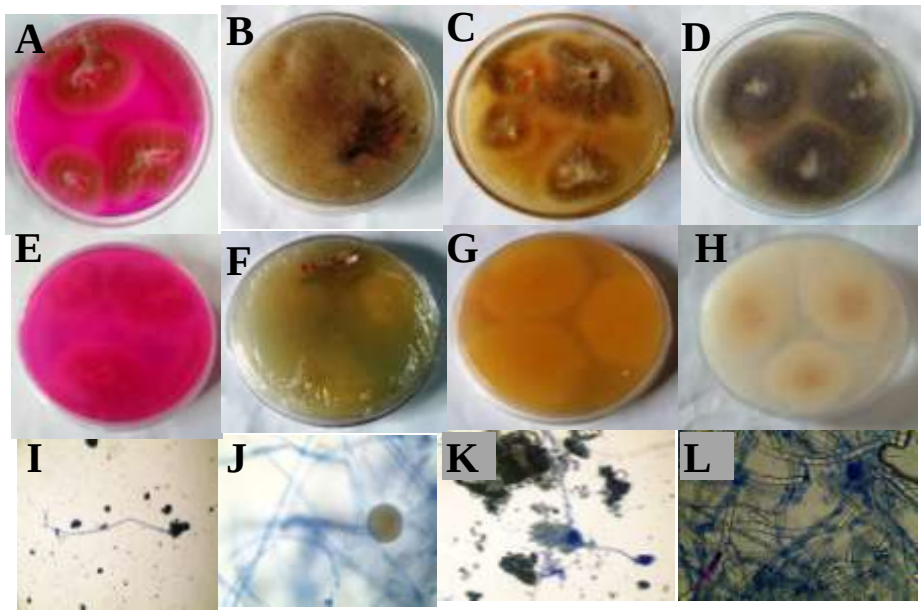

Figure 3.51. *Rhizopus microspores,* A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: sporangiophore with sporangium and rhizoid under 10x, J: sporangium under 40x, K: sporangiospores under 40x L: chlamydospores under 40x, scale bar = 10 µm

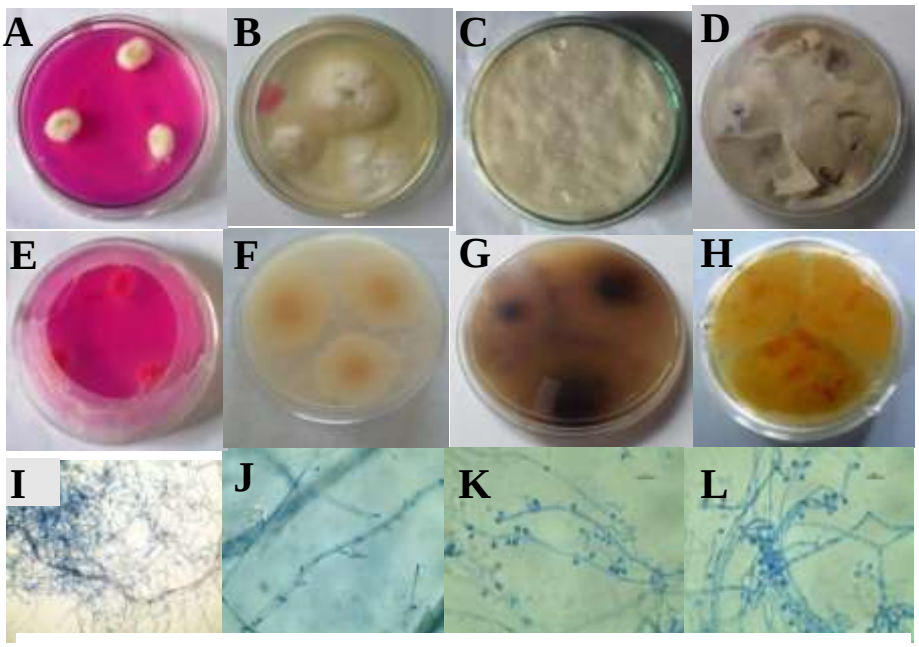

Figure 3.52. *Scedosporium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: hyphae under 10x, J: conidia on hyphae under 10x, K-L: conidia under 40x, scale bar = 10 µm

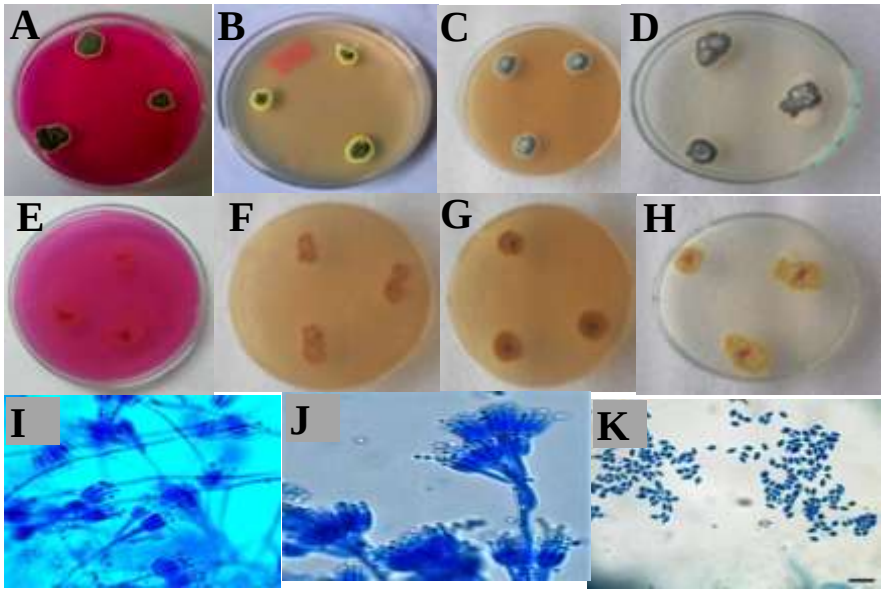

Figure 3.53. *Taloromyces* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: condiophores with phialides under 10x, J: phialides with conidia under 40x, K: conidia under 40x, scale bar = 10 µm

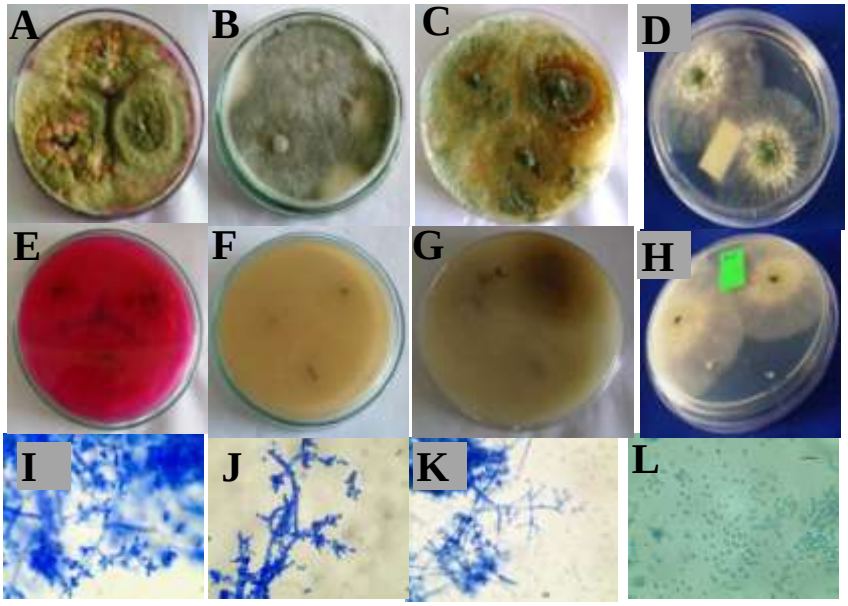

Figure 3.54. *Tricoderma hamatum*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: condiophores with phialides under 10x, J-K: phialides with conidia under 40x, L: conidia under 40x, scale bar = 10 µm

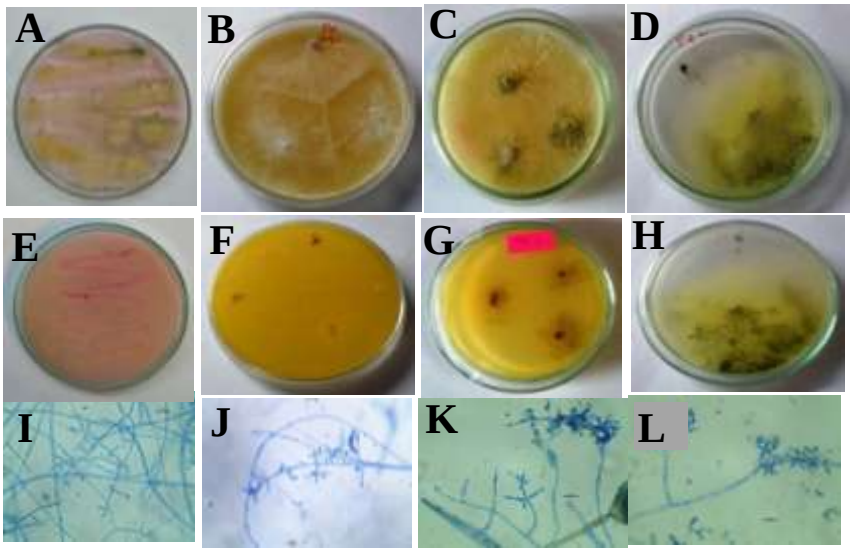

Figure 3.55. *Tricoderma longibrachiatum*, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-J: long condiophores under 10x, K-L: phialides and conidia under 40x, scale bar = 10 µm

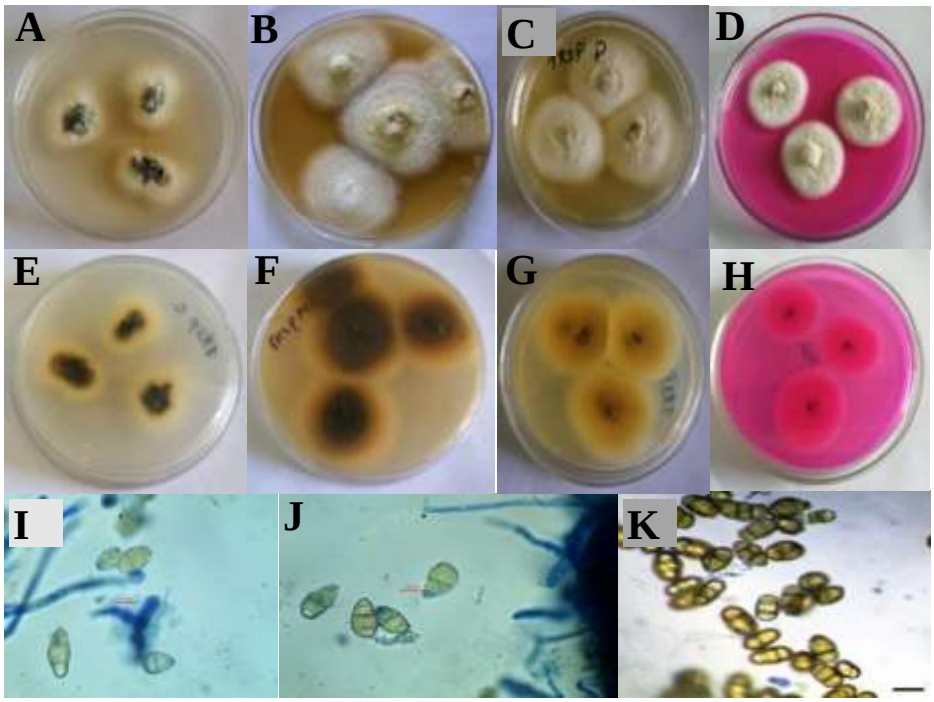

Figure 3.56. *Ulocladium* sp., A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I: hyphae with conidia, J-K:conidia under 10 and 40x, scale bar = 10 µm

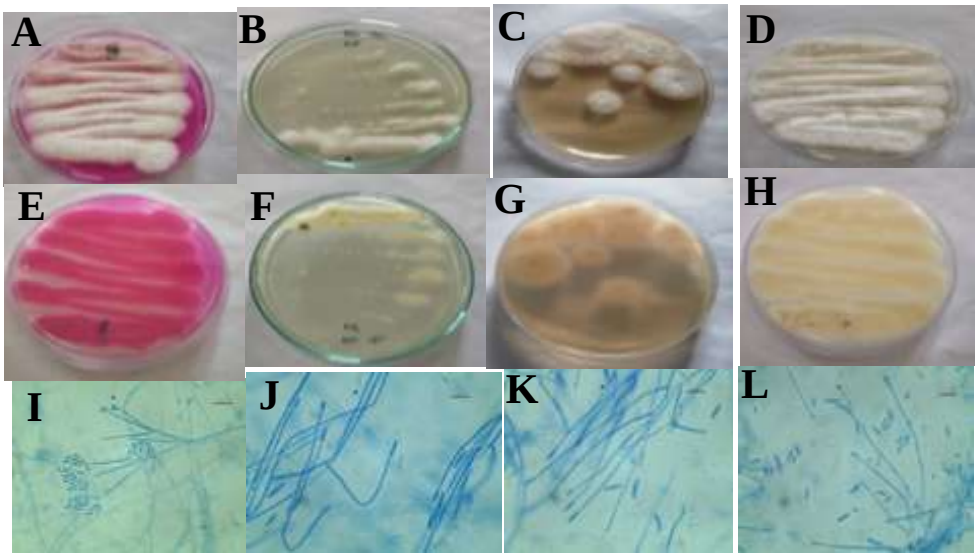

Figure 3.57. *Verticilllium* sp.1, A-D: colony top view, E-H: colony bottom view on RBA, PDA, MEA and CDA, I-L: conidiophores and conidia under 40x, scale bar = 10 µm

3.3.7. Seasonal and site variation of soil microbial diversity

Soil bacterial diversity

During the present study, a total of 133 bacterial isolates were identified through phenotypic and 16S *r*RNA characterization. Species were identified under the genera *Acinetobacter, Bacillus, Chryseobacterium, Lysinibacillus, Microbacterium* and *Ralstonia* were. The maximum number of isolates belonged to the genera *Bacillus* with 14 species during the present study. Out of the total 133 bacterial isolates, Minkong reserve forest recorded the highest bacteria isolates with 40 isolates followed by abandoned jhum land with 35 bacteria isolates and tea garden and cultivated jhum land both with 29 bacteria isolates together in the first and second year of the study period. Bacteria species such as *Acinetobacter seiferti*, *Lysinibacillus* sp., *Chryseobacterium takakiae*, *Bacillus tequilensis, Bacillus licheniformis, Bacillus aerophilus, Bacillus cereus, Bacillus stercoris* were restricted to Minkong reserve forest and *Ralstonia picketti* was restricted to tea garden. *Microbacterium imperiale, Bacilllus altitudinis* and *Bacillus amyloliquefaciens* were observed only in abandoned jhum land and tea garden while *Bacillus zhangzhouensis* occurred only in tea garden and Minkong reserve forest and *Bacillus halotolerans* was found only in cultivated jhum land and abandoned jhum land. In cultivated jhum land, *Bacillus halotolerans* and *Microbacterium* sp. were recorded only in the first year and absent in the second year. Species such as *Bacillus pumilus* and *Bacillus safensis* were isolated only during the spring while *Bacillus subtilis* occurred in all the seasons in both first and second year. Among the seasons, the maximum number of bacterial isolate was recorded during winter in the first year

and during spring in the second year. In the abandoned jhum land, *Bacillus altitudinis* and *Bacillus halotolerans* were recorded only in the second year during the study period. Among the seasons, spring season recorded the maximum number of species in both the first year and second year. In the first year, species such as *Bacillus velezensis* and *Bacillus safensis,* occurred only during the spring season while *Microbacterium* sp. was recorded only during the autumn season. In the second year, *Bacillus altitudinis* and *Microbacterium imperial* was recorded only during the spring season. In the tea garden, *Ralstonia picketti* was found to occur only during the second year. Among the seasons, the maximum bacterial isolate was found during spring season in both the first year and second year. *Bacillus altitudinis* was observed only in the spring season, *Bacillus safensis* in summer season and *Bacillus tequilensis* in autumn season during the first year whereas during the second year, *Bacillus licheniformis* and *Ralstonia picketti* was recorded only in autumn season while *Bacillus zhangzhouensis* occured only in summer season. In the Minkong reserve forest, species that occurred only during the first year was *Bacillus safensis* and *Lysinibacillus* sp. while species such as *Bacillus aerophilus, Bacillus halotolerans, Bacillus stercoris* and *Microbacterium* sp. occurred only during the second year. Among the seasons, the maximum bacterial isolate was recorded during spring season in both the first year and second year. Bacteria species such as *Acinetobacter seiferti*, *Bacillus cereus, Bacillus halotolerans, Bacillus safensis* and *Lysinibacillus* sp. occurred only during spring season, *Bacillus licheniformis*, *Bacillus pumilus, Bacillus stercoris and Bacillus zhangzhouensis* was recorded only during autumn season while *Microbacterium* sp. was found to occur only during summer season during the present study.

Table 3.12. Variations in soil bacterial isolates between sites and seasons during 2016-18

	2016-17				2017-18			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
CJL	*Bacillus halotolerans* AK17(ON158773), *Bacillus pumilus* AK13(ON150922), *Bacillus sp* AK45(ON171227), *Bacillus subtilis* AK5(ON140796)	*Bacillus halotolerans* AK110 (ON171376), *Bacillus safensis* AK58 (ON152402), *Bacillus sp* AK93 ON152421, *Bacillus subtilis* AK47 (ON152386)	*Bacillus pumilus* AK44(0N152313, *Bacillus safensis* AK39(ON15231), Bacillus sp AK65(ON15241), *Bacillus subtilis* AK46(ON15238), AK179(ON156015), AK186(ON1	*Bacillus sp* AK105 (ON155839), *Bacillus subtilis* AK56 (ON152399)	*Bacillus pumilus* AK146 (ON155993), Bacillus pumilus (AK134 ON158801)	*Bacillus pumilus* AK61 (ON152406) *Bacillus safensis* AK136 (ON155917), *Bacillus* sp AK137 (ON155920), *Bacillus subtilis* AK159 (ON156005), AK187 (ON156449), AK206 (ON156439)	*Bacillus* sp AK158 (ON155998), *Bacillus subtilis* AK171 (ON156006)	*Bacillus* sp AK207 (ON156455), *Bacillus subtilis* AK178 (ON156033)

			56400) *Microbacterium* sp AK11(ON15085)					
AJL	*Bacillus* sp. AK48 (ON166503), *Bacillus subtilis* AK96 (ON166812)	*Bacillus* safensis AK116 (ON170419), *Bacillus* sp AK87 (ON166709), *Bacillus subtilis* AK122 (ON171202)	*Bacillus pumilus* AK67 (ON166657), *Bacillus* sp AK95 (ON166784), *Bacillus subtilis* AK123 (ON171203), *Microbacterium* sp AK27 (ON166492	*Bacillus pumilus* AK63 (ON166708), Bacillus sp AK30 (ON141506), *Bacillus subtilis* AK100 (ON166825), *Bacillus velezensis* AK127 (ON171204)	*Bacillus* sp AK97 (ON166813), *Bacillus subtilis* AK129 (ON171216), *Bacillus velezensis* AK128 (ON171215)	*Bacillus amyloliquefaciens* AK118 (ON170420) *Bacillus pumilus* AK41 (ON166551), *Bacillus* sp AK150 (ON171234), *Bacillus subtilis* AK147 (ON171229)	*Bacillus altitudinis* AK181 (ON171373), *Bacillus halotolerans* AK91 (ON166768), *Bacillus pumilus* AK149 (ON171231), *Bacillus safensis* AK142 (ON171225), *Bacillus* sp AK130 (ON171220), *Bacilllus* sp	*Bacillus amyloliquefaciens* AK180 (ON171372), *Bacillus halotolerans* AK109 (ON166826) *Bacillus safensis* AK185 (ON166704), *Bacillus* sp AK164 (ON171318), *Bacillus subtilis* AK170 (ON171367),

							AK189 (ON166676), *Bacillus subtilis* AK131 (ON171219) AK177 (ON171369), *Microbacterium imperial* AK28 (ON159960)	*Bacillus velezensis* AK156 (ON171335)
TG	*Bacillus licheniformis* AK 4 (ON140985), *Bacillus safensis* AK139 (ON158803), *Bacillus* sp AK155 (ON158804), *Bacillus subtilis* AK204 (ON158808), *Ralstonia picketti*	*Bacillus pumilus* AK53 (ON158778), *Bacillus safensis* AK107 (ON158791), *Bacillus* sp AK33 (ON158777), *Bacillus subtilis* AK133 (ON158799, AK173 (ON158805),	*Bacillus safensis* AK135 (ON158802), *Bacillus* sp AK103 (ON075548), *Bacillus subtilis* AK203 (ON158807), *Bacillus tequilensis*	*Bacillus* sp. AK31 (ON158776), *Bacillus subtilis* AK102 (ON158789)	*Bacillus pumilus* AK66 (ON158788), *Bacillus subtilis* AK70 (ON158787)	*Bacillus amyloliquefaciens* AK157 (ON158806), *Bacillus atltitudinis* AK55 (ON171374), *Bacillus* sp AK16 (ON158774), *Bacillus subtilis* AK14	*Bacillus safensis* AK88 (ON158790), *Bacillus* sp AK6 (ON158766), *Bacillus subtilis* AK25 (ON158775)	*Bacillus amyloliquefaciens* AK59 (ON158781), *Bacillus subtilis* AK7 (ON158768)

	AK40 (ON158779)	*Microbacterium imperiale* AK12 (ON158772)	AK132 (ON158792), *Bacillus zhangzhouensis* AK49 (ON152390)			(ON158771)		
MRF	*Bacillus* sp. AK18 (ON159204), *Bacillus subtilis* AK21 (ON159205), *Bacillus velezensis* AK202 (ON159620)	*Acinetobacter seiferti* AK168 (ON159608), *Bacillus aerophilus* AK163 (ON159601), *Bacillus cereus* AK182 (ON159611) *Bacillus safensis* AK42 (ON159274), *Bacillus* sp. AK209 (ON159707), *Bacillus stercoris*	*Bacillus licheniformis* AK175 (ON159612), *Bacillus pumilus* AK36 (ON159260), *Bacillus* sp. AK161 (ON159599), *Bacillus subtilis* AK104 (ON159556), *Bacillus zhangzhouen*	*Bacillus* sp. AK108 (ON075533), *Bacillus stercoris* AK120 (ON159594), *Bacillus subtilis* AK52 (ON159275)	*Bacillus safensis* AK92 (ON159280), *Bacillus* sp AK165 (ON159602), *Bacillus subtilis* AK152 (ON159597), *Bacillus velezensis* AK201 (ON159621)	*Acinetobacter seiferti* AK169 (ON159609), *Bacillus aerophilus* AK43 (ON159276), *Bacillus cereus* AK183 (ON159610), *Bacillus* sp. AK166 (ON159607), *Bacillus subtilis* AK154 (ON159596), *Chryseobacteri*	*Bacillus licheniformis* AK195 (ON171412), *Bacillus pumilus* AK167 (ON159603) *Bacillus* sp AK208 (ON159706), *Bacillus stercoris* AK148 (ON171409), *Bacillus subtilis* AK193 (ON159619), *Chryseobacterium*	*Bacillus safensis* AK106 (ON159559), *Bacillus* sp AK191 (ON159616), *Bacillus subtilis* AK192 (ON159617), *Bacillus zhangzhouensis* AK162 (ON159598), *Microbacterium* sp AK23 (ON159258)

AK20 (ON159203), *Bacillus subtilis* AK29 (ON159273), *Lysinibacillus sp*. AK71 (ON159277)	*sis* AK64 (ON159279)	*um takakiae* AK26 (ON159259)	*takakiae* AK22 (ON159206)

Characters in brackets indicates NCBI GenBank accession number of each identifies bacterial isolate

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Soil fungal diversity

During the present study, altogether a total of 551 soil fungi were isolated from all the four study sites. The identification was carried out by morphological and microscopical studies, through which 551 fungal isolates were identified under 101 fungal species belonging to 45 fungal genera throughout the study period (Table 3.13-3.14). The number of species under each 45 fungal genera during the present study were *Absidia* (3 species), *Acremonium* (1 species), *Alternaria* (2 species), *Arthrographis* (1 species), *Apophysomyces* (1 species), *Aspergillus* 11 species), *Arthrinium* (1 species), *Botrysis* (1 species), *Chaetomium* (3 species), *Chrysosporium* (2 species), *Cladiophialophora* (1 species), *Cladosporium* (4 species), *Clonostachys* (1 species), *Colletotrichum* (1 species), *Cunninghamella* (1 species), *Emmonsia* (1 species), *Epicoccum* (1 species), *Exophiala* (1 species), *Fusarium* (5 species), *Geotrichum* (3 species), *Gliocladium* (1 species), *Humicola* (1 species), *Madurella* (1 species), *Microascus* (1 species), *Mortierella* (1 species), *Mucor* (5 species), *Neoscytalidum* (1 species), *Nigrospora* (1 species), *Paecilomyces* (2 species), *Penicillium* (12 species), *Periconia* (2 species), *Pestaliotopsis* (1 species), *Phaeocremonium* (1 species), *Phoma* (4 species), *Phomopsis* (1 species), *Rhizopus* (2 species), *Scedosporium* (2 species), *Sclerotinia* (1 species), *Scopulariopsis* (1 species), *Scytalidium* (1 species), *Talaromyces* (2 species), *Trichophyton* (2 species), *Tricoderma* (6 species), *Ulocladium* (1 species), *Verticillium* (2 species).

Some fungal genera were common as well as restricted to specific sampling site throughout the study period. Common fungal genera in all the four study sites

were *Aspergillus* and *Penicillium*. Whereas, fungal genera under *Humicola*, *Exophiala* and *Clonostachys* were restricted to cultivated jhum land, genera under *Arthrographis* and *Apophysomyces* were restricted to abandoned jhum land, genera under *Epicoccum, Nigrospora* and *Neoscytalidium* was restricted to tea garden, genera under *Cunninghamella, Scopulariopsis,* and *Phomopsis* was restricted to Minkong reserve forest. Between the study sites, Minkong reserve forest recorded the highest fungal isolates (173 isolates under 37 genera) followed by abandoned jhum land (143 isolates under 33 genera), tea garden (124 isolates under 29 genera) and cultivated jhum land (111 isolates under 26 genera).

Seasonal variation in soil fungal isolates was also observed between the study sites. The spring season recorded the highest soil fungi in Minkong reserve forest with 94 and 96 isolates, in tea garden with 71 and 58 isolates, in abandoned jhum land with 90 and 94 isolates respectively while in cultivated jhum land, winter and spring season showed the highest soil fungal isolates with 59 and 47 isolates during the first and second year respectively. Whereas some fungal genera were common to all the sampling season. Fungal genera that occurred constantly in all the sampling seaons were *Aspergillus, Penicillium, Mucor* and *Humicola* in cultivated jhum land, *Aspergillus, Absidia, Arthrographis, Apophysomyces, Chaetomium, Penicillium, Phoma, Mucor* and *Tricoderma* in abandoned jhum land, *Absidia, Aspergillus,Cladosporium, Epicoccum,Geotrichum, Mucor, Nigrospora, Neoscytalidium, Penicillium* and *Tricoderma* in tea garden, *Aspergillus, Chaetomium, Cunninghamella, Geotrichum, Mucor, Penicillium, Scopulariopsis* and *Tricoderma* in Minkong reserve forest.

The dominant fungal genera in cultivated jhum land was *Penicillium* with 10 species followed by *Aspergillus* with 9 species and *Tricoderma* with 4 species. In abandoned jhum land, *Aspergillus* was recorded as the most dominant genera with 10 spcecies followed by *Penicillium* with 9 species and *Tricoderma* with 4 species. In tea garden, the most dominant fungal genera were under *Penicillium* with 11 species followed by *Aspergillus* with 8 species and *Tricoderma* with 6 species. In Minkong reserve forest, *Aspergillus* and *Penicillium* with 11 species each was recorded as the most dominant fungal genera followed by *Tricoderma* with 6 species.

Table 3.13. Variation in soil fungal isolate between sites and seasons during 2016-17

	CJL				AJL				TG				MRF			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Absidia cylindrospora	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+
Absidia glaucas	-	-	+	-	+	-	+	+	-	+	-	+	-	+	+	+
***Absidia* sp1**	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+
***Acremonium* sp1**	-	-	+	-	+	+	-	+	-	+	-	+	-	-	+	+
***Acremonium* sp2**	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+
Alternaria alternata	-	-	-	+	+	-	-	+	+	+	-	+	-	-	+	+
Alternaria sp	-	-	-	-	+	+	+	+	-	-	-	+	-	-	+	+
***Arthtrographis* sp**	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Apophysomyces elegans	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus flavus	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Aspergillus fumigatus	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus heteromorphus	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus ochraceus	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus oculeatus	-	-	+	-	-	-	-	+	-	-	-	-	+	+	+	+
Aspergillus oryzae	+	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+
Aspergillus terreus	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+
Aspergillus versicolor	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+
***Aspergillus* sp1**	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+
***Aspergillus* sp2**	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+
***Athrinium* sp**	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-
***Botrytis* sp**	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-
Chaetomium globosum	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
***Chaetomium* sp1**	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+
***Chaetomium* sp2**	-	-	-	-	+	+	-	+	-	-	-	-	+	+	+	+

***chrysosporium* sp1**	-	-	-	-	+	+	-	+	+	+	-	-	+	+	+	-
***chrysosporium* sp2**	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-
***Cladophialophora* sp**	-	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-
Cladosporium cladosporioides	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Cladosporium oxysporum	+	+	-	-	-	-	+	+	-	+	-	-	+	-	-	+
Cladosporium herbarium	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-
***Cladosporium* sp**	-	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-
***clonostachys* sp**	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
***Colletotrichum* sp**	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	-
Cunninghamella echinulata	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
***Emmonsia* sp**	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	+
***Epicoccum* sp**	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Exophiala	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporium	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-
Fusarium solani	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
***Fusaruim* sp1**	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Fusarium* sp2**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
***Fusaruim* sp3**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Geotrichum candidum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***Geotrichum* sp1**	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-
***Geotrichum* sp2**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
***Gliocladium* sp**	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
Humicola sp	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
***Madurella* sp**	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+
***Microascus* sp**	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	+
Mortierella	-	+	-	-	+	-	+	+	-	+	-	-	+	+	+	-
Mucor circinelloides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucor heimalis	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Mucor plumbeus	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+

Mucor racemosus	-	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+
Mucor sp	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+
***Neoscytalidium* sp**	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Nigrospora osmanthi	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
***Paecomyciles* sp1**	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
***Paecomyciles* sp2**	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-
Penicillium chrysogenum	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Penicillium citrinum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium commune	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
Penicillium harzanium	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+
Penicillium italicum	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+
Penicillium janthinellum	+	-	+	-	-	+	-	+	+	+	+	+	-	-	-	-
Penicillium koningii	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+
Penicillium palitans	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Penicillium waksmani	-	-	+	-	+	+	+	+	-	+	+	-	+	-	+	+
***Penicillium* sp1**	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+
***Penicillium* sp2**	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-
***Penicillium* sp3**	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-
***Periconia* sp1**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
***Periconia* sp2**	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-
***Pestaliotopsis* sp**	+	-	-	-	-	+	-	+	-	-	+	+	+	+	+	-
Phaeoacremonium	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+
Phoma glomerata	+	+	-	-	+	+	+	+	+	-	-	+	-	-	+	-
***Phoma* sp1**	-	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-
***Phoma* sp2**	-	-	-	-	+	+	+	-	-	+	+	+	-	-	+	-
***Phoma* sp 3**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Phomopsis	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

amananthicola																
Rhizopus microspores	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Rhizopus stolonifer	-	-	-	+	+	+	-	-	-	+	+	-	-	+	+	-
***Scedosporium* sp 1**	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+
***Scedosporium* sp 2**	-	+	-	-	+	+	+	-	-	-	-	-	-	-	+	+
***Sclerotiana* sp**	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-
scopulariopsis brumtptii	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Scytalidium dimidiatum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
***Scytalidium* sp**	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
***Taloromyces* sp**	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-
***Trichophyton* sp1**	-	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-
***Trichopyton* sp2**	-	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-
Tricoderma hamatum	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+
Tricoderma harzianum	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Tricoderma longibrachiatum	-	+	-	+	-	+	-	+	+	+	+	+	-	+	+	+
Tricoderma viride	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+
***Tricoderma* sp1**	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+
***Tricoderma* sp2**	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+
***Ulocladium* sp**	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
***Verticillium* sp1**	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
***Verticillium* sp2**	-	-	-	-	+	+	+	-	-	-	+	+	-	+	+	-

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table 3.14. Variation in soil fungal isolate between sites and seasons during 2017-18

	CJL				AJL				TG				MRF			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Absidia cylindrospora	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
Absidia glaucas	+	+	-	-	-	-	-	+	-	+	-	-	+	+	+	+
***Absidia* sp1**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
***Acremonium* sp1**	-	-	+	+	-	-	-	+	-	+	-	-	+	+	+	+
***Acremonium* sp2**	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Alternaria alternata	-	-	+	-	-	+	-	+	+	+	-	-	+	+	+	+
Alternaria sp	-	+	-	-	-	-	-	+	-	-	-	+	+	+	-	+
***Arthtrographis* sp**	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Apophysomyces elegans	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus heteromorphus	-	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus ochraceus	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Aspergillus oculeatus	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+
Aspergillus oryzae	-	+	+	+	-	-	-	-	-	+	+	-	+	+	0	+
Aspergillus terreus	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+
Aspergillus versicolor	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+
***Aspergillus* sp1**	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+
***Aspergillus* sp2**	-	-	-	-	-	+	-	-	-	+	+	-	+	-	-	+
***Athrinium* sp**	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-
***Botrytis* sp**	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+
Chaetomium globosum	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-
***Chaetomium* sp1**	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+
***Chaetomium* sp2**	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-

***chrysosporium* sp1**	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-
***chrysosporium* sp2**	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-
***Cladophialophora* sp**	-	-	-	-	+	+	+	+	-	+	-	-	-	+	-	-
Cladosporium cladosporioides	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Cladosporium oxysporum	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Cladosporium herbarium	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
***Cladosporium* sp**	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
***clonostachys* sp**	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
***Colletotrichum* sp**	-	-	-	+	-	-	+	-	-	+	+	+	+	+	+	-
Cunninghamella echinulata	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
***Emmonsia* sp**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
***Epicoccum* sp**	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Exophiala	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporium	+	+	-	-	+	-	+	+	-	+	+	-	+	+	+	+
Fusarium solani	-	-	+	-	+	+	+	+	+	-	-	-	+	+	+	+
***Fusaruim* sp1**	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-
***Fusarium* sp2**	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-
***Fusaruim* sp3**	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+
Geotrichum candidum	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
***Geotrichum* sp1**	-	-	-	-	+	-	+	+	-	-	-	-	+	+	+	+
***Geotrichum* sp2**	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
***Gliocladium* sp**	-	-	-	+	-	+	-	-	-	+	-	-	+	+	+	-
Humicola sp	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
***Madurella* sp**	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+
***Microascus* sp**	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Mortierella	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+
Mucor circinelloides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Mucor heimalis	-	-	-	+	+	+	-	+	+	+	+	+	-	+	-	+
Mucor plumbeus	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
Mucor racemosus	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
Mucor sp	-	-	-	-	+	+	-	+	-	-	-	-	+	+	+	-

***Neoscytalidium* sp**	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Nigrospora osmanthi	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
***Paecomyciles* sp1**	-	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+
***Paecomyciles* sp2**	-	+	-	+	+	+	-	+	+	-	-	+	-	+	-	+
Penicillium chrysogenum	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
Penicillium citrinum	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+
Penicillium commune	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+
Penicillium harzanium	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+
Penicillium italicum	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	+
Penicillium janthinellum	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-
Penicillium koningii	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	+
Penicillium palitans	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+
Penicillium waksmani	-	+	-	-	+	+	+	-	-	-	-	-	-	+	-	+
***Penicillium* sp1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
***Penicillium* sp2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
***Penicillium* sp3**	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+
***Periconia* sp1**	-	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+
***Periconia* sp2**	-	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+
***Pestaliotopsis* sp**	-	-	-	+	+	+	-	-	-	+	-	-	-	-	+	+
Phaeoacremonium	-	-	-	-	-	+	+	+	-	+	+	-	-	-	+	+
Phoma glomerata	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+
***Phoma* sp1**	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	+
***Phoma* sp2**	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+
***Phoma* sp 3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Phomopsis amananthicola	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Rhizopus microspores	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-
Rhizopus	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+

stolonifer																
***Scedosporium* sp 1**	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-
***Scedosporium* sp 2**	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+
***Sclerotiana* sp**	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-
scopulariopsis brumtptii	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Scytalidium dimidiatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
***Scytalidium* sp**	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-
***Taloromyces* sp**	-	-	-	+	+	+	-	-	+	-	-	+	-	-	+	-
***Trichophyton* sp1**	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	-
***Trichopyton* sp2**	-	-	-	-	+	+	+	-	-	-	+	-	-	-	+	-
Tricoderma hamatum	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+
Tricoderma harzianum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tricoderma longibrachiatum	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	+
Tricoderma viride	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
***Tricoderma* sp1**	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
***Tricoderma* sp2**	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-
***Ulocladium* sp**	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-
***Verticillium* sp1**	+	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+
***Verticillium* sp2**	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

3.4. Discussions

In the present study, variation in bacterial counts ($x10^7$ CFU g^{-1}) was observed between seasons in all the study sites. One-way ANOVA analysis (Table 3.1) showed significant variation ($p<0.05$) of bacterial counts with seasonal fluctuations in all the four study sites in both first and second year. The higher bacterial counts during the spring and summer season indicate that the condition in spring and summer season was more suitable for the multiplication and activity of the soil bacteria. Correlation analysis (Table 3.7) showed positive significant correlation ($p<0.01$ and $p<0.05$) of bacterial counts with the soil moisture and temperature during the study period. Lower soil bacterial counts during winter and autumn season shows that the low soil moisture and temperature affected the fungal count as winter and autumn season recorded the lower soil *p*H, moisture and temperature during the present study. Chandini (2020) and Madegwa and Uchida (2021) also found similar results and reported that soil moisture and temperature was one of the important factors that influenced the soil bacterial population in their study. Therefore, an optimum soil moisture and temperature is of paramount importance for the multiplication and activity of the soil microbes.

Variations in bacterial counts between the study sites was observed through two-way ANOVA analysis (Table 3.2) which was found to significantly vary ($p<0.05$). Soil bacterial population varied between the study sites. Shannon diversity index (H') and Simpson's index of diversity (1-D) was higher in Minkong reserve forest (H'=2.29 and 1-D=0.92; H'=2.46 and 1-D=0.94) followed by abandoned jhum land (H'=1.69 and 1-D=0.86; H'=2.09 and 1-D=0.91), tea garden (H'=1.64 and 1-

D=0.88; H'=1.64 and 1-D=0.85) and cultivated jhum land (H'=1.61 and 1-D=0.83; H'=1.28 and 1-D=0.70) both the first and second year indicating that the bacterial diversity, evenness and richness was higher in Minkong reserve forest in comparision to the other study sites (Table 3.3). Soil bacterial counts were higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land (Figure 3.1). The results of correlation analysis (Table 3.7) revealed significant positive correlation ($p<0.01$ and $p<0.05$) of the soil bacterial counts with SOC, available N, available P, available K and significant negative correlation ($p<0.01$ and $p<0.05$) with sand throughout the study period, while the correaltion of silt and clay did not show a constant linear relationship with soil bacterial population. For instance, silt and clay showed significant negative ($p<0.01$ and $p<0.05$) correlation with bacterial population in abandoned jhum land, while it showed positive significant correlation ($p<0.01$ and $p<0.05$) between silt and clay with bacterial population in the other study sites. The correlation significance level varied between the study site, however these results indicated that the variation in soil bacterial counts in Minkong reserve forest, abandoned jhum land, tea garden and cultivated jhum land (Figure 3.1) were influenced by the soil physicochemical properties (Table 3.7).

The soil bacterial population was higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land. The possible reason for a higher bacterial population in Minkong reserve forest and abandoned jhum land than the other study sites may be due the accumulation of higher organic matter with minimal antropogenic disturbances and soil bacterial population actively contributes

in mineralization of the organic matter content. Which increases the microbial activity and multiplication of the microbial population that enhances the SOC and available N content in the soil. Therefore, a strong significant positive correlation ($p<0.01$ and $p<0.05$) of soil bacterial population with SOC and available N was observed in the present study (Table 3.7). Kavitha et al. (2020) in their study in forest soil microbial diversity and Temjen et al. (2021) in their study in a fallow regeneration site, also reported that the soil microbial population increases with an increase in organic matter content. Sharma et al. (2015) also found similar findings, that the abundance of the microbial population is influenced by the amount of the soil organic matter present which is required in the breakdown and decomposition of the organic matter. Whereas, in tea garden and cultivated jhum, the high degree of human intervention through agricultural activities such as tillage, regular weed removal, crop harvesting, and slashing and burning in jhum cultivation might have resulted in lower soil bacterial population. Such agricultural activities are associated with lower organic matter accumulation, land degradation, nutrient depletion and loss of vegetation cover that affects the soil microbial communities.

There was a strong significant positive correlation ($p<0.01$ and $p<0.05$) of soil bacterial population with SOC and available K in the present study which shows that the amount of the SOC and available K were important factors that influenced the bacterial population in the study site (Table 3.7).The pesent result concurs with the results reported by several other workers who also have observed that agricultural activities leads to a decrease in the soil organic matter content, thereby resulting in a reduced soil microbial population (Hosen et al., 2016; Bossio et al.,

2005; Miah et al, 2014; Kendawang et al., 2004). The higher soil bacterial counts in abandoned jhum land than the cultivated jhum land and tea cultivation also reveals that the length of fallow period is important for regeneration of vegetation cover, soil nutrients and accumulation of organic matter which leads to an increase in the microbial population. Similar results has been reported by several workers where jhum fallow length plays important role in the regeneration of soil fertility and increased microbial activity (Majumder et al., 2010; Temjen et al., 2021; Arunachalam and Pandey, 2003). Therefore, the result from the study shows that the variation in soil bacterial counts observed between the study sites were positively influenced by the temperature, moisture, SOC, available N, available P and available K in the present study.

The variations in soil bacterial species were observed between and within the study sites (Table 3.11). Some bacterial species were season specific while some species were restricted to particular study sites. The bacterial isolate under the genera *Bacillus* showed the highest isolates in all the study sites (Table 3.12) and Relative abundance(%) was recorded highest under *Bacillus* sp. and *Bacillus subtilis* in all the study site (Table3.5). The dominancy of the study sites by the *Bacillus* genera maybe because of its fungal-inhabiting ability, greater adaptability to environmental fluctuations. Renla and T, (2017) has also reported dominancy of tea garden soil by *Bacillus* genera under Mokokchung district, Nagaland. Panda, et al. (2017) also found *Bacillus* as the most dominating bacterial genra under tea garden soil in India. The dominance of the cultivated jhum land by *Bacillus* genera after use of fire for buring slashed vegetation shows the heat tolerant ability of the *Bacillus*

genera. Only the heat resistant bacterial species remain after the heat-intolerant ones are eliminateand (Pandey et al., 2011) which might have lead to the multiplication and rapid increase of *Bacillus* species in the cultivated jhum land. This result is supported by other workers, where they have also isolated and identified *Bacillus* bacterial species as the most dominant bacteria genera in their study in jhum land (Miah et al., 2014).

In the current research work, morphological, biochemical and and molecular method were used for the characterization of the soil bacteria. The study on the colonies morphology of the bacterial isolates with biochemical test aided in the determination of the bacterial strain. Gram-positive, gram-negative, rod and cocci-shaped bacteria were observed out of which bacterial isolates belonging to gram-positive and rod-shaped bacteria were higher in the present study. Furthermore, the molecular characterization of the bacterial isolates by 16S *r*RNA and BLAST analysis was done to identify the bacterial species. I6S rRNA is often employed for genotypic analysis since it is highly conserved in the evolutionary process of bacteria and other microorganisms (Clarridge, 2004). It aids in the identification of bacteria at the species level and helps distinguish between closely related bacterial species (Jay and Inskeep, 2015). In the present study out of the 133 bacterial isolates, 38 isolates were found to show <98% sequence similarity and 103 isolates showed 98-100% sequence similarity (Table 3.11). Futhermore, phylogentic trees (Figure 3.8) were established by using the sequences of the bacterial isolates in the present study to provide a visual representation of the genetic distance relationship between different bacterial genera isolated in the present study. Phylogenetic trees are

hypotheses on the evolutionary relationships between species. Closely related species have fewer sequence differences, whereas less related species have more. Gene or protein sequences may be compared between species and used to build phylogenetic trees (Baum, 2008). The phylogenetic tree revealed that the genera belonging to *Bacillus*, *Lysinibacillus*, *Microbacterium* was more closely related than with the other genra belonging to *Chryseobacterium*, *Acinetobacter* and *Ralstonia*, and genera under *Chryseobacterium* and *Acinetobacter* showed close relationship with each other from which it can be concluded that these two genera shares more common ancestry and genera under *Bacillus*, *Lysinibacillus*, *Microbacterium* shares more common ancestry while genera *Ralstonia* showed the most distanct relationship from the other bacterial genera in the present study.

Only few bacterial species were isolated on the agar plates. Among the isolated genera of bacteria, *Bacillus* was found to occur in all the season at all the four study sites during the study period which shows that the *Bacillus* genera was not much affected by the seasonal fluctuations and land use. The occurrence of similar common bacterial genera such as *Bacillus* has also been reported by other workers from different parts of the world in forest (Kavitha et al., 2020; Muñoz-Arenas, 2020), jhum land (Pandey et al., 2011; Sharma et al., 2015; Banerjee et al., 2017; Hosen et al., 2016) and tea cultivated land (Gomez et al., 2006; Banakar et al., 2012; Jamir and Ajungla, 2018; Renla and T, 2017). Whereas some bacterial species under the genera *Acinetobacter, Lysinibacillus, Chryseobacterium, Ralstonia picketti* and *Microbacterium* were confined to a particular season and site expressing high sensitivity to seasonal changes and different land use that can result from the

interaction of plant and microbes, agricultuaral systems, and environmental factors which exerts a selective pressure on the soil microbial community (Pandey et al., 1997; Temsurenla, 2021).

In the present study, variation in fungal counts ($x10^6$ CFU g^{-1}) was observed between seasons in all the study sites (Figure 3.3-3.14). One-way ANOVA analysis (Table 3.1) showed significant variation ($p<0.05$) of fungal counts with seasonal fluctuations in all the four study sites in both first and second year. The higher fungal counts during the spring season and summer indicate that soil moisture and temperature were one of the main factors among the soil parameters that affected the fungal counts in the present study. The correlation analysis (Table 3.8) showed positive significant correlation ($p<0.01$ and $p<0.05$) of fungal counts with soil *p*H, moisture and temperature during the present study. Lower soil fungal counts during winter and autumn season showed that the low soil moisture and temperature affected the fungal count. The present result concurs with several workers under different land use all over the world who have also also recorded higher soil fungal population in summer and spring season due to the favourable conditions and higher soil available nutrients during these seasons (Kaushal and Singh, 2013; Bhattacharyya and Jha, 2010; Díaz-Raviña et al., 1995). The affect on the soil microbial population by the variation in soil moisture and temperature between different study sites has also been reported by Gray et al., (2011).

Variations in fungal counts in the study sites was observed through two-way ANOVA analysis (Table 3.2) which was found to significantly vary ($p<0.05$). Soil fungal population varied between the study sites. Shannon diversity index (H') and

Simpson's index of diversity (1-D) was higher in Minkong reserve forest (H'=4.34and 1-D=0.97; H'=4.41 and 1-D=0.97) followed by abandoned jhum land (H'=4.14 and 1-D=0.94; H'=4.20 and 1-D=0.95), tea garden (H'=4.02 and 1-D=0.91; H'=4.03 and 1-D=0.90) and cultivated jhum land (H'=3.93 and 1-D=0.90; H'=3.79 and 1-D=0.54) in both first and second year repectively (Table 3.6) indicating higher fungal diversity, richness and evenein the Minkong reserve forest as compared with the other study sites. The results of correlation analysis revealed significant correlation of the soil fungal counts with one or more soil properties during the study period indicating that the soil fungal population in the study sites were influenced by the variation in soil properties (Table 3.8).

The higher soil fungal population in Minkong reserve forest may be attributed to the higher soil organic matter, moisture and available nutrients than the other study sites which supported the microbial activity and multiplication. The present finding is supported by studies of several other workers in forest ecosystem (Chandini, 2020; Lyngdoh and Karmakar, 2018). Correaltion analysis also showed positive significant relationship (p<0.01 and p<0.05) of soil fungal population with SOC and available N, indicating the importance of the number and type of soil fungal population in decomposition of organic matter which lead to an increase in soil avialible nutrients in the present study. The higher soil fungal counts in abandoned jhum land than the cultivated jhum land and tea cultivation showed that a long jhum fallow period is necessary for the organic matter deposition on the surface of the soil that will increase the microbial population and soil nutrients depleted during cropping period. Similar results have also been reported by workers in

shifting fallow land under Mokokchung district (Temjen et al., 2020). The lower soil fungal population in tea garden and cultivated jhum land is due to the agricultural practice such as tillage, use of herbicides, regular removal of weed, crop harvesting, that depletes soil nutrients and increases soil degradation which can have an impact on fungal communities by the disturbances caused in the soil (Frac et al., 2018; Chen et al., 2022). Prolonged or inappropriate use of pesticides chemically contaminates the soil and leads to destruction of soil biodiversity limiting the variety and abundance of soil microbes in tea cultivation. (Désiré et al., 2021). The results from the correction analysis showed positive significant relationship between soil fungal population with SOC and available P in tea garden which is similar with Sharma et al., (2014) who has also reported positive correation between fungal communities and SOC in tea cultivated soil which indicates that the fungal population was affected by the amount of the SOC in the present tea garden. Yu et al., (2022), Kanse et al., (2015) and Sharma et al., (2013) has reported that soil microbe significantly increases with the availability of soil organic phosphorus which helps in converting the organic phosphorus into available form for plants use. The current also showed dominance by phosphate solubilising soil fungi such as *Aspergillus*, *Penicillium*, *Trichoderma* in the present study which might have increased the mineralization of phosphorus in the soil. Elias (2016) reported that soil fungal species such as *Aspergillus*, *Penicillium and Fusarium* significantly increased available P in their study.

Hosen et al. (2016) and Joshi et al. (2019) has reported that use of fire in the jhum land affects the amount of the soil organic matter deposition that affects the

soil microbial communities. During the event of buring slashed vegetation in jhum fields, there is an increase in the soil temperature which can alter the soil microbial community (Kendawang et al., 2004). Several other workers have studied on the effect of fire on soil microbial population in different ecosystems who also found negative impact of fire on the soil microbial diversity (Köster et al., 2021; Singh et al., 2021; Barreiro and Díaz-Raviña, 2021). Fire activity results in rapid decomposition of soil organic matter with little time to incorporate it into the soil, and reduces the SOC and available nutrients content in burnt jhum fields (Saplalrinliana et al., 2016) which has a negative impact on the soil microbial population. As a result, cultivated jhum land recorded the lowest soil fungal population than the other study sites during the present study period. The decrease in SOC and available NPK had direct correlation with decrease soil fungal population.

From the total 101 soil fungal species under 45 genera, maximum number of species belong to the genera *Penicillium* with 12 species followed by *Aspergillus* with 11 species, *Tricoderma* with 6 species in the study sites. There were also some fungal genera which were found to occur only in specific season and study site. Relative abundance (%) was recorded highest under *Aspergillus* and *Penicillium* followed by Tricoderma in all the study sites (Table 3.6). The dominancy of the fungal species by the genera *Penicillium, Aspergillus* and *Tricoderma* may be because of their antagonistic nature to other fungal genera and also due to their greater ability to adapt better to seasonal fluctuations and variations in agricultural practices and high spore forming abilities (Pandey et al., 2001). Supporting the present study, several other workers has also reported dominancy by *Aspergillus*,

Penicillium and *Tricoderma* under Mokokchung district, Nagaland in tea garden of Ungma and Tuli village (Renla and T, 2017) and in shifting cultivation and fallow land of Longsa village (Temjen et al., 2021). Whereas, some fungal genera showed selectivity over a particular season and site that reveals the specificity of the bacterial species towards particular season, geographical location and vegetation type in the different study sites (Mishra and Sharma, 1977; Karaoglu and Ulker, 2006).

The fungal isolates were enumerated through colony count method by using different culture media (MEA, CDA, RBA and PDA) and identified by studying the culture colony characters, fungal morphological and microscopical characteristics which is a commonly employed approach (Jamir and Ajungla, 2018) and photography with decriptions of the soil fungal isolates is important for the identification of the soil fungi (Senanayake et al., 2020). Different study sites were dominated by diverse soil fungal group where the dominating soil fungal species according to relative abundance analysis (Table 3.6) in both the study year were *Penicillium chrysogenum, Penicillium citrinum, Tricoderma harzianum, Aspergillus niger, Aspergillus flavus,* and *Aspergillus fumigatus* in Minkong reserve forest, *Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Penicillium chrysogenum, Tricoderma harzianum* and *Tricoderma viride* in abandoned jhum land, *Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Penicillium janthinellum, Penicillium citrinum, Penicillium italicum, Tricoderma longibrachiatum, Tricoderma viride, Tricoderma harzianum* in tea garden and *Penicillium chrysogenum, Aspergillus niger, Aspergillus flavus, Aspergillus*

fumigates, Penicillium citrinum, and Tricoderma harzianum in cultivated jhum land. There are several reports from India and other parts of the world where fungal genera under *Aspergillus, Penicillium, Tricoderma, Mucor, Cladosporium, Fusarium* commonly occurred in abuandance in forest (Behera et al., 1991; Pal et al., 2006; Bhaghat and Pan, 2010; Lopez-Bucio et al., 2015; Aziz and Zainol, 2018; Chandini, 2020; Delgado, et al., 2021, Jufri et al., 2021), jhum land (Majunder et al., 2010; Sharma et al., 2015; Jian et al., 2016; Hosen et al., 2016; Banerjee et al., 2017; Temjen et al., 2021) and tea cultivated land (Jamir and Ajungla, 2018; Renla and T, 2017; Zhang et al., 2020; Jamir et al., 2022a).

There are other factors that influence soil microbial activity such as soil texture (Hamarashid et al., 2010) and soil *p*H (Ali et al., 2021). In the present study, soil microbial population was observed to be influenced by varying composition of soil texture (sand, silt and clay) and soil *p*H. The sand content was found to show negative significant correlation ($p<0.01$ and $p<0.05$) with the microbial population while silt and clay showed both negative and positive significant correlation ($p<0.01$ and $p<0.05$) with the microbial population. This results show that the soil with higher clay and silt content was preferred more by the microbial population as clay and silt sized particles have greater capacity to maintain more soil organic matter and nitrogen than in sand particles in the soil. The present results are comparable with results from other workers (Hemkemeyer, 2018; Seaton et al., 2020, Melaini, 2012). The soil *p*H showed significant positive correlation with the fungal population only in abandoned jhum land however the correlation was found to be positive between the soil *p*H and fungal population in the other study sites

throughout the study period. There are reports from other workers such Goswami et al. (2017) in tea garden of Assam found significant positive correlation of soil microbial population with soil *p*H wheras there are contasrting reports where workers such as Sharma et al. (2014) who found a negative significant correlation between soil fungal population and soil *p*H. Rousk et al. (2010) reported that the abundance and occurrence of soil fungal and bacterial population changed with the varying soil *p*H level. Therfore, soil *p*H is also an important factor that regulates microbial communities for thriving in the soil (Matthies et al., 1997) and the influence of the soil *p*H on the soil soil microbial communities depends upon several factors such as the study location, type of vegetation and soil type (Pietri et al., 2008)

3.5. Conclusion

In the present study, the seasonal effect on the soil microbial population showed significant variation between the study sites with spring season recording higher fungal CFU in cultivated jhum land, tea garden and Minkong reserve forest and summer in abandoned jhum land and lower fungal CFU during winter season in all the four study sites except for the first year in cultivated jhum land. Both fungal and bacterial CFU was higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land which shows that agricultural activities and vegetation type had an impact on the number and type of the soil microbes. Shannon-Weiner diversity index (H') and Simpon's index of diversity (1-D) with higher values was found in Minkong reserve forest indicating that higher diversity, evenness, and richness of soil microbes followed by abandoned jhum land,

tea garden and cultivated jhum land. The significance level of correlation between soil fungal CFU and bacterial CFU varied significantly between the study sites however both fungal and bacterial CFU showed positive significant correlation ($p<0.01$ and $p<0.05$) with available N, available P, available K, SOC, moisture, *p*H and a negative significant correlation ($p<0.01$ and $p<0.05$) with sand throughout the study period.The presented result shows that the fungal and bacterial population was significantly ($p<0.01$ and $p<0.05$) influenced by SOC, available nutrients, moisture, *p*H, silt and clay. The results suggest that minimial antropogenic disturbances, no tillage and long fallow period enhanced the soil properties and both the fungal and bacterial communities in Minkong reserve forest and abandoned jhum land. Whereas, monoculture like tea cultivation, overuse or improper application of chemicals like herbicides and pesticides can result in soil biodiversity loss, tillage and removal of vegetation cover reduce the soil fertility and SOC that leads to a decrease in the fungal and bacterial communities in tea garden and cultivated jhum land. This study provides an understanding of how land use type with seasonal fluctuations influence soil properties and diversity of soil fungal and bacterial population, and the relationship beween soil SOC, available N, available P, available K, *p*H, silt, clay and moisture with the soil fungal and bacterial population. Therefore, an interaction of seasonal fluctuation and different land use type together had an impact on the distribution and abundance of the microbial population. There is need for a wider study to increase our understanding on the effect of the different land use type on soil microbial community in species level in Mokokchung district, Nagaland.

Common fungal and bacterial genera were recorded from all the four study sites, out of which *Aspergillus*, *Tricoderma* and *Peniliium* were dominant fungal genera while *Bacillus* was found as the dominant bacterial genera among the microbial population which showed higher relative abundance percentage during the study period which can be attributed to their antagonistic nature, greater survival ability, higher resistance to extreme environmental changes, and better spore forming features that lead to the abundance of bacterial and fungal species under these genera. This study provides baseline information on the combined effects of the seasonal variation and land use on the distribution and abundance of fungal and microbial population. The recent finding gives a glimpse into the diverse native fungal and bacterial species including a possibly new or previously unrecorded strain from Mokokchung district Nagaland. The current finding shows that Mokokchung district can be considered as a valuable source of fungal and bacterial species with antagonistic and phosphate solubilising potentiality. Species of *Bacillus*, *Aspergillus*, *Penicillium*, *Tricoderma, Gliocladium*, has been reported as biocontrol agent and antifungal agent against plant disease for a sustainable agriculture (Agarwal et al. 2011; Hosen and Shamsi, 2019; Adebole and Amadi, 2010; Tariq et al., 2020) and as biofertilzer for economic crop-like tea (Kalayu, 2019; Swer et al., 2011). Therefore exploration of beneficial microflora is necessary to understand and determine their ability of these indigenous soil microbes as plant growth promoter, biocontrol and antimicrobial agent and for maintaining soil health. Furthermore, the local stakeholders should be made aware of the potential and rich biodiversity in the region and more studies are required to bring out the best agricultural management practice for sustainable use of the microbial ecosystem.

CHAPTER 4

Study on soil

Enzyme activity

4.1 Introduction

In the soil ecosystem, the enzymatic activities of the soil are crucial for both the mineralization and transformation of organic materials and the cycling of nutrients (Kumar et al., 2011). There are enzymes that solely assist in the breakdown of organic materials like hydrolase and glucosidase, while others are involved in the mineralization of nutrients like amidase, urease, phosphatase, sulphates (Dick, 1994; Ferreira et al., 2016). One of the most essential growth-restricting nutrients is phosphorus, and its availability to plants is restricted and its usage efficiency is only 15%–20%, despite its ample content in the soil (Dotaniya et al., 2019). Phosphorus is taken up in the form of orthophosphates, and its propensity to change into insoluble forms has a substantial influence on its availability (Šarapatka, 2003). Phosphatases are extracellular enzymes that catalyze the hydrolysis of phospho-ester linkages in substrates that are organically phosphorus-containing, releasing inorganic phosphorus in the form of orthophosphates that may be used by soil biota and plants (Margalef et al., 2017). Phosphatases are inductive adaptive enzyme, and therefore the concentration of soil phosphatase is controlled by the number of soil orthophosphates that are readily accessible and its activity is decreased when the amount of orthophosphates in the soil is increased (Nannipieri, 1994). To break the phospho-ester bonds in the organic matter, organic P is an important source to acquire enzymes (Dotaniya, 2012). Phosphatases are divided into several categories based on the number of bonds broken down to release the orthophosphate and depending upon the ideal *p*H (Šarapatka, 2003). Microbial activity can dissolve insoluble phosphorus in

their immediate surroundings by producing extracellular enzyme (phosphatase) and increases its availability which can be utilised readily by the plants and are therefore, essential for dictating the phosphorus availability in the soil (Pankhurst et al., 1995). The soil phosphatase activity are closely connected to a number of soil characteristics, such as soil temperature, moisture content, nutritional status, organic matter concentration, and soil *p*H (Neal, 1990; Tabatabai, 1994). Deforestation and agricultural intensification have resulted in considerable environmental changes, soil fertility-related quality and biological disturbances (Salam et al, 1998) and soil enzyme activity is considered as one of the crucial soil characteristics that determines soil health and is thought to be significantly impacted by forest conversion for different land use and agricultural activities (Liu et al., 2018).

Therefore, the present work was undertaken to study the variation in soil phosphatase activity between agricultural and forest ecosystem and the current study highlights the influence of seasonal fluctuation, variation in soil physicochemical properties and soil microbial activity on the soil phosphate enzyme in different land-use. The study sites selected for the present work were cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest under Mokokchung district, Nagaland as there is scanty information on soil enzyme activity under these study areas in the district.

4.2 Materials and methods

Estimation of the soil phosphatase

Estimation of phosphatase activity was done colorimetrically following the method given by Tabatabai and Bremner (1969). 1 g of air-dried soil (2 mm sieved) was taken in 50 ml conical flasks and 2.5 ml of Toluene, 4 ml of MUB (*p*H 6.5) and 1 ml of *p*-nitrophenyl phosphate solution was added, mixed by swirling for few seconds and incubated at 37°C for 1 hour. After incubating for 1 hour, 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH were added. After this the contents were mixed by swirling the flask was swirled for few seconds and the soil suspension was filtered with Whatman no. 1 filter paper. The intensity of the yellow colour filtrate was measured spectrophotometrically at 420 nm.

A standard curve was made by diluting 1 ml of standard *p*-nitrophenol solution upto a final volume of 100 ml. The *p*-nitrophenol contents of filtrate was estimated by reference to calibration graph plotted with standard containing 0, 10, 20, 30, 40 and 50 µg of *p*-nitrophenol. Then 0, 1, 2, 3, 4 and 5 ml of aliquots of diluted standard solution with pipette out and the final volume was made to 5 ml with sterilized distilled water. To each of the standard solution, 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH was added and filtered with the help of Whatman No. 1 filter paper. The absorbance of yellow coloured filtrate was measured at 420 nm. The phosphatase activity was expressed as µg PNP g^{-1} soil h^{-1}.

For calculation, a standard graph was plotted and the amount of phosphatase content in each of the sample was noted down from the graph. The following formula was used for calculation:

$$\text{Phosphatase activity } \mu g \text{ PNP } g^{-1} \text{ soil } h^{-1} = \frac{\text{Amount of p-nitrophenol from standard curve } (\mu g)}{\text{Time of incubation } (h) \times \text{dryweight of soil } (\mu g)}$$

Reagents required

The reagents required for the soil phosphatase assay is as given below:-

Reagents	Procedure
Modified universal buffer (MUB) stock solution	For 1000ml, 12.1 gm of Tris (hydroxymethyl) amino methane (THAM) 11.6 gm of maleic acid, 14.0 gm of citric acid and 6.3 gm of boric acid were dissolved in 488 ml of 1 N sodium hydroxide (NaOH) solution and diluted to one litre with sterilized distilled water and stored in refrigerator
Modified universal buffer (MUB) (pH 6.5)	For 1000ml, 200 ml of MUB stock solution was taken in 1000 ml conical flask and pH was adjusted to 6.5 using by 0.1 N HCL and the final volume was brought to 1 litre with sterilized distilled water
P-Nitrophenyl phosphate solution (0.025 M)	For 50ml, 420 mg of Disodium *p*-nitrophenyl phosphate tetrahydrate was dissolved in about 40 ml of modified universal buffer (*p*H 6.5) and it was further diluted up to 50 ml with MUB of same *p*H and stored in refrigerator
Calcium chloride solution (0.5 M)	For 1000 ml, 73.5 gm of CaCl2. H2O was made to dissolved in about 500 ml of sterilized distilled water and the final volume

	was brought to 1 litre with sterilized distilled water
Sodium hydroxide solution (0.5 M)	For 1000ml, 20 gm of NaOH was dissolved in about 500 ml of sterilized distilled water and the volume was made to 1 litre with sterilized distilled water
Standard *p*-Nitrophenol solution (1000 µg ml^{-1})	For 1000ml, 1 gm of *p*-nitrophenol (PNP) was dissolved in 100ml of sterilized distilled water and the final volume was made upto 1000 ml and stored in refrigerator

4.3 Statistical analysis

Significant differences in soil chemical properties and enzymatic activities between types of land use were evaluated using a one-way ANOVA at 5%. These statistical analyses were performed using the software SPSS 16.0 for test of significance. The significant seasonal variation for soil phosphatise enzyme was done by one-way ANOVA and if the results were significant Tukey's *Post hoc* test was carried out. Two-way ANOVA analysis was done to determine the effect of the season and study sites on the soil phosphatise enzyme. The significant difference was based with a probability of $p< 0.05$ and $p< 0.01$. Pearson's correlation was done to determine the relationship between the soil phosphatise enzyme, physicochemical parameters, fungal and bacterial population. The significant difference was based with a probability of $p< 0.05$ and $p< 0.01$. All the statistical analysis was done by using the software SPSS 16.0 for test of significance.

4.4 Results

The mean value of the soil phosphatase activity recorded in different sampling season in cultivated jhum land at Khensa village, abandoned jhum land at Mekuli village, in tea garden at Longmisa village and in Minkong reserve forest at Chuchuyimpang village during 2016-2017 is shown in figure 4.1 and during 2017-2018 is shown in figure 4.2.

In cultivated jhum land, the soil phosphatase activity ranged from 30.35-43.96 µg PNP g^{-1} soil h^{-1}in the first year and from 30.68-39.73 µg PNP g^{-1} soil h^{-1} during second year. In the first year, the soil phosphatase activity was found higher during the summer season (43.96±0.02) followed by autumn season (33.77±0.10), winter season (31.70±0.27) and spring season (30.35±0.10) while in the second year, the soil phosphatase activity was found higher during the summer season (39.73±0.10) followed by autumn season (35.39±0.02), spring season (39.73±0.10) and winter season (30.68±0.05).

In abandoned jhum land, the phosphatase activity ranged from 40.53-50.84 µg PNP g^{-1} soil h^{-1}in the first year and from 55.04-57.12 µg PNP g^{-1} soil h^{-1} during second year. The soil phosphatase was recorded during summer season (50.84±0.13; 57.12±0.02) and lowest during winter (40.53±0.10; 55.04±0.02) season in both the study year. In both the first year and second year, the soil phosphatase activity was found higher during the summer season (50.84±0.13; 57.12±0.02) followed by autumn season (45.49±0.07; 56.39±0.02), spring season (45.39±0.04; 56.15±0.02) and winter season (40.53±0.10; 55.04±0.02).

In tea garden, the phosphatase activity ranged from 35.79-48.01 µg PNP g^{-1} soil h^{-1}in the first year and from 38.20-48.82 µg PNP g^{-1} soil h^{-1}during second year. In the first year, the soil phosphatase activity was found higher during the winter season (48.01±0.01) followed by spring season (44.72±0.02), summer season (42.27±0.07) and autumn season (35.79±0.06) while in the second year, the soil phosphatase activity was found higher during the summer season (48.82±0.02) followed by winter season (48.64±0.05), spring season (43.41±0.01) and autumn season (38.20±0.30).

In Minkong reserve forest, the phosphatase activity ranged from 43.68-61.77 µg PNP g^{-1} soil h^{-1} in the first year and from 55.73-64.06 µg PNP g^{-1} soil h^{-1} during second year. In both the first year and second year, the soil phosphatase activity was found higher during the summer season (61.77±0.02; 64.06±0.02) followed by spring season (61.56±0.03; 61.07±0.04), autumn season (55.59±0.08; 58.46±0.09) and winter season (43.68±0.01; 55.73±0.01).

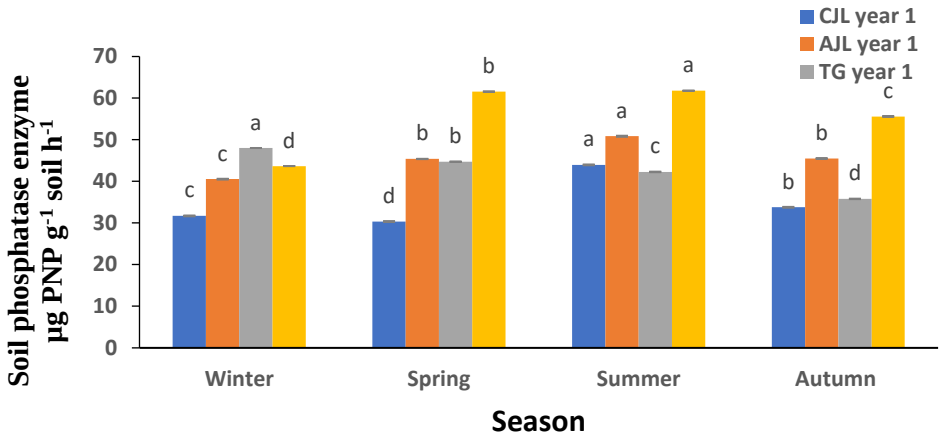

Figure 4.1. Comparision of soil phosphatase enzyme among different sampling seasons during 2016-2017 between cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean. Different letters [abcd] in each study site indicates the significant differences at $p<0.05$ as measured by Tukey's *post hoc* test.

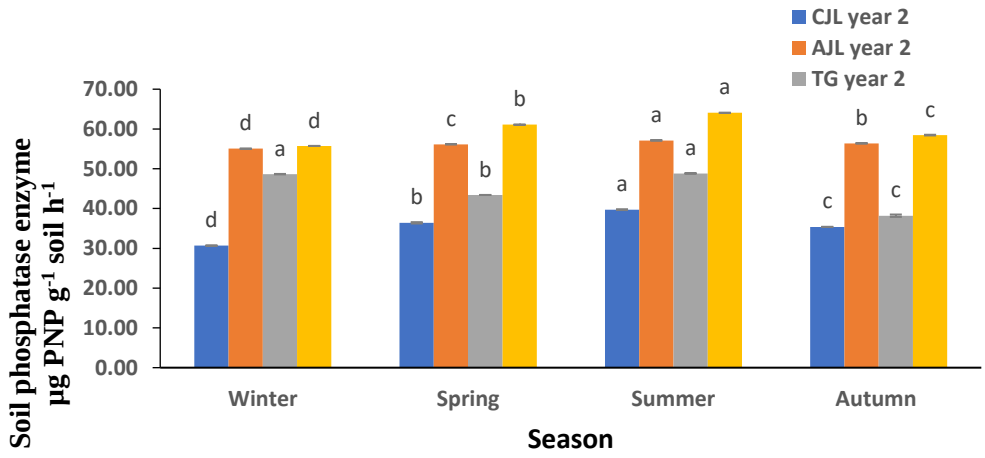

Figure 4.2. Comparision of soil phosphatase enzyme among different sampling seasons during 2017-2018 between cultivated jhum land (CJL), abandoned jhum land (AJL), tea garden (TG) and Minkong reserve forest (MRF). Error bar represents the ±standard deviation mean. Different letters [abcd] in each study site indicates the significant differences at $p<0.05$ as measured by Tukey's *post hoc* test.

Table 4.1: One-way ANOVA of soil physicochemical parameters of CJL, AJL, TG and MRF during 2016-2018

Parameters	CJL				AJL				TG				MRF			
	2016-17		2017-18		2016-17		2017-18		2016-17		2017-18		2016-17		2017-18	
	F(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)	*F*(3,8)	*p*(value)
Soil Phosphatase	5.11	.001	3.98	.001	7.03	.001	7.91	.001	4.13	.001	3.33	.001	1.19	.001	1.59	.001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

Table 4.2: Two-way ANOVA of soil physicochemical parameters of CJL, AJL, TG and MRF during 2016-2018

Parameters	Source	2016-17		2017-18	
		F(3,8)	*p*(value)	*F*(3,8)	*p*(value)
Soil Phosphatase	Site	2.08	.001	7.78	.001
	Season	1.04	.001	1.63	.001
	SitesxSeason	1.14	.001	3.26	.001

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest

The correlation coefficient between the soil phosphatase, physicochemical parameters, fungal population and bacterial population of cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest from 2016-2018 are presented below (Table 4.3). The correlation results showed both positive and significant relationship between the soil phosphatase, physicochemical parameters, fungal population and bacterial population. The soil phosphatase was found to show a positive correlation with soil moisture and SOC and a negative correlation with available phosphorus in all the four study sites throughout the study period while the relationship of soil phosphatase with other soil properties, fungal and bacterial population were found to vary between the study sites.

In cultivated jhum land, during the first year, correlation results showed positive significant correlation of soil phosphatase with soil moisture (r=.99**, *p*=.001), temperature (r=.70*, *p*=.011), sand (r=.60*, *p*=.044), fungal counts (r=.64*, *p*=.026) and bacterial counts (r =.66*, *p*=.020), while it showed a negative significant correlation with available K (r=.81**, *p*=.-002) and silt (r =.67*, *p*=-.018). In the second year, soil phosphatase showed positive significant correlation with soil *p*H (r=.89*, *p*=.001), moisture (r=.88*, *p*=.001) and temperature (r=.91**, *p*=.001).

In abandoned jhum land, during the first year, correlation results showed positive significant correlation of soil phosphatase with soil *p*H (r=.92**, *p*=.001), moisture (r=.95**, *p*=.001), temperature (r=.91**, *p*=.001), SOC (r=.53*; *p*=.004) fungal counts (r =.95**, *p*=.001) and bacterial counts (r=.92*, *p*=.001). In the second year, soil phosphatase showed positive significant correlation with soil pH (r=.76**, *p*=.004), moisture (r=.95**,

p=.001), temperature (r=.98**, *p*=.001), fungal counts (r=.88**, r=.001) and bacterial counts (r=.92**, *p*=.001) wheras a negative significant correlation existed between silt (r=-.58*, *p*=.048) and clay (r=-.65*, *p*=.001).

In tea garden, during the first year, correlation results showed positive significant correlation of soil phosphatase with clay (r=.88**, *p*=.001) and bacterial counts (r=.61*, *p*=.037) while the correlation was negative and significant with *p*H (r=-.88*, *p*=.001), moisture (r=-.66**, *p*=.019), temperature (r=-.76**, *p*=.004), bacterial counts (r=-.61*, *p*=.037),. In the second year, soil phosphatase showed negative significant correlation with available N (r=-.76*, *p*=.005), available P (r=-.71*, *p*=.010), available K (r=-.87*, *p*=.001) and fungal counts (r=-.59*, =.050),

In Minkong reserve forest, during the first year, correlation results showed positive significant correlation of soil phosphatase with soil *p*H (r=.72**, *p*=.009), moisture (r=.74**, *p*=.006), temperature (r=.94**, *p*=.001), available N (r=.88**, *p*=.001), fungal counts (r=.90**, *p*=.001), and bacterial counts (r=.78**, *p*=.003). In the second year, soil phosphatase showed positive significant correlation with soil pH (r=.67*, *p*=.018), temperature (r =.75**, *p*=.005), available N (r=.58*, *p*=.047), fungal counts (r=.88**, *p*=.001), and bacterial counts (r=.64*, *p*=.026), whereas a negative significant correlation existed between clay (r =-.79*, *p*=.002).

Table 4.3. Correlation between between the soil phosphatase enzyme, physicochemical parameters, fungal population and bacterial population at CJL, AJL, TG and MRF during 2016-18

	CJL		AJL		TG		MRF	
Parameters	**2016-17 Soil Phosphatase**	**2017-18 Soil Phosphatase**	**2016-17 Soil Phosphatase**	**2017-18 Soil Phosphatase**	**2016-17 Soil Phosphatase**	**2017-18 Soil Phosphatase**	**2016-17 Soil Phosphatase**	**2017-18 Soil Phosphatase**
pH	-.06	.89*	.92**	.76*	-.88*	-.44	.72**	.67*
Moist. (%)	.99**	.88*	.95**	.95**	-.66*	-.35	.74**	.55
Temp. (℃)	.70*	.91**	.91**	.98**	-.76**	-.34	.94**	.75**
SOC (%)	-.25	.39	.35	.53*	-.06	.23	.57	.54
AN (Kg/ha)	-.18	-.49	.20	.13	-.40	-.75*	.88**	.58*
AP (Kg/ha)	-.49	-.47	-.22	-.08	-.04	-.71*	-.09	-.39
AK (Kg/ha)	-.81**	-.28	-.56	-.40	-.74*	-.87*	.003	-.41
Sand (%)	.59*	.23	.49	.68*	-.40	.31	.31	.53
Silt (%)	-.67*	-.21	-.45	-.58*	-.21	-.17	-.31	-.52
Clay (%)	-.53	.001	-.52	-.65*	.88**	-.33	-.06	-.79*
Fungal count	.67*	.54	.95**	.88**	.18	.59*	.90**	.88**
Bacterial count	.66*	.50	.92**	.92**	.61*	.30	.78**	.64*

CJL-Cultvated jhum land, AJL-Abandoned jhum land, TG-Tea garden, MRF-Minkong reserve forest
Moist.-moisture, Temp.-temperature, SOC-soil organic carbon, AN-available nitrogen, AP-available phosphorus, AK-available potassium
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4.5 Discussions

The one-way ANOVA analysis (Table 4.1) revealed that the seasonal variation in soil phosphatase activity was significant (p<0.05). From the present result it was observed that among the sampling seasons, summer season recorded the highest soil phosphatase activity (Figure 4.1-4.2). The results from two-way ANOVA analysis estimated a

significant interaction effect of season and site (p<0.05) on the soil phosphatase activity (Table 4.2). Between the different study sites, Minkong reserve forest recorded the highest soil phosphatase activity followed by abandoned jhum land, tea garden and cultivated jhum land. This finding is in line with Dotaniya (2012) where the higher soil phosphatase activity during summer season may be linked to the higher moisture, *p*H, temperature, available nutrients, SOC content, and the enzyme that was produced by soil fungal and bacterial population in the soil, in all the four study sites which are the principle source of the phosphatases. The soil phosphatase showed a positive correlation with SOC, moisture, temperature, bacterial and fungal counts in the current study (Table 4.3). Ajungla et al. (2002) has also reported that the seasonal variation of soil phosphatise activity was influenced by the soil microbial population and organic matter content in their study. Matinizadeh et al. (2008) also found higher soil phosphatase activity during summer season and minimum during autumn season in forest soil where the temperature was reported as the main factor influencing the soil phosphatase activity which is similar with the present finding. Luo et al. (2020) reported that the soil phosphatase activity was higher in spring and lower in winter season, and soil moisture and temperature were the factors influencing the soil phosphatase activity. Shao et al. (2015) reported that the soil enzyme activity may be influenced by seasonal and climatic changes in their study carried out in different vegetation types. Cao et al. (2021) also reported that the soil enzyme activity varied in their study due to seasonal fluctuation and further added that soil microorganisms were an another important factor that influenced the variation in the soil enzyme activity. Sao et al. (2015) and Rao et al. (1990) in their investigation reported that the higher

phosphatase activities may be influenced by the type of vegetation that regulates the availability of P in the soil. Veeraragavan et al. (2018) also found significant correlation between soil enzyme and moisture. Chen et al. (2021) has reported that with a decrease in soil moisture and temperature there was a decrease in the soil enzyme activity. Boerner et al. (2005) observed in their work that soil enzyme activity was influenced by the organic C content. There was no significant correlation between the soil phosphatase activity with available phosphorus but showed a negative relation throughout the study period (Table 4.3). Wang et al. (2011) has reported that the soil phosphate activity decreases with the increase in available P indicating that the amount of P in the soil is an important factor that regulates the activity of the soil phosphatase. Dilly and Nannipieri (2001) has mentioned that the connection between nutritional status and enzyme activity is managed by a negative feedback mechanism and in reaction to the nutrient availability, the soil microbes and plants control the mineralization of soil nutrients. Nannipierri (1994) has reported that the enzymes are stimulated and nutrients are mineralized when the supply of nutrients is insufficient, however, when the supply of nutrients is large, enzymes are subdued and mineralization stops.

The soil phosphatase activity was found to be higher in the Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land. The reason for a higher soil phosphatase activity in the forest soil can be explained by the greater accumulation of the SOC and higher *p*H and moisture which is favoured by the microbial population for its growth and multiplication that lead to an increase in the soil phosphatase activity. This result is supported by findings from several other workers in forest soil who

also found that the higher soil phosphatase activity was related with the higher soil organic matter, and moisture (Ho, 1979; Silva-Olaya et al., 2021; Bueis et al., 2019; Margalef et al., 2017). Agricultural practice was found to have an effect on the soil phosphatase activity as the cultivated jhum land and tea garden recorded lower soil phosphatase activity. Agricultural activities causes disturbance in the soil which leads to a decrease in the organic matter and thereby decreases the soil phosphatase activity (Wang et al., 2011). Gonnety et al. (2012) in their study between different land-use types found that the soil phosphatase activity increased with an increase in soil organic carbon and reported that the different land-use affected the soil phosphatase activity. Huang et al. (2011) in their study on the activity of soil phosphatase in different forest ecosystem reported that the soil phosphatase activity was related with the vegetation type, soil moisture, microbial activity and the requirement for the available P in the forest soil. Cabugao et al. (2017) reported that the bacterial community and tree species regulated the activity of the soil phosphatase in response to the availability of the soil phosphorus. The soil phosphatase activity in abandoned jhum land was higher in than cultivated jhum land and tea garden which indicates that the long fallow period in the present study attributed to higher SOC, increased enzyme activity and enhanced microbial community. This finding concurs with the result from Singh et al. (2017) where the authors in their study between jhum fallow and jhum cultivated land on soil microbial biomass carbon and enzyme activity reported that an increase in the fallow time had a substantial impact on the enzyme activity, and SOC. Sardans et al. (2006) has reported that the soil phosphatase activity was regulated by the nutrient availability, that the microbial population increased with soil organic matter

and moisture which in turn contributed in the increase of the soil phosphatase activity for the mineralization of phosphorus depending upon the demand of available P by the plants. In the cultivated jhum land, the soil phosphatase activity was lowest as compared to the other study sites which can linked to the use of fire in jhum cultivation for burning the slashed vegetation as the soil phosphatase activity reduced after burning activity. The spring season recorded the lowest soil phosphatase activity which was after burning the slashed vegetation however, with the increase in crop cover, the soil organic matter increased due to the addition of the plants and leaf litter that enhanced the soil fungal and bacterial population leading to an increase in soil phosphatase activity. The soil phosphatase, soil fungal, and soil bacterial population were found to be highest during the summer season in the present cultivated jhum site while the soil available P was recorded lowest during the summer season, indicating that the soil phosphatase activity was regulated by the microbial population in response to the amount of the nutrient availability (Table 4.1). Zothansiam and Thakuria (2020) has reported that the disturbances caused by the use of fire in jhum cultivation alter the microbial community and affects the soil enzyme activity. Lungmuana et al. (2017) has also found in their findings that the phosphatise activity in jhum soil was lowered after the use of fire but it moderately increased with time. Deka et al. (2019) has also reported similar finding where the burning activity in jhum fields of Mokokchung district was found to affect the soil microbial community and soil enzyme activity. In tea garden, the inappriopriate application of herbicides can inhibit the enzyme activity in the soil and decrease soil health (Kumari et al. 2020). The soil phospatase activity was also found to be influenced by the availability of

soil nutrients and microbial population along with moisture and temperature in the tea garden. Jiang et al. (2021) has also reported similar results that with increased soil organic matter there is an increased microbial population and enzyme activity in the tea garden soil. There are also several other workers who have reported that the soil enzyme activity is regulated by the microbial community in response to nutrient availability in tea garden (Godishala and Kumari, 2019; Chen et al., 2021; Nath and Samantha, 2012).

4.6 Conclusion

The present finding shows that the activity of soil phosphatase was significantly higher in the Minkong reserve forest with higher soil microbial population than in the other three land-uses, indicating that the agricultural activities disturbed the growth and multiplication of the fungal and bacterial communities which were involved in the production of soil phosphatase enzyme. Between the four study sites, cultivated jhum land recorded the lowest soil phosphatase activity and, in abandoned jhum land the soil phosphatase activity was higher than cultivated jhum land and tea garden where the fallow period played was related with the increase in the soil phosphatase activity. This present result shows that the nutrient status of the Minkong reserve forest was higher followed by the abandoned jhum land, tea garden and cultivated jhum land because the higher the soil acid phosphatase activity would be, the better the soil nutritional condition. In all of the study sites, soil phosphatase activity was dependent on soil organic matter and with higher soil organic matter supporting a greater microbial population for its breakdown. Soil phosphatase activity was significantly influenced by the seasonal fluctuation with higher activity in rainy season than the dry season which correlated with the highest microbial

activity and lowest available P in the present study. The variation in soil nutrient status and vegetation type was also another cause of the differences in soil acid phosphatase activity between the four study sites. The current findings provide some insight on how soil characteristics, enzyme activity, and microbial communities are affected by seasonal changes coupled with different land-uses. Further investigation on the functions of soil ecosystem services for better knowledge of the underlying processes that cause seasonal fluctuations in different land-use type and its effect on microbial community and enzyme activity will be beneficial. Furthermore, studying the proper soil enzyme for a certain agricultural management system should be included in future studies as soil enzyme are sensitive to agricultural management practices and therefore can be used as an effective indicator to monitor soil health and sustainable use of soil under different land-use types.

CHAPTER 5

Summary and conclusion

The present investigation entitled "Studies on Soil Microbial Diversity in Mokokchung district, Nagaland" was carried out during the year 2016-2017 and 2017-2018. Four study sites with different land-use types were chosen for the current study viz: cultivated jhum land, abandoned jhum land, tea garden, and Minkong reserve forest based on the importance of forest cover, jhum and tea cultivation in the study area as jhum cultivation is one of the major agricultural practice and tea cultivation is a substantial cash crop in the study region. The intensive agricultural practices have led to deteriorating soil health and quality and a decline in agricultural productivity in the district. Thus enhancing or sustaining soil fertility should be the foremost objective in the region. The biological component, which drives the transformation and cycling of nutrients, is a key area that is mostly unexplored in the present study area. The major goal of this study was to compare the microbial flora, soil characteristics, and soil enzyme activity throughout the seasons and its correlation between various land uses in the Mokokchung district of Nagaland. The results of this study showed that the seasonal fluctuations and cultivation practice in the area had an impact on the soil health and microbial flora. The current study includes the findings of the influence of seasonal fluctuations on soil physicochemical properties (soil *p*H, texture, moisture, temperature, SOC, nutrients), soil microbial diversity (fungi and bacteria), and soil enzyme activity (soil phosphatase activity) under different land use.

i) Soil physicochemical properties

With respect to sampling seasonal, the variation in soil physicochemical properties were found to be significant ($p<.05$) due to seasonal fluctuations in all the study sites throughout the study period. The soil textural class remained the same as sandy loam to sandy clay loam in cultivated jhum land, Khensa village whereas in abandoned jhum land, Mekuli village and in tea garden, Longmisa village it remained as sandy clay loam, and in Minkong reserve forest, Chuchuyimpang village as loam throughout the study period in each of the respective study sites. Sand content was higher than the silt and clay in all the four study sites. Soil moisture and temperature was recorded to be highest during summer season and lowest during winter season in

all the four study sites throughout the study period. Soil *p*H was highest during the summer season except in tea garden where soil *p*H was highest during autumn season while winter season recorded the lowest soil *p*H in all the four study sites. SOC, available N, available P and available K in all the study sites in both the study year was found during the spring season except for available N in cultivated jhum land in the first year where its highest content was recorded during winter season. The lowest content of these soil properties varied between the study sites. Winter season recorded the lowest SOC content except for cultivated jhum land where it was recorded during the autumn season while available P and available K was recorded lowest during summer season except for tea garden during winter season. Available N was recorded lowest during winter season in abandoned jhum land and Minkong reserve forest, during summer season in tea garden, during autumn and summer in the first and second year respectively in cultivated jhum land. The correlation analysis showed mutual significant correlation among the soil physicochemical properties however the level of significance varied between the four study sites and the study period.

The soil physicochemical properties showed significant ($p<.05$) variation due to the interaction effects of study site with sampling season in both the study year. Between the study sites, the soil properties such as soil moisture, available nutrients, *p*H, silt and clay content were higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land. The soil temperature was highest in tea garden followed by cultivated jhum land, abandoned jhum land and lowest in Mingkong reserve forest. The SOC content was highest in Minkong reserve forest followed by abandoned jhum land throughout the study period however between the cultivated jhum land and tea garden the SOC content showed variation during the two study years. The cultivated jhum land showed higher SOC content than tea garden during the first year but in the second year the SOC content was lower in the cultivated jhum land than the tea garden. The soil available N, available P and available K was very low-medium low, low-medium low and very low to low respectively in cultivated jhum land, low to medium, low-medium, low to medium low respectively in tea garden, medium low-medium, low to medium and very low to low respectively in

abandoned jhum land, medium-high, medium low-medium and low-medium respectively in Minkong reserve forest. Soil *p*H was acidic throughout the study period in all the four study sites. The present finding shows that the different land-use type with seasonal fluctuations affected the soil properties and the essential nutrients.

ii) Soil microbial diversity

The seasonal effect on the soil microbial population showed significant variation between the study sites ($p<0.05$) by one-way ANOVA analysis. Spring season recorded the highest fungal and bacterial CFU in tea garden and Minkong reserve forest, summer season recorded the highest fungal and bacterial CFU in abandoned jhum land in both the study years while cultivated jhum land recorded the highest fungal and bacterial CFU in summer season the first year and during spring season in the second year. The lowest of the fungal and bacterial CFU was recorded during winter season in abandoned jhum land and Minkong reserve forest in both the study year. In cultivated jhum land during the first year, both the fungal and bacterial CFU was found lowest during autumn season wheras in the second year, both the fungal and bacterial CFU was found lowest during winter season. In the tea garden, the fungal and bacterial CFU was recorded lowest during winter and autumn season repectively in both the study years.

The observation on the microbial population between the study sites showed significant variation in fungal and bacterial population due to interaction effect of season and site ($p<0.05$) through two-way ANOVA analysis. Both fungal and bacterial CFU was higher in Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land which showed that different land-use had an impact on the number and type of the soil microbes. The physicochemical properties such as SOC, temperature, moisture, available nutrients, and *p*H may be responsible for the variation of the microbial activities.

Composition of the soil microbial population showed both common and restricted fungal and bacterial species between the four study sites. Fungal genera such as *Aspergillus*, *Penicillium*, and *Tricoderma* were most dominant with higher relative

abundance (%) in all the four study sites whereas *Bacillus* showed dominancy among the bacterial genera with higher relative abundance (%) in all the four study sites which may be due to the antagonistic nature of these microbial genera over other species, greater ability of these microbial genera to utilize the soil substrates more readily than the other microbes. After the use of fire in the cultivated jhum land, the microbial population decreased significantly. The fungal species under *Aspergillus*, *Trichoderma* and *Penicillium*, as well as bacterial species under the genera *Bacillus*, were largely isolated after the fire event which may be because these microbial genera are regarded as early colonizers of burnt soil. Additionally, it was found that the study areas were home to a variety of fungal and bacterial species, some of which may have novel or undiscovered strains. The Minkong reserve forest showed higher diversity, evenness and abundance of soil microbes than the other study sites with a higher Shannon diversity index(H') and Simpson's index of diversity(D), followed by abandoned jhum land, tea garden and cultivated jhum land. The finding from the present study highlights how the soil microflora may be significantly impacted by seasonal variation and different land-use along with the variation in soil physicochemical properties between different study sites.

iii) Soil enzyme activity

The present finding also shows that the seasonality had a significant effect on the soil phosphatase activity ($p<0.05$) by one-way ANOVA and the highest soil phosphatase activity was recorded in the Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land. The variation in the soil phosphatase activity due to interaction effect of season and site were also found to be significant ($p<0.05$) through two-way ANOVA. The higher soil phosphatase activity was found to be influenced by the soil microbial population in response to the availability of soil nutrients. The agricultural activities in the tea garden and cultivated jhum land disturbed the buildup of organic matter resulting in a lower fungal and bacterial community which in turn resulted in lower production of enzyme soil phosphatase. The greater bacterial and fungal population, which was associated with

the extended fallow period that increased soil organic matter, in the abandoned jhum land improved the soil phosphatase activity. Soil phosphatase activity was dependent on the soil organic matter in every study site, and more soil organic matter supported a larger microbial population for its decomposition.

Results indicated that the season and land use influenced the soil physicochemical, microbial population and soil phosphatase activity in the present study. It can be concluded that the soil health was greater in the Minkong reserve forest followed by abandoned jhum land, tea garden and cultivated jhum land during the study period. The microbial abundance, SOC, avavailble nutrients and soil enzyme activity was significantly reduced from forest to cultivated land depending on the sampling season, and the difference in agricultural management. Most of the studied parameters were interrelated which illustrates the presence of various regulating forces in the study region. The abandoned jhum land showed higher microbial diversity and higher nutrient status than the tea garden and cultivated jhum land which indicates that the fallow length significantly played important role in the increase of the microbial communities and ehnhancing the nutrient availability, however, it was still significantly lower as compared with the Minkong reserve forest even after a fallow period of 10 years which shows that jhum cultivation practices such as slashing and burning has a long-term effect on the soil microbial diversity which in turn influences the poor vegetation and nutrient recovery in abandoned jhum land even after a long period of time. The soil microbial diversity and nutrient availability was higher in tea garden than the cultivated jhum land however lower than the Minkong reserve forest and abandoned jhum land which shows that monocultural cultivation for a long time might lead to selectivity of microbial communities and elimination of several other microbes including the beneficial genera which could lead to a decrease in maintaining soil heath. Furthermore, monoculture practice like tea cultivation is associated with soil acidification and use of chemicals such as herbicides should be applied appropriately as it can lead to soil deterioration thus, the soil under such cultivation practice should be properly managed and monitored. Among the four different land use, Minkong reserve forest showed that the soil maintained its microbial diversity and

nutrient status throughout the two years of study period which should be better maintained and protected from further degradation and depletion from antropogenic disturbances. The present findings shows that overall biological quality of the cultivated soils has been significantly declined in the region as compared with the forest soil which is undesirable for a sustainable agro-ecosystem. Therefore, to sustain agricultural production, it is necessary to create integrated soil management techniques that might aid in restoring soil nutrients and so reduce the continued soil deterioration.

The current study establishes a baseline for the native soil microflora and soil physicochemical characteristics in different land use in the Mokokchung area of Nagaland. The results might aid in developing measures of soil degradation processes and enhance soil quality monitoring. Based on the findings of this study, the area may be advised to adopt an appropriate management approach, such as conservation agriculture, increase jhum fallow period with integrated soil management strategy, in order to preserve its sustainability. The present information can be used as baseline information for assessing the soil quality and for the better management of the soil in cultivated jhum land, abandoned jhum land, tea garden and Minkong reserve forest in the Mokokchung district of Nagaland. Further detailed studies are recommended to better understand the effect of agricultural practice on soil physicochemical properties along with the plant response and productivity and nutrient requirement by the plants. Further researches in jhum land with different fallow period, tea garden of different age and other natural forest with varying altitudes in the district should be studied to compare and examine its effect on soil physicochemical properties for a sustainable land management.

Recommendations for future work:

1. Further research is required to determine how the season and type of land use affect the genetic and taxonomic diversity found in the study area. It is necessary to assess and classify soil microbial communities using the culture-independent method.

2. A larger research area is needed to fully represent the diversity of soil microbes and their benefits in agricultural systems as plant growth promoters, biocontrol agents and antimicrobial agents for improving soil fertility. This will encourage and expand organic farming in Mokokchung district of Nagaland.
3. The dynamics of nutrient mineralization and management strategies in the tea cultivation area under Mokokchung district of Nagaland, and the outcome of repeated applications of different fertilizers, insecticides, and herbicides on the yield and quality of tea leaves may be investigated in future studies.
4. More in-depth study is suggested to compare and evaluate how agricultural methods impact the physicochemical characteristics of the soil, plant response, productivity, and nutrient requirements of the plants on the jhum land with different fallow periods, tea gardens of varying ages, and other natural forests with varying elevations is needed for sustainable land management in the district.
5. The potential and richness of the region's biodiversity should be made known to regional stakeholders.
6. Future research should also focus on determining the most appropriate soil enzyme for a particular agricultural management system because soil enzymes are sensitive to agricultural management practices and can be used as an efficient indicator to monitor soil health and sustainable use of soil under various land-use scenarios.

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Paper Publications

Kichu, A., Ajungla, T., Nyenthang, G., Yeptho, L. (2020). Colonial and morphological characteristics of soil fungi from jhum land. *Indian Journal of Agricultural Research,* 54(1),1-9. DOI:10.18805/IJARe.A-5265

Kichu, A., Ajungla, T., Walling, M., Yeptho, L., Temjen, W. (2022). Seasonal variation of soil fungi in tea garden soil of Mokokchung district, Nagaland. *The Journal of Plant Science Research,* 38(1). ISSN: 0970-2539

Kichu, A., Ajungla, T., Thsangla (2021). Morphological Identification of Fungi in Tea Soil at Zunheboto District, Nagaland. Bioresource and sustainable livelihood, 341-37 (Book chapter)

Paper Presentation in Conference/Symposium

1. The National Seminar on Bio-resource **"Exploration and utilization: Application in Modern Biology"** on the topic **"Influence on Carbon Content by Soil Fungi in Jhum Land, Mokokchung District, Nagaland, India"** organized by Bioinformatics Infrastructure Facility (BIF) Centre, Nagaland University, Lumami, sponsored by Department of Biotechnology, Ministry of science and Technology, Government of India, new Delhi, on 9th and 10th October 2018.
2. **10th Annual Interdisciplinary Symposium of Sree Ayyappa College, two-day virtual international conference on energy, environmental and health (VICEEH-2020)** on the topic **"Soil fungal population n Jhum land, Mokokchung district, Nagaland"**

organised by internal quality cell (IQAC) & IETE Students Forum of Sree Ayyappa College, Chengannur, Kerela, India held on 11th-12th September, 2020.

3. National e-Conference **"Bioresources and Sustainable Livelihood of Rural India"** on the topic **"Morphological Identification of Fungi in Tea Soil"** organised by Department of Botany, Nagaland University, Lumami on 28-29th September, 2020.
4. Online International Conference on **"Novel Approaches In Life Sciences"** on the paper titled **"Seasonal variation of soil fungi in tea garden of Mokokchung district, Nagaland"** organized by Department of Botany & IQAC (RUSA sponsored) held on 8-9th April, 2020, at GURU Nanak Khalsa College of Arts, Science & Commerce, Matunga, Mumbai
5. International Conference on **'Bioresources and Bioeconomy'** (ICBB-2022) organized by **Department of Botany, Nagaland University**, Lumami-798627, Nagaland, India in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. of Nagaland, India, on 19-21st September, 2022

Participation in Conference/ Workshop/Training

1. National seminar on **'Advances in Biological Science Research'** organized by **Department of Botany, Nagaland University,** Lumami-798627, Nagaland on 28th February to 1st March, 2017.
2. Hands on Training on **"Functional Genomics"** organized by **Department of Biotechnology, Government of India sponsored Institutional Biotech Hub, Nagaland University, Lumami and Department of Botany, Nagaland University sponsored by Institutional Biotech Hub,** on November 14-21, 2017.

3. Short–Term Skill Development Training Program in Biotechnology for Students of North-East India on **"Orchid Propagation"** sponsored by **Institute of Bioresources and Sustainable Development of Biotechnology, Government of India, jointly organized by Biotech Park, Lucknow and Institutional Biotech Hub, Nagaland University,** on 16th November to 15th December 2017.
4. Workshop and Training Programme on **'Standard Operating Procedures (SOPs) for the Isolation and Characterizaton of Bacteria and Fungi from Diverse Habitats'** under the project **"Establishment of a Culture Collection Centre in North East Regon of India, Phase-II"** organized by The North East Microbial Repository Centre (MRC), Institute of Bioresources and Sustainable Development (IBSD), a National Institute of Department of Biotechnology, Ministry of sciences and technology, Government of India, Takyelpat, Imphal, Manipur, India on 2-3 July, 2018.
5. Hands on Training on '**Genomics and Gene Expression Analysis'** organized by **Department of Biotechnology, Government of India sponsored Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami-798627, Nagaland** on 18th to 23rd July, 2018.
6. Workshop on **'Skill and Entrepreneurial Development of the Tribal Youth'** with the theme **'Value-additions to Rich Bio-Resources with Special Reference to Medicinal and Aromatic Plants'** jointly organized by Biotech Park, Lucknow and Institutional Biotech Hub, Department of Botany, Nagaland University under the aegis of The National Academy of Sciences, India on 25th to 28th July, 2018.

7. Hands on Training on **"Molecular Taxonomy of Microbes and Higher Plants"** organized by **Department of Biotechnology, Government of India sponsored Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami** on 17th to 23th July, 2019.
8. National Conference on **'Stakeholders on Conservation, Cultivation, Resource Development and Sustainable Utilization of Medical Plants of North-East India'** jointly organized by **Department of Botany, Nagaland University, Lumami, Nagaland, Society for Conservation and Resource Development of Medicinal Plants (SMP), New Delhi** on 6th to 7th March, 2019.