Investigation on Soil Microflora and Polyphenol Content of *Camellia Sinensis* (L.) O. Kuntze at Different Altitudes of Mokokchung District, Nagaland

by
Ms. Temsurenla

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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NAGALAND, INDIA

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DEDICATION

This thesis is dedicated to my Late brother Mr. **Akumtoshi Jamir**.

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Lumami 798 627, Nagaland, India

CERTIFICATE

This is to certify that the thesis entitled **"Investigation on Soil Microflora and Polyphenol Content of *Camellia Sinensis* (L.) O. Kuntze at Different Altitudes of Mokokchung District, Nagaland"** which is being submitted by Ms. Temsurenla, **726/2016, with effect from 06/08/2015**, 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.

Further, certify that Ms. Temsurenla has adhered to the rules and regulations of Nagaland University for the submission of this thesis.

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DECLARATION

I, Ms. Temsurenla, bearing Ph.D. registration number **726/2016, with effect from 06/08/2015**, hereby declare that, the thesis entitled **"Investigation on Soil Microflora and Polyphenol Content of *Camellia Sinensis* (L.) O. Kuntze at Different Altitudes of 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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The thesis titled **"Investigation on Soil Microflora and Polyphenol Content of *Camellia Sinensis* (L.) O. Kuntze at Different Altitudes of Mokokchung District, Nagaland"** has already been checked in URKUND (Plagiarism Checker)- Ouriginal.

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CHAPTER 1

INTRODUCTION

This chapter presents a brief introduction of the history of tea and its wide implications for the economy and human health. The important factors for optimum tea production are discussed. The chapter also presents the influence of season, altitude, geographical locations and soil properties in the tea plant growth, development, leaves yield and quality. Furthermore, the importance of soil microbes in tea cultivation is discussed. Glimpse on tea production in Nagaland and other northeastern states of India are also presented.

Chapter 1 *Introduction*

1.1. The tea plant and its history

It is a universal fact that humans rely on plants for sustenance. The importance of certain plants in the human diet, treatment in a wide array of diseases and environmental functioning makes them multipurpose plants. The tea plant, scientifically known as *Camellia sinensis* L. (O). Kuntze is one such multipurpose plant. The word 'tea' originated from the Chinese word 't'e' was first mentioned in a Chinese dictionary published in 350 B.C. The leaves and non-developed buds of *Camellia sinensis* are used for preparing a popular non-alcoholic beverage called tea, consume by millions of people throughout the globe. Looking at the plant itself, it is an evergreen tree or shrub under the Theaceae family. It has three races including *Camellia sinensis* L or the China tea plant, *Camellia assamica* (Masters) or the Assam tea plant and *Camellia assamica* sub sp. Lasiocalyx (Planch. MS) or the Southern or the Cambod type plant (Barua, 1989). The height of the tea plant is between 10 - 15 m in the wild whereas, in cultivation, it attains its height between 0.6 - 1.5 m tall only (Mahmood et al., 2010). The shape of the tea leaves may be lanceolate or elliptic with a width size 2 - 3 cm but the length of the leaves varies depending on the species and variety (Tounekti et al., 2013). For instance, the length of the China and Assam tea leaves are 5 - 12 cm and 15 - 20 cm respectively while, the Cambod tea has intermediate-length leaves (Tounekti et al., 2013). The tea plant is grown from seeds, tissue culture and vegetative propagation but for high-yielding clones and the development of uniform stands vegetative propagation is preferred (Hajiboland, 2017). The productive lifespan of the tea plant is 100 years or more (Hajiboland, 2017; Potom and Nimasow, 2019) however, tea plant as ancient as 1500 years still thrives in their original forest such as in the Yunnan province of China (Hara, 1995). Thus, if the tea plant is managed properly in a scientific way, it can create great investment in one time (Potom and Nimasow, 2019).

Tea has been known since time immemorial. One of the reasons for its popularity is its association with history. This suggests that the studies on the history of tea plant require information not only on the plant itself but also about the history of population migration along with time, geography and nationality of those who were first involved with the cultivation and drinking of wild tea (King-Ward, 1950). According to Baruah (2008), the origination of tea was associated with Daruma, the founder of Ch'an school of Buddhism in China by one group while, the other group associated tea origination with the Chinese emperor Shen Nung, also known as the 'Divine farmer' who lived around 2737 B.C. The

latter view is mentioned in other studies as well (Ukers, 1935; Harbowy and Balentine, 1997; Renla and T, 2017). Despite all these views, when it comes to authenticate literature, the first book that deals exclusively on tea, its types, origin, usage, horticultural and other aspects on tea plant were published only from A.D. 780 in the noted Chinese author and tea expert Lu Yu's Ch'a Ching or Tea Classic, (Ukers, 1935).

There is no doubt that tea is an important crop strongly attached to the history, culture and economy of human life however, the history, place of origin and taxonomic classification of the tea plant is a subject that creates lots of debate and controversy among plant scientists. Stuart (1919) reported two possible centres of the tea plant origin namely based on the tea leaf types: i) East and Southeast China (from where the small-leaf plant was originated) and ii) India and Yunnan in China (from where the large-leaf plant was originated). Hashimoto (1985) rejected Stuart's two possible centres of origin of the tea plant. Through his numerical taxonomy and multivariate analysis, the author argued that the Yunnan and Sichuan in China as the centre of tea plant origin. The author further stated that the tea plant is generally divided into the small-leaf plant of temperate regions and the large-leaf plant of tropics but various intermediate leaves types can occur due to environmental variations. Recently, Meegahakumbura et al., (2016) reported three independent domestication centres of the tea plant (China type, Chinese Assam type and Indian Assam type) including Southern China, Southwest Yunnan province of China and Assam of India. Another study divided tea plant based on their ecophysiological differences into two distinct ecotypes namely the China type (var. sinensis) and the Assam type (var. assamica) and their respective hybrids (Hajiboland, 2017). Through the combination of demographic modelling, new hybrids and neighbour-joining tree analysis Meegahakumbura et al., (2018) stated that China type and Assam type tea plant first diverged 22,000 years ago at the time of the last glacial maximum that later on divided into the Chinese Assam type and the Indian Assam tea.

1.2. Importance of tea

Tea is the most popularly consumed beverage and the second most popular drinks in the world. It is considered as the liquid next only to water in terms of consumption (Marcos et al., 1998; McKay and Blumberg, 2002). The popularity of tea is so overwhelming that its plantation occupies about 4.37 million hectares of the world's land (ITC, 2016) with global tea production of over 5 million tons (FAO, 2015; Tang et al., 2020). It is produced in 50 countries among which China, India, Kenya, Sri Lanka, Vietnam, Turkey, Indonesia and Iran

are the major tea producers (FAO, 2015). Tea is commercially available in various types such as black tea, oolong tea, green tea and white tea. These tea types vary greatly in their tastes and chemical compositions due to differences in oxidation and processing. Based on the various steps involved in the processing of tea leaves, Bizuayehu et al., (2016) differentiated tea into three types namely,

i) black tea which is the most processed and has the greatest oxidation
ii) oolong tea which undergoes half processing and half fermentation and
iii) green tea, the product of minute processing and no oxidation.

White tea is another tea type that is prepared from newly grown buds with tiny, silvery hairs. It is the product of the least processing among tea types with no or slight fermentation. Among these tea types, black tea represents about 78 % of the total tea produced and is the most commonly produced and consumed tea type in the world (McKay and Blumberg, 2002; Khan and Mukhtar, 2007; Sajilata et al., 2008). This is followed by green tea, oolong tea and white tea (Chan et al, 2007). Apart from this, tea types are also divided into blended and unblended based on the variety and conventional and cut, tear, curl (CTC) based on the processing.

Tea production as an agro-based industry is a significant contributor to the economy of many countries in the world. China has been the forerunner for the past few years in terms of tea production. Tea cultivation is also been practised extensively in India since its introduction by the British colonials around 1839. The Indian tea industry has a market of around $40.7 billion (Mukhopadhyay and Mondal, 2017) and the country stands out as one of the highest tea producing nations in the world. The tea industry is labour-intensive so it offers equal employment opportunities to a large number of poorly educated as well as well-educated people (Mukhopadhyay and Mondal, 2017). The importance of tea production is also associated with its involvement as a prominent earner of foreign currency and uplifter of the country's gross national product. Thus, tea is a good avenue of employment as well as a great contributor to the country's economy. Furthermore, the tea plant plays a key role in the maintenance of the terrestrial ecology by effectively prevent soil erosion, provide extensive land cover and a pollution-free atmosphere (Cao et al., 2018). Being a woody perennial plant that experiences multiple decadal effects of climate change, the tea plant is also a suitable study system to examine the effects of climate change (Ahmed et al., 2019).

Another important factor associated with tea is that it is a pleasant and refreshing aroma, flavour and taste with numerous health beneficial properties (Khokhar and Magnusdottir, 2002; Lin et al., 2003). Therefore, tea is not merely an economy booster but also an enjoyable, cost-effective, safe, and healthy drinks. Most of the tea leaves constituents are important from a human health perspective (Mondal et al., 2004) and provides a considerable amount of polyphenols and flavonoids, proteins, lipids, amino acids, carbohydrates, xanthic bases, pigments, volatile compounds, essential oils, riboflavin, niacin, folic acid, ascorbic acid, pantothenic acid, malic acid, oxalic acid, manganese, potassium, magnesium and fluoride (Boehm et al., 2009). All these valuable compounds as constituents of tea leaves make tea an asset to human health. It is therefore not surprising that besides being a traditional beverage, tea as a health drink has a more significant role (Hajiboland, 2017).

Studies have stated the importance of tea leaves for human health as potential antioxidants (Lee et al., 2002; Peng et al., 2008; Bizuayehu et al., 2016; Yang et al., 2016). Strong antioxidant properties and scavenging of free radicals by tea polyphenols are due to the attachment of phenolic hydroxyl group to the flavan- 3-ol structure (Nor and Mohd, 2013; Kaur et al., 2015). Thus, tea consumption can remove endogenously generated superoxide, peroxyl and hydroxyl radicals due to the free radical scavenging activity of potent antioxidant compounds in tea leaves. According to Mahmood et al., (2010), the main reason for the continuous increase in tea consumption is due to its preventive effects against certain human diseases. Tea leaves extracts have numerous potential health implications for instances, Katiyar and Mukhtar (1996) reviewed health protective effects of tea leaves concerning cancer and summarized that tea intake can provide preventive effects against various types of cancer such as cancer of stomach, liver, lung, breast, pancreas, colon, rectum, uterus, oesophagus, nasopharynx, bladder, kidney and urinary tract. Tea consumption has also been associated with protection and treatments of other health issues including protection against oxidative damage to human red blood cells, cytoskeletal protein degradation and overall disorganization of membrane structure (Halder and Bhaduri, 1998), prevention of HIV-1 virion, gp120 attachment to CD4 molecules (Kawai et al., 2003) and antihypertensive (Brown et al., 2009). Pan et al., (2000) observed that theasinensin A from oolong tea was involved in apoptosis induction through cytochrome *c* release and activation of human U-937 cells capases. Tea leaves as a boon to human health are well documented in

other works as well, where compounds such as epigallocatechin-3-gallate (EGCG) positively influenced the reduction of neurodegenerative disease and ageing (Reznichenko et al., 2005). Furthermore, tea consumption is associated with the improvement in beneficial intestinal microflora, immunity against intestinal disorders and protection of cell membranes against oxidative damage (Bhagat et al., 2010). Tea leaves are also great antipathogenic and the outcome of tea leaves extracts as an antimicrobial agent has been reported in many studies. Toda et al., (1989) reported bactericidal activity of tea extracts through its inhibitory action against gram-positive and gram-negative bacterial species including *Staphylococcus*, *Salmonella*, *Shigella*, *Vibrio*, *Aeromonas*, *Plesiomonas* and *Yersinia*. Fukai et al., (1991) revealed inhibition of commonly cultivated vegetables phytopathogenic bacteria *Agrobacterium*, *Clavibacter*, *Erwinia*, *Pseudomonas* and *Xanthomonas* by different polyphenols. Potential treatments against methicillin-resistant *Staphylococcus auerus* (MRSA) infections by tea extracts through its ability to prevent PBP2' synthesis and sensitivity to ß-lactan was reported by Yam et al., (1997). Sajilata et al., (2008) disclosed preventive action by polyphenols and polyphenol derivatives against gastric infection caused by *Helicobacter pylori*. Yang et al., (2013) observed the effect of tea polyphenol on nectarine grey mould decay. Likewise, Goswami et al., (2020) showed antibacterial activity of tea extracts against five different bacterial pathogens including *Staphylococcus mutans, S. aureus, Lactobacillus acidophilus, Klebsiella* spp. and *Escherichia coli*.

1.3. Tea leaves chemical compositions and quality attributes

Tea is a part of our daily dietary intake therefore, assessment of the content and distribution of tea leaves chemical composition is crucial not only for its colour or flavour but also for other perspectives including quality of nutrition and health. Tea leaves contain more than 700 chemical constituents Mondal et al., 2004) and about 4,000 bioactive compounds (Mahmood et al., 2010). Flavonoids, amino acids, vitamin C, E, K, caffeine and polysaccharides have been reported to be some of the important tea leaves constituents from human health perspective (Mondal et al., 2004). Consumption of tea is largely associated with leaves quality attributes such as aroma, flavour, attractive colour, liquor, taste and refreshing affect. There are several biochemicals in tea leaves responsible for these quality attributes among which, polyphenols are the most abundant group of constituents. Tea polyphenols account for 42% of the dry weight of the solids in green tea extract (Khan and Mukhtar, 2007; Sajilata et al., 2008). Tea polyphenols are considered important markers of

tea quality due to the sensory and health-promoting properties of these compounds (Tounekti et al., 2013). Furthermore, it contributes one-third of the bioactive compounds in tea (Mahmood et al., 2010). Another quality marker in tea leaves are varieties of tannin substances which are secondary plant metabolites with different biological activities. Tea tannins are known to affect the astringency and bitterness of the taste (Takeda, 1994). Tea leaves also contain other equally important quality markers such as thearubigens and theaflavins which are the result of extensive enzymatic oxidation (Mukhtar and Ahmad, 2000). These oxidative transformations products are also greatly responsible for the taste, liquor, aroma and flavour.

1.4. Conditions necessary for optimum tea production

The tea plant is widely adaptable to areas with different climatic and environmental conditions under a broad altitudinal range. The cultivations of tea plant have been carried out in altitudes ranging from sea level in Japan to as high as 2700 m above mean sea level in Kenya and Rwanda (Owuor et al., 2008; Han et al., 2017). Although the tea plant is not over particular about the climatic and environmental conditions for its growth, however, it requires certain conditions for its optimum growth, development, maximum yield and for obtaining the best quality leaves. The important conditions for optimum tea production have been reported in some studies and these include moderately hot and humid climate (Nath and Bhattacharya, 2014), long hours of sunshine, excessive air and leaf temperature, optimum air temperature in the range of 18 - 30 °C, low air humidity and adequate nutrients particularly nitrogen (Carr, 1972). The growth of tea plant also depends on soil conditions such as soil structure, *p*H and depth (Otheino 1992; Nath and Bhattacharya, 2014). According to (FAO, 2015) requirements for proper growth of tea plant includes hot, moist climate, acidic soil, 10 - 30 ℃ temperature, minimum annual precipitation of 1250 mm, 0.5 - 10 degree slopes and elevations up to 2000 m.

1.5. Important factors that affect tea plant

It is well established that the tea plant can adapt to diverse climatic and environmental conditions. However, it is very important to understand that, the tea growth, yield, can vary remarkably due to differences in one or more of these conditions. This can affect the tea leaves chemical compositions and quality because the chemical compositions in tea leaves and consequent tea quality is directly related to the tea growth and yield performance.

According to Owuor et al., (2010), factors responsible for tea growth and yield bring about changes in the production of tea plant metabolites, which eventually leads to variations in the chemical composition and quality of resultant black teas. Studies have demonstrated that the leaves compositions depend on many factors and they can vary with climatic conditions, season, horticultural practices, plant varieties and leaf age (Katiyar and Mukhtar, 1996; Lin et al., 1996; Chan et al., 2007; Owuor et al., 2008), soil properties, nutrient availability and cultural operations imposed on it. Ahmed et al., (2019) highlighted that the factors that impact tea quality are seasonality including change of harvest seasons (shifts between spring, summer, autumn, and winter harvest seasons or shifts between dry and wet seasons), bud-burst, water stress, geography, light factors including solar radiation, altitude, herbivory and microbes, temperature, soil and nutrient factors.

1.5.1. Tea plant variety

Variation in tea compositions and quality due to plant varieties were presented by Owuor et al., (1990) where volatile flavour compounds responsible for sweet flowery aroma and improvement in flavour index (qualitative order of flavour characteristic) decreased with an increase in altitude in some tea clones while in other clones the same compounds increased with increase in altitude. Likewise, Cherotich et al., (2013) observed differences in the accumulation of catechins among the tea clones cultivated in East Africa and concluded that the level of chemical constituents in tea plants was varietal dependent.

1.5.2. Climatic and other conditions between geographical locations

The importance of differences in climatic and other conditions between tea growing areas have been highlighted in many studies. According to Owuor et al., (2010), the adaptability of tea to wide geographical regions with diverse climates conditions cause differences in photosynthetic and growth rates resulting in differences in yields and quality of the resultant made tea. Carr and Stephens, (1992) mentioned that when nutrients are not limiting the important climate and its day-to-day variables (weather variables) for the growth and the potential yield of tea are solar radiation, temperature, saturation deficit of the air and soil water availability while, Dutta (2011) observed that tea yield is influenced by age, pruning, and fertilizer application. Hajiboland (2017) reviewed light, CO_2, temperature and water availability as the most important factors for determining tea productivity. Zhang (2018) stated that environmental parameters such as light heterogeneity, diseases and pests, temperature, nutrients and water in time and space affect tea growth and development but,

Jayasinghe et al., (2020) opined that solar radiation followed by slope and aspect provide a stronger explanatory environmental variable for the geographic variation in tea cultivation.

Several studies have demonstrated the response of tea plants to changing climatic conditions and geographical locations of the tea plantation. The differences in tea leaves constituents total catechin due to location was observed to be tremendous and far larger than among cultivars (Wei et al., 2011). According to Nyabundi et al., (2019), differences in geographical location can influence productivity, growth rates, leaf nutrients levels, quality parameters precursors and the resultant quality of black tea. Even minor climatic or geographical changes can provoke significant changes in tea chemical compositions and quality (Owuor et al., 1990). This is because the geographical environment can affect the distribution of sunshine duration, rainfall, temperature, soil moisture and fertility in tea plantations which may ultimately result in the constituents variation of secondary metabolites in tea leaves between plantation areas (Wen et al., 2020). Kwach et al., (2016) was observed that it was not possible to produce the same quality of black tea even within the same tea cultivar due to differences in tea production locations. The same authors mentioned that the same tea cultivar (TRFK 6/8) with identical agronomic inputs cultivated in Eastern Africa had variation in caffeine, total polyphenols and flavan-3-ols concentration due to differences in location of production (Kwach et al., 2016). The effect of geographical locations on tea chemical compositions and quality were elucidated in other studies as well. Owuor et al., (2008) compared the quality of CTC black tea from Malawi and Kenya using the same clonal tea plants. The authors observed that the quality variations in tea leaves occurred due to climatic differences in the geographical areas of production and/or agronomic practices. Similarly, Jayasekera et al., (2014) reported a significant effect of climatic variability in total polyphenols, as well as related constituent compounds in Sri Lankan tea.

1.5.3. Altitude

Another factor that deserves special mention not only in studies related to tea leaves composition and quality but overall productivity of tea is the altitude. The altitudinal impact on tea quality is a significant factor that requires investigation to develop strategies for sustaining tea quality in a particular region (Han et al., 2017). Altitude is one of the main factors governing the yield of tea in many parts of the world (Squire et al., 1993). It has been reported that the altitudinal change can bring major differences in the teas (Owuor et al., 1990) even when other agronomic or cultural practices are the same (Owuor et al., 2010).

Likewise, the variations in cultivation altitudes within the same geographical location could result in differences in the chemical composition as well as tea taste (Han et al., 2017). Furthermore, altitudinal variations can result in tea quality difference even when the cultivar is the same. For instance, Owuor et al., (2008) had observed variations in quality difference within the same cultivars of black teas grown in Malawi (altitude 650 m amsl) and Kenya, (altitude 2180 m amsl). The authors attributed altitudinal variation as one of the factors responsible for such quality difference. This is because tea plants cultivated at different altitudes may experience different climatic, soil and other conditions. The altitudinal effect in tea concerning its growth, yield and chemical constituents are mentioned in many different studies conducted in different parts of the world (Owuor et al., 1990; Squire et al., 1993; Owuor et al., 2010; Han et al., 2017). Elevation or altitude is one among the largest local or regional influencers of climate because as elevation increases, temperatures become more variable, rainfall generally becomes higher and humidity becomes lower (Kavitha et al., 2015). According to Othieno et al., (1992), there is an estimated loss of 1 kg made tea ha^{-1} for every 100 m rise in altitude because the rates of shoot growth are greater in low altitudes than in high altitudes (Mutuku et al., 2016). Contrary to this, the quality of tea improves with altitude (Owuor et al., 1990). It is, therefore, assumed that changes in yield and quality will vary with altitude in an expected manner, though it may not be at the same rates for different cultivars (Owuor et al., 2010; Tounekti et al., 2013).

1.5.4. Season

When it comes to factors affecting tea production, the study on seasonal or periodic fluctuations in soil and environmental conditions is crucial. Change of seasons and day length can affect many indispensable processes of the tea plant including flowering initiation, leaves and fruits abscission, induction of vegetative growth or dormancy and seeds germination (Bhagat et al., 2010). According to Yu et al., (2020), the specific compounds present in tea leaves differ seasonally which may significantly influence taste quality. Similarly, Gulati and Ravindranath (1996) related seasonal variations in concentrations of tea biochemicals such as total phenols, particularly catechins and enzymatic oxidation products theaflavin and thearubigin to seasonal variations in tea quality. Zhao and Zhao, (2019) also mentioned that the harvest season is crucial among the factors responsible for tea quality. Thus, the seasonal change can influence soil and environmental conditions, which ultimately affect tea quality by influencing plant metabolism, leaf physiology and biochemical makeup.

Additionally, tea yield and quality are affected by fluctuations in weather factors within and between the years in a single location (Nyabundi et al., 2016). Yamanishi (1981), found that the tea made during January/February in the Dimbula district and in July/Sept in the Uva district of Sri Lanka are more flavoury than other seasons. According to Zhao and Zhao, (2019), tea types were divided into the spring, summer and autumn tea based on their harvest seasons, out of these spring tea harvested and processed before late May had a less bitter taste and increased flavour complexity while summer tea and autumn tea were acknowledged as more astringent and bitter than spring tea. In Kangra valley at Himachal Pradesh (India), tea yield is distributed over early flush, rains (main) flush and backend flush among which the early flush collected during mid-April are valued for high flavour and liquor characteristics (Gulati and Ravindranath, 1996). Theaflavins, thearubigins, caffeine, aroma complex, flavour components and several unidentified components recorded to be maximum during early flush undergo quality reduction with progress in season (Gulati and Ravindranath, 1996). Likewise, Le Gall et al., (2004) reported the highest quality of tea in the first flush during April and May. According to Laddi et al., (2014), the tea leaves are young and fresh with high levels of polyphenols and catechins at the beginning of the tea harvesting season which results in the finest quality of made tea whereas, during the end of the tea harvesting season, quality of leaves degrades as they become older leading to the lowest quality of made tea. It is therefore evident that plant genotype, geographical locations, one or more of the multiple conditions prevailing in the plantation site and season of the year are responsible for tea growth, synthesis and accumulation of the chemical compounds responsible for the quality characteristics.

1.6. Soil properties under tea cultivation

Soil is a complex and dynamic natural medium composed of organic matter, water, air, nutrients and a wide array of microbes. All these soil properties along with other factors determine soil fertility and soil productivity, which are crucial for plant growth and agriculture sustainability. As far as the soil for tea cultivation is concerned, the tea plant has been reported to flourish in a wide range of soils of different geological origin ranging from those found in tropics to sub-tropics and temperate developed from diverse parent rock materials and under different climatic conditions (Othieno, 1992). The soil under tea cultivation includes latosols of southern India, red-yellow podzolic and reddish-brown laterite of Bangladesh, China, Srilanka and Taiwan, alluvial of Assam, sedimentary types of

Darjeeling, bheel or peaty of Cachar, andosols of Indonesia, podzolic of USSR, red-yellow podzolic and volcanic of Japan, volcanic of Kenya, parts of Tanzania and Uganda, acrisols and ferrasols of East African countries (Othieno, 1992). Thus, the successful cultivation of tea in a wide variety of soil types makes it difficult to put tea soils into any general classification (Nyabundi et al., 2019).

Although tea cultivations are being established successfully and commercially in a wide variety of soil types, specific soil characteristics are required in a particular soil type for economic tea production. Furthermore, the physical, chemical and biological properties and interaction among these properties can vary not only between soil types but also between seasons of the year within a particular soil type, which further adds to the complications. This is because there is none among tropical crops other than tea that demands more precisely a soil with special characters if the economic yield is to be obtained (Othieno, 1992) and the variations in soil types contribute significantly to locational yield variations (Nyabundi et al., 2016). Bhagat et al., (2010) mentioned that there was a wide variation in the soil and climatic conditions even within a small district and in the soil conditions within a section of tea. The outcome of such soil variations can be reflected in tea growth performance, quality, yield and overall production.

Many researchers have stated the optimum soil conditions for tea plant based on the areas where it is cultivated. According to Otheino (1992), a tea plant requires optimum soil *p*H from 5.0 - 5.6, a minimum of 2 m depth, aggregated or crumb structure with 50% pore spaces, drainage system with a minimum of 1 m depth. Nath and Bhattacharya (2014) stated that for good growth, the tea plant requires well-drained soils of highland with good depth, *p*H value ranging from 4.5 - 5.5, groundwater table with 90 cm or more depth and with 2% organic matter. Some other important soil conditions to be taken into consideration for economic tea production include field slope, topsoil gravel and rock percentage because conditions such as soil depth of less than 50 cm, more than 50% soil gravel in the top 50 cm of the soil layer and rockiness of 20% can negatively affect tea growth (Jayasinghe et al., 2019).

Being a monoculture and economic crop with agronomy management practices, the soil under tea cultivation has specific natural characteristics that can change the tea soil properties (Chen et al., 2006). The areas under plantations are prone to depletion of minerals, change in soil structure and toxic substances accumulation and pathogen attack (Owuor,

1996). Therefore, research dealing with tea soil properties is crucial for economic production. Along with soil properties, nutrient status and fertilizer application are key for the growth of tea plant (Tongsiri et al., 2020). Like in any economic crop productivity, the soil with suitable physical, chemical and biological conditions are crucial for the tea plant. Soil is also a key factor that affects the biological synthesis compounds responsible for tea quality. Therefore, the smooth operation of soil is of the utmost importance for the tea industry. This can be achieved only when the soil quality and health are good which are reflected in its physicochemical characteristics (Baruah et al., 2013). Soil physicochemical properties include organic as well as inorganic materials. Deficiency or excessive concentration of these materials affect soil physicochemical characteristics and eventually harm living organisms in the soil and above-ground vegetation. While the soil physical properties determine the manner, in which it can be applied for cultivation and provide a support system for biological process, the soil chemical properties, on the other hand, deals with its different chemical processes that determine its ability to supply nutrients and other growth requirements for microbial populations and plant species. Soil physical properties include its colour, texture, structure, porosity, density, consistency, temperature and air (Osman, 2013). Good soil physical properties in tea growing areas are very much necessary for stabilizing the water, gas and heat relations in soil that are conducive to the growth and development of the root system and promote tea quality improvement (Chen, 2019). Important indices of soil chemical properties include ion exchange properties, *p*H and redox potential along with varieties of chemical elements occurring as oxides and hydroxides, mineral and solution or organic and inorganic forms (Osman, 2013). Among soil chemical properties *p*H, total organic carbon, total nitrogen, available potassium and sulfur play a major role in the optimization of tea production (Karak et al., 2015). Soil moisture is another important parameter that can affect tea yield and quality (Lemmesa, 1996). Apart from these, nutrients are also crucial for tea production. The most important minerals among essential elements in the tea soil are nitrogen, phosphorus and potassium (Meena et al., 2014) which together constitute the trio know as NPK (Salim et al., 2015).

The physicochemical characteristics of tea soils including texture, nutrients availability, cation exchange capacity and organic carbon content of the soils are influenced by different pedogenic processes and related physiography of the tea-growing areas such as differences in plant age, elevation, organic and non-organic inputs (Ray and Mukhopadhyay,

2012). Tea garden soil properties and fertility are also affected by weather, unscientific method of reclamation and fertilizers that result in soil compaction, ageing and a serious imbalance in plant nutrients such as nitrogen, phosphorus and potassium.

1.6.1. Soil texture

The inorganic component in the solid phase of the soil consists of particles of different sizes ranging from fine clay to rocks. Soil texture is the degree of fineness or coarseness created by these various sized particles closely packed in soil that is originated through weathering and pedogenic processes from inherent parent materials (Osman, 2013). Sand, clay and silt are the three major soil minerals and the relative proportions of these particles constitute soil texture. Sand constitutes coarse size soil particles where leaching of water and nutrients occurs at the maximum. Sandy soils are well-drained soils that allow good aeration and are more susceptible to erosion than other types of soil. Clay predominantly contains a secondary mineral formed by weathering of primary minerals with phyllosilicate and silicate clay minerals as the major secondary mineral. It constitutes the smallest particle size that restricts the movement of water and nutrients through the soil. Contrary to sandy soil, clay soil does not allow good aeration as they have more micropores. Silt includes those soil particles sizes that are between clay and sand. There is also another type of soil called loam, which contains a mixture of sand, clay and silt and exhibits properties of all three soil particles.

Soil texture also called soil mechanical composition is one of the most stable properties. It is a qualitative classification tool used in laboratory and fields for class determination of agricultural soils based on their physical texture (Bhuyan and Sharma, 2015). It provides a useful index of several other properties that determines the soil agricultural potential (Nath, 2014). Soil texture is involved in soil quality screening with functions including retention and transportation of water and chemicals, estimation of soil erosion and variability (El-Ramady et al., 2014). It governs soil properties including soil aeration, the ability of soil to support permanent plant cover, withstand mechanical working and the ability for the availability of nutrient stored in the clay-humus complex for plant usage (Wang et al., 2010). Soil texture can affect plant productivity by influencing moisture availability, soil temperature, nutrient supply and the accessibility of soil organic matter to microbial decomposition (Schimel et al., 1996), water holding capacity, infiltration and permeability.

The best soil texture type for tea production has been reported to be sandy clay loam soil (Nath, 2015). However, tea cultivated soils of the world are of different origin and range from the lightest of sand to heavily silt loam or even silt clay loam types (Nath, 2014). This suggests that tea can grow in a wide variety of soil textural types (Othieno1992; Nyabundi e et al., 2016). Ray and Mukhopadhyay (2012) observed diverse soil texture types including sandy loam, sandy clay, sandy clay loam, clay loam and clay from two districts of West Bengal in India. The authors mentioned that the soil characteristics in the tea-growing areas were influences by different pedogenic processes and related physiography. Nyabundi et al., (2016) demonstrated the ability of tea to grow in a wide variety of soil textural types (sandy loam to clay) through their study in three different tea growing geographic regions in Kenya. The natures of soil texture observed by Khan et al., (2017) in four experimental plots of Bangladesh Tea Research Institute were sandy clay loam or sandy loam. Huu Chien et al., (2019) observed that soil texture classes in the tea production area of the northern region in Vietnam varied from sandy loam to light clay. The authors pointed out the effect of different terrains along the transect lines as well as severe disturbance such as terracing and earth excavation on the soil texture.

1.6.2. Soil *p*H

*p*H is one of the most important soil properties, which immensely influence nutrients availability (Akenga et al., 2014; Arevalo-Gardini et al., 2015; Ranganathan and Natesan, 2015). In an agricultural system, appropriate soil *p*H is necessary for sustainable production. Soil *p*H is crucial for screening soil quality and its functions involve defining soil biological and chemical activity thresholds, nutrient availability, toxicities and deficiencies, pesticides absorption and mobility, models processing, microbial habitat and growth and development of plant (El-Ramady et al., 2014). The optimum soil *p*H of different plants differs for their productivity. Soil with very low or high *p*H value is deleterious for plants because under the high acidic condition there is an abnormal rise in levels of metals like soluble aluminium and manganese while, high alkaline soil condition there is a problem of low solubility for plants nutrients availability thereby, creating conditions such as leave chlorosis.

The most important feature in tea cultivated soils is the soil *p*H that differs from one country to another (Hajiboland, 2017). An unusual characteristic of the tea plant is the requirement of acidic *p*H for its growth and acidification of the soil where it grows (Wang et al., 2010; Wan et al., 2012; Yan et al., 2018). The tea plant growth is arrested slowly when

the soil *p*H exceeds 6.5 and the plant dies when the *p*H is greater than 7.0 (Li et al., 2016). According to Ranganathan and Natesan (1985), the optimum soil *p*H is the most important condition for the better growth of tea plant and this can vary with the nature of the soil, particularly the organic matter content (Otheino, 1992). Although in many countries, the optimum *p*H for good growth and better utilization of nutrients is 4.5 to 5.0 (Ranganathan and Natesan, 1985), different studies have reported various optimum soil *p*H for the tea plant. For instances, Otheino, (1992) speculated the optimum soil *p*H for tea plant ranged from 5.0 - 5.6 while, Nath and Bhattacharya, (2014) mentioned that the soil *p*H from 4.5 - 5.5 is an ideal value for the tea plant. Another study mentioned that the optimum soil *p*H value for the tea plant ranged from 4.5 - 5.6 (Hajiboland, 2017). Despite slight differences in the optimum soil *p*H values between the aforementioned studies, it is evident that the tea plant prefers acidic soil for its proper growth. However, it has been reported that when the *p*H value goes down beyond 4.0 the tea plant growth is inhibited consequently affecting tea quality as well as quantity (Li et al., 2016). Therefore to rectify this problem, it has been suggested to minimize acid-based fertilizers and adopt soil improvement measures when tea soil *p*H decreases beyond 4.8 (Zhang, 2018).

The decrease in soil *p*H occurs due to factors such as years of mono-cropping (Owino and Othieno, 1991) and age factor which means decreasing trend in the soil *p*H with the increase in age of cultivation (Pansombat, 1997; Dong et al., 2005; Xue et al., 2006; Wang et al., 2010; Karak et al., 2015; Yan et al., 2018). This decrease in *p*H with the increase in plantation age was also observed in other plantations such as cashew plantation (Ogeh and Ipinmoroti, 2015), alder-cardamom plantation (Sharma et al., 2009). Apart from the age factor, the important pathways that accelerate soil acidification are the nitrification of NH^{+4} in the fertilizers supplied to tea plants and the biogeochemical cycling of aluminium in tea litter (Karak et al., 2015). The other factors that enhance soil acidification under tea plantation include high aluminium, iron and manganese contents, parent material, heavy rainfall and weathering processes (Karak et al., 2011), the use of chemical fertilizers, root exudation and biological cycling of tea plants (Cao et al., 2018), soil and plant processes associated with the carbon and N cycles (Yan et al., 2018).

1.6.3. Soil moisture

The definition for soil moisture varies depending upon the context which may be relative, absolute or indirect and reference storage. Jin and Mullens (2014) defined soil moisture as "the measure of water content in terms of water content in terms of volume and mass among soil probes that determine various soil thermodynamic properties" while, Seneviratne et al., (2010) referred to soil moisture as "the amount of water store in the unsaturated soil zone". Data on soil moisture is also crucial for many fields of studies such as agriculture and soil science, forest ecology, engineering, water resources and meteorology (Wetzel and Woodward, 1987). Soil moisture is one of the essential climate variables that have a prominent control on the hydrosphere, biosphere and atmosphere and the interactions that take place between them (Chaube et al., 2019). It determines the growth and development of the biota, the rate of soil processes, accumulation and removal of organic and inorganic compounds, the efficiency of plants and regulates species diversity phyto-, zoo-, and microbial complexes (Lvova and Nadporozhskaya, 2017). Soil moisture is also involved with litter production in different environments (Rigobelo and Nahas, 2004). It is crucial for plant growth and development because plant take up water and nutrients only through the medium of soil water available in the soil. The abundance of soil moisture is responsible for the promotion of vegetative growth, contrary to this, low soil moisture for long period can reduce vegetation (Melliger and Niemann, 2010).

The tea plant is sensitive to change in soil moisture because excess or shortage of soil moisture can affect the tea plant. The growth, development and yield of tea are determined by the soil moisture status (Bhagat et al., 2010). The scarcity of soil moisture decreases tea branches growth and results in very hard leaves with some sterile buds (Lemmesa, 1996). It can also induce changes in leaves constituents involved with tea quality (Jeyaramraja et al., 2003). Soil moisture is governed by factors such as climate (Longobardi, 2008), land use, rainfall, soil texture, vegetation, topography (Slave and Allen-Diaz, 2001; Niu et al., 2015), water redistribution and spatial variation of evaporation in different seasons (Li et al., 2016). Variation of soil moisture is the game-changer for evapotranspiration and surface and subsurface runoff (Wilson et al., 2005), water-energy balance, water supply to plants by the soil and moisture gradient between the land surface and atmosphere (Song et al., 2009).

1.6.4. Soil temperature

Soil temperature is generally expressed as the thermal regime of soils including heat flux into the soil, the thermal characteristics of the soil and heat exchange between soil and air (Chiemeka, 2010). Soil temperature is an important factor that affects plant right from its seed germination to its influence in microbiological activities, which are crucial for plant nutrient availability. Organic matter decomposition and release of nutrients, their uptake by plant roots, growth, development and lifecycle of the plant and characteristics of soil organisms can all be related, at least in part, to soil temperature (Maclean and Ayres, 1985). Soil temperature affects plant growth in terms of its physio-chemical and biological processes (Tale and Ingole, 2015) by influencing the growth of root system components, initiation, branching, orientation and direction of growth and root turnover (Bhagat et al., 2010). Some crops require specific temperature ranges as well as the certain intensity of solar radiation, which directly affect harvest quality and yield (Ashardiono and Cassim, 2014). Although tea plants are known to withstand a wide range of temperatures from -16 to 40°C (Bhagat et al., 2010) however, it is the major environmental variable that controls tea yield (Tanton, 1982) because it influences photosynthesis and control plant growth and dormancy (Nath and Bhattacharyya, 2014). Temperature is also among the most important variables that determined the suitability of an area for tea cultivation (Jayasinghe et al., 2020). The most favourable soil temperature range for plant growth is 19 °C - 22 °C and beyond that range, a linear relationship is maintained (Mukhopadhyay and Mondal., 2017). The effects of soil temperature on the tea plant are well documented in some studies. Temperature variations have been observed in one study to explain the occurrence of tea production peaks through their influence on shoot growth rates (Squire, 1979). Carr and Stephens (1992) mentioned that shoot extension rates in tea were reduced and the duration of the shoot replacement cycle was extended from soil temperatures of 16 °C and below. Othieno (1982) remarked on the direct effect of soil temperature on the production rates and quality of growth-regulating substances necessary for bud initiation and development in tea. Reduction in vegetative growth and induction of early flowering of the tea plant because of respective high and low soil temperature during the daytime and night was stated by Hajiboland (2017). The decrease in tea yield under sustained periods of higher temperatures was revealed in some studies (Hajiboland, 2017; Jayasinghe et al., 2019). Soil temperature is one important property that fluctuates with the season, time and local climatic conditions. While solar

radiation is the major source of heat energy for the soil, water content and other soil properties can influence soil temperature. Soil temperature is governed by several factors including soil colour, mulch and organic matter content, land surface slope, vegetative cover, evaporation of water from the soil, solar radiation, soil moisture content and bulk density (Onwuka and Mang, 2018).

1.6.5. Soil organic carbon

Soil organic carbon (SOC) refers to the carbon in the soil as its organic matter. The amount and quality of SOC are crucial for sustaining soil quality, health and productivity. It is a good source of soil fertility and an energy source for soil microorganisms, which acts as a storehouse for nutrients, contributes to soil aeration, reduces soil compaction, improves infiltration rates and increases water storage capacity (Munson et al. 2012). It can increase the availability of nutrients (Misra et al., 2018), influence soil-water relations, soil aerations and soil workability by influencing the soil colours, nutrient holding capacity, nutrient turnover and stability (Nath, 2014). SOC is, therefore, a key determiner for the availability of essential plant nutrients, plant productivity, quality and yield.

The knowledge of SOC status in tea cultivated soil is important since the relative long rotational life cycle of the tea plant could potentially represent storage and sequestration of large amounts of carbon (Li et al., 2011). SOC is crucial for determining the factors that affect carbon storage in tea plantations to investigate methods for increasing SOC sequestration, decreasing loss of soil carbon and enhancing low-carbon planting in tea cultivated areas (Di et al., 2020). Although healthy management practices like conservation tillage, proper shade, surface mulching, compost application, maintenance of floor litter mass etc. have been reported to be crucial for maintaining a sizable amount of SOC under the tea agroforestry system (Kalita et al., 2016), it is necessary to determine the factors that control SOC to apply the appropriate management practices. Di et al., (2020) studied the effects of the soil environment on SOC in the largest Pu'er tea plantation area in southwestern China. The result from their study showed that the soil environmental factors such as water content, total N, oxalate-extractable iron, sulfur, total P, calcium, redox potential, clay, bromine, and manganese affect SOC. The authors speculated that interactions between water content and elements including iron, carbon, N, sulphur, and P concentrations were the main mechanisms underlying the effects of these factors on SOC. Additionally, SOC in tea garden soil could also be influenced by factors such as tea leaf littering, branches cutting during pruning,

organic matter application, shade tree leaf littering (Karak et al., 2011), soil *p*H (Haorongbam et al., 2014), soil depth (Amanuel et al., 2018) and plantation age (Zhengzhong et al., 2018).

1.6.6. Soil nutrients

Successful cultivation relies on soil's potential and constraint, as a medium for the crops but very often it becomes a heavy task because of limited knowledge on nutrient requirements by crops. Soil nutrients deficiency has been the major hindrance for any agricultural productivity as it brings great loss to the farmers as well as the country's economy and it can affect consumers as well. The situation worsens when farmers rampantly use chemical fertilizers without prior information on the soil status or physio-chemical and the nutrient requirements of the crop plant. Plant nutrient requirement varies among crop types, environmental parameters, soil characteristics and soil properties all of which differ from place to place. Thus, for sustainable agriculture production, it is imperative to routinely check soil properties and evaluate the fertility status of a plantation area for appropriate recommendations on agricultural activities and to tackle problems related to agricultural issues.

It is well established that tea, like any other crop, requires many nutrients for its growth (Karak et al., 2015). The tea industry rely on the plant leaves and to meet the demands of the industry tea leaves are being continuously plucked, this results in macronutrients mining making replenishment of nutrients necessary to the plant (Tabu et al., 2015). Furthermore, the evaluation of nutrient status in tea soil is crucial because the chemical composition of tea leaves is inextricably linked to available nutrient status and its absorption by the plant. Although tea cultivation requires micro- as well as macro-nutrients, however, nitrogen, phosphorus and potassium have been reported to be the main nutrients for tea production (Omwoyo, 2017; Ruto et al., 2019) due to their importance in tea leaves constituents such as contents of flavone, total polyphenols, free amino acids catechins, and theanine (Li et al., 2005).

Nitrogen

Nitrogen (N) is the most important among major macronutrients required in substantial amount by the tea plant during its lifespan because it plays a key role in the physiology of the tea plant. N constitutes about 5% of the dry weight of harvested tea shoots (Barooah et al., 2005). It is an important constituent of tea leaves chlorophyll, proteins, enzymes, vitamins, nucleic acids, alkaloids, amino acids and various types of hormones (Xiao et al., 2018).

Being a major part of amino acids N control all life processes (Kumar and Seema, 2016), N is associated with tea yield and quality improvement (Oh et al., 2006). N nutrition enhance tea yield provided, the conditions including temperature, rainfall, relative humidity and evaporation are favourable (Owuor, 1997). Owuor et al., (2010) pointed out that tea shows easily demonstrable quality responses for N. Likewise, Hamid et al., (2014) also demonstrated that increasing the supply of available N in tea soil increased the concentrations of some quality determinants in tea leaves up to certain level. However, it is of utmost importance to avoid excessive amounts of N in tea gardens because this will reduce tea quality as well as bring about tea plantation soil acidification, cause environmental pollution and harm to human health (Zicheng et al., 2012).

Tea cultivation relies on harvesting its leaves during where a great amount of N is being removed from the plant. According to Karak et al., (2015), an average harvest of 3000 kg ha^{-1} tea presumably withdraws around 150 kg N kg ha^{-1} from the soil annually. Therefore, information on available N status for plant usage is crucial for the tea industry. N is available in the soil in different forms however, the most important plant-available forms of soil nitrogen include ammonium and nitrate. Plants respond to these forms of N depending on variability and species differences (Britto and Kronzucker, 2002). As per the findings in some studies, the response of the tea plant is better when provided with N in ammonium form than in nitrate form (Ishigaki, 1974; Morita et al., 1998).

Plant-available N can vary between sites and seasons depending on which, the requirement by plants may differ. Available N is influenced by diverse factors including soil and environmental properties, microbial processes such as decomposition, nitrogen fixation and mineralization, types of N fertilizer used and management practices. For instance, temperature facilitates or reduces the N availability in soil (Geng et al., 2017) and soils with high sand, high CEC and low clay contents can stimulate the N mineralization process (Khalil et al., 2005). One study mentioned the respective positive and negative influence of the SOC and sand content in tea soils available N content (Misra et al., 2018). Another study showed that the soil moisture and temperature were the major environmental factors that affected N availability from organic N materials and the influence of these factors differed based on the source of N (Agehara and Warncke, 2005). Misra et al., (2018) observed that the available N status of all the soils under different tea growing regions of North Bengal was low to medium. The authors opined that leaching loss, emission of nitrous oxide of N from

the surface soil and less moisture content were the probable reason for such a result. Eventually, one or more factors that influence N availability will *bring* about *changes* in the tea plant growth, yield and quality components. Watanabe (1995) reported that the tea shoots growth and quality components were affected by N dressing at least three weeks before plucking time. Likewise, Owuor (1997) mentioned that N nutrition can enhance the tea yield when conditions including temperature, rainfall, relative humidity and evaporation are favourable, provided there are no adverse effects of a large N supply.

Phosphorus

P is a macronutrient that is considered the master key to agriculture due to its multifarious roles. It is the most important essential element after N when it comes to influencing agricultural and natural ecosystems (Desta, 2015). It is the most important nutrients for tea production after N and K. This macronutrient is crucial for physiological and biological process in living organisms because it is a constituent of nucleic acid and is indispensable for the energy transport system. P is vital for the development of tea root and normal growth of tea (Ahmed et al., 2005; Hajiboland, 2017), seed formation, early maturation of crops and contributes significantly towards disease resistance (Sharma et al, 2013). P is also linked intimately with yield and tea quality. According to Lin et al., (2012), the sensory, biochemical qualities and antioxidant activity in tea enhanced when the concentrations of parameters responsible for these properties (total polyphenols, total free amino acids and flavonoids) increased with increasing P supply and with its deficiency, the concentration of parameters for tea quality also decreased.

The chemistry of soil P is a complex one due to the presence of its different forms and its possible transformation depending on the physical, chemical and biological environment (Kiflu et al., 2017). Generally, P occur as soil solution P, readily available or labile P which includes easily organic P that is mineralizable, low energy sorbed P and soluble mineral P and non-labile P which include inorganic P, high energy sorbed P and phosphate minerals that are relatively insoluble (Desta, 2015). Both organic and inorganic forms of P are present in the soil and are interconvertible through mineralization (conversion of organic P to inorganic P) and immobilization (conversion of inorganic P to organic P). The P available for plant usage is limited in many agricultural soils despite the necessity for adequate P nutrition in crops. This is because P diffuses slowly through the soil solution toward roots and the migrating P ions become susceptible to chemical fixation in most agricultural soils (Ziadi et

al., 2013) which consequently form complexes with aluminium, iron and calcium, occluded and organic P (Bhagowati and Das, 2016).

The availability of P to plant root has been reported to be influenced by several factors such as the concentration of phosphate ions in the soil solution and buffer capacity of the soil (the ability of the soil to substitute these ions when plant roots remove it) (Syers et al., 2008), the combination of biological and chemical (adsorption/desorption and dissolution) processes (Geng et al., 2017) and management practices (Huu Chien et al., 2019). Studies have reported various level of available P in tea soils depending on one or more of the aforementioned factors. Zhang (2018) recorded a high concentration of available P in the tea plantation of Qishan Mountain in China due to the application of compound fertilizer. Huu Chien et al., (2019) observed low values to high values of available P (54 to 1110 mg kg^{-1} and 6 to 660 mg kg^{-1}) at the surface and subsurface in tea production areas in the northern region of Vietnam. In another study at the Rungicherra tea estate in Bangladesh, the available P ranged from 2.31 - 4.02 ppm (Ahmed et al., 2005). Such low content of available P was presumed to be the result of several factors including its conversion to unavailable form, fixation by aluminium and differences in organic P compounds formation. According to Zoysa et al., (1999), there was a reduction in the availability of P from both native and fertilizer P sources in highly weathered tea soils due to aluminium-P precipitation and the fixation of P with large amounts of iron aluminium and hydroxyl oxides. It is interesting to note that even though P fixation in tea soils is high, tea plants generally do not suffer from P deficiencies due to some mechanisms in tea plants through which they can utilize the fixed soil P (Zoysa et al., 1999).

Potassium

K plays an important role in crop production because of its involvement in different physiological processes of the plant (Bhuyan and Sharma, 2015). Unlike other macronutrients that are assimilated into organic compounds, K remains as a free K^+ in the plant cell cytosol, vacuoles, and vascular tissues. It is required in moderate to a high level by tea plant (Sitienei et al., 2013) and is the second most important nutrient next only to N for tea plant growth, development and yield (Ranganathan and Natesan, 1985). K holds a key position in tea nutrition because it interacts with other nutrients as well as with many cultural factors (Ranganathan and Natesan, 1985). K is crucial in photosynthesis for sugars translocation within the plants and xylem transportation of water and nutrients such as

nitrates, phosphates, calcium and magnesium and is involved in making crops tolerant to water stress, confer winter hardiness, disease resistance, improvement in quality and yield of crops (Sekhon, 1999). It is also essential in several biochemical processes, for plant water retention, maintenance of ion homeostasis, osmotic balance regulation, movement of stomata and resistance to harsh climatic condition (Hasanuzzaman et al., 2018), counteracting the effect of excess N (Sitienei et al., 2013) and development of robust bush frames in the tea plant, which is necessary for sustaining productivity over a longer period (Singh and Pathak, 2018). Apart from maximizing crop productivity, a good supply of K favours the production of quality tea, improves the water-use efficiency under stress conditions and reduces the susceptibility of the plant to certain diseases (Ranganathan and Natesan, 2015). It has been reported that the ability of a plant to absorb K^+ from the soil is a prerequisite for its survival and the ability to efficiently absorb, transport, and utilize K^+ is directly linked to the quality and yield of crops (Shin, 2014). A study conducted by Ruan et al., (1999) revealed an increase in the polyphenol contents and important quality parameters in black tea including theaflavin and thearubigins with the application of K fertilizers. In addition to increased total polyphenol contents, concentrations of free amino acids and water-extractable dry matter were considerably increased with K-fertilizer application (Ruan et al., 2013).

Depending on its availability to plants, soil K is grouped into four forms namely exchangeable, non-exchangeable, structural and water-soluble form (Singh and Pathak, 2018). Despite the large potassium reserve in the soil, the average concentration of potassium present as an immediate source for plants in the soil solution ranges from 1% to 2% (Meena et al., 2014). This is because water-soluble form and exchangeable K are the only forms of K that are readily available for plant usage. Furthermore, K in tea leaf is removed at a large amount which depletes its soil reserves (Sitienei et al., 2013). According to Karak et al., (2015), average harvests of 3000 kg ha^{-1} tea withdraw 60 kg K_2O ha^{-1} from the soil in a year. The amount of potassium in the soil is determined by many factors including the parent material, degree of weathering, increase through manures and fertilizers and decrease through crop removal, erosion and leaching (Sekhon, 1999) soil, plant, nutrient and cultural factors (Ranganathan and Natesan, 1985). The availability of K for crop usage over an extended period depends on the K content of and the rate of K^+ released by the primary minerals, the nature or type and the quantity of clay (secondary) minerals present in the soil (Ranganathan and Natesan, 1985). It also relies on factors such as its intensity, quantity and

renewal rate in the soil (Ruan et al., 2013), soil *p*H (Singh and Pathak, 2018) and SOC (Misra et al., 2018). Adiloglu and Adiloglu (2006) reported 37% of tea plantation soils at Trabzon province of Turkey were deficient in K content due to the application of excess N fertilizers, tea plant vegetation and an increase in K uptake from soil. Zhang (2018) observed great variations in available potassium content at different tea plantation soils in Qishan mountain and nearly half of the areas was lower than the soil requirements for high quality, high-yield and high-efficiency tea gardens. Contrary to the above studies, Karak et al., (2015) reported a high level of plant-available K varying from 150.89 mg kg^{-1} and 186.63 mg kg^{-1} in the tea soils from two districts in Assam.

1.7. Importance of soil microflora

Soil is a living habitat for varieties of biotic components. Due to the availability of nutrients, water, organic and inorganic matter, it is an excellent culture medium for the growth and development of various microorganisms (Bisi-Johnson et al., 2010). The wide arrays of microbes that the soil harbours interact among themselves and with other parts of the soil biota. These interactions can beneficially or detrimentally influence each other and the aboveground plants. The major function of the soil microbes undoubtedly is their involvement in the cycling of elements. However, their roles are not limited to this alone but are quite diverse, extending right from organic matter decomposition to direct or indirect effect on plant performance. The contribution of soil microbial communities towards organic matter decomposition, nutrient fertility and soil structural stability has led the researchers to scrutinize it in soil management and perturbation studies (McGrath et al., 2001). The role of soil microbes as nutrient drivers and cyclers of organic matter makes them of utmost importance for soil fertility, soil restoration and plant health in agriculture**.** Soil microbes also have a profound impact on plant growth and development concerning plant growth hormone productions and antagonistic activities. Some of the mechanisms through which plant growth can be boasted by microbial activity include manipulation of plants hormonal signalling, repelling or outcompeting microbial strains that are pathogenic and increasing nutrients availability (Jacoby et al., 2017). It is noteworthy that there are negative effects from these microbes where they hamper plant growth, development and yield or may even kill the plant. Additionally, some microbes are helpful as early warning signals of changes in soil quality because of their ability to respond more rapidly to changes in soil quality than soil carbon or N (Kennedy and Papendick, 1995).

The growth, development, quality and yield of any plant rely heavily on the existing soil biological properties among which, microflora are the most important group. Soil microflora includes various microbial groups of bacteria, actinomycetes, and fungi. These are the main agents of nutrient cycling (Kibunja et al., 2010). Researches addressing the identifications of soil bacteria and fungi are crucial because they are integral components for soil and ecosystem functioning and are capable of carrying out almost all known biological reactions. Bacteria are the smallest prokaryotes and are among the most common and ubiquitous organism found on earth. They constitute the most abundant group among the soil microflora (Kibunja et al., 2010) with a population of up to several billion per gram of soil. The existence of such a huge number of genomes is a reflection of the presence of niches richness in soil and its role as drivers of different soil processes and many ecological functions. Soil bacteria can reproduce even under nutrient limitation or water stress condition in soil and are called bags of enzymes and fertilizers (Dick et al., 1996). Soil bacteria also promote soil ecosystem sustainability and productivity by releasing nutrients from litter and humus in soils. This group of microbes are also involved in the production and absorption of greenhouse gases (Lladó et al., 2017). Fungi are a group of non-photosynthetic eukaryotic organisms and comprise both single-celled and multicellular forms. Among the soil microflora, fungi are the main decomposers of organic matter in soils because of their essential role in the processes that form humus (Christensen, 1989). They constitute more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Karaoglu and Ulker, 2006). This microfloral group plays a significant role in human, plant and animals life. The fact that true fungi underlie major evolutionary and ecological transitions such as being instrumental in the colonization of land by the ancestors of terrestrial plants as well as the termination of carbon deposition into geological reserves illustrate their important roles in soil (Taylor and Sinsabaugh, 2015).

1.8. Tea garden soil microflora

Tea garden soil provides a unique place for the growth and multiplication of diverse microflora. Tea soil has its own set of physicochemical properties, which are regulatory parameters and shape the microflora composition owing to some other characteristic features associated with tea cultivation. These include: i) monoculture nature ii) intense management practices, iii) frequent pruning of tea leaves and iv) release of various organic acids in the form of exudates from tea root. Some other characteristic features associated with tea soil

includes negative rhizospheric effect and antagonist's microbial colonization capable of antibiosis, competition, parasitism and predation (Pandey et al., 2013). Additionally, the major problem restriction of microbial growth by the rhizosphere of tea bushes are associated with tea growing areas (Pandey and Palni, 1996). Thus, the microbial activity in the tea rhizosphere is very intense and dynamic (Pandey et al., 2001). Nevertheless, tea soil is considered as an excellent site for isolation of biocontrol agents (Pandey et al., 1997), efficient inoculants for hardening of tissue culture raised tea plants as well as for growth promotion (Pandey et al. 2000) and for studying microbial interactions under natural conditions in a specific environment (Pandey et al., 2013). According to Goswami et al., (2017), microbial population in tea plantation soil is the key to understanding its soil processes and plant growth performance because of their diverse roles such as promotion of plant growth, inhibition of plant pathogens and tea pests, mineral acquisition and maintenance of the biogeochemical cycles in tea plantation soil. Worldwide studies in different tea growing soils recorded the presence of one or more of such soil microbes. Hayatsu and Kosuge (1993) detected the presence of acid-tolerant autotrophic nitrifying bacteria in acidic soil of tea growing areas in Japan. Pandey et al., (2000) reported higher survivability rates in tissue culture raised tea plants after inoculation with bacterial isolates from different tea rhizospheres. The authors attributed this increase in survivability to the antagonism towards pathogens by the introduced bacterial strains. *Bacillus* and *Pseudomonas* species capable of producing IAA-like substance, siderophore, and solubilize phosphate as well as inhibit *Rhizoctonia solani invitro* was isolated from tea rhizosphere by Mazumdar et al., (2007). In another study, at the tea rhizosphere of Kangra valley in Himachal Pradesh, thirteen fungal phosphate solubilizer belonging to genera *Aspergillus*, *Eupenicillium*, *Gliocladium*, *Penicillium*, *Discosia* and *Trichoderma* were isolated (Rahi et al., 2009). It was further observed that the *Discosia* sp. possessed multiple growth-promoting substances and enhanced dry matter, root and shoot length of maize, pea and chickpea. *Azotobacter* species from tea gardens of West Doar regions and Darjeeling showed N-fixing ability along with salt tolerance and antibiotic resistance (Bhaduri et al., 2016). Thus, all these studies are indicative of the importance of tea soil microbial studies. Such studies will not only provide useful information about the type of microorganisms that can adapt, exploit and persist in such monoculture plantation soil but also offer tremendous scope for many potential biofertilizers and plant growth promoters for the upliftment of the tea industry. Furthermore,

such studies are crucial to abandon the dependence on synthetic fertilizers that are detrimental to the environment (Renla and T, 2017).

Agricultural soil microbes including those under monoculture plantations like tea soils are subjected to several changes with time. The effect of seasonality, soil physicochemical properties and geographical locations of the tea plantation sites are among the major factors responsible for such changes and or differences because microfloral population and activities are directly or indirectly influenced by these factors. Such complex interactions between soil microbial communities and environmental factors in the tea orchard ecosystem have attracted the attention of many researchers (Li et al., 2016). According to Graystone et al., (1998), soil microbial population growth and their actions are dependent on various factors such as soil type, nutrient status, *p*H, moisture and plant factors such as species and age. Other studies have attributed agricultural inputs, agricultural management (Buyer and Kaufman**,** 1997) and fertilization practices (Qiu et al., 2014) to soil microbial growth and diversity. The importance and effects of one or more of these factors on tea soil microbial populations and activities have been well studied. Hayatsu (1993) reported that soil acidification reduced the microbial population and lowered the cellulose degradation rate and the nitrification activity of the tea field soil. Pandey et al., (2001) observed that the interactions among various factors, the plant, the microbial communities, and the environmental parameters resulted in the dominancy of *Penicillium* and *Trichoderma* species in the rhizosphere of established tea bushes in the Indian Himalayan region. Xue et al., (2006) observed positive and negative effects of fertilization, root exudates and leaf litter on the microbial community in tea orchard ecosystems. Li et al., (2016) revealed that the length of continuous cropping age of tea orchards in China significantly affected the composition and structure of its soil bacterial community. The authors further stated that the SOC and *p*H exhibited the strongest effect on the bacterial community structure among the analyzed soil properties. It is therefore evident that, apart from giving importance to microfloral activities, it is crucial to understand the factors influencing the microfloral population because any change in their population and activity in tea soil can affect the cycling of nutrients as well as the availability of soil nutrients consequently affecting soil functions and tea plant performance.

1.9. Tea in Nagaland and other northeastern states of India

India is second among the largest producer and exporter of tea in the world. The tea plant was introduced in India by the British with the seeds and plants brought from China in 1839. Interestingly, even before its introduction by the British, some tribals among the local population of Assam were habituated with drinking tea in their indigenous method of preparation (Baruah, 2017b) indicating the presence of wild tea plant indigenous to India (Das et al., 2012). However, the production of commercial tea in the country started only after its introduction by the British after and since then, the Indian tea industry has been unstoppable. The demand for Indian teas both within and outside of the country is overwhelming. This is because the finest liquoring quality of black teas in the world is produced in Assam (Baruah, 2017a). Apart from this, Indian teas such as Darjeeling, Assam Orthodox and high-range Nilgiri tea have a distinct aroma, strength, colour and flavour (Benla and Sharma, 2019). At present, tea production in India is confined to geographic regions including Bihar and West Bengal and Assam, Tripura, Sikkim, Manipur, Nagaland, Meghalaya, Arunachal Pradesh and Mizoram under the East and Northeast respectively, Kerala, Karnataka and Tamil Nadu under South and hills of Himachal and Uttarkhand under Northwest (Sharma et al., 2010). Assam, West Bengal, Tamil Nadu and Kerala are the four major tea-growing states that altogether account for 96% of the country's total tea production (Potom and Nimasow, 2019).

Northeast of India is transversed by the Himalayan and Indo-Burma biodiversity hotspots of the world. Its unique climatic and physiographic conditions varying from tropical to alpine zones make this region very rich in plant diversity. This part of the country has a rich floristic composition, rare and endemic species and varying types of forest ecosystems. Tea is one of the most popular perennial crops that are extensively cultivated in northeast India since the beginning of the 19th century (Haorongbam et al., 2014). In northeast India, most of the tea plantations have alluvial soils and tea plants are grown from foothills down to fairly flat or gently sloping valley beds (Mann and Gokhale, 1960). The tea industry in northeast India provides employment opportunities to many and contributes to the region's economy. The northeastern states represent 54% of the country's total tea production and Assam alone contribute 51% of India's total and about one-sixth of the tea produced in the world (Saikia et al., 2013).

Nagaland is a mountainous state situated at an altitude ranging from 150 m to 3800 m above sea level in the easternmost parts of northeast India. It is located between 25º6' and 27º4'N latitude and between 93º 20' and 95º15'E longitude covering an area of 16,579 square km. The state is blessed with a sub-tropical to temperate climate. Tea consumption among the Naga people is as old as its introduction by the British to India if not earlier than that. In the past, red tea was immensely popular among the Nagas that a utensil was kept specifically for red tea in almost every household. Nowadays, consumptions of other tea types like green and white teas are gradually increasing and becoming a favourite among the Nagas. The history of tea in Nagaland is associated with the Konyak tribe of Nagaland serving tea brews to the Britishers during the British rule in India. As far as the tea production in Nagaland is concerned, Late Mr Sashimeren Aier of the Mokokchung district pioneered this area of agriculture. Despite favourable agroclimatic, topographic and natural conditions for tea cultivation, the production of tea in Nagaland is a handful as compared to other tea producing states and the state relies heavily on its neighbour Assam for tea (Benla and Sharma, 2019). However, for the past few years, Naga people are giving due importance to tea cultivation with farmers switching from jhum and terrace farming to exclusive tea cultivation. In areas Mokokchung, Kohima, Zunheboto, Mon, Phek, Tuensang, Dimapur and Wokha districts acreage of lands under are being cleared specifically for tea cultivations. Tea factories are also being set up in many places and despite on a small scale, the state has started tea leaves export.

1.10. Scope of the present work

Looking at the popularity of tea in the global market for beverages, it has become a necessity to determine the quality and quantity of tea leaves constituents with regard to the place of cultivation as well as the tea leaves harvesting seasons. For a tea plant to have maximum yield and produce the best tea leaves quality, it requires proper growth and development which can be achieved only when it is provided with all the necessary conditions. Furthermore, these conditions can vary among seasons and between the places of cultivation. Therefore, it is imperative to evaluate the seasonal or periodic influence on factors that are crucial for the growth and development of tea plants such as soil physicochemical properties and nutrient status. It is also necessary to give importance to soil microflora as their populations and activities are intimately linked with soil health and above-ground plants. Thus, the present research attempts to determine the extent to which chemical compositions of tea leaves can be influenced by the seasonal and altitudinal mediated differences in soil properties and microbial populations. This may lead to the identification of suitable management strategies, which will accelerate the tea leaves chemical compositions without compromising the environment and further improve the status of the tea industry.

1.11. Aims and objectives of the present work

The proposed study on "Investigation on Soil Microflora and Polyphenol Content of *Camellia sinensis* (L.) O. Kuntze at Different Altitude of Mokokchung District, Nagaland" aims at exploring seasonal differences in soil physicochemical properties, the population of soil microflora, types of indigenous soil microflora and chemical profile of tea leaves in tea gardens situated at different altitudes under the Mokokchung district of Nagaland. The objectives of the proposed study are as follows:

i) To determine soil properties such as texture, temperature, *p*H, moisture content and organic carbon content.

ii) To estimate the concentrations of available nutrients such as nitrogen, phosphorus and potassium.

iii) Enumeration of tea soil microflora.

iv) To determine the chemical compositions in tea leaves.

v) Evaluation of seasonal variations in soil physicochemical properties, the population of soil microflora and tea leaves chemical compositions at different altitudes.

vi) To observe the relationships between soil physicochemical properties, the population of soil microflora and tea leaves chemical compositions.

The outline of the thesis is presented in Table 1.1.

Chapter 1	Introduction
Chapter 2	Study on Tea Soil Physicochemical Properties
Chapter 3	Enumeration of Tea Soil Microflora
Chapter 4	Analysis of Tea Leaves Chemical Compositions
Chapter 5	Summary and Conclusions
References	

Fig.1.1. Outline of thesis

CHAPTER 2

STUDY ON TEA SOIL PHYSICOCHEMICAL PROPERTIES[1]

This chapter gave an account of sampling sites, sampling frequency, sample collections and experimental methodology for soil physiochemical parameters. The seasonal and altitudinal differences in soil physicochemical properties in tea gardens under the Mokokchung district of Nagaland are discussed. Significant relationships between soil physicochemical properties are also investigated.

[1] A section of this chapter is already published as:

Temsurenla., Ajungla, T., 2017. Soil physio-chemical analysis in tea growing areas of Mokokchung district, Nagaland. International Journal for Science and Advance Research in Technology 3(12), 1016-1021.

2.1. Introduction

Soil is a medium for plant growth and the means of sustenance for any agricultural productivity. Soil properties influence the capacity of soil to perform effectively. It is directly related to agricultural production and overall ecosystem stability. If soil properties vary over time and differ between areas, changes may be wrongly attributed to agricultural management practices when they are occurring naturally and independently of the treatment imposed (Haines and Cleveland, 1981). A clear understanding of the seasonal and site distribution of soil properties and their relationships is imperative to evaluate potential soil properties for the yield of the plant as well as for the sustainable development of soil.

Tea growing soil has been a hot topic for researchers all over owing to the numerous contribution of the plant to the world. Tea is the liquid next to the water in human consumption and the plant's attribute such as its taste and flavour, along with positive health benefits make it the most popular non-alcoholic beverage. It is an important economic and cash crop of tropical and subtropical regions with more than 50 countries as active tea growers, among which India is one. The tea producing areas in India is confined to major geographic regions namely the east and northeast represented by Bihar and West Bengal, and Assam, Tripura, Sikkim, Manipur, Nagaland, Meghalaya, Arunachal Pradesh, and Mizoram respectively, the south represented by Kerala, Karnataka, and Tamil Nadu and the northwest represented by the hills of Himachal Pradesh and Uttarakhand (Sharma et al., 2010).

Cultivation of tea successfully on varieties of soil types makes it very difficult to fit the soils into a general classification (Jayasinghe et al., 2019). It is universal that tea cultivation involves monoculture practice where tea plants remain rooted in the same soil for many years with no crop rotation or inter-cropping except for some tea plant shades. Furthermore, tea leaves quality and yield are directly associated with the soil nutrient status along with other physiochemical properties and climatic conditions. All this calls for management practices such as heavy fertilization, pesticides spray and weeding which can subsequently affect microbial processes and alter one or more physicochemical properties of the soil environment. Such changes can have an impact on the other soil properties and the consequences of such conditions are borne by the plant in its growth, yield and quality. The present study was conducted with the following objectives: (i) to determine the soil physicochemical properties in tea gardens varying in altitudes (ii) to investigate significant seasonal differences in these soil physicochemical properties in each of the tea gardens and

(iii) to correlate these soil physicochemical properties to study significant relationships between one another.

2.2. Materials and methods

Details on the tea gardens, sampling frequency, sample collections and an outline of the experimental methodology for soil physicochemical properties are discussed in this section.

2.2.1. Sampling sites

Mokokchung is a major district located in the north-western part 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 which is dominated by hilly areas but there are few plain lands as well. The climate of the areas under the Mokokchung district varies from mild to warm climate throughout the year.

Private tea gardens in Tuli and Ungma under the Mokokchung district of Nagaland were selected for this research. Prior permission was taken from the owners. Both tea gardens are 11 years old and are cultivating the same variety of tea plant (TV7, Tocklai Vegetative 7). Geographical coordinates of the selected tea gardens were determined by a global positioning system (Garmin, 12H). The study map was generated using ArcGIS Desktop 10.8. Details on the sampling sites are indicated in Fig. 2.1, Fig. 2.2 and Table 2.1.

Table 2.1. Geographical location of the tea gardens

Location	Coordinates	Altitude (masl)
Tuli	N 26°39'19.3E 094°39'22.7	174.04
Ungma	N 26°17'30.6 E 094°28'29.2	1365.2

2.2.2. Sampling frequency

Samples were collected from both the tea gardens at a seasonal interval over 2 years from 2016 - 2018. April, July, October and January were categorized as spring, summer, autumn and winter season respectively. April 2016 - January 2017 was considered as the first year/study period whereas, April 2017 - January 2018 was considered as the second year/study period.

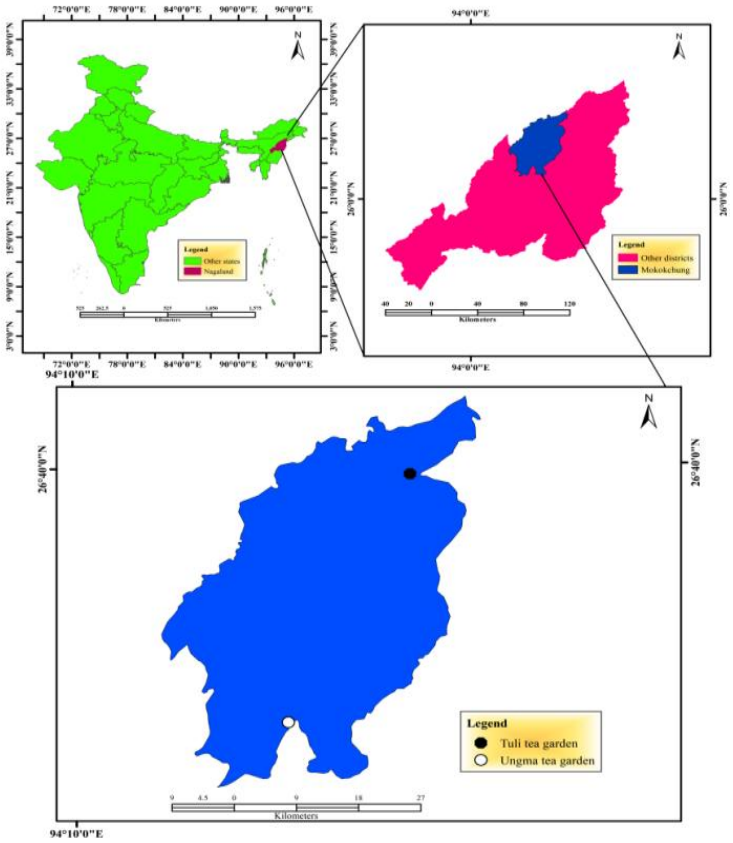

Fig. 2.1. Study map depicting an outline map of India, Nagaland state and sampling sites under Mokokchung district

2.2.3. Sample collection

Soil samples were collected from rhizosphere and non-rhizosphere in all seasons from both tea gardens during 2016 - 2018. Rhizospheric soil samples were collected by uprooting tea roots from the soil. The roots were then shaken gently to remove as much of the adhering soil as possible. Non-rhizospheric soil samples were collected from 0 - 15cm soil depth using a soil auger. Nine soil samples were collected from the rhizosphere as well as the non-rhizosphere at both sites. Composite samples of triplicates for each rhizosphere and non-rhizosphere in the corresponding season were used for analyses. The collected soil samples were transferred to the laboratory under sterile condition. Fractions of the soil samples were processed immediately in the laboratory for soil *p*H, moisture content and microbiological analysis. The remaining soil samples were air-dried, crushed to aggregates with the help of mortar and pestle to pass through a 2mm sieve. Soil samples were collected in sterile polypropylene bottles, marked and stored in dark condition at room temperature until analysis.

Fig. 2.2. Image of study areas. (A-B) Tuli tea garden and (C-D) Ungma tea garden

2.2.4. Soil physicochemical analysis

The physicochemical properties of the soil samples were determined following standard laboratory methods. Analysis of soil physical characteristics such as soil texture (% sand, silt and clay) and moisture content were carried out. Among chemical analysis, soil temperature, soil *p*H, SOC, available N, available P and available K were estimated. Analysis of each soil properties was carried out in triplicates.

2.2.4.1. Determination of soil texture

Soil texture is originated from parent materials and does not depend on the physical or chemical properties of the soil but it does determine these soil properties depending upon the particles size variations. Soil texture was determined by the hydrometer method (Anderson and Ingram, 1993). 50 ± 0.5g of air-dried soil sample was transferred into a 250ml calibrated screw lid bottle. The sample was diluted with 125 ml distilled water and 20ml of 30% H_2O_2 was added. This was followed by gentle swirling and the addition of 3 drops of amyl alcohol. The mixture bottle was transferred to a boiling water bath to complete the reaction. The mixture was allowed to cool down to room temperature. To this, 5 ml of sodium hexametaphosphate was added. The volume was brought up to the 250 ml mark with distilled water and the suspension was stored overnight. This was then transferred to a 1000 ml measuring cylinder and the final volume was brought up to the mark with distilled water. The cylinder was transferred to a water bath maintained at 20 ºC temperature. The sample was mixed vigorously and the stopwatch was started immediately. Blank was also run simultaneously following the same method but without soil sample. Readings were recorded after 40 seconds and after 5 hours with Bouyoucos hydrometer (ASTM D422). Particle sizes were calculated as follows:

40 sec (corr) = 2 (sample reading after 40 sec – blank reading after 40 sec + T)

5 hr (corr) = 2 (sample reading after 5 hr– blank reading after 5 hr + T)

Where,

sec = second

T = temperature corrections

For every ºC above 20 (d), T = 0.3 x d and

For every ºC below 20 (d), T = - 0.3 x d

% sand = 100 - 40 sec (corr)

% silt = 40 sec (corr) - 5 hr (corr)

% clay = 5 hr (corr)

Soil texture class was calculated in USDA soil textural calculator (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167).

2.2.4.2. Determination of soil moisture content, soil *p*H and soil temperature

20g of fresh soil sample was weighed accurately into pre-weighed porcelain using an analytical balance (Wensar, PGB 200)**.** The weighed sample was oven-dried at 105 ºC for 24 hours. The oven-dried sample was allowed to cooled down to room temperature and reweighed. Moisture content was calculated by the formula given below:

$$\text{Moisture content (\%)} = \frac{\textbf{fresh weight – dry weight}}{\textbf{dry weight}} \text{ X } 100$$

Soil temperature was recorded from the tea gardens at the time of soil sampling using a soil thermometer (MAXTECH, DT-9).

The *p*H of any solution is the negative logarithm of the hydrogen ion activity that measures the alkalinity or acidity of any solution. Soil *p*H value was determined in *p*H meter (Systronics microcontroller-based *p*H meter system, 361). Before proceeding with the experiment, the *p*H meter was calibrated with 4.0 *p*H and 9.2/8 *p*H buffers. Soil *p*H was recorded from soil paste prepared using soil: distilled water suspensions (1:2.5). 5g of soil sample was transferred to a 100 ml beaker filled with 12.5 ml distilled water, which was then shaken for 15 minutes. The suspension was allowed to stand for 5 minutes and a *p*H reading was taken. Every *p*H reading was followed by washing the electrode with distilled water and drying it in tissue paper.

2.2.4.3. Determination of soil organic carbon content

The SOC content of the soil sample was determined by the volumetric method of Walkey and Black as given by Motsara and Roy (2008). This method involves the oxidation of carbon to CO_2 by the action of chromic acid formed by the combination of potassium dichromate and sulphuric acid.

1g of air-dried soil sample was transferred to a 500 ml conical flask. To this, 10 ml $K_2Cr_2O_7$, 20 ml concentrated H_2SO_4 and 0.25g silver sulphate was added. The mixture was swirled for some time and allowed to react for 30 minutes. The reaction mixture was diluted with 200 ml distilled water and 10ml of orthophosphoric acid was added. This was followed

by the addition of 1 ml of diphenylamine indicator during which the solution turned blue-violet colour. The solution was titrated with standard ferrous ammonium sulphate (FAS) solution. Reading was recorded on the colour change from blue-violet to brilliant green. Blank without soil sample was also prepared and titrated with standard FAS solution. The experiment including the blank was carried out in triplicate. The organic carbon content of the soil sample was calculated using the following formula:

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

Where,

S = ml of FAS used in the blank

T = ml of FAS used in the soil sample

2.2.4.4. Determination of available nutrients

Available nitrogen

Available N was determined in the N distillation apparatus (Kelpus, DISTYL EM VA). In this experiment, 5g of air-dried soil sample was added to the digestion tube. The digestion tube was loaded in the distillation unit using a slider mechanism. 25 ml of 0.32 % $KMNO_4$ and 25 ml of 2.5 % NaOH were added to the digestion tube using the control panel. Distillation was carried out for 9 minutes. Before distillation, a 250 ml conical flask containing 25 ml of 2.5 % boric acid mixed with an indicator was placed at the receiver end of the distillation unit to collect the liberated ammonia. The collected green colour distillate was titrated against 0.02N H_2SO_4 until the colour changed to the original pinkish shade. Simultaneously blank without soil sample was run following the same procedure. The amount of available N content in the soil sample was calculated using the following formula:

$$\text{Available N (kg/ha)} = \frac{14\ X\ (\text{Normality of acid})\ X\ (\text{Titrant value of the sample})\ X\ 2.24\ X\ 1000000}{\text{Weight of the soil sample X } 1000}$$

Available phosphorus

Available P was determined following the Bray and Kurtz method as given by Motsara and Roy (2008). This method is used as an index of available P, especially for acidic soil. The combination of ammonium fluoride and hydrochloric acid results in the removal of P forms that are easily acid-soluble, calcium phosphate, aluminium phosphate and iron phosphate. 5g of air-dried soil sample was transferred to a 250 ml conical flask. To this, 50 ml of Bray's P-1 was added. The suspension was shaken for 5 minutes on a reciprocating

shaker and filtered through Whatman filter paper No.42 until the clear filtrate was obtained. 5 ml of the filtered was pipetted into a 50 ml conical flask followed by the addition of 5 ml of molybdate reagent. The solution was diluted up to 20 ml with distilled water and 1ml of the working $SnCl_2.2H_2O$ solution was added. The contents were then diluted up to 25 ml with distilled water. Blank was also prepared without the soil extract.

For standard P, stock P solution was prepared by dissolving 0.2195g of oven-dried KH_2PO_4 in 1 litre of distilled water. 10 ml of the stock solution was 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. To these, 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 spectrophotometrically 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:

$$P\ (kg/ha) = \frac{A}{1{,}000{,}000} \times \frac{\text{Volume of the extract}}{\text{Volume of extract taken for estimation}} \times \frac{2{,}000{,}000}{\text{Weight of the soil}}$$

Where,

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

Available potassium

Available K was determined following Toth and Prince method as given by Motsara and Roy (2008). Available K in soil constitutes exchangeable as well as water-soluble forms of soil K which are determined by flame photometry after extraction of the soil with 1N neutral ammonium acetate. Emissions spectral at characteristic wavelength due to excitation of the element in the flame determine the emission intensity of the solution to the known K concentration in the solution.

5 g of air-dried soil sample was transferred to a 50 ml conical flask. To this, 25 ml of ammonium acetate extractant was added. The suspension was shaken for 5 minutes on a reciprocating shaker and filtered through Whatman filter paper No.42 until the clear filtrate was obtained. 1N ammonium acetate without soil was used as blank.

For standard K solution, stock K solution of 1000 µg/ml was prepared by dissolving 1.908 g of oven-dried potassium chloride (KCl) in 1000 ml of 1N ammonium acetate solution. Working K standard solutions were prepared by transferring 0, 2, 4, 6, 8 and 10 ml of the

stock solution into a series of 100 ml conical flask and diluted up to the marks with 1N ammonium acetate solution.

Available K content in the filtrate was determined photometrically in 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 using the following formula:

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

Where,

A = content of K in the sample, as read from the standard curve.

2.2.5. Statistical analysis

Microsoft Excel 2010 version was used for arranging the collected data. Data were expressed as the mean values of triplicated tests of each of the six sites ± standard error of the mean. All statistical analyses were performed using SPSS for Windows, version 22.0 (SPSS, Chicago, IL). One-way Analysis of variance (ANOVA) was used to identify statistically significant differences ($p \leq 0.05$) in each of the soil physicochemical properties. In cases where ANOVA results were significant ($p < 0.05$), Tukey's HSD post hoc analysis was performed to detect the differences in the studied parameters between the seasons. Two-way ANOVA was performed to test the effects of seasons and sites on the studied parameters. Pearson correlation of mean of each tea garden was used to detect the relationships among soil physicochemical properties. The correlations were considered significant if $p < 0.01$ and $p < 0.05$.

2.3. Results

Soil physicochemical properties exhibited seasonal variations at both tea gardens throughout the study period. The average of different physicochemical parameters considered in rhizospheric and non-rhizospheric soil monitored in different seasons during 2016 - 2018 at Tuli and Ungma tea garden is presented in tabular format. Further, this is being supported by appropriate figures and statistical analysis.

2.3.1. Soil texture

The Tuli tea garden soils were predominantly sandy. It followed the patterned sand > clay > silt and belonged to sandy clay loam of the textural class (Table 2.2; Figure 2.3a and Figure 2.3b).

In the first year, the content of sand, clay and silt in the rhizospheric soil ranged from 50.7 - 56.40%, 25.53 - 29.67% and 17.60 - 22.13% respectively and in the non-rhizospheric soil, it ranged from 50.67- 54.73%, 25.73 - 29.80% and 17.47 - 21.80% respectively. Overall, the respective mean of the sand, clay and silt content in the first year was 50.70 - 55.57%, 25.63 - 29.73% and 17.54 - 21.97% (Table 2.2). The highest content of sand, clay and silt were observed during spring, autumn and summer season respectively whereas, the lowest content of these soil properties were observed during summer, winter and spring season. In the second year the sand, clay and silt content in the rhizospheric soil ranged from 53.13 - 60.47%, 21.47 - 30.27% and 15.27 - 19.53% respectively and in the non-rhizospheric soil, it ranged from 52.93 - 58.60%, 21.80 - 29.67% and 16.13 - 19.67% respectively. Overall, the respective mean of the sand, clay and silt content in the second year was 53.03 - 59.53%, 21.63 - 29.97% and 15.70 - 19.60% (Table 2.2). The highest content of sand, clay and silt were observed during spring, autumn and summer season while, the lowest content of these soil properties were observed during summer, spring and autumn season.

Table 2.2. Soil texture of Tuli tea garden during 2016-2018

Soil properties	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Sand (%)	R	56.40 ±0.23	50.73 ±0.18	51.47 ±0.29	54.40 ±0.64	60.47 ±0.24	53.13 ±0.13	54.47 ±0.35	55.47 ±0.29
	NR	54.73 ±0.37	50.67 ±0.57	52.07 ±0.24	53.53 ±0.71	58.60 ±0.12	52.93 ±0.24	54.20 ±0.35	56.53 ±0.24
	M	**55.57 ±0.42^{a}**	**50.70 ±0.27^{c}**	**51.77 ±0.22^{c}**	**53.97 ±0.47^{b}**	**59.53 ±0.43^{a}**	**53.03 ±0.13^{d}**	**54.33 ±0.23^{c}**	**56.00 ±0.29^{b}**
Clay (%)	R	26.13 ±0.44	27.47 ±0.44	29.67 ±0.29	25.53 ±0.52	21.47 ±0.18	27.33 ±0.24	30.27 ±0.24	26.07 ±0.41
	NR	27.67 ±0.18	27.20 ±0.35	29.80 ±0.20	25.73 ±0.48	21.80 ±0.42	27.40 ±0.20	29.67 ±0.24	23.87 ±0.47
	M	**26.90 ±0.40^{b}**	**27.33 ±0.26^{b}**	**29.74 ±0.16^{a}**	**25.63 ±0.32^{c}**	**21.63 ±0.22^{d}**	**27.37 ±0.14^{b}**	**29.97 ±0.20^{a}**	**24.97 ±0.56^{c}**
Silt (%)	R	17.60 ±0.20	22.13 ±0.29	18.13 ±0.35	20.73 ±0.35	18.07 ±0.07	19.53 ±0.24	15.27 ±0.44	18.47 ±0.64
	NR	17.47 ±0.24	21.80 ±0.35	18.87 ±0.07	20.07 ±0.13	19.60 ±0.40	19.67 ±0.27	16.13 ±0.37	19.60 ±0.64
	M	**17.54**	**21.97**	**18.50**	**20.40**	**18.84**	**19.60**	**15.70**	**19.04**

	±0.14[d]	±0.22[a]	±0.23[c]	±0.23[b]	±0.39[a]	±0.16[a]	±0.32[b]	±0.48[a]
Texture class	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**	**Sandy clay loam**

R - rhizosphere; NR - non-rhizosphere.
Mean ± standard error mean.
Different letters ([a, b, c] and [d]) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

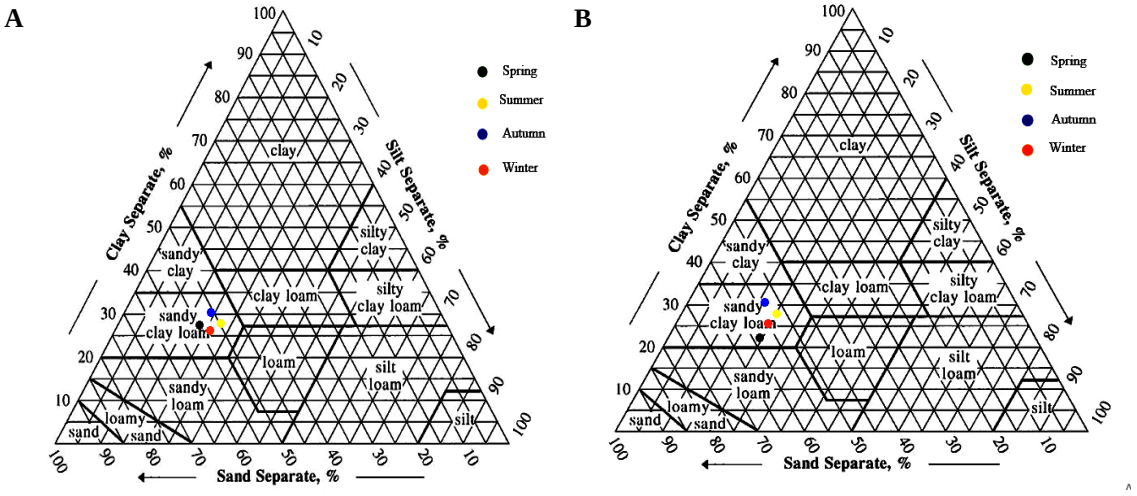

Figure 2.3a. Soil textural classification triangle of the Tuli tea garden during (A) 2016 - 2017 and (B) 2017 - 2018

The soil from the tea garden at Ungma was also predominantly sandy. It followed the pattern sand > clay > silt and belonged to sandy clay and clay loam (Table 2.3; Figure 2.4a and Figure 2.4b).

In the first year, the sand, clay and silt content in the rhizospheric soil ranged from 44.00 - 48.13%, 34.00 - 35.33% and 16.53 - 22.00% respectively and in the non-rhizospheric soil, it ranged from 43.00 - 48.60%, 35.07 - 36.67% and 16.20 - 20.33 %. Overall, the respective mean of the sand, clay and silt content in the first year of study at of Ungma tea garden was 43.50 - 48.27%, 35.03 - 35.37% and 16.37 - 21.17% (Table 2.3). The highest content of sand, clay and silt were observed during the spring, spring and summer season respectively whereas, the lowest content of these soil properties was observed during the summer, winter and spring season.

In the second year, the sand, clay and silt content in the rhizospheric soil ranged from 45.87 - 50.53%, 35.00 - 37.00% and 13.67 - 18.13% respectively and in the non-rhizospheric soil, it ranged from 44.60 - 51.13%, 35.00 - 36.13% and 13.87 - 19.27%. Overall, the respective mean of the sand, clay and silt content was 45.24 - 50.83 %, 35.00 - 36.34 % and

14.17 - 18.70 % (Table 2.3). The highest content of sand, clay and silt were observed during the spring, autumn and summer respectively whereas, the lowest content of these soil properties were observed during the summer, spring and winter.

Table 2.3. Soil texture of Ungma tea garden during 2016 - 2018

Soil properties	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Sand (%)	R	48.13 ±0.18	44.00 ±0.12	46.13 ±0.31	47.73 ±0.37	50.53 ±0.24	45.87 ±0.47	47.60 ±0.23	49.93 ±0.24
	NR	48.40 ±0.12	43.00 ±0.53	46.07 ±0.18	48.60 ±0.42	51.13 ±0.29	44.60 ±0.42	47.40 ±0.31	50.33 ±0.57
	M	**48.27 ±0.11^{a}**	**43.50 ±0.33^{c}**	**46.10 ±0.15^{b}**	**48.17 ±0.32^{a}**	**50.83 ±0.22^{a}**	**45.24 ±0.40^{b}**	**47.50 ±0.18^{b}**	**50.13 ±0.29^{a}**
Clay (%)	R	35.33 ±0.18	34.00 ±0.35	35.27 ±0.07	35.00 ±0.12	35.00 ±0.12	36.00 ±0.23	37.00 ±0.53	36.40 ±0.53
	NR	35.40 ±0.20	36.67 ±0.18	35.33 ±0.18	35.07 ±0.41	35.00 ±0.12	36.13 ±0.24	35.67 ±0.24	35.20 ±0.31
	M	**35.37 ±0.12^{a}**	**35.34 ±0.62^{a}**	**35.30 ±0.09^{a}**	**35.04 ±0.19^{a}**	**35.00 ±0.07^{b}**	**36.07 ±0.15^{ab}**	**36.34 ±0.40^{a}**	**35.80 ±0.38^{ab}**
Silt (%)	R	16.53 ±0.27	22.00 ±0.46	18.60 ±0.31	17.27 ±0.48	14.47 ±0.24	18.13 ±0.24	15.40 ±0.31	13.67 ±0.35
	NR	16.20 ±0.12	20.33 ±0.35	18.60 ±0.31	16.33 ±0.13	13.87 ±0.27	19.27 ±0.35	16.93 ±0.18	14.47 ±0.27
	M	**16.37 ±0.15^{c}**	**21.17 ±0.45^{a}**	**18.60 ±0.19^{b}**	**16.80 ±0.31^{c}**	**14.17 ±0.21^{c}**	**18.70 ±0.32^{a}**	**16.17 ±0.38^{b}**	**14.07 ±0.27^{c}**
Texture class		**Sandy clay**	**Sandy clay**	**Clay loam**	**Sandy clay**	**Sandy clay**	**Sandy clay**	**Sandy clay**	**Sandy clay**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

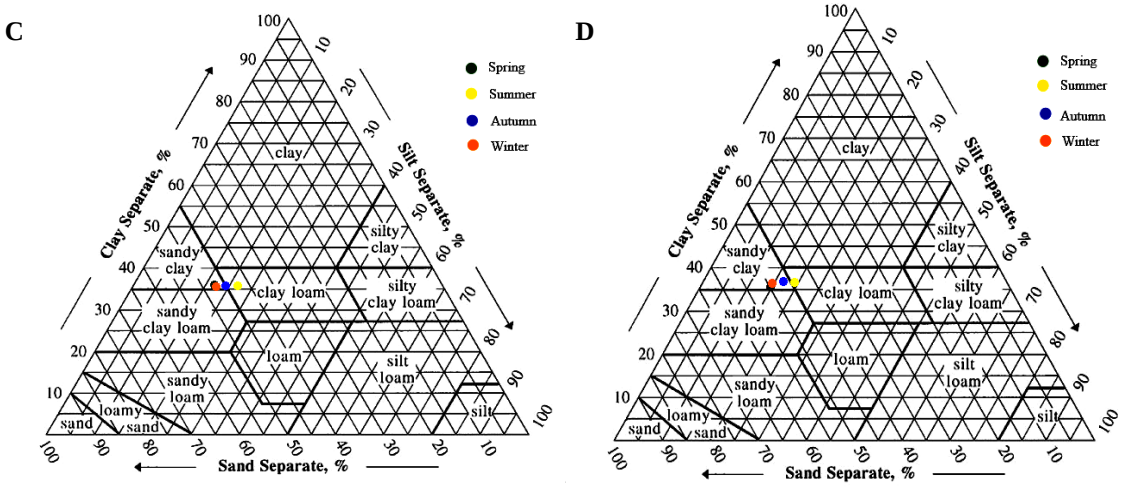

Fig. 2.3b. Soil textural classification triangle of the Ungma tea garden during (A) 2016 - 2017 and (B) 2017 - 2018

2.3.2. Soil *p*H

The *p*H values of the soil samples collected from rhizospheric and non-rhizospheric soil at Tuli tea garden in the first year ranged from 5.03 - 6.30 and 4.96 - 6.24 and in the second year, it ranged from 5.04 - 5.64 and 5.07 - 5.52 (Table 2.4). The mean of the soil *p*H in the first and second year ranged from 5.00 - 6.27 and from 5.05 - 5.58 respectively. Among the seasons, the highest and the lowest soil *p*H was recorded during autumn and winter in the first year however, in the second year the highest and lowest soil *p*H was recorded during winter and spring respectively. The soil *p*H value at Tuli tea garden was slightly acidic to acidic.

The *p*H values of soil samples collected from tea gardens at Ungma ranged from 4.98 - 6.11 and 5.02 - 6.24 in the first year at the rhizospheric and non-rhizospheric soil respectively (Table 2.4). The recorded soil *p*H in the second year varied from 5.16 - 5.72 and 5.22 - 5.75 (Table 2.4). The mean soil *p*H in the first and second year ranged from 5.00 - 6.18 and from 5.19 - 5.74 respectively. Among the seasons, the highest and the lowest soil *p*H were recorded during summer and winter in both the study years. The soil *p*H at Ungma was also found to be slightly acidic to acidic.

Table 2.4. Soil *p*H of Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	5.46 ±0.01	5.92 ±0.01	6.30 ±0.01	5.03 ±0.01	5.64 ±0.01	5.40 ±0.07	5.23 ±0.06	5.04 ±0.04
	NR	5.61 ±0.01	5.97 ±0.00	6.24 ±0.01	4.96 ±0.01	5.52 ±0.00	5.43 ±0.07	5.30 ±0.06	5.07 ±0.05
	Mean	**5.53 ±0.02^{c}**	**5.95 ±0.01^{b}**	**6.27 ±0.02^{a}**	**5.00 ±0.02^{d}**	**5.58 ±0.03^{b}**	**5.42 ±0.04^{c}**	**5.27 ±0.04^{a}**	**5.05 ±0.03^{d}**
Ungma	R	5.67 ±0.00	6.11 ±0.01	5.72 ±0.00	4.98 ±0.00	5.58 ±0.00	5.72 ±0.06	5.38 ±0.04	5.16 ±0.03
	NR	5.72 ±0.01	6.24 ±0.01	5.79 ±0.01	5.02 ±0.01	5.63 ±0.00	5.75 ±0.03	5.45 ±0.07	5.22 ±0.02
	Mean	**5.69 ±0.01^{b}**	**6.18 ±0.03^{a}**	**5.76 ±0.01^{b}**	**5.00 ±0.01^{c}**	**5.61 ±0.01^{c}**	**5.74 ±0.03^{b}**	**5.41 ±0.04^{a}**	**5.19 ±0.02^{d}**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

2.3.3. Soil moisture content

The soil moisture content at the rhizospheric and non-rhizospheric soil of Tuli tea garden ranged from 22.18 - 43.58% and 21.62 - 41.85% in the first year depicting the mean value ranging from 21.90 - 42.72% (Table 2.5). In the second year, it ranged from 25.14 - 44.98% at the rhizospheric and 24.15 - 42.44% at non-rhizospheric soil, depicting the mean soil moisture content ranging from 24.65 - 43.71% (Table 2.5). The highest soil moisture content in the first and second year was recorded during the autumn and winter season respectively whereas, the lowest soil moisture content was recorded during the winter season in both years.

In the Ungma tea garden, the soil moisture content ranged from 29.84 - 48.49% and 32.45 - 45.61% in the rhizospheric and non-rhizospheric soil depicting the mean value ranging from 31.15 - 47.05% (Table 2.5). In the second year, it ranged from 29.56 - 42.65% at the rhizospheric and 28.11 - 41.94% at non-rhizospheric soil, depicting the mean value ranging from 28.83 - 42.28% (Table 2.5). In both study years, the highest and the lowest soil moisture content were recorded during the summer and winter season respectively.

Table 2.5. Soil moisture content of Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	29.28 ±0.20	37.98 ±0.08	43.58 ±0.08	22.18 ±0.08	39.49 ±0.32	44.98 ±0.64	33.62 ±0.35	25.14 ±0.47
	NR	30.35 ±0.04	37.3 9±0.07	41.85 ±0.06	21.62 ±0.23	37.22 ±0.06	42.44 ±0.55	32.09 ±0.64	24.15 ±0.42
	Mean	**29.81 ±0.27^{c}**	**37.68 ±0.15^{b}**	**42.72 ±0.39^{a}**	**21.90 ±0.15^{d}**	**38.35 ±0.53^{b}**	**43.71 ±0.68^{a}**	**32.85 ±0.47^{c}**	**24.65 ±0.36^{d}**
Ungma	R	38.94 ±0.16	48.49 ±0.28	40.12 ±0.11	29.84 ±0.06	40.10 ±0.06	42.62 ±0.47	35.62 ±1.35	29.56 ±0.37
	NR	38.42 ±0.09	45.61 ±0.18	42.33 ±0.06	32.45 ±0.07	37.70 ±0.23	41.94 ±0.08	34.00 ±0.48	28.11 ±0.55
	Mean	**38.68 ±0.13^{c}**	**47.05 ±0.65^{a}**	**41.23 ±0.49^{b}**	**31.15 ±0.58^{d}**	**38.90 ±0.55^{b}**	**42.28 ±0.26^{b}**	**34.81 ±0.73^{c}**	**28.83 ±0.44^{d}**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

2.3.4. Soil organic carbon

SOC content at the rhizospheric and non-rhizospheric soil of Tuli tea garden ranged from 0.63 - 1.56% and 0.59 - 1.51% respectively in the first year depicting the mean value ranging from 0.61 - 1.54% (Table 2.6). In the second year, it ranged from 0.91 - 1.45% at the

rhizospheric and 0.89 - 1.44% at non-rhizospheric soil, depicting the mean value ranging from 0.90 - 1.45% (Table 2.6). In both years, the highest and the lowest SOC content was recorded during the spring and winter season respectively.

In the Ungma tea garden, SOC content ranged from 0.99 - 1.92% and 0.95 - 1.91% in rhizospheric and non-rhizospheric soil depicting the mean value ranging from 0.97 - 1.91% (Table 2.6). In the second year, it ranged from 0.97 - 1.69% at the rhizospheric and 0.91 - 1.66% at non-rhizospheric soil depicting the mean value ranging from 0.94 - 1.68% (Table 2.6). Among the seasons, the highest SOC was recorded during spring and summer in the first year and the second year respectively whereas, the lowest SOC content was recorded during the winter season in both years.

Table 2.6. Soil organic carbon content (%) of Tuli and Ungma tea garden 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	1.56 ±0.01	1.19 ±0.01	1.29 ±0.02	0.63 ±0.01	1.45 ±0.01	1.30 ±0.01	1.21 ±0.01	0.91 ±0.01
	NR	1.51 ±0.01	0.93 ±0.01	1.26 ±0.03	0.59 ±0.01	1.44 ±0.01	1.27 ±0.01	1.17 ±0.01	0.89 ±0.01
	Mean	**1.54 ±0.01^{a}**	**1.06 ±0.06^{c}**	**1.28 ±0.02^{b}**	**0.61 ±0.01^{d}**	**1.45 ±0.01^{a}**	**1.29 ±0.01^{b}**	**1.19 ±0.01^{c}**	**0.90 ±0.01^{d}**
Ungma	R	1.92 ±0.06	1.56 ±0.00	1.70 ±0.018	0.99 ±0.01	1.44 ±0.01	1.69 ±0.01	1.35 ±0.01	0.97 ±0.01
	NR	1.90 ±0.08	1.47 ±0.01	1.62 ±0.01	0.95 ±0.00	1.52 ±0.01	1.66 ±0.01	1.31 ±0.01	0.91 ±0.01
	Mean	**1.91 ±0.04^{a}**	**1.51 ±0.02^{c}**	**1.66 ±0.02^{b}**	**0.97 ±0.01^{d}**	**1.48 ±0.02^{c}**	**1.68 ±0.01^{b}**	**1.33 ±0.01^{a}**	**0.94 ±0.02^{d}**

R - rhizosphere; NR - non-rhizosphere.
Mean ± standard error mean.
Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

2.3.5. Soil temperature

Soil temperature at the Tuli tea garden ranged from 17.53 - 35.57 ºC and 18.70 - 36.63 ºC in the first year at the rhizospheric and non-rhizospheric soil respectively. The recorded soil temperature in the second year varied from 18.92 - 36.63 ºC and 19.18 - 36.94 ºC at the respective soil. The mean soil temperature in the first and second year ranged from 18.12 - 36.10 ºC and 19.05 - 36.78 ºC (Table 2.7). Among the seasons, the highest and the lowest soil temperature were recorded during summer and winter in both years.

Soil temperature at the Ungma tea garden ranged from 13.37 - 32.10 ºC and 13.90 - 34.10 ºC in the first year at the rhizospheric and non-rhizospheric soil respectively. The

recorded soil temperature in the second year varied from 15.01 - 29.07 ºC and 15.40 - 29.93 ºC at the respective soil. The mean soil temperature in the first and second year ranged from 13.63 - 33.10 ºC and 15.50 - 29.50 ºC (Table 2.7). Among the seasons, the highest and the lowest soil temperature were recorded during summer and winter in both years.

Table 2.7. Soil temperature (°C) of Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	28.57 ±0.18	35.57 ±0.12	33.47 ±0.26	17.53 ±0.09	24.97 ±0.09	36.63 ±0.28	33.76 ±0.19	18.92 ±0.11
	NR	27.43 ±0.20	36.63 ±0.45	35.10 ±0.12	18.70 ±0.12	25.87 ±0.03	36.94 ±0.35	34.00 ±0.35	19.18 ±0.13
	Mean	**28.00 ±0.28^{c}**	**36.10 ±0.32^{a}**	**34.28 ±0.39^{b}**	**18.12 ±0.27^{d}**	**25.42 ±0.21^{c}**	**36.78 ±0.21^{a}**	**33.88 ±0.19^{b}**	**19.05 ±0.10^{d}**
Ungma	R	24.10 ±0.12	32.10 ±0.10	30.17 ±0.15	13.37 ±0.09	21.23 ±0.18	29.07 ±0.17	25.88 ±0.10	15.61 ±0.27
	NR	22.33 ±0.15	34.10 ±0.10	29.60 ±0.12	13.90 ±0.06	22.13 ±0.09	29.93 ±0.03	26.26 ±0.07	15.40 ±0.22
	Mean	**23.22 ±0.15^{c}**	**33.10 ±0.40^{a}**	**29.88 ±0.45^{b}**	**13.63 ±0.15^{d}**	**21.68 ±0.22^{c}**	**29.50 ±0.21^{a}**	**26.07 ±0.10^{b}**	**15.50 ±0.16^{d}**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

2.3.6. Available nutrients

Available nitrogen

The available N content in the first year at the rhizospheric and non-rhizospheric soil of Tuli tea garden ranged from 413.13 - 673.19 kg/ha and 397.23 - 593.75 kg/ha respectively, with an average ranging from 405.59 - 619.79 kg/ha (Table 2.8). The season with the highest and the lowest available N was represented by the autumn and winter respectively. In the second year, the available N content at the respective soil ranged from 413.95 - 657.42 kg/ha and 405.59 - 621.69 kg/ha, with an average that ranged from 409.77 - 639.55 kg/ha (Table 2.8). The season with the highest and the lowest available N was represented by the summer and winter respectively. As per the Methods Manual Soil Testing in India (2011), that categorized available N under low (< 272 kg/ha), medium (272 - 544 kg/ha) and high (> 544 kg/ha) category, the available N content in Tuli falls under the medium and high category.

The available N content in the first year at the rhizospheric and non-rhizospheric soil of Ungma tea garden ranged from 238.34 - 510.12 kg/ha and 196.52 - 451.58 kg/ha respectively, with an average that ranged from 217.45 - 480.85 kg/ha (Table 2.8). In the

second year, the available N content ranged from 313.60 - 480.85 kg/ha and 275.97 - 443.22 kg/ha in the rhizospheric and non-rhizospheric soil, with an average that ranged from 294.78 - 544.77 kg/ha (Table 2.8). In both years, the season with the highest and the lowest available N was represented by spring and summer respectively. As per the Methods Manual Soil Testing in India (2011), the available N content in Ungma falls under the low and medium category.

Table 2.8. Available nitrogen content (kg/ha) at Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	673.19 ±4.18	593.75 ±11.06	645.83 ±3.23	413.95 ±7.24	480.85 ±7.43	657.42 ±10.36	601.93 ±4.82	413.95 ±8.08
	NR	564.48 ±7.24	547.75 ±15.08	593.75 ±11.06	397.23 ±4.18	430.68 ±4.18	621.69 ±9.10	575.99 ±6.49	405.59± 4.18
	Mean	**618.84 ±24.60^{a}**	**570.75 ±13.26^{a}**	**619.79 ±12.73^{a}**	**405.59 ±5.29^{b}**	**455.77 ±11.85^{c}**	**639.55 ±10.09^{a}**	**588.96 ±6.83^{b}**	**409.77 ±4.48^{d}**
Ungma	R	510.12 ±11.06	359.59 ±4.18	393.11 ±4.15	238.34 ±7.24	480.85 ±11.06	313.60 ±7.24	363.78 ±7.24	426.5 ±7.24
	NR	451.58 ±7.24	338.69 ±7.24	384.75 ±4.21	196.52 ±8.36	430.68 ±4.18	275.97 ±7.24	376.32 ±7.24	443.22 ±4.18
	Mean	**480.85 ±14.36^{a}**	**349.14 ±5.99^{c}**	**388.93 ±3.24^{b}**	**217.43 ±10.58^{d}**	**455.77 ±12.40^{a}**	**294.78 ±9.58^{c}**	**370.05 ±5.37^{b}**	**434.86 ±5.29^{a}**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

Available phosphorus

The available P content in the first year at the rhizospheric and non-rhizospheric soil of the Tuli tea garden ranged from 12.31 - 34.90 kg/ha and 9.86 - 34.04 kg/ha respectively, with an average that ranged from 11.08 - 34.47 kg/ha (Table 2.9). In the second year, the available P content at the respective soil ranged from 11.13 - 24.39 kg/ha and 10.09 - 27.06 kg/ha, with an average that ranged from 10.61 - 24.73 kg/ha (Table 2.9). In both years, the season with the highest and the lowest available P was represented by the spring and winter respectively. Based on the available P limit in soil by Baruah and Barthakur (1997), who categorized available P into low (< 22.5 kg/ha), medium (22.5 - 56 kg/ha) and high (>56 kg/ha), the available P content in Tuli tea garden falls under the low and medium category.

The available P content in the first year at the rhizospheric and non-rhizospheric soil of the Ungma tea garden ranged from 16.71 - 46.45 kg/ha and 15.69 - 45.57 kg/ha respectively, with an average that ranged from 16.20 - 46.01 kg/ha (Table 2.9). Spring and winter

represented the season with the highest and the lowest available P. In the second year, the available P content at the respective soil ranged from 28.61 - 47.06 kg/ha and 24.81 - 47.93 kg/ha, with an average that ranged from 26.71 - 47.49 kg/ha (Table 2.9). The season with the highest and the lowest available P was represented by the autumn and winter respectively. Based on the available P limit in soil by Baruah and Barthakur (1997), the available P content in Ungma tea garden falls under the low and medium category.

Table 2.9. Available phosphorus content (kg/ha) at Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	34.90 ±0.16	29.41 ±0.24	22.37 ±0.53	12.31 ±0.21	24.39 ±0.08	22.25 ±0.24	20.67 ±0.23	11.13 ±0.34
	NR	34.04 ±0.28	25.71 ±0.09	19.61 ±0.28	9.86 ±0.22	27.06 ±0.52	23.26 ±0.34	19.02 ±0.31	10.09 ±0.32
	Mean	**34.47 ±0.24^{a}**	**27.56 ±0.84^{b}**	**20.99 ±0.67^{c}**	**11.08 ±0.57^{d}**	**24.73 ±0.28^{a}**	**22.76 ±0.29^{b}**	**19.85 ±0.41^{c}**	**10.61 ±0.31^{d}**
Ungma	R	46.45 ±0.30	38.67 ±0.31	42.12 ±0.27	16.71 ±0.41	33.02 ±0.34	41.20 ±0.59	47.06 ±0.23	28.61 ±0.69
	NR	45.57 ±0.31	36.63 ±0.08	40.63 ±0.28	15.69 ±0.08	31.09 ±0.25	43.65 ±0.37	47.93 ±0.36	24.81 ±0.41
	Mean	**46.01 ±0.28^{a}**	**37.65 ±0.48^{c}**	**41.37 ±0.38^{b}**	**16.20 ±0.29^{d}**	**32.05^{c} ±0.47**	**42.43^{b} ±0.63**	**47.49^{a} ±0.27**	**26.71^{d} ±0.92**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

Available potassium

The available K content in the first year at the rhizospheric and non-rhizospheric soil of the Tuli tea garden ranged from 71.49 - 227.88 kg/ha and 55.87 - 224.19 kg/ha respectively, with an average that ranged from 63.68 - 225.92 kg/ha (Table 2.10). The season with the highest and the lowest available K was represented by the autumn and winter respectively. In the second year, the available K content at the respective soil ranged from 97.75 - 168.71 kg/ha and 103.21 - 175.50 kg/ha, with an average that ranged from 100.48 - 172.11 kg/ha (Table 2.10). The season with the highest and the lowest available K was represented by the autumn and winter respectively. As per the limits suggested by Methods Manual Soil Testing in India (2011), that categorized available K under low (<108 kg/ha), medium (108 - 280 kg/ha) and high (>280 kg/ha) category, the available K content in the Tuli tea garden falls under the low and medium category.

The available K content in the first year at the rhizospheric and non-rhizospheric soil of the Ungma tea garden ranged from 94.05 - 265.83 kg/ha and 92.31 - 253.69 kg/ha respectively, with an average that ranged from 93.18 kg/ha - 259.76 kg/ha (Table 2.10). The season with the highest and the lowest available K was represented by the autumn and winter respectively. In the second year, the available K content at the respective soil ranged from 93.92 - 157.42 kg/ha and 90.45 - 150.48 kg/ha, with an average that ranged from 92.19 - 153.95 kg/ha (Table 2.10). The season with the highest and the lowest available K was represented by the autumn and winter respectively. As per the Methods Manual Soil Testing in India (2011), the available K content in the Ungma tea garden falls under the low and medium category.

Table 2.10. Available potassium content (kg/ha) at Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	199.90 ±3.01	161.70 ±1.75	227.66 ±1.74	71.49 ±3.47	135.69 ±2.51	97.75 ±2.38	168.71 ±2.60	118.94 ±1.73
	NR	192.96 ±1.73	144.42 ±1.71	224.19 ±1.74	55.87 ±4.59	120.08 ±4.00	103.21 ±1.98	175.50 ±3.20	128.99 ±2.99
	Mean	**196.43 ±2.19^{b}**	**153.06 ±4.02^{c}**	**225.92 ±1.34^{a}**	**63.68 ±4.34^{d}**	**127.89 ±4.08^{b}**	**100.48 ±1.85^{c}**	**172.11 ±2.39^{a}**	**123.96 ±2.73^{b}**
Ungma	R	177.34 ±1.74	192.96 ±4.59	265.83 ±4.59	94.05 ±1.74	142.60 ±2.95	116.60 ±3.01	157.42 ±2.30	93.92 ±1.86
	NR	173.87 ±3.01	188.08 ±1.90	253.69 ±3.47	92.31 ±1.74	139.20 ±1.73	114.87 ±1.74	150.48 ±1.50	90.45 ±1.51
	Mean	**175.60 ±1.74^{c}**	**190.52 ±2.48^{b}**	**259.76 ±3.74^{a}**	**93.18 ±1.16^{d}**	**140.90 ±1.71^{b}**	**114.00 ±2.23^{c}**	**153.95 ±1.98^{a}**	**92.19 ±1.32^{d}**

R - rhizosphere; NR - non-rhizosphere.

Mean ± standard error mean.

Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

2.3.7. Statistical Analysis

ANOVA determined the association between two variables. Considering different sampling seasons as one variable and different soil properties as the other one, the association between the two can be determined by variance analysis. It detects whether the change in the properties of the soil is dependent on the sampling seasons. The season-wise data of soil physicochemical properties were pooled for one-way ANOVA. Except for clay in the first year at Ungma, the result revealed the significant effects of the sampling seasons ($p < 0.05$) in all parameters at the studied tea gardens (Table 2.11). Further, Tukey's post hoc test of significance for mean differences between the spring-summer, spring - autumn, spring

- winter, summer-autumn, summer - winter and autumn-winter revealed statistically significant differences in parameters including *p*H, moisture, SOC, temperature, available P at Tuli and in moisture, SOC, temperature, available P and available K at Ungma in both the years.

Two-way ANOVA conducted between the study sites and sampling seasons revealed statistically significant ($p < 0.05$) interaction effects of season and site in all the soil properties (Table 2.12).

Table 2.11. One-way ANOVA of soil physicochemical properties at Tuli and Ungma tea garden during 2016 - 2018

Parameter	**Tuli**				**Ungma**			
	2016 - 2017		**2017 - 2018**		**2016 - 2017**		**2017 - 2018**	
	F (3, 20)	*p* value	F (3, 20)	*p* value	F (3, 20)	*p* value	F (3, 20)	*p* value
Sand (%)	37.13	0.00	92.28	0.00	82.21	0.00	82.17	0.00
Clay (%)	33.03	0.00	118.17	0.00	0.21	0.89	4.00	0.02
Silt (%)	91.82	0.00	24.33	0.00	52.95	0.00	52.94	0.00
***p*H**	673.41	0.00	38.57	0.00	692.31	0.00	77.66	0.00
Moisture (%)	1226.55	0.00	241.07	0.00	168.99	0.00	121.63	0.00
Temperature (°C)	658.11	0.00	1997.23	0.00	727.91	0.00	1141.17	0.00
SOC (%)	163.07	0.00	911.17	0.00	212.88	0.00	470.54	0.00
Available N (kg/ha)	42.36	0.00	152.18	0.00	131.25	0.00	69.63	0.00
Available P (kg/ha)	258.65	0.00	365.23	0.00	1305.32	0.00	233.06	0.00
Available K (kg/ha)	480.80	0.00	107.59	0.00	763.72	0.00	225.22	0.00

Table 2.12. Two-way ANOVA of soil physicochemical properties during 2016-2018

Parameter	**Source**	**2016 - 2017**		**2017 - 2018**	
		F (3, 20)	*p* value	F (3, 20)	*p* value
Sand (%)	Site	888.49	0.00	1284.10	0.00
	Season	99.22	0.00	165.79	0.00
	Site x Season	4.07	0.01	9.00	0.00
Clay (%)	Site	1236.10	0.00	2035.92	0.00
	Season	16.28	0.00	89.22	0.00
	Site x Season	13.38	0.00	47.16	0.00
Silt (%)	Site	56.41	0.00	443.17	0.00
	Season	112.00	0.00	108.12	0.00
	Site x Season	18.88	0.00	22.82	0.00
***p*H**	Site	4.93	0.03	53.80	0.00
	Season	1220.47	0.00	96.46	0.00
	Site x Season	142.76	0.00	7.55	0.00
Moisture (%)	Site	26.84	0.00	12.56	0.00
	Season	944.78	0.00	352.47	0.00
	Site x Season	120.62	0.00	10.17	0.00
Temperature (°C)	Site	344.26	0.00	1934.46	0.00
	Season	1383.39	0.00	3068.90	0.00
	Site x Season	3.13	0.04	79.51	0.00

	Site	366.86	0.00	33.39	0.00
SOC (%)	Season	368.70	0.00	658.32	0.00
	Site x Season	1.01	0.40	12.85	0.00
	Site	454.00	0.00	474.36	0.00
Available N (kg/ha)	Season	127.96	0.00	15.80	0.00
	Site x Season	5.28	0.00	206.91	0.00
	Site	1075.02	0.00	2539.48	0.00
Available P (kg/ha)	Season	979.31	0.00	379.41	0.00
	Site x Season	78.37	0.00	144.06	0.00
	Site	97.04	0.00	11.71	0.00
Available K (kg/ha)	Season	1128.02	0.00	239.01	0.00
	Site x Season	45.56	0.00	44.32	0.00

Pearson's correlation coefficient was analyzed to understand the relationship between different physicochemical parameters. Most of the calculated values were established as strong correlations. The correlation pairs with a high positive '*r*' value between them indicate the dependency on one another whereas, pairs having a negative correlation value indicate an inverse relation between them. The correlation coefficient between the physicochemical properties at studied tea gardens are presented below (Table 2.13 - Table 2.16).

In the first year, the correlation between soil properties at Tuli tea garden revealed a mutually significant and positive correlation ($p < 0.01$ and $p < 0.05$) between clay, moisture, *p*H, temperature and available K (Table 2.13). SOC, available N and available K were also mutually correlated with one another while available P established a significant positive correlation with SOC and available N (Table 2.13). Likewise in the second year, a mutually significant and positive correlation ($p < 0.01$ and $p < 0.05$) existed between the moisture, *p*H, SOC and available P and between temperature, moisture and available N (Table 2.14). The relation between sand and clay and between silt and available K were significant and negative (Table 2.14).

As presented in Table 2.15, correlation studies of soil properties in the first year at the Ungma tea garden revealed a mutually significant and positive correlation ($p < 0.01$ and $p < 0.05$) between silt, moisture, temperature and *p*H. A mutually significant and positive correlation ($p < 0.01$ and $p < 0.05$) was also observed between SOC, available N and available P. Further, significant and positive correlation of temperature with available P and available K and significant and positive correlation of available P with temperature and *p*H were established. Contrary to this, sand was negatively correlated with almost all the studied soil properties. In the second year, a mutually significant and positive correlation ($p < 0.01$

and $p < 0.05$) existed between moisture, temperature and *p*H (Table 2.16). Further, correlations of parameters like SOC with moisture, *p*H and temperature, temperature with silt and available P, available N with sand were significant and positive ($p < 0.01$ and $p < 0.05$) however, the correlation of sand with silt, temperature and available P was significant and negative ($p < 0.01$ and $p < 0.05$) Table 2.16.

Table 2.13. Correlation between soil properties at Tuli tea garden during 2016 - 2017

Parameters	**Sand (%)**	**Clay (%)**	**Silt (%)**	**Moisture (%)**	***p*H**	**Temp. (°C)**	**SOC (%)**	**AN (kg/ha)**	**AP (kg/ha)**	**AK (kg/ha)**
Sand (%)	1.00									
Clay (%)	-0.53	1.00								
Silt (%)	-0.61	-0.30	1.00							
Moisture (%)	-0.68	.903**	-0.04	1.00						
***p*H**	-0.65	.915**	-0.08	.996**	1.00					
Temp. (°C)	-0.64	.719*	0.09	.927**	.926**	1.00				
SOC (%)	0.20	0.50	-0.65	0.51	0.55	0.56	1.00			
AN (kg/ha)	-0.07	0.59	-0.44	.729*	.736*	.760*	.901**	1.00		
AP (kg/ha)	0.15	0.19	-0.33	0.36	0.39	0.56	.875**	.786*	1.00	
AK (kg/ha)	-0.12	.804*	-0.56	.803*	.828*	.750*	.896**	.927**	0.67	1.00

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

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

Table 2.14. Correlation between soil properties at Tuli tea garden during 2017 - 2018

Parameter	**Sand (%)**	**Clay (%)**	**Silt (%)**	**Moisture (%)**	***p*H**	**Temp. (°C)**	**SOC (%)**	**AN (kg/ha)**	**AP (kg/ha)**	**AK (kg/ha)**
Sand (%)	1.00									
Clay (%)	-.858**	1.00								
Silt (%)	0.12	-0.61	1.00							
Moisture (%)	-0.13	0.00	0.21	1.00						
***p*H**	0.41	-0.40	0.15	.818*	1.00					
Temp. (°C)	-0.64	0.64	-0.25	.739*	0.39	1.00				
SOC (%)	0.32	-0.27	0.02	.837**	.965**	0.49	1.00			
AN (kg/ha)	-0.70	0.70	-0.27	0.69	0.28	.965**	0.36	1.00		
AP (kg/ha)	0.14	-0.10	-0.02	.885**	.924**	0.64	.979**	0.51	1.00	
AK (kg/ha)	0.08	0.41	-.911**	-0.39	-0.16	0.06	-0.09	0.02	-0.08	1.00

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

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

Table 2.15. Correlation between soil properties at Ungma tea garden during 2016 - 2017

Parameter	**Sand (%)**	**Clay (%)**	**Silt (%)**	**Moisture (%)**	***p*H**	**Temp. (°C)**	**SOC (%)**	**AN (kg/ha)**	**AP (kg/ha)**	**AK (kg/ha)**
Sand (%)	1.00									
Clay (%)	0.04	1.00								
Silt (%)	-.967**	-0.15	1.00							
Moisture (%)	-.872**	0.04	.813*	1.00						
***p*H**	-.777*	0.25	.713*	.964**	1.00					

Temp. (°C)	-.816*	0.22	.760*	.947**	.956**	1.00				
SOC (%)	-0.11	0.11	0.05	0.56	0.65	0.60	1.00			
AN (kg/ha)	0.00	0.11	-0.03	0.47	0.57	0.51	.981**	1.00		
AP (kg/ha)	-0.25	0.14	0.20	0.67	.756*	.719*	.986**	.951**	1.00	
AK (kg/ha)	-0.47	0.12	0.42	0.68	0.69	.828*	0.70	0.62	.784*	1.00

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

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

Table 2.16. Correlation between soil properties at Ungma tea garden during 2017 - 2018

Parameter	**Sand (%)**	**Clay (%)**	**Silt (%)**	**Moisture (%)**	***p*H**	**Temp. (°C)**	**SOC (%)**	**AN (kg/ha)**	**AP (kg/ha)**	**AK (kg/ha)**
Sand (%)	1.00									
Clay (%)	-0.56	1.00								
Silt (%)	-.959**	0.30	1.00							
Moisture (%)	-0.55	-0.04	0.64	1.00						
***p*H**	-0.51	-0.19	0.65	.965**	1.00					
Temp. (°C)	-.841**	0.31	.860**	.819*	.802*	1.00				
SOC (%)	-0.57	0.00	0.65	.980**	.972**	.872**	1.00			
AN (kg/ha)	.961**	-0.57	-.910**	-0.53	-0.52	-.823*	-0.59	1.00		
AP (kg/ha)	-.775*	0.54	0.71	0.49	0.44	.869**	0.57	-0.71	1.00	
AK (kg/ha)	-0.04	0.05	0.03	0.36	0.33	0.48	0.44	0.02	0.63	1.00

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

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

2.4. Discussions

Soil properties vary due to differences in soil parent material, climate, irrigation and biological activity (Zheng et al., 2012). Such variations can have a pronounced effect on plant growth and production. Seasonal or periodic evaluation of soil physicochemical properties in agricultural soil like tea soil is imperative for improvement in the nutrient management system and sustainable production. Although the tea plant is widely adaptable to areas with a wide altitudinal range, however differences in soil physicochemical properties between tea growing altitudes can have a pronounced impact on tea growth, yield and quality. Therefore, comparative studies in soil physicochemical properties between tea gardens especially those differing in altitudes are crucial for developing strategies for sustaining tea quality in a particular region. The effect of seasons and altitudes for some important physicochemical parameters including soil texture, *p*H, temperature, moisture content, SOC and available nutrients have been discussed in this section. This is intended to create a better understanding of the dynamic nature of the soil properties as well as to establish a significant relationship between these properties in the tea gardens studied.

2.4.1. Variations in soil texture

The soil texture depends on the relative proportion of sand, silt and clay particles. Estimation of sand, silt and clay content is crucial for soil and agricultural engineering researches particularly, for the determination of draft requirements in specified soil (Al-Hamed, 2014). The studied tea garden soils have a high proportion of sand and lesser content in silt and clay, which indicates the dominance of sand forming minerals in parent materials. This result is consistent with prior studies in tea soils (Li et al. 2012; Nath 2014 and Ruto et al., 2019) but contradicts with Haorongbam et al., (2014) who reported dominancy of clay in their study. Generally, tea plantation involves management practices such as frequent removal of vegetation cover therefore lack of plant cover might have accelerated soil erosion leading to dominancy of sand in the studied tea gardens. The present study showed the ability of tea plant to grow in a wide variety of soil textural types which agrees with the previous study (Nyabundi et al., 2016). The textural classes of the study areas fall under sandy clay loam in Tuli to sandy clay and clay loam in Ungma which is partially in alignment with prior researches in tea growing soils (Baishya and Sharma 2017; Misra et al. 2018; Haorongbam et al. 2018). Additionally, the present result is more or less similar to the results regarding the soils in other districts of Nagaland. For instance, soils in Kohima, Longleng and Tuensang districts of Nagaland fall under sandy clay and sandy clay loam (Bier and Singh, 2018). Furthermore, the study indicates that when comes to its soil texture tea plant does not seem to be over-particular rather, it can thrive on the soil of nearly any texture (Othieno, 1992). However, for optimum tea production, the soil texture at the lower altitude of Tuli tea garden can be considered better than that of Ungma tea garden which is situated at a higher altitude because sandy clay loam soil observed in the former is considered best for tea plant (Nath, 2015).

The significant effect of the season ($p < 0.01$) on the sand, silt and clay content were observed in each of the tea gardens except for clay content in the first year at Ungma tea garden. However, the soil textural class remained the same throughout the study period in each of the tea gardens except in one season at Ungma tea garden (Table 2.3). Such consistency of soil textural class is because soil texture does not change by management practices except by processes such as erosion, deposition, truncation and some other human interventions (Osman, 2013). Even though the sand, silt and clay content exhibited different pattern over the season, the highest sand content was observed in the spring season in both

years likewise, the highest content of silt observed in the summer season in both years. However, clay content exhibited a different and uneven relationship over the season with higher content for the autumn season in Tuli and spring and autumn in Ungma (first and second year). Such variation can be attributed to the uneven distribution of environmental conditions over the study period.

2.4.2. Variations in soil temperature

Soil temperature determines most of the physiochemical and biological factors including moisture regime, the metabolic activity of soil microorganisms, seed germination and growth of plant roots. Being a major factor that controls growth rates and yields of tea shoot (Carr, 1970), it is crucial for tea production. The soil temperature distribution is a major factor that determines the lengths of growing seasons in the tea plant. Significant changes in soil temperature due to season were established in the studied tea gardens which agree with other studies (Wang et al., 2018). Seasonal and daily variation in soil temperature occurs because of change in radiant energy and energy changes that take place through the soil surface (Chiemeka, 2010). It is also greatly influenced by air temperature (Wang et al., 2018). The seasonal fluctuation of the soil temperature at both tea gardens followed the same pattern throughout the study period with the maximum and minimum soil temperature in the same season in both years. The soil temperature peaked during summer at both the tea gardens. Higher soil temperature in summers could probably be due to the increase in solar radiation during this season. According to Geiger et al., (2003), the soil temperature increases with the increase in solar radiation reaching the soil surface. Additionally, higher soil temperature can also be attributed to a more perpendicular position of the sun (Jayasinghe et al., 2020). Lower soil temperature in winter can be related to a lower intensity of radiation and an increase in evaporation rates. At both the tea garden, non-rhizospheric soil exhibited higher temperature than rhizospheric soil in almost all seasons, which probably because the rhizospheric soil is closer to the root with greater soil moisture content.

Significant effect of season and site interaction was established in the present study indicative of the fact that the amount of solar radiation that soil receives, absorbs and transmits can vary with geographic, climatic, edaphic and topographic conditions (Osman, 2013). Between the tea gardens, higher soil temperature was recorded at the Tuli tea garden than that at the Ungma tea garden in all seasons. The altitudes of the study sites can best

explain this because with every increase in altitude, there is always a decrease in temperature and thus, the soil temperatures of higher altitude are cooler than the lower altitude (Osman, 2013).

2.4.3. Variations in soil moisture content

Soil moisture is an important property that controls the rate of chemical and biological processes in the soil. Plants take up water from the soil for their survival and development therefore, a preferred range of soil water content within an area is crucial. Excessive or limited soil moisture is harmful to plants as the former result in leaching of nutrients from the soil, poor aeration of the soil and root rot while the latter, effect nutrient movements and photosynthesis process. Seasonal variations in soil moisture content were well documented from both tea gardens. Similar results were observed in other studies (Roxy et al., 2010; Li et al., 2016). As far as the moisture content at the study sites is concerned, no definite pattern was observed however, the highest moisture content was recorded either in summer or in the autumn season. Such differences can be attributed to seasonal changes in precipitation. In the present study, higher moisture content during summer and autumn coincided with the respective maximum rainfall period at the study areas in the corresponding years while the lowest soil moisture during winter coincided with the cold dry period of January month at the Mokokchung district. It is not surprising to get such a result because, under maximum rainfall, moisture levels in soil tend to increase. The soil moisture content after heavy rain often reaches its highest value but decrease sharply for few days after that when the rain stops due to evapotranspiration absorbed by roots and runoffs (Niu et al., 2015). Low soil moisture content in winter could also be attributed to a higher precipitation rate as well as the use of soil water by plants for transpiration (El Masri, 2017). Additionally, the importance of soil temperature as one of the contributing factors cannot be ignored because moisture flows due to temperature gradient, which falls downward in summer and goes upward in winter. In both tea gardens, the higher moisture content in rhizospheric soil as compared to non-rhizospheric soil occurred probably due to mucilage exuded by the roots that increased the water holding capacity of the rhizosphere (Young, 1995).

Significant effect of site and their interaction ($p < 0.05$) with sampling season were observed for soil moisture. Furthermore, the mean soil moisture level was slightly higher in Ungma compared to Tuli throughout the study period, which could be due to greater

moisture retention and shade trees, among other factors in the former study site. While lower moisture content at Tuli tea garden could be due to rapid run-off from the slopes and low water retention capacity of the soil. It could also be because the Ungma tea garden is inherently wetter than the Tuli tea garden.

2.4.4. Variations in soil *p*H

The essentiality of *p*H study in any agricultural soil is well understood from the fact that it is one of the important determiners for soil health. The majority of the soil nutrients are affected by *p*H indicating its importance in the nutrient management system. Optimum *p*H is, therefore, the most important condition for better growth of tea (Jayasinghe et al., 2019). Irrespective of the location and season, the *p*H of the soil samples studied were slightly acidic to acidic. Such acidic nature of the soil *p*H can be seen in other areas of Mokokchung district and other parts of Nagaland state (Patton et al. 2005; Amenla et al. 2010). Furthermore, the acidic nature of tea soil has been reported to be common in tea soils (Baruah et al. 2013; Gogoi et al. 2016; Misra et al. 2018). Such low *p*H in tea growing soils has been attributed to several factors including acceleration of the net loss of base cations through leaching (Igwe et al. 1999), parent material, heavy rainfall, weathering processes and high Al, iron and manganese contents (Karak et al., 2011), monocropping of tea for a very long period (Gogoi et al., 2016) and use of nitrogenous fertilizers (Huu Chien et al., 2019; Ruan et al., 2000).

Seasonal fluctuations in soil *p*H were evident from the study where the autumn (represented by October) and spring (represented by April) season depicted the highest soil *p*H in the Tuli tea garden while the summer (represented by July) seasons depicted the highest soil *p*H in the Ungma tea garden (Table 2.4). As for the lowest soil *p*H, it was recorded in the winter (represented by January) seasons in both the tea gardens. The variability in *p*H could be related to differences in rainfall, depletion of exchangeable bases between seasons and time of fertilizers used in the study areas. Contrary to the present work, Barauh et al., (2013) observed lower soil *p*H during April-October as compared to November-March in tea garden belts of Golaghat district, Assam. The present study revealed a lower soil *p*H value in rhizospheric than in non-rhizospheric soils in both the tea gardens in almost all seasons. which agrees with Pandey and Palni (1996), who mentioned that the *p*H of rhizospheric soil is in general lower than that of the non-rhizospheric soil. Comparatively,

lower soil *p*H value in rhizospheric soil in the present study could probably be due to the excretion of organic acids from the tea roots and differences in microbial activities.

Between the tea gardens, the soil *p*H at Tuli (situated at lower altitude) was lower than that of Ungma tea garden (situated at higher altitude) in almost all the seasons indicating the significance of inherit parent material, altitudinal and agricultural activity differences in controlling the *p*H level in the tea soils of Mokokchung district. The present study more or less agrees with Lin et al., (2020) who observed an increase in soil *p*H with altitude but contradicts Mathew et al. (2016) who reported higher soil *p*H at the lower elevation at Mount Kilimanjaro of northern Tanzania. Such contradictory results with the present study could be due to difference in other soil properties and environmental conditions. In the present study also the significant correlations of soil *p*H with the various parameters such as moisture, temperature, organic carbon and available nutrients were established at both the tea gardens which could mean that a combination of these factors is involved in influencing the soil. Furthermore, the soil *p*H values and its correlation level with other parameters differed between the seasons as well as sites. Therefore, the acidic nature of the tea garden soils in the present study can be attributed to inherit of parent material, monocropping of tea, one or more environmental parameters, fertilizers application and other management practices mediated change in soil properties. Despite all these, it is noteworthy that, the tea plant grows best in acidic soil, with a *p*H between 4.0 and 6.0 as ideal values (Othieno, 1992). Therefore, it can be concluded that the studied tea garden soils are favourable for tea plant as far as their soil *p*H is concerned.

2.4.5. Variations in soil organic carbon

SOC is the basis of the soil system because the physical, chemical and biological properties and productivity of soil are closely related to the content and character of organic carbon (Zhangliang, 2016). SOC plays an important role in nutrient regulation under tea-growing soil (Misra et al. 2018) and it is controlled by complex interactions of many factors (Burke et al., 1989). This agrees with the correlation analysis of SOC with other soil properties in the present study (Table 2.13 - Table 2.16). SOC content changed remarkably with the season in both tea gardens. Similar results were observed in other tea garden soils (Gogoi et al., 2016). Karak et al. (2011) attributed higher SOC in tea gardens to the incorporation of a higher dose of organic matter in the soil due to tea leaf littering, cutting of

tea branches, leaf littering of shade tree and application of organic matter. A significantly ($p < 0.05$) higher SOC was observed in the spring seasons at Tuli tea garden whereas, at Ungma tea garden, it was observed in the spring and summer season (first and second year). This could be associated with more incorporation of tea leaves and branches debris, mineralization process under favourable conditions such as moisture content and temperature at these seasons in each tea gardens because the content of SOC depends on the balance between the rate of inflow of fresh debris and the decomposition of organic matter (Dluzewski et al. 2019). According to Wang et al. (2000), an increase in soil moisture content enhances the decomposition of soil organic matter until an optimal soil moisture level is reached and then inhibits decomposition when more pores in soils become saturated with water and oxygen availability for microbes becomes limited. Likewise, Buschiazzo et al., (2004) mentioned that the potential deterioration of both temperature and soil water regimes can diminish the capacity of the soil to accumulate soil organic matter. Therefore, an increase in soil temperature and moisture content after winter might have paved the way for higher microbiological activity while the decrease in the same in winters might have limited microbiological activity ultimately resulting in higher SOC in the studied tea gardens. Additionally, management practices can also result in substantial fluctuations of SOC over a year in seasonal patterns (Wuest, 2014). Thus, the factors responsible for seasonal variations in SOC in the tea gardens studied are attributed to conditions such as management practices, tea leaves production, amount of leaves litter, decomposition of biomass and microbial activity under favourable moisture and temperature.

The pattern of variation in SOC differed between the tea gardens and there was a significant effect of site and significant interaction between site and season at $p < 0.05$ (Table 2.12) affirming the relation of SOC with altitude (Anandacoomaraswamy and Ananthacumaraswamy, 1999), climatic condition, soil type, mineralization process and land use type and management (Zhangliang, 2016). Comparatively higher SOC at Ungma tea garden was observed throughout the study periods which agrees with Saeed et al., (2014). The result observed in the present study could due to specific characteristics of each tea gardens such as interactions between changes in litter inputs and subsequent immobilization mediated by soil microorganisms. Homann et al. (1995) observed that up to 50% of the variation in SOC at the western portion of the Oregon state in the USA was explained by site characteristics, such as climatic variables, soil texture and slope. It can also be the result of

the congenial temperature and faster decay of the organic matter favouring new microbial growth in the Ungma tea garden.

2.4.6. Variations in available nitrogen

N is the most important among the macronutrients for yield and quality improvements of tea (OH et al., 2006). According to Owuor (1997), N nutrition enhance the tea yield under favourable conditions of temperature, rainfall, relative humidity and evaporation but without adverse effects of large amounts of N supply. Tea cultivation focus on regular harvesting of young shoots therefore, the N requirement in tea is comparably higher than other crops (Varmazyari and Cakmakci, 2018) and amounts to about 5% of harvested shoots in dry weight (Barooah et al., 2005). Available N represents part of the total N in soil accessible by plants for usage. Knowledge of N availability in soil is a prerequisite for optimizing N fertilizer recommendations. In the present study, seasonal variations in available N content were observed (Table 2.8), which is consistent with studies conducted in other tea garden soils (Barauh et al., 2012; Gogoi et al., 2016). According to Sulkava et al., (1996), the change of environmental factors, in general, affect the seasonal patterns of N mineralization and this can create difficulty in optimizing N fertilizer applications (Sitienei et al., 2013). The highest available N content at Tuli was observed during the autumn and summer season (first and second year) while, the lowest values were recorded during the winter seasons. Contrary to this, the highest values at Ungma were recorded during the spring seasons while the lowest value was observed during the winter and summer season (first and second year). These seasonal variations within and between tea garden probably occurred due to differences in the mineralization rates, plants and microbial uptake, management practices, soil erosion, leaching and runoff between the tea gardens as well as between the seasons. Furthermore, it also indicates the importance of N recycling in response to seasonal fluctuation of other soil properties such as soil moisture, *p*H and temperature on available N content. An increase in soil moisture after dry winter and favourable temperature resulting in a higher rate of soil organic matter decomposition and favourable condition for mineralization and nitrification can be accounted for comparably higher available N content during summer and autumn season in Tuli as well as during spring seasons in Ungma. Li et al., (2004) reported that soil moisture was one of the important factors influencing N mineralization in the Ailao mountain region. Because of their importance in soil aeration, soil temperature and moisture probably

have the greatest influence on nitrification (Grundmann et al., 1995). Additionally, the input of large amounts of N fertilizers to agricultural fields influences microbiological processes, especially nitrification and denitrification that subsequently resulted in increased production of N_2O (Akiyama et al., 2006). On the other hand, with the exception in the second year at Ungma tea garden, the lowest available N content was recorded in winter seasons and this can be attributed to cool and dry conditions leading to slow decomposition rate and other properties such as soil *p*H. Rao (2001) also made similar observations where different forms of N were less available to plants because of low soil *p*H. The variations in available N were also observed between rhizosphere and non-rhizosphere in both the tea gardens, which perhaps can be due to differences in nitrifying microbial populations along with other factors.

Significant effect of the sampling sites and interaction between sites and seasons ($p < 0.05$) for available N content was evident from the study. It is noteworthy that, the pattern of variations differed between the study years as well as between the tea gardens. The available N content fluctuated between a medium to high amount at Tuli tea garden and between low to a medium amount at Ungma tea garden. Despite this, the values recorded in this study (217.43 - 639.55 kg/ha) was comparatively higher than those reported from other tea garden soils (Barauh et al., 2013; Gogoi et al., 2016). The differences observed between tea gardens in the present study as well as with others work probably might have been the result of differences in soil physicochemical properties acting on soil microbial activities, differences in the efficiency of the nitrification process or substantial microbial assimilation between these tea gardens. Grundmann et al., (1995) suggested that temperature either was a selecting factor for the microbial population composition or inducer of physiological adaptation of the nitrifiers. Additionally, the type of fertilizers used and fertilizer application methods can partially explain the observed differences.

2.4.7. Variations in available phosphorus

P is a decisive role player among macronutrients that stimulates root development and aid in normal tea growth (Ahmed et al., 2005). High P concentration in soil enhances the tea shoots catechins and caffeine and theaflavins, thearubigins as well as brightness and total colour of made tea (Gogoi et al., 1993). Available P constitutes only a fraction of total P in soil that is readily available for absorption by plant root and its utilization efficiency in the soil is very low due to its solubility and mobility thereby making it the second most limiting

factors for tea productivity (Varmazyari and Cakmakci, 2018). The seasonal variations in available P observed in the present study agrees with others (Saunders and Metson, 1971; Wang et al., 2018). Seasonal variation in P content occurs due to differences in the degree of response to phosphate fertilizer (Saunders and Metson, 1971) and physiochemical changes in soil due to management practices (Sharpley et al., 1995). In the tea gardens studied, the spring and autumn seasons favoured available P content whereas, the winter season was the least favourable. Higher values in spring were also observed by Scott and Cullen (1965), who hypothesized that build-up of available phosphate during the period of slow growth in winter resulted in higher values of available P. Similarly, Saunders and Metson (1971) reported higher available P during spring and suggested the release of phosphate from organic residues and soil organic matter might have been the reason. Higher accumulation of available P in autumn during the second year at Ungma might be related to the plant senescence and microorganism degradation (Bardgett et al., 2005; Jefferies et al., 2010; Wang et al., 2018). Lower available P in winter was expected because soils with low temperature are related to low available P owing to the fact that the release of P from organic material is hindered by low temperature (Gahoornia and Nielsen, 2003). Additionally, low available P in winter seasons might be the result of poor mineralization or fixation with metals in soil under low soil *p*H and moisture content. Bonheure and Wilson (1992), mentioned that the available P content is at its highest when the soil *p*H is between 5.5 and 7. However, this does not seem to be entirely true in the present study because the season with the highest as well as the lowest available P content falls under this *p*H value. Nevertheless, in lower soil *p*H, large quantities of soil P stocks can fix as free oxides and hydroxides of aluminium and iron, which subsequently reduce the P availability to plants. Therefore, it is reasonable to suggest that the lowest available P content observed in the study areas during winter seasons were partially due to the lowest soil *p*H. Seasonal variations in available P content in this study, therefore, occurred as are a result of multiple factors relationships of available P with other soil properties, environmental conditions and human interference especially through fertilization that modify the soil properties which subsequently create such differences in available P content. At both the tea gardens available P was observed to be slightly more in rhizospheric soil in most sampling seasons because chemicals such as protons, carboxylates, phosphatases, and phytases released from the rhizosphere are important in solubilizing P from sparingly available pools in soil (Shen et al., 2011).

The significant effect of sites and season interaction at $p < 0.05$ (Table 2.12) for available P content was revealed by two-way ANOVA. Furthermore, the concentrations of available P recorded in the present study were comparatively lower than those recorded in the studies of Barauh et al., (2013) and Gogoi et al., (2016) but higher than those recorded in the study of Baishya and Sharma (2017). All these indicate the importance of the altitude and geographical location of tea gardens in determining the tea soil available P content. Comparatively, the lower amount of available P content at Tuli may be due to a lesser amount of inherent soil P content and a high degree of P fixation (Kiflu et al., 2017). While higher available P content at Ungma can be due to greater solubilization of native P owing to phosphate solubilizers. Another possible explanation for this result would be the parent material because weathering of primary minerals contributes to the soil available P pool (Cross and Schlesinger, 1995).

2.4.8. Variations in available K

K is the second most important nutrient next only to N for tea plant growth, metabolism and development and it interacts with other nutrients as well as with many cultural factors (Ranganathan and Natesan, 1985). In the present study, low to medium level of available K was observed, which show consistency with Saha (2004) who also observed similar result in about 86 % of tea soils in Darjeeling hills. The values recorded from the present study were comparatively higher than those recorded in other tea soils (Barauh et al., 2013; Baishya and Sharma, 2017). Although the tea plant requires K from a moderate to a high level (Sitienei et al., 2013), agricultural soils including tea plantations are commonly low in plant-available K (Bagyalakshmi et al., 2013). Furthermore, the soil reserves of this nutrient deplete because of its removal in a large amount in the leaf (Sitienei et al., 2013).

Seasonal studies revealed significantly higher ($p < 0.05$) available K content during autumn seasons at both tea gardens (Table 2.10). The higher available K content in autumn seasons probably occurred due to the influence of soil properties such as temperature, moisture content and SOC as indicated in correlation studies (Table 2.13 - Table 2.16). Soil moisture content is one of the important factors that create a favourable soil environment for available K in tea soil (Meena et al., 2006). Similarly, Mouhamad et al., (2016) observed that an increase in soil moisture and temperature increase greater availability of K. Additionally, it could also be due to the reduction in the plant uptake and nutrient requirements (Omer et

al., 2018). The lowest available K content was observed in winters at the Ungma tea garden however, it varied from winter to summer at Tuli tea garden indicating the dynamic nature of soil and influence of other parameters in this nutrient. Low available K content during winter can be due to low cations exchange and replacement of K by NH^+ arising due to high accumulation of aluminium (Ruto et al., 2019) and root exudates (Shin, 2014). It could also be due to other soil properties such as a decrease in soil *p*H. According to previous studies, the efficiency of K decreases with a decrease in soil *p*H from 5.2 to 4.5 due to a decrease in binding strength of K^+ ions on the soil clay complex with the increase in soil acidity (Ranganathan and Natesan, 1985). This seems to partially agree in the present study at least in the case of the Ungma tea garden because the lowest soil *p*H which was observed in winters fall under the stated range. Available K content varied slightly between the rhizosphere and non-rhizosphere soil at both the tea gardens. Higher content in rhizospheric soil could be associated with secretions of various compounds from tea roots and microbial activity.

The present study showed that the available K content varied between tea gardens and the effect of sites and interaction between sites and seasons were significant ($p < 0.05$) as shown in two-way ANOVA study (Table 2.12). Comparison between tea gardens revealed higher available K content in Ungma as compared to that of Tuli indicating the influence of altitude. K variation due to altitude was also carried out in one study where the nutrient release was found to be highest at a higher elevation (Sheikh and Kumar, 2010). Another possible explanation associated with this could be differences in the important cultural operations performed on the tea plant such as young tea training, pruning, tipping and plucking (Ranganathan and Natesan, 1985), potash solubilizing microbes and variation in the degree of leaching of K.

2.4.9. Relationships between soil physicochemical properties

Pearson's correlation analysis revealed that there were significant correlations ($p <$ 0.01 and 0.05) between most of the soil physicochemical properties in both tea gardens. The soil *p*H and moisture were significantly positively correlated with each other in both tea gardens (Table 2.13 – Table 2.16). A mutual significant positive correlation between other soil physicochemical properties was also observed depicting its importance in mapping the tea ecosystem. For instance, SOC was positively correlated with soil temperature and

moisture (Table 2.14 and Table 2.16) suggesting that these soil properties are one of the main driving factors for accelerating SOC mineralization. Furthermore, the significant correlation shown by soil temperature with other soil physicochemical properties like *p*H, moisture, SOC and nutrients in the present study may be explained in terms of the fact that temperature determines nutrient availability through its impact on microbial activities under favourable moisture and *p*H. The present result finds an agreement with others (Grundmann et al. 1995; Vigil and Kissel, 1995) who reported that change in soil temperature significantly affects the rate of decomposition and movements of nutrients and chemical in the soil profile.

The correlations of soil textural particles with other soil properties were also established in the present study. A significant or non-significant negative correlation of sand with almost all other soil physicochemical properties is because sand is a dominating soil physical property that affects other soil properties especially silt, soil acidity and temperature and soil water. The negative correlation of sand with parameters like soil moisture was expected because water drains quickly in the sand than in clay and silt. Additionally, large and numerous pores of sand size make the soil water easily evaporates into the air, weaken the soil suction and draw the soil water easily (Liu et al., 2016). This also indicates the importance of soil texture in determining the rate of soil moisture change, soil water retention and transmission capacity.

The present study also demonstrated the correlation between available nutrients as well as their corresponding correlation with other soil physicochemical properties. A significant positive correlation was observed between N, P and K in the tea gardens (Table 2.13 and Table 2.15), which shows that these crucial soil elements are intimately interlinked. A high correlation between available P and soil *p*H were established in other studies (Jusoff, 2004) which corroborate the present work. Positive correlations between available P and soil moisture, *p*H and SOC depicts that available P is derived from organic matter and soil reaction and soil water governs the P availability in the studied tea gardens.

It is noteworthy that the correlation between soil physicochemical properties was not consistent between tea gardens. For instance, in the Tuli tea garden, the clay content was positively correlated with soil moisture, *p*H and temperature in the first year whereas, the silt content established strong positive correlations with these soil properties in the Ungma tea garden in the second year which confirms that climate and parent material profoundly influence soil characteristics (Schinner 1982; Yang et al. 2008; Charan 2013). Likewise, the

level of significance in correlations ($p < 0.01$ and 0.05) between other soil physicochemical properties differed between tea gardens which indicate that altitude is an important factor that determines the spatial heterogeneity of soil physicochemical properties. Different altitudes can result in different influencing factors such as light, humidity, air and hence, soil physicochemical properties may also show differences. Additionally, this also indicates multiple controlling factors for tea growth.

2.5. Conclusion

The present study demonstrated differences in the soil physicochemical properties and available nutrients in tea gardens at the Mokokchung district of Nagaland occur due to the sampling season and altitude. The interactions of tea gardens with sampling season showed a significant influence for all soil properties except soil texture. Seasonal difference in sand, silt and clay content were significant in the Tuli tea garden throughout the study period. Likewise, in the Ungma tea garden, the seasonal difference in the sand, silt and clay content were significant in both years except for clay content in the first year. The soil textural class remained the same as sandy clay loam throughout the study period in the Tuli tea garden whereas, in the Ungma tea garden, the soil textural class remained the same as sandy clay in almost all the sampling seasons.

At the Tuli tea garden, soil *p*H and soil moisture content were higher in the autumn season in the first year but in the second year, soil *p*H was higher in the spring season while soil moisture content was higher in the summer season. In both years, soil properties like SOC and available P were highest in the spring season while soil temperature and available K was highest in summer and autumn season respectively. At the Ungma tea garden, SOC and available P higher in the spring season in the first year however, SOC was higher in the summer season while available P in the autumn season in the second year. In both years, soil *p*H, soil moisture content and soil temperature were highest in the summer season while available N content and available K was highest in the spring and autumn season respectively.

The correlation studies showed a significant mutual correlation among the soil physicochemical properties however, the level of significance vary between years in each of the tea gardens as well as between the tea gardens. The differences in correlation between soil physicochemical properties in each tea gardens showed the multiple controlling factors

for tea growth while, the correlation differences between soil physicochemical between the Tuli and Ungma tea garden in between indicate the influence of altitude, an area's geographical conditions and climatic factors on tea soil.

Between the tea gardens, soil physicochemical properties like soil temperature, available P were higher at the lower altitude whereas, soil *p*H, SOC, available N were higher at the higher altitude in all seasons. However, available K content has not shown any trend with altitude. Available N content at the Tuli tea garden was medium to high whereas, the Ungma tea garden had low to medium available N content. Available P and available K content at both tea gardens fall under the low to medium category. As far as the soil *p*H is concerned, the studied tea garden soils are favourable for the tea plant however, when it comes to soil texture, the soil texture of the Tuli tea garden was better than that of the Ungma tea garden for optimum tea production.

The study shows that the nature of tea gardens soil physicochemical properties are dynamic mainly due to seasonal and altitudinal influence. Each of these properties influences one or more properties directly or indirectly. Therefore, it can be concluded that depending on the altitude of the tea gardens and season of the year in each tea gardens, change in one or more soil properties can affect the corresponding soil properties. Furthermore, management practices such as differences or unbalanced fertilizers usage in tea gardens might also have resulted in differences in soil properties between seasons and tea gardens. Soil variability especially in soil nutrients between seasons inflicted by fertilization can affect the yield and quality of tea. This infers for an integrated approach toward soil management practices in tea gardens studied.

The present study will provide baseline information for soil quality assessment and monitoring of tea growing areas in the Mokokchung district of Nagaland. This study was conducted in only two tea gardens for two years. Therefore, long-term studies on these soil properties in more tea gardens varying in altitudes along with fertilization effects on tea plants are required to have more clarity on patterns of seasonal variation.

CHAPTER 3

ENUMERATION OF TEA SOIL MICROFLORA [2]

This chapter presents the seasonal differences in the population and isolates of soil microflora (bacteria and fungi) in the tea gardens. This chapter also compares bacterial and fungal population and types of isolates between tea gardens. The correlation study between the soil physicochemical properties and the bacterial and fungal population was carried out to establish the relationship between these parameters.

[2] A section of this chapter has already been published as:

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3.1. Introduction

Soil an excellent culture medium for the growth and development of various microorganisms due to the availability of nutrients, water, organic and inorganic matter (Bisi-Johnson et al., 2010). Soil and its microbial communities are requisites for biogeochemical cycles of soil nutrients, improvement of soil crop quality and sustenance of agroecosystem (Stark et al., 2008). Soil harbours wide arrays of microflora that interact among themselves and with other soil properties. The outcome of such interactions can be beneficial or detrimental for soil microflora, soil properties and aboveground plants. Researches addressing these soil microflora population and their identifications are crucial because they are integral components for soil functioning and are capable of carrying out almost all known biological reactions which can influence plant growth performance. Microbial activity can enhance plant growth through mechanisms such as manipulation of plants' hormonal signalling, repelling or outcompeting pathogenic microbial strains and increasing nutrients availability (Jacoby et al., 2017).

Bacteria and fungi are among important groups of soil microflora with a diverse role that extent from organic matter decomposition to inflicting diseases in growing plants. Bacteria constitute the most abundant group among the soil microflora (Kibunja et al., 2010) with numbers are as huge as 1.5×10^{10} per gram of soil (Torsvik et al., 1990). The existence of such a huge number of genomes is a reflection of the presence of niches richness in soil and its role as drivers of many ecological functions. Fungi are another important group of microflora and play a significant role in human, plant and animals life. This microbial group is the main decomposers of organic matter in soils because of their essential role in the processes that form humus (Christensen, 1989).

Tea garden soil provides a unique place for the growth and multiplication of diverse microflora. It is an excellent site for isolation of biocontrol agents (Pandey et al., 1997), efficient inoculants for hardening of tissue culture raised tea plants as well as for growth promotion (Pandey et al. 2000) and for studying microbial interactions under natural conditions in a specific environment (Pandey et al., 2013). However, populations and activities of agricultural soil microflora including those under tea soils are subjected to several changes with time. Furthermore, the microflora population and activities can vary between altitudes due to differences in soil and other environmental conditions. It is therefore imperative to understand the factors influencing the microfloral population in tea soil because any change in their population can affect the cycling of nutrients as well as the availability of

soil nutrients consequently affecting soil functions and tea production. Keeping all these in mind, the present study was conducted to seasonally isolate soil bacteria and fungi from tea gardens differing in altitudes. For bacterial and fungal population, colony-forming units (CFU) were enumerated on agar plates following the serial dilution technique. Bacterial isolates were identified using two approaches namely, phenotypic characterization and molecular characterization using the 16S *r*RNA sequencing technique. For fungal isolates, identifications were carried out based on their colony features in agar plates and microscopic characteristics. Furthermore, a correlation study between the soil physicochemical properties and the bacterial and fungal CFU was carried out to establish the relationships of soil properties with these microflora populations.

3.2. Materials and method

3.2.1. Study sites and sampling

Details on tea gardens, sample collection and sampling frequency have already been provided in Chapter 2 (2.2.1 - 2.2.3).

3.2.2. Sterilization

Borosil glasswares and Tarson's disposable petriplates and microtips were used throughout the experiment. All the reusable glasswares and inoculation loop, needles and scalpel were washed with labolene (Fisher Scientific). After rinsing thoroughly, they were oven-dried at 105 ºC. Prior to use in experiments, materials were autoclaved at 121 ºC for 30 minutes and kept inside a laminar airflow chamber. UV irradiation was used to sterilize equipment every time before inoculation.

3.2.3. Chemicals used and reagent preparation

The chemicals and reagents used are indicated below. Except for the consumable with its manufacturer and product code indicated in parenthesis, all other consumables for phenotypic characterization of bacteria and fungal characterization were purchased from Sisco Research Laboratories Pvt. Ltd., India.

Durham tubes (HiMedia, GW163), Gram's crystal violet solution (HiMedia, S012), Gram's iodine solution (HiMedia, S057), Gram's safranine solution (HiMedia, S027), Immersion oil (RM225), oxidase discs (HiMedia, DD018), phenol red indicator (HiMedia, I010).

Barritt's reagent

Barritt's reagent A: 5 g of α-naphthol was transferred to a 100 ml conical flask containing 10 ml of absolute ethanol and the solution was brought up to the mark with absolute alcohol.

Barritt's reagent B: 40 g of KOH was transferred to a 100 ml conical flask containing 50 ml of distilled water. The solution was then made up to 100 ml with distilled water.

Nitrate reagent

Solution A: 0.8 g of sulphanilic acid was dissolved in 100 ml of 5N Acetic acid.

Solution B: 0.5 g of α-naphthylamine was dissolved in 100 ml of 5 N Acetic acid.

Methyl red reagent

0.1 g of methyl red was dissolved into 300 ml of 95% ethyl alcohol followed by bringing up the final volume to 500 ml with distilled autoclaved water.

For molecular characterization of bacteria, all chemicals were purchased from Himedia, India except for PCR master mix, Taq DNA polymerase, 6X Blue/Orange loading dye and DNA ladders (500 bp and 1 kb) which was purchased from Promega, India. Preparation of buffer and other solutions used in this study are given below:

C:I (24:1)

For 250 ml**,** 10 ml of isoamyl alcohol was mixed with 240 ml of chloroform and stored under refrigeration.

0.5 M EDTA, *p*H 8.0

For 1000ml, 186.12g EDTA.Na_2 was dissolved in 800ml sterilized distilled water. To this, 50 ml of NaOH was added. The solution was stirred vigorously and the final volume was brought to 1000 ml with sterilized distilled water. The solution was autoclaved at 121°C for 15 minutes and stored in a refrigerator.

70% ethanol

For 100ml of 70% ethanol, 70 ml of ethanol was transferred to a reagent bottle containing 30 ml of distilled water.

Ethidium bromide

10 mg of ethidium bromide (EtBr) powder was dissolved in a bottle containing 1 ml of distilled water. The bottle with the solution was wrapped in aluminium foil and stored in a refrigerator.

Proteinase K (20 mg/ml)

20 mg of Proteinase K was dissolved in 1ml of a solution containing 50mM Tris-Cl (*p*H 8.0), 1.5 mM calcium acetate. The stock solution was stored at -20 ºC.

RNase (10 mg/ml

10 mg of RNase was dissolved in 1ml of 0.01 M sodium acetate (pH 5.2) and heated to 100 °C for 15 minutes. The solution was allowed to cool slowly to room temperature followed by the addition of 0.1 volume of 1 M Tris-Cl (*p*H 7.4). RNase solution was stored at -20 °C.

Running buffer (Stock 50x TAE), *p*H 8.0

For 1000 ml buffer, 242 g of Tris base, 57.1 ml glacial acetic acid and 100 ml 0.5 M EDTA (*p*H-8.0) and final volume was adjusted with distilled water. Stock buffer was used for the preparation of 1X TAE (working buffer).

10% SDS

10 g of SDS was dissolved in 100 ml of distilled water.

3M Sodium acetate, *p*H 5.2

24.6 g of sodium acetate (anhydrous) was dissolved in a bottle of 70 ml of distilled water. *p*H was maintained using glacial acetic acid. The volume was brought up to 100 ml with distilled water.

1x Tris-EDTA/ 1x TE buffer, *p*H 8.0

For 1000ml buffer, 1.21 g of tris base and 0.292 g of EDTA were dissolved in 500 ml distilled water. The *p*H of the solution was adjusted with concentrated HCl and the volume of buffer was brought to 1000 ml with sterilized distilled water.The buffer solutions were autoclaved at 121°C for 15 minutes and stored in a refrigerator.

3.2.4. Culture media used

The agar media and broth used for bacterial analysis in the present study were purchased from HiMedia, India. Culture media with the code number in parenthesis are shown below: Eosin methylene blue agar (M317), McConkey agar (M082), MR-VP medium (M070), nitrate broth (M439S), nutrient agar (M1269A), nutrient broth (M002), Simmons citrate agar (M099), Triple sugar iron agar (MM021), tryptone water (GM463), urea agar base (M112). Media preparations were carried out as per the manufacturer's instruction.

For fungal studies, four different media namely, Czapex dox agar MEA, PDA and Rose Bengal agar were used. PDA (Himedia, India) and RBA (Himedia, India) were prepared from a dehydrated base according to the manufacturer's instruction.

The chemical composition of CDA and MEA used in the study were prepared following the standard protocol (Atlas 2004) as given below:

Composition of CDA and MEA

CDA	
Ingredient	g/l
Sucrose	30
K_2HPO_4	1
$MgSO_4.7H_2O$	0.5
KCl	0.5
$FeSO_4.\ 7H_2O$	0.01
$NaNO_3$	3
Agar	15
Distilled water	1000
Final *p*H	7.3 ± 0.2

RBA	
Ingredient	g/l
Glucose	20
Malt Extract	20
Mycological peptone	1
Agar	16
Distilled water	1000
Final *p*H	6.8 ± 0.2

All the ingredients for each media except Rose Bengal Agar (RBA) media were dissolved in 500 ml distilled water by swirling the flasks. Flasks were filled up to the final 1000 ml with distilled water and the *p*H of respective fungal media was maintained. Media were then boiled with interval shaking to avoid sticking in the bottom of the flask while melting the agar. The media were autoclaved at 121ºC for 15 minutes and transferred to laminar airflow. In RBA, Rose Bengal was added before autoclave.

Autoclaved media were allowed to cool down to about 40°C and filter sterilized 30 µg/ml streptomycin sulphate (to inhibit bacterial growth) was added in each of the fungal media before pouring the medium on petriplates.

About 25 ml of agar medium was poured on sterilized petriplates and allowed to cool down to room temperature. Agar slants and culture broths were poured into test tubes prior to autoclave. The solidified agar plates and broth were treated with UV for 5 minutes. Agar plates, slants and culture broths were kept inside the laminar airflow cabinet overnight.

3.2.5. Estimation of bacterial colony-forming units

1 g of soil sample was added to 10 ml of distilled and autoclaved water to make 10^{-1} dilution. The suspension was kept in a shaker for 10 minutes at room temperature. 10^{-1} dilution was then serially diluted up to 10^{-7} with distilled and autoclaved water. 100 µl of each of the 10^{-6} and 10^{-7} dilution were spreaded on nutrient agar (NA) and eosin methylene blue agar (EMBA) plates with an L-shaped spreader. Plates with inoculums were sealed with parafilm, marked and incubated in IKON (IK-120) 37°C ± 2°C for up to 48 hours. Following incubation, culture plates with well-developed colonies were selected and counted. An average of three replicates from both the plates was recorded and combined. Colony-forming units per g of soil (CFU g^{-1}) were calculated using the formula (Johnson and Case, 2007):

$$\text{CFU } g^{-1} = \frac{\text{No.of colonies}}{\text{Volume plated (ml)}} \text{ x Dilution factor}$$

3.2.6. Purification and maintenance of isolated bacteria

For pure culture, a loopful of each bacteria was transferred on separate nutrient agar plates with a sterile loop and streaked with the same to obtain discrete bacterial colonies. Colonies were purified by streaking and re-streaking onto nutrient agar plates. Purified colonies of all the isolates obtained were used for different phenotypic characterizations, inoculated in nutrient broth for genomic DNA extraction and preparation of glycerol stocks. Inoculated bacterial isolates were incubated in shaking condition at 250 rpm maintaining the same temperature and time as that of mixed culture. Glycerol stock (20%) was made and stored at -20°C.

3.2.7. Bacterial identification

Bacterial identification was based on phenotypic characterization and molecular characterization using the 16S *r*RNA sequencing technique.

3.2.7.1. Phenotypic characterization

Bacterial isolates were characterized and identified employing traditionally used morphological tests such as colony characters, gram characters, motility test and biochemical characters following Cappuccino and Sherman (2002) and Godkar, 1999. The results were analyzed as per standard protocol (Bergey et al., 2012; Garrity et al., 2005; Holt et al., 1994; Ludwig et al., 2009) and in ABIS online - bacterial identification software version 12. For each test that involves media preparation control was maintained without bacterial isolates.

Colony characteristics

The pure culture isolates were preliminarily identified based on culture characteristics like colour, margin, form, elevation and texture.

Gram staining

The Gram staining differentiates gram-positive and gram-negative bacteria. A bacterial smear for gram staining was prepared by placing a drop of water with the help of a sterilized dropper on an autoclaved slide. 18-24 hours old bacteria was transferred to the slide with a heat sterilized loop and spreaded by the circular motion of the inoculating loop. The smear was allowed to air-dry and heat-fixed by passing the slide through the bunsen burner flame. The smear was flooded with crystal violet and allowed to stand for 1 minute. The stained slide was washed gently with distilled water followed by flooding with gram's iodine mordant, followed by allowing the slide to stand for 1 minute and washing with distilled water. This was followed by decolourizing where 95% of ethyl alcohol was added until the slide showed only a blue tinge. The decolourization step was followed by counterstaining with safranin for 0.45 minutes after washing the slide with distilled water. Excess safranin was washed with distilled water and the slide was dried with blotting paper. The slide was examined under a microscope at 100X after adding a drop of oil immersion. Gram-positive bacteria retain the primary stain (crystal violet) and iodine complex and appear violet under microscope while, gram-negative bacteria lose crystal violet and iodine complex upon alcohol washing and gave pink colouration of safranin counterstain. Observation of bacterial shape was carried out simultaneously.

Motility test

The motility test medium was prepared according to the manufacturer's protocol. Before autoclaving the medium, triphenyl tetrazolium chloride (0.5g/l) was added to enhance the visibility of bacterial growth. Bacterial isolates were inoculated on agar tubes by stabbing the agar with a needle in one straight line through the centre of the medium. Tubes were incubated at 37ºC for 24 hours. Results were recorded as positive if the medium turned red along with outward radiation of the bacterial growth from the inoculation line while confinement of the bacterial growth only to the inoculation line indicated a negative result.

Catalase test

The catalase test determines the presence of a protective enzyme called catalase that cleaves hydrogen peroxide (H_2O_2) into water and oxygen gas. A loopful of 24 hours old

bacteria from the nutrient broth was transferred on an autoclaved slide. To this, a drop of 3% H_2O_2 was added and mixed using the loop. The effervescence after the addition of H_2O_2 indicated the catalase activity of the bacteria.

Oxidase test

Oxidase test was carried out to identify microorganisms containing the enzyme cytochrome oxidase which catalyses the oxidation of reduced cytochrome by oxygen. Oxidase disc soaked in sterilized water was transferred on to an autoclaved slide. To this, 24 hours old bacteria from the nutrient broth was transferred with the help of a sterilized inoculation loop. The result was recorded on the appearance of a purple colour on the disc after a few seconds of bacterial transfer.

Citrate utilization test

This test detects the ability of bacteria to utilize sodium citrate as the sole carbon and energy source and ammonium salt as the sole nitrogen source. The simmon-citrate medium was prepared according to the manufacturer's instruction. The isolate was streaked in the test tubes containing autoclaved citrate medium. Incubation was carried out at 37 ºC for 48 - 72 hours. Test tubes were recorded for colour change of the medium from deep forest green to a prussian blue colour. The control tube was maintained without bacterial isolates.

Methyl Red -Voges Proskauer test

These tests detect the ability of the microorganisms to ferment glucose. While in methyl red (MR) test glucose is fermented to produce acid, the Voges Proskauer (VP) test determines the organism's capability to produce acetyl methyl carbinol from glucose fermentation.

For the MR test, a loopful of the isolate was transferred into a 10 ml MR-VP broth (glucose phosphate broth) and incubated at 37 ºC for 48 hours. Following incubation, 3-5 drops of methyl red indicator was added to the bacterial broth. Colour change in the upper layer of the broth from yellow to red was recorded in positive isolates while methyl red negative bacteria and control tube was detected by no change in the yellow colour of culture broth.

For the VP test, a loopful of the isolate was transferred into a 10 ml MR-VP broth (glucose phosphate broth) and incubated at 37 ºC for 48 hours. Following incubation Barritt's reagent A and Barritt's reagent B were added to the bacterial broth. The test tube was shaken

for 3 minutes, allowed to stand at room temperature for 15 minutes. Colour change to pink was recorded in test tubes with positive isolates while VP negative bacteria and control tubes were detected with no change in colour of culture broth.

Indole test

Indole test detects the presence of the tryptophan degrading enzyme tryptophanase. The degradation of tryptophan release indole. Each isolate was inoculated into test tubes containing 5 ml of tryptone-water broth followed by incubation at 37 ºC for 24 hours. Following incubation, 0.5 ml of Kovac's reagent was added to the bacterial broth. Test tubes with a cherry-red ring on the surface of the bacterial broth indicated a positive reaction while tubes without colour development on the surface of the bacterial broth indicated a negative reaction.

Nitrate reduction test

Nitrate reduction test was conducted to determine the ability of the microbe to reduce nitrate to nitrite, which is the source of nitrogen for many bacteria. Each isolate was inoculated into a test tube containing 5 ml nitrate broth. Incubation was carried out at 37 ºC for 48 hours. Following incubation, 5 drops of solution A and B were added to the bacterial broth. Colour change from colourless to deep-red colour was recorded in test tubes with positive isolates.

Triple sugar iron test

The triple sugar iron (TSI) test determines the ability of a microorganism to ferment sugars lactose, sucrose and glucose and to produce hydrogen sulfide. Bacteria were streaked in the test tubes containing autoclaved TSI agar for multi-test including fermentation reaction, gas and H_2S production. Incubation was carried out at 37 ºC for 48 - 72 hours. Test tubes were recorded for the following results:

i) Glucose fermentation indicated by an alkaline slant (red) and acid butt (yellow), with or without breaks in agar butt.

ii) Lactose and or sucrose fermentation indicated by an acid slant (yellow) and acid butt (yellow), with or without breaks in agar butt.

iii) No fermentation indicated by an alkaline slant (red) and alkaline butt (red), with no change in agar butt.

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

Urease production

Urease test identifies organisms that are capable of producing carbon dioxide and the weak base, ammonia by hydrolyzing urea. The ammonia so formed increases the *p*H of the media and the *p*H indicator, phenol red, change from yellow to pink. Each test tube containing autoclaved urea agar base (Christensen) was inoculated with the inoculums followed by incubation at 37 ºC for 24 hours. The observation was carried out for a colour change from the yellow indicator to the bright pink colour.

3.2.7.2.Molecular characterization of bacterial isolates

16S *r*RNA sequencing technique was used for molecular characterization of the bacterial isolates. 16S *r*RNA sequence is a valuable tool for identifying and characterizing bacteria at different levels of taxonomic specificity because it has conserved regions, with the same sequence in many microorganisms and variable regions with a species-specific sequence. In this method, genomic DNA of the bacteria was extracted followed by PCR amplification of the 16S *r*RNA gene of the bacteria, sequencing of amplicon and analysis of the sequence.

Genomic DNA extraction

Extraction of bacterial genomic DNA was carried out following standard protocols (Sambrook and Russell, 2001; Wilson, 2001) with minor modifications. In this method, 2 ml of 18 to 24 hours old bacteria was pelleted at 4ºC for 5 minutes at 10,000 rpm. The supernatant was decanted and the pellet was re-suspended in 485µl of 1X TE buffer by repeated pipetting. 30 µl of 10% SDS and 3µl of 30 mg/ml proteinase K solution were added to the suspension, which was followed by inverting the centrifuge tube several times and incubation at 60 ºC for 1 hour or more in a water bath until the suspension becomes clear. Extraction was carried out by adding equal volume (500 µl) of chloroform: isoamyl alcohol (C:I) in the ratio 24:1 to the suspension. The supernatant was extracted by centrifugation at 12000rpm for 10 minutes and the aqueous layer containing nucleic acids were transferred to a new eppendorf tube. To this, 1 µl of RNase solution was added followed by incubation at 35 ºC for 10 minutes. Extraction was repeated by adding equal volume C:I to the suspension and centrifugation at 12000 rpm for 10 minutes. To the supernatant, 10 µl of 3M sodium

acetate was added followed by filling the tube with ice-chilled isopropanol. The tube was inverted several times and incubated at -20ºC for 5 minutes. This was followed by subsequent centrifugation at 12, 000 rpm, decanting the supernatant, filling the tube with 70% ethanol, centrifugation at 12, 000 rpm, decanting the supernatant. The tube was kept inverted on tissue paper under a laminar airflow chamber until the alcohol dried up. The bacterial DNA was then dissolved in 50 µl of 1X TE and stored at 4°C.

Agarose gel electrophoresis

The quality checked of isolated genomic DNA was analyzed by electrophoresis (TARSONS, MC-01) on 1% agarose gel. For this, 0.6g of agarose was melted in 60 ml of autoclaved 1X TAE buffer (*p*H 8.5) in a hotplate. Agarose was cooled to around 40°C and 2 µl of 10 mg/ml EtBr was added. The molten agarose on cooling was poured into a gel-casting tray. The comb was inserted carefully and the tray was allowed to set for 30 minutes at room temperature followed by, 5 minutes at 4°C for proper setting. The tray was then transferred to 1X TAE buffer filled electrophoresis chamber presetted at ~60 volts and the comb was removed slowly. 3-5 µl of each extracted DNA sample was mixed with 2 µl of 6X loading dye and loaded in the wells. For size estimation of DNA samples, 1 kb DNA marker was used as a size marker. The gel electrophoresis was continued until the dye bromophenol and xylene cyanol FF had migrated to the desired distance in the gel. The DNA bands were visualized under UV transilluminator and the images were documented through Gel Doc System (BIOSTEP, UST-20M-BE).

PCR amplification, sequencing and sequence submission

Polymerase chain reaction (PCR) is the basic technique for the molecular method. Specific primers provide amplification of nucleotide sequences in this technique. Bacterial 16S *r*RNA gene universal primer pairs used for the PCR amplification of cultured bacterial isolates in the study were as follows:

i) 27F (5′-AGAGTTTGATCMTGGCTCAG-3′)
511R (5′-GCGGCTGCTGGCACRKAGT -3′) and

ii) 9-20F (5’CGCGGGATCCGAGTTTGATCCTGGCTC-3’)
1492R (5′-GGCCGTCGACACGGATACCTTGTTACGACTT-3′)

PCR amplification was performed in a thermal cycler (ThermoFisher, 5020). The volume of each reagent used for the reaction mix is provided below**.**

PCR reagent	Volume (µl)	Final concentration
5X PCR buffer	12	1X
25 mM $MgCl_2$	3.6	1.5 mM
10 mM dNTPs	1.5	250 µM
10 µM forward primer	1.5	0.25 µM
10 µM 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	

Reaction mix with water but without DNA was used as a negative control. Sample and control tubes were quick spin in a microcentrifuge to bring the solution to the bottom of the tube. The PCR program was performed by the following cycling conditions used: an initial step of 10 minutes at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 45 seconds and elongation at 72 °C for 1 minute. A final extension was performed at 72 °C for 10 minutes. PCR amplicons were observed by electrophoresis on 1.2 % agarose gel containing EtBr, visualized under UV light and photographed.

Amplified products were outsourced to Chromous Biotech Pvt. Ltd. (Bangalore, India) and Biokart Pvt. Ltd. (Bangalore, India) for purification and sequencing.

The sequences obtained were compared with those present in NCBI GenBank using the BLASTN tool to confirm identity between species. Ambiguous sequences were removed using the Bioedit software and trimmed sequences were BLAST searched for similarity. Query sequences with the highest similarity were considered as the same species. The nucleotide sequences were submitted to NCBI GenBank (www.ncbi.nlm.nih.gov).

3.2.11. Estimation of fungal colony-forming units

To estimate the CFU of fungi, PDA and RBA were used. Colonies were counted based on the serial dilution method of Waksman (1922). 1 g of soil sample was added to 10 ml of distilled and autoclaved water to make 10^{-1} dilution. The suspension was kept in a shaker for 10 minutes at room temperature. 10^{-1} dilution was then serially diluted up to 10^{-6} using the distilled and autoclaved water. Aliquots of 100 µl each from 10^{-5} and 10^{-6} dilution were spreaded on PDA and RBA plates with an L-shaped spreader. Plates with diluents were then sealed with parafilm, marked and incubated at in IKON (IK-120) 27°C ± 2 °C for 120 - 168

hours. Following incubation, an average of three replicates from both the plates was recorded and combined. $CFUg^{-1}$ was calculated using the formula (Johnson and Case, 2007):

$$CFUg^{-1} = \frac{\text{No.of colonies}}{\text{Volume plated (ml)}} \text{ x Dilution factor}$$

3.2.9. Purification and maintenance of isolated fungi

Fungal colonies were picked from each plate, taking into account to include all colonies with distinct features in each of the plates. Purification was carried out by sub-culturing each colony on CDA, MEA, PDA and RBA plates. Three points inoculation and streaking of the fungal colony were done with a flame sterilized scalpel on each of the four different media plates to record variation in colony morphologies of the isolates. Plates were incubated following the same condition as that of the mixed culture or by increasing growth temperature and incubation time for some isolates. Following incubation, pure culture isolates were stored in the PDA slant at 4 ºC.

3.2.10. Identification of fungal isolates

Identification of purified fungi was carried out using colony features in the agar plates and microscopic characteristics. Identification was based on review and consultation of available literature and taxonomic keys (Bamett 1965; Domsch et al., 1980; Frisvad and Samson, 2004; Gilman, 2001; Giraldo et al., 2015; Jayasiri et al., 2019; Klich, 2002; Nelson et al., 1983; Pitt and Hocking, 2009; Raper and Thom, 1949; Rifai, 1969; Samson, 1974; Samson et al., 2004; Samson et al., 2014; Siddiquee, 2017; Watanabe, 2002).

3.2.10.1. Colony morphology features based identification

Culture characteristics including reverse and obverse colony colour, size of the colony, growth pattern, surface texture, margin character, pigmentation etc., were recorded from different culture media.

3.2.10.2. Microscopic features based identification

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 teased 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 in the stained slides were carried

out in microscope (Moitic, BA210LED) at 10X, 40X and 100X magnification under oil immersion. Micrographs were captured using the camera equipped with the microscope.

3.2.11. Statistical analysis

Data arrangement and statistical analysis were performed in the same software used for soil physicochemical analysis. Significance differences for multiple comparisons of soil microfloral population (fungal $CFUg^{-1}$ and bacterial $CFUg^{-1}$) were determined using one-way ANOVA. The significant difference was based on $p < 0.05$. Two-way ANOVA was performed to test the effects of seasons and sites on the fungal $CFUg^{-1}$ and bacterial $CFUg^{-1}$. Pearson correlation of mean of each sampling area was used to detect the relationships between soil physicochemical properties and microfloral population. The correlations were considered significant if $p < 0.01$ and $p < 0.05$.

3.3. Results

3.3.1. Bacterial analysis

3.3.1.1. Variation in bacterial counts

The soil samples analyzed for bacterial counts as well as isolates during the year 2016 - 2018 revealed marked seasonal variations at Tuli and Ungma tea garden. Furthermore, variations were observed between the tea gardens as well as between the sampling years. Bacterial counts recorded from soil samples at both tea gardens are presented in Table 3.1.

The bacterial counts in the first year at the rhizospheric and non-rhizospheric soil at Tuli tea garden ranged from 6.27 x 10^5 $CFUg^{-1}$ to 12.93 x 10^5 $CFUg^{-1}$ and 5.87 x 10^5 to 13.60 x 10^5 $CFUg^{-1}$. The overall mean of the bacterial counts were from 6.07 x 10^5 to 13.27 x 10^5 $CFUg^{-1}$. In the second year, the bacterial count at the rhizospheric and non-rhizospheric ranged from 4.83 x 10^5 to 10.30 x 10^5 $CFUg^{-1}$ and 5.10 x 10^5 to 11.33 x 10^5 $CFUg^{-1}$. The overall mean of the bacterial counts were from 4.97 x 10^5 to 10.82 x 10^5 $CFUg^{-1}$. The highest and the lowest bacterial counts in both years were recorded in the summer and winter season respectively. Based on the results from both the years, seasonal studies of bacterial counts at the Tuli tea garden showed the following trend of decreasing order summer > spring > autumn > winter.

The bacterial count in the first year at the rhizospheric and non-rhizospheric soil at Ungma tea garden ranged from 4.20 x 10^5 to 9.10 x 10^5 $CFUg^{-1}$ and 4.70 x 10^5 to 9.47 x 10^5 $CFUg^{-1}$. The overall mean of the bacterial counts were from 64.45 x 10^5 to 9.28 x 10^5 $CFUg^{-1}$. In the second year, the bacterial count at the rhizospheric and non-rhizospheric soil ranged

from 2.90 x 10^5 to 8.63 x 10^5 $CFUg^{-1}$ and 3.13 x 10^5 $cfug^{-1}$ to 8.80 x 10^5 $CFUg^{-1}$. The overall mean of the bacterial counts were from 3.02 x 10^5 to 8.72 x 10^5 $CFUg^{-1}$. In both years, the summer and winter season had the highest and the lowest bacterial counts respectively. In the Ungma tea garden, bacterial counts in both years showed the following trend of decreasing order summer > autumn > spring > winter.

Table 3.1. Bacterial counts (x 10^5 $CFUg^{-1}$) at Tuli and Ungma tea garden during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	11.47 ± 0.20	12.93 ± 0.26	10.97 ± 0.26	6.27 ± 0.12	9.63 ± 0.18	10.30 ± 0.12	9.23 ± 0.20	4.83 ± 0.18
	NR	12.10 ± 0.12	13.60 ± 0.15	11.23 ± 0.29	5.87 ± 0.12	10.30 ± 0.31	11.33 ± 0.30	9.43 ± 0.18	5.10 ± 0.23
	Mean	**11.78 ± 0.18^b**	**13.27 ± 0.20^a**	**11.10 ± 0.18^b**	**6.07 ± 0.12^c**	**9.97 ± 0.22^b**	**10.82 ± 0.22^a**	**9.33 ± 0.13^b**	**4.97 ± 0.14^c**
Ungma	R	6.33 ± 0.20	9.10 ± 0.17	8.73 ± 0.20	4.20 ±0.21	6.57 ± 0.20	8.63 ± 0.18	5.83 ± 0.15	2.90 ± 0.15
	NR	7.10 ± 0.23	9.47 ± 0.20	8.30 ± 0.23	4.70 ± 0.27	6.87 ± 0.18	8.80 ± 0.17	6.20 ± 0.21	3.13 ± 0.17
	Mean	**6.72 ± 0.22^a**	**9.28 ± 0.15^b**	**8.52 ± 0.17^c**	**4.45 ± 0.19^d**	**6.02 ± 0.14^a**	**8.72 ± 0.12^b**	**6.72 ± 0.14^c**	**3.02 ± 0.11^d**

R - rhizosphere; NR - non-rhizosphere.
Mean ± standard error mean.
Different letters ($^{a, b, c}$ and ^{d}) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

3.3.1.2 Statistical analysis

The season-wise data of soil bacterial count pooled for one-way ANOVA revealed significant effects of the sampling seasons ($p < 0.05$) at both the tea gardens (Table 3.2). Further, Tukey's post hoc test of significance for mean differences revealed statistically significant differences between most of the seasons in both years. Similarly, two-way ANOVA conducted between the study sites and sampling seasons revealed statistically significant interaction ($p < 0.05$) between the effects of season and site (Table 3.3).

The correlation between soil bacterial count and soil properties is presented in Table 3.4. A significant positive correlation was established between bacterial counts and moisture, *p*H, temperature, SOC, available N and available P at Tuli tea garden in both years. At Ungma tea garden, a significantly positive correlation was established between bacterial counts and soil properties such as silt, *p*H, moisture and temperature whereas, the correlation of bacterial counts was significantly negative with sand in both the years. The correlation of bacterial counts with SOC and available nutrients revealed an uneven relationship between the first and second year at the Ungma tea garden.

Table 3.2. One-way ANOVA of bacterial count

Parameter	Tuli				Ungma			
	2016 - 2017		**2017 - 2018**		**2016 - 2017**		**2017 - 2018**	
	F (3, 20)	*p* value	F (3, 20)	*p* value	F (3, 20)	*p* value	F (3, 20)	*p* value
Bacterial CFU	327.88	0.00	172.61	0.00	139.33	0.00	342.80	0.00

Table 3.3. Two-way ANOVA of bacterial count between tea gardens and seasons

Parameter	Source	2016 - 2017		2017 - 2018	
		F	*p* value	F	*p* value
Bacterial CFU	Site	697.14	0.00	506.07	0.00
	Season	420.38	0.00	429.85	0.00
	Site X Season	36.71	0.01	14.87	0.00

Table 3.4. Correlation analysis of soil bacterial counts with soil physicochemical properties

Soil parameter	Tuli		Ungma	
	2016 - 2017	**2017 - 2018**	**2016 - 2017**	**2017 - 2018**
	Bacterial CFU		**Bacterial CFU**	
Sand (%)	-0.37	-0.11	-.826*	-.779*
Clay (%)	0.49	0.13	0.10	0.23
Silt (%)	-0.02	-0.09	.802*	.817*
Moisture (%)	.742*	.942**	.951**	.918**
*p*H	.749*	.830*	.934**	.880**
Temperature (°C)	.909**	.813*	.986**	.976**
SOC (%)	.737*	.891**	0.58	.941**
Available N (kg/ha)	.838**	.741*	0.51	-.752*
Available P (kg/ha)	.838**	.941**	0.70	.778*
Available K (kg/ha)	.742*	-0.07	.824*	0.46

SOC - soil organic carbon, Available N - available nitrogen, Available P- available phosphorus, Available K- available potassium.

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

3.3.1.3 Identification of the bacterial isolates

Phenotypic characterization

Phenotypic characterization of isolates showed variation in their culture characteristics, gram morphology and biochemical test results. Fig. 3.1 and Fig. 3.2 represent the pure culture and phenotypic studies of some of these bacteria isolates. Altogether, 128 bacterial isolates from Tuli tea garden and Ungma tea garden that exhibited variations in culture plates were selected for phenotypic characterization. Culture characteristics of the isolates on NA showed diverse colony shape, colours, elevations, margins and opacity (Table 3.5). Isolates were gram-positive, gram-negative as well as gram variable and varied from rod to cocci shape in their gram morphology. Further, the ability of these isolates to excrete enzymes tested through tests on catalase, citrate utilization, hydrogen sulphide production, Lactose,

methyl red, motility, nitrate reduction, oxidase, TSI, urease and V-P test differed greatly (Table 3.6).

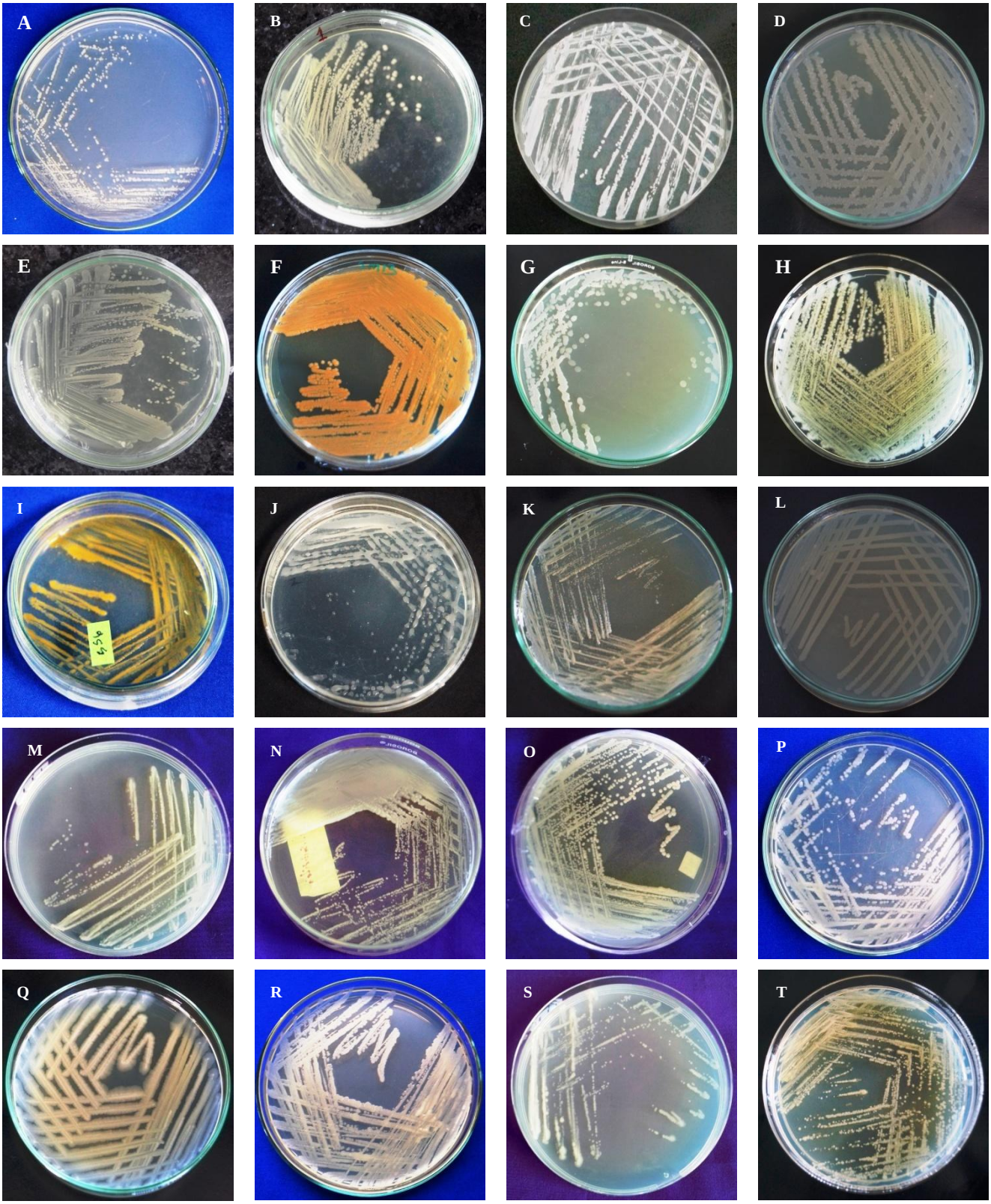

Fig. 3.1. Pure culture of some bacterial isolates from Tuli and Ungma tea garden. A- ARENTL-NU37, B- ARENTL-NU72, C- ARENTL-NU14, D- ARENTL-NU6, E- ARENTL-NU1, F- ARENTL-NU23 G- ARENTL-NU21, H- TLTENU18, I-ARENTL-NU32, J- TREN-NU11, K- ARENTL-NU35, L-TLTENU11, M- ARENTL-NU36, N-AREN-NU43, O- ARENTL-NU69, P-ARENTL- NU84, Q- TLTENU19, R- TREN-NU1, S- TREN-NU6, T- ARENTL-NU11.

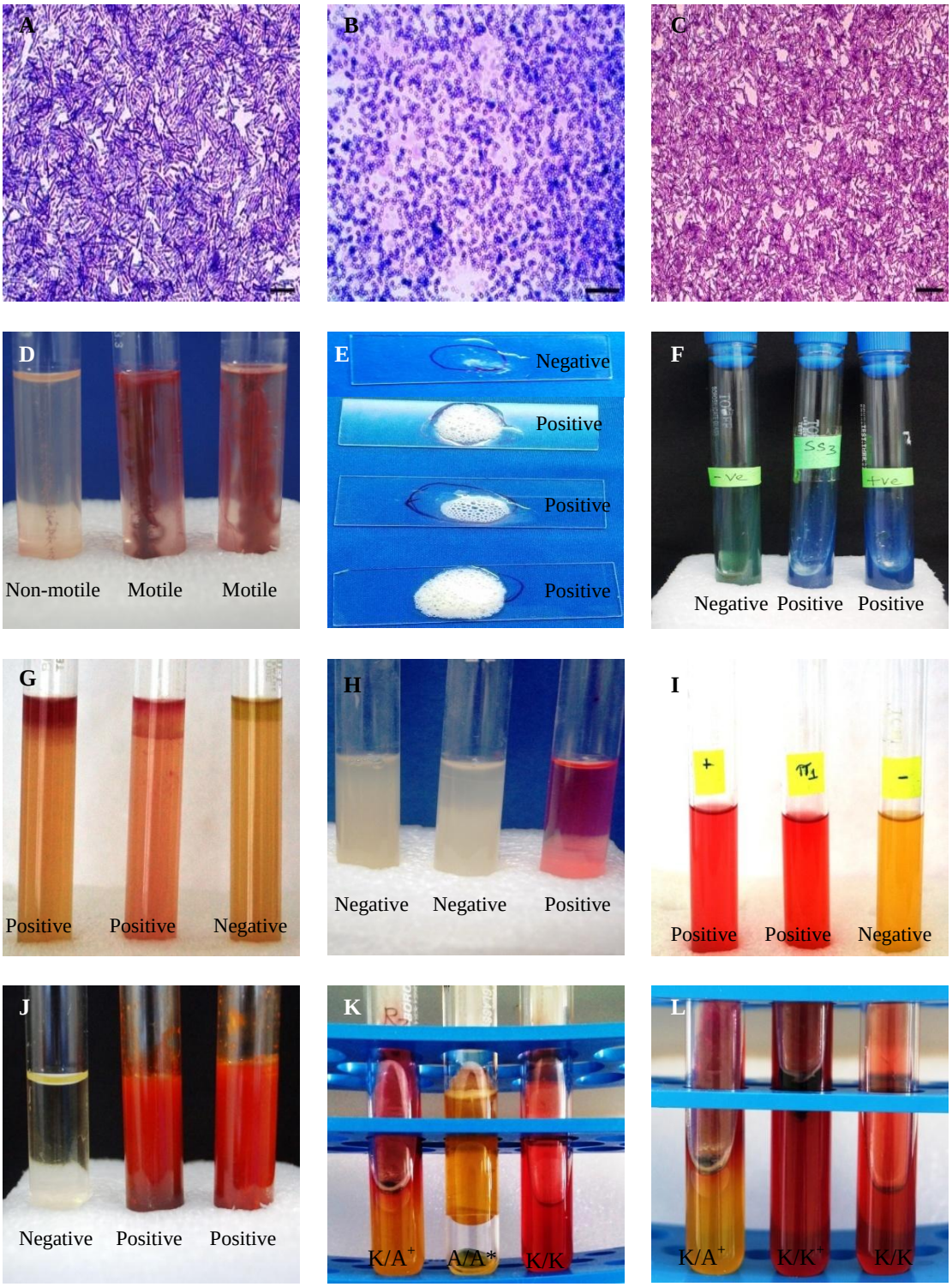

Fig.3.2. Gram test, motility test and biochemical characterization of bacterial isolates. A- Gram-positive, rod-shaped, B- Gram-positive, cocci shaped, C- Gram-negative, rod-shaped, D- Motility test, E- Catalase positive, F- Citrate utilization test, G- Indole test, H- Methyl red test, I- Voges Proskauer test, J- Nitrate reductase test, K, L- Triple sugar iron agar test. Scale bar represents 10µm.

K/K (Alkaline/Alkaline) i.e., Red slant /Red butt indicates no fermentation.

A/A (Acidic/Acidic) i.e., Yellow slant/Yellow butt indicates fermentation of glucose, lactose and/or sucrose.

+ indicates blackening of the medium due to the presence of H_2S.

*indicates bubbles or cracks due to the production of gas.

Table 3.5. Morphological characterization of bacterial isolates through culture plate

Bacterial strain	Shape	Margin	Elevation	Texture	Surface	Colour	Opacity
ARENTL-NU72	Circular	Entire	Convex	Moist	Smooth	Cream	Translucent
ARENTL-NU96	Circular	Entire	Convex	Moist	Smooth	Cream	Opaque
TREN-NU12	Circular	Entire	Flat	Moist	Smooth	Yellowish white	Translucent
ARENTL-NU37	Circular	Entire	Convex	Mucoid	Smooth	White	Opaque
TREN-NU8	Circular	Entire	Convex	Mucoid	Smooth	White	Opaque
TLTENU12	Circular	Entire	Convex	Mucoid	Smooth	Yellowish white	Opaque
ARENTL-NU45	Circular	Entire	Convex	Mucoid	Smooth	Cream	Opaque
ARENTL-NU91	Circular	Entire	Raised	Mucoid	Smooth	Yellowish white	Opaque
ARENTL-NU93	Irregular	Entire	Raised	Moist	Smooth	White	Opaque
ARENTL-NU59	Irregular	Undulate	Flat	Dry	Rough	Yellow	Opaque
ARENTL-NU 89	Wrinkled	Fimbriate	Raised	Moist	Shiny	Cream	Opaque
ARENTL-NU	Circular	Undulate	Raised	Butyrous	Smooth	Cream	Opaque
ARENTL-NU1	Circular	Entire	Flat	Butyrous	Smooth	Yellow	Opaque
ARENTL-NU2	Irregular	Fimbriate	Flat	Butyrous	Smooth	Yellowish white	Translucent
ARENTL-NU2	Irregular	Undulate	Flat	Butyrous	Smooth	Yellow	Opaque
ARENTL-NU9	Circular	Undulate	Slightly convex	Dry	Rough	Cream	Translucent
ARENTL-NU39	Circular	Undulate	Convex	Dry	Rough	Cream	Opaque
ARENTL-NU55	Irregular	Undulate	Convex	Dry	Rough	Pinkish white	Opaque
ARENTL-NU57	Circular	Entire	Flat	Dry	Rough	Grayish white	Opaque
ARENTL-NU62	Circular	Undulate	Raised	Moist	Smooth	Cream	Opaque
ARENTL-NU2	Irregular	Undulate	Flat	Moist	Rough	Yellowish white	Opaque
ARENTL-NU2	Irregular	Lobate	Raised	Mucoid	Smooth and Shiny	White	Opaque
ARENTL-NU5	Circular	Entire	Umbolate	Butyrous	Smooth and Shiny	Orange	Opaque
ARENTL-NU6	Irregular	Undulate	Flat	Moist	Shiny	Yellow	Opaque
ARENTL-NU12	Irregular	Undulate	Raised	Dry	Smooth	Pinkish white	Opaque
ARENTL-NU13	Irregular	Lobate	Raised	Mucoid	Smooth and Shiny	White	Opaque
ARENTL-NU13	Circular	Entire	Convex	Dry	Smooth	White	Opaque
ARENTL-NU14	Irregular	Undulate	Raised	Moist	Smooth	White	Translucent
ARENTL-NU15	Wrinkled	Fimbriate	Raised	Dry	Smooth	Cream	Opaque
ARENTL-NU16	Irregular	Undulate	Raised	Moist	Shiny	Orange	Opaque
ARENTL-NU17	Circular	Entire	Convex	Mucoid	Smooth	Yellow	Opaque
ARENTL-NU18	Irregular	Undulate	Raised	Moist	Smooth	Yellow	Opaque
ARENTL-NU19	Irregular	Entire	Convex	Mucoid	Smooth	White	Opaque Translucent
ARENTL-NU20	Irregular	Undulate	Flat	Moist	Shiny	Cream	

.ARENTL-NU23	Irregular	Undulate	Raised	Moist	Smooth and Shiny	Orange	Opaque
ARENTL-NU25	Circular	Entire	Flat	Moist	Smooth and Shiny	Creamish white	Translucent
ARENTL-NU29	Irregular	Undulate	Flat	Dry	Rough	Yellow	Opaque
ARENTL-NU44	Irregular	Entire	Raised	Mucoid	Smooth	White	Opaque
ARENTL-NU61	Irregular	Undulate	Flat	Mucoid	Smooth	White	Opaque
ARENTL-NU77	Irregular	Crenate	Convex	Mucoid	Smooth	Cream	Opaque
ARENTL-NU78	Circular	Entire	Convex	Mucoid	Smooth	Cream	Translucent
ARENTL-NU79	Circular	Lobate	Raised	Moist	Smooth and Shiny	White	Translucent
ARENTL-NU80	Irregular	Undulate	Convex	Moist	Smooth and Shiny	Cream	Translucent
ARENTL-NU82	Irregular	Undulate	Convex	Dry	Rough	Cream	Opaque
ARENTL-NU86	Irregular	Crenate	Convex	Moist	Smooth	White	Opaque
ARENTL-NU90	Irregular	Undulate	Convex	Moist	Smooth	Cream	Translucent
ARENTL-NU92	Irregular	Undulate	Flat	Mucoid	Smooth	Cream	Opaque
ARENTL-NU94	Wrinkled	Crenate	Raised	Moist	Smooth and Shiny	Pinkish white	Opaque
ARENTL-NU99	Irregular	Undulate	Flat	Dry	Rough	Cream	Opaque
TREN-NU4	Circular	Entire	Raised	Moist	Smooth	Cream	Opaque
TREN-NU6	Irregular	Undulate	Raised	Dry	Rough	Pinkish white	Opaque
TREN-NU7	Irregular	Undulate	Raised	Moist	Shiny	Orange	Opaque
TREN-NU13	Irregular	Undulate	Raised	Moist	Smooth	Cream	Opaque
TREN-NU17	Irregular	Undulate	Convex	Moist	Smooth and Shiny	Cream	Translucent
TREN-NU18	Irregular	Fimbriate	Flat	Mucoid	Smooth	White	Opaque
TREN-NU19	Circular	Entire	Flat	Dry	Rough	Grayish white	Opaque
TREN-NU20	Irregular	Undulate	Convex	Moist	Smooth	Cream	Opaque
TREN-NU24	Circular	Entire	Convex	Mucoid	Smooth	Cream	Translucent
TREN-NU25	Circular	Undulate	Slightly convex	Dry	Rough	Cream	Translucent
TLTENU5	Irregular	Entire	Raised	Moist	Smooth	White	Opaque
TLTENU13	Circular	Entire	Convex	Moist	Smooth	Cream	Opaque
TLTENU17	Irregular	Undulate	Slightly raised	Butyrous	Smooth	White	Translucent
TLTENU20	Irregular	Undulate	Slightly raised	Butyrous	Smooth	White	Translucent
ARENTL-NU21	Circular	Entire	Convex	Mucoid	Smooth	White	Opaque
ARENTL-NU58	Circular	Entire	Flat	Mucoid	Smooth	White	Opaque
ARENTL-NU34	Irregular	Entire	Convex	Mucoid	Smooth	White	Opaque
ARENTL-NU60	Irregular	Entire	Slightly convex	Mucoid	Smooth	White	Opaque
TLTENU1	Circular	Entire	Convex	Moist	Shiny	Yellow	Translucent
TLTENU7	Circular	Entire	Convex	Moist	Shiny	Yellow	Translucent
TLTENU8	Circular	Undulate	Convex	Mucoid	Smooth	Yellowish white	Translucent
TLTENU18	Circular	Entire	Convex	Mucoid	Shiny	Yellowish white	Translucent

TLTENU2	Circular	Undulate	Convex	Mucoid	Smooth	Yellowish white	Translucent
ARENTL-NU32	Circular	Entire	Convex	Mucoid	Shiny	Yellow	Opaque
ARENTL-NU87	Circular	Lobate	Convex	Mucoid	Smooth	Cream	Opaque
TREN-NU11	Circular	Lobate	Convex	Mucoid	Smooth	Cream	Opaque
TREN-NU16	Circular	Lobate	Convex	Mucoid	Smooth	Cream	Opaque
ARENTL-NU35	Circular	Entire	Raised	Moist	Smooth	Cream	Translucent
ARENTL-NU41	Circular	Entire	Convex	Moist	Smooth	White	Opaque
TREN-NU3	Circular	Entire	Raised	Moist	Smooth	Cream	Translucent
ARENTL-NU66	Circular	Entire	Flat	Dry	Rough	White	Translucent
TLTENU3	Circular	Entire	Raised	Butyrous	Smooth	Creamish white	Opaque
TLTENU11	Circular	Entire	Raised	Butyrous	Smooth	Creamish white	Opaque
AREN-NU1	Irregular	Entire	Flat	Moist	Smooth	Creamish white	Translucent
ARENTL-NU36	Circular	Entire	Convex	Moist	Smooth	Yellow	Translucent
ARENTL-NU65	Irregular	Undulate	Convex	Moist	Smooth	Yellow	Translucent
ARENTL-NU98	Circular	Entire	Flat	Moist	Smooth	Yellow	Translucent
AREN-NU70	Irregular	Entire	Raised	Mucoid	Smooth and Shiny	White	Translucent
TREN-NU23	Irregular	Entire	Flat	Mucoid	Smooth and Shiny	White	Translucent
AREN-NU23	Irregular	Entire	Flat	Mucoid	Smooth	Creamish white	Translucent
ARENTL-NU24	Irregular	Entire	Flat	Mucoid	Smooth and Shiny	White	Translucent
AREN-NU43	Circular	Entire	Convex	Moist	Smooth and Shiny	White	Opaque
AREN-NU38	Circular	Entire	Raised	Moist	Smooth	Cream	Opaque
AREN-NU45	Irregular	Entire	Convex	Moist	Smooth	Yellow	Translucent
ARENTL-NU54	Circular	Entire	Raised	Moist	Smooth	Cream	Translucent
ARENTL-NU69	Circular	Entire	Raised	Moist	Smooth	Cream	Opaque
AREN-NU71	Circular	Entire	Raised	Moist	Smooth	Cream	Opaque
ARENTL-NU100	Circular	Entire	Raised	Moist	Smooth	Cream	Translucent
ARENTL-NU73	Circular	Undulate	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque
TLTENU4	Circular	Undulate	Convex	Butyrous	Smooth and Shiny	Brownish yellow	Translucent
ARENTL-NU46	Circular	Entire	Convex	Dry	Rough	Yellow	Translucent
ARENTL-67	Circular	Entire	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque
AREN-NU74	Circular	Entire	Flat	Moist	Smooth	Yellowish white	Translucent
ARENTL-NU97	Circular	Undulate	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque
TREN-NU2	Circular	Entire	Slightly raised	Butyrous	Smooth and Shiny	Yellow	Translucent
TREN-NU9	Circular	Entire	Convex	Butyrous	Smooth and Shiny	White	Opaque
TREN-NU21	Filamentous	Entire	Umbolate	Butyrous	Smooth and Shiny	Brownish yellow	Opaque
TREN-NU22	Circular	Entire	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque
TLTENU6	Circular	Entire	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque

TLTENU9	Circular	Undulate	Raised	Butyrous	Smooth and Shiny	Yellow	Opaque
TLTENU10	Circular	Entire	Raised	Moist	Smooth	Yellow	Translucent
TLTENU14	Filamentous	Undulate	Convex	Butyrous	Smooth and Shiny	Yellow	Opaque
ARENTL-NU76	Circular	Entire	Raised	Moist	Smooth and Shiny	White	Opaque
ARENTL-NU84	Circular	Entire	Raised	Moist	Smooth and Shiny	White	Opaque
TLTENU19	Filamentous	Lobate	Flat	Moist	Smooth	Creamish white	Translucent
ARENTL-NU50	Circular	Entire	Raised	Moist	Shiny and smooth	Cream	Opaque
ARENTL-NU68	Circular	Undulate	Flat	Dry	Smooth	Cream	Opaque
TREN-NU1	Circular	Entire	Flat	Dry	Smooth	Cream	Opaque
ARENTL-NU44	Circular	Entire	Convex	Moist	Smooth	Cream	Opaque
TREN-NU6	Circular	Entire	Convex	Moist	Smooth	Cream	Opaque
ARENTL-NU11	Circular	Entire	Flat	Moist	Shiny and smooth	Yellow	Opaque
ARENTL-NU56	Circular	Entire	Flat	Moist	Shiny and smooth	Yellow	Opaque
ARENTL-NU75	Circular	Entire	Convex	Mucoid	Smooth	Yellow	Translucent
ARENTL-NU85	Circular	Entire	Convex	Moist	Smooth	Yellow	Opaque
ARENTL-NU88	Circular	Entire	Convex	Moist	Smooth	Yellow	Translucent
ARENTL-NU95	Circular	Entire	Convex	Mucoid	Smooth	Yellow	Translucent
TREN-NU15	Circular	Entire	Convex	Moist	Smooth	Yellow	Opaque
TLTENU18	Circular	Entire	Flat	Moist	Smooth	Cream	Translucent
TLTENU20	Circular	Entire	Flat	Moist	Smooth	Cream	Translucent

Table 3.6. Gram characters, motility and biochemical characterization of bacterial isolates

Bacterial strain	GC	Shape	Mo	IP	MR	VP	CU	Ca	Ur	Ox	TSI	NR	H_2S	La	Presumptive identification
AENTL-NU72	-	R	M+	+	-	+	+	+	-	+	K/A	+	+	-	*Aeromonas* sp.
ARENTL-NU96	-	R	M+	+	+	+	-	+	-	+	K/A	-	-	-	*Aeromonas* sp.
TREN-NU12	-	R	M+	+	+	+	+	+	-	+	K/A	+	-	+	*Aeromonas* sp.
ARENTL-NU37	-	R/C	M-	-	+	-	+	+	-	-	K/A	-	-	-	*Acinetobacter* sp.
TREN-NU8	-	R/C	M-	-	-	-	+	+	-	-	K/A	-	-	-	*Acinetobacter* sp.
TLTENU12	-	R/C	M-	-	-	-	+	+	-	-	K/A	-	-	+	*Acinetobacter* sp.
ARENTL-NU45	-	C	M-	-	-	-	+	+	-	+	K/A	-	-	-	*Acinetobacter* sp.
ARENTL-NU91	+	R	M+	+	+	-	+	-	-	+	K/A	-	-	+	*Bacillus cereus*
ARENTL-NU93	+	R	M+	-	+	-	+	+	-	+	K/A	-	-	+	*Bacillus cereus*
ARENTL-NU59	+	R	M+	-	-	-	+	+	-	+	K/A*	-	+	+	*Bacillus licheniformis*
ARENTL-NU89	+	R	M+	-	-	+	+	+	-	-	K/A*	+	-	+	*Bacillus licheniformis*
ARENTL-NU	+	R	M+	-	-	+	+	+	-	+	A/A	+	+	-	*Bacillus subtilis*
ARENTL-NU1	+	R	M+	+	-	+	+	+	-	+	A/A	+	-	-	*Bacillus subtilis*
ARENTL-NU2	+	R	M+	-	-	+	+	+	-	+	K/K	+	-	-	*Bacillus subtilis*
ARENTL-NU2	+	R	M+	-	-	+	+	+	-	+	A/A	+	+	-	*Bacillus subtilis*
ARENTL-NU9	+	R	M+	-	+	-	+	+	-	-	K/K	+	-	-	*Bacillus subtilis*
ARENTL-NU39	+	R	M+	+	+	-	+	+	-	+	K/A	+	+	-	*Bacillus subtilis*
ARENTL-NU55	+	R	M+	-	-	+	+	+	-	+	K/K	+	+	-	*Bacillus subtilis*
ARENTL-NU57	+	R	M+	-	-	-	+	+	-	+	K/K	+	+	+	*Bacillus subtilis*
ARENTL-NU62	+	R	M+	-	-	+	+	+	-	+	K/A	+	+	+	*Bacillus subtilis*
ARENTL-NU2	+	R	M+	+	-	+	-	+	-	+	K/A	+	-	+	*Bacillus* sp.
ARENTL-NU2	+	R	M+	-	-	-	-	+	+	+	K/A	+	+	-	*Bacillus* sp.
ARENTL-NU5	+	R	M+	-	-	-	+	+	-	+	K/A	-	+	-	*Bacillus* sp.
ARENTL-NU6	+	R	M+	-	-	+	+	+	-	-	K/A	+	-	+	*Bacillus* sp.
ARENTL-NU12	+	R	M+	-	+	-	+	-	-	-	K/K	+	+	+	*Bacillus* sp.
ARENTL-NU13	+	R	M+	+	-	+	-	+	-	+	K/A	+	+	+	*Bacillus* sp.
ARENTL-NU13	+	R	M+	-	-	+	-	+	-	+	K/A	-	-	+	*Bacillus* sp.
ARENTL-NU14	+	R	M+	-	-	+	-	+	-	+	K/K	+	+	-	*Bacillus* sp.
ARENTL-NU15	+	R	M+	-	-	+	-	+	-	+	K/A	+	+	+	*Bacillus* sp.
ARENTL-NU16	+	R	M+	-	+	+	+	+	+	-	A/A	+	-	-	*Bacillus* sp.
ARENTL-NU17	+	R	M+	-	-	+	+	+	+	-	A/A	-	+	+	*Bacillus* sp.
ARENTL-NU18	+	R	M+	-	+	+	+	+	+	+	K/A	+	+	+	*Bacillus* sp.
ARENTL-NU19	+	R	M+	-	-	-	+	+	-	+	K/A	+	+	+	*Bacillus* sp.
ARENTL-NU20	+	R	M-	-	-	+	+	+	-	-	A/A	+	-	+	*Bacillus* sp.
ARENTL-NU23	+	R	M+	-	+	+	+	+	-	+	K/A	+	-	-	*Bacillus* sp.

ARENTL-NU25	+	R	M+	-	-	+	+	+	-	+	K/A	-	+	-	*Bacillus* sp.
ARENTL-NU29	+	R	M+	-	-	-	+	+	-	+	K/A*	-	+	-	*Bacillus* sp.
ARENTL-NU44	+	R	M+	-	-	-	+	+	-	+	K/K	-	-	+	*Bacillus* sp.
ARENTL-NU61	+	R	M+	-	-	+	+	+	+	-	A/A	-	+	+	*Bacillus* sp.
ARENTL-NU77	+	R	M+	+	+	+	+	+	-	-	A/A	-	-	-	*Bacillus* sp.
ARENTL-NU78	+	R	M+	-	-	+	+	+	-	+	K/A	+	-	-	*Bacillus* sp.
ARENTL-NU79	+	R	M+	-	-	-	+	+	-	-	K/A*	+	+	+	*Bacillus* sp.
ARENTL-NU80	+	R	M+	-	-	-	+	+	-	+	K/A*	+	-	+	*Bacillus* sp.
ARENTL-NU82	+	R	M+	-	-	-	-	+	+	-	A/A	+	-	+	*Bacillus* sp.
ARENTL-NU86	+	R	M+	-	+	-	-	+	-	-	A/A	+	-	-	*Bacillus* sp.
ARENTL-NU90	+	R	M+	-	-	+	+	+	+	+	K/A	+	-	-	*Bacillus* sp.
ARENTL-NU92	+	R	M+	-	+	-	+	+	-	-	A/A	+	-	-	*Bacillus* sp.
ARENTL-NU94	+/-	R	M+	-	-	+	+	+	-	+	K/A*	-	+	-	*Bacillus* sp.
ARENTL-NU99	+	R	M+	-	-	+	+	+	+	+	K/A	+	+	-	*Bacillus* sp.
TREN-NU4	+	R	M+	-	-	+	+	+	-	-	K/K	+	-	+	*Bacillus* sp.
TREN-NU6	+	R	M+	-	+	-	+	-	-	-	K/K	+	+	+	*Bacillus* sp.
TREN-NU7	+	R	M+	-	+	+	+	+	+	-	A/A	+	-	-	*Bacillus* sp.
TREN-NU13	+	R	M+	+	+	-	+	+	-	+	K/A	-	-	+	*Bacillus* sp.
TREN-NU17	+	R	M+	-	-	-	+	+	-	+	K/A*	+	+	+	*Bacillus* sp.
TREN-NU18	+	R	M+	-	+	+	+	+	-	-	K/A	+	-	-	*Bacillus* sp.
TREN-NU19	+	R	M+	-	-	-	+	+	+	-	K/A	+	+	+	*Bacillus* sp.
TREN-NU20	+	R	M+	-	+	+	+	+	+		K/A	-	-	-	*Bacillus* sp.
TREN-NU24	+	R	M+	+	+	+	+	-	-	-	K/A	-	-	-	*Bacillus* sp.
TREN-NU25	+	R	M+	-	+	+	+	+	-	-	A/A	+	+	+	*Bacillus* sp.
TLTENU5	+	R	M+	-	+	+	+	+	+	+	K/K	-	-	-	*Bacillus* sp.
TLTENU13	+	R	M+	-	-	+	+	+	-	+	K/K	+	-	-	*Bacillus* sp.
TLTENU17	+	R	M+	-	-	-	+	+	-	+	K/A	-	+	-	*Bacillus* sp.
TLTENU20	+	R	M+	-	-	-	+	+	-	+	A/A	-	+	-	*Bacillus* sp.
ARENTL-NU21	+	R	M+	-	-	-	-	+	-	-	K/K	+	-	-	*Brevibacillus*sp.
ARENTL-NU58	+	R	M+	-	-	-	-	+	-	-	K/K	-	-	+	*Brevibacillus*sp.
ARENTL-NU34	+	C	M-	-	-	-	+	+	+	-	K/A	+	-	-	[*Brevibacterium*]sp.
ARENTL-NU60	+	C	M-	-	+	-	+	+	+	-	K/A	+	-	-	[*Brevibacterium*] sp.
TLTENU1	-	R	M+	-	-	-	+	+	-	+	A/A	-	-	-	*Burkholderiacepacia*
TLTENU7	-	R	M+	-	-	-	-	+	+	-	**A/A**	-	-	-	*Burkholderiacepacia*
TLTENU8	-	R	M+	-	-	-	-	+	-	-	K/K	+	-	+	*Burkholderiacepacia*
TLTENU18	-	R	M+	-	-	-	+	+	+	+	K/K	+	-	+	*Burkholderiacepacia*
TLTENU2	-	R	M+	-	-	-	+	+	-	+	K/K	+	-	+	*Burkholderias*p.

ARENTL-NU32	-	R	M-	+	-	+	-	+	-	+	K/K	-	-	-	*Chryseobacterium*sp.
ARENTL-NU87	-	R	M+	-	-	-	+	+	+	-	K/A	-	+	+	*Comamonas*sp.
TREN-NU11	-	R	M+	-	-	-	-	+	-	+	K/K	+	-	+	*Comamonas* sp.
TREN-NU16	-	R	M+	-	-	-	-	+	-	+	K/K	+	-	+	*Comamonas* sp.
ARENTL-NU35	-	R	M+	-	-	+	+	-	-	-	K/K	+	-	-	*Enterobacter* sp.
ARENTL-NU41	-	R	M+	-	-	+	+	-	-	-	K/K	+	-	-	*Enterobacter* sp.
TREN-NU3	-	R	M+	-	-	+	+	+	-	-	**A/A**	+	-	+	*Enterobacter* sp.
ARENTL-NU66	+	R	M+	-	-	+	+	+	+	+	A/A	-	+	+	*Lysinibacillus*sp.
TLTENU3	+	R	M+	-	-	-	+	+	-	+	A/A	-	-	-	*Lysinibacillus*sp.
TLTENU11	+	R	M+	-	-	-	+	+	-	+	K/A	-	-	-	*Lysinibacillus*sp.
AREN-NU1	+/-	R	M+	+	-	-	-	+	+	+	K/K	-	+	-	*Paenibacillus*sp.
ARENTL-NU36	+	R	M+	-	-	-	-	+	-	+	A/A	-	-	-	*Paenibacillus*sp.
ARENTL-NU65	+	R	M+	-	-	-	-	+	-	+	A/A	-	-	-	*Paenibacillus*sp.
ARENTL-NU98	+	R	M+	-	-	-	-	+	-	-	K/K	+	+	-	*Paenibacillus*sp.
AREN-NU70	+	R	M+	-	-	-	-	+	-	-	K/K	-	+	-	*Paenibacillus*sp.
TREN-NU23	+	R	M+	-	-	-	+	+	-	+	K/A	+	+	-	*Paenibacillus*sp.
AREN-NU23	+/-	R	M+	-	-	-	-	-	+	-	K/K	+	+	+	*Paenibacillus*sp.
ARENTL-NU24	+	R	M+	-	-	-	-	+	-	-	K/K	-	+	-	*Paenibacillus*sp.
AREN-NU43	-	R	M+	+	+	-	+	+	-	-	K/K	+	-	-	*Providencia* sp.
AREN-NU38	-	R	M+	-	-	-	+	-	-	+	K/K	-	-	-	*Pseudomonas monteilii*
AREN-NU45	-	R	M-	-	+	-	+	+	-	-	K/K	+	-	-	*Pseudomonas monteilii*
ARENTL-NU54	-	R	M-	-	+	-	+	+	-	-	K/K	+	-	-	*Pseudomonas monteilii*
ARENTL-NU69	-	R	M+	-	+	-	+	+	-	+	K/K	+	-	+	*Pseudomonasmonteilii*
AREN-NU71	-	R	M+	-	-	-	+	+	-	+	K/K	+	-	+	*Pseudomonas monteilii*
ARENTL-NU100	-	R	M-	-	+	-	+	+	-	-	K/K	+	-	-	*Pseudomonas monteilii*
ARENTL-NU73	-	R	M+	-	+	-	+	+	-	+	K/A	+	-	-	*Pseudomonas putida*
TLTENU4	-	R	M+	-	-	-	+	+	-	+	K/A	-	-	-	*Pseudomonas putida*
ARENTL-NU46	-	R	M+	-	-	-	+	+	-	-	K/K	+	-	-	*Pseudomonas* sp.
ARENTL-67	-	R	M+	-	-	-	+	+	-	+	K/K	+	-	-	*Pseudomonas* sp.
AREN-NU74	-	R	M+	+	+	+	+	+	-	+	K/K	+	-	-	*Pseudomonas* sp.
ARENTL-NU97	-	R	M+	-	-	-	+	+	-	+	K/A	+	-	-	*Pseudomonas sp.*
TREN-NU2	-	R	M+	-	-	-	+	+	-	+	K/K	+	-	-	*Pseudomonas* sp.
TREN-NU9	-	R	M-	+	-	-	+	+	-	+	K/A	+	-	+	*Pseudomonas* sp.
TREN-NU21	-	R	M+	+	+	+	+	+	-	+	K/K	-	-	-	*Pseudomonas* sp.
TREN-NU22	-	R	M+	-	+	+	+	+	-	+	K/A	+	+	-	*Pseudomonas* sp
TLTENU6	-	R	M+	-	-	-	+	+	-	-	K/A	-	+	+	*Pseudomonas* sp.
TLTENU9	-	R	M+	+	-	-	+	+	-	+	K/A	+	-	-	*Pseudomonas* sp.

TLTENU10	-	R	M+	-	+	+	+	+	-	-	K/K	-	-	-	*Pseudomonas* sp.
TLTENU14	-	R	M+	-	-	-	+	+	-	+	K/A	+	-	-	*Pseudomonas* sp,
ARENTL-NU76	-	R	M-	+	+	+	+	+	+	-	A/A	+	-	+	*Raoultella*sp.
ARENTL-NU84	-	R	M-	-	-	+	+	+	+	-	A/A	+	-	+	*Raoultella* sp.
TLTENU19	-	R	M-	-	-	+	+	-	-	-	K/A	-	-	-	*Romboutsia* sp.
ARENTL-NU50	-	R	M+	-	-	+	+	+	-	-	K/A	+	-	-	*Serratia* sp.
ARENTL-NU68	-	R	M+	-	+	+	+	+	+	-	K/A	+	-	-	*Serratia* sp.
TREN-NU1	-	R	M+	-	+	+	+	+	-	-	K/A	+	-	-	*Serratia* sp.
ARENTL-NU44	-	R	M-	-	+	-	-	+	-	-	K/K	+	-	-	*Shigella* sp.
TREN-NU6	-	R	M-	-	+	-	-	+	-	-	K/K	+	-	-	*Shigella* sp.
ARENTL-NU11	+	C	M-	-	+	+	+	+	+	-	K/A	+	+	-	*Staphylococcus* sp.
ARENTL-NU56	-	C	M+	-	-	-	+	+	-	-	K/A	+	-	-	*Staphylococcus* sp.
ARENTL-NU75	-	R	M+	-	-	-	+	+	-	-	K/K	+	-	+	*Stenotrophomonas* sp.
ARENTL-NU85	-	R	M+	-	-	-	-	+	-	-	K/A	+	+	+	*Stenotrophomonas* sp.
ARENTL-NU88	-	R	M+	-	-	-	-	+	-	-	K/K	+	-	+	*Stenotrophomonas* sp.
ARENTL-NU95	-	R	M+	-	-	-	+	+	-	-	K/A	+	-	+	*Stenotrophomonas* sp.
TREN-NU15	-	R	M+	-	-	-	+	+	-	-	K/A	+	-	+	*Stenotrophomonas* sp.
TLTENU18	-	R	M+	-	-	-	+	+	+	+	K/A	+	-	+	*Stenotrophomonas* sp.
TLTENU20	-	R	M+	-	-	-	+	+	+	+	K/A	+	-	+	*Stenotrophomonas* sp.

GC- Gram character, M- Motility test, IP: Indole production test, MR-Methyl red test, VP-Voges-Proskauer, CU- Citrate utilization test, Ca- Catalase test, Ur: Urease test, Ox-Oxidase test, TSI- Triple sugar iron test, NR-Nitrate reductase test; H_2S-Hydrogen sulphide test, La- Lactose test, +- Positive, - -Negative, +/- -Variable, R- Rod shape, C- Cocci shape, R/C- Rod, slightly cocci shape, M+-Motile, M- - Non-motile.

16S *r*RNA basedcharacterization

Genomic DNA extracted from all isolates were successfully amplified using universal primer pairs. The banding pattern produced by isolates on 1.2% agarose gel is shown in Fig. 3.3. The BLAST search of the aligned sequences of the bacterial isolates carried out against the NCBI GenBank database had similarity to sequences of primer regions of the respective bacteria. The markers used provided high accuracy for the identification of bacterial species from NCBI GenBank. All the isolates along with their GenBank accession number are presented in Table 3.7. The identified isolates were labelled with respective strain name and deposited at the Microbiology laboratory, Nagaland University, Lumami, India.

Out of the total 128 bacterial isolates, 35 isolates were identified up to generic level whereas, 93 isolates were identified up to species level. The isolates were assigned to 19 genera out of which, *Bacillus* was predominantly noted with 56 isolates followed by *Pseudomonas* with 20 isolates whereas, *Chryseobacterium*, *Providencia* and *Romboutsia*each with only 1 isolate represent the genera with the lowest number of the isolate.

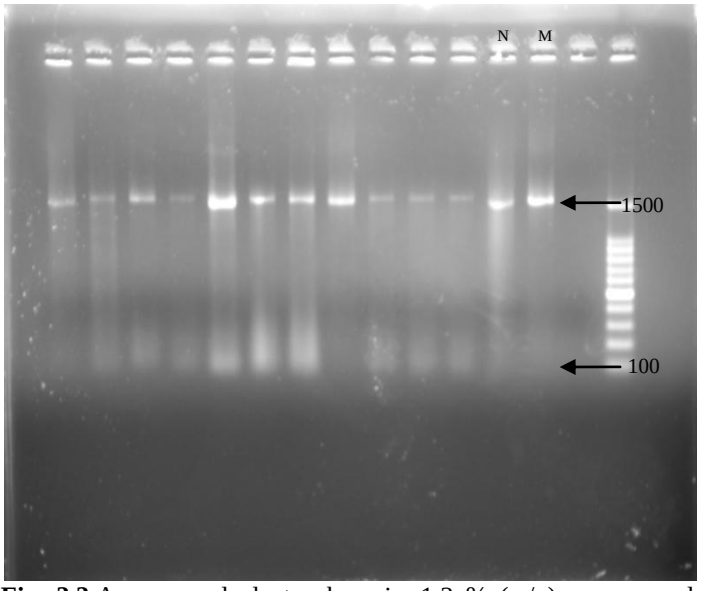

Fig. 3.3. Agarose gel electrophoresis. 1.2 % (w/v) agarose gel with PCR amplified 16S *r*RNA gene product and DNA ladder, lane M denote molecular weight marker, lane N denote negative control and the remaining lane denote PCR amplified 16S *r*RNA gene product of some representative bacteria.

Table 3.7. 16S *r*RNA based identification of bacteria from tea gardens with NCBI GenBank accession number

Representative Strain	Organism	Percent Similarity	Query cover	NCBI GenBank Accession number
ARENTL-NU72	*Aeromonas hydrophila*	100	100	MH503889
ARENTL-NU96	*Aeromonas hydrophila*	98.31	99	MH979999
TREN-NU12	*Aeromonas hydrophila*	99.16	99	MK780823
ARENTL-NU37	*Acinetobacter calcoaceticus*	96.24	92	MG589482
TREN-NU8	*Acinetobacter calcoaceticus*	86.94	94	MK775344
TLTENU12	*Acinetobacter johnsonii*	100	100	MN218075
ARENTL-NU45	*Acinetobacter tandoii*	97.19	98	MG597043
ARENTL-NU14	*Bacillus amyloliquefaciens*	99.58	100	MG252263
ARENTL-NU78	*Bacillus cereus*	99.29	99	MH628528
ARENTL-NU90	*Bacillus cereus*	99.77	100	MH979640
ARENTL-NU91	*Bacillus cereus*	100	100	MH979629
ARENTL-NU93	*Bacillus cereus*	100	100	MH979630
TREN-NU13	*Bacillus cereus*	99.28	100	MK784809
TREN-NU20	*Bacillus cereus*	98.26	100	MK480667
TREN-NU24	*Bacillus cereus*	100	98	MK484111
ARENTL-NU82	*Bacillus circulans*	99.38	100	MH635412
ARENTL-NU6	*Bacillus licheniformis*	99.79	99	MG232647
ARENTL-NU15	*Bacillus licheniformis*	99.8	98	MG252281
ARENTL-NU16	*Bacillus licheniformis*	99	98.76	MG252373
ARENTL-NU29	*Bacillus licheniformis*	98.30	99	MG573070
ARENTL-NU59	*Bacillus licheniformis*	99.04	100	MH145368
ARENTL-NU89	*Bacillus licheniformis*	100	98	MH974113
TREN-NU7	*Bacillus licheniformis*	99.34	99	MK775303
ARENTL-NU79	*Bacillus magaterium*	98.80	99	MH628532
ARENTL-NU80	*Bacillus magaterium*	100	100	MH628585
TREN-NU17	*Bacillus magaterium*	99.85	100	MK478850
ARENTL-20	*Bacillus paralicheniformis*	98.94	99	MG252778
ARENTL-NU17	*Bacillus pumilus*	96.88	98	MG252475
ARENTL-NU61	*Bacillus pumilus*	99.20	98	MH145385
ARENTL-NU44	*Bacillus stratosphericus*	98.38	99	MG597042
ARENTL-NU	*Bacillus subtilis*	99.59	99	MG233399
ARENTL-NU1	*Bacillus subtilis*	100	100	MG209570
ARENTL-NU2	*Bacillus subtilis*	98.95	97	MG231260
ARENTL-NU2	*Bacillus subtilis*	98.76	98	MG243404
ARENTL-NU9	*Bacillus subtilis*	96	99	MG250170
ARENTL-NU39	*Bacillus subtilis*	99.58	99	MG589507
ARENTL-NU55	*Bacillus subtilis*	100	100	MG738717
ARENTL-NU57	*Bacillus subtilis*	100	100	MG739223
ARENTL-NU62	*Bacillus subtilis*	100	100	MH145386
ARENTL-NU99	*Bacillus subtilis*	98.97	98	MH990797
TREN-NU4	*Bacillus subtilis*	99	98.95	MK770353
TREN-NU6	*Bacillus subtilis*	96.49	99	MK770448
TREN-NU18	*Bacillus subtilis*	96.89	99	MK478845
TREN-N19	*Bacillus subtilis*	97	99.16	MK480665
TREN-NU25	*Bacillus subtilis*	100	99	MK484113
ARENTL-NU2	*Bacillus* sp.	97.88	99	MG209800
ARENTL-NU2	*Bacillus* sp.	98.06	99	MG245865
ARENTL-NU5	*Bacillus* sp.	86	88.26	MG231427
ARENTL-NU12	*Bacillus* sp.	96.82	96	MG250175
ARENTL-NU13	*Bacillus* sp.	88.83	97	MG252233
ARENTL-NU13	*Bacillus* sp.	90.02	97	MG250197
ARENTL-NU18	*Bacillus* sp.	84.49	90	MG252499
ARENTL-NU19	*Bacillus* sp.	87	98	MG252504
ARENTL-NU23	*Bacillus* sp.	92	98	MG256759
ARENTL-NU25	*Bacillus* sp.	90.30	98	MG257790

ARENTL-NU94	*Bacillus* sp.	99.33	100	MH979642
TLTENU5	*Bacillus* sp.	99.53	100	MN206973
TLTENU13	*Bacillus* sp.	97.80	100	MN218189
TLTENU17	*Bacillus* sp.	100	100	MT786391
TLTENU20	*Bacillus* sp.	99	100	MT786397
ARENTL-NU77	*Bacillus thuringiensis*	98.32	99	MH628464
ARENTL-NU86	*Bacillus thuringiensis*	99.36	99	MH973699
ARENTL-NU92	*Bacillus thuringiensis*	99.88	100	MH979639
ARENTL-NU21	*Brevibacilluspanacihumi*	99.15	98	MG257922
ARENTL-NU58	*Brevibacilluspanacihumi*	98.77	100	MG742312
ARENTL-NU34	[*Brevibacterium*] *frigoritolerans*	100	100	MG581181
ARENTL-NU60	[*Brevibacterium*] *frigoritolerans*	95.36	100	MH145367
TLTENU1	*Burkholderiacepacia*	99.7	100	MN206032
TLTENU7	*Burkholderiacepacia*	99.86	100	MN211555
TLTENU8	*Burkholderiacepacia*	99.87	100	MN211554
TLTENU18	*Burkholderiacepacia*	99.88	100	MT786392
TLTENU2	*Burkholderialatens*	100	100	MN207011
ARENTL-NU32	*Chryseobacterium*sp.	89.32	95	MG576135
ARENTL-NU87	*Comamonas* sp.	97.58	100	MH973727
TREN-NU11	*Comamonas* sp.	100	100	MK455794
TREN-NU16	*Comamonas* sp.	100	100	MK478848
TREN-NU3	*Enterobacter* sp.	99.35	97	MH997487
ARENTL-NU35	*Enterobacter* sp.	99	97.74	MG581286
ARENTL-NU41	*Enterobacter* sp.	96.07	84	MG596988
ARENTL-NU66	*Lysinibacillusboronitolerans*	92.76	97	MH458789
TLTENU11	*Lysinibacillusfusiformis*	100	100	MN217682
TLTENU3	*Lysinibacillusfusiformis*	100	100	MN207012
AREN-NU1	*Paenibacillusdentritiformis*	98.88	99	MG209370
ARENTL-NU36	*Paenibacillusillinoisensis*	97.30	97	MG581458
ARENTL-NU65	*Paenibacillusillinoisensis*	98.60	100	MH458788
ARENTL-NU98	*Paenibacillusillinoisensis*	96.49	94	MH986195
AREN-NU70	*Paenibacilluspabuli*	98.06	100	MH479559
TREN-NU23	*Paenibacilluspabuli*	98.34	100	MK484109
AREN-NU23	*Paenibacillus* sp.	92.13	98	MG256172
ARENTL-NU24	*Paenibacillus*sp.	95.66	98	MG256494
AREN-NU43	*Providencia alcalifaciens*	99.37	100	MG597034
AREN-NU74	*Pseudomonas cedrina*	98.73	99	MH985302
TLTENU10	*Pseudomonas cremoricolorata*	99.50	100	MN218450
ARENTL-NU46	*Pseudomonas lutea*	92.42	98	MG738688
ARENTL-NU38	*Pseudomonas monteilii*	99.35	98	MG589483
AREN-NU45	*Pseudomonas monteilii*	95.75	98	MG738473
ARENTL-NU54	*Pseudomonas monteilii*	98.07	99	MG738711
ARENTL-NU69	*Pseudomonas monteilii*	100	100	MH479554
AREN-NU71	*Pseudomonas monteilii*	99.58	100	MH484604
ARENTL-NU100	*Pseudomonas monteilii*	98	100	MH990861
ARENTL-NU73	*Pseudomonas putida*	100	98	MH503890
TLTENU4	*Pseudomonas putida*	99.83	100	MN206776
ARENTL-67	*Pseudomonas* sp.	88.57	100	MH475141
ARENTL-NU97	*Pseudomonas* sp.	98.28	89	MH986193
TREN-NU2	*Pseudomonas* sp.	99.88	100	MH990907
TREN-NU9	*Pseudomonas* sp.	98.30	99	MK780822
TREN-NU21	*Pseudomonas* sp.	90.14	100	MK483978
TREN-NU22	*Pseudomonas* sp.	99.86	100	MK484032
TLTENU6	*Pseudomonas* sp.	99.39	100	MN207013
TLTENU9	*Pseudomonas* sp.	100	99	MN211557
TLTENU14	*Pseudomonas* sp.	100	100	MN224046
ARENTL-NU76	*Raoultellaplanticola*	97.05	99	MH626065
ARENTL-NU84	*Raoultella* sp.	94.21	98	MH651756

TLTENU19	*Romboutsia* sp.	98.96	100	MT786394
ARENTL-NU50	*Serratia marcescens*	99.58	100	MG738710
ARENTL-NU68	*Serratia marcescens*	99.50	100	MH475145
TREN-NU1	*Serratia marcescens*	99.37	99	MH990862
ARENTL-NU44	*Shigella sonnei*	99.78	100	MG597038
TREN-NU6	*Shigella sonnei*	98.92	100	MK775462
ARENTL-NU11	*Staphylococcus equorum*	96.19	97	MG250169
ARENTL-NU56	*Staphylococcus sciuri*	99.98	99	MG738790
ARENTL-NU75	*Stenotrophomonasmaltophilia*	99	98.61	MH626043
ARENTL-NU85	*Stenotrophomonasmaltophilia*	88.81	99	MH973664
ARENTL-NU88	*Stenotrophomonasmaltophilia*	94.68	99	MH974229
ARENTL-NU95	*Stenotrophomonasmaltophilia*	100	100	MH979650
TREN-NU15	*Stenotrophomonasmaltophilia*	100	100	MK478846
TLTENU18	*Stenotrophomonas maltophilia*	98.57	100	MT786393
TLTENU20	*Stenotrophomonas maltophilia*	98.90	100	MT786396

3.3.1.4. Variation in bacterial isolates

The seasonal screening of bacterial isolates at the rhizospheric and non-rhizospheric soil at both the tea gardens is presented in Table 3.8. The taxonomic classification of the bacterial isolates performed using the phenotypic method and further confirmed by 16S *r*RNA based identification placed the isolatesto 19 genera namely, *Aeromonas*, *Acinetobacter*, *Bacillus*, *Brevibacillus*, [*Brevibacterium*], *Burkholderia*, *Chryseobacterium*, *Comamonas*, *Enterobacter*, *Lysinibacillus*, *Paenibacillus*, *Providencia*, *Pseudomonas*, *Raoultella*, *Romboutsia*, *Serratia*, *Shigella*, *Staphylococcus* and *Stenotrophomonas*.Among these genera, the maximum number of isolates at both tea gardens were under *Bacillus* followed by *Pseudomonas*. 37 bacterial isolates under 11 genera and 33 bacterial isolates under 10 genera were recorded fromthe Tuli tea garden in the first and second year respectively. In both the years, the highest number of isolates were recorded in the spring season. Species of *Brevibacillus*, *Providencia* and *Staphylococcus* were observed only in the first year whereas, species of *Raoultella*, *Serratia* and *Shigella* were observed only in the second year. 30 bacterial isolates under 10 genera and 28 bacterial isolates under 9 genera were recorded from theUngma tea garden in the first and second year respectively. In both the years, the highest number of isolates were recorded in the summer season. Species of [*Brevibacterium*], *Chryseobacterium*, *Serratia* and *Staphylococcus* were observed only in the first year whereas, species of *Comamonas*, *Romboutsia* and *Stenotrophomonas* were observed only in the second year. Bacterial genera *Aeromonas*, *Brevibacillus*, *Enterobacter*, *Providencia*, *Raoultella* and *Shigella* were restricted to the Tuli tea garden whereas, [*Brevibacterium*], *Chryseobacterium*, *Lysinibacillus* and *Romboutsia* were restricted to the Ungma tea garden.

Table 3.8. Seasonal and site variations of bacterial isolates during 2016-2018

Site	Spring	Summer	Autumn	Winter
Tuli	*Aeromonas hydrophila*(MH503889-TR1, MK780823-TNR1, MH979999-TR2), *Bacillus licheniformis* (MG252373-TR1, MH974113-TR2, MK775303-TNR2), *Bacillus* sp. (MG250175-TR1 MG252504-TNR1),*Bacillus subtilis* (MK770448-TR1, MG589507-TNR1, MG739223-TR2, MH990797-TNR2)*Brevibacilluspanacihumi*(MG742312-TR1 MG257922-TNR1), *Enterobacter* sp. (MH997487-TR2), *Pseudomonas cedrina*(MH985302-TR1), *Pseudomonas lutea* (MG738688-TNR1), *Pseudomonas monteilii*(MH479554-TNR1), *Pseudomonas putida* (MH503890-TNR2), *Pseudomonas* sp. (MH990907-TR1 MH475141-TR2), *Raoultellaplanticola* (MH626065-TR2),*Raoultella* sp. (MH651756-TNR2)	*Bacillus magaterium* (MH628532-TR2), *Bacillus pumilus*(MG252475-TR1, MH145385-TNR1),*Bacillus subtilis* (MK484113-TR2, MK770353-TNR2), *Bacillus* sp. (MG256759-TR1), *Bacillus stratosphericus*(MG597042-TNR1), *Comamonas*sp (MK478848-TR1), *Enterobacter* sp. (MG596988-TR1, MG581286-TNR1), *Providencia alcalifaciens*(MG597034-TR1), *Pseudomonas monteilii*(MH484604-TR1 MG589483-TNR1), *Pseudomonas putida* (MN206776-TNR2), *Pseudomonas* sp. (MK484032-TR2), *Serratia marcescens* (MH475145-TR2, MH990862-TNR2), *Shigella sonnei* (MG597038-TR2, MK775462-TNR2), *Staphylococcus sciuri*(MG738790-TNR1)	*Acinetobacter calcoaceticus* (MG589482-TR1, MK775344-TNR1), *Bacillus magaterium* (MH628585-TR2, MK478850-TNR2),*Bacillus* sp. (MG245865-TR1), *Bacillus subtilis* (MH145386-TR1), *Bacillus thuringiensis* (MH628464-TR1),*Paenibacillus*sp. (MG256494-TR1), *Pseudomonas* sp. (MN207013-TR2, MK483978-TNR2), *Stenotrophomonas maltophilia*(MH979650-TNR1, MH973664-TR2, MH626043-TNR2)	*Bacillus cereus* (MK480667-TR1, MH979640-TNR1, MH628528-TR2, MK484111- TNR2), *Bacillus subtilis* (MG738717-TNR1, MK480665-TNR2), *Bacillus thuringiensis* (MH973699-TR1), *Comamonas* sp. (MK455794-TR2),*Paenibacilluspabuli*(MH479559-TR1 MK484109-TNR1),*Paenibacillusillinoisensis* (MH986195-TR2 MH458788-TNR2), *Stenotrophomonas maltophilia*(MK478846-TR2, MH974229-TNR2)
Ungma	*Acinetobacter johnsonii* (MN218075-UR2), *Acinetobacter tandoii* (MG597043-UR1), *Bacillus licheniformis* ARENTL-NU15 (MG252281-UR1 MG232647-UNR1, MH145368-UR2, MG573070-UNR2),	*Bacillus subtilis*(MG209570-UR1, MG233399-UNR1), *Bacillus* sp (MG231427-UNR1, MN206973-UR2, MG257790-UNR2), *Burkholderiacepacia*	*Bacillus circulans* (MH635412-UR1), *Bacillus paralicheniformis* (MG252778-UR1), *Bacillus subtilis*(MG243404-UNR1), *Bacillus* sp (MG250197-UR1, MG252499-	*Bacillus amyloliquefaciens* (MG252263-UR1), *Bacillus cereus* (MH979629-UNR1, MK784809-UR2, MH979630UNR2), *Bacillus* sp (MG252233-UNR1, MN218189-UR2,

	Bacillus subtilis (MG250170-UR1, MG231260-UNR1, MK478845-UNR2), *Bacillus* sp. (MG209800-UR1, MT786397-UR2), [*Brevibacterium*] *frigoritolerans* (MH145367-UR1, MG581181-UNR1), *Pseudomonas monteilii* (MG738473-UR1, MG738711-UNR1), *Pseudomonas* sp. (MN224046-UR2, MN211557-UNR2)	(MN211554-UR2, MT786392-UNR2), *Chryseobacterium*sp. (MG576135-UNR1), *Lysinibacillusboronitolerans* (MH458789-UR1), *Pseudomonas* sp. (MK780822-UNR1), *Serratia marcescens* (MG738710-UR1), *Staphylococcus equorum* (MG250169-UR1), *Pseudomonas monteilii* (MH990861-UR2), *Pseudomonas* sp. (MH986193-UNR2), *Romboutsia* sp. (MT786394-UR2), *Stenotrophomonas maltophilia* (MT786396-UR2, MT786393-UNR2)	UNR1, MT786391-UNR2), *Bacillus thuringiensis* (MH979639-UR2), *Burkholderiacepacia* (MN206032-UR1, MN211555-UNR1), *Burkholderialatens* (MN207011-UR2), *Comamonas* sp. (MH973727-UNR2), *Lysinibacillusfusiformis* (MN217682-UR2), *Paenibacillusillinoisensis*(MG581458-UNR2), *Pseudomonas cremoricolorata*(MN218450-UR2)	MH979642-UNR2), *Paenibacillusdentritiformis* (MG209370-UR1), *Paenibacillus* sp. AREN-NU23 (MG256172-UNR1), *Lysinibacillusfusiformis* (MN207012-UR2)

Characters in parenthesis indicate NCBI GenBank accession numbers and sampling sites. TR1-Rhizospheric soil at Tuli during the year 2016-2017, TNR1- Non-rhizospheric soil at Tuli during the year 2016-2017, TR2-Rhizospheric soil at Tuli during the year 2017-2018, TNR2-Rhizospheric soil at Tuli during the year 2017-2018, UR1-Rhizospheric soil at Ungma during the year 2016-2017, UNR1-Non-rhizospheric soil at Ungma during the year 2016-2017, UR2-Rhizospheric soil at Ungma during the year 2017-2018, UR2- Non-rhizospheric soil at Ungma during the year 2017-2018.

3.3.2. Fungal analysis

3.3.2.1. Variations in fungal counts

Fungal counts recorded seasonally at Tuli and Ungma tea garden over the two study years are presented in Table 3.9.

In the first year, the fungal counts at the rhizospheric and non-rhizospheric soil at the Tuli tea garden ranged from 2.73 x 10^4 - 5.70 x 10^4 $CFUg^{-1}$ and 2.57 x 10^4 - 5.43 x 10^4 $CFUg^{-1}$ with the mean value from 2.65 x 10^4 - 5.57 x 10^4 $CFUg^{-1}$. In the second year, the fungal counts ranged from 2.67 x 10^4 - 5.03 x 10^4 $CFUg^{-1}$ and 2.93 x 10^4 - 4.83 x 10^4 $CFUg^{-1}$ at the rhizospheric and non-rhizospheric soil with the mean value from 2.80 x 10^4 - 4.93 x 10^4 $CFUg^{-1}$. Thehighest fungal counts were recorded during the spring seasons whereas, the lowest fungal counts were recorded during winter seasons in both years.

The fungal counts in the first year at the rhizospheric and non-rhizospheric soil at the Ungma tea garden ranged from 2.13 x 10^4-4.17 x 10^4 $CFUg^{-1}$ and 2.00 x 10^4 - 4.07 x 10^4 $CFUg^{-1}$ with the mean value from 2.07 x 10^4 to 4.12 x 10^4 $CFUg^{-1}$. In the second year, the fungal counts ranged from 1.77 x 10^4 - 3.77 x 10^4 $CFUg^{-1}$ and 1.63 x 10^4 - 3.70 x 10^4 $CFUg^{-1}$ at the rhizospheric and non-rhizospheric soil with the mean value from 1.70 x 10^4 - 3.73 x 10^4 $CFUg^{-1}$. Thehighest fungal counts were recorded during the spring seasons whereas the lowest fungal counts were recorded during winter seasons in both years.

Based on the results from both the years, seasonal studies of fungal counts in the Tuli tea garden showed the following trend of decreasing order spring > autumn > summer > winter whereas, in the Ungma tea garden, it showed the following trend of decreasing order spring > summer > autumn > winter.

Table 3.9. Fungal counts (x 10^4 $cfug^{-1}$) from the tea garden of Tuli and Ungma during 2016 - 2018

Site	Soil	2016 - 2017				2017 - 2018			
		Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Tuli	R	5.70 ± .12	3.93 ± .07	4.63 ± .09	2.73 ± .09	5.03 ± .12	3.40 ± .06	4.07 ± .09	2.67 ± .03
	NR	5.43 ± .15	3.67 ± .03	4.50 ± .06	2.57 ± .09	4.83 ± .12	3.13 .09	3.87 ± .08	2.93 ± .09
	Mean	**5.57 ± 1.02^a**	**3.80 ± .068^c**	**4.57 ± .06^b**	**2.65 ± .07^d**	**4.93 ± .09^a**	**3.27 ± .08^c**	**3.97 ± .08^b**	**2.80 ± .07^d**
Ungma	R	4.17 ± .08	3.20 ± .06	2.67 ± .07	2.13 ± .03	3.77 ± .07	3.03 ± .03	2.30 ± .06	1.77 ± .03
	NR	4.07 ± .07	3.03 ± .03	2.50 ± .06	20.00 ± .06	3.70 ± .06	2.80 ± .06	2.17 ± .07	1.63 ± .03
	Mean	**4.12 ± .05^a**	**3.11 ± .05^b**	**2.58 ± .05^c**	**2.07 ± .04^d**	**3.73 ± .04^a**	**2.92 ± .06^b**	**2.23 ± .05^c**	**1.70 ± .04^d**

R - rhizosphere; NR - non-rhizosphere.
Mean ± standard error mean.

Different letters ([a, b, c] and [d]) in each year indicate significant differences between seasons ($p < 0.05$) after a Tukey *post hoc* test.

3.3.2.2 Statistical analysis

The effects of the sampling seasons on fungal counts were statistically significant ($p < 0.05$) at the tea gardens as revealed by one-way ANOVA (Table 3.10). Further, Tukey's post hoc test of significance for mean differences revealed statistically significant differences in fungal counts between the seasons in both years. Two-way ANOVA conducted between the study sites and sampling seasons also revealed statistically significant interaction ($p < 0.05$) between the effects of season and site (Table 3.11).

The correlation study of the fungal counts revealed an uneven relationship with soil properties between the tea gardens as well as between the sampling years (Table 3.12). In the first year at Tuli tea garden, the correlation of fungal counts was statistically significant and positive with SOC and all the available nutrients but negative with silt whereas, in the second year correlation was statistically significant and positive with *p*H, SOC and available P. At Ungma tea garden fungal counts in the first year established statistically significant and positive correlation with SOC, available N and available P whereas, the correlation was significant and positive with moisture, *p*H and SOC in the second year.

Table 3.10. One-way ANOVA of fungal count

Parameter	**Tuli**				**Ungma**			
	2016 - 2017		**2017 - 2018**		**2016 - 2017**		**2017 - 2018**	
Fungal CFU	F (3,20)	*p* value	F (3,20)	*p* value	F (3,20)	*p* value	F (3,20)	*p* value
	267.17	0.00	135.95	0.00	308.63	0.00	337.57	0.00

Table 3.11. Two-way ANOVA of fungal count between tea gardens and seasons

Parameter	**Source**	**2016 - 2017**		**2017 - 2018**	
		F	*p* value	F	*p* value
Fungal CFU	Site	676.22	0.00	556.92	0.00
	Season	505.67	0.00	341.41	0.00
	Site X Season	53.91	0.00	37.63	0.00

Table 3.12. Correlation analysis of soil fungal count with soil physicochemical properties

Soil parameter	**Tuli**		**Ungma**	
	2016-2017	**2017-2018**	**2016-2017**	**2017-2018**
	Fungal CFU	**Fungal CFU**	**Fungal CFU**	**Fungal CFU**
Sand (%)	0.31	0.67	0.04	0.08
Clay (%)	0.43	-0.41	0.10	-0.46
Silt (%)	-.724*	-0.25	-0.08	0.06
Moisture (%)	0.43	0.41	0.40	.778*
*p*H	0.47	.789*	0.52	.787*
Temperature (°C)	0.48	0.11	0.33	0.38
SOC (%)	.986**	.829*	.852**	.758*
Available N (kg/ha)	.868**	-0.03	.879**	0.07
Available P (kg/ha)	.861**	.738*	.795*	0.06
Available K (kg/ha)	.865**	0.32	0.25	0.46

SOC - soil organic carbon, Available N - available nitrogen, Available P- available phosphorus, Available K- available potassium.

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

3.3.2.3. Culture and microscopic characterization of fungal isolates

The morphological characterization for the identification of fungal isolates was carried out from culture plates and microscopic studies. The majority of the isolates belonged to genera *Aspergillus*, *Penicillium* and *Trichoderma* represented by 11, 9 and 9 speciesrespectively. Fig.3.4-Fig.3.37 represents some of these fungal isolates identified in the present study.

***Acremonium* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA in 5 days were 2.7-3.2 cm, 2.5-3 cm, 1.8-3 cm and 1.1-1.7 cm respectively. Colonies on the obverse of these media plates were initially white which turned to greyish brown (CDA and RBA) and greyish orange (MEA and PDA). On the reverse of theseplates**,** colony colour varied frombrown, dark brown and colourless. Colonies were compact tufts to powdery, wrinkled and convoluted, slightly raised in the centre, irregular and entire to slightly undulate.

Microscopic features: Hyphae hyaline, septate, branched and produced phialides. Phialides 1.8-3 µm wide and 16-40 µm long, awl-shaped, erect, septate, unbranched, hyaline, with a basal septum, tapered towards the apex with terminal slimy conidial masses. Conidia 2.7-3.5 µm x 1.2-2 µm, phialosporous, 1-celled, solitary, hyaline, cylindrical to oval and ellipsoidal.

***Acremonium* sp.2**

Culture characteristics: Colony diameters on CZA, PDA and RBA in 7 days were 0.8-1cm, 1.1-1.3 cm and 1-1.2 cm respectively. Colonies on the obverse on these media plates were pinkish white. On the reverse of theseplates**,** colony colour varied fromsalmon to colourless. Colonies were cottony, felty and flat.

Microscopic features: Hyphae hyaline, septate, branched and produced phialides. Conidiophores reduced to conidiogenous cells, raised laterally from hyphae. Phialides 1.5-2.3 µm wide and 28-45 µm long, awl-shaped, erect, septate, unbranched, hyaline, with a basal septum, tapered towards the apex with terminal slimy conidial masses. Conidia 3.3-5.5 µm x 1.5-2 µm, phialosporous, grouped in slimy heads, 1-celled, solitary, hyaline and cylindrical to ellipsoidal.

Apophysomyces viriabilis

Culture characteristics: Colonies developed fast on all the culture plates and within 3 days it reached 4.5-5.3 cm, 4.1-5cm, 3.9-4.8 cm and 4-4.9 cm on CZA, MEA, PDA and RBA respectively. Colony colours on the obverse of these media plates varied from whitish-grey (CZA, MEA, PDA), to creamy white (RBA). On the reverse of theseplates**,** colony colour waspale brown (CZA and MEA), yellow (PDA) and colourless (RBA). Colonies were floccose, cottony, raised, irregular and entire to undulate.

Microscopic features: Hyphae hyaline, septate to aseptate, branched, 3.3-5.3 µm, irregular. Foot cells at the base of sporangiophore. Sporangia 15.7-53.6µm wide, multispored, terminal, pyriform, apophysate. Columellae hemispheric. Apophyses funnel-shaped. Sporangiophore 2.6-4µm wide and 106.7-397µm long, erect, slightly tapered towards the apex, subhyaline, ellipsoidal, unbranched. Rhizoids subhyaline, unbranched. Sporangiospores 3.3-5.8µm wide, subhyaline, ellipsoid, trapezoid and claviform.

***Arthrinium* kogelbergense**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.5-4.1cm, 3.8-4.6cm, 3.5-4.4cm and 3.5-4cm respectively. Colony colours on the obverse and reverse of these media plates varied from greyish-white and greyish-yellow, dirty white and pale yellow, dirty white and yellow, yellowish-white and colourless. Colonies were slightly raised in the centre, spreading, cottony, sparse aerial mycelium and circular.

Microscopic features: Mycelium with hyaline, smooth, branched and septate, 1.8-4.3µm diameter hyphae. Conidiophores reduced to conidiogenous cells, which were clustered in aggregation on hyphae, sympodial or polyblastic, doliiform to ampulliform, 5.2-10.5µm long and 3.8-5.7µm wide. Conidia 8.7-13.3µm x 6.4-11.8µm,brown to dark brown, pale equatorial slit, central scar, smooth and globose to ellipsoidal.

Aspergillus flavus

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.1-4.5cm, 4.1- 5.2cm, 4.1-5.2cm and 4-5cm in 5 days. Colony colours were yellowish-green (CZA), light green (MEA and RBA) and deep green (PDA) the obverse of these media plates whereas, on the reverse of the plates colony colours were yellow (CZA and PDA), brownish-yellow (MEA) and colourless (RBA).Yellow and tiny transparent exudates were released from colonies on MEA and RBA plates. The colonies were with white mycelium, floccose at

the centre, granular, raised, plane to sulcate on obverse and plane to wrinkle on the reverse of the plates.

Microscopic features: Conidiophores raised separately from the substratum, 500-700µm long, rough, hyaline. Phialides 5.5 -7.2µm long and 2.3 -3.4µm wide, 2 series of phialides covered the 35-60µm diameter globose to sub-globose vesicles. sclerotia, large and globose. Conidia 4.8-8.7µm x 4.1-4.8µm, rough, more or less pyriform and elliptical.

Aspergillus heteromorphous

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.3-3.9cm, 3.8-4.5cm, 4.1-5.3cm and 4-5cm respectively. Colony colours on the obverse and reverse of these media plates varied from black and pale brown (CZA), brownish-black and brown (MEA), black and pale brown (PDA), brownish-black and colourless (RBA). Colonies were granular, surrounded by white floccose mycelium.

Microscopic features: Conidiophores raised from sub-surface and substratum, 423-790 µm long and 9-12µm wide, smooth, aseptate hyphae, biserrate. Vesicles 33-47µm diameter, globose. Phialides 5.9 -7.7 µm long and 2.5 -3.3 µm wide. Conidia 3-3.8µm diameter, rough and globose.

Aspergillus niger

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.5-4.6cm, 3.3-3.9cm, 4.8-5.2cm and 2.1-2.6cm respectively. Colony colours were black on the obverse of these plates whereas, on the reverse of these plates colony colours were cream (CZA), yellow (PDA and MEA) and colourless (RBA). Colonies. Black exudates were released from colonies on PDA plates. Colonies were effuse and velvety to powdery, radially sulcate on obverse and reverse of culture plates, flat and entire.

Microscopic features: Conidiophores raised directly from the substratum, 8- 15µm wide and 310-450µm long, smooth, hyaline, septate, biseriate. Phialides 6.9 -8.3µm long and 2.9-3.5µm wide, 2 series of phialides covered the entire vesicles which were 15-40 µm in diameter. Conidia 2.5-4µm diameter, smooth and globose.

Aspergillus ochraceus

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.2-4.1cm, 3-3.7cm, 3.2-3.9cm, 0.9-2.8cm. Colony colours were pale yellow (CZA), brownish-yellow (MEA) and golden yellow (PDA and RBA) on the obverse of these media plates whereas, on the reverse of these plates colony colours were yellow (CZA), pale yellow

(MEA), pale brown (PDA) and colourless (RBA). Bright yellow pigmentswere observed around the colonies on CZA plate. The colonies were with white or creamy mycelium, powdery, velvety to floccose, slightly raised in the centre, plane to diametrically sulcate on the obverse and wrinkled to plane on the reverse of the plate, entire.

Microscopic features: Conidiophores raised directly from the substratum, 7.7-14.3µm wide, 398-615µm long stipes, biseriate. Phialides 7.9-12µm long and 2.4-2.9µm wide, 2 series of phialides covered the entire globose vesicles which were 22.8-30.3µm in diameter. Conidia 5-3.8µm in diameter, rough and globose.

Aspergillus versicolor

Colony features: Colony sizes on CZA, MEA, PDA and RBA varied from 3.6-4.6cm, 2.1-2.9cm, 3.6-4.8cm, 2.3-3.2cm. Colony colours on obverse and reverse of these media plates were brownish green and yellow to deep brown (CZA), deep green and yellow-brown (MEA), dark bluish with a brown centre and yellow (PDA), bluish-green and colourless (RBA). Brown coloured exudates were released from the colonies on MEA, PDA and RBA plates. Colonies were surrounded with white mycelium, velvety, floccose, sulcate on the obverse and wrinkled on the reverse of the plates.

Microscopic features: Conidiophores raised from the substratum, 220-750µm long, aerial and closely interwoven mycelium, smooth-walled, sub-globose, biseriate. Phialides 5.3-8.3µm long and 2.1-2. µm wide. Vesicles 9.1-16µm diameter, pyriform. Conidia 2.7-3.6µm diameter, ellipsoidal and rough. Hyaline, smooth-walled and monoverticillate*Penicillium*-like conidiophores were also observed.

***Aspergillus* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 2.1-2.9cm, 2.5-3.4cm, 3.1-4cm and 2.9-3.7cm respectively. Colony colours on obverse and reverse of these media plates were greyish yellow and yellowish to brown (CZA), greenish-grey and brown (MEA), greenish-grey and yellow to brown (PDA), cream and colourless (RBA). Brown and transparent exudates were released from colonies on MEA and RBA plates. Colonies were velvety, with off white mycelium, diametrically sulcate on the obverse and heavily wrinkled on the reverse of the plate, entire.

Microscopic features: Conidiophores raised from the substratum, 5.3-6.9µm wide, 315-622µm long, smooth-walled, hyaline, septate, biseriate. Phialides, 6.1-7.9µm long and 4.3-

7.8µm wide. Vesicles9.5-20.9µm diameter, pyriform. Conidia 1.8-2.5µm x 1.9-3.3µm, sub-globose.

***Aspergillus* sp.2**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 2.5-3.4cm, 2.1-4.3cm, 3.5-4.1cm and 3.2-3.9cm respectively. Colony colours on obverse and reverse of these media plates were light yellow to pale brown colonies and yellow to brown (CZA), greyish-yellow and brown (MEA), greyish to yellow and yellow (PDA), pale brown and colourless (RBA). Brown and transparent exudates were released from colonies on PDA and RBA plates. Colonies were velvety, with off white mycelium, diametrically sulcate on the obverse and heavily wrinkled on the reverse of the plate.

Microscopic features: Conidiophores raised from the substratum, 159-270µm long, 5.1-8.3µm wide, smooth-walled, hyaline, septate, biseriate. Phialides 3.8-6.9µm wide and 5.3-9µm long. Vesicles 10-19µm diameter, sub-globose. Conidia 1.8-2.3µm in diameter and globose.

***Aspergillus* sp.3**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 4.5-5.3cm, 3.6-4.3cm, 4.1-5.1cm and 3.8-4.8cm in 5 days. Colony colours on the obverse and reverse of these media plates were bright yellow and orange (CZA), greenish-yellow and brown (MEA), yellowish-green and pale yellow to brown (PDA), yellowish-green and colourless (RBA). Colonies were raised in the centre on PDA and RBA plates, cottony, granular, with white mycelium, sulcate from the edge to the outer circumference of the centre on the obverse of PDA and varied from heavily wrinkled to plane and sulcate on the reverse of the plates.

Microscopic features: Conidiophores raised from the substratum, 165-390µm long, rough-walled, hyaline, septate, 5.6-8.8µm wide, biseriate. Phialides 4.8-10µm long and 3.9-7.3µm wide. Vesicles 12-21µm diameter,globose to subglobose. Conidia 2-2.7µm in diameter and globose to subglobose.

***Aspergillus* sp.4**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 1.9-2.3cm, 2.1-2.8cm, 2.9-3.3cm and 2.2-3.4cm in 5 days. Colony colours on obverse and reverse of these media plates varied from bluish-white and brownish (CZA, MEA and RBA)

to brownish-white and pale yellow (PDA). Colonies were lanose, radially sulcate on the obverse and slightly wrinkled on the reverse of the plates, entire.

Microscopic features: Conidiophores raised from the substratum, 4.8-7.9µm wide, smooth-walled, hyaline, aseptate. Vesicles uniseriate to biseriate, 4.8-5.3µm wide and 10-14 µm long, sub-globose. Conidia 1.5-2.7 µm in diameter and globose.

***Aspergillus* sp.5**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.5-3cm, 3.1-3.6cm, 4.2-5cm and 2.8-3.3cm in 5 days. Colony colours on the obverse and reverse of these media plates varied from yellowish-white and bright yellow (CZA and MEA) to yellowish-grey and pale yellow (PDA and RBA). Colonies on all plates released transparent exudates, cottony, granular, smooth to slightly sulcate from the edge to the outer circumference of the centre, slightly wrinkled and flat.

Microscopic features: Conidiophores raised from the substratum, rough-walled, hyaline to sub-hyaline, septate. Vesicles 7.6-10µm wide,subglobose to slightly oval, biseriate. Conidia 1.67-1.89µm diameter and globose.

***Aspergillus* sp.6**

Culture characteristics: Colony sizes on CZA, MEA, PDA and RBA reached 2.1-2.9cm, 2.7-3.2cm, 3.8-4.4cm and 3-4.2cm in 7 days. Colony colours on obverse and reverse of these media plates varied from pale yellow and colourless (CZA and RBA), bluish-grey and pale yellow (MEA), yellowish-white and pale yellow (PDA). Colonies on MEA and PDA plates released yellowish pigments. Colonies were lanose, sulcate, wrinkled, slightly raised and entire.

Microscopic features: Conidiophores raised from the substratum, rough-walled, hyaline to sub-hyaline, septate. Vesicles 6.6-8µm wide and 8.9-13µm long, oval, biseriate. Conidia 1.9-2.1µm x 2-2.5 µm and sub-globose to ovoid.

***Botrytis* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 4.3-5cm, 4.6-5.5cm, 4.5-5.3cm and 4.5-5.5cm in 5 days. Colony colours on the obverse of these media plates varied from creamy white (CZA MEA and RBA) to greyish-white (PDA) whereas, on the reverse of these plates colony colours varied from brown, whitish and colourless. Colonies were cottony, floccose to effuse, raised to slightly raised, regular to irregular and wavy to entire.

Microscopic features: Mycelium hyaline, filamentous, branched and septate. Conidiophore 6-9µm wide, raised directly from mycelium, irregularly branched, constricted at the base, septate, erect. Conidia 6.1-8.2µm wide and 8.9-10.7µm long, hyaline, 1-celled and ovoid to ellipsoidal.

Chaetomium globosum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.2-3.6cm, 4.3-4.8cm, 4.3-4.9cm and 3.4-4cm. The obverse and reverse of these media plates showed greyish-yellow and pale yellow (CZA), white to grey and brownish yellow (MEA), olivaceous buff and brown (PDA), yellowish-grey and colourless (RBA) colony colours. Colonies were floccose, granulated, cottony and lobate to entire.

Microscopic features: Ascomata 150-188µm wide and 180-270µm long, superficial, attached to the substratum by short rhizoid like hyphae subglobose, ellipsoidal. Ascomatal wall radiated in all directions. Terminal hairs 3.3-5.6µm wide, undulate. Asci hyaline, fasciculate, clavate. Ascospores 6-8.3µm wide, ellipsoidal, apiculate ends.

***Chaetomium* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.9-3.7cm, 3.1-4.2cm, 4.1-4.9cm and 2.7-3.5cm in 7 days. The obverse and reverse of these media plates showed whitish-grey and yellowish-brown (CZA), greyish brown and colourless (MEA), yellowish-white and pale yellow (PDA), greyish-white and colourless (RBA) colony colours. Colonies were floccose, cottony, granulated, irregular, entire to slightly lobate and crenate.

Microscopic features: Ascomata 190-250µm wide and 210-340µm long, globose to obovate. Ascomatal wall radiate in all directions. Terminal hairs 3.1-5µm wide, branched, undulate. Asci hyaline, fasciculate, clavate. Ascospores 7.2-10.5µm wide, sub-globose to ellipsoidal, apiculate ends.

***Chaetomium* sp.2**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.5-4.4cm, 3.3-4.2cm, 3.2-4cm and 2.7-3.5cm in 7 days. The obverse and reverse of these media plates showed white and pale yellow (CZA), greyish-white and pale brown (MEA), whitish olivaceous grey and pale brown (PDA), white and colourless (RBA) colony colours. Colonies were floccose, cottony, irregular, hairy, entire to crenate.

Microscopic features: Ascomata 200-350µm wide and 240-380µm long, globose to sub-globose. Peridium dark brown, rough. Ascomatal wall radiate in all directions. Terminal hairs 3.3-4.8µm wide, branched, undulate. Asci hyaline, fasciculate, clavate. Ascospores 6.8-9 µm wide, sub-globose and irregular.

***Chrysosporium* sp.**

Culture characteristics: Colonies developed fast on CZA, MEA and PDA but the growth was poor on RBA. Colony diameters on CZA, MEA and PDA reached 3.8-4.3cm, 3.4-4cm, and 3.6-4.2cm within 3 days. Colony colours on the obverse of these media plates were white whereas, the colours on the reverse of these plates varied from colourless (CZA) to yellowish-brown (MEA and PDA). Colonies were fluffy to powdery to granular, circular, flat and entire.

Microscopic features: Hyphae 1.1-3.3µm wide, hyaline to arthroconidia like septations, straight to racquet, branched. Conidia 3.7-5µm x 1.6-2.6µm, hyaline, aleuriosporus globose to clavate and pyriform, 1-celled, basal scars, terminal, intercalary as well as lateral.

Cladosporium cladosporiodes

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.7-3.8cm, 2.4-3.5cm, 2.5-3.6cm and 2.2-3.2cm in 5 days. Colony colours on the obverse of these media plates were initially whitish-grey then changed to olivaceous brown whereas, the colours on the reverse of these plates varied from black (CZA and RBA) to brown (MEA and PDA). Colonies were floccose-felty to floccose, slightly raised to raised, round and entire to scalloped.

Microscopic features: Hyphae branched and septate. Condiophore 290-340µm long and 3.4-6.2µm wide, straight, branched, solitary with 3 to 4 cylindrical ramoconidia. Conidia numerous, 3-4.9µm long and 1.9-2.1µm wide, 1 to 2-celled, branched, acropetal chains, olivaceous brown, limoniform and ovoid.

Cladosporium herbarum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 4.8-5.5cm, 3.1-4.2cm, 2.4-3.9cm and 3-4cm in 7 days. Colony colours on the obverse of these media plates were initially whitish-grey then changed to grey (CZA), pale olivaceous grey with a white margin (MEA), olivaceous green (PDA and RBA) whereas, the colours on the reverse of these plates varied from olivaceous black (CZA and MEA) and iron-grey (PDA

and RBA). Colonies were glabrous, floccose to velvety, raised, radially furrowed, folded and wrinkled in the centre, slightly wrinkled, irregular to regular and undulate to entire.

Microscopic features: Hyphae 2.1-5µm wide, branched, creeping and septate. Conidiophore 243-270µm long, solitary, branched, septate, erect, cylindrical, terminal and intercalary swelling. Ramoconidia cylindrical, ampulliform, septate. Conidia numerous, 1.8-2.5µm wide and 4.5-7.2µm long, branched chains, 1 to 2-celled and ovoid to ellipsoidal.

Cladosporium oxysporum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.2-2.8cm, 2.1-2.5cm, 2.2-2.9cm and 1.2-2.2cm in 5-7 days. Colony colours on the obverse of these media plates varied from pale grey (CZA and MEA), smoke grey (PDA) and bright grey (RBA) whereas, the colours on the reverse of these plates varied from leaden grey (CZA, MEA and PDA) to colourless (RBA). Colonies were floccose, velvety, slightly raised to flat, irregular to regular and undulate to entire.

Microscopic features: Hyphae 2.3-5.6µm wide, branched and septate. Conidiophore 240-260µm long, unbranched, cylindrical to filiform, intercalary swelling. Ramoconidia cylindrical, ampulliform, septate. Conidia numerous, 1.5- 2.2µm long and 1.8-3.9µm wide, greyish brown, 1-celled and ovoid to cylindrical.

***Cladosporium* sp.1**

Culture characteristics: Colony diameter on CZA, MEA, PDA and RBA reached 2.4-3.8cm, 1.5-2.2cm, 2-3cm and 1.2-1.7cm in 5 days. Colony colours on the obverse of these media plates varied from olivaceous grey (CZA and PDA), dark green (MEA), bright green (RBA) whereas, the colours on the reverse of these plates varied from dark green (CZA, MEA and PDA) to black (RBA). Colonies were convoluted, powdery, slightly raised to raised, round, floccose-felty and scalloped to entire.

Microscopic features: Hyphae hyaline, septate, with branched conidiophores. Conidiophores 320-410µm long and 4.3-7.8µm wide, erect, solitary with 3 to 4 cylindrical ramoconidia. Conidia 3.8-5.2µm long and 1.6-2.4µm wide, 1 to 2-celled, chains, sympodial, hyaline to pale brown, limoniform and ovoid.

***Cladosporium* sp.2**

Culture characteristics: Colony diameter on CZA, MEA, PDA and RBA reached 2.1-2.8cm, 3.8-4.5cm, 2-2.5cm and 3.5-4.7cm in 5 days. Colony colours on the obverse of these media plates varied from olivaceous grey (CZA, MEA and PDA) and bright green (RBA)

whereas, the colours on the reverse of these plates varied from dark brown (MEA and PDA) to black (CZA and RBA). Colonies were convoluted, powdery, flat, floccose-felty, irregular, wrinkled and scalloped to entire.

Microscopic features: Hyphae hyaline, septate, with branched conidiophores. Conidiophores 280-380µm long and 4-6.6µm wide, slightly erect, solitary with 3 cylindrical ramoconidia. Conidia 3.1-4.6µm long, hyaline and ellipsoidal.

***Clonostachys* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached4.1-5cm, 3.8-4.2cm, 4-4.6cm and 4.3-5.1cm in 7 days. Colony colours on the obverse of all these media plates were yellowish-white then changed to greenish-white however, on the reverse of these plates the colours varied from pale yellow (CZA and MEA), brown (PDA) and colourless (RBA). Colonies were cottony, raised, granular to felty, entire.

Microscopic features: Hyphae 1.4–4.7µm wide, hyaline. Conidiophores primary and secondary, hyaline, erect and branched. Primary conidiophore 11-393µm x 2-3µm, verticillium-like branches. Secondary conidiophore with 10.8-14.7µm long phialides, *Penicillium*-type, solitary or aggregated, hyaline and divergent phialides. Chlamydospores 7.3-15µm in diameter, intercalary to terminal. Conidia 2.8-3.5 µm x 2.2-3.1µm, 1-celled, hyaline and globose to ellipsoidal.

***Clonostachys* sp.2**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.8-4.3cm, 4.1-4.7cm, 3.8-4.1cm and 4-4.5 cm. Theobverse and reverse of these media plates were yellowish-white to white and pale yellow (CZA, MEA and PDA), yellow to colourless (RBA). Colonies were cottony, raised, granular to felty, entire.

Microscopic features: Hyphae 1.1–3.9µm wide, hyaline. Conidiophores hyaline and branched. Primary conidiophore with verticillium-like branches, 73-113µm long stipe. Secondary conidiophore with 14.4-26.7µm long phialides. Chlamydospores 6.7-9.5µm in diameter. Conidia 2.2-3.8µm wide, 1-celled and globose to ellipsoidal.

***Colletotrichum* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.8-4.6cm, 4.4-5cm, 4.8-5.3cm and 4.2-4.9cm respectively in 5 days. Colony colours on the obverse and reverse of these media plates were white and creamy yellow (CZA, MEA and

PDA) to off-white and colourless (RBA). Colonies were cottony, floccose, smooth and entire.

Microscopic features: Conidiophores septate, conidiogenous cells hyaline. Appresoria obovate, entire. Conidia 4.7-6.6µm wide and 13-17µm long, hyaline, aseptate to 1-septa and fusiform to slightly cylindrical with an obtuse end.

Cunninghamella echinulata

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA varied from 3.4-4.3cm, 4.6-5.4cm, 4-4.9cm and 3.9-4.8cm respectively in 3 days. Colony colours on the obverse of all media plates were initially white which changed to pale cream and then to brownish-grey however, the reverse of the media plates showed colony colours varying from cream (PDA) to colourless (CZA, MEA and RBA). Colonies were dense, floccose, cottony, raised and entire.

Microscopic features: Hyphae branched, aseptate. Sporangiophores 3-8µm wide, erect, branched, vesicles at the terminal end and braches in the apical region. Terminal vesicles 33-48µm in diameter, vesicles on branches 13-30µm in diameter, hyaline, pyriform, covered with sporangioles. Spores 8.5-13µm in diameter, hyaline to brownish, 1-celled and globose.

***Emmonsia* sp.**

Culture characteristics: Colony diameters on MEA, PDA and RBA reached 3.5-4.1cm, 3-3.7cm and 3.6-4.3cm in 7 days however, growth was not observed on CZA. Colony colours on the obverse on media plates were white with tan whereas the reverse of these plates was brown (MEA and PDA) and colourless (RBA). Colonies were velvety to cottony, irregular, raised to flat. Brown “pepper-like” spots develop in the centre of the colonies after some days.

Microscopic features: Hyphae septate, hyaline to bamboo-like with darkening at septa. Conidiophore branched. Conidia 2.3-5µm in diameter, hyaline, in chains. Adiaspores 24-50 µm in diameter.

***Exophiala* sp.**

Culture characteristics: Colony diameters on MEA, PDA and RBA were 2.4-3.3cm and 2.8-3.5cm after 7 days however, growth was not observed on CZA. Obverse and reverse on these media plates were olivaceous black and black (MEA and PDA), olivaceous brown and black (RBA). Colonies were soft, slightly floccose, velvety, slightly raised, radially sulcate.

Microscopic features: Hyphae septate. Conidiogenous cells flask-shaped, terminal. Conidiophores short, erect, brown, cylindrical. Conidia 4.9-8.6µm × 3.5–4.3µm, 1-celled, hyaline to subhyaline and cylindrical to slightly ellipsoidal.

Fusarium oxysporum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 4.3-4.9cm, 4.5-5.3cm, 3.5-4.3cm and 3.8-4.5cm in 7 days. Colony colours on obverse and reverse of these media plates were brownish grey and brown (CZA), creamy white and dull brown (MEA), yellowish-white with orange centre and yellow (PDA) and creamy white and colourless (RBA). Colonies in MEA and PDA plates developed distinct zonation. Colonies were floccose, cottony with abundant mycelium, slightly raised to flat, irregular to regular and undulate to entire.

Microscopic features: Mycelium septate, hyaline and sporulating. Conidiophore hyaline, branched and septate. Macroconidia 3.1-4.6µm wide and 21.5-43.7µm long, hyaline, 3-5 septa but mostly 3, tapered and curved apical cells, foot-shaped basal cells, short and hooked apical cell. Microconidia 5-14.8µm long and 2.7-3.8µm wide, produced on short monophialides, 0-1 septa, oval to bean-shaped. Chlamydospores abundant and solitary.

Fusarium solani

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA with its diameters in media plates from 4.7-5.2cm, 4.5-5cm, 4.8-5.5cm and 4.3-5cm within 3-5 days. Colony colours on the obverse and reverse on these media plates werecream and pale yellow (CZA), pinkish-white and brown (MEA), bluish-white and brown (PDA) andcream and colourless (RBA). Colonies were floccose, cottony, slightly raised to flat, irregular to regular and entire.

Microscopic features: Mycelium septate, hyaline and sporulating. Hyphae septate, hyaline and branched. Macroconidia 4.8-7.3µm wide and 46.8-75.6µm long, 3-5 septa, smooth, hyaline, cylindrical, tapering ends, apical cell short and blunt. Microconidia 3.4-5.1µm wide and 26.1-33.7µm long, 0-1 septa, hyaline, oval, ellipsoid, conidiogenous cell monophialidic. Chlamydospore abundant, 6.3-7.8µm in diameter, hyaline, intercalary to terminal and globose.

Fusarium proliferatum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached4.4-5.1cm, 4.1-4.9cm, 3.8-4.5cm and 4-4.7cm in 7 days. Colony colours on obverse and reverse

of these media plates were brownish grey and brown (CZA), off white and brown (MEA), white to purple and yellow (PDA) and creamy white and colourless (RBA). Colonies were floccose, cottony, abundant, slightly raised to raised, irregular and undulate to entire.

Microscopic features: Conidiophore hyaline, branched and septate. Macroconidia 3-4.3µm wide and 24.7-45.1µm long, hyaline, 3-5 septa, straight to slightly curved, poorly developed basal cells, curved apical cell. Microconidia 2.1-3.2µm wide and 5.2-11.3µm long, produced on monophialides and polyphialides, 0 septa, oval to bean-shaped. Chlamydospore absent.

***Fusarium* sp.**

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA with its diameters in media plates from 2.3-2.7cm, 3.7-4.4cm, 3.9-4.7cm and 2.7-3.5cm within 3-5 days. Colony colours on obverse and reverse on these media plates were pinkish-white and pale yellow (CZA), brownish-grey and yellow (MEA), pinkish and yellow (PDA), white and colourless (RBA). Colonies were floccose, cottony, slightly raised to flat, irregular to regular, smooth to radially sulcate, circular and entire.

Microscopic features: Hyphae septate, hyaline and branched. Macroconidia 3.8-5µm wide and 27-40µm long, 1-3 septa, smooth, hyaline, cylindrical, slightly curved, apical cell short and blunt. Microconidia 2.4-3.5µm wide, 0-1 septa, hyaline, ellipsoid. Chlamydospore hyaline, solitary and globose.

***Geosmithia* sp.**

Culture characteristics: Colonies developed poorly on CZA, MEA, PDA and RBA at 27°C however, growth increased when the incubation temperature was raised to 35°C. Colony diameters on CZA, MEA, PDA and RBA media varied from were 1-1.4cm, 2.3-3.4cm, 2-3.1cm and 3-4cm in 7 days. Colony colours on obverse of these media plates varied from creamy white (CZA, PDA and RBA) to beige (MEA) wheras, on reverse of these plates the colony colours were pale yellow (CZA and PDA), brown (PDA) and colourless (RBA). Soluble pigments were observed in PDA and RBA plates. Colonies were floccose, slightly velvety, and powdery, flat to slightly raised and undulate to lobate.

Microscopic: Hyphae 2.8-6µm wide, hyaline. Conidiophores 52-230µm long, hyaline, from substratum or aerial mycelium, septate, branched, biverticillate to terverticillate. Stipe septate. Metulae cylindrical. Phialides cylindrical, whorls 2-4, tapered towards the apex. Conidia 2.3-2.8µm wide and 3.9-5.2µm long, hyaline, cylindrical to ellipsoidal.

Geotrichum candidum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 1.9-2.5cm, 4.1-4.6cm, 3.5-3.9cm and 3.6-4cm. Colony colours on obverse and reverse on these media plates were creamy white and cream (CZA), creamy brown and brown (MEA), creamy white and yellowish (PDA), creamy brown and colourless (RBA). Colonies were soft, yeast-like texture to slightly floccose, dry and regular.

Microscopic features: Mycelium septate, hyaline and sporulating. Hyphae 3.3-4.9µm wide, hyaline, septate and branched. Chalamydospores 4-5.7µm in diameter, solitary, subglobose. Conidia 5-15µm long and 3.2-5.8µm wide, arthosporous, in chains, smooth, 1-celled and cylindrical.

***Geotrichum* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 2.3-3.5cm, 3.8-4.1cm, 3.7-4cm and 3.1-4cm. Colony colours on obverse and reverse on these media plates were white and pale yellow. Colonies were soft, yeast-like texture, velvety, dry, slightly granular, regular and entire.

Microscopic features: Mycelium septate, hyaline and sporulating. Hyphae 3.9-5.1µm wide, hyaline, septate and branched. Chalamydospores 4.8-6µm in diameter, solitary, subglobose. Conidia 4.5-13µm long and 3-5.1µm wide, arthosporous, in chains, smooth, 1-celled and cylindrical with blunt ends.

***Lichtheimia* sp.1**

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA plates with diameters of 4.5-5.3cm, 4.1-5cm, 3.9-4.8cm and 4-4.9cm within 3 days. Colony colours on the obverse of these media plates were white and however, on the reverse of the plates the colours varied from pale yellow (CZA, MEA and PDA) to colourless (RBA). Colonies were fluffy, cottony, raised, irregular and entire to undulate.

Microscopic features: Hyphae aseptate, branched, hyaline, 6.3-8.3µm, irregular. Sporangia 12-45µm in diameter, multispored, terminal, pyriform, apophysate. Columellae hemispheric. Apophyses funnel-shaped. Sporangiophores raised from the stolons, 35-130µm wide, branched, solitary to cluster. Rhizoids subhyaline. Giant cells grouped. Sporangiospores 2.8-4.8µm wide, hyaline and ellipsoid.

***Lichtheimia* sp.2**

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA plates with diameters above 5 cm within 5 days. Colony colours on the obverse of these media plates were initially white then changed to greyish-white however, colony colours on the reverse of these plates varied from yellow (CZA, MEA and PDA) to colourless (RBA). Colonies were compact tufts, floccose, woolly to cottony, released yellowish pigments (PDA), raised, irregular, plane to wrinkled on the reverse, entire.

Microscopic features: Hyphae aseptate, branched, hyaline, irregular. Sporangia 20-65µm in diameter, hyaline, multispored, terminal, pyriform, apophysate. Columellae dome shape. Apophyses funnel-shaped. Sporangiophores arose from the stolons, 28-80µm wide, erect, branched, solitary to cluster. Sporangiospores 2.3-4µm wide, 1-celled, hyaline and subglobose to ellipsoid.

***Microascus* sp**.

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.7-4 cm, 3.9-4.2cm, 4-4.5cm and 4.1-4.7cm in 7 days. Colony colours on the obverse of these media plates varied from grey (CZA, MEA and PDA) to brown (RBA) while on the reverse of the plates, the colours were black. Colonies were with white mycelium, velvety to floccose, granular, radially sulcate to slightly sulcate.

Microscopic features: Hyphae septate, hyaline. Conidiophoressimple to branched.Conidiogenous cells flask-shaped, solitary or in group of 2-5. Conidia in chains, 2.8-4.7µm x 1.8-2.7µm, hyaline, 1-celled and globose to obovate.

Mucor circinelloides

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA varied from 3.3-3.9cm, 4.2-4.9cm, 4-4.9cm and 3.7-4.1cm respectively. Colony colours on the obverse and reverse of these media plates varied from whitish-grey and grey (CZA), brownish-grey and pale yellow (MEA), white to yellowish-grey and pale yellow (PDA), white to yellowish-grey and colourless (RBA). Colonies were floccose, turf thick, cottony, raised and irregular.

Microscopic features: Hyphae sparsely septate. Sporangiophores 2.9-4.7µm wide and 4.7-9.5µm long, erect, hyaline, subglobose, sympodial branching, slightly curved terminated by sporangium. Sporangia 37.9-69.7µm wide, erect, echinulate, globose, columellate on dehiscence. Columellae ellipsoidal, hyaline, with a collar. Chlamydospores 11.6-21.9µm wide, subglobose. Spores 3.1-4.7 µm x 2.9-3.3µm, 1-celled and subglobose.

Mucor hiemalis

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA varied from 4.4-4.7cm, 4.5-5.3cm, 4.1-4.6cm and 3.9-4.2 cm in 5 days. Colony colours on obverse and reverse of these media plates varied from greyish-yellow and yellow (CZA and MEA), yellow and brownish-yellow (PDA), and grey and colourless (RBA). Colonies were floccose, cottony, slightly raised to raised and irregular.

Microscopic features: Sporangiophores 3.4-13.9µm in diameter, erect, branched sympodially. Sporangia 47.3-71.8µm wide and 62.3-89.6µm long, globose, columellate on dehiscence. Columellae 15.8-32.5µm wide, globose to subglobose, truncate base, with a collar. Chlamydospores 9.3-16.8µm wide and globose. Spores 7.7-2.9µm, 1-celled and subglobose to ellipsoidal.

***Mucor* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.7-4.1cm, 3.6-4.1cm, 4.2-4.7cm and 3.4-3.9cm respectively in 5 days. Colony colours on obverse and reverse of these media plates varied from brownish-grey and pale yellow (CZA and MEA), dark brown and pale brown (PDA) and yellowish-grey and colourless (RBA). Colonies were floccose, turf thick, cottony, flat to slightly raised and irregular.

Microscopic features: Hyphae sparsely septate. Sporangiophores 3.2-5µm wide and 4.9-11.2µm long, erect, hyaline to sub-hyaline, globose to subglobose, sympodial branching, slightly curved terminated by sporangium. Sporangia 33-71µm wide, erect, echinulate, globose, columellate on dehiscence. Columellae sub-globose, hyaline, with a collar. Chlamydospores 9-19.9µm wide. Spores 6.3-10.8µm, 1-celled and ellipsoidal.

Nigrospora osmanthi

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA varied from 3.5-3.9cm, 4.1-4.8cm, 3.7-4.4cm and 3.4-4cm in 5 days but covered the entire 15 cm diameter petriplates within 7 days. Colony colours on obverse of CZA, MEA, PDA and RBA plates were initially white but turned grey (MEA and PDA) and off white (CZA and RBA) in 5 days. After 7 seven days, colony colours in all the plates turned black. On reverse of these plates, colony colours were pale yellow, brown, yellow and colourless. Colonies were flat, flocosse, cottony, sparse aerial mycelia, irregular to regular and fimbriate.

Microscopic features: Mycelium with hyaline, smooth, micronematous, branched and septate, 2.2-4.3µm diameter hyphae. Conidiophores reduced to conidiogenous cells which

were monoblastic, discrete, solitary, ampulliform, 5.7-12.5µm long and 4.8-8.7µm wide. Conidia 12.7-17.3µm in diameter, black, shiny, aseptate, solitary, smooth and globose to sub-globose.

***Nigrospora* sp.1**

Culture characteristics: Colony showed poor growth on CZA but on MEA, PDA and RBA, colony diameters varied from 4.6-5.1cm, 3.9-4.4cm and 3.1-3.9cm in 7 days. Colony colours on the obverse of all media plates were grey whereas, on reverse of these plates colony colours varied from dark brown (CZA), brown (MEA), black (PDA) and colourless (RBA). Colonies were flat, sparse sporulation, spreading, floccose, cottony, aerial mycelia, regular and fimbriate.

Microscopic features: Mycelium with hyaline, smooth, micronematous, branched and septate, 2.5-4.1µm diameter hyphae. Conidiophores reduced to conidiogenous cells which were monoblastic, scattered, discrete, solitary, ampulliform to doliiform, 5.3-11.8µm long and 4.1-8.3µm wide. Conidia 11.9-17.1µm in diameter, black, shiny, aseptate, solitary, smooth, granular and globose to ellipsoid.

***Nigrospora* sp.2**

Culture characteristics: Colony showed poor growth on CZA but on MEA, PDA and RBA colony diameters varied from 2.2-2.8cm, 3.2-3.9cm and 3.4-3.9cm in 7 days. Colony colours on obverse of these media plates were olive green (MEA and RBA) and grey (PDA). On the reverse of these plates, colony colours were brown (MEA), dark brown (PDA) and black (RBA). Colonies on PDA plate released yellow pigments. Colonies were flat, sparse sporulation, spreading, floccose, slightly velvety, aerial mycelia, regular to slightly irregular and entire.

Microscopic: Mycelium with hyaline, smooth, micronematous, branched and septate, 2.8-4.7µm wide hyphae. Conidiophores reduced to conidiogenous cells, monoblastic, scattered, discrete, solitary, ampulliform to doliiform, 6-12.3µm long and 4.4-8.9µm wide. Conidia 12.3-17.7µm in diameter, aseptate, solitary, smooth, granular and sub-globose.

***Paecilomyces* sp. 1**

Colony characteristics: Colony diameters on PDA and RBA reached 3.4 -3.9cm and 3-4.3cm in 5 days. Colony colours on the obverse of these media plates were yellowish-brown and light brown whereas on the reverse of these plates, colony colours were yellowish and colourless. Colonies were powdery, flat and entire.

Microscopic features: Hyphae septate, hyaline. Conidiophores 2.3-2.9µm wide, loosely branched. Phialides swollen at the base and tapered towards the apex, solitary to pairs. Conidia 1-1.5µm x 3.8-4µm, phialosporous, 1-celled, hyaline, ellipsoid to ovoid, slightly apiculate at one end.

***Paecilomyces* sp. 2**

Colony characteristics: Colony diameters on PDA and RBA reached 3.6-4cm and 3.4-4cm in 5 days. Colony colours on the obverse of these media plates were yellowish-white and white whereas on the reverse of these plates, colony colours were brown. Colonies were velvety, flat and entire.

Microscopic features: Conidiophores 2.2-2.7µm wide, loosely branched, hyaline, erect. Hyphae septate, hyaline. Phialides swollen at the base and tapered towards the apex, solitary to pairs. Conidia 3.2µm x 4.1µm, phialosporous 1-celled, terminal and subglobose to ovate.

Periconia byssoides

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.2-3.9 cm, 3.5-4.1cm, 4.3-5.2cm and 3.7-4.3cm respectively in 7 days. Colony colours on the obverse of these media plates were pinkish-grey (CZA, MEA and RBA) and off-white (PDA). Colonies were surrounded by deeper shade (CZA, MEA and RBA) and pale orange (PDA) margin. The reverse of these plates showed colony colours of grayish-brown (CZA, MEA and PDA) and colourless (RBA). Colonies were cottony, effuse, with concentric rings, cracks in reverse of PDA, raised in the centre and flat at margins, entire.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore 4.5-5.5µm wide and 345-420µm long, micronematous and macronematous, dark brown, verruculose, solitary to small groups, slightly bent, bore globose spore heads at the apex. Conidiogenouscells monoblastic. Stipe verruculose. Conidia 8.8-15.6µm in diameter, blastosporous, catenate, brown, aseptate, 1-celled and globose.

Penicillium citrinum

Colony characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.3-3.4cm, 2.3-3.1cm, 2.5-3.6cm and 2.4-3.3cm respectively in 7 days. Colony colours the on obverse of CZA and MEA plates were green with white mycelia, green with grey edge on PDA, orange with white mycelia at the margins on RBA. On the reverse of these plates, colony colours were yellowish-orange or orangecolours. Brown exudates were released from

colonies on CZA and PDA plates. Colonies were velvety, sulcate on CZA and PDA, fasciculate on MEA and RBA.

Microscopic features: Conidiophores 110–300µm long, raised from surface or subsurface hyphae. Hyphae smooth-walled stipes, monoverticilate to terverticilate.Metulae cylindrical. Phialides 7–9.3x2-2.5µm, ampulliform. Conidia 2.5-3.1µm and globose to sub-globose.

P. commune

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.1-3.2cm, 2.6-3.7cm, 3.5-3.9cm, 3.9-4.1cm in 7 days. Colony colour on the obverse of these media plates varied from initial pale brown which changed to grey colour (CZA), pale green (MEA), pale grey with yellow centre (PDA), dark green with yellow centre (RBA). Colonies were surrounded by white mycelium at the margin. On the reverse of these plates, colony colours were reddish brown (CZA), brown (MEA), yellow (PDA).Colonies were velvety to floccose, granular, radially sulcate to slightly sulcate.

Microscopic features: Conidiophores raised from the subsurface. Hyphae 188-380µm long smooth-walled stipes, terverticillate. Metulae13-17µm wide, cylindrical. Phialides 7.9-9.2µm long, ampulliform. Conidia 3.1-3.8µm wide and ellipsoidal to globose.

P. italicum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 1-1.9cm, 2.5-3.7cm, 2.3-3.4cm and 0.8-1.3cm in 5 days. Colony colour on the obverse and reverse of these media plates were grey and grey (CZA), greyish blue and pale brown (MEA), deep green and yellow (PDA), grey and colourless(RBA). Transparent exudates were released from colonies on PDA plate. Colonies were radially sulcate with white edge (PDA), velvety to floccose.

Microscopic features: Conidiophores raised from the subsurface hyphae,terverticillate, 100-335µm long, smooth-walled stipe. Metulae13.5-14.8µm, cylindrical. Philiades 8-10 x 2-4.3µm, cylindrical. Conidia 3.1-3.7µm wide and ellipsoidal.

P. palitans

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 1.3-3.9cm, 0.9-3.7cm, 0.5-3.4cm and 0.5-3.3cm in 5 days. Colony colours on the obverse of these media plates were light green however, the colony colours on the reverse of the plates varied from cream (CZA), pale brown (MEA), yellow (PDA) and brownish green (RBA).

Colonies on CZA, MEA and PDA plates released transparent exudates. Colonies were velvety, radially sulcate to plane.

Microscopic features: Conidiophores raised from the subsurface hyphae, 250-380µm long rough-walled stipe, terverticillate. Metulae10-14µm, cylindrical. Philiade 9.9-10.3 x 2.1-3.3µm. Conidia with 2.8- 4µm in diameter and globose.

P. waksmanii

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2-2.8cm, 2.1-3cm, 1.6-2.5cm and 1.4-2.3cm in 5 days. Colony colours on the obverse of these media plates were dull green however, the colony colours on the reverse of the plates varied from cream (CZA), brown (MEA), yellow (PDA) and colourless (RBA). Colonies were surrounded with white, pale green or pale yellow mycelium. Colonies on MEA and RBA plates released transparent and yellow exudates. Colonies were velvety to floccose, plane to radially sulcate.

Microscopic features: Conidiophore raised from the subsurface hyphae, biverticillate, smooth-walled and 210-440µm long stipes. Metulae 5-6µm long, cylindrical, ampuliform. Phialids 6.5-8.8µm long. Conidia with 2.6-3.2µm diameter and globose.

***Penicillium* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA ranged from 1.7-2cm, 1.9-2.5cm, 2.5-3.5cm, and 2.4-3.6cm respectively in 5 days. Colony colours on obverse of these media plates were initially white then changed to yellowish-green (CZA), deep green (MEA), sage green (PDA) and greyish-green (RBA). Colonies were surrounded by white mycelium at the margin. On the reverse of these plates, colony colours were brown (CZA, MEA and PDA) and colourless (RBA). Colonies on PDA and RBA plates released colourless exudates. Colonies were granular, powdery to velvety, flat to umbonate centrally, irregular and entire to slightly undulate, radially sulcate to slightly sulcate.

Microscopic features: Hyphae hyaline, septate. Conidiophore hyaline, straight to erect, septate, monoverticillate, unbranched. Metulae absent. Phialides 3.2-4.5µm wide and 9.1-12.3µm long, hyaline, ampulliform. Conidia 3.7-4µm wide, hyaline, 1-celled and globose.

***Penicillium* sp.2**

Colony characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.2-3cm, 2.5-3.7cm, 2.5-3.6cm and 2.5-3.9cm in 7 days. Colony colours on the obverse of these media plates were initially white then, changed to yellowish whereas on the reverse of these

plates colony colours varied from bright yellow (CZA and PDA) and dark brown (MEA and RBA). Yellow pigments were released from PDA and RBA plates. Colonies were slightly velvety, sulcate on some plates (PDA and RBA), entire.

Microscopic features: Conidiophores raised from the surface or subsurface. Hyphae smooth-walled, stipes simple, smooth,terverticilate.Metulae cylindrical. Phialides ampulliform. Conidia 3.1µm wide, short chains, 1-celled and globose to sub-globose.

***Penicillium* sp.3**

Culture characteristics: Colony diameters on CZA, MEA, PDA and PDA reached 1.5-2cm, 2.1-2.5cm, 1.6-2.2cm and 0.7-1.1cm in 7 days. Colony colours on the obverse and reverse of these media plates varied from green and pale yellow (CZA), blue-green and brown (MEA), green and yellow (PDA) and yellowish-green and colourless (PDA). Colonies on RBA plate released black colourexudates. Colonies were velvety to floccose, radially sulcate to plane.

Microscopic features: Conidiophores raised from the subsurface hyphae. Hyphae 180-710µm long, stipes smooth-walled, terverticilate. Metulae7.8-13.3µm x 3.2-3.9µm, cylindrical. Philiades ampulliform. Conidia 2.2-3.3µm wide and globose to sub-globose.

***Penicillium* sp.4**

Culture characteristics: Colony diameters on CZA, MEA, PDA and PDA reached 2.7-3.7cm, 2.5-3.1cm, 2.2-2.8cm and 2.9-3.7cm in 7 days. Colony colours on the obverse were green (CZA and PDA) and orange (MEA and RBA). Colony colours were white in margins and in centre. On the reverse of these plates, colony colours were yellowish orange (CZA and MEA), orange (PDA) and colourless (RBA). Transparent exudates were observed from colonieson PDA plate. Colonies were velvety, floccose, sulcate.

Microscopic features: Conidiophores raised from the surface or subsurface hyphae with 90–185µm long, smooth-walled stipes.Metulae8-13.7 x 2.9-3.5µm, cylindrical. Phialides 6.4-8.7x8.3-10.5µm, ampulliform. Conidia 2.2-3.5µm in diameterand globose to sub-globose.

***Pestalotiopsis* sp.1**

Culture characteristics: Colonies on CZA, PDA, MEA and RBA were 4.5-5.1cm, 4.7-5.3cm, 4.7-5.5cm and 3.3-4cm in 7 days. Colony colours on obverse of these media plates were white (CZA) and yellow surrounded by white from inside out on (MEA, PDA and RBA). Reverse of these plates showed colours varying from yellowish-brown, brown and colourless. Black fruiting bodies were observed in all plates. Colonies were slightly dense,

fluffy, slightly raised aerial mycelium on the central surface, wrinkled to smooth, filamentous to circular and undulate.

Microscopic features: Conidiomata pycnidial. Conidiophores not distinct rather reduced to discrete conidiogenous cells. Conidiogenous cells 7-10.8µm long and 2.2-3.4µm wide, cylindrical, smooth, hyaline, 1-2 times per currently. Conidia 22-27.7 x 4.9-7.2µm, fusiform, straight to slightly curved, 4 septate. Basal cells 3.5-6µm long, hyaline, smooth, thin-walled, 1 unbranched basal appendage at the centre, 3 median cells, brown, darker than other cells. Apical cells 3.8-5.8µm long, subcylindrical, hyaline, thin-walled, 2-3 appendages and unbranched.

***Pestalotiopsis* sp.2**

Culture characteristics: Colonies on CZA, PDA, MEA and RBA were 3.9-4.6cm, 4.3-4.7cm, 4.1-4.7cm and 3-4.4cm in 7 days. Colony colours on obverse of these media plates were greyish white (CZA and MEA), pinkish-white (PDA) and white (RBA). Reverse of these plates showed colours varying from yellowish-brown and colourless. Colonies were slightly dense, floccose, cottony, flat to slightly raised aerial mycelium on the central surface, slightly wrinkled to smooth, filamentous to undulate.

Microscopic features: Conidiomata pycnidial. Conidiophores reduced to lageniform or ampulliform conidiogenous cells. Conidia 20–28.9 × 4.5.5–7.9µm, fusiform, slightly curved, 4 septate, 5-celled. Apical and basal cells conical shape. Basal cells 3.3-9µm long, hyaline, smooth, thin-walled, 1 unbranched basal appendage at the centre, 3 median cells, brown, darker than other cells. Apical cells 8.5-12.5µm long, subcylindrical, hyaline, thin-walled, 2-4 appendages and unbranched.

***Phoma* sp.1**

Culture characteristics: Colony diameters on CZA, MEA, PDA and PDA reached 4.5-4.9cm, 4.2-5cm, 4.5-5cm and 4.2-4.9cm in 5 days. Colony colourson the obverse of these media plates were greyish-white with a pinkish tinge whereas on the reverse of these plates colony colours varied from pinkish (CZA), reddish-brown (MEA) red (PDA) and colourless (RBA). Colonies were cottony, fluffy, compact tufts, slightly raised in the centre, irregular and slightly undulate to entire.

Microscopic features: Hyphae hyaline, septate. Pycnidia 93-153µm wide, sub-globose to pyriform. Conidia 3.9-7.5 x 2.1-2.8µm, hyaline, aseptate 1-celled and ellipsoid to ovoid.

***Phoma* sp.2**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.3-3 cm, 2.5-3cm, 1.8-3 cm and 1.1-1.7 cm in 5 days. Colony colourson the obverse of these media plates were yellow whereas on the reverse of theseplatescolony colours varied from pale yellow (CZA and PDA), brown (MEA) and colourless (RBA). Colonies were white at the margin, slightly velvety, powdery, flat, circular regular and entire.

Microscopic features: Hyphae hyaline, septate. Pycnidia 78-137µm wide, sub-globose to pyriform and irregular. Conidia 3.4-7 x 1.5-2.9µm, hyaline, aseptate, 1-celled, ellipsoid to ovoid. Chlamydospores globose.

***Phomopsis* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3.9-4.4cm, 3.6-4.2cm, 3.6-4.1cm and 3.5-4cm in 7 days. Colony colours on the obverse of these media plates were white whereas on the reverse colony colours were brown (CZA, MEA and PDA) and colourless (RBA). Colonies were turf thick, slightly woolly, with prominent concentric rings, irregular, flat to slightly raised in the centre and wavy.

Microscopic features: Conidiophore hyaline, branched, septate. Conidiomata cylindrical. Alpha conidia 5.4 x 2.1µm, hyaline, aseptate, 1-celled, ellipsoidal. Beta conidia 11.33 x 1.41µm, hyaline, filiform and slightly curved.

Rhizopus microspores

Culture characteristics: Colonies developed fast on CZA, MEA, PDA and RBA. Within 5 days, colony diameters on these media reached 4.7-5.3cm, 4.4-5.3cm, 4-4.8cm and 3.5-4.3cm respectively. Colony colours on the obverse and reverse of these media plates varied from grey and yellowish-grey (CZA), dark brown and dull brown (MEA), off-white to grey and brown (PDA), and offwhite to grey and colourless (RBA). Colonies were floccose, cottony, slightly raised to raised, irregular and entire.

Microscopic features: Hyphae hyaline, branched, irregular. Sporangia 83.9- 107.8µm in diameter, globose to sub-globose, columellate on dehiscence. Sporangiophores 7.5-16.1µm wide, erect, unbranched, solitary to groups, connected by stolons. Apophyses noticeable. Stolons 3.8-7.2µm wide, hyaline. Rhizoids subhyaline. Columellae 23.7-65µm wide, globose to obovoid, with collar. Sporangiospores 3.7-4.6µm wide and 5.5-6.9µm long, subglobose to ovoid, striated. Chlamydospores 7.3-16.8µm wide, in short chains or in mass and globose to irregular.

Rhizopus stolonifer

Culture characteristics: Colonies developed fast on CZA, MEA, PDA and RBA. Within 5 days, colony diameters on these media reached 4.3-5cm, 4.5-5.3cm, 4.35.3cm and 4.4-4.9cm in 3 days. Colony colours on obverse of the plates were initially white which then changed to off-white(CZA) and pale yellow, grey (MEA and RBA), pale yellowish-brown (PDA). Colony colours on the reverse of these media plates varied from dull brown (CZA and MEA), blackish brown (PDA) and colourless (RBA). Colonies were floccose, cottony and irregular.

Microscopic features: Hyphae 4.3-13.8µm, hyaline, branched, irregular. Sporangia 99.8-177.9µm wide, erect, echinulate, globose to subglobose, columellate on dehiscence. Columellae 45.2-77.9µm wide, globose to sub-globose, hyaline, hemispheric. Sporangiophores 7.8-17.5µm wide, erect, hyaline, globose to ellipsoidal, unbranched, septate to aseptate, striated, connected by stolons. Stolons well developed, septate, hyaline. Rhizoids radiate along the sporangiophore, hyaline, branched, septate to aseptate. Sporangiospores 3.3-4.8µm wide, sub-globose to oblong and striated.

***Sarocladium* sp.**

Culture characteristics: Colonies diameters on CZA, MEA, PDA and RBA reached 1.2-1.9cm, 2.2-3cm, 1.6-2cm and 1.8-2.9cm in 7 days. Colonies on the obverse of these media plates were initially white which turned to greyish (CDA) and yellowish-white (MEA, PDA and RBA). On the reverse of these plates colony colours varied from black (CDA), dark brown (MEA), brownish-yellow (PDA) and colourless (RBA). Colonies were slightly velvety to powdery, radially folded, wrinkled, umbonated, irregular and entire to slightly undulate.

Microscopic features: Hyphae 1.3-1.8µm wide hyaline, septate, branched. Conidiophores erect, slightly bent, simple to loosely branched. Phialides 1.2-1.8µm wide and 19-45µm long, hyaline, tapered towards the apex with terminal slimy conidial masses. Conidia 1.2-2µm wide and 3-4.5 x 1.2-2.4µm, 1-celled, grouped in slimy heads, hyaline to sub-hyaline and oval to ellipsoidal.

***Scedosporium* sp.**

Culture characteristics: Colonies diameters on CZA, MEA, PDA and RBA ranged from 3.7-4.2cm, 3.5-4cm, 3.5-4.2cm and 3.8-4.4cm in 5 days. Colony colours on the obverse of these media plates were greyish-white and smoky brown whereas, on the reverse of these

plates, colours were brown. Colonies were cottony, forming mycelia tufts as the colony grows.

Microscopic: Hyphae hyaline and septate. Conidiogenous cells cylindrical, produced along the hyphae, flask shape extending an elongated neck. Conidia 3µm wide, solitary or in small groups at the apex of the neck, borne laterally in hyphae, smooth, 1-celled, ovoid and clavate.

***Sclerotinia* sp.**

Culture characteristics: Colonies on CZA, PDA, MEA and RBA were 4.2-5.3cm, 5.1-5.5cm, 5-5.3cm and 3.5-4.7cm in 5 days. Colonies on the obverse of these media plates were initially off- white then, changed to brownish hereas, on the reverse of these plates, colours were dark brown. Colonies were slightly dense, fluffy, flat, circular to irregular colourless to black sclerotia were formed on the mycelium. Sclerotia released white to yellow drops and were more concentrated at the periphery than in the centre of petriplates.

Microscopic features: Hyphae 3-4.9µm in wide, hyaline, septate branched and multinucleate.

***Scytalidium* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 3.7-4.2cm, 3.5-4cm, 2.4-3.2cm, 2.6-3.4cm in 7 days. Colony colours on obverse of these media plates were pale grey (CZA and PDA), grey (MEA) and greenish-grey (RBA). On reverse of these media plates, colony colours were dark brown, yellowish-brown and colourless. Colonies were lanose, diametrically to radially sulcate, flat to slightly raised in the centre, regular and entire.

Microscopic features: Hyphae 2-4µm wide, septate, hyaline to sub-hyaline. Conidiophores absent. Conidiogenous cells hyaline. Fertile hyphae, irregularly arising from the main hyphae, septate in basipetal succession, produced arthroconidia. Arthroconidia 2.6-6.9 x 1.5-2.3 µm, hyaline, aseptate, square to short-cylindrical. Vegetative hyphae formed globose, lobed or irregularly shaped cells and solitary or in chains.

***Taloromyces* sp.**

Culture characteristics: Colonies diameters on CZA, MEA, PDA and RBA were 1.2-1.5cm, 3.3-4cm, 3.5-4.2cm and 2.3-3.1cm in 5 days. Colony colours on the obverse of these media plates varied from lime green (CZA, MEA and PDA) to bluish green (RBA). Colonies were surrounded by yellow margin. On the reverse of these plates colony colours were pale

yellow (CZA), brownish-yellow (MEA and PDA) and colourless (RBA). Clear exudate was released from colonies on PDA plate. Colonies were floccose to velvety, bright yellow mycelium, slightly raised at the point of inoculation, slightly folded at the centre, sulcate, regular and entire.

Microscopic: Conidiophores hyaline, raised from substratum, biverticillate. Stipe septate, smooth. Metulae 11-13.5 x 2.5-3.2µm, 3 to 8, divergent. Phialides 11-13 x 2-2.8µm, 3 to 6 per metulae, acerose. Conidia 2.8-3.5 x 1.5-2.3µm, hyaline and ellipsoidal to fusiform.

Trichophyton rubrum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 2.5-3.3cm, 2.2-3.1cm, 3.2-3.4cm, and 3.3-3.7cm in 5-7 days. Colony colours on the obverse of these media plates varied from white (CZA and PDA) to creamy-white (MEA and RBA). On the reverse of these plates colony colours were pale yellow (CZA, MEA and PDA) and colourless (RBA). Colonies on MEA plate produced red-wine pigments. Colonies were cottony, granular, flat to slightly raised and filamentous.

Microscopic features: Hyphae hyaline, spiral and septate. Macroconidia 4.8-7.8 x 8.3-48µm, borne laterally directly on the hyphae or on short pedicels, multicellular and cigar or club-shaped. Microconidia 2.2-3.8 x 2.3-4.5µm borne on hyphae, 1-celled, clavate to pyriform.

***Trichophyton* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.9-3.1cm, 3.1-3.5cm, 3.2-3.5cm, and 3.5-3.7cm in 5-7 days. Colony colours on the obverse of these media plates varied from white to yellowish. The reverse of these plates were pale yellow (CZA and PDA), yellow (MEA) and colourless (RBA). Colonies were fluffy, irregular, flat to slightly raised and entire.

Microscopic features: Hyphae hyaline and septate. Macroconidia multicellular and cigar or club-shaped. Microconidia borne on hyphae, 1-celled, clavate to pyriform.

Trichoderma hamatum

Culture characteristics: Colonies developed moderately on CZA, MEA, PDA and RBA. Colony diameters on these media plates reached 4.6-5.1cm, 4.5-5cm, 4.4-5.1cm and 4.3-5.3cm respectively in 5 days. Colonies on obverse of these media plates were initially white then, changed to bluish green (CZA, MEA and PDA) and yellowish-green (RBA).

Colony colours on reverse of these plates were pale brown and colourless. Colonies were compact tufts, effuse, pustules distributed irregularly and flat to slightly raised.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, upper part undulate to hamate, erect, highly branched, irregular, short side branches, 3-6 phialides on each branch arise in 1-3 whorls. Phialides 2.9-3.9µm wide and 4.6-8.9µm long, hyaline, flask-shaped, tapered towards the apex, septate, densely clustered on board. Conidia 2.3-3µm wide and 3.3-4.3µm long, hyaline, 1- celled, oblong to ellipsoidal. Chlamydospore 7.5-11.3µm in diameter, terminal and intercalary, sub-globose to ellipsoidal.

Trichoderma harzianum

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA. Colony diameters on these media plates reached 4.7-5.3cm, 4.9-5.5cm, 4.8-5.6cm and 4.3-5.1cm respectively within 4 days. Colonies on the obverse of these media plates were white and yellowish green while on reverse, colony colours were pale yellow and colourless. Colonies were floccose, powdery, white pustules and green conidia distributed irregularly over the surface, 1-2 concentric rings, slightly raised.

Microscopic features: Hyphae hyaline, septate. Conidiophore 64-108.9µm long, hyaline, erect, highly branched, side branches stand at right angles to the bear tip. Phialides 3.7-6.5µm long, ampulliform, short and board in the middle, convergent. Conidia 2.7µm in diameter, hyaline, 1-celled, globose. Chlamydospore 6-8.4µm in diameter, sub-globose.

Trichoderma koningii

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA. Colony diameters on these media plates reached 3.9-4.3cm, 4.1-4.8cm, 4.3-5.1cm and 4.2-4.9cm in 5 days. Colonies on the obverse of these media plates were initially white then changed to greenish-yellow (CZA) and bluish green (MEA, PDA and RBA). Reverse of these plates showed colours varying from brown, pale yellow and colourless. Colonies were floccose to compact tufts, greenish pustules and conidia distributed irregularly over the surface, smooth and flat to slightly raised.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, erect, highly branched, side branches stand at right angles to the bear tip, 2 or 3 phialides on each branch arise in opposite pairs. Phialides 2.1-2.9µm wide and 7.7-9.8µm long, 3 or 4 whorls, hyaline, flask-shaped, board in the middle, tapered towards the apex. Conidia 2.7-3.3µm

wide and 3.8-4.2µm long, hyaline, 1-celled, subglobose to ellipsoidal. Chlamydospore 9.2-12.8µm in diameter, terminal and sub-globose.

Trichoderma longibrachiatum

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 2.8-3.1cm, 4.4-5cm, 4.5-5.2cm and 4.4-5.2cm respectively in 5 days. Colony colours on the obverse of these media plates were initially watery white then, changed to greenish-yellow (CZA and PDA), yellowish-white (MEA) and yellowish-white (RBA). Reverse of these plates showed colours varying from greenish-yellow (CZA), pale yellow (PDA and MEA) and colourless (RBA). Colonies were cottony to compact tufts, white pustules distributed over the surface, green and yellowish conidia around the inoculation point, flat and undulate.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, long main axis and shorter side branches, erect, weakly branched, phialides arise along the main axis and along branches from the main axis. Phialides 2.2-3.8µm wide and 7.1-9µm long, hyaline, slender, solitary, alternate, slightly bent at the apex, lageniform. Conidia 3.2µm wide and 6µm long, hyaline, 1-celled, obovoid to ellipsoidal. Chlamydospore terminal and intercalary and globose.

Trichoderma viride

Culture characteristics: Colonies developed rapidly on CZA, MEA, PDA and RBA and reached 4.5-5.1cm, 4.7-5.4cm, 4.6-5.4cm and 4.4-5.2cm respectively within 3 days. Colony colours on the obverse of these media plates were initially white then changed to yellowish-white and light-green whereas the reverse of these plates showed colours varying from pale yellow (CZA), brown (MEA) and colourless (RBA). Colonies were floccose, powdery, white pustules and green conidia distributed irregularly over the surface, with a yellowish ring, slightly raised to flat and undulate.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, erect, highly branched, side branches stand at right angles to the bear tip, 2 to 3 phialides on each branch arise in opposite pairs. Phialides 2.2-3.2µm wide and 6.7-10.9µm long, hyaline, solitary or in whorls of 2-3, flask-shaped, cylindrical to board in the middle, ampulliform. Conidia 3-4.5µm wide and 4µm long, hyaline, 1-celled, globose to obovoid. Chlamydospore 5.5-7.3µm in diameter, intercalary and globose to sub-globose.

***Trichoderma* sp.1**

Culture characteristics: Colony developed poorly on CZA but developed rapidly on MEA, PDA and RBA. Colony diameters on these media plates reached 0.8-1.3cm, 4.3-5.2cm, 4.7-5.5cm and 4.2-4.9cm respectively in 5 days. Colony colours on the obverse of these media plates were initially white then changed to light green (MEA) and bluish-green (CZA, PDA and RBA) whereas on the reverse of the plates colonies were colourless. Colonies were floccose, powdery, pustules white, abundant and dense at the edge of the plates, green conidia distributed irregularly at the edge of the plates, flat to slightly raised.

Microscopic features: Hyphae hyaline, septate. Conidiophore 64-108.9µm long, hyaline, erect, sparingly branched, side branches short. Phialides 2.9-3.5µm wide and 6.4-8.9µm long, solitary to 2-5, irregular, ampulliform to lageniform, board in the middle, bent at the tip. Conidia 1.8-2.7µm wide and 2.9-4µm long, hyaline, 1- celled, oval to ellipsoidal. Chlamydospore 7.9-10.4µm in diameter and globose.

***Trichoderma* sp.2**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA plates reached 3.8-4.6cm, 4.3-5cm, 4.5-5.2cm and 4.6-5.3cm respectively in 5 days. Colony colours on the obverse of these media plates were initially watery white then changed to greenish-yellow (CZA), bluish-green (MEA and PDA) and green (RBA). Reverse of these plates showed colours varying from pale yellow (CZA and PDA) and colourless (MEA and RBA). Colonies were cottony to compact tufts, white pustules distributed over the surface, flat, irregular, undulate.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, long main axis and shorter side branches, side branches stand at right angles to the bear tip, erect, weakly branched, phialides arise along the main axis and branches from the main axis. Phialides 1.3-2.8µm wide and 4.2-5.8µm long, hyaline, slender, solitary, slightly bent at the apex, ampulliform. Conidia 2.3-3µm wide and 3.4-4.1µm long, hyaline, 1-celled, ellipsoidal. Chlamydospore terminal to intercalary and sub-globose.

***Trichoderma* sp.3**

Culture characteristics: Colonies developed poorly on CZA but developed rapidly on MEA, PDA and RBA. Colony diameters on these media plates reached 1.1-1.7cm, 4.9-5.4cm, 4.9-5.5cm and 4.4-5cm respectively in 5 days. Colony colours on the obverse of these media plates were initially white then changed to green (CZA), and yellowish (MEA, PDA

and RBA). Reverse of these plates showed colours varying from yellow (CZA, MEA and PDA) and colourless (RBA). Colonies were floccose to compact tufts, whitish pustules and green conidia distributed irregularly over the surface, flat to slightly raised, irregular.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore hyaline, slightly erect, branched, side branches stand at right angles to the bear tip, 3 phialides on each branch arise in opposite pairs. Phialides 2-2.7µm wide and 7.1-8.3µm long, hyaline, ampulliform, board in the middle, tapered towards the apex. Conidia 1.8-3µm wide and 2.9-3.8µm long, hyaline, 1-celled, sub-globose. Chlamydospore intercalary and sub-globose.

***Trichoderma* sp.4**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA reached 4.8-5.5cm, 4.9-5.5cm, 4.9-5.5cm and 4.3-5cm respectively in 5 days. Colonies on the obverse of these media plates were yellowish-white. Reverse of these plates showed colours varying from pale brown (CZA, MEA and PDA) and colourless (RBA). Colonies were cottony, compact tufts, green conidia distributed irregularly and flat to slightly raised.

Microscopic features: Hyphae hyaline, septate, branched. Conidiophore 64-108.9µm long, hyaline, erect, highly branched, side branches with 3-5 phialides on each branch arise in opposite pairs or solitary. Phialides 3.5-5µm long, hyaline, ampulliform, tapered towards the apex, septate, convergent. Conidia 2.5-3.3µm in diameter, hyaline, 1-celled and globose to sub-globose.

***Ulocladium* sp.**

Culture characteristics: Colony diameters on CZA, MEA, PDA and RBA were 3-3.5cm, 4.5-4.9cm, 4.7-5.6cm and 3.5-4.1cm in 7 days. Colony colours on the obverse and reverse of these media plates were white and yellowish-brown (CZA), white and yellow (MEA), yellowish-white and brown (PDA), white and colourless (RBA). Colony colours turned grey after 10 days of incubation. Colonies were cottony with superficial mycelium and regular edge.

Microscopic features: Conidiophores 3.3-4.5µm wide, sub-hyaline, septate to aseptate, smooth. Conidiogenous cells solitary to fascicles of a few hyphae. Conidia 27-35 x 17-20 µm, solitary, dark brown, obovoid to ellipsoidal, slightly verrucose, muriform, 1-3 septa, septa transverse and longitudinal, constricted at or near septa.

3.3.2.4 Seasonal and site variations in fungal isolates

Altogether 393 fungal isolates under 35 genera were identified in the present study (Table 3.13). Based on their cultural and microscopic characteristics, the identified 35 fungal genera in the present study were *Acremonium,Arthrinium,Apophysomyces, Aspergillus, Botrytis, Chaetomium, Chrysosporium, Cladosporium, Clonostachys,Colletotrichum, Cunninghamella, Emmonsia, Exophiala, Fusarium, Geosmithia, Geotrichum, Lichtheimia, Microascus, Mucor, Nigrospora, Paecilomyces, Periconia, Penicillium, Pestalotiopsis, Phoma, Phomopsis, Rhizopus, Sarocladium, Scedosporium, Sclerotinia, Scytalidium, Taloromyces, Trichoderma, Trichophyton* and *Ulocladium*. Among the fungal genera, *Aspergillus* with 11 species and *Penicillium* and *Trichoderma* each with 9 species was found to be dominant.

Between the tea gardens, the highest number of fungal isolates was recorded from Tuli tea garden (223 isolates under 29 genera) was recorded from Tuli tea garden as compared with Ungma tea garden (171 isolates under 25 genera). Fungal genera selective toTuli tea garden were *Acremonium, Arthrinium, Chrysosporium, Clonostachys, Emmonsia, Exophiala, Lichtheimia, Periconia, Sarocladium* and *Sclerotinia* whereas, fungal genera selective to Ungma tea garden were *Botrytis, Geosmithia, Microascus, Phomopsis, Scedosporium, Taloromyces* and *Trichophyton*.

Among the seasons, the highest fungal isolates in the Tuli tea garden was recorded in the spring season with 40 and 35 fungal isolates in the first and second sampling year respectively. Likewise, in the Ungma tea garden, the highest fungal isolates were recorded in the spring season with 33 and 29 fungal isolates in the first and second sampling year respectively.

At the Tuli tea garden, species of *Aspergillus, Penicillium* and *Trichoderma*were recorded in all the sampling seasons whereas, *Arthrinium, Emmonsia, Cunninghamella, Geotrichum, Periconia, Sarocladium* and *Sclerotinia* were restricted to the specific season only. At the Ungma tea garden, species of *Aspergillus* and *Penicillium* were recorded in almost all the sampling seasons whereas, *Apophysomyces, Botrytis, Chaetomium, Nigrospora, Scedosporium* and *Scytalidium* were restricted to the specific season only.

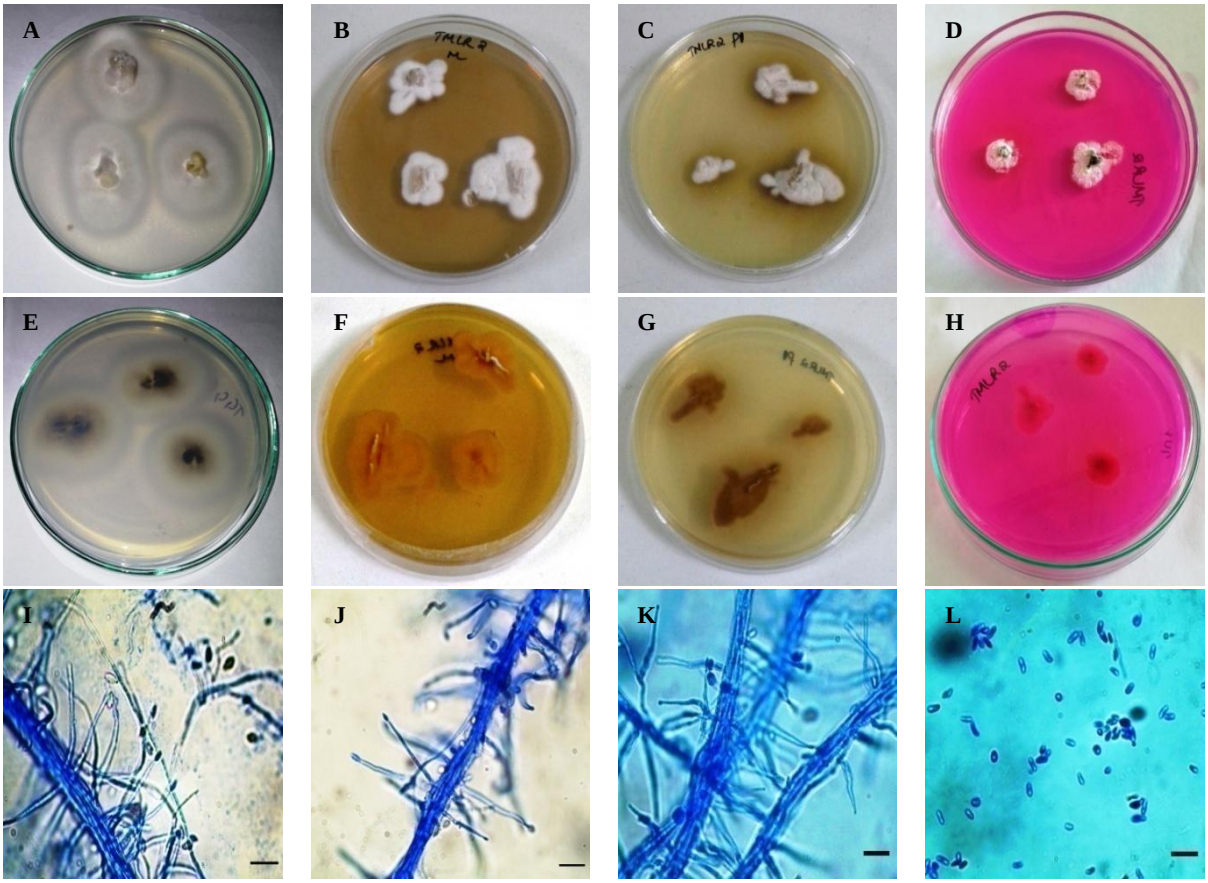

Fig. 3.4. Culture and microscopic features of *Acremonium* sp.1. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA, I-K- conidiophores and conidia under 100X, L- conidia under 100X. Scale bars represent 10µm

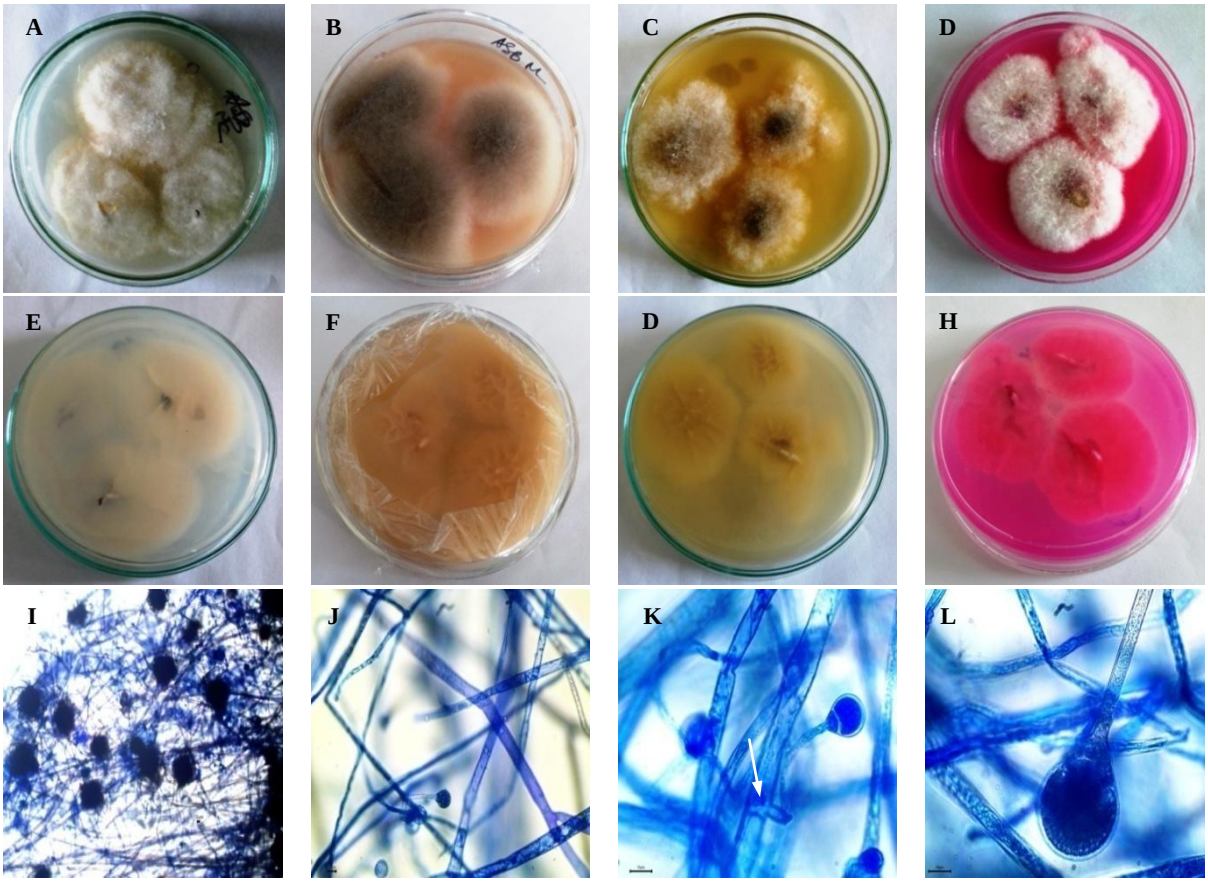

Fig. 3.5. Culture and microscopic features of *Apophysomyces viriabilis*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA, I-J- branching hyphae and sporangia under 10X and 40X, K- foot cell (arrow), L- sporangia and sporangiophore under 100X. Scale bars represent 10µm

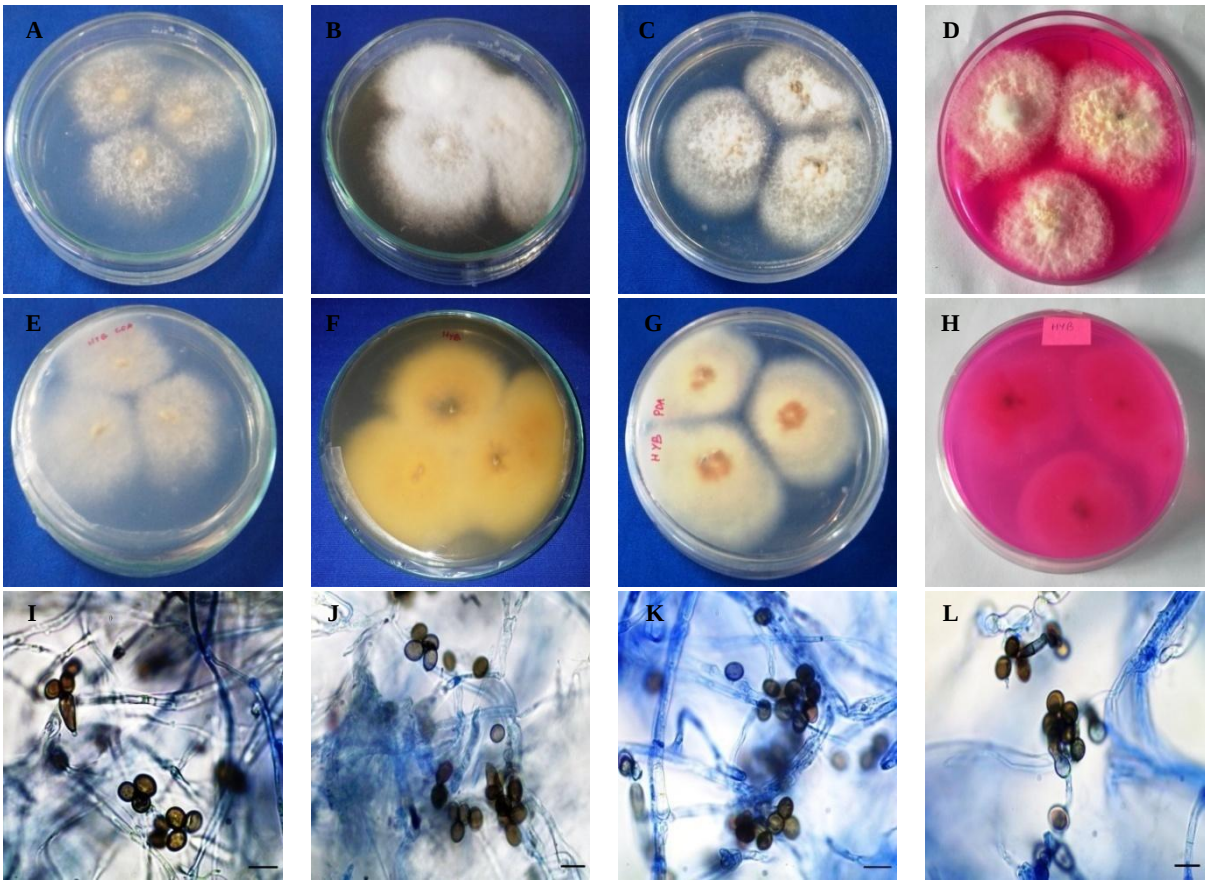

Fig.3.6. Culture and microscopic features of *Arthrinium kogelbergense*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-K- conidia under 100X, L-conidiogenous cells giving rise to conidia as observed under 100X. Scale bars represent 10µm

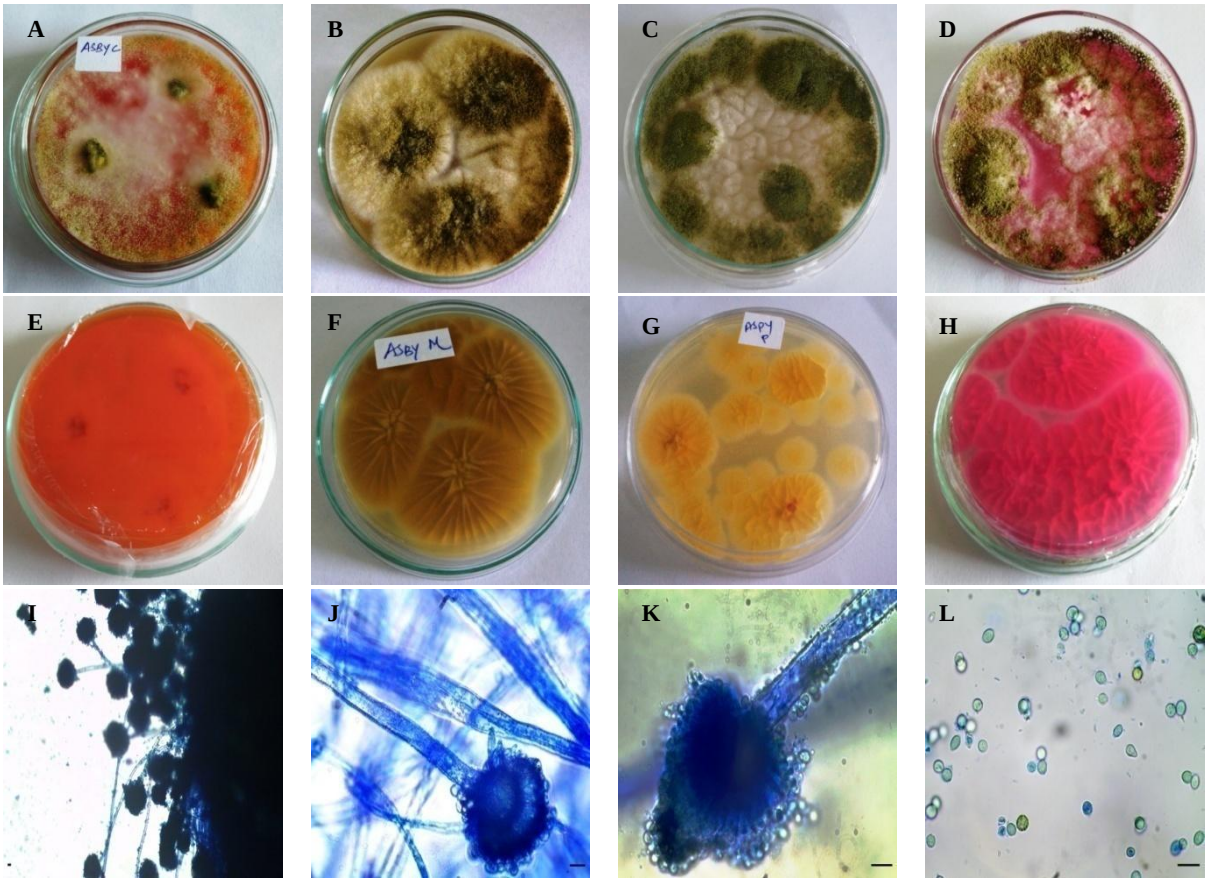

Fig. 3.7. Culture and microscopic features of *Aspergillus flavus*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- conidiophores with conidial heads under 10X, J-K- conidiophores with conidial heads under 40X and 100X, L- conidia under 100X. Scale bars represent 10µm

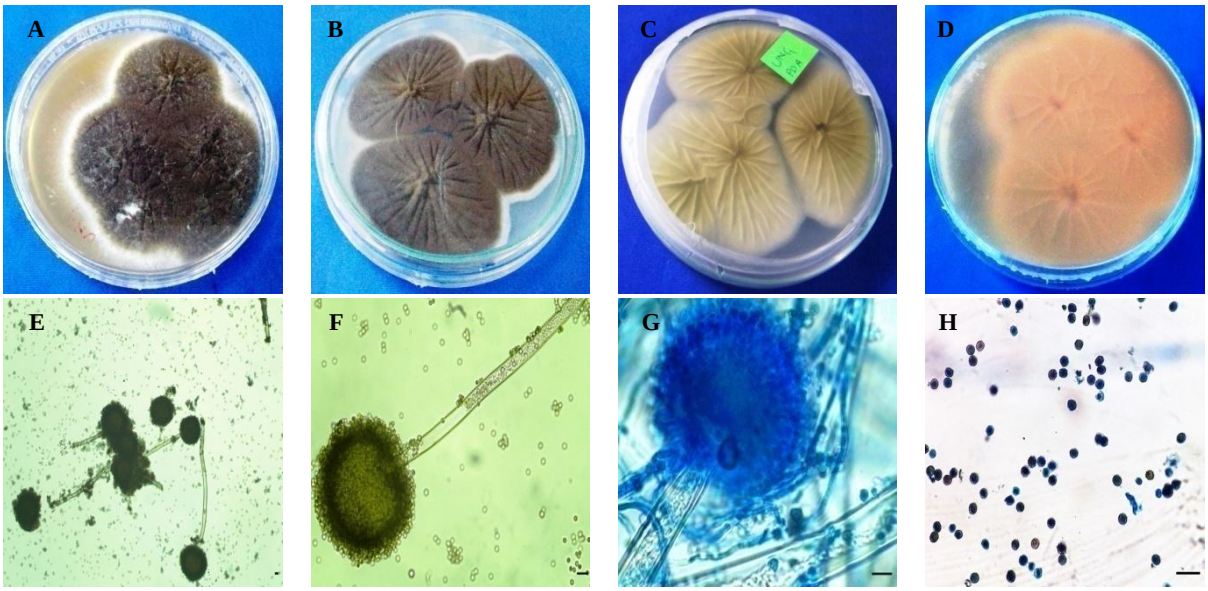

Fig.3.8. Culture and microscopic features of *Aspergillus heteromorphous*. A-D- Obverse and reverse view on MEA and PDA plates, E- conidiophores with conidial heads under 10X, F- conidiophore with conidial head under 100X, G- conidiophore with conidial head under 100X, H- conidia under 100X. Scale bars represent 10µm

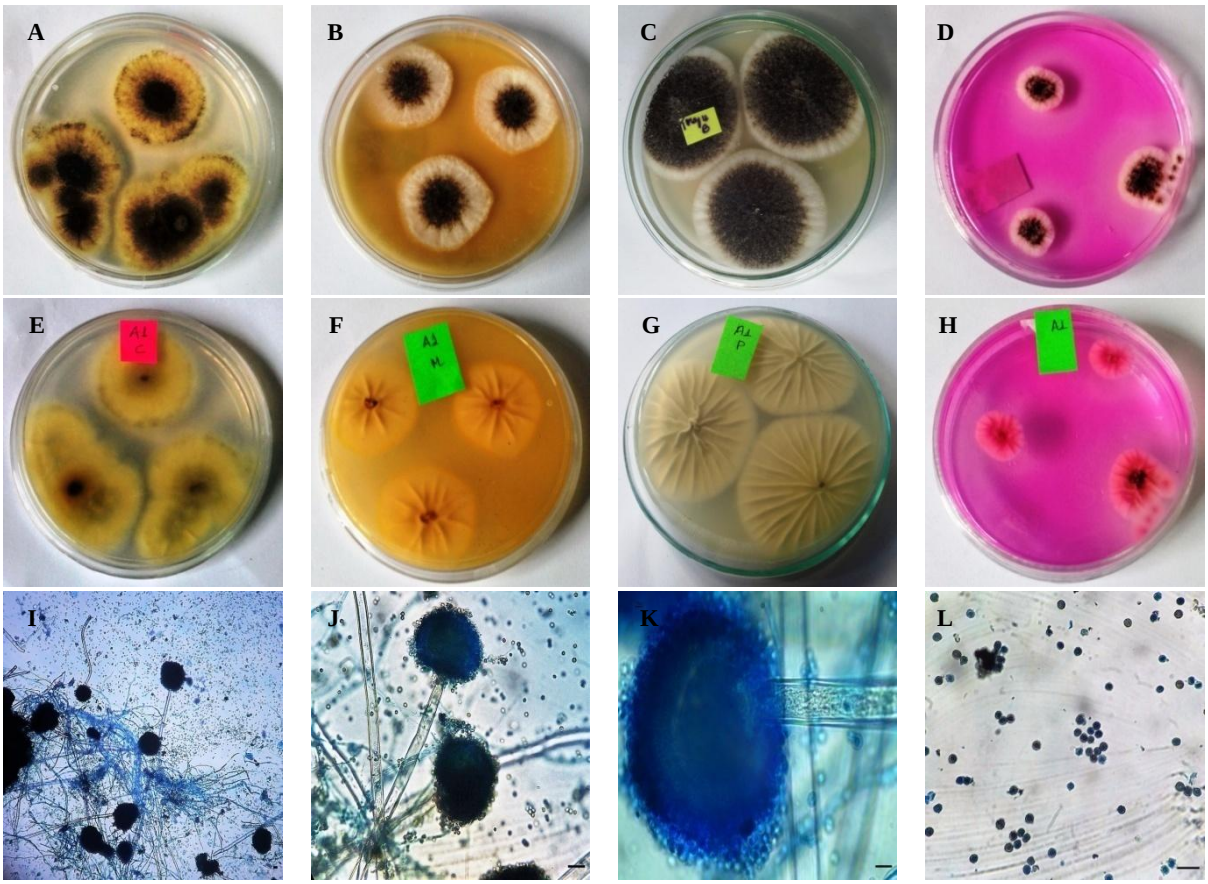

Fig.3.9. Culture and microscopic features of *Aspergillus niger*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- conidiophores with conidial heads under 10X, J- conidiophores with conidial heads under 40X, K- conidiophore with conidial head under 100X, L- conidia under 100X. Scale bars represent 10µm

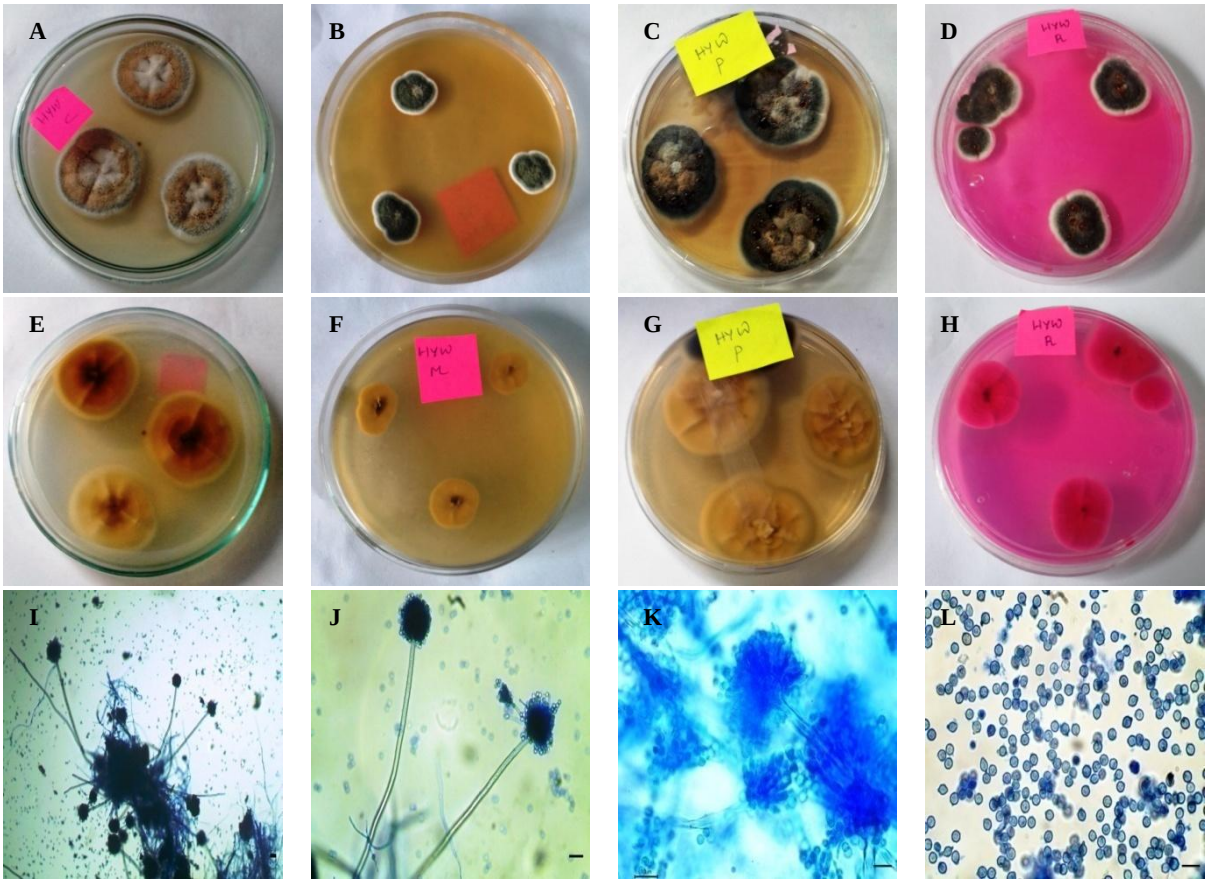

Fig.3.10. Culture and microscopic features of *Aspergillus versicolor*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- conidiophores with conidial heads under 10X, J- conidiophores with conidial headsand *Penicillium*-like conidiophores under 40X, K- conidiophore with conidial head under 100X, L- conidia under 100X. Scale bars represent 10µm

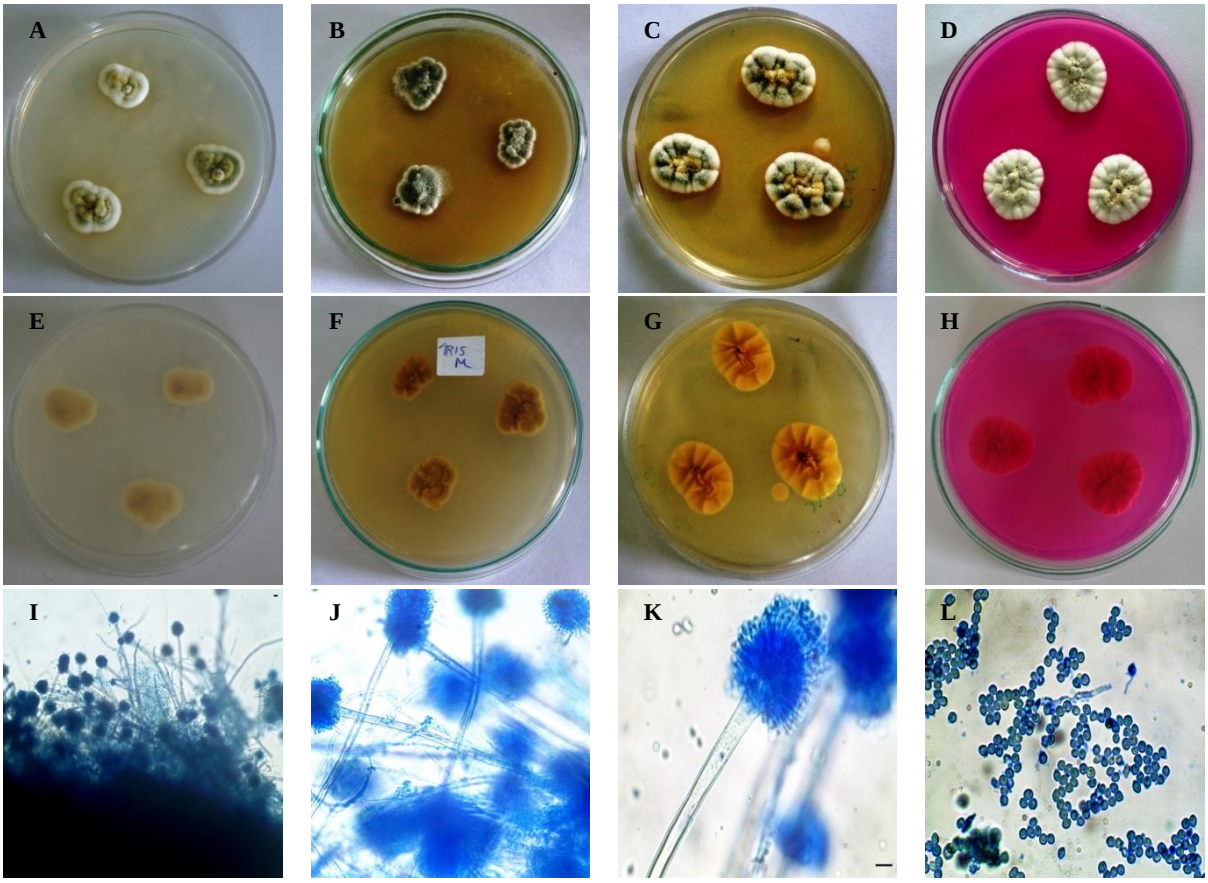

Fig.3.11. Culture and microscopic features of *Aspergillus* sp.2. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- conidiophores with conidial heads under 10X, J- conidiophores with conidial headsunder 40X, K- conidiophore with conidial head under 100X, L- conidia under 100X. Scale bars represent 10µm

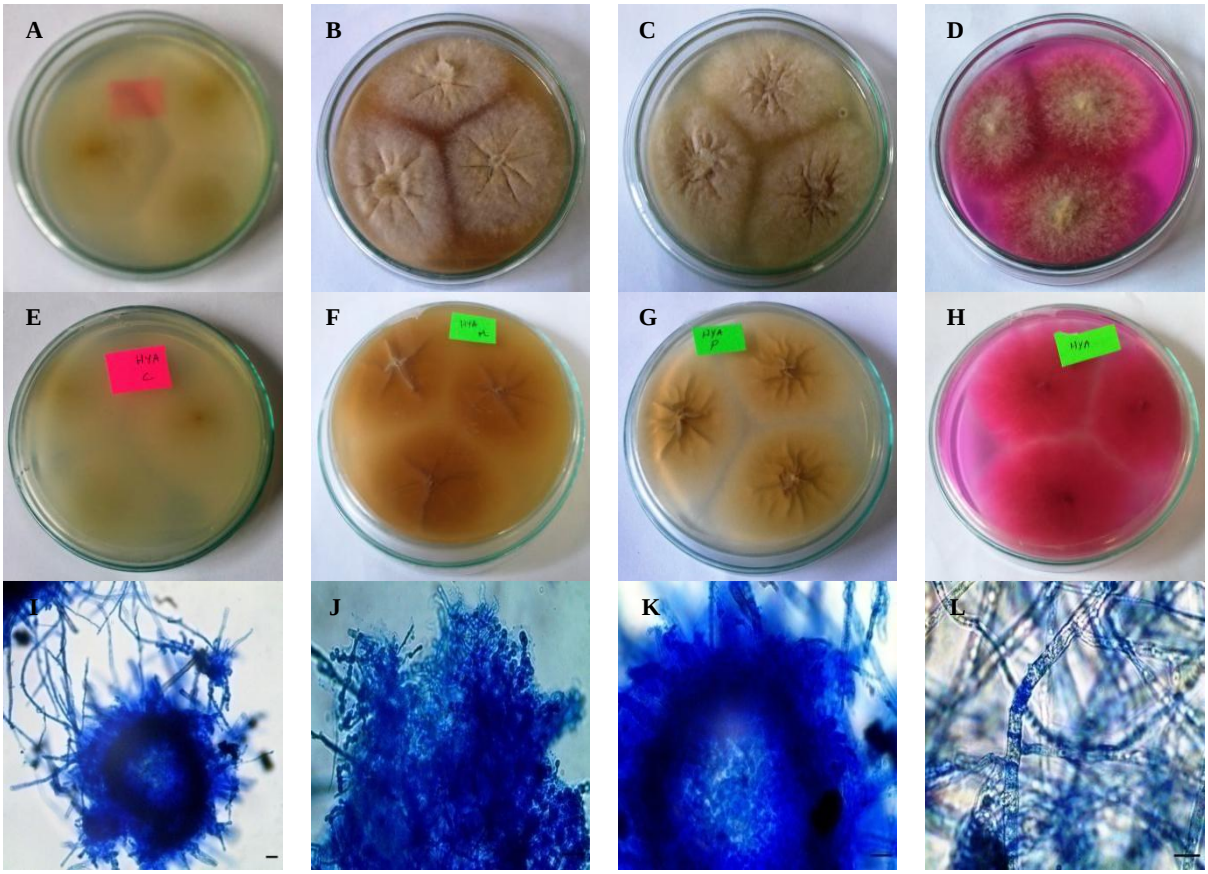

Fig.3.12. Culture and microscopic features of *Chaetomium globosum*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- ascomata under 40X, J-K- ascomata with ascospore masses 100X, L- ascomatal hairs under 100X. Scale bars represent 10µm

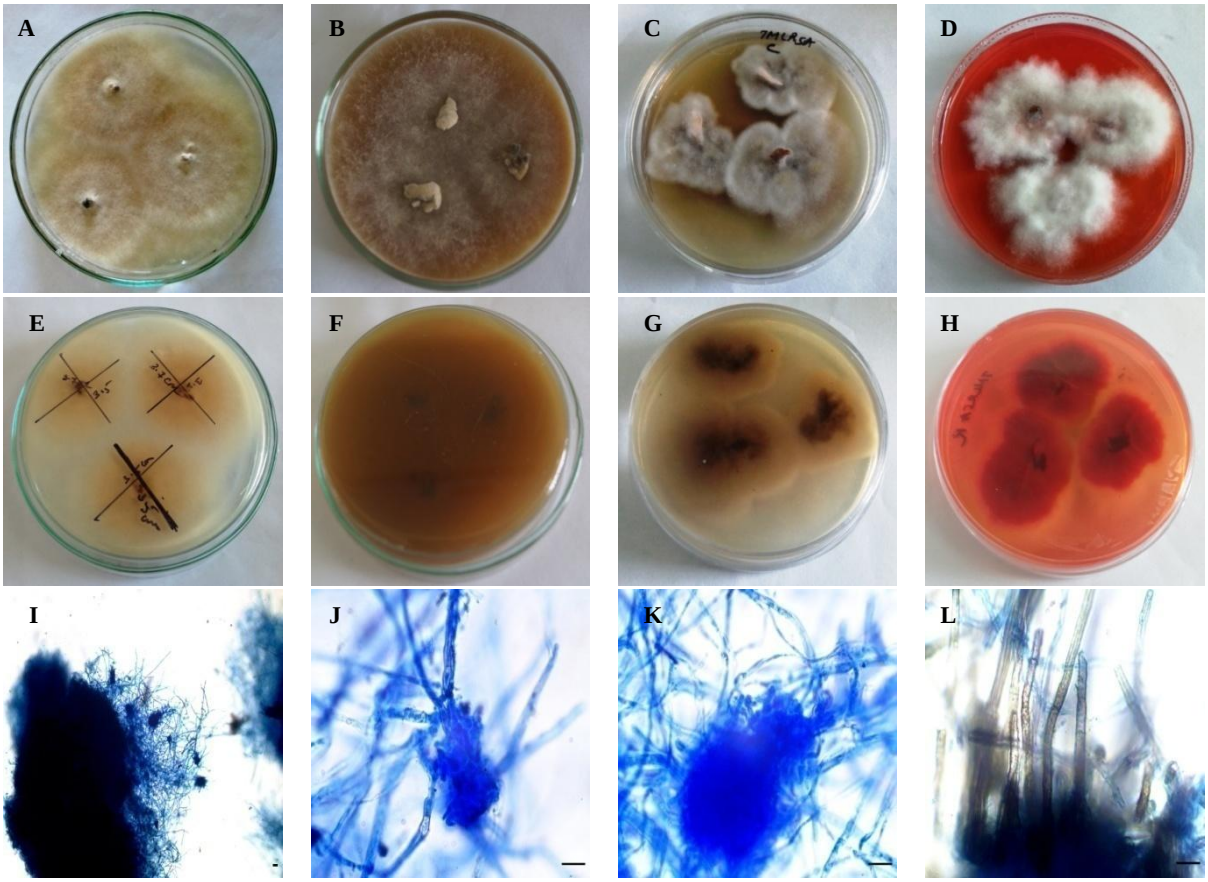

Fig.3.13. Culture and microscopic features of *Chaetomium* sp.1.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- ascomata under 10X, J: ascomatal hair under 100X, K- ascomata under 100X, L-ascomatal hairs under 100X. Scale bars represent 10µm

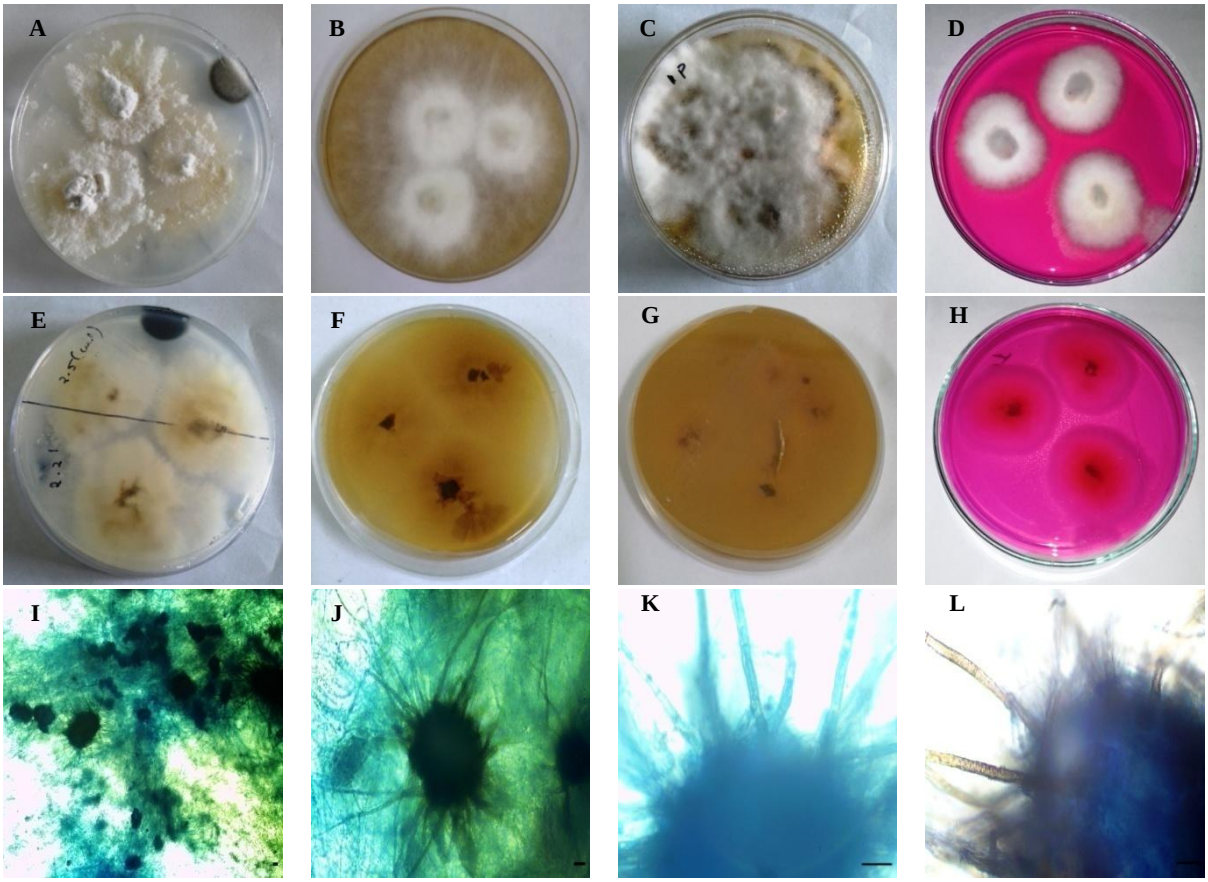

Fig.3.14. Culture and microscopic features of *Chaetomium* sp.2. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- ascomata under 10X, J- ascomata under 40X, K-L- ascomata with ascomatal hairs under 100X. Scale bars represent 10µm

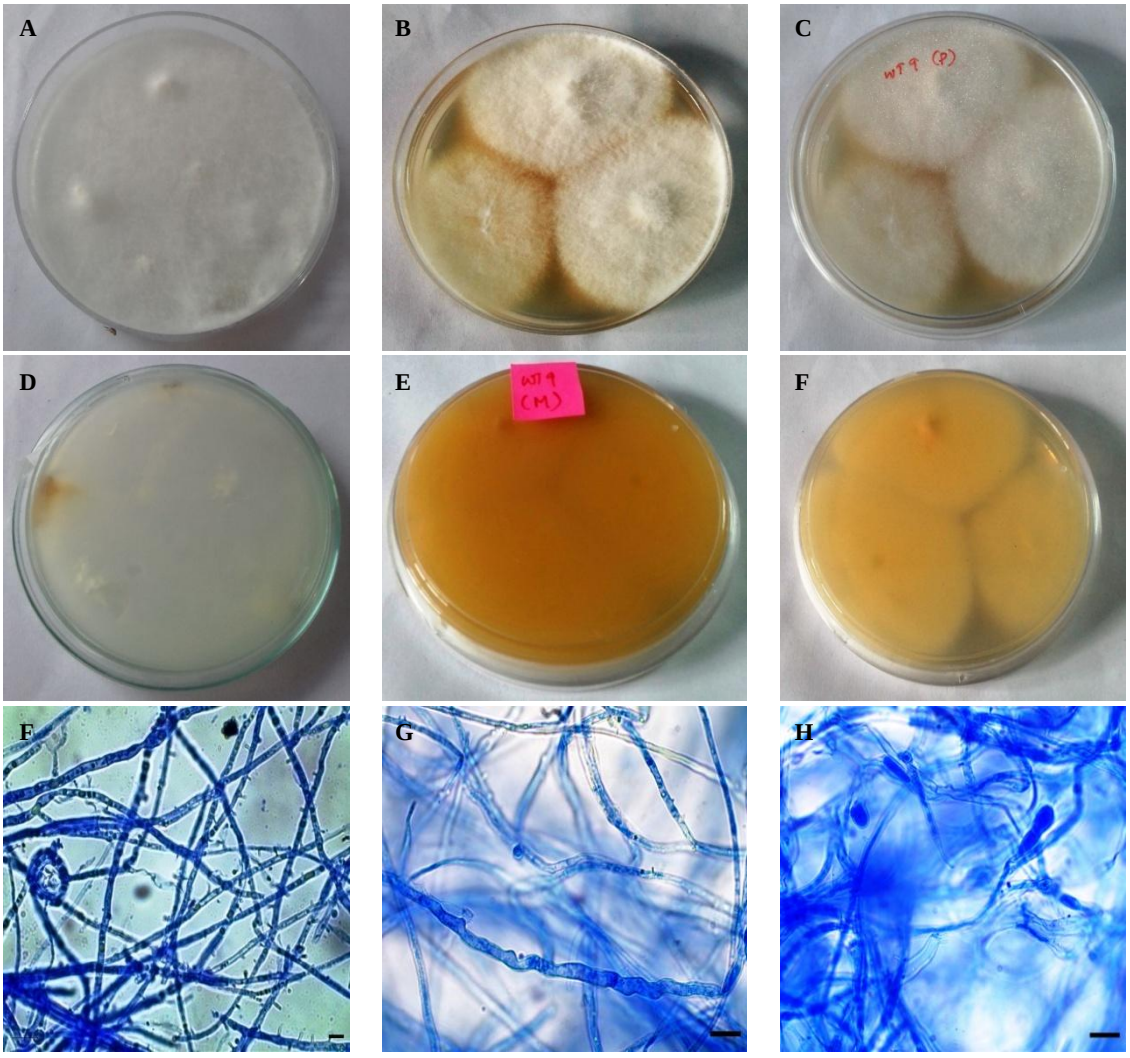

Fig.3.15. Culture and microscopic features of *Chrysosporium* sp. A-C and D-F- Obverse and reverse view on CZA, MEA and PDA plates, G: septate hyphae with branching under 40X, H-hyaline to arthroconidia like septations hyphae under 100X, I- hyphae bearing conidia under 100X. Scale bars represent 10µm

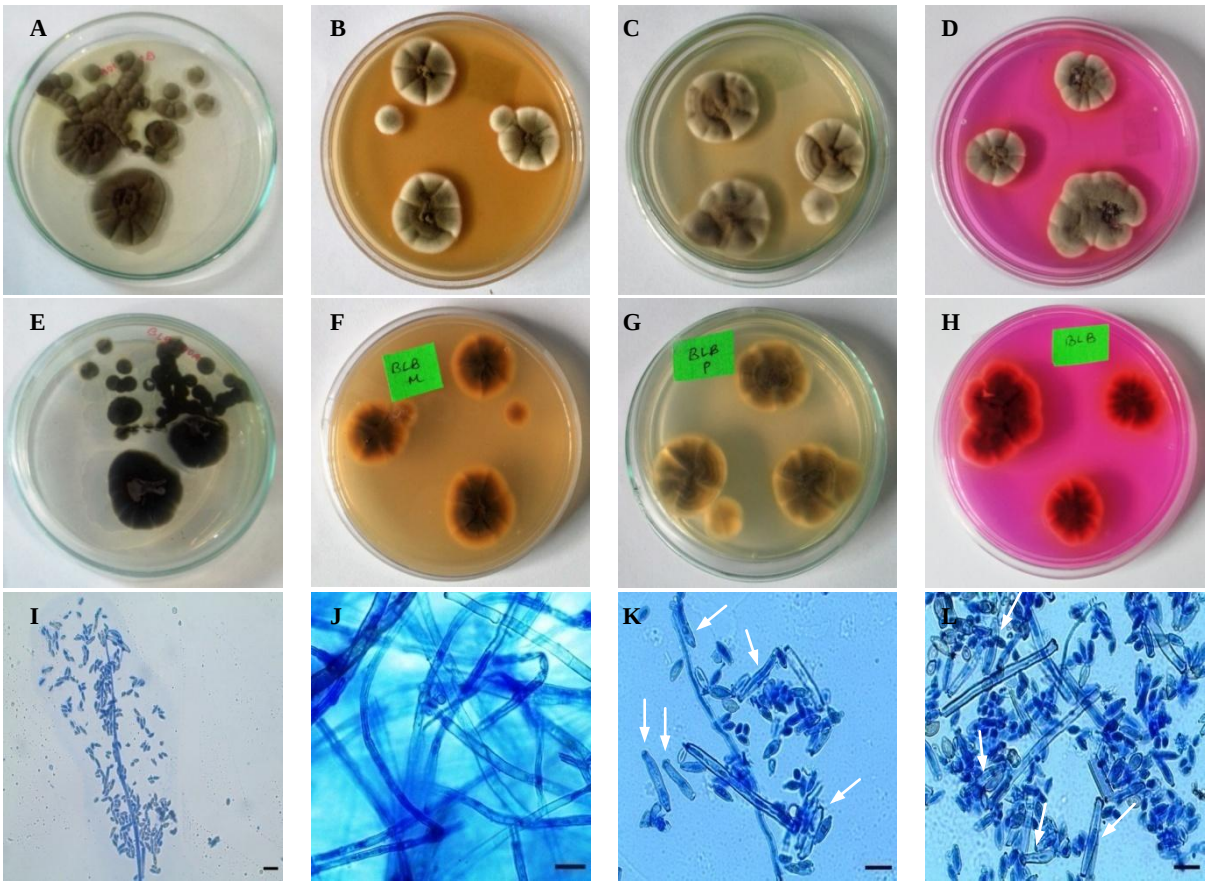

Fig.3.16. Culture and microscopic features of *Cladosporium cladosporiodes*.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- Conidiophore with conidia 40X, J- hyphae under 100X, K-L- conidia and ramoconidia (arrow) under 100X. Scale bars represent 10µm

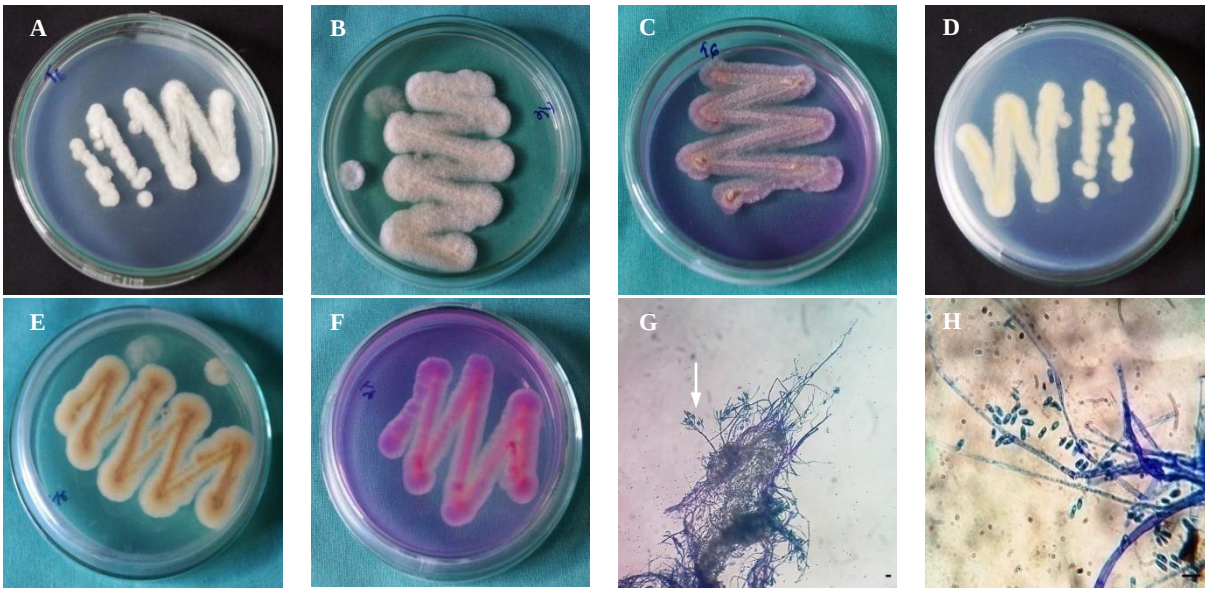

Fig.3.17. Culture and microscopic features of *Clonostachys*sp.1.A-C and D-F- Obverse and reverse view on CZA, PDA and RBA plates, G- *Penicillium*-like conidiophores (arrow) under 10X, H-conidia under 100X. Scale bars represent 10µm

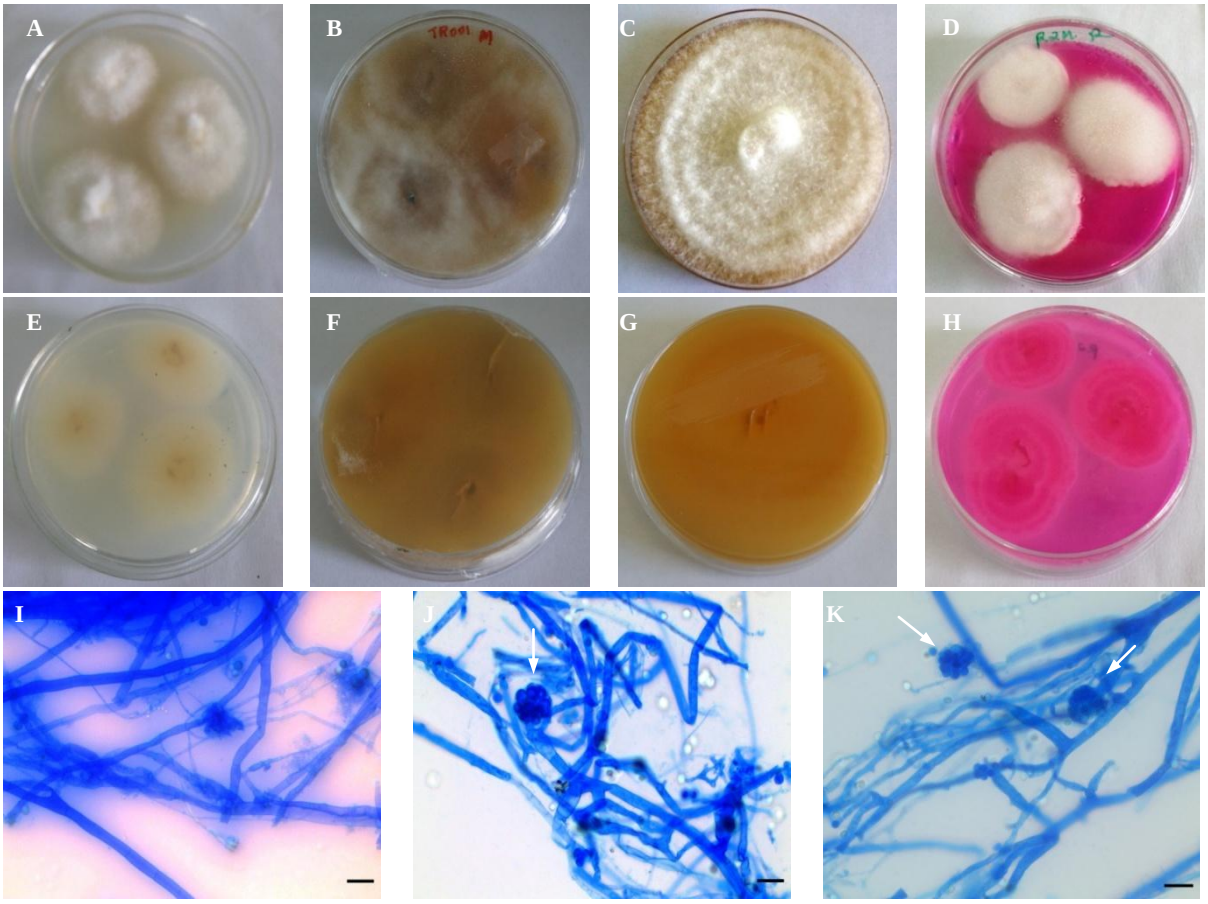

Fig.3.18. Culture and microscopic features of *Cunninghamellaechinulata*. A-D and E-H- Obverse and reverse view on CZA, PDA and RBA plates, I- hyphae under 100X, J-K- sporangiophores with sporangiola (arrow) under 100X. Scale bars represent 10µm

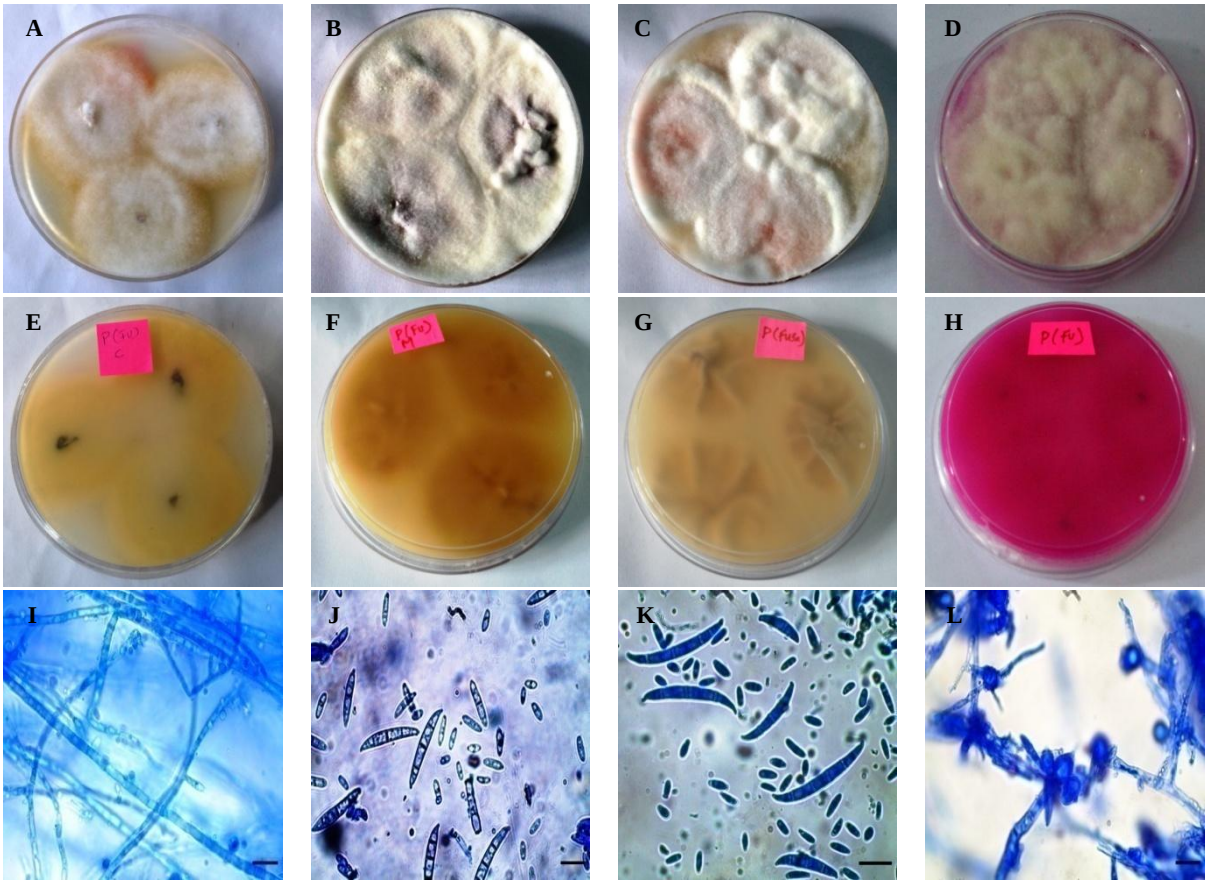

Fig.3.19. Culture and microscopic features of *Fusarium solani***.**A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- hyphae under 100X, J-K- macroconidia and microconidia under 100X, L- terminal and intercalary chlamydospore under 100X. Scale bars represent 10µm

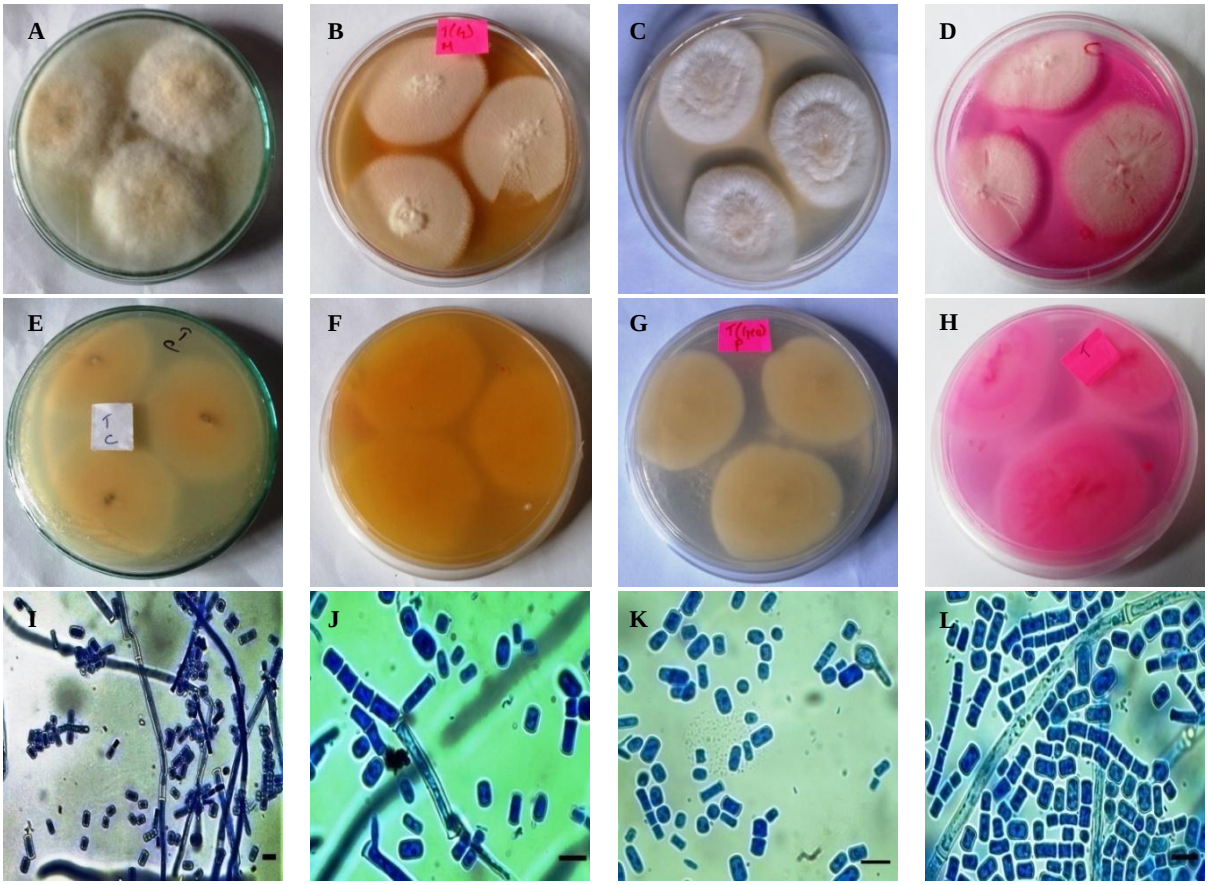

Fig.3.20. Culture and microscopic features of *Geotrichumcandidum*.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- hyphae and arthroconidia under 40X, J-L-1-celled arthosporous conidia under 100X. Scale bars represent 10µm

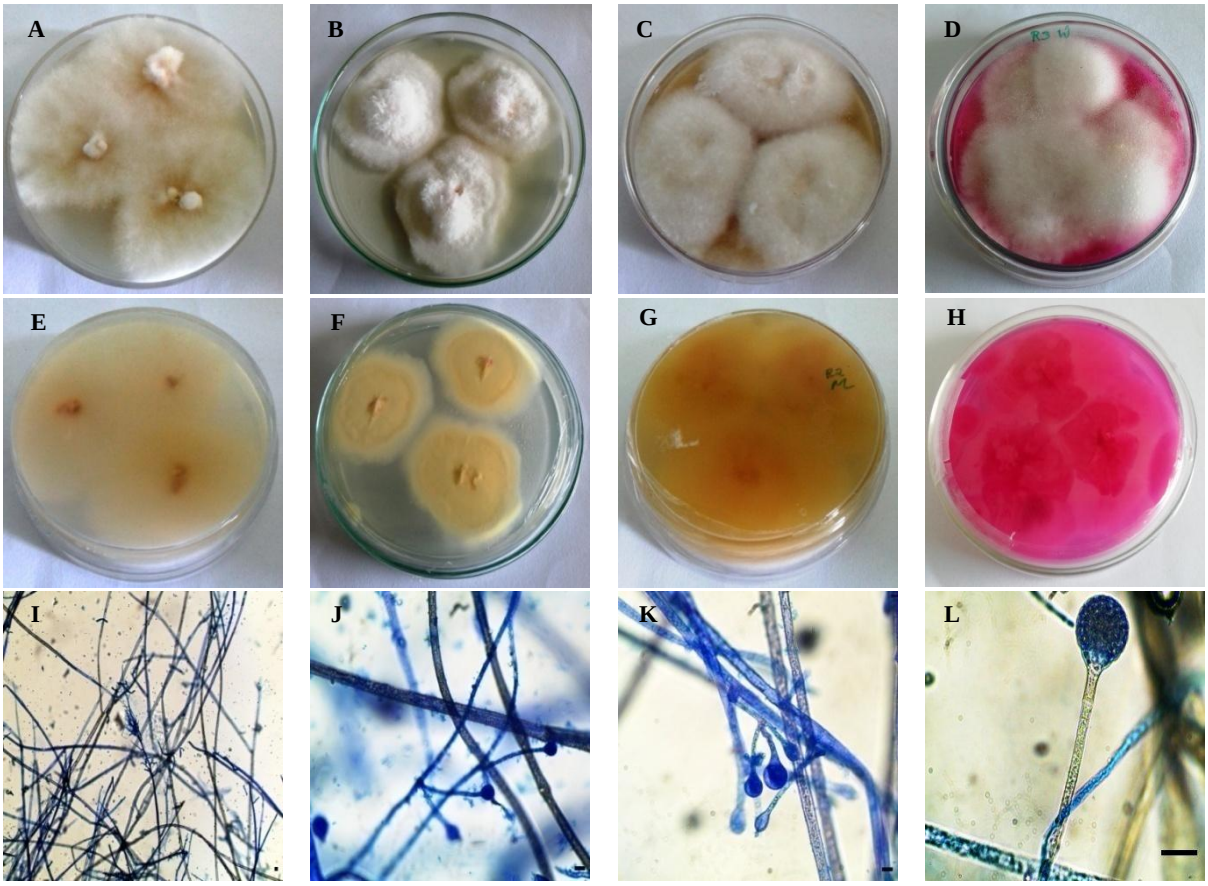

Fig.3.21. Culture and microscopic features of *Lichtheimia*sp.1.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-nonseptatehyphae under 10X, J-K stolons with branching sporangiophores under 40X, L- sporangiophores with sporangiospores filled pyriform sporangia under 100X. Scale bars represent 10µm

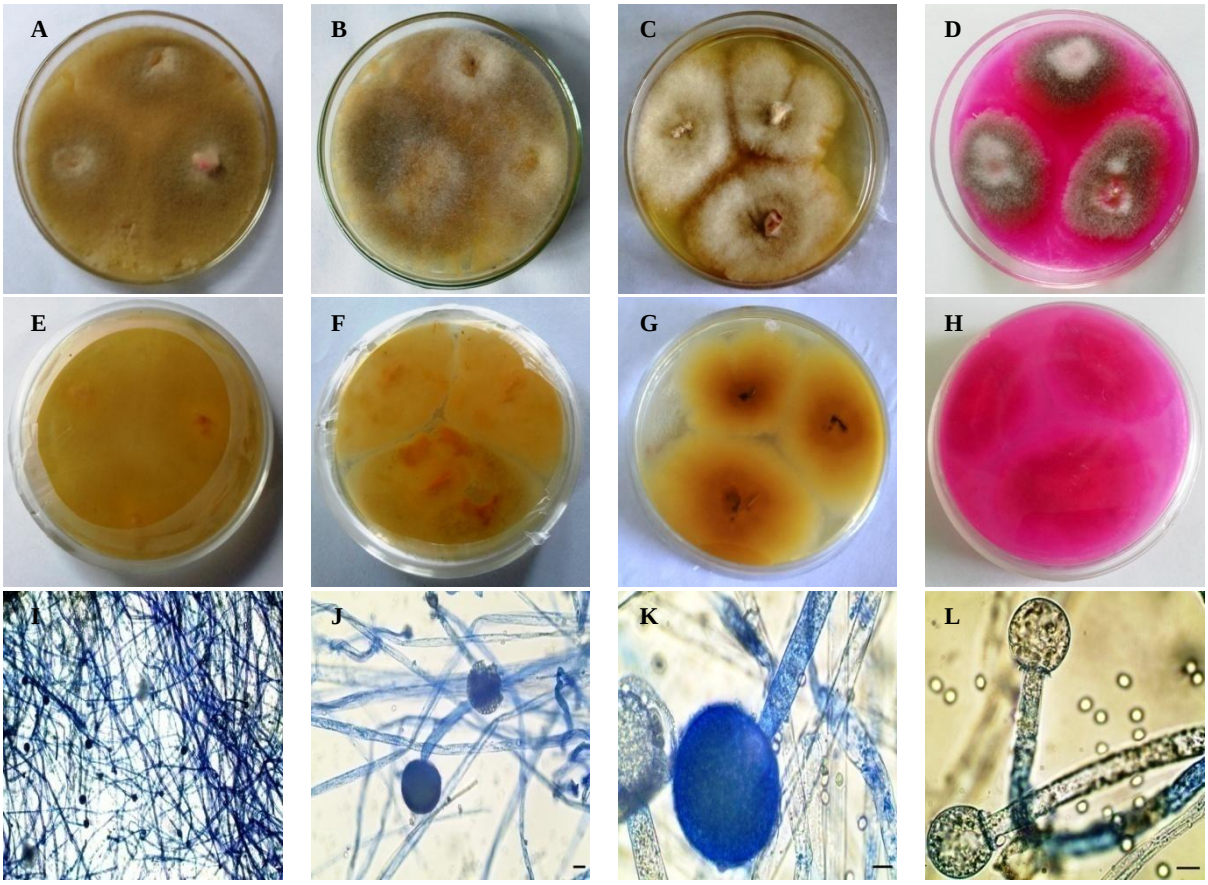

Fig.3.22. Culture and microscopic features of *Mucor hiemalis*.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- sparsely septate hyphae and sporangiophores with sporangia under 10X, J- sporangiophores with sporangia under 40X, K- sporangiophores with sporangia under 100X, L- columellae with collar under100X. Scale bars represent 10µm

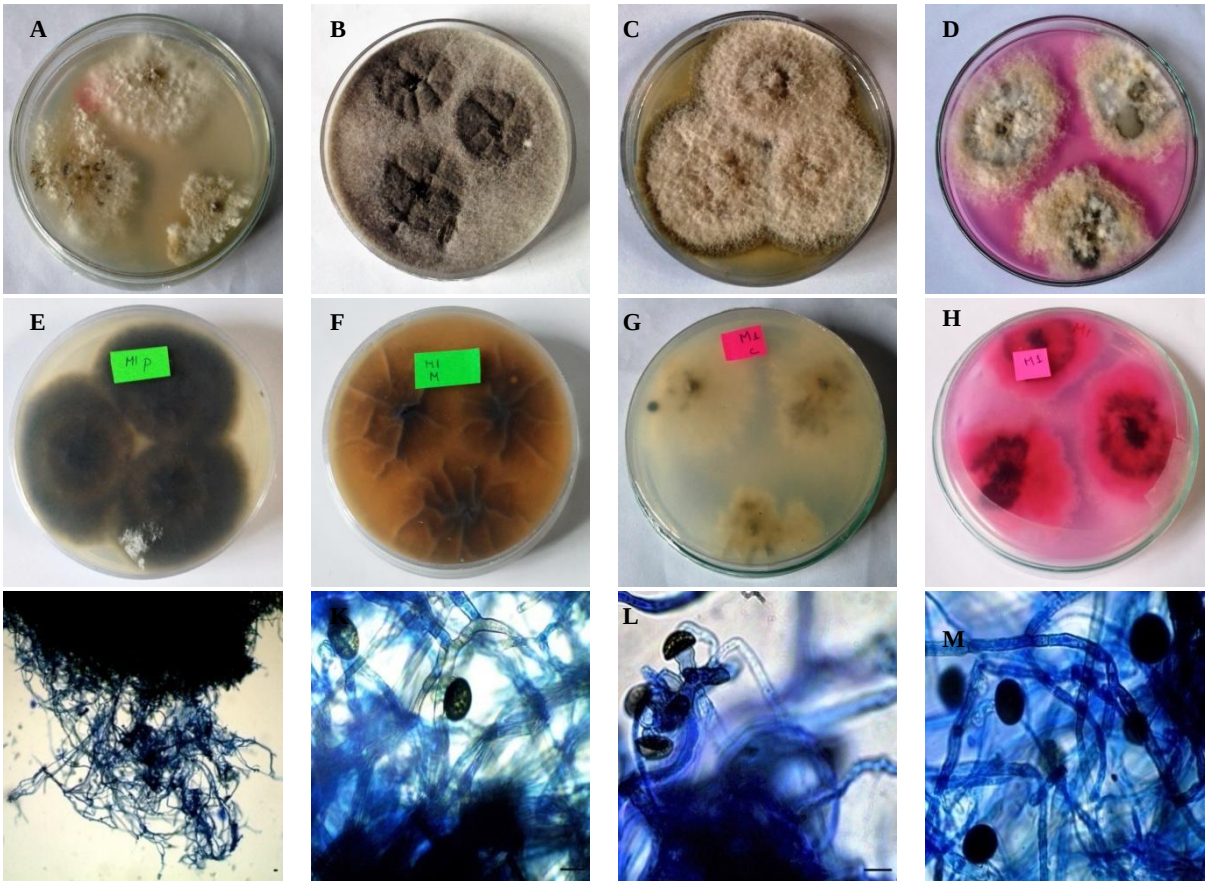

Fig.3.23. Culture and microscopic features of *Nigrospora osmanthi*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- mycelium under 10X, J-K-conidiogenous cells (giving rise to conidia) under 100X, L- black, shinyconidia under100X. Scale bars represent 10µm

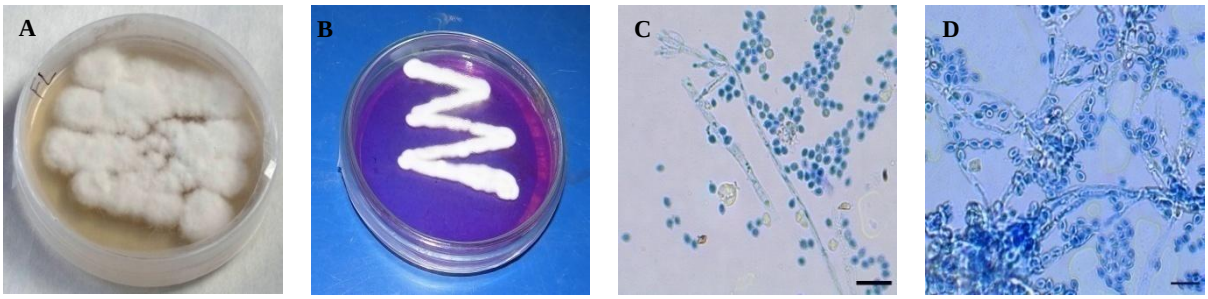

Fig.3.24. Culture and microscopic features of *Paecilomyces* sp. 2. A-B- Obverse view on PDA and RBA plates, C-D-conidiophores with phialides and conidia under 100X. Scale bars represent 10µm

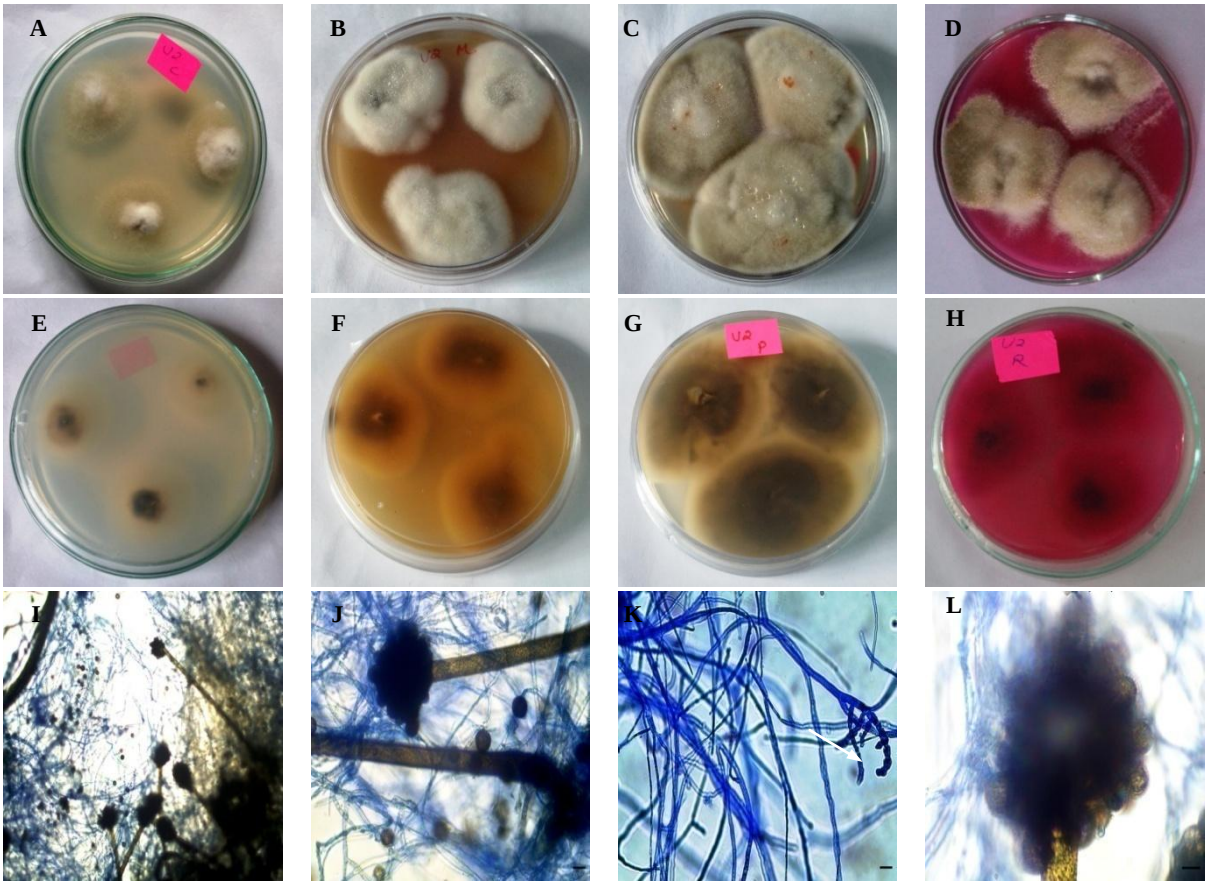

Fig.3.25. Culture and microscopic features of *Periconia byssoides*.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-J-conidiophore with conidia under 10X and 40X, K-conidiogenous cells (arrow) under 40X, L- apical part of conidiophores with heads of conidia under 100X. Scale bars represent 10µm

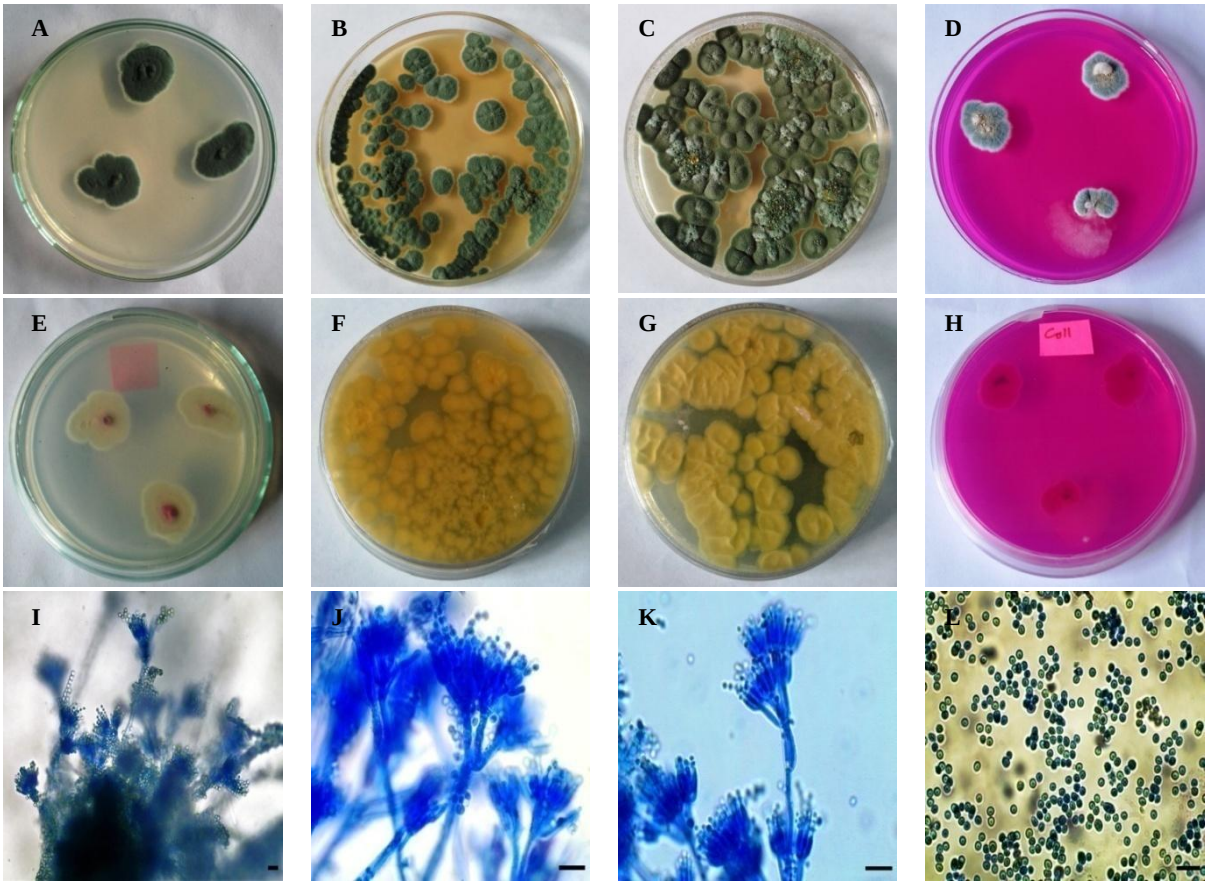

Fig.3.26. Culture and microscopic features of *Penicillium citrinum*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-K-conidiophore with conidia under 40X and 100X, L- conidia under 100X. Scale bars represent 10µm

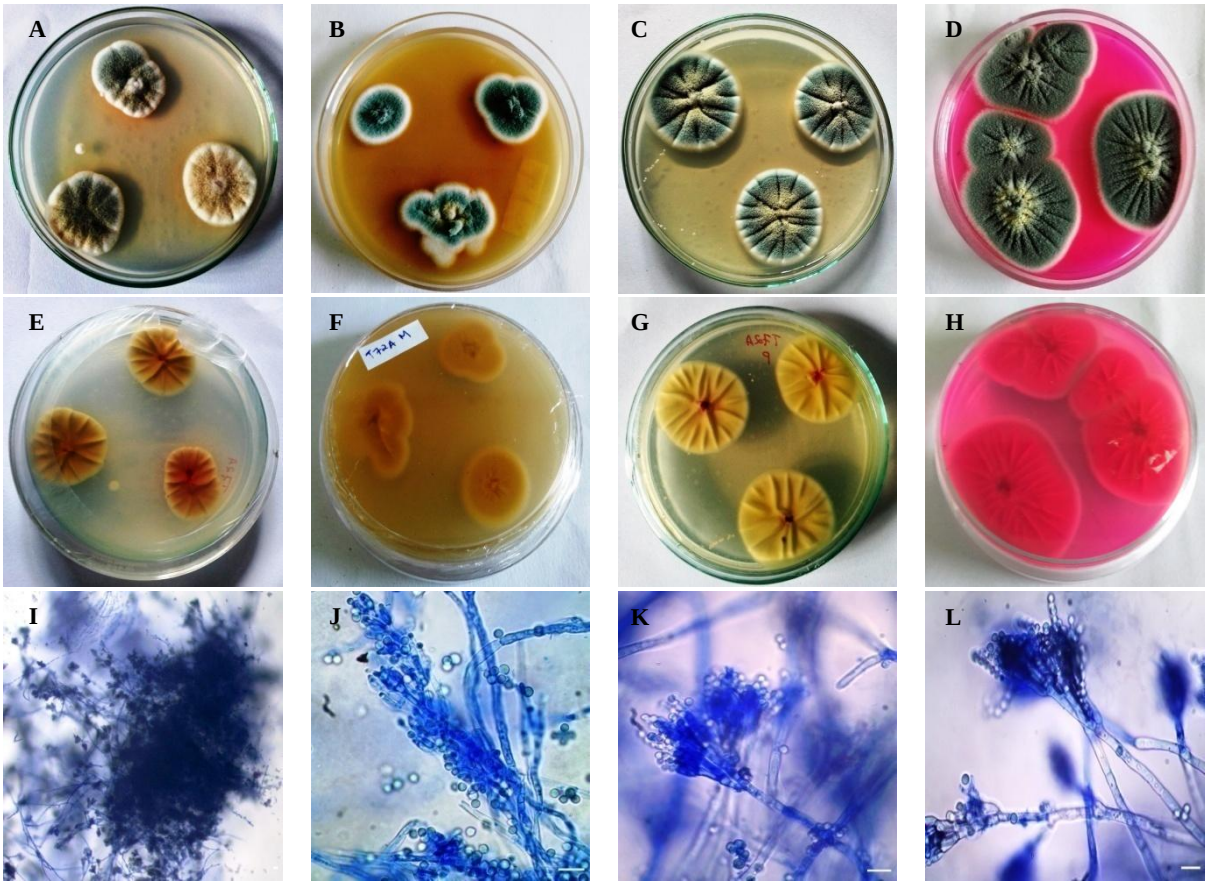

Fig.3.27. Culture and microscopic features of *Penicillium commune*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- mycelium and condiophore with conidia under 10X, J-L-conidiophore with conidia under 100X. Scale bars represent 10µm

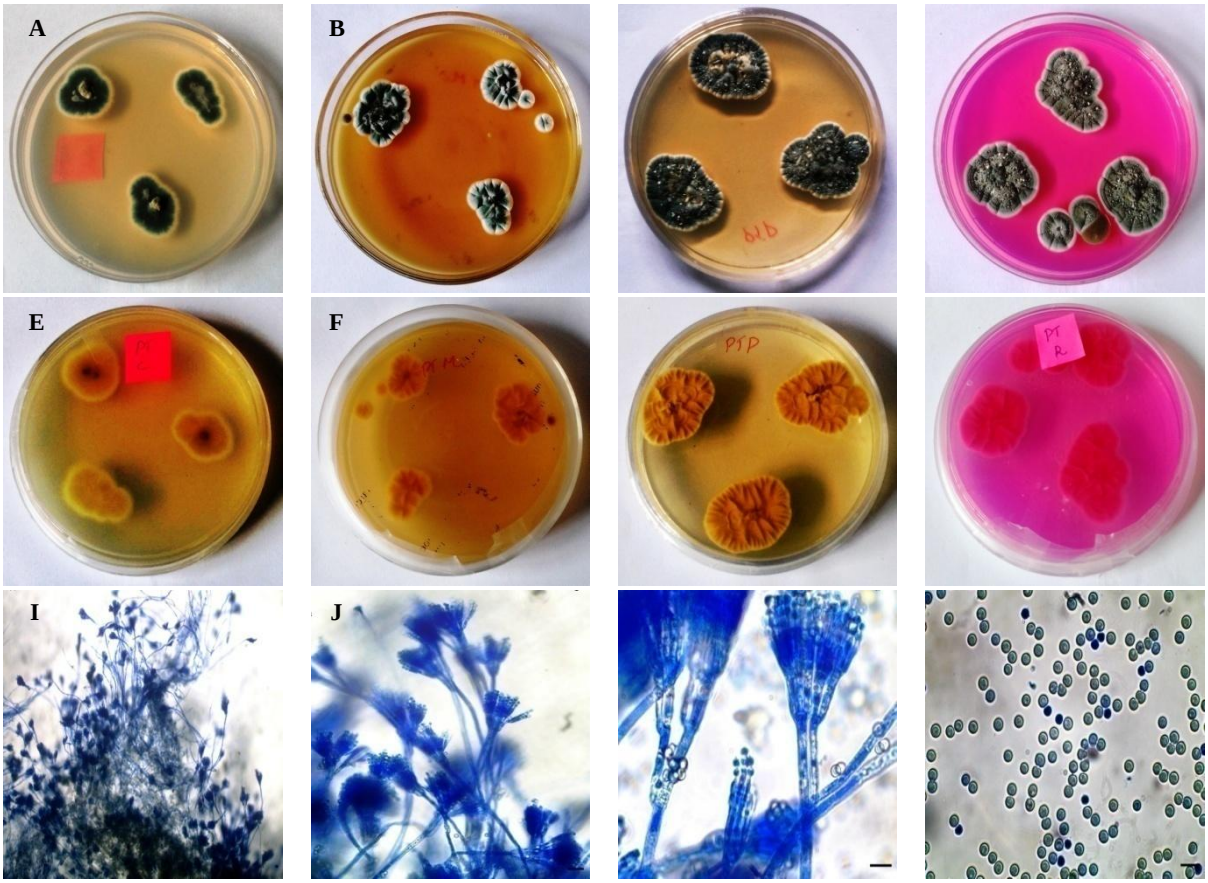

Fig.3.28. Culture and microscopic features of *Penicillium* sp.1. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- mycelium and condiophore with conidia under 10X, J-K-conidiophore with conidia under 40 and 100X, L- conidia under 100X. Scale bars represent 10µm

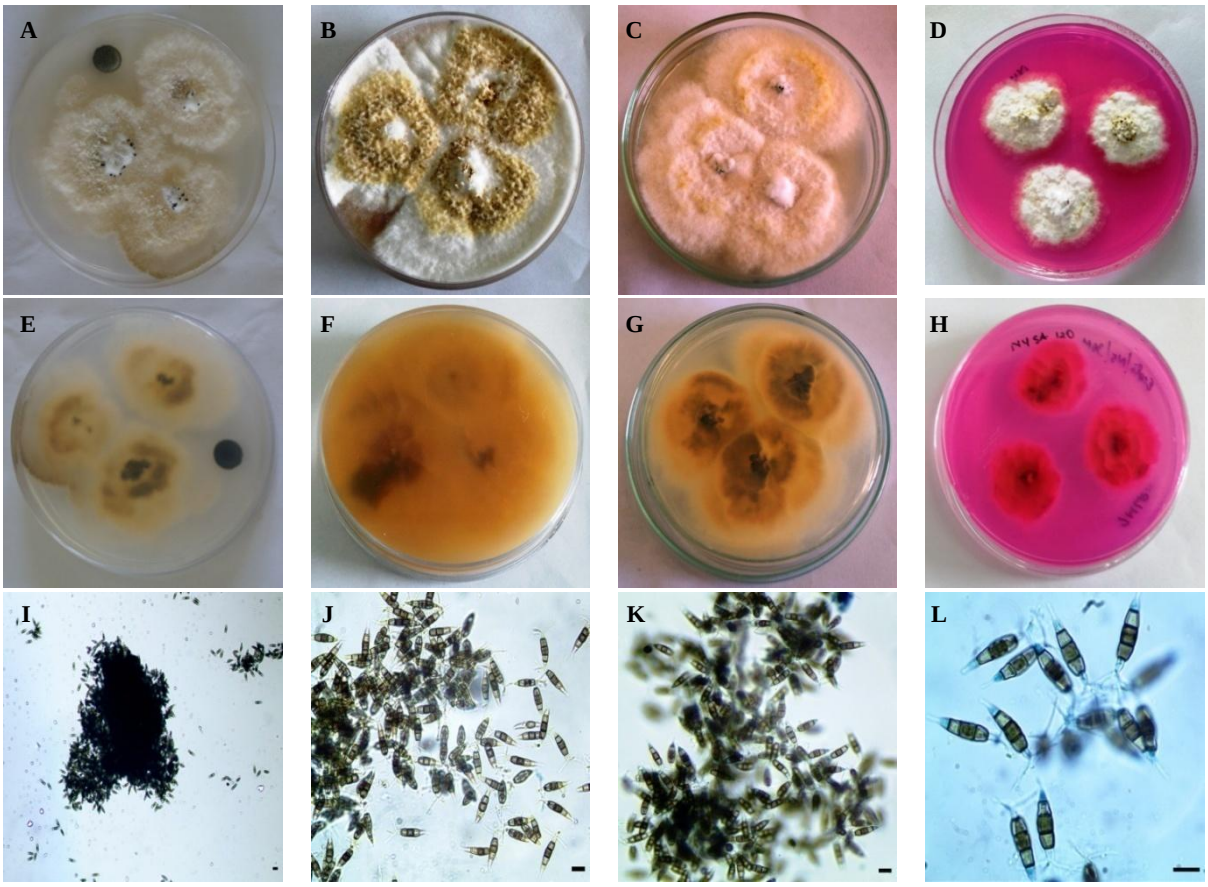

Fig.3.29. Culture and microscopic features of *Pestalotiopsis* sp.1.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- conidia under 10X, J-K-conidia under 40, L- conidia with clear basal and apical appendages under 100X. Scale bars represent 10µm

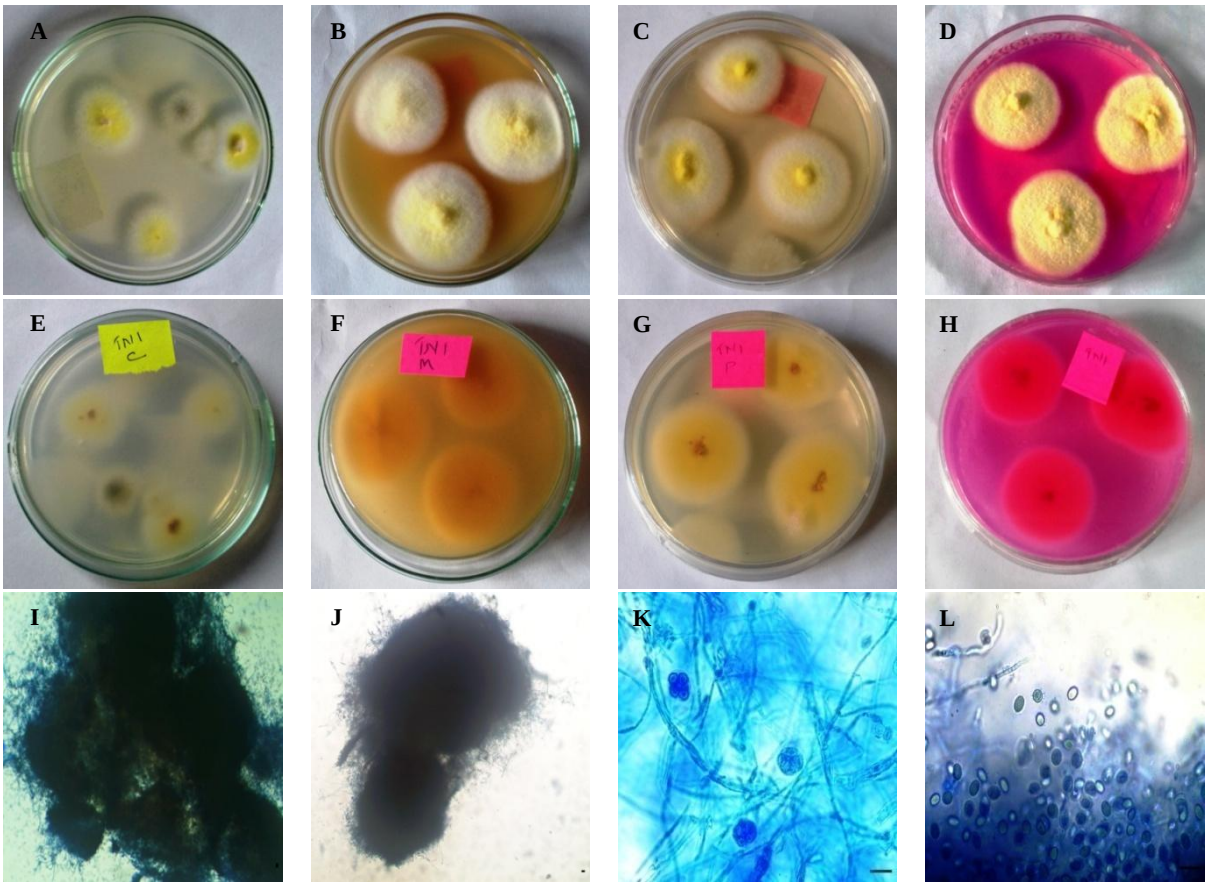

Fig.3.30. Culture and microscopic features of *Phoma* sp.2. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-J- Pycnidia under 10X, K- chlamydospores under 100X, L- conidia under 100X. Scale bars represent 10µm

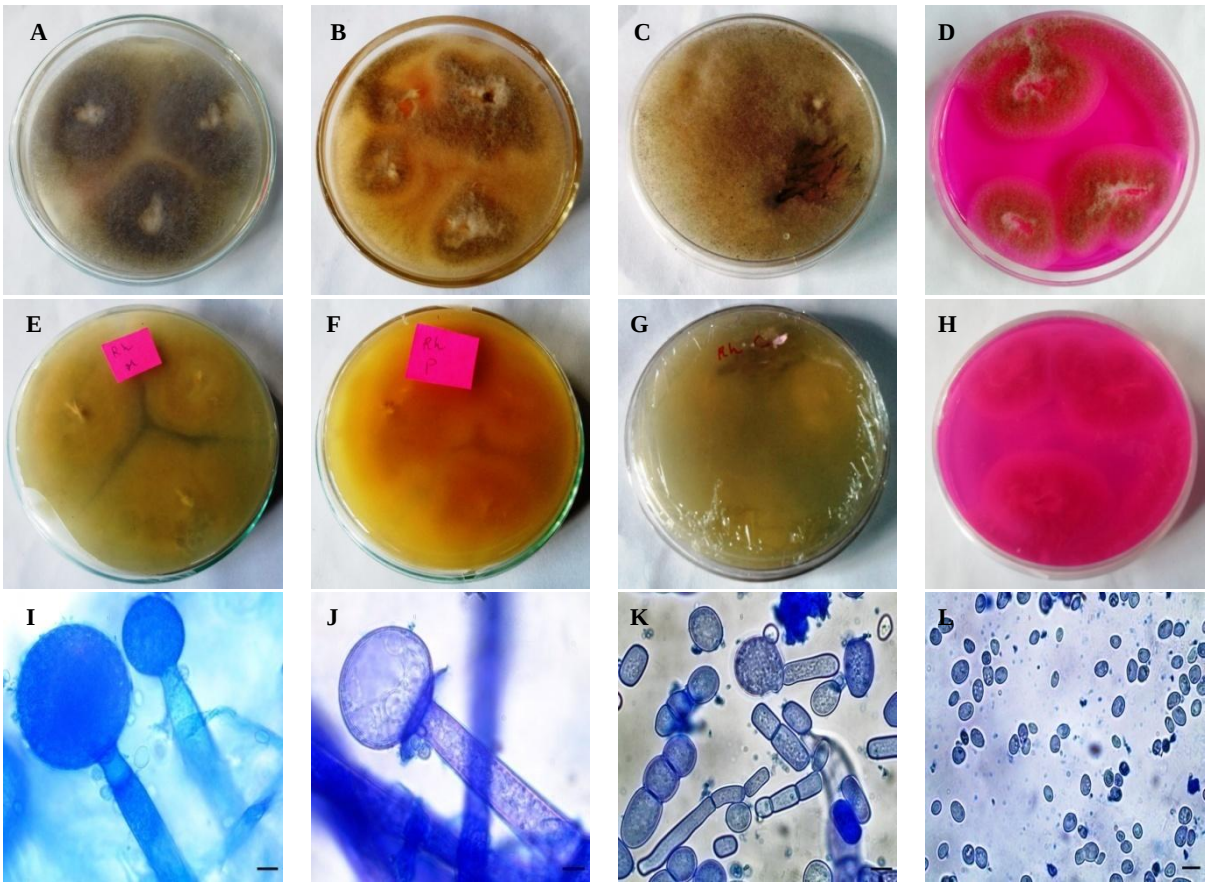

Fig.3.31. Culture and microscopic features of *Rhizopus microspores*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- sporangia and sporangiophore under 100X, J- columellae with collar under 100X, K- chlamydospores under 100X, L- sporangiospores under 100X. Scale bars represent 10µm

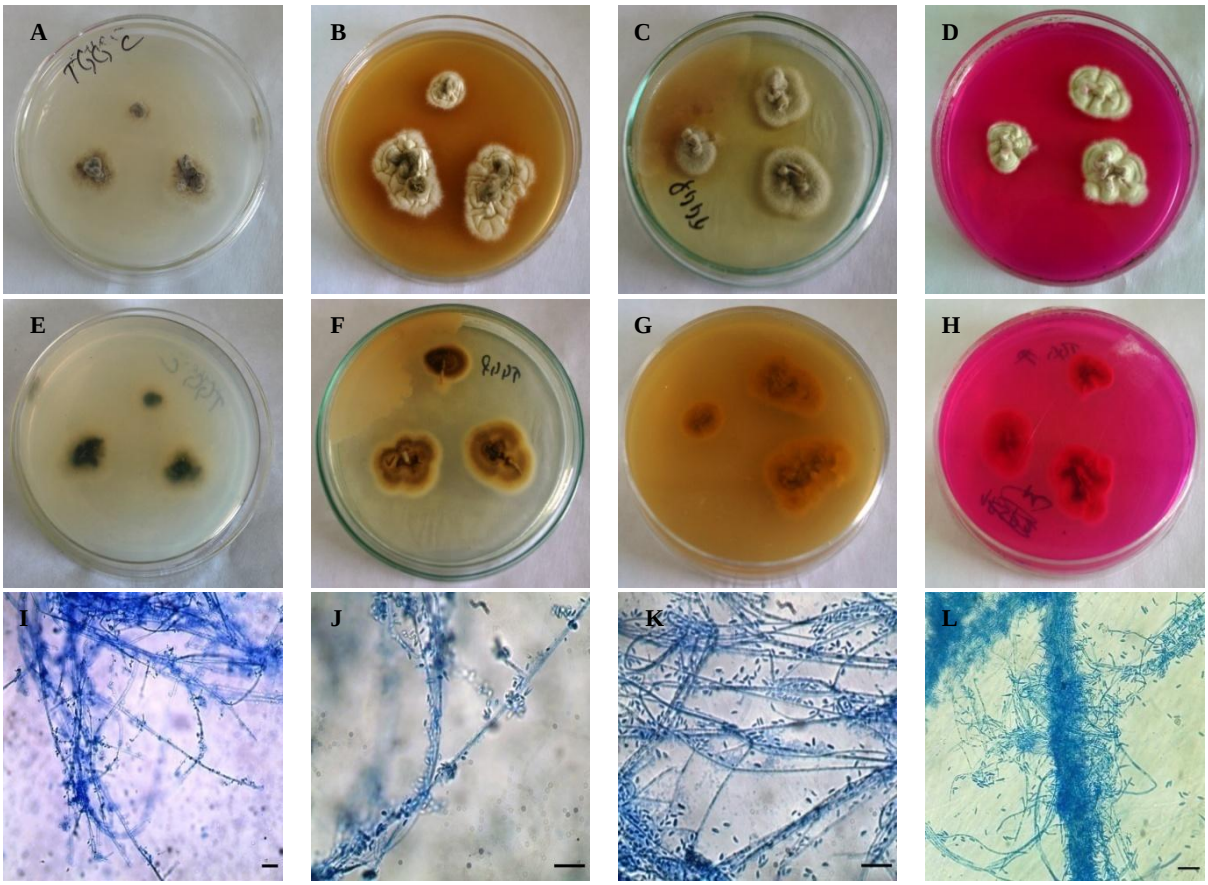

Fig.3.32. Culture and microscopic features of *Sarocladium sp*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- hyphae and aerial conidiophores 40X, J- conidia arranged in slimy heads as observed under 100X, K-L- branched conidiophores with conidia under 100X. Scale bars represent 10µm

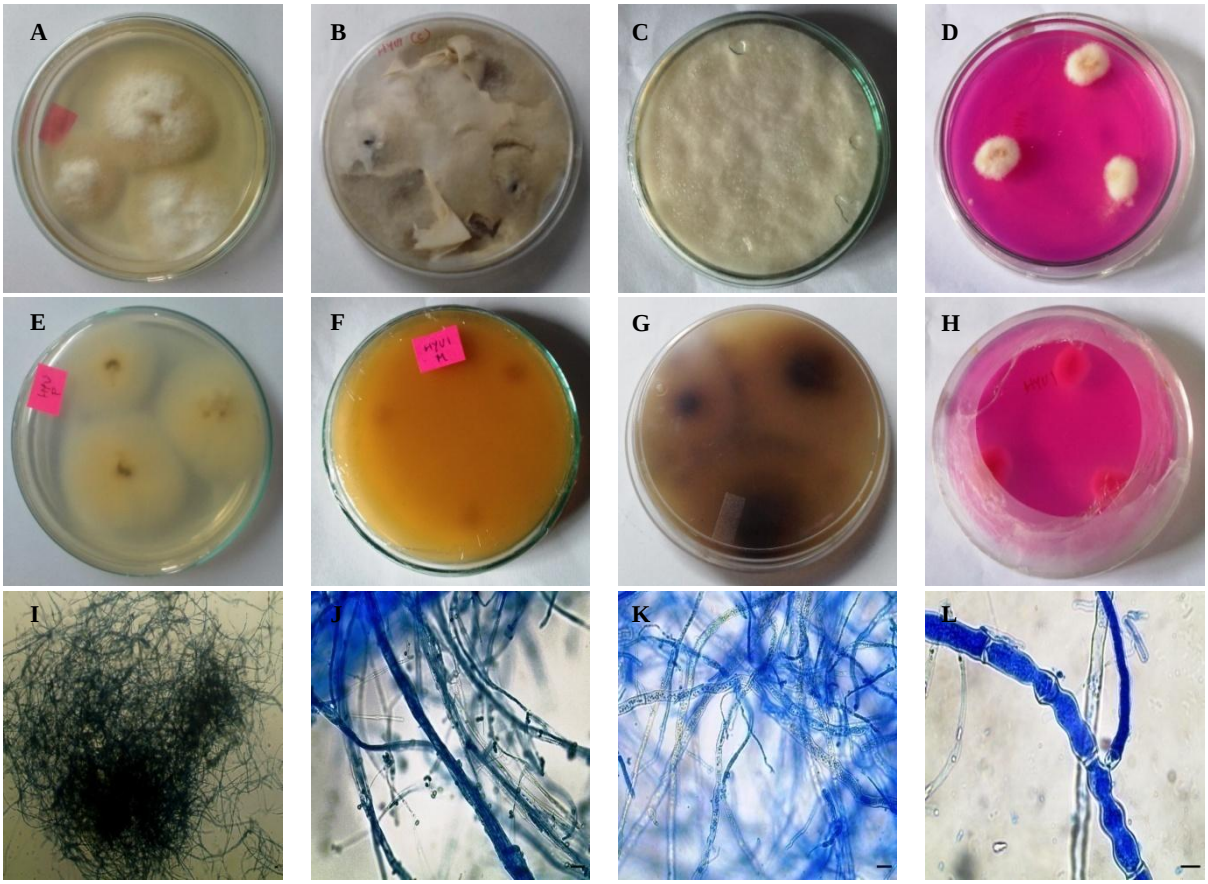

Fig.3.33. Culture and microscopic features of *Scytalidium sp* .A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- hyphae under 10X, J-K- septate hyphae and arthroconidiaunder 40X, L- hyphae and arthroconidiaunder 100X. Scale bars represent 10µm

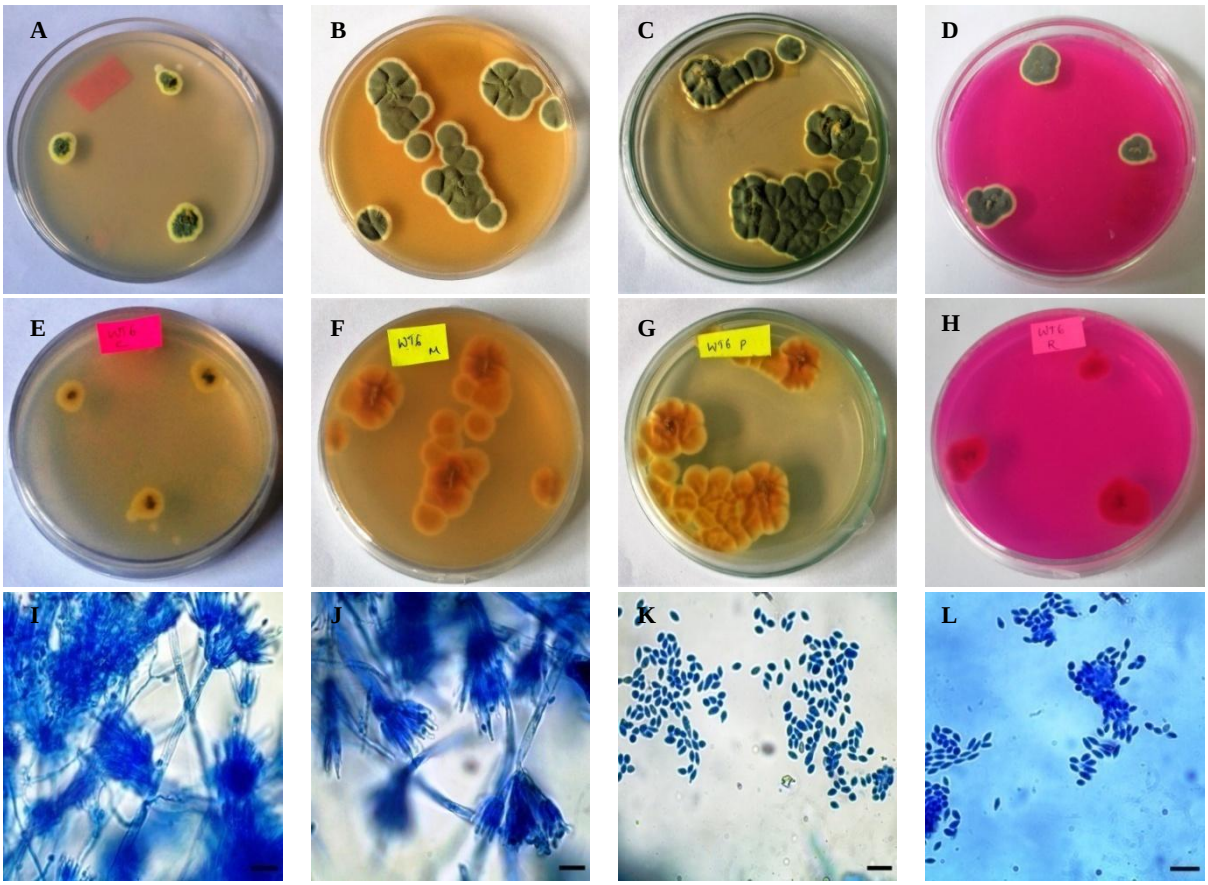

Fig.3.34. Culture and microscopic features of *Taloromyces* sp.A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-J- conidiophores with phialides and conidia, K-L- conidiaunder 100X. Scale bars represent 10µm

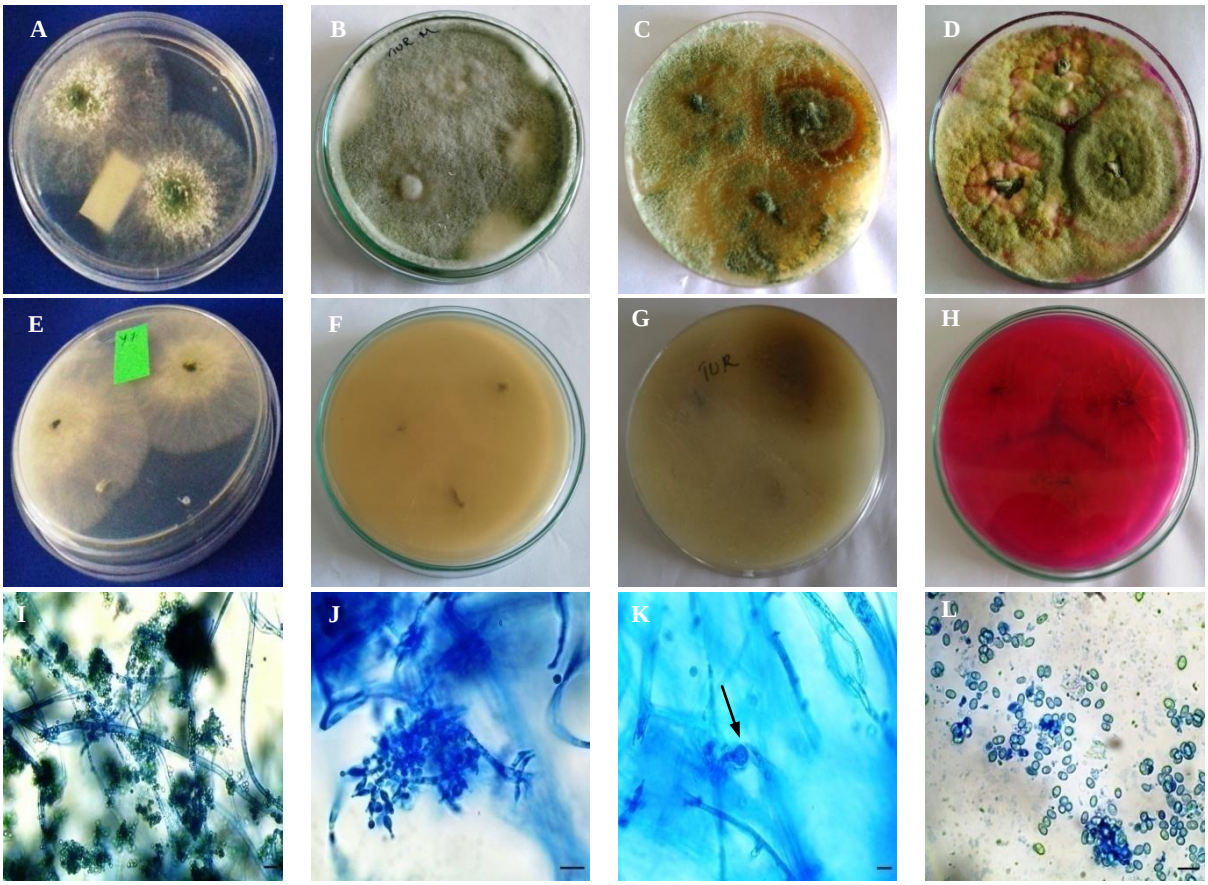

Fig.3.35. Culture and microscopic features of *Trichoderma hamatum*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-J- conidiophores with phialides and conidia under 40X and 100X, K- chlamydospore (arrow) under 40X, L- conidia under 100X. Scale bars represent 10µm

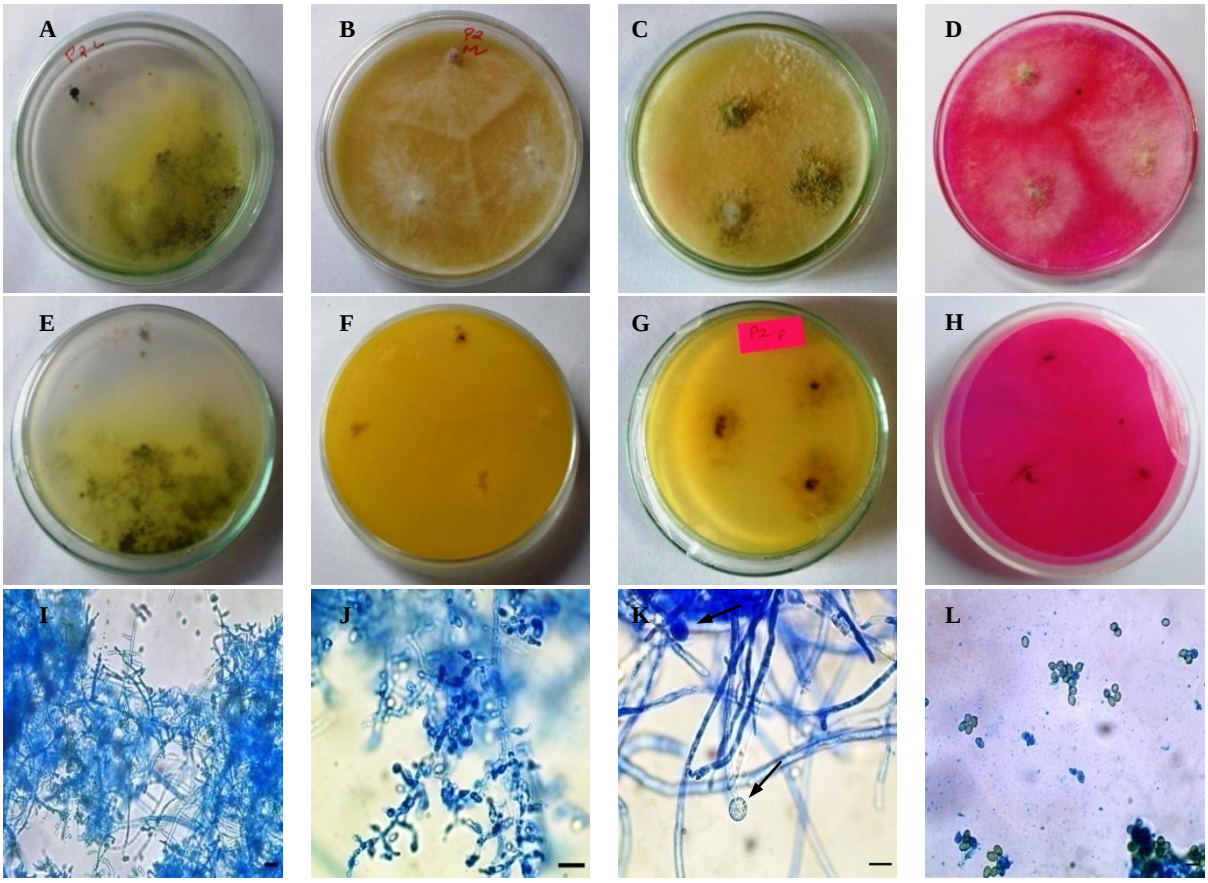

Fig.3.36. Culture and microscopic features of *Trichoderma longibrachiatum*. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I-J- conidiophores with phialides and conidia under 40X and 100X, K-chlamydospore (arrow)under 100X, L- conidia under 40X. Scale bars represent 10µm

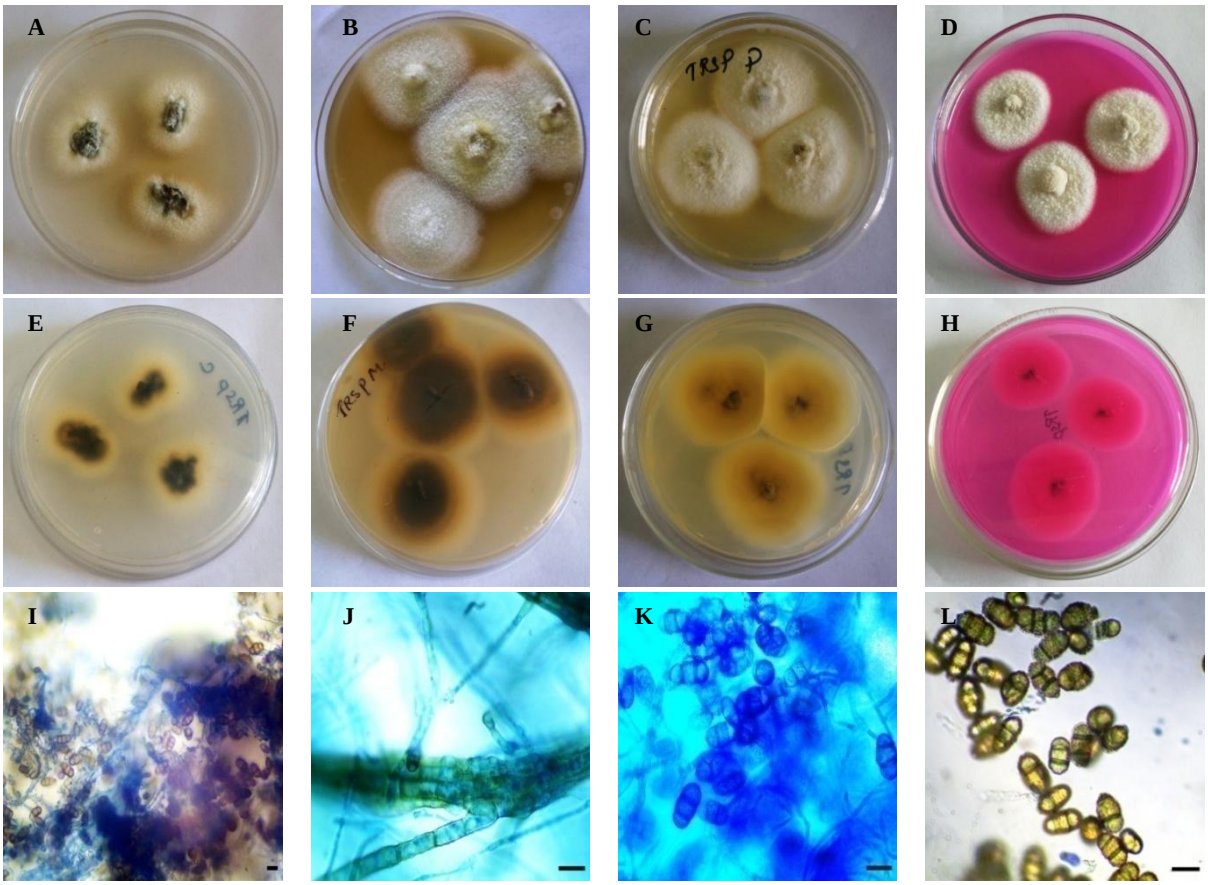

Fig.3.37. Culture and microscopic features of *Ulocladium* sp. A-D and E-H- Obverse and reverse view on CZA, MEA, PDA and RBA plates, I- hyphae and conidia under 40X, J-conidiogenous cells under 100X, K-L- conidia under 100X. Scale bars represent 10µm

Table 3.13. Fungal isolates at the Tuli and Ungma tea garden in different seasons during 2016 - 2018

Fungal isolates	Tuli				Ungma			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Acremonium sp.1	R1,NR1	R1,NR1	ND	ND	ND	ND	ND	ND
Acremonium sp.2	R1,NR1	R1, NR1	ND	ND	ND	ND	ND	ND
Arthrinium kogelbergense	R2, NR2	ND	ND	ND	ND	ND	ND	ND
Apophysomycesviriabilis	R1,R2	ND	R1,R2	ND	R1	ND	ND	ND
Aspergillus flavus	R1,NR1	ND	ND	R1,NR1	R2,NR2	ND	ND	ND
A. heteromorphus	ND	R1,NR1	R1,NR1	ND	ND	ND	ND	ND
A.niger	R1,NR1, R2,NR2	ND	ND	R1,NR, R2,NR2	ND	R2,NR2	R1,NR1, R2,NR2	R1,NR1, R2,NR2
A.ochraceus	R2, NR2	ND	ND	ND	ND	ND	ND	ND
A. versicolor	ND	ND	ND	ND	R1,NR1, R2,NR2	ND	R1,NR1	R1,NR1
Aspergillus sp.1	ND	ND	ND	ND	R1, NR1	ND	ND	R1, NR1
Aspergillus sp.2	ND	R2,NR2	ND	ND	R2, NR2	R2, NR2	ND	ND
Aspergillus sp.3	ND	ND	R2, NR2	R2,NR2	ND	ND	ND	R2
Aspergillus sp.4	R1,NR1	ND	ND	ND	ND	ND	ND	ND
Aspergillus sp.5	ND	ND	R1,NR1	R1,NR1	R1, NR1	R1,NR1	ND	ND
Aspergillus sp.6	ND	ND	R2,NR2	R1,NR1	R2, NR2	ND	ND	ND
Botrytis sp.	ND	ND	ND	ND	ND	R2,NR2	ND	ND
Chaetomium globosum	ND	ND	R1,NR1	ND	ND	R2,NR2	ND	ND
Chaetomium sp.1	ND	R1,NR1	ND	ND	ND	ND	ND	ND
Chaetomium sp.2	ND	R1,NR1	ND	R1,NR1	ND	R2, NR2	ND	ND
*Chrysosporium*sp.	R2,NR2	R2,NR2	R2, NR2	ND	ND	ND	ND	ND
Cladosporium cladosporiodes	R2,NR2	R2,NR2	ND	ND	R2,NR2	ND	R1,NR1, R2,NR2	ND
Cladosporium herbarum	ND	R1, NR1	R1, NR1	ND	R1,R2	R1	ND	ND
Cladosporium oxysporum	R2,NR2	R2, NR2	ND	ND	ND	R2, NR2	R2,NR2	ND
Cladosporium	R1,NR1	R1,NR1	ND	ND	R1,NR1	ND	ND	ND

sp.1								
Cladosporium sp.2	ND	ND	R1,NR1	ND	ND	NR2	ND	ND
*Clonostachys*sp.1	R1,NR1	ND	ND	ND	ND	ND	ND	ND
Clonostachys sp.2	ND	ND	ND	R1,NR1	ND	ND	ND	ND
Colletotrichum sp.	R1,NR1, R2,NR2	ND	R1,NR1, R2,NR2	ND	R1,NR1, R2,NR2	R1,NR1, R2,NR2	ND	ND
Cunninghamella echinulata	ND	ND	ND	R1,NR, R2,NR2	R2,NR2	ND	ND	R1,NR1, R2,NR2
Emmonsia sp.	R2,NR2	ND	ND	ND	ND	ND	ND	ND
Exophiala sp.	R1,NR1	ND	R1,NR1	ND	ND	ND	ND	ND
Fusarium oxysporum	ND	ND	ND	ND	R1,NR1, R2,NR2	R1,NR1, R2,NR2	R1,NR1, R2,NR2	ND
Fusarium solani	ND	R1,NR1, R2, NR2	R1,R2, NR2	ND	ND	ND	ND	ND
Fusarium proliferatum	R1	ND	R1,NR1	R1,NR1	R1,NR1	R1,NR1	ND	ND
Fusarium sp.	ND	ND	ND	R1	ND	ND	ND	ND
*Geosmithia*sp.	ND	ND	ND	ND	R2,NR2	ND	R2,NR2	ND
Geotrichum candidum	R1,NR1	ND	ND	ND	R1,NR1	ND	ND	ND
Geotrichum sp.	ND	ND	ND	ND	ND	R1,NR1	ND	ND
Lichtheimia sp.1	ND	R1,NR1	ND	ND	ND	ND	ND	ND
Lichtheimia sp.2	ND	R1,NR1	ND	ND	ND	ND	ND	ND
Microascus sp.	ND	ND	ND	ND	R1	R1	ND	ND
Mucor circinelloides	R2,NR2	ND	ND	R2,NR2	R2,NR2	ND	ND	ND
Mucor hiemalis	R1,NR1	ND	ND	R1,NR, R2,NR2	ND	ND	ND	ND
Mucor sp.	ND	ND	ND	ND	R1,NR1	R1,NR1	ND	ND
Nigrospora osmanthi	R1,NR1	ND	R1,NR1	ND	ND	ND	ND	ND
Nigrospora sp.1	R1,NR1	ND	ND	ND	ND	ND	ND	ND

Nigrospora sp.2	ND	R1,NR1	ND	ND	ND	R1,NR1	ND	ND
*Paecilomyces*sp. 1	ND	ND	ND	ND	R2,NR2	ND	ND	ND
*Paecilomyces*sp. 2	R2,NR2	ND	R1,NR1	R2, NR2	ND	ND	ND	R1,NR1
Periconia byssoides	R1,NR1, R2,NR2	ND	ND	ND	ND	ND	ND	ND
Penicillium citrinum	ND	R2	R2	ND	ND	R1,R2	ND	ND
P. commune	R2,NR2	ND	ND	NR2	ND	ND	ND	ND
P. italicum	R1	R1	R1	R1	ND	ND	R1	ND
P.palitans	ND	R1,NR1	ND	ND	ND	ND	ND	ND
P. waksmanii	R2, NR2	R2,NR2	ND	R2,NR2	R1,NR1, R2,NR2	ND	R2,NR2	R2,NR2
Penicillium sp.1	ND	ND	ND	ND	NR2	ND	NR2	ND
Penicillium sp.2	NR2	ND	R2,NR2	ND	ND	ND	ND	ND
Penicillium sp.3	ND	ND	ND	ND	ND	R1,NR1	ND	R1,NR1
Penicillium sp.4	R1,NR1	ND	ND	R1	ND	ND	ND	ND
Pestalotiopsis egyptiaca	ND	R2	R2	ND	ND	ND	R2,NR2	ND
Pestalotiopsis sp.	ND	ND	R2,NR2	ND	ND	R2	R2,NR2	ND
Phoma sp.1	ND	ND	ND	ND	R1,NR1	ND	R1,NR1	ND
Phoma sp.2	R1	ND	R1,NR1	ND	ND	ND	ND	ND
Phomopsis sp.	ND	ND	ND	ND	R1	R1	R1	ND
Rhizopus microspores	ND	NR1	NR1	ND	ND	ND	ND	ND
Rhizopus stolonifer	ND	ND	ND	R1,NR1	R2,NR2	ND	ND	R2,NR2
Sarocladium gamsii	R1	ND	ND	ND	ND	ND	ND	ND
Scedosporium sp.	ND	ND	ND	ND	ND	R1,NR1	ND	ND
Sclerotinia sp.	ND	R1,NR1, NR2	ND	ND	ND	ND	ND	ND
Scytalidium sp.	R1,R2	R1,R2	ND	ND	R1	ND	ND	ND
Taloromyces sp.	ND	ND	ND	ND	ND	R1,NR1	R1,NR1	ND

Trichoderma hamatum	R2, NR2	ND	R1,NR1, R2,NR2	ND	ND	ND	ND	ND
Trichoderma harzianum	R1,NR1, R2,NR2	NR1,R2	ND	R1,NR, R2	ND	ND	R1,NR1, R2,NR2	R1,NR1, R2,NR2
Trichoderma koningii	R2	R1,R2, NR2	ND	ND	R2	ND	ND	ND
Trichoderma longibrachiatum	ND	R1	R1	ND	ND	ND	ND	ND
Trichoderma viride	ND	R2,NR2	ND	ND	R1,NR1	ND	R1,NR1	ND
Trichoderma sp.1	ND	R2,NR2	ND	R2,NR2	ND	ND	ND	ND
Trichoderma sp.2	R2	ND	ND	ND	ND	ND	ND	ND
Trichoderma sp.3	ND	ND	ND	ND	ND	ND	ND	R2,NR2
Trichoderma sp.4	NR1	NR1	ND	ND	ND	ND	ND	ND
Trichophyton rubrum	ND	ND	ND	ND	R1,NR1	ND	ND	ND
Trichophyton sp.1	ND	ND	ND	ND	ND	ND	R2,NR2	ND
Ulocladium sp.	R1,NR1	R1,NR1	ND	ND	R1,NR1	R1,NR1	ND	ND

R1, R2, NR1 and NR2 indicates rhizospheric and non-rhizospheric soil fungal isolates in the first and second sampling year respectively; ND- not detected

3.3. Discussions

The understanding of the seasonal distribution of bacteria is a prerequisite in tea soil because soil bacteria is one of the most sensitive organisms to seasonal fluctuations and variation in their population and activity in response to seasonal fluctuation can have an adverse effect to plant productivity. Seasonality on the bacterial population in the form of CFU was evident from the study, which agrees with other studies (Buyer and Kaufman, 1997). Soil bacterial counts showed a steady increase from spring to summer and then there was a sharp decline in autumn, which further declined in winter in the tea gardensstudied. This suggests that the conditions in summer were more favourable to this microbial group. Buyer and Kaufman (1997) also reported a similar result with the highest bacterial counts in July as compared to other months. At both the tea gardens studied, soil temperature was higher in the summer season therefore, an increase in soil temperature might have accelerated the nutrient mineralization and the decomposition of litter and soil organic matter, thereby, providing a more favourable condition for the bacteria. Contrary to this, the bacterial counts at both the tea gardens were lowest during the winter seasons, which coincides with limited moisture, low *p*H and low-temperature period of the year. Therefore, the decrease in temperature, moisture and *p*H may be responsible for the lowest bacterial counts in the winter season. Similarly, Pandey et al., (2001) also mentioned that low soil *p*H was one of the possible reasons for the inhibition of the bacterial population in the rhizosphere of established tea bushes.

It was also observed that the bacterial counts differed between the tea gardens and there wasa statistically significant effect of site and season interaction ($p < 0.05$) on the bacterial count (Table 3.3). Furthermore, the correlation of bacterial population with soil properties revealed that soil factors accounted for a significant amount of variability in soil bacterial composition in the study areas. However, the correlation of bacterial population with these properties and the level of correlation differed between the tea gardens as well as the sampling years. As indicated in Table 3.4, almost all the soil properties showed a significant positive correlation ($p < 0.01$ and $p < 0.05$) at Tuli tea garden however, an uneven or negative correlation was established with certain soil parameters such as SOC, available nutrients and sandat Ungma tea garden. Furthermore, the correlation of bacterial counts and soil properties differed between sampling years in each tea garden. These differences in the correlation of bacterial counts with soil properties between the tea gardens and sampling

years indicate the influenced of geographical location as well as sampling years mediated differences in soil properties bacterial populations.

The study highlighted in this section also discusses the identification of culture-dependent tea soil bacteria with reliance on conventional microbiological methods and molecular approaches. Nowadays culture-independent methods are being used for bacterial studies however culturable bacterial studies are of paramount importance to understand the relationship between soil properties and bacterial population. Culture methods might be more sensitive to retrieve changes in the microbial community because the culturable fraction of the microbial community might react more rapidly to changes in biotic and abiotic factors (Smit et al., 2001; Franca et al., 2016). Furthermore, culture-dependent studies of bacteria significantly contributed to our understanding of live bacteria. The phenotypic characterization involving morphological and biochemical method is a traditional and commonly used method of culture-dependent bacterial characterization. The bacterial isolates were studied for their morphological and biochemical characteristics to obtain a preliminary idea regarding the probable strain of bacteria that possess the ability to thrive in the studied tea gardens. Phenotypic characterization revealed bacteria differing in their culture morphologies, gram characters, motility and biochemical characters. For instance, in gram characters, both gram-positive and gram-negative bacteria, rod-shaped, cocci-shaped and gram variable bacteria were recorded.Agreater number of gram-positive and rod-shaped bacteria was recorded as compared with other bacteria suggesting that gram-positive and rod-shaped bacteria can adapt better to environmental fluctuation. Based on their phenotypic characters, the highest number of isolate belonged to the genus *Bacillus* (59 isolates) followed by *Pseudomonas* (20 isolates) while genera with the lowest number of isolate (each with only 1 isolate) were *Chryseobacterium*, *Providencia* and *Romboutsia*.

The phenotypic characterization was supplemented by the use of genotypic analysis in this study to validate the result. In this study, the 16S *r*RNA and BLAST analysis were employed to assess the molecular identity of the tea garden soil bacteria. The closest affiliation of a new isolate or molecular sequence was assigned by evaluating the phylogenetic relatedness to known bacteria based on the homology of 16S *r*RNA sequences. The 16S *r*RNA is the most widely employed genotypic analysis because of it'shighly conserved nature. The sequence of 16S *r*RNA gene being universal in bacteria measures relationships among all bacteria and is an excellent and extensively used choice for

identifying an unknown organism based on no prior knowledge (Clarridge, 2004). It can also mark the evolutionary distance and relatedness of organisms. Out of 128 bacterial isolates, 35 isolates showed sequence similarity <98 % (Table 3.7) which indicated that presence of unknown or previously unexplored bacterial community in tea gardens of Mokokchung.

The results from the phenotypic method and corresponding molecular characterization for bacterial identification revealed isolates under genera *Aeromonas*, *Acinetobacter*, *Bacillus*, *Brevibacillus*, [*Brevibacterium*], *Burkholderia*, *Chryseobacterium*, *Comamonas*, *Enterobacter*, *Lysinibacillus*, *Paenibacillus*, *Providencia*, *Pseudomonas*, *Raoultella*, *Romboutsia*, *Serratia*, *Shigella*, *Staphylococcus*and *Stenotrophomonas*depicting the diverse and complex soil bacterial community in the studied tea gardens.The presence of one or more of these bacterial genera can be seen in other tea garden soils, for instance, *Bacillus*, *Burkholderia*, *Enterobacter* and *Pseudomonas* were reported from different tea estates of Assam in India (Dutta et al., 2015) while in another tea soil *Aeromonas*, *Bacillus*, *Enterobacter*, *Lysinibacillus*, *Brevibacillus*, *Paenibacillus*, *Pseudomonas* and *Staphylococcus* were recorded (Goswami et al., 2017).The highest number of isolates recorded in the present study were under the genus *Bacillus* followed by *Pseudomonas* which agrees with others who worked in different tea growing soils of the world (Pandey and Palni 1997; Pandey and Palni, 2001; Goswami et al., 2017; Varmazyari and Cakmakci, 2018; Cakmakci, 2019). The maximum number ofisolates under these two genera (*Bacillus* and *Pseudomonas*) was because *Bacillus* and *Pseudomonas* are common soil-inhabiting bacteria however, in the studied tea garden soils species of*Bacillus* probably adapted better for survival as compared to *Pseudomonas*. The dominance of *Bacillus* in the present study might be due to their antifungal activity and tolerance of stress under unfavourable environmental condition (Pandey and Palni 1997), a*bility to form*endospores within cells that provide high resistance to survive under adverse conditions for an extended at dormant stage (Garbeva et al., 2003). This could also be because of their ubiquitous nature, ability to adapt to fluctuations in soil and environmental conditions and the innate ability to produce various enzymes that inhibit or antagonize the growth and multiplication of other bacteria.

Seasonal identification of bacterial isolates revealed the highest number in the summer season at Ungma tea garden and spring seasons at Tuli tea garden which is an indication of differences in favourable conditions for bacterial multiplication between the two tea gardens. The lowest number of bacterial isolates, however, was recorded in winter

seasons at both the tea gardens with the majority of isolates under *Bacillus*. This result corroborates the findings of Pandey and Palni, (2001) whoreported that most tea soil bacterial species fail to grow during the winter months due to low soil temperature. The authors were able to isolate only a couple of *Bacillus*species on agar plates.

Differences in bacterial isolates were also observed between the two tea gardens with a higher number as well genera of isolates recorded from the Tuli tea garden as compared with the Ungmatea garden which indicates that the conditions at lower altitude were more suitable for bacteria. It could also be attributed to the presence of lessantagonist andmore resource availability along with one or more environmental factors in the Tuli tea garden. Additionally, the present result suggests the importance of altitudes, soil types and other soil properties, chemical nutrient elements and management practices for the type and number ofbacterial isolates.

Among the bacterial genera, *Bacillus* occurred consistently throughout the sampling seasonsat both the tea gardens which suggest that thisbacterial genus did not suffer much from seasonal fluctuations. It also indicates the non-selective nature of the tea garden soil for this genus. Contrary to this, some genera restricted themselves to a specific season which suggeststhehigh susceptibility of these generatowardsseasonal fluctuations in their growth conditions as well as the selective nature of the tea garden soil for these bacterial genera. This selective nature may be attributed to plant-microbe interaction, microbe-microbe interaction, and environmental factors (Pandey et al., 1997; Renla and T, 2017) and other soil factors such as soil acidity which exerts selective pressure on tea soil microbial diversity (Goswami et al., 2017).

Soil fungi are an extensive range of organisms that may be actively free growing, closely associated with other organisms or can even be dormant (Bridge and Spooner, 2001). This group of microscopic organisms are fundamental for nutrient cycling and various types of biogeochemical cycling. Soil fungi also contribute significantly to the agricultural system through their diverse roles including plant root diseases suppression, enhances plant growth and health by producing many vitamins and by attacking plants pathogens with fungal enzymes and antagonize competitors by producing antibodies (Raja et al., 2017). The dynamic nature of the soil or any other environmental parameters can greatly influence soil fungal population and activities which can subsequently affect the above-ground plants. Therefore, periodic or seasonal studies on the fungal population are necessary from an agricultural perspective. In the present study, fungal populations at two tea gardens were estimated seasonally using the colony counting method. Although a range of alternative high-throughput gained popularity in recent years, quantitative population analysis of microbes using colony counting is still a widely applied methodology (Sieuwerts et al., 2008).

As presented in Table 3.10, seasonal variations on fungal counts at both the tea gardens were statistically significant ($p< 0.05$) which indicates that seasonality is one factor that affects the fungal community structure (Seephueak et al., 2010). Several other workers have also reported seasonality on fungal counts in different soils (Rao, 1970; Kaushal and Singh, 2013; Abdel-Sater et al., 2016). Such seasonal variations in fungal populations occur as a result of changes in factors such as micro and macronutrients, water holding capacity, temperature and *p*H (Banakar et al., 2012). Among the seasons, the fungal counts were significantly higher in the spring seasons at both the tea gardens. Bhattacharyya and Jha (2011) also observed that the fungal population flourished greater in the spring season as compared with other seasons. Similarly,Shigyo et al., (2019) reported that fungal communities were higher in April than in other months. Higher fungal counts in spring seasons as observed at the studied tea gardens might be due to favourable conditions such as favourable soil moisture and temperature that accounted for greater fungal decomposition rate and proliferation. Furthermore, the correlation studies (Table 3.10) showed that the fungal counts were positively correlated with one or more soil nutrients. Therefore, an increase in the soil nutrient budget in the spring due to higher soil macronutrients might also have resulted in a greater number of fungal counts. Saravanakumar and Kaviyarasan (2010)

observed a marked decrease in the number of fungal colonies with decreasing soil micro and macronutrients. Similarly, Bhattacharyya and Jha, (2011) pointed out that seasonal variations of parameters such as soil *p*H, moisture, organic carbon, total Nitrogen and available K were the affective factors associated with variations in the surface and subsurface fungal population. The lowest fungal count recorded during winters in both the tea gardens during the sampling years corroborates the findings of other workers (Pandey and Palni 1996; Bhattacharyya and Jha 2011). Contrary tothis, Rao (1970) observed the lowest fungal count in summer. The lowest fungal counts observed in the present study could be due to unfavourable temperatures and limitations of natural soil moisture that hampered the fungal growth and multiplication. In agreement with this, Ilhan and Asan, (2001) mentioned that the low temperature and rainfall were the main reason for the lowest number of soil fungi in the winter season.

At both the tea gardens, fungal counts were slightly higher in the rhizospheric soil as compared with the non-rhizospheric soil. Pandey and Palni (1996) also reported differences in fungal counts between rhizosphere and non-rhizosphere of tea soils despite the differences being statistically non-significant. The higher fungal counts in the rhizospheric soil are because the rhizosphere contains many organic substrates that harbour high microbial count, especially fungi (Noveriza and Quimio, 2004). The rhizosphere area of soil has intense fungal and other microbial activity where the fungi help the plant root in nutrient uptake and return, are benefitted through nutrient release by the plant. The energy for the development of an active fungal population in the rhizosphere is provided through the loss of organic materials from roots (Noveriza and Quimio, 2004). The result from the present investigation showed consistency in the observations of others who worked in different types of soil (Pandey and Palni, 2007).

There were marked variations in the fungal counts between the two tea gardens indicating the altitudinal effect for soil fungal growth and multiplication. This could also be due to differences in plant species and other factors specific to each tea garden.A significant variation in the total number of fungal colonies isolated in soil samples from various sites was observed in another study (Noveriza and Quimio, 2004). Between the studied tea gardens, the highest fungal counts were recorded in the Tuli tea garden indicating that the conditions necessary for the fungal growth and multiplication were more favourable at the lower altitude than that of the higher altitude. Similarly, Pandey and Palni (2007) also

observed that the microbial population including fungi decreased with a concomitant increase in altitude.

Apart from establishing seasonality and site differences in fungal counts, differences in the types and number of fungal isolates were also observed in the present study. This was revealed by their growth and morphologies in different culture plates and microscopic observations. The identifications of fungal isolates and study on its variations between seasons or sites is a very fascinating subject that aid and expand knowledge on indigenous fungi and their various roles. Despite common improvement and rapid availability of molecular methods, traditional methods involving microscopy and culture plate are still the commonly used and essential tools for fungal identification (Bandh et al., 2011). This is because there are serious limitations associated with molecular methods for fungal studies such as lack of discrimination in the technique between living and dead material, or active and dormant organisms, relatively small amount of reference data available for comparison of sequence data that create uncertainty surrounding some fungal species concepts (Bridge and Spooner, 2001). The present study revealed that the seasonal differences brought about notable changes in the number and types of fungi. Irrespective of the tea gardens, the number of fungal isolates was more in the spring seasons throughout the sampling seasons. Thus, conditions in the spring season at the studied tea gardens seem to be the best for its fungal growth, development and activities. At both the tea gardens, the number and types of fungi were more at the rhizospheric soils in almost all the sampling seasons which indicate that the rhizospheric fungal species are more diverse and they seem to benefit from different types of exudates released from tea roots. The present result more or less agrees with Pandey and Palni (2001) who observed variations in the number and types of fungal species between the rhizospheric and non-rhizospheric soil.

Despite differences in fungal types and numbers, some fungal genera were constant throughout the sampling seasons. *Aspergillus*, *Penicillium* and *Trichoderma* were constant in the Tuli tea garden throughout the sampling seasons whereas, in the Ungma tea garden *Aspergillus* and *Penicillium* were constant throughout the sampling seasons. Furthermore, the highest number of isolates were also recorded under these three genera thereby, showing their versatile nature and depicting a clear picture that, species of these genera can adapt easily to a different environment or seasonal fluctuations. Moreover, in tea soil, fungi that can adapt better to environmental changes are expected to dominate other species because tea

plantation includes monoculture practice with more or less the same cultivation practices for years. Somewhat similar to the present study, *Pandey et al., (2001) also reported dominancy of Penicillium* and *Trichoderma* in the tea rhizospheric and non-rhizospheric soil from various tea-growing locations in India. The dominance of *Aspergillus, Penicillium* and *Trichoderma* in the present study may be due to their ability to utilize different substrates in the soil more readily over other species (Gomez et al., 2006), a greater rate of spore production and dispersal and partly due to their resistance to extreme environmental conditions (Banakar et al., 2012). It could also be ascribed to the antagonistic activity exhibited by these genera against other fungi. It is noteworthy that some fungal genera some were restricted to a particular season in each of the tea gardens indicating the sensitivity of fungal genera towards changes brought through seasonal influence on soil parameters. It can also be due to the selectivity of fungi over time by tea soil owing to its monoculture plantation. Thus, the tea garden soil fungi belong to a restricted range of taxonomic groups with a few dominant and some rare species (Karaoglu and Ulker, 2006).

Differences in the number and types of fungi were also observed between the tea gardens indicating the influence of altitude, soil type, geographical locations and climatic conditions of the tea garden. Additionally, the composition of the soil fungi can be greatly influenced by the plant species growing on the soil (Mishra and Sharma, 1977). Therefore, it could be speculated that the major influencing factor to soil fungi was probably different in each of the tea gardens.When the comparison was made between the fungal genera from both the tea gardens, the majority of the fungal genera were common to both the tea gardens while species of some genera were site-specific. Species under the genera *Apophysomyces, Aspergillus, Chaetomium, Cladosporium, Colletotrichum, Cunninghamella, Fusarium, Geotrichum, Mucor, Nigrospora, Paecilomyces, Penicillium, Pestalotiopsis, Phoma, Rhizopus, Scytalidium, Taloromyces, Trichoderma* and *Ulocladium* were common to both the tea gardens whereas, *Acremonium, Arthrinium, Botrytis, Chrysosporium,Clonostachys, Emmonsia, Exophiala, Geosmithia, Lichtheimia, Microascus, Periconia, Phomopsis, Sarocladium* and *Sclerotinia* showed their restricted distributions to a particular tea garden. Rao (1970) also observed that 39 out of 101 fungal species isolated from different soils were common to all soils while some fungi were restricted in their distribution. Similarly, Abdel-Sater et al., (2016) recovered 74 genera and 208 species of fungi from two plantation soils in

the Assiut region of Egypt and observed many fungal species common to both sites and only some species were specific to a particular soil.

3.5. Conclusion

The altitude and seasonal influence mediated change in soil physicochemical properties accounted for a significant amount of variability in bacterial and fungal population in the tea gardens studied. In both tea gardens, the CFU of soil bacteria were significantly higher in the summer seasons whereas, the CFU of soil fungi were during the spring seasons. This indicates that the conditions in summer seasons favoured bacterial growth and multiplication while the fungal growth and multiplication are positively influenced by conditions in spring seasons in both tea gardens. The lowest CFU of both fungi and bacteria were recorded in the winter seasons at both tea gardens mainly due to a decrease in soil physicochemical properties such as temperature, moisture and *p*H.

The bacterial and fungal CFU and isolates differed between tea gardens. A significant effect ($p < 0.01$) of tea gardens and seasons interaction for both fungi and bacteria was also established. This suggest that the differences in the growth conditions especially in soil physicochemical properties between altitudes can tremendously affect the soil microflora.

The correlation of soil properties with bacteria and fungi differed significantly ($p < 0.01$ and $p < 0.05$) between tea gardens.In both years, the correlation between bacterial CFU and moisture, *p*H, temperature, SOC, available N and available P were significantly positive at the Tuli tea garden whereas, in Ungma tea garden correlation was significantly positive between bacterial CFU andsilt, *p*H, moisture and temperature. The correlations were significantly positive between fungal CFU and SOC and available P, and between fungal CFU and SOC for both years at the Tuli and Ungma tea garden respectively. Furthermore, the correlation of both bacterial and fungal CFU in Tuli and Ungma tea garden with corresponding soil properties revealed an uneven relationship between two years.

Among the bacterial genera, *Bacillus* and *Pseudomonas* dominated the tea garden soils indicating the non-selective nature of the tea garden soil for this genera. Between these genera, *Bacillus* adapted better for survival as compared to *Pseudomonas* probably due to their antifungal activity and tolerance of stress under unfavourable environmental condition, ubiquitous nature, ability to adapt to fluctuations in soil and environmental conditions and ability to produce various enzymes that inhibit or antagonize the growth and multiplication of other bacteria. Among the fungi, *Aspergillus, Penicillium* and *Trichoderma*dominated the tea

soils probably due to their greater ability to utilize different substrates in the soil, antagonistic activity against other fungi, a greater rate of spore production and dispersal and resistance against extreme environmental conditions.

The present study on the *culturable bacteria and fungi*in tea growing areas of the Mokokchung district of Nagaland presented novel information with an invaluable foundation for evaluating diverse microflora in tea growing areas of Mokokchung district in Nagaland. An insight into the diverse bacterial and fungal genera and many isolates with potential new or previously unexplored strain inhabiting the Tuli and Ungma tea soils were observed. The importance of seasons, altitudes, soil types and other soil properties, chemical nutrient elements and management practices for the type and number of bacterial isolates were also indicated. Further experiments with these indigenous soil microflora are required for their role in plant growth promotion, biocontrol, antimicrobial, soil fertility, etc. for enhancing the tea plantation and tea industry in the Mokokchung district of Nagaland.

CHAPTER 4

ANALYSIS OF TEA LEAVES CHEMICAL COMPOSITIONS[3]

In this chapter, the concentrations of important leave constituents including total polyphenol, total flavonoid and tannin content were estimated in tea leaves collected from the Tuli and Ungma tea garden in the Mokokchung district, Nagaland. The analyses were carried out to observe variations in these chemical compositions among seasons. Comparisons in these chemical compositions were also carried out between tea gardens. Furthermore, the studied tea leaves constituents in each tea garden were correlated with corresponding soil physicochemical properties and population of soil microflora.

[3] A section of this chapter using one sampling sitefrom both tea gardens is already published as:

Jamir, T., Ajungla, T., 2020. Seasonal variations in antioxidant capacities and phenolic contents of tea leave extracts. Asian Journal of Pharmaceutical and Clinical Research 13(4), 108-112. doi:10.22159/ajpcr.2020.v13i4.36866.

4.1. Introduction

Tea is the most popular beverages and an important economic crop of the world. It is a plant with immense medicinal and therapeutic value. There are different types of processed tea leaves consumed in the world among which the most commonly available ones include green tea, black tea and oolong tea (Bizuayehu et al., 2006; Chan et al., 2011). Aroma, test and various positive physiological functions of tea leaves make it desirable for consumption (Hajimahmoodi et al., 2008). Additionally, tea consumption has numerous health benefits such as protective effect against many different types of cancer (Katiyar and Mukhtar, 1996; Bushman, 1998), reduction of neurodegenerative disease and ageing (Reznichenko et al., 2005), antimicrobial activities against human pathogens (Yam et al., 1997; Sajilata et al., 2008) and preventive effect of tea against cellular DNA damage (Akter et al., 2015). The important factors that determine the taste, flavour and health benefits of a specific type of tea are its various leave compositions (Hara et al., 1995).

Although the tea plant is being cultivated worldwide under different climatic and environmental conditions within a broad altitudinal range, however, the growth, development and yield of tea leaves can be influenced by differences in these factors. Since the chemical compositions in tea leaves and quality of resultant teas are directly related to the tea growth and yield performance (Owuor et al., 2010), therefore, any change in the tea growth and yield will ultimately affect the tea quality. This will also affect the health implications of the made tea. Studies have associated the variations in the chemical compositions of tea leaves with factors such as altitude (Owuor et al., 1990), climate, season, variety, horticultural practices and the age of the leaf (Owuor et al., 2008).

Tea polyphenols, flavonoids and tannins are some important tea leaves constituents responsible for the quality of resultant teas. Among these, tea polyphenols are the most abundant groups which account for 42% of the dry weight of the solids in green tea extract (Khan and Mukhtar, 2007; Sajilata et al., 2008). Therefore, evaluations of these tea leaves constituents are necessary for the quality control as well as potential health implications of manufactured tea products.

In the present study, the concentrations of important tea leave constituents including total flavonoid contents (TFCs), total polyphenol contents (TPCs) and tannin contents (TCs) were determined using metabolites including gallic acid, catechin and tannic acid to observe variations in these constituents among seasons and between altitudes. This will provide

valuable information on the quality of tea leaves cultivated in the Mokokchung district of Nagaland.

4.2. Materials and methods

4.2.1. Sampling

Details on tea gardens and sampling frequency have already been provided in Chapter 2. Tea leaves sampling was carried out by manually plucking two leaves and a bud on the same day of soil collection. In each tea garden, tea leaves were collected from six sites to include leaves of the tea plants that are growing in or near to the area from where the soil sampling was done. Tea leaves were transferred to the laboratory under sterile condition. Tea leaves were kept at room temperature overnight, hand-rolled for 20 minutes, cut into pieces and allowed to dry in shade at room temperature. Shade-dried leaves samples were grounded to a fine powder and stored in an airtight sterile container for analyses. All analyses discussed below were carried out in triplicates.

4.2.2. Preparation of tea leaves solution

Preparation of water extract was done according to the method described in ISO 14502-1 (E) (2004). Before starting the experiment, a 70% methanol/water extraction mixture was allowed to equilibrate for 30 minutes in a water bath set at 70°C. 0.200 ± 0.001g of each sample was weighed in an extraction tube and 5 ml of 70% methanol/water extraction mixture was dispensed. The extract was mixed thoroughly and heated at 70°C for 10 minutes. Mixing was done after 5 and 10 minutes to ensure complete extraction. The extract was allowed to cool to room temperature and centrifuged for 10 minutes at 3,500 rpm. The supernatant was decanted into a 10 ml test tube. The extraction step was repeated twice. The extracts were combined and the final volume of the extract was made up to 10 ml with a cold methanol/water extraction mixture. Dilution of the extract was done as required.

4.2.3. Estimation of total flavonoid contents

TFCs were determined using aluminium chloride colorimetric assay (Zhishen et al, 1999). In this method, aluminium chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.

1 ml of tea leaves extract was transferred to 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ was added. The solution was allowed to stand for 5 minutes and 0.3 ml of 10% $AlCl_3$

was added. This was followed by incubation for 1 minute at room temperature. Following incubation, 2 ml of 1 M NaOH was added. The total volume was brought up to 10 ml with distilled water. The solution was mixed well and allowed to stand for 15 minutes at room temperature. Blank was prepared using the same procedure but with distilled water instead of sample extract.

The standard solution was prepared using catechin in concentrations of .10, .20, .30, .40, .50 and .60 mg/l following the same procedure used in the sample.

The absorbance was measured at 510nm against blank in UV–visible spectrophotometer. The calibration line was constructed using standard. Based on the measured absorbance, the concentration of total phenols was measured (mg/ml) using regression equation of Y = 0.659x - 0.011; R2 = 0.998 from the standard curve. The TFCs of tea leaves was then expressed as mg CE/g dry weight of tea leaves.

4.2.4. Estimation of total polyphenol contents

TPCs were determined by the Folin-Ciocalteu reagent assay (Singleton et al., 1965). In this method, the products of the reduction of a mixture of tungsten and molybdenum oxides develop a blue colour that exhibits a broad light absorption with a maximum at 765nm. The intensity of light absorption at 765nm is proportional to the concentration of phenols (Singelton et al., 1999).

1 ml of tea leaves extract was transferred to 9 ml of distilled water. To this, 1 ml of Folin-Ciocalteu reagent was added. The mixture was shaken and kept at room temperature for 5 minutes. 10 ml of 7% Na_2CO_3 solution was added to the mixture and the total volume was brought up to 25 ml with distilled water. The sample solution was incubated at room temperature for 90 minutes. Blank was prepared using the same procedure but with distilled water instead of sample extract.

The standard solution was prepared using gallic acid in concentrations of .10, .20, .30, .40 and .50 mg/ml following the same procedure.

The absorbance was measured at 750nm with UV–visible spectrophotometer. The calibration line was constructed using standards. Based on the measured absorbance, the concentration of total phenols was measured (mg/ml) using regression equation of Y = 0.746x + 0.029; R2 = 0.997 from the standard curve. The TPCs of tea leaves was then expressed as mg GAE/g dry weight of tea leaves.

4.2.5. Estimation of tannin contents

TCs of the leaves extract were determined following Mythili et al., (2014). 1 ml of tea leaves extract was transferred to 7.5 ml of distilled water. To this, 0.5 ml Folin-Ciocalteu reagent and 1 ml of 35% Na_2CO_3 solution were added. The solution was mixed properly and the total volume was brought up to 10 ml with distilled water. Blank was prepared using the same procedure but without sample extracts.

The standard solution was prepared using tannic acid in concentrations of .20, .40, .60, 0.80 and 1 mg/l. Standard solution, blank, and sample solution were incubated at room temperature for 30 minutes.

The absorbance was measured at 725nm against blank in UV–visible spectrophotometer. The calibration line was constructed using standards. Based on the measured absorbance, the concentration of tannin content was measured (mg/ml) using regression equation of Y = 0.025x + 0.016; R2 = 0.992 from the standard curve. The TCs was expressed as mg TAE/g dried weight of tea leaves.

The absorbance was measured spectrophotometrically at 725nm. The TC was expressed as mg tannic acid equivalent (TAE)/g weight of tea leaves.

4.2.6. Statistical analysis

Data arrangement and statistical analysis were performed in the same software used for soil physicochemical analysis. Graphs were plotted in OriginPro 8.5 version. One-way Analysis of variance (ANOVA) was used to identify statistically significant differences ($p < 0.05$) in each of the tea leaves chemical compositions. In cases where ANOVA results were significant ($p < 0.05$), Tukey's HSD post hoc analysis was performed to detect the differences in the studied parameters between the seasons. Two-way ANOVA was performed to test the interaction effect of seasons and sites on the studied parameters.

Pearson correlation was used to detect the relationships of tea leaves compositions with soil physicochemical properties and soil microflora counts in each of the tea gardens. The correlations were considered significant if $p < 0.01$ and $p < 0.05$.

4.3. Results

The tea leaves extracts sampled from Tuli and Ungma tea garden were used for the determination of their TPCs, TFCs and TCs. The TPCs in tea extracts was expressed in terms of GAE (mg/g) whereas the TFCs and TCs in tea extracts were expressed in terms of CE (mg/g) and tannic acid equivalent (mg/g) respectively. All these properties showed seasonal variations for both the tea gardens studied (Fig. 4.1 - 4.6 and Table 4.1). The season and site interaction effect as per two-way ANOVA was significant at $p < 0.05$ for TPCs and TCs in both the years but it was not significant for TFCs (Table 4.2).

4.3.1. Total flavonoid contents

The TFCs in tea leaves collected from the Tuli tea garden showed variations ranging from 149.26 to 215.07 mg/g and 124.77 to 197.46 mg/g in the first and second year respectively (Fig. 4.1). Significant effect ($p < 0.05$) of the season was observed in both years (Table 4.1). In the first year, the TFCs in the spring season (215.07 ± 5.51 mg/g) was significantly higher ($p < 0.05$) than summer (190.06 ± 3.91 mg/g), autumn (163.72 ± 3.83 mg/g) and winter season (149.26 ± 3.67 mg/g) (Fig. 4.1). Similarly, in the second year the TFCs in the spring season (197.46 ± 3.33 mg/g) was significantly higher ($p < 0.05$) than in summer (166.15 ± 3.82 mg/g), autumn (145.23 ± 3.21 mg/g) and winter season (124.77 ± 3.23 mg/g) (Fig. 4.1). Significant differences ($p < 0.05$) were established between seasons except between autumn and winter in the first year.

The TFCs in tea leaves collected from the Ungma tea garden showed variations ranging from 136.97 to 208.16 mg/g and 111.78 to 184.89 mg/g in the first year and second year respectively (Fig. 4.2). The TFCs in both years were significantly higher ($p < 0.05$) in spring seasons (208.16 ± 5.72 mg/g and 184.89 ± 3.80 mg/g) when compared with summer (165.81 ± 2.87 mg/g and 150.49 ± 2.67 mg/g), autumn (154.22 ± 3.31 and 132.46 ± 2.70 mg/g) and winter seasons (136.97 ± 3.02 and 111.78 ± 2.39 mg/g). Significant differences ($p < 0.05$) were established between seasons in both the years except between summer and autumn in the first year.

Tea leaves sampled from Tuli and Ungma tea garden showed comparable TFCs in each season. The effect of site was significant however interaction effect of site and season was not significant at $p < 0.05$ in both years (Table 4.2).

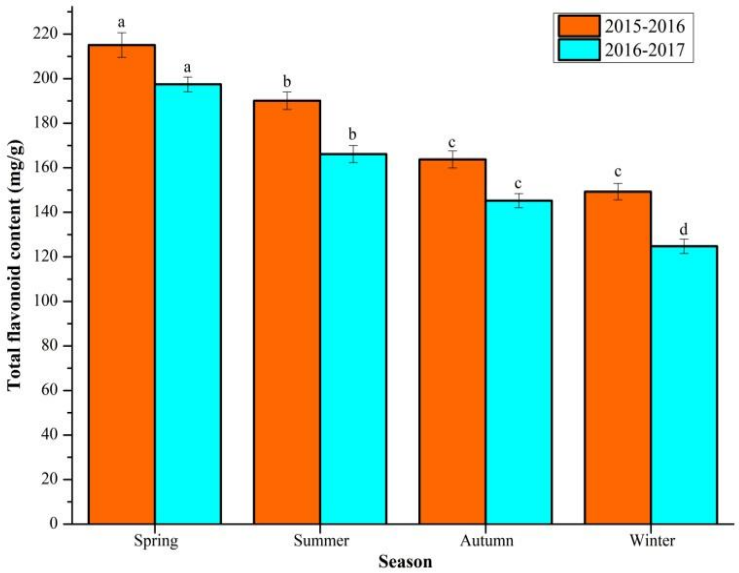

Fig. 4.1. Comparison of tea leaves TFCs among different seasons in the Tuli tea garden.
Error bar represents the mean ± standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

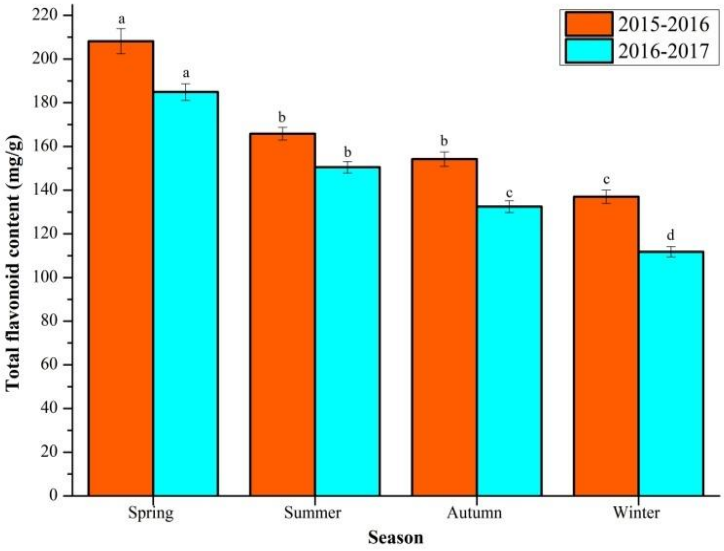

Fig. 4.2. Comparison of tea leaves TFCs among different seasons in the Ungma tea garden.
Error bar represents the mean ± standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

4.3.2. Total polyphenol contents

The TPCs in tea leaves collected from the Tuli tea garden varied from 374.13 to 499.77 mg/g and 315.02 to 452.78 mg/g in the first and second year respectively (Fig. 4.3). There was a significant effect ($p < 0.05$) of the season on TPCs in both years (Table 4.1). Among the seasons, the concentration of tea leaves total polyphenols were highest in the spring

seasons in both years however, the differences were not significant ($p > 0.05$) with summer seasons. The TPCs in the first and second year descended in the following order: spring (499.77 ± 13.77 and 452.78 ± 9.03 mg/g) > summer (494.01 ± 12.83 and 429.02 ± 9.18 mg/g) > autumn (440.15 ± 13.22 and 380.01 ± 10.15 mg/g) > winter season (374.13 ± 14.29 and 315.02 ± 9.38 mg/g).

The TPCs in the tea leaves collected from the Ungma tea garden varied from 300.26 to 478.53 mg/g and 298.10 to 403.72 mg/g in the first and second respectively (Fig. 4.4). There was a significant effect ($p < 0.05$) of the season on tea leaves TPCs in both years (Table 4.1). Among the seasons, tea leaves collected in the spring seasons in both years contained a significantly higher amount of total polyphenols ($p < 0.05$) compared to other seasons. The TPCs in the first and second year descended in the following order: spring (478.53 ± 6.64 and 403.72 ± 7.98 mg/g) > summer (412.29 ± 7.80 and 360.17 ± 6.80 mg/g) > autumn (381.33 ± 7.48 and 335.43 ± 6.49 mg/g) > winter season (300.26 ± 7.13 and 298.10 ± 6.57 mg/g).

Between tea gardens, tea leaves collected from Tuli had a higher concentration of TPCs throughout the sampling seasons (Fig 4.3 and Fig. 4.2). The effect of site, as well as the interaction effect of site and season, were significant (Table 4.2).

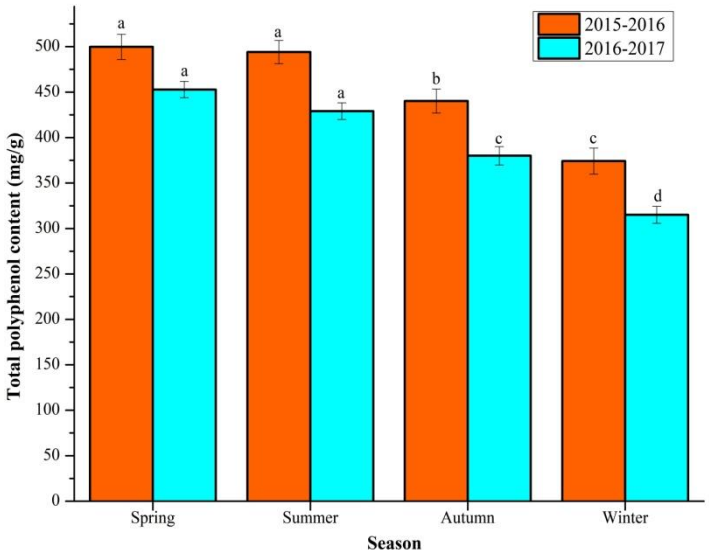

Fig.4.3. Comparison of tea leaves TPCs among different seasons in the Tuli tea garden.
Error bar represents the mean±standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

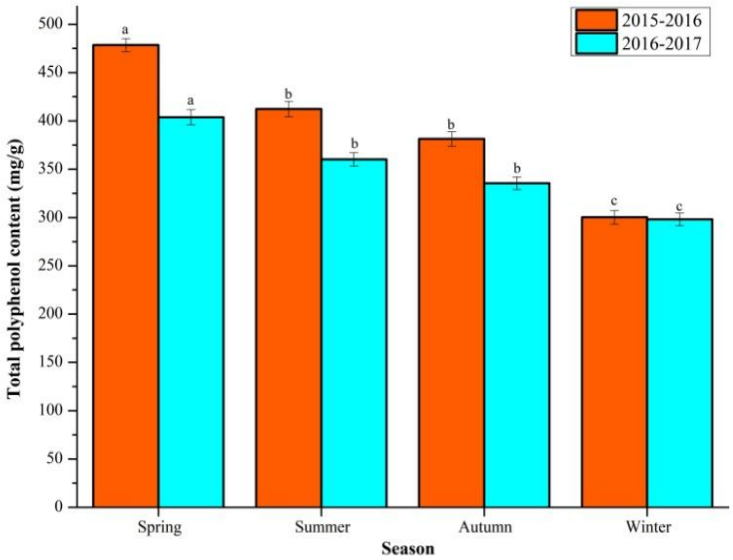

Fig.4.4. Comparison of tea leaves TPCs among different seasons in the Ungma tea garden.
Error bar represents the mean±standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

4.3.3. Tannin contents

The comparative analysis of tea leaves TCs collected from the Tuli tea garden in different seasons is shown in Fig. 4.5. In the first year, the TCs in summer season (24.66 ± 0.46 mg/g) was higher than spring (22.90 ± 0.39 mg/g), autumn (20.49 ± 0.53 mg/g) and winter season (18.20 ± 0.51 mg/g). Similarly, in the second year, the TCs in summer season (21.97 ± 0.39 mg/g) was higher than spring (19.55 ± 0.44 mg/g), autumn (17.35 ± 0.43 mg/g) and winter season (14.97 ± 0.50 mg/g). There was a significant effect ($p < 0.05$) of the season on TCs in both years (Table 4.1) and the difference between each season was also significant ($p < 0.05$) except between spring and summer and between autumn and winter in the first year.

The result of TCs in tea leaves collected from the Ungma tea garden analysed seasonally for two years is shown in Fig. 4.6. In the first and second year, the TCs in spring (25.79 ± 0.38 mg/g and 19.69 ± 0.39 mg/g), summer (24.07 ± 0.20 mg/g and 17.04 ± 0.47 mg/g), autumn (20.90 ± 0.55 mg/g and 15.06 ± 0.44 mg/g) and winter seasons (18.19 ± 0.49 mg/g and 13.82 ± 0.31 mg/g) differed significantly from each other ($p < 0.05$) except between autumn and winter season in the second year. From the results presented, spring seasons had the highest TCs, whereas winter seasons showed the lowest TCs among the seasons studied in both years.

Differences in the TCs were observed between tea gardens across all sampling seasons. The site effect, as well as site and season interaction, were significant at $p < 0.05$ for both years (Table 4.2).

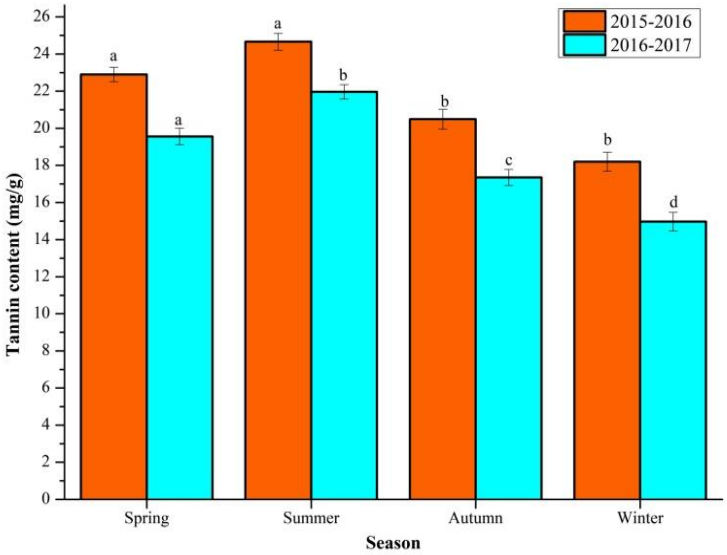

Fig. 4.5. Comparison of tea leaves TCs among different seasons in the Tuli tea garden.
Error bar represents the mean±standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

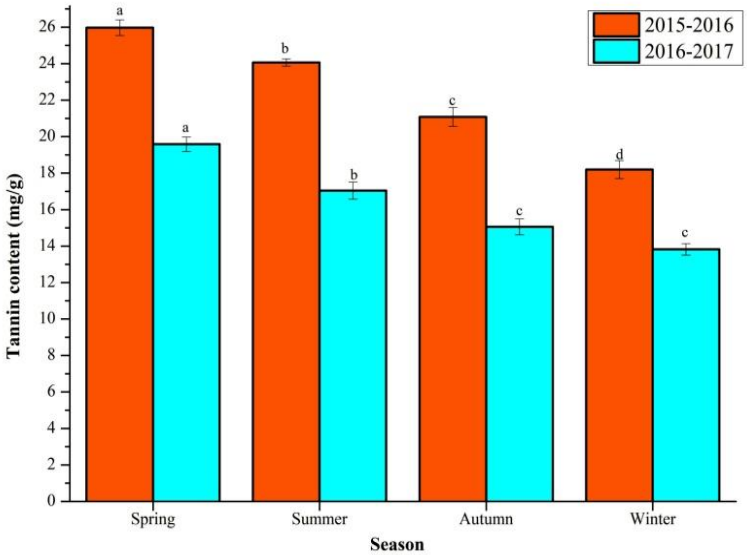

Fig. 4.6. Comparison of tea leaves TCs among different seasons in the Ungma tea garden.
Error bar represents the mean±standard error mean of six locations within the tea garden.
Different[abc] in each year indicates significant differences at $p < 0.05$ as measured by Tukey's post hoc test.

Table 4.1. One way ANOVA of tea leaves biochemicals during 2016 - 2018

Parameter	Tuli				Ungma			
	2015 - 2016		2016 - 2017		2015 - 2016		2016 - 2017	
	F	*p* value	F	*p*-value	F	*p* value	F	*p*-value
TPC	18.65	0.00	41.54	0.00	103.25	0.00	40.22	0.00
TFC	45.90	0.00	82.98	0.00	60.21	0.00	111.21	0.00
TC	35.57	0.00	45.94	0.00	63.902	0.00	38.18	0.00

Table 4.2. Two way ANOVA of tea leaves biochemicals during 2016- 2018

Parameter	Source	2015 - 2016		2016 - 2017	
		F	*p*-value	F	*p*-value
TPC	Site	58.75	0.00	58.29	0.00
	Season	72.21	0.00	78.82	0.00
	Site X Season	3.05	0.04	3.32	0.03
TFC	Site	20.79	0.00	36.00	0.00
	Season	103.02	0.00	185.95	0.00
	Site X Season	1.74	0.17	0.10	0.96
TC	Site	4.12	0.05	34.91	0.00
	Season	4.65	0.01	35.75	0.00
	Site X Season	3.27	0.03	3.83	0.02

4.3.4. Correlation analysis

The Pearson's correlation coefficient of tea leaves TPCs, TFCs and TCs with soil properties and microfloral population are presented in Table 4.3a and Table 4.3b.

A positive correlation was established between tea leaves constituents and parameters including moisture, *p*H, temperature, SOC, available N, available P, fungal counts and bacterial counts at Tuli tea garden in both years. However, tea leaves constituents were significantly correlated at $p < 0.05$ and $p < 0.01$ with some parameters only. In the first year, available P significantly correlated with TPCs (.969*) and TFCs (.976*) whereas, in the second year significant positive correlation were established between TPCs and *p*H (.991**), TPCs and SOC (.988*), TPCs and available P (.981*), TFCs and *p*H (.984*), TFCs and OC (.957*), TCs and moisture content (.994**).

The correlation between the tea leaves constituents and parameters including moisture, *p*H, temperature, SOC, available N, available P, available K fungal counts and bacterial counts at Ungma tea garden were positive in both years however, correlations of TPCs, TFCs and TCs were significant only with fungal counts ($p < 0.05$ and $p < 0.01$).

Table 4.3a. Correlation analysis of tea leaves constituents with soil properties and microfloral population in Tuli tea garden during the year 2016 - 2018

Year	LC	Sand (%)	Clay (%)	Silt (%)	Moisture (%)	*p*H	Temp. (°C)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	F	B
2015	**TPCs**	-0.09	0.28	-0.14	0.50	0.52	0.73	0.80	0.82	.969*	0.66	0.76	0.94
-	**TFCs**	0.30	-0.01	-0.32	0.15	0.18	0.40	0.79	0.67	.976*	0.50	0.79	0.73
2016	**TCs**	-0.3	0.18	0.18	0.48	0.49	0.75	0.58	0.66	0.85	0.47	0.52	0.93
2016	**TPCs**	0.25	-0.28	0.17	0.91	.991**	0.53	.988*	0.41	.981*	-0.23	0.74	0.91
-	**TFCs**	0.52	-0.54	0.25	0.76	.984*	0.25	.957*	0.12	0.90	-0.24	0.83	0.75
2017	**TCs**	-0.19	-0.03	0.35	.994**	0.81	0.73	0.80	0.67	0.85	-0.49	0.32	0.90

LC- tea leaves constituents, TPCs- total polyphenol contents, TFCs- total flavonoid contents, TCs- tannin contents, Temp.- temperature, SOC- soil organic carbon, AN- available nitrogen, AP- available phosphorus, AK- available potassium, F- fungal CFU, B- bacterial CFU.

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

Table 4.3b. Correlation analysis of tea leaves constituents with soil properties and microfloral population in Ungma tea garden during the year 2016 - 2018

Year	LC	Sand (%)	Clay (%)	Silt (%)	Moisture (%)	*p*H	Temp. (°C)	SOC (%)	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)	F	B
2015	**TPCs**	-0.11	0.92	0.05	0.57	0.67	0.53	0.94	0.95	0.91	0.46	.973*	0.50
-	**TFCs**	0.15	0.77	-0.21	0.31	0.43	0.25	0.85	0.89	0.78	0.22	.993**	0.22
2016	**TCs**	-0.23	0.90	0.17	0.63	0.72	0.55	0.86	0.87	0.85	0.37	.964*	0.53
2016	**TPCs**	0.06	-0.54	-0.13	0.79	0.81	0.42	0.77	0.05	0.14	0.55	.994**	0.55
-	**TFCs**	0.14	-0.61	-0.21	0.75	0.77	0.35	0.72	0.13	0.05	0.51	.996**	0.48
2017	**TCs**	0.13	-0.59	-0.27	0.76	0.78	0.33	0.73	0.11	0.00	0.43	.998**	0.48

LC- tea leaves constituents, TPCs- total polyphenol contents, TFCs- total flavonoid contents, TCs- tannin contents, Temp.- temperature, SOC- soil organic carbon, AN- available nitrogen, AP- available phosphorus, AK- available potassium, F- fungal CFU, B- bacterial CFU.

**: Correlation is significant at the 0.01 level.

*: Correlation is significant at the 0.05 level.

4.4. Discussions

The fact that tea is a pleasant drink together with numerous health benefits increases its demand worldwide. The tea quality attributes such as taste, colour, flavour and aroma and its health implications are intimately associated with the tea leaves chemical constituents. The determination of total polyphenols, total flavonoids and total tannins content of tea is very important in assessing the standard and quality of tea as well as any potential implications to health (Bizuayehu et al., 2016). Tea polyphenols account for about 42% of the dry weight of the solids in tea extracts (Khan and Mukhtar, 2007; Sajilata et al., 2008) and contribute one-third of the bioactive compounds in tea (Mahmood et al., 2010). Tea flavonoids control various cellular pathways and help to provide antioxidant effects by detoxifying free radicals and preventing cell damages in the body. Tea tannins are a type of bioactive compounds with

antioxidant and antitumoral activities and are directly associated with higher functional properties of tea (Orak et al., 2013).

4.4.1. Variation in tea leaves chemical composition between seasons

There are considerable differences in the content and distribution of tea leaves chemical compositions even within the same cultivars due to differences in seasons of the year (Zhang et al., 2020). This ultimately results in different qualities of made tea. Therefore, the study on seasonality in tea leaves chemical composition has been an area of interest for many researchers. Moreover, determining the best harvesting seasons with maximum concentrations of important tea leaves constituents is crucial for tea growers, daily tea beverage consumers, pharmaceutical and therapeutic industry. The present study shows a significant seasonal influence on the tea leaves chemical composition at both tea gardens studied (Figure 4.1 - 4.6 and Table 4.1) indicating that the harvesting season of tea leaves is crucial for tea quality in the Mokokchung district. Seasonal influence on tea leaves constituents and corresponding tea quality were reported on various tea types worldwide (Yao et al., 2005; Chen et al., 2010; Jayasekera et al., 2011; Zhang et al., 2020). According to previous studies, the possible reasons that led to differences in the tea leaves chemical compositions among seasons were variations in environmental conditions (Yao et al., 2005), physiological activities and growth behaviour of tea plants (Patel et al., 2019) and an array of biotic and abiotic factors (Hazra et al., 2021).

Among the seasons studied, except for TCs in Tuli tea leaves, the tea leaves constituents were highest during spring seasons (Figure 4.1 - 4.6). Further, the differences in Tuli tea leaves TPCs recorded in spring seasons were not significant with summer seasons ($p > 0.05$). These suggest that the plant growth and metabolic activities were higher during spring and summer seasons at Tuli tea garden but at Ungma tea garden, these were significantly higher ($p < 0.05$) in spring seasons. On the contrary, all the chemical compositions of the tea leaves studied drastically lowered in the winter seasons because the tea plant remains dormant during the winter season due to shorter day length and low temperature in northeast India (Patel et al., 2019). The present observation corroborates the findings in other studies (Yao et al., 2005). As seasonal variations in tea leave chemical compositions are related to seasonal variations in tea quality (Gulati and Ravindranath, 1996) therefore, the present study on TPCs and TFCs seems to agree with Le Gall et al., (2004)

who mentioned that the best green teas were usually plucked during the first flush in April or May. Gulati and Ravindranath, (1996) also recorded maximum content in tea leaves chemical composition and flavour quality during early flush which gradually decreased with progress in season. Similarly, Ahmed et al., (2019) reviewed that out of 18 studies, 78% demonstrated a decrease in phenolic compounds or their bioactivity concentrations with a seasonal shift from the spring and or first tea harvest to other seasons. Contrary to the present study, observations from other studies reported higher leaves constituents in the second flush or summer seasons due to stronger sunlight, higher temperature and longer daytime length (Ercisli et al., 2008). Although the concentrations of the studied tea leaves constituents were the lowest in winter seasons, the concentrations of TPCs and TFCs were highest during the spring seasons (even though the soil temperature was at its highest during summer seasons). Therefore, the temperature effect on tea leaves chemical composition seems to be partially true in the present study. Instead, a combination of the temperature with other factors or other factors alone might be responsible for the observed seasonal variations in tea leaves chemical compositions. It has been reported that supplementation of balanced nutrition including the N and K ratio can significantly improve tea quality (Venkatesan and Ganapathy, 2004). However, unbalanced nutrient status in tea soil can hinder the plant growth and formation of biochemical ingredients in tea (Ma et al., 2013). The results from the present study seem to agree with the aforementioned statement. It was observed that seasons with the highest available nutrient contents (Table 2.8 - Table 2.9) were not always the seasons with the highest tea leaves constituents probably resulting from unbalanced nutrients supply. Thus, optimum available nutrients are crucial for the increase in the synthesis of tea leaves TPCs, TFCs and TCs. Additionally, a significant and non-significant positive correlation of tea leaves constituents were established with soil properties and microfloral counts (Table 4.3a and Table 4.3b) indicating the direct or indirect influence of these parameters.

Variations in leaves constituents were observed between the two years as well. The soil conditions of the same season in each year varied so this along with other conditions might have resulted in such differences. Similarly, Zhao and Zhao, (2018) opined that the tea leaves elemental concentrations of the same season differed in different years due to change in climatic conditions. Therefore, it is reasonable to suggest that the level of concentrations in the studied tea leaves constituents differed seasonally due to multiple factors including soil properties, available nutrients and other environmental conditions.

4.4.2. Differences in tea leave chemical compositions between tea gardens

The results from the present study show that the tea leaves from study areas contained a huge amount of bioactive compounds including total polyphenols (298.10 to 499.77 mg/g), total flavonoids (111.78 to 215.07 mg/g) and tannins (13.82 to 25.79 ± 0.38 mg/g) (Figure 4.1 - 4.6). This reveals that the phenolics are the most abundant group among tea leaves constituents. A huge concentration of tea leaves total polyphenols varying from 210.9 to 450.6 mg GAE g^{-1} was also reported in the study of Savsatli et al., (2021). These values were lower when compared with those recorded in the present study. Such discrepancy between the present values and other results signifies the dependence of tea leaves constituents on tea genotypes, leafage, growing and climatic conditions, geographical locations of the tea garden (Turkmen et al., 2009). In the present study also, tea leaves sampled from Tuli and Ungma tea garden showed differences in the concentration of the chemical compositions between tea gardens. However, this difference probably occurred due to differences in altitude, geographical locations of the tea garden, tea garden management practices and soil conditions because the tea plant in both study areas was of the same strain. Likewise, the tea gardens were also of the same age. Similar to the present result Han et al., (2018), opined that altitudinal variations could result in differences in the chemical composition as well as tea taste even in the same varieties of tea plants.

Based on the average of six separate samplings, the tea leaves TPCs and TFCs were higher at the Tuli tea garden which is situated at a lower altitude than at the Ungma tea garden which is situated at a higher altitude in each of the seasons studied (Figure 4.1 - Figure 4.4). Interestingly, however, TCs were higher in Ungma tea leaves in almost all seasons in the first year (Figure 4.5 and Figure 4.6). The present results are in partial agreement with the observations made by Han et al., (2017) where there was decreased in tea leaves total polyphenols with an increase in altitude on Lushan mountain in eastern China. Contrary to this, Owuor et al., (1990) reported decreases in tea quality with a decline in altitude. Although their observation agrees with the result on TCs in the first year it contradicts with the result from the second year. The differences between the present results and observations made by others may be due to differences in cultivation altitudes, tea genotype, seasons, geographical locations etc. (Han et al., 2017). It is well established that the tea plant flourishes in humid and warm environmental conditions (Nath and Bhattacharya, 2014). Furthermore, the biosynthesis of phenolic compounds can be greatly

induced by sunlight (Harbowy et al., 1997). In the present study, Tuli tea garden had lesser shade trees which probably paved way for full penetration of sunlight in the tea garden resulting in greater accumulation of tea leaves constituents especially total polyphenols and flavonoids whereas, the Ungma tea garden had more shade trees which might have reduced sunlight exposure time and strength consequently lowering the accumulation of compounds in tea leaves. Furthermore, the influence of other factors including site-specific soil properties, microbial processes and management practices on the studied tea leaves chemical compositions is also considered in the present study. Although correlation studies of tea leaves constituents with soil properties, available nutrients and microbial population resulted in positive relationships with almost all these parameters, however, there were differences in correlation level between the tea gardens studied (Table 4.3a and Table 4.3b) suggesting that the influence of these parameters on tea leaves TPCs, TFCs and TCs varied between the sites. In Tuli, a significant positive correlation ($p < 0.05$ and $p < 0.01$) of tea leaves constituents were established with available P, soil moisture and *p*H depending on the years thereby, underlining the profound effect of these parameters on the formation of tea leaves constituents. Furthermore, a significant correlation of available P with tea leaves constituents signifies that high P concentration in soil enhances the tea shoots constituents (Gogoi et al., 1993). At Ungma, a correlation between tea leaves constituents and fungal counts were positively significant ($p < 0.05$ and $p < 0.01$) indicating the influence of indigenous fungi in tea leaves TPCs, TFCs and TCs. Tea soil harbours varieties of biofertilizers (Renla and T, 2017) therefore, the presence of biofertilizer fungi probably influence nutrient exhibited improvement in the tea leaves constituents. The influence of fungi on tea leaves constituents and consequent quality have also been reported in other studies (Singh et al., 2010).

4.4.3. The interaction effect between tea gardens and seasons

The interaction effect between site and season for TPCs and TFCs in both years ($p < 0.05$) were significant. This shows that the differences in the TPCs and TFCs between tea gardens were not consistent for each season. Jayasekera et al., (2014) also observed similar results in high-grown unfermented and fermented Sri Lankan teas where the interaction between plantation sites and seasons had a significant effect on phenolic compounds. As for TCs, there were no significant interaction effects for both years ($p > 0.05$) indicating an independent effect of both site and season. Thus, these factors need to be taken into

consideration in future investigations on the constituents of tea leaves grown in the Mokokchung district of Nagaland. The observation made from the present study suggests that the leaves constituents are controlled by several factors including altitudes, season, soil properties and appropriate nutrient ratio.

4.5. Conclusion

The present study highlights the information regarding the effect of seasons and sites on the tea leaves quality determining constituents. The result demonstrated that tea leaves chemical compositions including TPCs, TFCs and TCs were significantly affected by seasonal variations. At Tuli tea garden, the concentration of tannin was highest during summer seasons whereas, total polyphenol and total flavonoid were highest in the spring season although the difference was not significant with the summer season. At Ungma tea garden, all the studied tea leaves constituents were highest in spring seasons. To obtain the best tea quality from the Mokokchung district tea gardens leaves harvested from Tuli during the spring and summer seasons and from Ungma, the leaves harvested during the spring season is advisable. The relationship between tea leaves constituents and soil physicochemical properties, as well as bacterial and fungal CFU, was established. This indicates that the mechanisms responsible for seasonal variations in the levels of tea leaves constituents occur due to one or more soil properties that varied markedly between seasons.

The differences in tea growing altitude and growing conditions also affected tea leaves constituents. Variation in tea leaves constituents between tea gardens showed the profound effect of altitude and soil properties along with other factors. The contents of studied the tea leaves constituents grown at Tuli (low altitude) were comparably higher than those grown at Ungma (high altitude), at least for TPCs and TFCs. Based on correlation study, soil moisture, *p*H and available P could be considered the most important parameters for tea leaves constituents in Tuli tea garden whereas, in Ungma tea garden, the soil fungal activities probably influenced the nutrient exhibited improvement in the tea leaves constituents.

The present study could be useful for assessing tea quality in the Mokokchung district of Nagaland. A further study, however, is recommended to seasonally determine other compounds in tea leaves with the inclusion of more tea varieties cultivated at varying altitudes.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This chapter summarizes the overall thesis and presents some conclusions of it. The future scope of the work is also highlighted in this chapter.

Tea is an everyday beverage with numerous health benefits. It is also a great contributor to the world's economy. There are various studies related to tea plants such as their health benefits, the soil and other environmental conditions under tea cultivations, factors controlling tea growth and development, leaves yield and quality of made tea. However, these studies have numerous conflicting pieces of evidence on the effect of the geographical location and soil physicochemical properties of an area on tea production. This suggests that the observations made in a particular area may not be reproducible in another. The present study thus shed some light on the variation of soil microflora, soil properties and tea leaves constituents among seasons and also between two tea gardens located in the Mokokchung district of Nagaland. A summary of the present study is given below:

Season and altitude mediated influence on soil physicochemical properties (soil texture, *p*H, moisture content, temperature, SOC and available nutrients), soil microflora (bacteria and fungi) and tea leave chemical compositions (TFCs, TPCs and TCs) were observed in the present study.

i) Tea soil physicochemical properties

The tea gardens showed dominancy in sand content as compared to clay and silt content. The soil texture in the Tuli tea garden was sandy clay loam but in the Ungma tea garden, it was sandy clay and clay loam. The soil *p*H was found to be slightly acidic in both tea gardens and hence, favourable for the tea plant. The available N content falls under the medium to high category whereas, the available P and available K content were under the low to the medium category in the Tuli tea garden. In the Ungma tea garden, all these nutrients were under the low to medium category.

The influence of season in both tea gardens soil physicochemical properties was significant ($p < 0.05$) as revealed by the one-way ANOVA result. Soil temperature was highest in the summer and lowest in the winter among the sampling seasons in both tea gardens. The season with the highest and the lowest content of other soil properties differed between tea gardens and it was uneven for soil properties in both tea gardens. At the Tuli tea garden, SOC and available P were highest in the spring season while soil temperature and available K was highest in summer and autumn season respectively in both years. For other soil properties including soil *p*H, moisture and available N content, the season with the highest and the lowest content differed between years. At the Ungma tea garden, soil *p*H and moisture content were highest in the summer season while the available N content and

available K was highest in the spring and autumn season respectively in both years. For SOC and available P, the season with the highest and the lowest content differed between years.

The significant effect of sites as well as interaction effect of seasons and sites at $p < 0.05$ were observed for almost all the soil physicochemical properties. Furthermore, multiple controlling factors for tea garden soils was shown by the differences in correlation among soil physicochemical properties. Differences in the correlation were also observed between the Tuli and Ungma tea garden. All these suggest the influence of altitude, an area's geographical conditions and climatic factors on tea soil. Comparatively, soil physicochemical properties like soil temperature, available P was higher at the lower altitude whereas, soil *p*H, SOC, available N were higher at the higher altitude in all seasons. However, soil moisture content and available K content did not show any trend with altitude.

ii) Tea soil microflora

Seasonality on the population of soil microflora in the form of CFU was observed in the study. The seasonal differences were significant between each sampling seasons. One-way ANOVA revealed that the CFU of soil bacteria was significantly higher ($p < 0.05$) in the summer seasons whereas, the CFU of soil fungi was significantly higher ($p < 0.05$) in the spring seasons. The lowest CFU of both groups of soil microflora was significantly lower ($p < 0.05$) in the winter seasons. Likewise, the highest number of bacterial and fungal isolates were recorded during the summer and spring seasons respectively. It can be speculated that the conditions in summer seasons favoured bacterial growth and multiplication while the fungal growth and multiplication are positively influenced by conditions in spring seasons. The decrease in soil physicochemical properties such as temperature, moisture and *p*H might have hindered the activities of soil microflora during winter seasons and hence, can be accounted for the low CFU of bacteria and fungi in the tea gardens studied.

Among the bacterial genera, *Bacillus* and *Pseudomonas* were found to be dominant whereas, *Aspergillus*, *Penicillium* and *Trichoderma* were found to be dominant among the fungal genera due to their better adaption for survival in the tea gardens studied. Furthermore, many bacterial and fungal isolates with potential new or previously unexplored strains inhabiting the Tuli and Ungma tea soils were observed.

The effect of site, as well as interaction effect of seasons and sites, were significant ($p < 0.05$) for both bacterial and fungal CFU. Furthermore, there were differences in the type as well as the number of bacterial and fungal isolates. This shows that the differences in the

growth conditions especially in soil physicochemical properties between altitudes can tremendously affect the soil microflora.

iii) Tea leaves chemical compositions

The present study also gave an account of the variation of tea leaves chemical composition among seasons and between tea gardens. Seasonal evaluation on the Tuli tea leaves TPCs and TFCs revealed the highest concentrations during the spring seasons but the difference was not significant ($p > 0.05$). The lea leaves TCs was highest during the summer seasons. Tea leaves sampled from the Ungma tea garden showed the highest TPCs, TFCs and TCs in the spring seasons. Therefore, to obtain the best tea quality from the Mokokchung district tea gardens, it is advisable to use the tea leaves harvested during the spring and summer seasons in Tuli and the leaves harvested during the spring season in Ungma. The relationship between the concentrations in tea leaves chemical compositions and soil physicochemical properties, as well as bacterial and fungal CFU, was established. Thus, the mechanisms responsible for seasonal variations in the levels of tea leaves chemical compositions occurs due to one or more of these properties.

Tea leaves chemical compositions were also found to differ between tea gardens. The concentrations of tea leaves TPCs and TFCs were comparably higher in leaves harvested at Tuli (lower altitude) than at Ungma (higher altitude). However, TCs contents did not show any trend with altitude. The correlation study explained that the most important parameters for tea leaves constituents in the Tuli tea garden were soil moisture, *p*H and available P whereas, in the Ungma tea garden, the soil fungal activities probably influenced the nutrient exhibited improvement in the tea leaves chemical compositions.

Thus, it can be concluded from the present work that the soil physicochemical properties, the population as well as the types of soil microflora and tea leaves chemical compositions varied tremendously depending on the seasons and geographical locations with altitudinal differences. Differences in management practices and fertilization can also be accounted for in the present result. Many of the studied parameters are mutually dependent on one another depicting the multiple controlling factors in the study areas. The present study provides a baseline on soil physicochemical properties and indigenous soil microflora at tea gardens in the Mokokchung district of Nagaland. The present work also provides valuable information on the quality of the tea leaves cultivated in the district. This may serve as the

starting point for many future studies that can address specific issues related to not only tea growing areas but also other plantation areas in this part of the country.

Future scope

Some of the future scopes of the present work include:

- Future studies can initiate on evaluation of heavy metals and trace elements, details on management practices and nutrient mineralization rates in tea garden soil.
- Impact of long-term application of various fertilizers, pesticides and herbicides.
- Further experiments with these indigenous soil microflora for their role in plant growth promotion, biocontrol, antimicrobial activities, soil fertility, etc., for enhancing the tea industry in the Mokokchung district of Nagaland.
- Assessment and identifications of soil microbial communities using the culture-independent method.
- Study on genetic and species diversity of the soil microflora inhabiting the study areas.
- Assessment of other compounds in tea leaves with the inclusion of more tea varieties cultivated in these areas.

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Temsurenla and Ajungla T. 2017. Soil physio-chemical analysis in tea growing areas of Mokokchung district, Nagaland. International Journal for Science and Advance Research in Technology 3(12), 1016-1021.

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Paper Presentation in Conference/Symposium

'Variations in tea soil mycofloral populations under different altitudes'. In: National seminar on **'Advances in Biological Science Research'**, Department of Botany, Nagaland University, Lumami, February 28 - March 01, 2017.

'Microbial influence on carbon content in tea soil'. **In:** National seminar on **'Chemistry in Interdisciplinary Research'**, Department of Chemistry, Nagaland University, Lumami, March 16-17, 2017.

'Prevelant *Bacillus* species associated with tea growing areas of Mokokchung district, Nagaland, India'. **In:** National seminar on **"Bio-resource Exploration and Utilization: Applications in Modern Biology"**, Bioinformatics Infrastructure Facility Centre, Nagaland University, Lumami, October 9-10, 2018.

'Comparative assessment of tea, *Camellia sinensis* (L.) O. Kuntze leaves phytoconstituents of Mokokchung district, Nagaland, India'. In: International Conference on '**Energy, Environment and Health'** Sree Ayyappa College, Alappuzha district, Kerala, September 11-12, 2020.

Participation in Conference/Workshop/Training

National Workshop on **'Computational Drug Designing-I'** organized by Bioinformatics Infrastructure Facility Centre, Nagaland University, Lumami, October 05-06, 2015.

National Seminar on **"Inventory, Sustainable Utilization & Conservation of Bioresources"** jointly organized by Department of Botany, Nagaland University, Lumami, Nagaland and Institutional Biotech Hub, Nagaland University, Lumami-798627, Nagaland, February 26 - 27, 2016.

International Conference on **"Natural resources Management and Technology Trends (ICNRM-17)"** at Manipur University, Imphal, Manipur, India , March 27-29, 2017.

Hands on Training on **'Functional Genomics'** organized by Department of Biotechnology, Govt. of India sponsored Institutional Biotech Hub, Nagaland University, Lumami and Department of Botany, Nagaland University Sponsored by 'Institutional Biotech Hub', November 14-21, 2017.

Hands on Training on **"Genomics and Gene Expression Analysis"** organized by Department of Biotechnology, Govt. of India sponsored Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami 798627, July 18-23, 2018.

National Conference on **"Skill and Entrepreneurial Development of the Tribal Youth"** 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, July 25-28, 2018.

National Conference on **"Stakeholders on Conservation, Cultivation, Resource Development and Sustainable Utilization of Medicinal Plants of North-Eastern India"** jointly organized by Department of Botany, Nagaland University, Lumami, Nagaland and Society for Conservation and Resource Development of Medicinal Plants (SMP), New Delhi, March 06-07, 2019.

Hands on Training on **"Molecular taxonomy of microbes and higher plants"** organized and sponsored by Department of Biotechnology, Govt. of India sponsored Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami, July 17-23, 2019.