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Record on dominant microfungi and their potential phosphate solubilization in tea garden soils

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Abstract

Microfungi are one of the important microbial groups in agriculture due to their positive, mutualistic and negative effect on plant growth and productivity. The roles of fungi extend from organic matter decomposition and mineral cycling to plant growth promotion. Considering these indispensable roles of this microbial group, the present research was undertaken to investigate the indigenous dominant microfungi in tea garden soils of Mokokchung district, Nagaland in India. The dominant microfungi were screened for their phosphate solubilization activity in PVK agar medium using tri-calcium phosphate as the sole phosphate source. Microfungal isolates showed significant differences in culture plates as well as microscopic studies. A total of 110 fungal isolates under 19 genera were identified in the present study. Among the soil microfungi, *Aspergillus*, *Penicillium* and *Trichoderma* were found to dominate the studied tea garden soils. The highest phosphate solubilization activities were observed for species under *Aspergillus* (1.95 cm to 1.71 cm) followed by *Penicillium* (1.57 cm to 1.18 cm) and *Trichoderma* species (1.13 cm to1.06 cm). The present study offers a glimpse of indigenous microfungi as well as provide information on the dominant microfungi in tea garden soils of Mokokchung district, Nagaland and hence, will aid and expand knowledge on indigenous fungi and their various roles. Also, the applications of potent phosphate-solubilizers isolated in this study can be a future source of biofertilizers consortium for tea and other plants.

Keywords: *Aspergillus*; microfungi; Mokokchung; *Penicillium*; tea gardens; *Trichoderma*

Introduction

Soil is a living habitat for wide arrays of biotic components. It is an excellent culture medium for the growth and development of various microorganisms (Bisi-Johnson *et al.*, 2010) including fungi. Fungi are an important group of non-photosynthetic eukaryotic organisms that comprise both single-celled and multicellular forms that play a significant role in human, plant and animals' life. They are a source of numerous antibiotics, enzymes and medicines (Jamir and Ajungla, 2018) and are intimately linked with soil properties, nutrient cycling, ecosystem restoration and plant growth. Fungi constitute more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Karaoglu and Ulker, 2006). This group of microbes are the main decomposers of organic matter in soils because of their essential role in humus formation (Christensen, 1989) and are also responsible for the improvement or deterioration of plant health. Being an organism of immense value with a wide array of ecological and environmental importance, isolation and

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identification of soil fungi are very necessary from an agricultural perspective. As many as 11.7-13.2 million fungal species reside on the earth (Wu *et al.*, 2019) however, described fungi account for only 5-13% of the total estimated fungal species (Wang *et al.*, 2008). Thus, despite their importance and diversity, fungi constitute under described, poorly documented organisms on earth.

Fungal identifications and classifications are based on different methods such as morphological and molecular methods. Fungal studies using the morphological method is still the most common and reliable procedure (Senanayake *et al.*, 2020). This is because there are serious limitations associated with molecular methods 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). Microfungal identification by morphological methods involves the application of culture media and microscopic studies. Microfungal studies from culture plates can provide detailed information on their morphological features, growth characteristics and their interactions with each other which are of immense value especially for isolation of potential organisms that are capable of degrading heavy metals, solubilizing inorganic fertilizers and for studying antagonistic activities against many diseases causing pathogens. Traditional plate culture methods using different artificial culture media also give guidance in microfungal identification since their mycelia growth and sporulation vary on different media differing in nutrient types and compositions. Apart from this, morphological methods are more cost-effective therefore require less specialized equipment which is important for many, especially those researchers in developing nations of the world who are unable to obtain large amounts of molecular data (Senanayake *et al.*, 2020).

Tea is an economic crop and a healthy drink consumed by millions throughout the world. The tea industry relies heavily on soil nutrients especially macronutrients because it is intimately linked with tea growth, development, yield and quality. Among the soil macronutrient, phosphorus (P) is considered the master key to agriculture due to its multifarious roles. It is the most important nutrients for tea production after nitrogen and potassium. This macronutrient is crucial for physiological and biological processes 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 roots and normal growth of tea (Hajiboland, 2017), seed formation, early maturation of crops and contributes significantly towards disease resistance (Sharma *et al.*, 2013). Despite its importance for agricultural productivity, P availability for plant usage is limited in agricultural soils including tea plantations due to its association and formation of complexes with different compounds in soil. To meet the P demands of plants, P nutrition is supplied in the form of expensive chemical phosphate fertilization can also consequently deteriorate the soil health as well as result in environmental pollution. Therefore, there is an immediate need to substitute these chemicals with potent environmentally friendly phosphate fertilizers such as microbial-based fertilizers.

Nagaland state situated in the north-eastern part of India is in Indo-Burma biodiversity hotspots of the world however, vast areas of the state remain unexplored concerning its microbial richness and biodiversity. In recent years, several researchers have been exploring and documenting macrofungi of the state (Kumar *et al.*, 2013; Ao *et al.*, 2016; Wabang and Ajungla, 2016) however, work on microfungi of the state is barely a handful.

In this paper, the microfungal inhabitants and dominant species among these microfungi in tea garden soils of Mokokchung district, Nagaland, India was documented using plate culture and microscopic methods. The dominant fungi were further screened for phosphate solubilization ability. This study was undertaken to unveil and provide insights into the morphologies of the residential microfungi. Furthermore, it will provide useful information about the type of microorganisms that can adapt, exploit and persist in such monoculture plantation soil as well as offer tremendous scope for many potential biofertilizers and plant growth promoters for the upliftment of the tea industry.

Materials and Methods

Sampling

Soil samples of tea rhizosphere and non-rhizosphere were collected from Mokokchung district, Nagaland, India during July 2017-April 2018. Soil collections were made from tea gardens at Tuli (N 26°39'19.3 E 094°39'22.7) and Ungma (N 26°17'30.6 E 094°28'29.2). Visible debris and fauna were removed manually and soils were combined to composite samples in both cases. Soils were then transferred to the laboratory under sterile conditions.

Culture media

Czapek dox agar (CDA), malt extract agar (MEA), potato dextrose agar (PDA) and rose bengal agar (RBA) were used in the present study. PDA (Himedia, India) and RBA (Himedia, India) were prepared from a dehydrated base according to the manufacturer's instructions.

The chemical composition of CDA and MEA used in the study were prepared following the standard protocol (Atlas, 2004) as given below in Table 1.

Table1. Composition of CDA and MEA

CDA		MEA	
Ingredient	g/l	Ingredient	g/l
Sucrose	30	Glucose	20
K_2HPO_4	1	Malt Extract	20
$MgSO_4.7H_2O$	0.5	Mycological peptone	1
KCl	0.5	Agar	16
$FeSO_4.7H_2O$	0.01	Distilled water	1000
$NaNO_3$	3	Final *p*H	6.8 ± 0.2
Agar	15		
Distilled water	1000		
Final pH	7.3 ± 0.2		

All the ingredients for each 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 pH 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 121 °C for 15 minutes and transferred to laminar airflow. 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 Petri plates.

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

Sterilization

Glassware's, inoculation loop, needles and scalpel were washed with labolene and oven-dried at 105 °C. Prior to use in experiments, materials were autoclaved at 1200 rpm for 30 minutes and kept inside a laminar airflow chamber followed by UV irradiation.

Isolation and purification

Soil fungi were isolated following the serial dilution method of Waksman (1922) in PDA and RBA. 1 gm of soil sample was suspended in 10 ml of distilled and autoclaved water to make 10^{-1} dilution. Suspensions were incubated at room temperature in a shaker incubator for 10 minutes. Serial dilution was carried out from

this and aliquots of 100 µl each from 10^{-5} and 10^{-6} dilution were plated onto Petri dishes. For each dilution, plating's were carried out in triplicates to prevent any possible error. Plates with diluents were sealed, marked and incubated (IK-120) at 27 °C ± 2 °C for 120-168 hours.

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 CZA, MEA, RBA and PDA to record variation in colony morphologies of the isolates. 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.

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 (Raper and Thom, 1949; Bamett 1965; Rifai, 1969; Domsch *et al.*, 1980; Nelson *et al.*, 1983; Gilman, 2001; Klich, 2002; Watanabe, 2002; Asan, 2004; Frisvad and Samson, 2004; Pitt and Hocking, 2009; Samson *et al.*, 2014; Siddiquee, 2017).

Culture characterization

After three days of incubation, culture plates were checked every next day to record their colony morphologies. Culture characteristics including reverse and obverse colony colour, size of the colony, growth pattern, surface texture, margin character, pigmentation etc., were recorded from each culture media.

Microscopic characterization of isolates

Purified fungal isolates were transferred to a clean glass slide with a flame sterilized needle and mounted on lactophenol cotton blue. Microscopic examination including mycelium, hyphae shape, conidial development, conidial shape, conidiophore dimension, size and shape of metulae, philiade, chlamydospores spores and other special fungal structures 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 microscope.

Screening for phosphate solubilizing fungi

Dominant fungi were screened for their phosphate solubilizing ability. Fungal isolates were inoculated in Pikovskaya (PVK) agar plates supplemented with 0.5% tri-calcium phosphate. Isolates were incubated at 27 ± 2 °C for 5 days. Potential phosphate solubilizers were detected through clear halozones in the PVK plates after 5 days of incubation. Phosphate solubilization index (SI) was calculated using the formula:

$$\text{SI} = \frac{\text{Colony diameter + clearing zone}}{\text{Colony diameter}}$$

Results

Dominant fungi

A total of 110 fungal isolates under 19 genera were identified in the present study (Table 2). Based on their colony and microscopic characteristics, the identified 19 fungal genera were *Apophysomyces*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Colletotrichum*, *Cunninghamella*, *Fusarium*, *Geosmithia*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Rhizopus*, *Sclerotinia*, *Scytalidium*, *Trichoderma* and *Trichophyton*. Among the fungal genera, *Aspergillus* with 4 species, *Penicillium* with 5 species and *Trichoderma* with 6 species were found to be dominant. Furthermore, the highest of the isolates were under these genera. The morphological characterization of these dominant fungi is presented in Table 3.

The colony diameters and colours of *Aspergillus* and *Penicillium* were recorded in 7 days whereas, for *Trichoderma*, these were recorded in 3-5 days. Figures 1-5 represent some of the dominant fungi isolated in the present study.

Table 2. Genera and number of fungal isolates in tea garden soils

Fungal genera	Species	Number of isolates
Apophysomyces	*Apophysomyces viriabilis*	1
Aspergillus	*Aspergillus niger*	8
	Aspergillus sp.1	4
	Aspergillus sp.2	5
	Aspergillus sp.3	2
Botrytis	*Botrytis* sp.	2
Chaetomium	*Chaetomium globosum*	2
	Chaetomium sp.	2
Chrysosporium	*Chrysosporium* sp.	4
Cladosporium	*Cladosporium cladosporiodes*	4
	C. oxysporum	4
	Cladosporium sp.	1
Colletotrichum	*Colletotrichum* sp.	4
Cunninghamella	*Cunninghamella echinulata*	4
Fusarium	*Fusarium oxysporum*	4
	F. solani	4
Geosmithia	*Geosmithia* sp.	2
Mucor	*Mucor circinelloides*	2
	M. hiemalis	2
Paecilomyces	*Paecilomyces* sp.	2
Penicillium	*Penicillium citrinum*	2
	P. commune	1
	P. waksmanii	8
	Penicillium sp.1	1
	Penicillium sp.2	2
Pestalotiopsis	*Pestalotiopsis egyptiaca*	4
	Pestalotiopsis sp.	5
Rhizopus	*Rhizopus stolonifer*	2
Sclerotinia	*Sclerotinia* sp.	1
Scytalidium	*Scytalidium* sp.	1
Trichoderma	*Trichoderma hamatum*	2
	T. harzianum	6
	T. koningii	2
	T. viride	2
	Trichoderma sp.1	4
	Trichoderma sp.2	2
Trichophyton	*Trichophyton* sp.1	2

Table 3. Morphological characterization of dominant fungi

Isolates	Culture characteristics							Microscopic characteristics
	Media	CD (cm)	CCO	CCR	Exudates	Pigment	Other culture characteristics	
Aspergillus niger	CZA	3.5-4.6	Black	Cream			Effuse, velvety to powdery, radially sulcate, flat and entire	Conidiophores developed directly from the substratum, 15 µm wide and 310-450 µm long, smooth, hyaline, septate, biseriate. Phialides, 6.9-8.3 µm long, 2.9-
	MEA	3.3-3.9	Black	Yellow				

	PDA	4.8-5.2	Black	Yellow	Black			3.5 µm wide. Vesicles covered by 2 series of phialides, 15-40 µm in diameter. Conidia 2.5-4 µm diameter, smooth, globose
	RBA	2.1-2.6	Black	Colourless				
Aspergillus sp.1	CZA	2.5-3.4	Light yellow to pale brown	Yellow to brown			Velvety, off-white mycelium, diametrically sulcate on the obverse and heavily wrinkled on the reverse of the plate	Conidiophores developed directly from the substratum, 159-270 µm long, 5.1-8.3 µm wide, smooth-walled, hyaline, septate, biseriate. Phialides 3.8-6.9 µm wide, 5.3-9 µm long. Vesicles 10-19 µm diameter, sub-globose. Conidia 1.8-2.3 µm in diameter, globose
	MEA	2.1-4.3	Greyish-yellow	Brown				
	PDA	3.5-4.1	Greyish to yellow	Yellow	Brown			
	RBA	3.2-3.9	Pale brown	Colourless	Transparent			
Aspergillus sp.2	CZA	4.5-5.3	Bright yellow	Orange			Raised to flat, cottony, granular, with white mycelium, sulcate from the edge to the outer circumference of the centre on PDA, heavily wrinkled to plane and sulcate on the reverse of the plates	Conidiophores developed directly from the substratum, 165-390 µm long, 5.6-8.8 µm wide, rough-walled, hyaline, septate, biseriate. Phialides 4.8-10 µm long, 3.9-7.3 µm wide. Vesicles 12-21 µm diameter, globose to subglobose. Conidia 2-2.7 µm in diameter, globose to sub-globose
	MEA	3.6-4.3	Greenish-yellow	Brown				
	PDA	4.1-5.1	Yellowish-green	Pale yellow to brown				
	RBA	3.8-4.8	Yellowish-green	Colourless				
Aspergillus sp.3	CZA	2.1-2.9	Pale yellow	Colourless			Slightly raised, lanose, sulcate, wrinkled, entire	Conidiophores developed directly from the substratum, rough-walled, hyaline to sub-hyaline, septate. Vesicles6.6-8 µm wide, 8.9-13 µm long, oval, biseriate. Conidia 1.9-2.1 µm x 2-2.5 µm, sub-globose to ovoid
	MEA	2.7-3.2	Bluish-grey	Pale yellow		Yellowish		
	PDA	3.8-4.4	Yellowish-white	Pale yellow		Yellowish		
	RBA	3-4.2cm	Pale yellow	Colourless				
Penicillium citrinum	CZA	2.3-3.4	Green, white mycelia	Yellowish-orange	Brown		Velvety, sulcate to fasciculate	Conidiophores developed from surface or subsurface hyphae, 110-300 µm long. Stipes smooth-walled, monoverticilate to terverticilate. Metulae cylindrical. Phialides 7-9.3 x 2-2.5 µm, ampulliform. Conidia 2.5-3.1 µm, globose to sub-globose
	MEA	2.3-3.1	Green, white mycelia	Yellowish-orange				
	PDA	2.5-3.6	green, grey mycelia	Orange	Brown			
	RBA	2.4-3.3	Orange, white mycelia	Yellowish-orange				
P. commune	CZA	2.1-3.2	Grey, white mycelia	Reddish-brown			Velvety to floccose, granular, radially sulcate to slightly sulcate	Conidiophores developed from surface or subsurface hyphae. Stipes smooth-walled, terverticilate. Metulae 13-17 µm wide, cylindrical. Phialides 7.9-9.2 µm long, ampulliform. Conidia 3.1-3.8 µm wide, ellipsoidal to globose.
	MEA	2.6-3.7	pale green, white mycelia	brown				
	PDA	3.5-3.9	Pale grey, yellow centre, white mycelia	Yellow				
	RBA	3.9-4	Dark green, yellow centre, white mycelia	Colourless				
P. waksmanii	CZA	2.0-2.8	Dull green, white mycelium	Cream			Velvety to floccose, plane to radially sulcate	Conidiophores developed from the subsurface hyphae. Stipes smooth-walled, biverticillate and 210-440 µm long. Metulae 5-6 µm long, cylindrical, ampuliform. Phialids 6.5-8.8 µm long. Conidia 2.6-3.2 µm diameter, globose.
	MEA	2.1-3.0	Dull green, pale green mycelium	Brown	Transparent			

	PDA	1.6-2.5	Dull green, pale yellow mycelium	Yellow				
	RBA	1.4-2.3	Dull green, pale yellow mycelium	Colourless	Yellow			
Penicillium sp.1	CZA	1.7-2.0	Yellowish-green, white mycelium	Brown			Granular, powdery to velvety, flat to umbonate centrally, irregular and entire to slightly undulate, radially sulcate to slightly sulcate	Conidiophore hyaline, straight to erect, septate, monoverticillate, unbranched. Metulae absent. Phialides 3.2-4.5 µm wide and 9.1-12.3 µm long, hyaline, ampulliform. Conidia 3.7-4 µm wide, hyaline, 1-celled and globose
	MEA	1.9-2.5	Deep green, white mycelium	Brown				
	PDA	2.5-3.5	Sage green, white mycelium	Brown	Colourless			
	RBA	2.4-3.6	Greyish-green, white mycelium	Colourless	Colourless			
Penicillium sp.2	CZA	2.2-3	Yellowish	Bright yellow			Slightly velvety, sulcate to smooth, entire.	Conidiophores developed from the surface or subsurface. Stipes simple, smooth, terverticilate. Metulae cylindrical. Phialides ampulliform. Conidia 3.9-3.3 µm wide, short chains, 1-celled and globose to sub-globose
	MEA	2.5-3.7	Yellowish	Dark brown				
	PDA	2.5-3.6	Yellowish	Bright yellow		Yellow		
	RBA	2.5-3.9	Yellowish	Dark brown		Yellow		
Trichoderma hamatum	CZA	4.6-5.1	Bluish-green	Pale brown			Compact tufts, effuse, flat to slightly raised, pustules distributed irregularly	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 2.9-3.9 µm wide, 4.6-8.9 µm long, hyaline, flask-shaped, tapered towards the apex, septate, densely clustered on board. Conidia 2.3-3 µm wide, 3.3-4.3µm long, hyaline, 1-celled, oblong to ellipsoidal. Chlamydospore 7.5-11.3 µm in diameter, terminal and intercalary, sub-globose to ellipsoidal
	MEA	4.5-5.0	Bluish-green	Pale brown				
	PDA	4.4-5.1	Bluish-green	Pale brown				
	RBA	4.3-5.3	Yellowish-green	colourless				
T. harzianum	CZA	4.7-5.3	Yellowish green	Pale yellow			Floccose, powdery, slightly raised, 1-2 concentric rings, white pustules and green conidia distributed irregularly over the surface	Conidiophore 64-108.9 µm long, hyaline, erect, highly branched, side branches stand at right angles to the bear tip. Phialides 3.7-6.5 µm long, ampulliform, short and board in the middle, convergent. Conidia 2.4-2.9 µm in diameter, hyaline, 1-celled, globose. Chlamydospore 6-8.4µm in diameter, sub-globose
	MEA	4.9-5.5	Yellowish green	Pale yellow				
	PDA	4.8-5.6	White	Pale yellow				
	RBA	4.3-5.1	Yellowish green	Colourless				
T. koningii	CZA	3.9-4.3	Greenish-yellow	Brown			Floccose to compact tufts, flat to slightly raised, greenish pustules and conidia distributed irregularly over the surface, smooth	Conidiophore hyaline, erect, highly branched, side branches stand at right angles to the bear tip, 2 to 3 phialides on each branch developed in opposite pairs. Phialides 2.1-2.9 µm wide and 7.7-9.8 µm long, 3 or 4 whorls, hyaline, flask-shaped, board in the middle, tapered towards the apex. Conidia 2.7-3.3 µm wide and 3.8-4.2 µm long, hyaline, 1-celled, subglobose to ellipsoidal. Chlamydospore 9.2-12.8 µm in diameter, terminal and sub-globose
	MEA	4.1-4.8	Bluish-green	Pale yellow				
	PDA	4.3-5.1	Bluish-green	Pale yellow				
	RBA	4.2-4.9	Bluish-green	Colourless				

T. viride	CZA	4.5-5.1	Yellowish-white	Pale yellow			Floccose, powdery, white pustules and green conidia distributed irregularly over the surface, with a yellowish ring, slightly raised to flat and undulate	Conidiophore hyaline, erect, highly branched, side branches stand at right angles to the bear tip, 2 to 3 phialides developed on each branch in opposite pairs. Phialides 2.2-3.2 µm wide and 6.7-10.9 µm long, hyaline, solitary or in whorls of 2-3, flask-shaped, cylindrical to board in the middle, ampulliform. Conidia 3-4.5 µm wide and 4 µm long, hyaline, 1-celled, globose to obovoid. Chlamydospore 5.5-7.3 µm in diameter, intercalary and globose to sub-globose
	MEA	4.7-5.4	Light-green	Brownish				
	PDA	4.6-5.4	Yellowish-white	Pale yellow				
	RBA	4.4-5.2	Yellowish-white	Colourless				
Trichoderma sp.1	CZA	0.8-1.3	Bluish-green	Colourless			Floccose, powdery, flat to slightly raised, pustules white, abundant and dense at the edge of the plates, green conidia distributed irregularly at the edge of the plates	Conidiophore 64-108.9 µm long, hyaline, erect, sparingly branched, side branches short. Phialides 2.9-3.5 µm wide and 6.4-8.9 µm long, solitary to 2-5, irregular, ampulliform to lageniform, board in the middle, bent at the tip. Conidia 1.8-2.7µ m wide and 2.9-4 µm long, hyaline, 1- celled, oval to ellipsoidal. Chlamydospore 7.9-10.4 µm in diameter and globose
	MEA	4.3-5.2	Light-green	Colourless				
	PDA	4.7-5.5	Bluish-green	Colourless				
	RBA	4.2-4.9	Bluish-green	Colourless				
Trichoderma sp.2	CZA	1.1-1.7	Green	Yellow			Floccose to compact tufts, whitish pustules and green conidia distributed irregularly over the surface, flat to umbonate, irregular	Conidiophore 51-98.8 µm long, hyaline, slightly erect, branched, side branches stand at right angles to the bear tip, 3 phialides on each branch arise in opposite pairs. Phialides 2-2.7 µm wide and 7.1-8.3 µm long, hyaline, ampulliform, board in the middle, tapered towards the apex. Conidia 1.8-3 µm wide and 2.9-3.8 µm long, hyaline, 1-celled, sub-globose. Chlamydospore 6.3-8.1µm wide, intercalary and sub-globose.
	MEA	4.9-5.4	Yellowish	Yellow				
	PDA	4.9-5.5	Yellowish	Yellow				
	RBA	4.4-5	Yellowish	Colourless				

CD-colony diameter, CCO-colony colours in obverse, CCR-colony colours in reverse

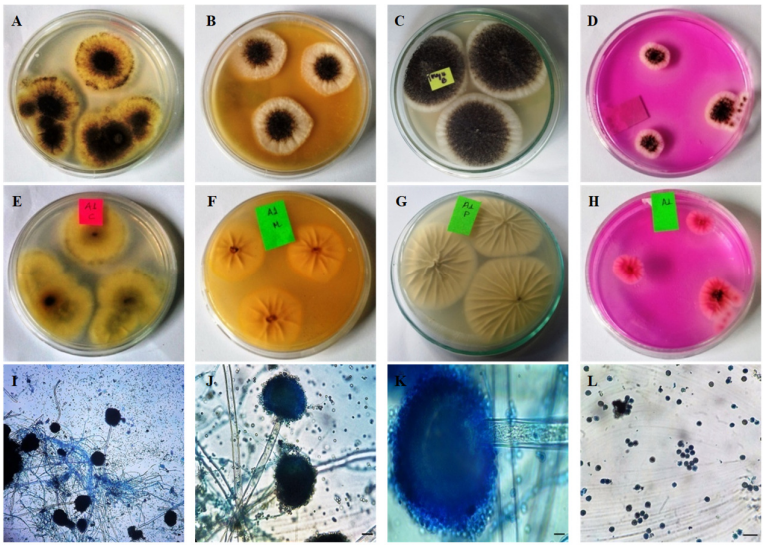

Figure 1. Culture and microscopic features of *Aspergillus niger*
(A-D) Obverse view of isolate on CZA, MEA, PDA and RBA plates; (E-H) Reverse view of isolate on CZA, MEA, PDA and RBA plates; (I) Conidiophores with conidial heads under 10X; (J) Conidiophores with conidial heads under 40X; (K) Conidiophore with conidial head under 100X; (L) Conidia under 100X; Scale bars represent 10 µm

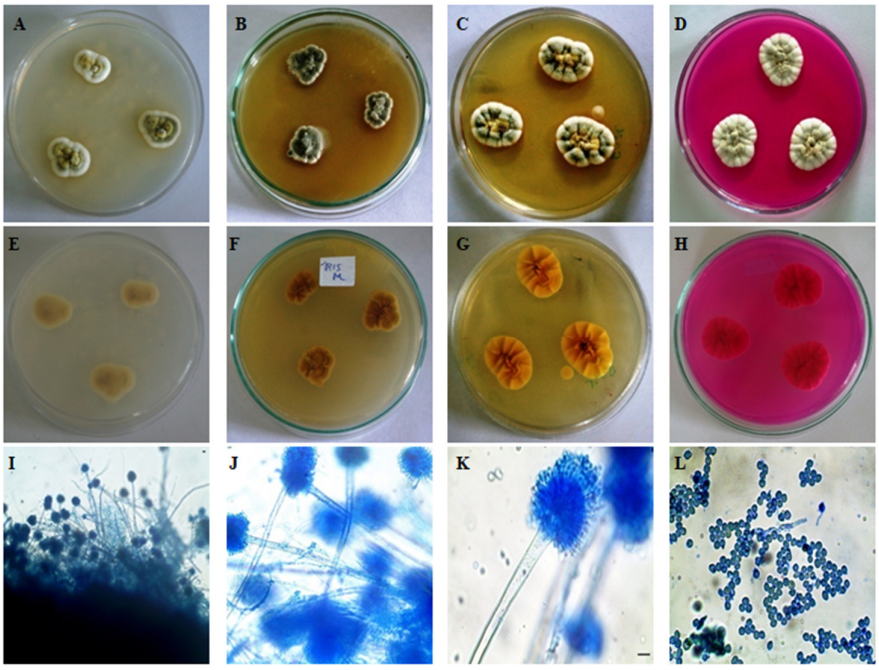

Figure 2. Culture and microscopic features of *Aspergillus* sp.2
(A-D) Obverse view of isolate on CZA, MEA, PDA and RBA plates; (E-H) Reverse view of isolate on CZA, MEA, PDA and RBA plates; (I) Conidiophores with conidial heads under 10X; (J) Conidiophores with conidial heads under 40X; (K) Conidiophore with conidial head under 100X; (L) Conidia under 100X; Scale bars represent 10 µm

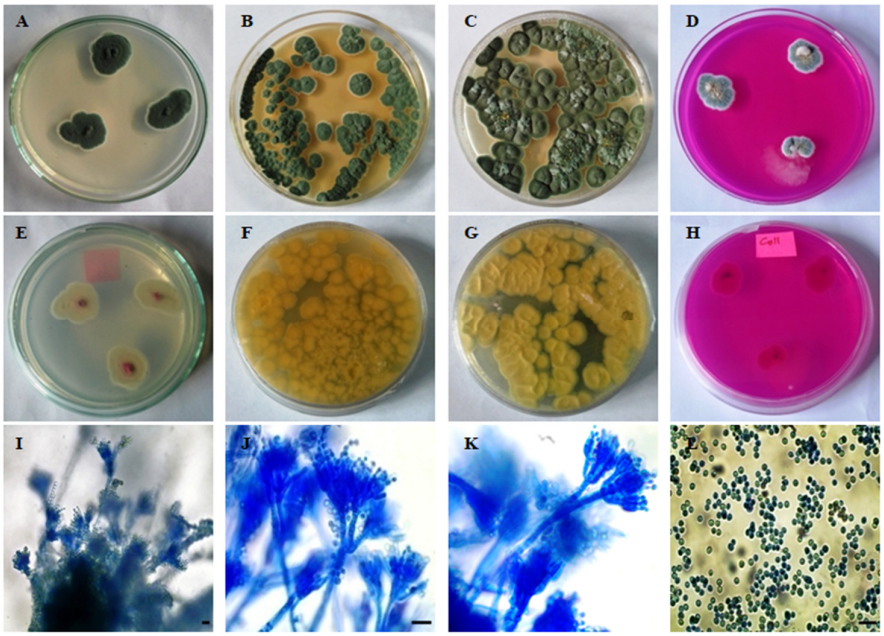

Figure 3. Culture and microscopic features of *Penicillium citrinum*
(A-D) Obverse view of isolate on CZA, MEA, PDA and RBA plates; (E-H) Reverse view of isolate on CZA, MEA, PDA and RBA plates; (I-K) Conidiophore with conidia under 40X and 100X; (L) Conidia under 100X; Scale bars represent 10µm

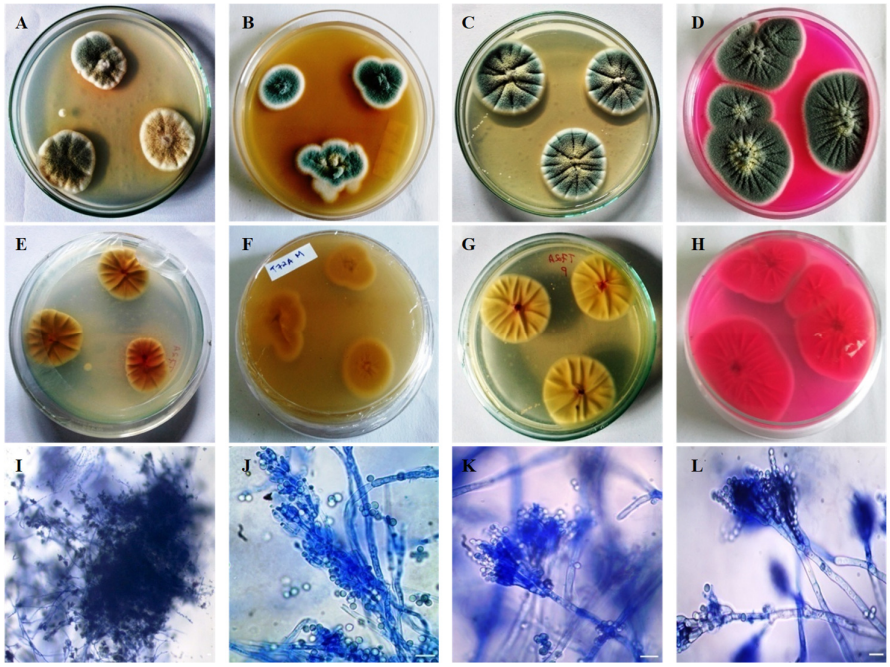

Figure 4. Culture and microscopic features of *Penicillium commune*
(A-D) Obverse view of isolate on CZA, MEA, PDA and RBA plates; (E-H) Reverse view of isolate on CZA, MEA, PDA and RBA plates; (I) Mycelium and conidiophore with conidia under 10X; (J-L) Conidiophore with conidia under 100X; Scale bars represent 10 µm

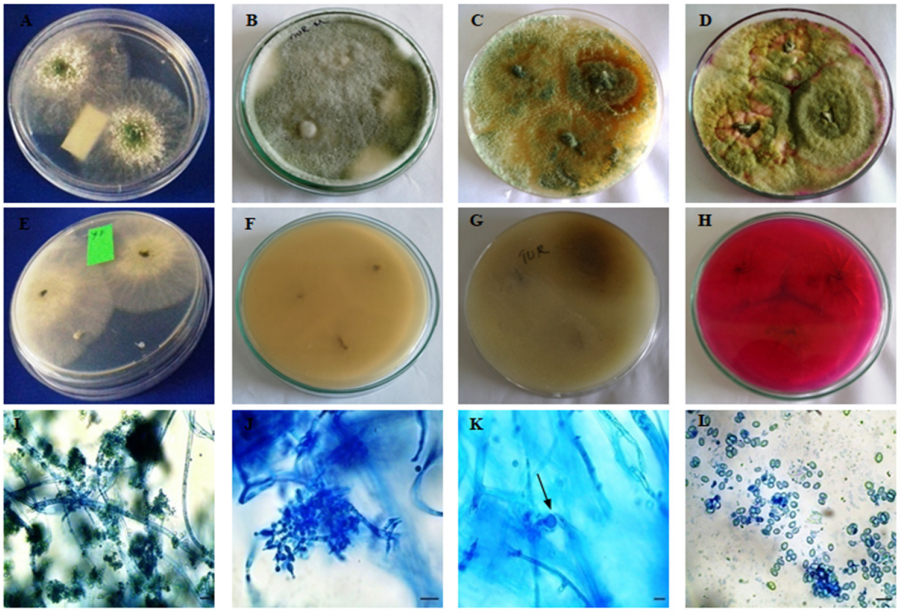

Figure 5. Culture and microscopic features of *Trichoderma hamatum*
(A-D) Obverse view of isolate on CZA, MEA, PDA and RBA plates; (E-H) Reverse view of isolate on CZA, MEA, PDA and RBA plates; (I-J) Conidiophores with phialides and conidia under 40X and 100X; (K) Chlamydospore (arrow) under 40X; (L) Conidia under 100X; Scale bars represent10 µm

Formation of halo zones and phosphate solubilization index

Fungal genera that dominated the study areas were screened for phosphate solubilization ability. In total, 15 isolates belonging to the dominant genera were selected. These isolates showed a clear halo zone on PVK agar plates after five days, which indicated that all these isolates exhibited the desired phosphate solubilizing ability. As presented in Table 4, the highest SI was recorded in plates inoculated with *Aspergillus* species (1.95 cm to 1.71 cm) followed by *Penicillium* species (1.57 cm to 1.18 cm). Contrary to this, the lowest SI was recorded in plates inoculated with *Trichoderma* species (1.13 cm to1.06 cm).

Table 4. Assessment of phosphate solubilization of dominant fungi

Fungal isolates	SI (in cm)
Aspergillus niger	1.95 ±0.03
Aspergillus sp.1	1.81 ± 0.02
Aspergillus sp.2	1.71 ± 0.03
Aspergillus sp.3	1.75 ± 0.03
Penicillium citrinum	1.57 ± 0.02
P. commune	1.39 ± 0.01
P. waksmanii	1.18 ± 0.01
Penicillium sp.1	1.20 ± 0.01
Penicillium sp.2	1.22 ± 0.01
Trichoderma hamatum	1.10 ± 0.01
T. harzianum	1.06 ± 0.01
T. koningii	1.08 ± 0.01
T. viride	1.09 ± 0.01
Trichoderma sp.1	1.13 ± 0.01
Trichoderma sp.2	1.09 ± 0.01

Discussion

A number of microfungal isolates under different genera were identified in the present study as revealed by their growth and morphologies in different culture plates and microscopic observations. The morphological characterization from culture plates and microscopic studies is crucial for the identification of fungi. Culture plate studies using different media provide valuable information on fungal mycelia growth, sporulation and release of exudates as well as pigments which vary between culture media differing in nutrient types and compositions. Likewise, photomicrography through microscopic study is an important and the most commonly used descriptive illustrating method for morphological characterization of fungi (Senanayake *et al.*, 2020). Although several microfungi were isolated from the tea garden soils, however, species of *Aspergillus*, *Penicillium* and *Trichoderma* dominated the studied tea garden soils. This agrees with Karaoglu and Ulker (2006) who reported that the tea garden soil fungi belong to a restricted range of taxonomic groups with a few dominant and some rare species. More or less similar observation with the present study was made by Pandey *et al.* (2001) who reported the dominancy of *Penicillium* and *Trichoderma* among the fungi in the rhizosphere of established tea bushes. The dominancy of *Aspergillus*, *Penicillium* and *Trichoderma* indicates their versatile nature and depicts 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. It could also be due to the ability of these fungi to utilize different substrates in the soil more readily over other species (Gomez *et al.*, 2006), antagonistic activity exhibited by these genera against other fungi and the tea plant itself. According to Pandey *et al.* (2001), tea roots exhibited selectivity

towards fungal species like *Penicillium*, *Trichoderma*, *Paecilomyces* and *Cladosporium* in comparison to other species.

The dominant microfungi were able to solubilize phosphate in the culture plate assay revealing that the tea garden soils in the Mokokchung district of Nagaland support a diverse group of phosphate solubilizers. Species of *Aspergillus*, *Penicillium* and *Trichoderma* showed great phosphate SI which indicates that the presence of these microfungal genera in the soil might be beneficial to P nutrition and tea growth. These microfungi are also among the economically important fungal genera serving plant and soil in tea and other types of agricultural fields (Pandya *et al.*, 2018; Thiep *et al.*, 2019). In this study, species of *Aspergillus* were the most effective phosphate solubilizers than other species tested as shown by phosphate SI on the agar plate. The present result corroborates the findings by Yadav *et al.* (2011) who found significantly higher SI by species of *Aspergillus* followed by *Penicillium* and *Trichoderma* species. This may be due to the higher production of organic acids by *Aspergillus* species (Yadav *et al.*, 2011). Additionally, the type and diffusion rates of organic acids by the phosphate solubilizers can be accounted for in the present result.

Conclusions

The morphological characterization revealed that the tea garden soils in the Mokokchung district of Nagaland has diverse fungal species however, there was selectivity towards several fungal genera. *Aspergillus*, *Penicillium* and *Trichoderma* dominated among the tea soil fungi probably due to their greater ability to utilize different substrates in the soil, antagonistic activity against other fungi, a greater rate of spore production as well as greater spore dispersal rate and resistance against extreme environmental conditions. Phosphate solubilization assay carried out with these dominant fungal genera showed that species under *Aspergillus* were the most effective phosphate solubilizers which were followed by *Penicillium* and *Trichoderma* species. The present study will aid and expand knowledge on indigenous fungi and their various roles. The potent phosphate-solubilizers isolated in this study can be a future source of biofertilizers consortium for tea and other plants and hence, provide economically and environmentally viable strategy towards plant growth-promoting strategies. In the present study, fungal identifications were carried out using morphological methods however, further studies employing molecular, biochemical, and physiological methods are important. The experiment was carried on solid media under laboratory conditions therefore, detailed investigation under field conditions must be considered to observe the potentialities of these fungi in improving tea productivity.

Authors' Contributions

TJ - Concept development, sample collection, methodology, writing original draft; TA and AK - Validation supervision, review and editing of the original draft. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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