Colonial and Morphological Characteristics of Soil Fungi from Jhum Land

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ABSTRACT

Soil fungi represent one of the important microbial groups that are actively involved in enhancement of environmental quality and plant nutrient supply. Studying native soil fungal species and their accurate identification is crucial for contribution to the checklist of fungi. The present study aimed at isolation and identification of some soil fungi from Jhum land, Mokokchung district, Nagaland. Serial dilution method was used to isolate soil fungi on RBA (Rose Bengal Agar) and PDA (Potato Dextrose Agar) plates. Altogether fourteen fungal species belonging to genera Absidia, Alternaria, Aspergillus, Cladosporium, Fusarium, Geotrichum, Mortierella, Mucor, Penicillium and Trichoderma were identified by studying their macro- and micro-morphological characteristics.

Key words: Fungi, Jhum, Morphological, Serial dilution, Soil.

INTRODUCTION

Shifting cultivation locally called jhum is a traditional cultivation practice in the hilly regions of North East India basically involved in slashing and burning of an area followed by cropping over the burnt area for a year or two followed by variable fallow period allowing natural processes of soil regeneration (Devi and Choudhury, 2013) however at present, jhum fallow period is drastically shortened to 2-3yrs from 20-30 years which leads to various environmental deterioration and degradation of soil and microbial properties (Arunachalam and Pandey, 2003). Along with environmental factors such as soil depth and nature, pH, temperature, season of the year, state of the cultivation, moisture and aeration Gaddeyya et al. (2012), slashing and burning practices involved in jhum cultivation effects soil properties, diversity and abundance of soil microbial population Miah et al., (2014). Also, microbial activities in the soil are found to be substantially affected by crop management practices Dhull et al., (2005). Agricultural practice such as long-term usage of chemical fertilizers instigates soil degradation thereby creating an imbalance in soil microflora (Jamir and Ajungla, 2018) which over a period of time will create significant changes in the level of soil organic matter and soil productivity (Manna and Ganguly, 2001; Kumar et al., 2012).

Among the soil microbes, fungi constitute an important part of the soil ecosystem. Owing to their degradation abilities fungi play major role in decomposition of organic matter. They are active role players in nutrient cycling which give positive impact on soil fertility, plant productivity and biodiversity of an area. Apart from being active nutrient cyclers, soil fungi also act as biofertilizers and bioremediaters as they play major role in soil activities like biochemical transformation and mineralization Magnet et al. (2013) which influence the structure and functioning of an ecosystem and thus play a key role in many ecological services Ramkumar et al. (2017). In addition, fungi are of immense value in agriculture, food and industries as they are source of many enzyme, antibiotics and medicine Abed et al. (2017) that makes fungi an important group of microbe to study. Only 5-10% of the overall estimated 1.5 million worldwide fungal species is considered to be formally characterized so far Seth et al. (2016) and out of total 1.5 million fungal species 1/3 exists in India indicating the need to explore and identify the unexplored native fungal communities Chandrashekar et al. (2014). Although molecular methods, physiological and biochemical methods are important for fungal identifications, morphological properties basing on culture and microscopical characteristics are still commonly used method and hence, they are essential for identification of fungal species (Chandini and Rajeshwari, 2017). Morphological characterizations of fungal species which are generally done on the basis of their colony features and microscopical examinations such as their colony characters, size, colour changes, exudates secretions, mycelia arrangements, spores orientations etc. all of which gives visible information on the studied fungal species. The present study aims to isolate and identify microfungi from selected jhum land of Mokokchung district in Nagaland, India.

MATERIALS AND METHODS

Soil sampling: Soil samples were collected from cultivated and abandoned jhum land under Mokokchung district, Nagaland during the month of January 2017.

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Physico-chemical parameters of soil sample: Soil moisture content was estimated on the same day by oven-dried method (Anderson and Ingram, 1993). The pH value was measured using soil pH meter in 1:2 soil and water ratio Jackson (1967). Soil organic carbon was determined following (Walkley and Black, 1934).

Isolation of Fungi: Fungi were isolated in Potato dextrose agar (PDA) and Rose bengal agar (RBA) plates following serial dilution method. PDA used was from Himedia, India while composition of RBA prepared in the laboratory were as follows: Dextrose 10g, Peptone 5g, Potassium dihydrogen ortho-phosphate 1g, Magnesium sulphate 0.5g, Agar 20g, Rose bengal 0.033g, Distilled water upto 1000ml. Antibiotic streptomycin of 0.03g was added in each media. One gram of soil samples was suspended to 9ml of sterile distilled water to obtain 10⁻¹ dilution and a series of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ diluents were placed in triplicates onto RBA and PDA plates. Plates were incubated at 25±1°C for 5-7 days. For fungal population, colony forming units (CFU) were estimated by counting the number of the colonies after five days and CFU was calculated on per gram dry soil basis. Resulting fungal colonies were observed and transferred to appropriate identification media.

Morphological and microscopic characterization of fungi: Macroscopic characters such as colony diameter, exudates, colony reverse and microscopic characteristics like coniodophore, vesicle, metulæ, phialides and conidia for all isolates were observed. For microscopic characteristics, slides were prepared, stained with cotton blue and studied under compound and light (Moitic) microscope. Detailed examination was done following relevant literatures for identification of fungi (Afzal et al., 2013; Domsch, 1980; Duss and Laane, 1984; Hauser, 2006; Ho et al., 2004; Thilagam, 2018; Thathana et al., 2017, Wagner et al., 2013, Watanabe, 1937; Webster and Weber, 2007).

RESULTS AND DISCUSSION
Physicochemical properties of soil: Soil physico-chemical analysis showed that moisture content, carbon content and pH level were higher in CJL than in AJL (Table 1). Increase in soil pH in CJL was attributed by fire used for burning slashed vegetation. Similar findings where slashing and burning cause an increase in pH in jhum land was also recorded by Osman et al., (2017), Devi and Choudhury (2013) and Tawnenga et al., (1997). Higher organic carbon
content in CJL (9-year-old jhum fallow) than in AJL (1-year-old jhum fallow) can be due to the length of the fallow period as also reported by other workers through their studies that length of the fallow period often plays a crucial role in conserving soil organic carbon and maintaining soil health (Osman et al., 2013, Devi and Choudhury 2013 and Arunachalam, 2003).

**Morphological and microscopic characterization of fungi:** Results of this study demonstrated that jhum land can be considered as a valuable natural source of soil fungi. Comparing the two jhum lands, fungal population and diversity was comparatively higher in CJL as compared to AJL. Fungal CFU was 4.8x10^5/grams soil while in AJL it was 2.5x10^5/grams soil. Higher soil organic matter could have favoured the growth of fungal population in CJL as soil pH, moisture and organic carbon content influences fungal population and diversity in jhum land (Gaddeyya et al., 2012). Nine fungal species was isolated and identified from cultivated jhum soil (*Absidia cylindrospora*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium* sp., *Geotrichum candidum*, *Mortierella* sp., *Mucor circinelloides*, *Trichoderma harzianum*) and five fungal species from abandoned jhum soil (*Absidia glauca*, *Alternaria* sp., *Aspergillus* sp., *Mucor racemosus*, *Penicillium* sp.).

*Absidia cylindrospora* (Fig 1): Colony morphology at first white becoming grayish brown with age in both RBA and PDA, fast growing, reverse pale yellow. Zygospores covered with stellate projections, finger-like appendages arising from suspensors. Sporangiophores arising singly or branched, 2-3 from the same place of the stolon bearing apophysate sporangia and with a septum, upto 200µm long and 1.8-2.5µm wide. Sporangia 10-12 µm in diameter, pyriform, smooth, columella present. Cylindrical spores, 2.1-2.4x3.5-4.1 µm with rounded ends.

*Absidia glauca* (Fig 2): Colony color initially white turned bluish green in PDA and brownish olive in RBA, reverse colorless and fast growing. Sporangiophores erect, 2-3 arising from the same place on stolons, 260-300µm long, 4.3-6µm wide, with a septum and rhizoids present. Sporangia pyriform, 22.2-48.3µm in diameter with columellae above apophysis, smooth with pointed spine at top.

*Alternaria* sp. (Fig 3): Colony appeared olive and velvety in both PDA and RBA reverse brown in PDA and fast growing. Hyphae septate, conidiophore 2-4 µm wide, septate, curved, light golden brown in color. Conidia solitary with short conical beak with germ tubes, golden brown, two to seven transverse septa with one or two longitudinal septa, 3-10 x 10-20 µm.

*Aspergillus flavus* (Fig 4): Colony greenish-yellow, granular in RBA and PDA, reverse pale yellow in PDA. Conidiophores colourless, rough walled bearing vesicle. Vesicles globose to subglobose. Conidial heads radiating, biseriate and uniseriate. Phialides bearing conidia measuring 3-4 µm in diameter.

*Aspergillus niger* (Fig 5): Colonies brownish black, grow fast reaching on RBA and PDA. Reverse grayish black in RBA and creamish in PDA. Conidiophores sub-hyaline and pale brown, erect, smooth, unbranched, and asceptate. Conidial heads biseriate, brown black with globular, asceptate unbranched chain of conidia. Conidia mostly measured 3-3.4 µm in diameter and appeared brownish, globose and rough.

*Aspergillus* sp. (Fig 6): Colony slow growing, greenish-brown with exudate and white margins on both PDA and RBA plates, velvety, reverse brown in PDA. Conidiophores unbranched and sub-hyaline, straight to flexuous, smooth and asceptate, 2-3µm wide. Vesicles globose to sub-globose, conidial heads sub-spherical, phialides borne directly on the vesicle and uniseriate. Conidia globose, smooth and 1.5-2µm in diameter.
Fig 3: Pictures of Alternaria sp.

A: Colony on PDA (top)
B: Colony on PDA (reverse)
C: Colony on RBA (top)
D: Colony on RBA (reverse)
E: Chains of macroconidia under 40x, bar = 50µm
F: Septate conidia under 100x, bar = 50µm

Fig 4: Pictures of Aspergillus flavus.

A: Colony on PDA (top)
B: Colony on PDA (reverse)
C: Colony on RBA (top)
D: Colony on RBA (reverse)
E: Conidiophore with globose vesicles under 40x, bar = 10µm
F: Globose vesicle under 100x, bar = 10µm

Fig 5: Pictures of Aspergillus niger.

A: Colony on PDA (top)
B: Colony on PDA (reverse)
C: Colony on RBA (top)
D: Colony on RBA (reverse)
E: Conidiophore with globose vesicles under 40x, bar = 10µm
F: Globose conidial head under 40x, bar = 10µm
**Cladosporium cladosporioides** (Fig 7): Colonies were olivaceous-gray, velvety and reverse brown on RBA and PDA plates. Aerial mycelium sparse, mycelium septate mostly unbranched, sub-hyaline. Conidiophores erect (2-3µm wide), cylindrical to cylindrical-oblong bearing numerous conidial chains arising below septa. Conidia ellipsoidal to lemon-shaped, mostly smooth walled, 3-7x2-4 µm.

**Fusarium sp.** (Fig 8): Colonies were slow growing, white colony in RBA and PDA. Hyphae, conidiophores, phialides and macroconidia present. Conidiophores consisting of simple lateral monophialides, irregularly branched (10-20µm long). Macro-conidia varied in size, straight to slightly curved in the extremities, 3-4 septate.

**Geotrichum candidum** (Fig 9): Colony morphology appeared yeast-like spreading across the culture plate. Growth non-aerial, creamish in color with a fruity fragrance. Conidiophore absent, arthroconidia was observed which were rectangular in shape with thick walls and 1-celled, 3-10x2-5µm.

**Mortierella sp.** (Fig 10): Colony produced a concentric pattern of growth and milky white, cottony, have a thin spreading mycelium in RBA and PDA plates, reverse cream colored in PDA. Conidiophores unbranched, less than 100 µm in length, aseptate, slightly widened below sporangium. Sporangia globose, smooth, 9-11µm in diameter, small chlamydospores abundantly present, thick walled, irregular in shape, 9-10µm in diameter. Conidia globose, 9-11 m in diameter.

**Mucor circinelloides** (Fig 11): Colonies first white turning pale yellowish PDA and RBA. Reverse is pale yellow.
**Fig 8:** Pictures of *Fusarium sp.*

A: Colony on PDA (top)  
C: Colony on RBA (top)  
E: Conidiophore and conidia under 40x, bar=10µm

B: Colony on PDA (reverse)  
D: Colony on RBA (reverse)  
F: Macroconidia under 100x, bar=10µm

**Fig 9:** Pictures of *Geotrichum candidum.*

A: Colony on PDA (top)  
C: Colony on RBA (top)  
E: Anthroconidia under 40x, bar=10µm

B: Colony on PDA (reverse)  
D: Colony on RBA (reverse)  
F: Anthroconidia under 100x, bar=10µm

**Fig 10:** Pictures of *Mortiella sp.*

A: Colony on PDA (top)  
C: Colony on RBA (top)  
E: Conidiophore under 40x, bar=50µm

B: Colony on PDA (reverse)  
D: Colony on RBA (reverse)  
F: Chlymadospore under 100x, bar = 10µm
Fig 11: Pictures of *Mucor circinelloides*.

A: Colony on PDA (top)  
B: Colony on PDA (reverse)  
C: Colony on RBA (top)  
D: Colony on RBA (reverse)  
E: Sporangiohores under 10x, bar=50µm  
F: Globose sporangiospore under 40x, bar=50µm

Fig 12: Pictures of *Mucor racemosus*.

A: Colony on PDA (top)  
B: Colony on PDA (reverse)  
C: Colony on RBA (top)  
D: Colony on RBA (reverse)  
E: Sporangiohores with chlamydospore under 40x, bar=10µm  
F: Chlamydospores under 100x, bar=10µm

Fig 13: Pictures of *Penicillium sp.*

A: Colony on PDA (top)  
B: Colony on PDA (reverse)  
C: Colony on RBA (top)  
D: Colony on RBA (reverse)  
E: Branching of conidiophore under 40x, bar=10µm  
F: Smooth conidia under 100x, bar=10µm
Colony was fast growing. Sporangiophores were branched sympodially, hyaline terminated by sporangium. Columellae present. Sporangiospores were globose, echinulate and 20-23.5 µm in diameter.

**Mucor racemosus** (Fig 12): Colonies on PDA and RBA smoke-grey, fast growing. Reverse pale yellow. Sporangiophores branched sympodially and monopodially, columellae subglobose. Numerous chlamydospores present in the sporangiophores, barrel-shaped to subglobose, 20-25µm in diameter.

**Penicillium sp.** (Fig 13): Colonies initially white turned bluish green in both PDA and RBA plates, velvety, fast growing, colourless exudate in PDA. Hyphae septate, branched conidiophores, less than 100µm in length, 2-3 metula, phialides swollen at the base, conidia produced in chains from phialides, conidia smooth and round, 3.4-4µm in diameter.

**Tricoderma harzianum** (Fig 14): Colonies fast growing in PDA and RBA, green yellowish in PDA and green whitish in RBA. Reverse brown in PDA. Conidiophores hyaline, bearing right angled branches to the tip. Phialides 2-3 in each branch, flask shaped and appeared in pairs. Conidia short, 2.6µm in diameter, globose, smooth and 1-celled.

**CONCLUSION**
The present study showed variations among soil properties between two different jhum land in Mokokchung district, Nagaland. A total number of 14 fungal species belonging to 10 different genera were identified from the study areas. Highest number of species recorded in the present study was *Aspergillus* with a total of 3 species followed by *Absidia* and *Mucor* each with 2 species. Diversity of fungal genera can be observed in the study areas therefore, further diversity analysis and molecular identifications using molecular techniques are recommended.

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**REFERENCES**
Afzal, H., Shazad, S. and Un Nisa, S.Q. (2013). Morphological identification of *Aspergillus* species from the soil of Larkana district (Sindh Pakistan). *Asian Journal of Agriculture and Biology*. 1:105-117.
Anderson, J.M. and Ingram, J.S.I. (Eds.) (1993). Tropical soil biology and fertility: A handbook of methods (2nd ed.). Wallingford:CAB international.
Arunachalam, A. (2003). Role of microbial biomass in soil nutrient dynamics along jhum cycle gradient. *Journal of Tropical Forest Science*. 15:279-288.
Arunachalam, A. and Pandey, H.N. (2003). Ecosystem restoration of jhum fallows in Northeastern India: Microbial C and N along altitudinal and successional gradient. *Restoration Ecology*. 11:168-173.
Chandini, K.C. and Rajeshwari, N. (2017). Isolation and identification of soil fungi in Mattavara forest, Chikamagalur, Karnataka. *Journal of Pharmacognosy and Phytochemistry*. 6: 721-726.
Chandrashkekar, M.A., Pai, K.S. and Raju, N.S. (2014). Fungal diversity of rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore district, Karnataka, India. *International Journal of Current Microbiology and Applied Science*. 3: 559-566.
Devi, N.L. and Choudhury, B.U. (2013). Soil fertility status in relation to fallow cycles and landuse practices in shifting cultivated areas of Chandel district Manipur, India. *IOSR Journal of Agriculture and Veterinary Science*. 4:01-09.
Dhull, S.K., Goyal, S., Kapoor, K.K. and Mundra, M.C. (2005). Crop rotation effects on soil organic matter and soil microbial properties. *Indian Journal of Agricultural Research. 39*:128-132.

Domsch, KH (1980). Compendium of Soil Fungi. Academic Press Ltd (London).

Duss, R. and Laane, M.M. (1984). Cytological studies on the life-cycle in *Absidia glauca*, Harem (Mucorales) *Cytologia. 49*: 457-472.

El Abed, N., Salem, I.B., Khedher, M.B., M’Hamdi, M, M’Hamdi, N.B. (2017). Isolation and identification of fungal communities in organic and conventional soils. *International Journal of Current Microbiology and Applied Sciences. 6*:111-1123.

Gaddeyya, G, Niharika, P.S., Bharathi, P. and Kumar, P.K. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Advances in Applied Science Research. 3*:2020-2026.

Hauser, J. T. (2006). Techniques for studying fungi and bacteria. Microbiology Department, Carolina Biological Supply Company, printed in USA.

Ho, H., Chuang, S., and Chen, S., (2004). Notes on zygomycetes of Taiwan (IV): three *Absidia* species (Mucoraceae). *Fungal Science. 19*:125-131.

Jackson, M.L. (1967). Soil chemical analysis. Prentice-Hall of India, pvt. Ltd., New Delhi, 498.

Jamir, T. and Ajungla, T. (2018). Morphological characterization of Fungi in Tea Garden. *International Journal of Basic and Applied Research. 8*:296-303.

Kumar, S., Dahlia, R., Kumar, P., Jhorar, B.S., Phogat, V.K. (2012). Long-term effect of organic materials and fertilizers on soil properties in pear millet-wheat cropping system. *Indian Journal of Agricultural Research. 46*:161-166.

Manna, M.C. and Ganguly, T.K. (2001). Influence of FYM and fertilizer N on soil microbial biomass dynamics, turn over and activity of enzymes in a typic haplustert under soyabean-wheat-fallow system. *Indian Journal of Agricultural Research. 35*:48-51.

Md. Magnet, M.H., Sarkar, D. and Ahmed, Z. (2013). Isolation and identification of bacteria and fungi from soil samples of different industry side in Dhaka city, Bangladesh. *International Journal of Innovative Research & Development. 2*:338-339.

Miah, S., Haque, S.M.S., Sumi, W. and Hossain, M.M. (2014). Effects of shifting cultivation on biological and biochemical characteristics of soil microorganisms in Khagrachari hill district, Bangladesh. *Journal of Forestry Research. 25*:689-694.

Osman, K.J., Jashimuddin, M., Haque, S.M.S. and Miah, S. (2013). Effect of shifting cultivation on soil physical and chemical properties in Bandarban hill district, Bangladesh. *Journal of Forest Research. 24*:791.

Ramkumar, R., Naya, B.K. and Nanda, A. (2017). Studies on the diversity and incidence of soil fungal communities in different cultivated lands. *Journal of Chemical and Pharmaceutical Research. 9*:165-169.

Seth, R.K., Alam, S. and D.N. (2016). Isolation and identification of soil fungi from wheat cultivated area of Uttar Pradesh. *Journal of Plant Pathology and Microbiology. 7*:1-3.

Tawnenga, Shankar, U. and Tripathi, R.S. (1997). Evaluating second year cropping on jhum fallows in Mizoram, North-eastern India: Soil fertility. *Journal of Biosciences. 22*:615-625.

Thathana, M.G., Murage, H., Abia, AL., Pillay, H. (2017). Morphological characterization and determination of aflatoxin-production potentials of *Aspergillus flavus* isolated from maize and soil in Kenya. *Agriculture. 7*: 1-14.

Thilagam, R., Kalaivani, G. and Hemalatha, N. (2018). Isolation and identification of phytopathogenic fungi from infected plant parts. *International Journal of Current Pharmaceutical Research. 10*:26-28.

Wagner, L., Stielow, B., Hoffmann, K., Petkovits, T., Papp, T., Vágólgýi, C., de Hoog, G.S., Verkley, G. and Voigt, K. (2013). A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. *Persoonia. 30*: 77-93.

Walkley, A. and Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil science. 37*:29-38.

Watanabe, T. (1937). Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species (second edition). CRC Press, LLC.

Webster, J. and Weber, R.W.S. (2007). Introduction to fungi (Third edition). Published in the United States of America by Cambridge University press, New York.