



Isolation Of Natural Fungal Microflora From Coffee Fields, Pulp And Husk For Sustainable Management

Kiranmae.P.Nagwand, Associate Professor of Botany, Maharani Science College, Mysuru 570005.

Abstract:

A total of twelve strains of filamentous fungi were isolated from soil, and berries collected in coffee-growing areas of Chikmagalur. Fungal growth and isolation made on PDA culture medium. Most of the isolated microorganisms belong to *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, and *Humicola* genera. A comparative study on the evaluation of natural microflora present in coffee growing soils, wet pulp and coffee husk (dry) revealed the presence of a wide variety of microorganisms. Coffee pulp and husks are the major products in coffee curing industry that usually thrown as waste, because of caffeine and tannins, on land causes pollution. But these mycoflora degrade completely in an ecofriendly manner. Exploration of naturally occurring fungi on the different samples gives the idea of natural decomposition of coffee waste in bulk, in an eco-friendly manner.

Difficult words: PDA, Eco-friendly, Microflora, Caffeine, sustainable, eco-friendly, Natural degradation

Introduction:

Coffee pulp is some of the most abundantly available agro-industrial waste produced during the pulping operation of the coffee cherries to obtain coffee beans in many coffee-producing areas of the tropics (Zuluaga 1981). According to the data available, the world green coffee and coffee pulp production during 1989-1990 reached a maximum of 5.52 x 10⁶ tons (5.61 x 10⁹ kg) of green coffee and 2.76 x 10⁶ tons (2.8 x 10⁹ kg) of coffee pulp (Roussos et al. 1993). Thus, for every 2 tons coffee cherries processed, nearly 1 ton pulp is generated, whereas in the dry process 0.18 ton coffee husk is generated for every ton of fresh coffee cherries (Adams and Dougan 1981). Coffee pulp is essentially rich in carbohydrates, proteins and minerals (especially potassium) and it also contains appreciable amounts of tannins, polyphenols and caffeine (Bressani, 1979). As this product of the coffee industry does not find any commercial application, it is considered to be the major polluting agent of rivers and lakes located near the coffee-processing regions. The presence of proteins, sugars and minerals in coffee pulp and its high humidity favors the rapid growth of microorganisms and, if it is not utilized immediately, it causes environmental pollution. For environmental protection and economic gain, attempts have been made in the past to utilize coffee pulp as an animal feed. However such attempts met with limited success because of the presence of antiphysiological factors such as caffeine, tannins, chlorogenic acid and high levels of potassium (Bressani 1979; Adams and Dougan 1981). Even though there have been many reports describing the composition, conservation, up-grading and utilization of coffee pulp, there is not a single

report on the natural microflora it contains, the agent responsible for causing environmental pollution if not conserved properly (Calle 1951, 1954; Ledger and Tilman 1972; Bressani 1979; Christensen 1981; Orue and Bahar 1985; Martinez-Carrera 1987). Accurate knowledge about the nature and characteristics of coffee pulp natural microflora is of utmost importance for its further utilization.

Materials and method:

Study area:

Chikmagalur is famous for its commercial major crop Coffee, hence named as “Land of Coffee”. Coffee in Chikmagalur district occupies about 85,465 ha spread over in 7 revenue taluks, while Arabica is major variety in hilly areas; Robusta is major in lower altitude. There are about 15,000 growers of which 96% are small growers having holdings up to 4 Ha, or less than 4 Ha. The average production is 55,000 MT comprising of 35,000 MT of Arabica and 20,000 MT of Robusta. The average productivity per ha is 810 Kgs for Arabica and 1110 Kgs of Robusta, which are higher than the national average (www.chickmagalur.nic.in/htmls/cb_main.htm). There are forty five Coffee curing works in Chikmagalur (www.chickmagalur.nic.in/htmls/stati_coftea.htm).

Samples:

The microflora of fungal population cultured from soil, wet beans and husk (dry) samples and compared. The soil samples were taken from Aricinaguppe coffee estate, wet and dry samples from Malnad coffee curing center, Chikmagalur.

The soil samples are taken as 10^{-2} concentration, berries of 10^{-1} , and husk as 10^{-2} . To obtain data on the description and identification of the strains, cultures were grown on potato/dextrose/agar (PDA) medium, observed under a microscope for morphological characteristics and compared by reference to classical keys reported in the literature (Raper and Fennell 1965; Barnet and Hunter 1972; Ainsworth 1973; von Arx 1974).

Methods and materials:

PDA medium has prepared by using the materials as

Composition	
Potato infusion	200gm
Dextrose	20gm
Agar Agar	20gm
Distilled water	1 liter

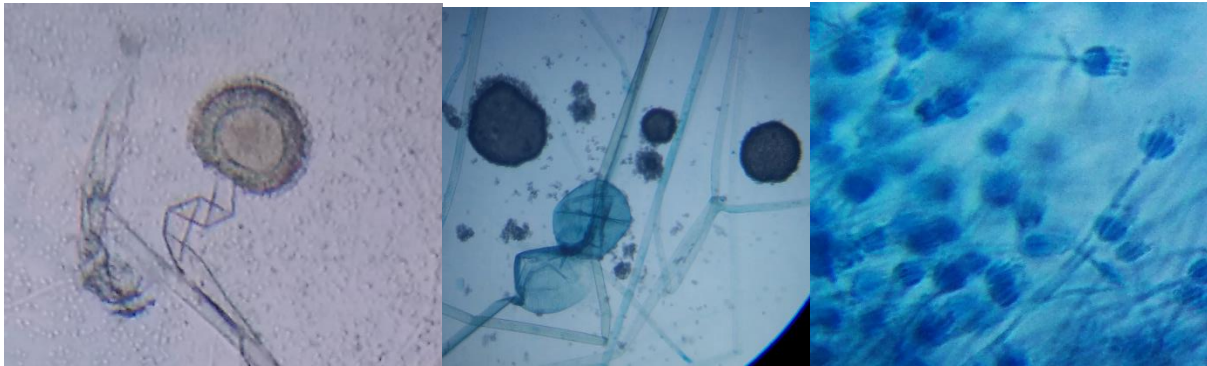
The PDA medium prepared as per standard procedures Agar Agar and Dextrose separately mixed with 400ml of distilled water and autoclaved at 121 °C for 15 min, added 20 gm of potato infusion before distributing the hot liquid to 10 cm plates, up to a depth of 0.5 cm, resulting in approximately 39.3 mL of PDA in each plate.

After solidification of medium, the soil concentrations prepared and inoculated on PDA media.

Result and Conclusion:

There are overall twelve different species of filamentous fungi identified up to genera level and isolated from the soil sample1, soil sample2, wet berries and dry husks. Two species of *Aspergillus*, two species of *Penicillium*, *Phytophthora*, *Curvularia*, *Trichodesma*, *Mucor*, *Humicola*, *Rhizopus*, *Alternaria* and *Fusarium* are the common fungi obtained from the soil sample one and two. But in the dry and wet coffee waste material contains heavy *Aspergillus* species like *A.niger*, *A.flavus*. These are the aflatoxin producing fungi usually appear on the berries

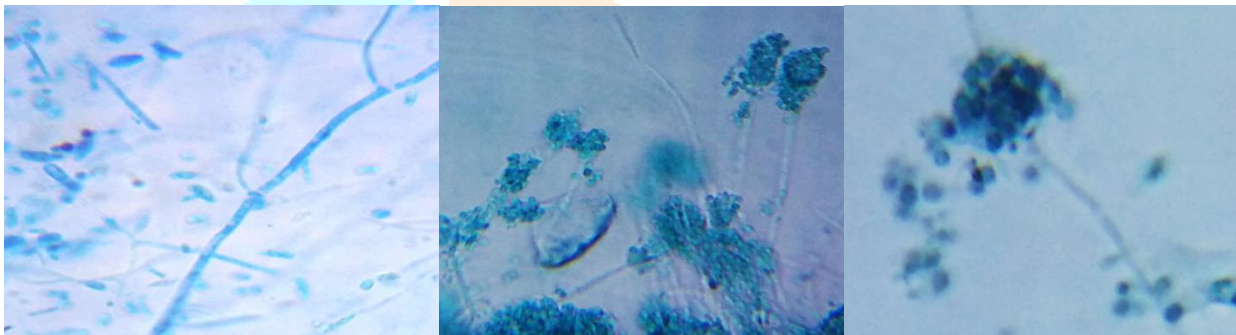
and dry husk causing environmental pollution. Immediate degradation needed for these effluents. Hence, the soil near to humus is (coffee plant debris, dead parts also with caffeine) taken for observing the naturally occurring fungi in the humus, isolated the above said species. All these may have caffeine degradation property. But on effluent *Aspergillus* is found to be predominant. One can spray the isolated fungi on the wet and dry coffee effluent, so that in an eco-friendly manner, the caffeine removal, degradation of it becomes faster.



A.niger

Rhizopus

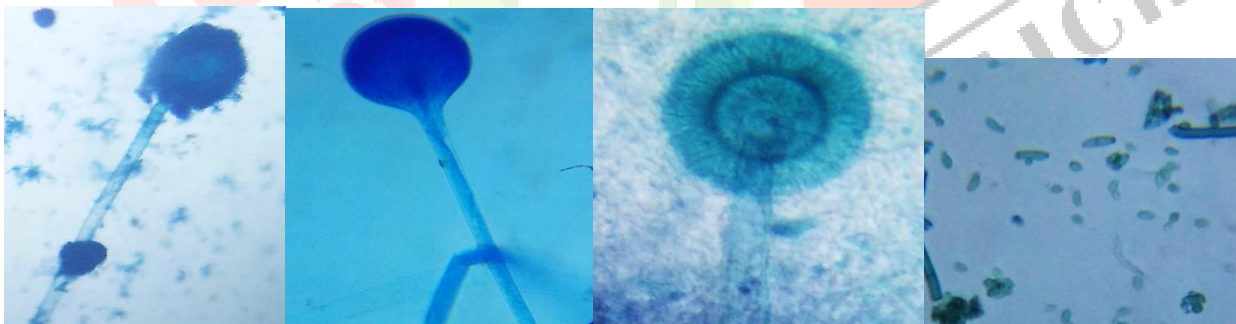
Penicillium



Alternaria

Penicillium

Trichoderma

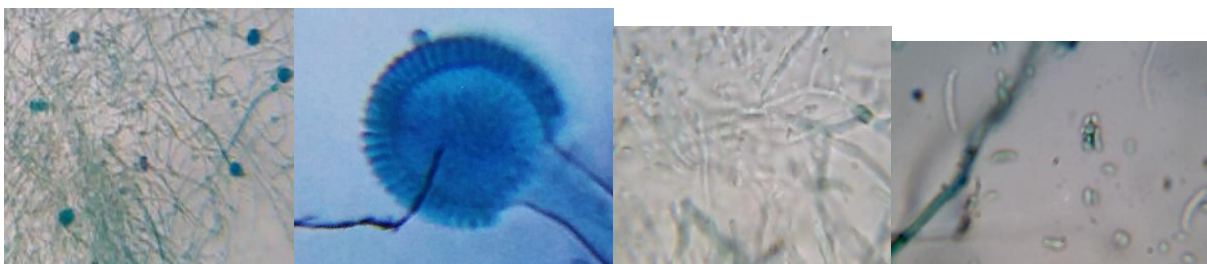


Mucor

Humicola

A.flavus

Alternaria



Phytophthora

Aspergillus

Fusarium

Curvularia

Acknowledgement:

For the analysis I heartily thankful to Department of Botany, IDSG Govt College provided lab facilities and references. Photos are taken through computer images from microscope and photos taken from mobile through microscopic eye-piece.

References: Net

1. www.chickmagalur.nic.in/htmls/stati_coftea.html
2. www.chickmagalur.nic.in/htmls/cb_main.html

Books articles and references:

1. Adams MR, Dougan J (1981) Biological management of coffee processing. *Trop sci* 123:178-196
2. Ainsworth G.C(1973) Introduction and key to the higher taxa In *The fungi, An advanced treatise vol 4a, A taxonomic review keys*
3. Arx von JA *The genera of fungi sporulating in pure culture*, Cramer J, Vaduj.2nd ED
4. Abedinzadeh M., Etesami H., Alikhani H.A. Characterization of rhizosphere and endophytic bacteria from roots of maize (*Zea mays* L.) plant irrigated with wastewater with biotechnological potential in agriculture. *Biotechnol. Rep.* 2019;21:e00305. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
5. Devi N., Prabakaran J. Bioactive metabolites from an endophytic fungus penicillium sp. isolated from *Centella asiatica*. *Current Research in Environmental & Applied Mycology*. 2014;4(1):34–43. doi: 10.5943/cream/4/1/3. [[CrossRef](#)] [[Google Scholar](#)]
6. Eid A.M., Salim S.S., Hassan S.E.-D., Ismail M.A., Fouda A. Role of Endophytes in Plant Health and Abiotic Stress Management. In: Kumar V., Prasad R., Kumar M., Choudhary D.K., editors. *Microbiome in Plant Health and Disease: Challenges and Opportunities*. Springer; Singapore: 2019. pp. 119–144. [[Google Scholar](#)]
7. Hassan S.E.-D. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *J. Adv. Res.* 2017;8:687–695. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
7. Hashim A.M., Alharbi B.M., Abdulmajeed A.M., Elkelish A., Hozzein W.N., Hassan H.M. Oxidative Stress Responses of Some Endemic Plants to High Altitudes by Intensifying Antioxidants and Secondary Metabolites Content. *Plants*. 2020;9:869. doi: 10.3390/plants9070869. [[PMC free article](#)] [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]
8. El-Esawi M.A., Al-Ghamdi A.A., Ali H.M., Ahmad M. Overexpression of AtWRKY30 Transcription Factor Enhances Heat and Drought Stress Tolerance in Wheat (*Triticum Aestivum* L.) *Genes*. 2019;10:163. doi: 10.3390/genes10020163. [[PMC free article](#)] [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]
9. Fouda A.H., Hassan S.E.-D., Eid A.M., Ewais E.E.-D. Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.) *Ann. Agric. Sci.* 2015;60:95–104. [[Google Scholar](#)]
10. Murali M., Mahendra C., Hema P., Rajashekar N., Nataraju A., Sudarshana M., Amruthesh K. Molecular profiling and bioactive potential of an endophytic fungus *Aspergillus sulphureus* isolated from *Sida acuta*: A medicinal plant. *Pharm. Biol.* 2017;55:1623–1630. doi: 10.1080/13880209.2017.1315435. [[PMC free article](#)] [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]
11. Fungal biodiversity: Distribution, conservation and prospecting of fungi from India by C. Manoharachary1,*, K. Sridhar2 , Reena Singh3 , Alok Adholeya3 , T. S. Suryanarayanan4 , Seema Rawat5 and B. N. Johri5 in Microbial diversity.

12. Mehrotra B., Dhar M., Dhar M., Dhawan B., Bhakuni D. S. Screening of Indian plants for biological activity: part II. *Indian Journal of Experimental Biology* . 1969;6 [[PubMed](#)] [[Google Scholar](#)]
13. ALKahtani M.D., Fouda A., Attia K.A., Al-Otaibi F., Eid A.M., Ewais E.E.-D., Hijri M., St-Arnaud M., Hassan S.E.-D., Khan N. Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. *Agronomy*. 2020;10:1325. doi: 10.3390/agronomy10091325. [[CrossRef](#)] [[Google Scholar](#)]
14. Moubasher A., Moustafa A. A survey of Egyptian soil fungi with special reference to *Aspergillus*, *Penicillium* and *Penicillium*-related genera. *Trans. Br. Mycol. Soc.* 1970;54:35–44. doi: 10.1016/S0007-1536(70)80121-8. [[CrossRef](#)] [[Google Scholar](#)]
15. Woudenberg J., Groenewald J., Binder M., Crous P. *Alternaria* redefined. *Stud. Mycol.* 2013;75:171–212. doi: 10.3114/sim0015. [[PMC free article](#)] [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]
16. Fungi in the bioremediation of toxic effluents by M. Maria M. de Wet, Hendrik G. Brink, in *Fungi Bio-Prospect in Sustainable Agriculture, Environment and Nano-technology*, 2021

