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Mycodiversity in *Phyllanthus Emblica* L. fruits

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Abstract

Phyllanthus emblica fruit is an important herb with the richest natural source of Vitamin C and antioxidant properties. This is a rasayan used as therapeutic agents for prevention of diseases and ailments as mentioned in Charak sanhita. A variety of relationship exists between the endophytic fungi and the host plants, ranging from mutuality or symbiotic to antagonistic or slightly pathogenic because of what appears to be their contribution to the host. Total 19 different fungi of two divisions Ascomycota and Zygomycota followed by four classes' viz. Dothideomycetes, Eurotiomycetes, Sordariomycetes, Mucoromycetes and six families i.e. Pleosporaceae, Davidiellaceae, Trichocomaceae, Hypocreaceae, Nectriaceae, Mucoraceae were identified in amla fruits sampled from Bitthal sabji market, Govindpura sabji market, Vindhya Harbal Garden, Mangalwara Market Mandideep, Piplani Hatt, Sunday Market TT Nagar, Bangrasia Sunday Market and Sehore Bajar during fruit harvesting season.

Keywords: - *Phyllanthus emblica*, endophytes, mycodiversity, ailments, immunity.

Introduction:

Emblica officinalis (*Phyllanthus emblica* L.) is also known as Amla/aola/ Indian gooseberry is an important herb with richest natural source of high content of vitamin C, constitute of phyllemblin, gallic acid, ascorbic acid, tannins etc (Ghoshal *et al.*, 1996). This can rejuvenate the organ system of body and provide strength and wellness for improvisation of immunity. This has been useful in amelioration of cold, cough, influenza, diabetes, lungs, heart, and many chronic infections. Amla is also used as a tonic to build up lost energy and vigor, blood purifier, cardio-protective, diuretic, laxative. Ayurvedic practitioners believed regular takes of amla with other product or alone can prevent from many diseases. This is much useful in treating diabetes, asthma, jaundice, cough, inflammation etc. and highly valued in Indian traditional medicines (Scartezzini *et al.*, 2006). A variety of relationship exists between the endophytic fungi and the host plants, ranging from mutuality or symbiotic to antagonistic or slightly pathogenic because of what appears to be their

contribution to the host (Arnold, 2007). Medicinal plants are reported to harbour of endophytes (Strobel, 2002). Endophytic fungi produce a number of substances such as antioxidants, novel antibiotics, antimycotics, immunosuppressant and anticancer compounds, and thus rich source of biologically active metabolites that find wide-ranging exploitation in medicine, agriculture, and industry (Strobel *et al.*, 2003; Bhagobaty *et al.*, 2011). Generally, plant diseases on the basis of causal factors mainly grouped into three types namely parasitic (fungi, bacteria, mycoplasma and spiroplasmas, nematodes, algae, protozoa, parasitic flowering plants) nonparasitic (a biotic agents such as unfavorable weather conditions, nutritional deficiency, air pollution and many edaphic conditions) and viral diseases (Mehrotra & Aggarwal, 2003). In other study due to risk of contamination of herbal medicinal plants a study conducted on isolation and identification of fungal contamination in stored medicinal plants such as *Saracaindica, Terminaliaarjuna, Hemidesmusindicus.* Thirteen different fungal species was isolated from all the three medicinal plant samples. The predominant mycoflora obtained was distributed in five different genera comprised of *Aspergillus, Penicillium, Alternaria, Rhizopus* and *Syncephalastrum.* A total of 19 genera representing 42 fungal species were isolated from the collected 48 fruits samples. Fungal speciesgenera*Aspergillus*,*Penicillium*, *Fusarium,Alternaria*, *Cladosporium* and *Curvularia*are the prominent isolated genera (Akhund*et al.*, 2010). Sharma & Sharma, 2018An investigation of mycoflora was carried out from market samples of fresh fruit of *Phyllanthusemblica* L. showed presence of 25 fungal species belonging to 12 genera. The other fungal genera isolated are *A.fumigatus, A.parasiticus, Fusariummoniliforme, F.oxysporum, P.islandicum, P.italicum, Rhizopusnigricans and R. stolonifer.* (Das and Sharma, 2012). Post harvest diasease of amla fruit like *Penicillum*rot*; Aspergillusniger*rot, *Rhizopus* rot, *Phomopsis*rot and *Cladosporium*rot were also reported from the survey of different fruits market of Marathwada regions Maharashtra state (Rathod, 2010).

Materials and Methods:

Dull greenish yellow Amla / Aola (*Phyllanthus emblica* L.) fruits were collected from Bitthal sabji market, Badkheda sabji market and Piplani sabji market Bhopal (India) during their fruit harvesting season in month of February, 2017. Collected fruits were washed with tap water and leave for air dried in laboratory at room temperature. Dried samples were stored in a clean zipper poly bag for further studies. Collected fruits samples were randomly pick and surface sterilized with 2% aqueous solution of sodium hypochlorite for two minutes followed by rinsing with sterile distilled water thrice. Samples were ready to use for further studies after air dry. Dried fruits were sliced with the help of a sterile scalpel and plated on PDA (Potato Dextrose Agar) media with an antibacterial agent Chloramphenicol (50ppm). Fruits slices were placed by there inner surfaces turned up and turned down randomly. Inoculated plates were incubated at 28℃ ± 2ºC for 7 days

and produces colonies were noted. Noted colonies were enumerate and subculture. Repeated subcultures were performed for purification and further identification (Akhund *et al.,* 2010). Isolated fungi were identified in order to morphological characteristics viz. colony growth, aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc. followed by stained with Lactophenol cotton blue and the reference methods of Gilman (1957), Domsch *et al.*, (1980), Barnett, (1992). Molecular characterization was performed followed by Sequencing of ITS gene, Sequence confirmation and Sequence submission.

Results and Discussion:

Total 19 different fungi i.e. *Alternaria alternata, Cladosporium oxysporum, Cladosporium cladosporioides, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Aspergillus parasiticus, Aspergillus Versicolor, Aspergillus nomius, Penicillum rubrum, Penicillum citrinum, Penicillum chrysogenum, Penicillum funiculosum, Acremonium implicatum, Aspergillus terreus, Fusarium solani, Fusarium verticilloides, Mucor microspores, Rhizopus stolonifer* were identified from sampled amla fruits. All those were reported in two divisions Ascomycota and Zygomycota followed by four classes' viz. Dothideomycetes, Eurotiomycetes, Sordariomycetes, Mucoromycetes and six families i.e. Pleosporaceae, Davidiellaceae, Trichocomaceae, Hypocreaceae, Nectriaceae, Mucoraceae. Out of 19 identified fungal species only 4 species i.e. *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Fusarium verticilloides* reported as mycotoxin producers. All fungal species identified in this study belongs to two different divisions i.e. Ascomycota and Zygomycota but the species reported for mycotoxins production belongs to Ascomycota division only whereas those are distributed in four different class i.e Dothideomycetes, Eurotiomycetes, Sordariomycetes, Mucoromycetes but mycotoxin producing species limit to two classes i.e. Eurotiomycetes, Sordariomycetes. Extensively reported fungal species distributed in six different families i.e. Pleosporaceae, Davidiellaceae, Trichocomaceae, Hypocreaceae, Nectriaceae, Mucoraceae; and mycotoxin producing fungi limit to two families i.e. Trichocomaceae, Nectriaceae. Maximum numbers of contaminated samples and as well mycotoxins were found species of *Aspergillus* in Emblica powder samples (Gautam and Bhaduria, 2009). This study was also supported earlier reports of Hitokoto *et al.,* 1978, Aziz *et al.,* 1998, Bugno *et al.,* 2006. A study of Sharma and Sharma (2018) reported the presence of 25 fungal species representing 12 different genera with the 09 total number of samples colonised, 16 grand total colonies recovered, 45.00 colony forming unit (CF) %, 2.86 abundance and 0.03 A/F ratio in *Fusarium verticilloides*; 03, 06, 04 total number of samples colonised, 05, 11, 13 grand total colonies recovered, 15.00, 30.00, 20.00 colony forming unit (CF) %, 1.67, 1.86, 3.25 abundance and 0.11, 0.06, 0.16 A/F ratio in *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* respectively. Results reported in this study revealed the above study of Sharma and Sharma (2018).

Table: Diversity of species

Division	Class	Family	Species	Family	Species
Ascomycota	Dothideomycetes	Pleosporaceae	Altenaria alternata	29	29
648	63	Davidiellaceae	Cladosporium oxysporum	34	4
			Cladosporium cladosporioides		30
	Eurotiomycetes	Trichocomaceae	Aspergillus niger	429	48
	429		Aspergillus flavus		48
			Aspergillus fumigatus		48
			Aspergillus parasiticus		48
			Aspergillus Versicolor		23
			Aspergillus nomius		22
			Penicillum rubrum		48
			Penicillum citrinum		48
			Penicillum chrysogenum		48
			Penicillum funiculosum		48
	Sordariomycetes	Hypocreaceae	Acremonium implicatum	95	48
	156		Aspergillus terreus		47
		Nectriaceae	Fusarium solani	61	30
			Fusarium verticilloides		31
Zygomycota	Mucoromycetes	Mucoraceae	Mucor microsporus	44	17
44	44		Rhizopus stolonifer		27

692 total number of samples colonised, 2310 grand total colonies recovered, 75.88 colony forming unit (CF) %, 2.86 abundance and 0.04 A/F ratio in total isolated fungi whereas 149 total number of samples colonised, 574 grand total colonies recovered, 77.60 colony forming unit (CF) %, 3.24 abundance and 0.04 A/F ratio in all identified from different sampling stations. Division wise study reported in all species for division.

CONCLUSIONS

This study stated that fruit surface is most valuable habitat that influences the microorganism to grow and evolve. Some species are grown well whereas some are killed due to antagonistic properties of fruits. 19 different species of fungus are detected from samples of amla (*Phyllanthus emblica*) fruits collected from different location of Bhopal and adjoin areas. This study suggests that there are urgent need to make awareness for proper collection and storage of amla fruit. Due to lack of proper storage and transportation, mycotoxin contamination occur in fruits leads diseases to consumer and also loss the economy followed by rot.

References:

Akhund S, Suhail M, Rani I, Memon FI, Abro H. (2010) Fruit borne Mycoflora of amla (*Phyllanthus emblica* L.). Pak J Bot. 42:4229-33.

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews* ;21:55-66.

Aziz, N.H., Youssef, Y.A., Moheye, Z. EL-F and Lofty, A.M. 1998. Contamination of some medicinal plant samples and spices by fungi and their mycotoxins. Botanical Bulletin of Academia Sinica, **39**:279-285.

Barton, R. (2013) Know Your Garden Soil: How to Make the Most of Your Soil Type. Eartheasy. http://learn.eartheasy. com/2013/06/how-tomake-the-most-of-your-soil-type/ accessed on January 8, 2019.

Bhagobaty RK, Joshi SR (2011) Metabolite profiling of endophytic fungal isolates of five ethno-pharmacologically important plants of Meghalaya, India. J Metabolomics Syst Biol; 2:20-31.

Bugno A, Adriana ABA, Tatiana CP, Terezinha AP & Myrna S (2006) Occurrence of toxigenic fungi. Journal of Microbiology, 37: 47-51.

Das A, Sarma TC (2012) Post harvest decay of *Averrhoa carambola* L. and *Phyllanthus emblica* L. due to some microfungi. International quarterly journal of environemntal sciences. 1:41-44.

Domsch, K.H., Gams, W. and Anderson, T.H. (1980) Compendium of Soil Fungi. Academic Press, New York, USA, **pp**. 1156.

Gautam AK, Bhadauria R. Mycoflora and mycotoxins in some imported stored crude and powdered herbal drugs. Biol Forum Int J 2009;1:1-7.

Ghoshal S, Tripathi VK, Chauhan S. (1996) Active constituents of *Emblica officinalis*. Part I, the Chemistry and antioxidative effects of two hydrolysable tannins, emblicanin A and B.Indian J Chem, 35 (B): 941-948.

Gilman, J.C. and Abbot, E.V. (1957) A summary of the soil fungi. Iowa State Coll. J. Sci. **1:**225–344.

Hitokoto, H., Morozumi, S., Wauke, T., Saka, S., and Kurata, H. 1978. Fungal contamination & mycotoxin detection of powdered herbal drugs. Applied and Environmental Microbiology, **36**: 252-256.

Lee, Y.J. and Hagler, W.M., Jr. (1991). Aflatoxin and Cyclopiazonic acid produced by Aspergillus flavus isolated from contaminated maize. J. Food Sci., 56, 871-72.

Mehrotra RS, Aggarwal A (2003) Fundamentals of Plant Pathology. New Delhi-110 006: McGraw Hill Education Pvt. Ltd; 433 p.

Mirhendi, Hossein & Diba, Kambiz & Rezaei, A & Jalalizand, N & Hosseinpur, L & Khodadadi, Hossein. (2007). Colony-PCR Is a Rapid and Sensitive Method for DNA Amplifi- cation in Yeasts. Iranian Journal of Public Health. 36.

Morton SG, Eadie T, Llewellyn GC (1979) Alflatoxigenic potential of dried figs, apricots, pineapples, and raisins. J. AOAC, 62, 958-62.

Rathod GM (2010) Survey of Post-Harvest Fungal Diseases of Some Fruits from Marathwada Regions of Maharashtra. Indian Journal of Ecobiotechnology; 2(6):7-10.

Sambrook, J., Fritsch, E. R., & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Scartezzini P, Antognoni F, Raggi MA, Poli F,Sabbioni C. (2006) Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn, Journal of Ethnopharmacology, 104(1-2), 113-8.

Senyuva HZ, Gilbert J, Ulken U (2007) Aflatoxins in Turkish dried figs intended for export to the European Union. Journal of Food Protection, 70, 1029-32.

Sharma R, Sharma V (2018) Investigation on the mycodiversity of fresh fruits of phyllanthus emblica l.,an important medicinal plant, from jammu province, india. International journal of Advanced Research 6(1): 168-173.

Sharma R, Sharma V (2018) Investigation on the mycodiversity of fresh fruits of phyllanthus emblica l., an important medicinal plant, from jammu province (INDIA). International Journal of Advanced Research, 6(1): 168-173.

Strobel GA (2002) Rainforest endophytes and bioactive products. Crit Rev Biotechnol; 22:315-33.

Strobel GA, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev; 67:491-502.

White, & Bruns, Tom & Lee, Steven & Taylor, John. (1990). White, T. J., T. D. Bruns, S. B. Lee, and J. W. Taylor. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics.