Isolation Of Fungi From Emblica officinalis (Amla) Fruit.

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ABSTRACT:

Fruits of *Emblica officinalis* Gaertn. (Amla), are used in traditional Ayurvedic medicine. Fruits sold in the market of Jalna (M.S.), were collected. Potato Dextrose Agar and CZA media were used for the isolation of fungi. During morphological examination of collected samples a remarkable change was observed in the colour, cracks, sizes and appearance of fruit. A total 13 fungal species belonging to the eight genera were isolated from amla fruits. The mostly dominating genera were *Aspergillus, Penicillium, Helminthosporium, Rhizopus, Alternaria,* and *Curvularia.* These tocxigenic fungi which produce mycotoxins may make these crude as well as powdered herbal drugs hazardous for human health. Thus it is needs to assure the quality control and decontamination of these herbal drug preparations. Therefore in the present investigation attempt were made to understand the association of fungi to the amla fruits.

Key words: Amla fruits, fungal flora, Incidence, Aspergillus, Penicillium, mycotoxin.

INTRODUCTION

In Ayurveda different parts of the medicinal plants as well as the powdered, mixture of various parts of different plants are used as herbal drug as medicines for general health disorders and various ailments. Amla (Emblica officinalis) fruit contain Vitamin C and used as a tonic, blood purifier, diuretic and laxative. It is also used as hair-dye & shampoo. It cures insomnia. It is also a cardio protective. Fruits make important diet for human beings. Amla fruits contain minerals, vitamins, amino acids and various sugars which provide the platform for the growth of microbes. During post harvest period diseases can affect the quality of fruits because the number of microbes attacks on the fruits. Unscientific storage and handling processes make post harvest deterioration of fruits. These deteriorated fruits may be used for the preparation of amla powder which is used in 'Triphala Churn''.

Various part of medicinal plant like stem, bark, root, rhizome, leaves, flowers, fruits and seeds were, used to prevent and treat diseases of humankind. Indian and also western populations are now looking for natural, herbal drugs, which are safe and effective (Shivanna, 2004). Essono et al(2007)were reported ,the unscientific methods of harvesting, collection, storage of fruits, raw materials, processing and poor storage of herbal drugs, responsible for fungal infections. When fungal contamination take place, they alter the

chemical composition of the raw materials and therefore medicinal potency of the herbal drugs were reduced this was reported by Roy (2003). Mostly these saprophytic fungi secretes secondary metabolites and contaminated with drugs and show adverse effect on liver, kidney, nervous system, , respiratory organs, digestive system, and genital organs. (Durakovic *et al.*, 1989, Purchase, 1974 and Muntanola, 1987). The present study is an effort to identify the especially fungal flora which can be toxic to human health.

MATERIALS AND METHODS

Sample Collection

Total 100 fruit samples of amla were collected from the market of Jalna city. These samples were stored at room temperature after the collection. Collected fruit samples were incubated for 4-5 days after these fruits examined morphologically with the help of hand lens and observe natural infected and damaged fruits .Fungal flora was isolated on culture media.

Isolaton of Fungi

Firstly amla fruits, surface sterilized with solution of Sodium hypochlorite. 2-3 mm pieces of fruit samples were inoculated, on potato dextrose agar (PDA) and Czapek dox agar media (Mandeel, 2005). The culture plates were incubated at 28 °C for 5-7 days and observations were recorded. Pure cultures of different isolated fungi were maintained by transferring the isolated colony on CZD media with the help of inoculating needle and this was used for fungal identification.

Identification of fungi : After pure culture, fungi identified primarily on the basis of their morphological and cultural characteristics and then stained with lactophenol cotton blue and identified microscopically with reference to standard literature (Ananthanarayan and Paniker, 1999).



RESULTS AND DISCUSSION

Fungi associated with Fruit samples

It is interesting that in India people are used amla fruits for making pickles and jellies. It is also used in making dyes and shampoos. In addition this fruits are used in Ayurvedic medicine (Triphala Churn). The fungal infected fruits rejected by the customers and medicine industries because these deteriorated fruits decrease the potency of drug.

At the time of collection it was found that fruits are mostly transported without taking any care about the fruit damage and these fruits are not stored in clean containers in the market, therefore there are chances to microbial infection. Most of the identified dominating fungal species like *Aspergillus, Penicillium* and *Alternaria* was reported. These saprophytic fungi ability to produce mycotoxins like aflatoxins, ochratoxins and citrinine ,reported by Bugno et al. 2006, Aziz et al. 1998, Hitokoto et al. 1978.

From the fruit of *E. officinalis*,(Fig.1) total of 13 fungal species classified under 8 genera were isolated from the fruit samples. The mzximum number of species belonged to the genus *Aspergillus*, with four species viz. *A. niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, while three species of *Penicillium* namely *P. citrinum* and *P. chrysogenum P.rubrum* and only one species of each viz. *Cladosporium sp*, *Helminthosporium sp*,

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Curvularaia lunata, Alternaria sp, Fusarium oxysporum and *Rhizopus* were observed. The highest percentage of incidance was shown by *A. niger* (60%), followed by *A. flavus* (50%) and *A. fumigatus* (30%), *A.parasiticus* and *Penicillium rubrum* (20% each) among the fruit samples. The lowest relative density was recorded in the range of 5-10%. The highest number of fungal species was obtained on PDA than Czapk's Dox Agar.

CONCLUSION

Roy et al. (1988), reported that aflatoxin contamination take place in medicinal plants. Efuntoyer (2004) had mentioned that the medicinal plants which are used in churn preparation have been contaminated with funagi.Simillar report also made by Choursia 1990, and Kumar & Roy1994. Therefore it is need to study the fungal flora associated with fruits. Benkee Thiyam & Sharma(2013).

The presence of mycotoxin producing fungal species of *Aspergillus, Penicillium, Alternaria, Helminthosporium,* in stored fruit samples of *Emblica officinalis* (Amla), revealed that these herbal drugs are not acceptable for human consumption. Even routine boiling processes will not able to detoxify. Aflatoxins have been reported to be heat stable up to 269°C reported by Frazier and Westhoff, 1988. The persons who are involved in harvesting, storage, processing and post processed storage of these herbal drugs are required well trained person to take stringent precautions in order to present the user with healthy and potential herbal drugs.

RFEREANCE

Essono G, Ayodele M, Akoa A, Foko J, Olembo S and Gock wski J (2007). Aspergillus species o cassava chips in storage in rural areas of southern.Cameroon: their relationship with storage duration, moisture content. and processing methods African Journal of Microbiology 001-008.

Ananthanarayan and Paniker (1999). A Text Book of Practical Microbiology. 6th Edit.

Bugno A, Adriana A B A, TatianaCP, TerezinhaAP, Myrna S (2006). Occurance of toxigenic fungi in herbal drugs. Brazillian Jr Microbio 37: 47-51.

Aziz NH, Youssef YA, Moheye Z EL-F, Lofty A M (1998). Contamination of some medicinal plant samples and spices by fungi and their mycotoxins. Bot. Bull Acad. Sin. 39: 279-285.

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

Roy A K, Sinha KK and choursia H K(1988). Aflatoxin contamination of common medicinal plants. Applied and Environmental Microbiology 54 : 842-843.

Efuntoye MO(2004). Fungi associated with herbal drug plants during storage. Mycopathologia 136: 115-118.

Choursia H K(1990). Aflatoxin contamination in drug yielding plants. J. Indian Bot. Soc. 69: 281-283.

Kumar S and Roy A K (1994). Mycotoxin contamination of some kidney curative herbal drugs.

Frazier, W.C. and Westhoff, D.C.1988. Food Microbiology. 4th ed. International edition,

Purchase, I.F.H. 1974. Mycotoxins. Amsterdam. Elsevier. 1–28.

Shivanna, K.R. 2004. Quality standards of Indian medicinal plants. Current Sci., 86: 13-28.

Roy, A.K. 2003. Mycological problems of crude herbal drugs-Overview and Challenges. *Indian Phytopathology*, **56**: 1–13.

Mandeel, Q.A. 2005. Fungal contamination of some imported species. *Mycopathologia*, 159: 291–298.

Muntanola, M. 1987. General mycology. Beograd: NIRO. Knjez evne novine, 257–269.

Durakovic, S., Galic, J. and Pajnovic, P. 1989. Toxic and cancer metabolites of moulds in food and fodder. *Hrana I ishrana,* **30**(2): 71–100.

Benkee Thiyam and G.D. Sharma (2013). Isolation and Identification of Fungal Associated with local Fruits of Bank Valley Assam. Current World Environment. Vol.8(2)319-322