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RESEARCH ARTICLE

INVESTIGATION ON THE MYCODIVERSITY OF FRESH FRUITS OF PHYLLANTHUS EMBLICA L., AN IMPORTANT MEDICINAL PLANT, FROM JAMMU PROVINCE (INDIA).

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Abstract

An investigation of mycoflora was carried out from market samples of fresh fruit of *Phyllanthus emblica* L., an important medicinal plant of India. The samples were collected from various wholesale and retail shops as well as orchards of Jammu province. A total of 25 fungal species representing 12 genera were recovered by using surface washing technique. Presence of low pH and sugars and high content of moisture and vitamin C and other nutrients in the fresh fruit of *P. emblica* were the prominent factors which made it prone to such a large number of mycopathogens. Assessment of mycobial load of *P. emblica* showed the presence of many such fungal species that are widely acknowledged as the most important mycotoxin producers. Lack of proper knowledge and use of unscientific methods of collection and storage of these fresh fruits of amla leads to their contamination. In view of the detected mycobial contamination, an urgent need for proper collection and storage of amla fruit is ardently required.

Introduction:-

India is endowed with a rich wealth of medicinal plants and a strong base of many systems of medicines including Ayurveda, Unani, Sidha and other local health practices. The curative properties of medicinal plants are due to the presence of complex chemical substances including alkaloids, glycosides, corticosteriods and essential oils (Silva *et al*,. 2011). It has been observed that despite spectacular advancement in modern synthetic drugs, the viability and popularity of indigenous systems of medicine and use of medicinal plants in curing various minor and major health ailments is still intact in our country. However, despite the rich heritage of medicinal plants, India has not been able to capitalize on its medicinal wealth, chiefly because the quality of crude botanicals and finished medicinal plant products is adversely affected by a number of internal and external factors right from harvesting to processing (Dubey *et al*., 2004). Another effect is that these medicinal plants also undergo deterioration even before they are used in making drugs. Realizing the fact that mycopathogenic contamination of medicinal plants has not received the attention that the magnitude of the problem warrants and since no such work has been attempted from Jammu and Kashmir State, which is a large reservoir of medicinal plants, an investigation was undertaken on one such important medicinal plant, *Phyllanthus emblica* L.

Phyllanthus emblica, which is known by the common name amla, belongs to family Euphorbiaceae (Hooker, 1897). It is reported to be native of India and is therefore, commonly called as Indian gooseberry. Commercial cultivation of amla is done in Uttar Pradesh (Bajpai, 1963) but in other Indian states its cultivation is increasing rapidly because of its high medicinal value and great commercial potential. Some of the cultivated varieties of amla, well-known in

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our country for their large fruits are Banarasi, Chakaiya and Hathijhool. Fruit of amla is very rich source of vitamin C (400-600mg/100g) and also contain calcium, phosphorous, iron, nicotinic acid and various amino acids like asparatic acid, alanine, glutamic acid, lysine and proline (Hanif, 1966). An inventiorization of medicinal plants shows that amla fruits are cardioprotective, antiscorbutic, diuretic and laxative (Bajpai *et al*., 1985). In view of its medicinal importance, many pharmaceutical industries are engaged in using amla fruit for the preparation of famous 'Triphala', 'Chawanprash' and many other ayurvedic formulations like Brahmarasayan, Sanjivani-vati, Agnitundi-vati and Arogyavardhini etc.

According to Sharma and Koul, (1999), almost every horticultural produce is high in moisture and nutrients and thus susceptible to injury and subsequent attack by variety of mycopathogens. Like others fruits, *Phyllanthus emblica* is also susceptible to mycobial infections at the pre- and post-harvest stage. These fruits have very low pH and therefore, may be attacked by a unique group of fungi, which have specific nutritional requirement and enzymatic capabilities that permit them to develop fruit rot. Thus, an investigation was undertaken to isolate, purify and identify the mycopathogens associated with the market samples of fresh fruit of this plant and their possible role in causing fruit rot.

Materials and Methods:-

(i) Isolation of mycoflora and their identification

Fresh fruit samples of selected medicinal plant was collected in pre-sterilized polythene bags from various wholesale and retail shops as well as various orchards of Jammu province. Sample bags were brought to the laboratory, sealed over flame to avoid external contamination and kept in the refrigerator at 5-7°C to prevent undesirable changes till further studies were conducted. Surface mycoflora associated with market samples of *Phyllanthus emblica* was determined by using surface washing technique (Singh and Kainsa, 1983). Isolation of mycopathogens from the samples were made on Czapek Dox agar (CDA) medium. The petriplates were incubated for 7 days at 28 ± 2ºC till proper growth of the fungal colonies was obtained. The recovered fungal species were identified by studying their macro and micro-morphological characters. For the purpose of identification, the isolated fungal species were grown and made to sporulate on different culture media, such as potato dextrose agar medium (PDA), malt extract agar medium (MEA), Czapek yeast agar medium (CYA), potato carrot agar medium (PCA) and water agar medium (WA). Identification of fungal species was done by using various keys and other relevant literature given by Brown and Smith (1957), Rapper and Fennel (1965), Tandon (1968), Rifai (1969), Booth (1971), Ellis (1971, 1976), Barron (1972), Pitt (1979), Domsch *et al*. (1980), Onions *et al*. (1981) Schipper (1984), Pitt and Hocking (1985), Gams (1997) and Chowdhary *et al*. (*2000*).

(ii) Percentage colonization frequency (CF%) and A/F ratios of the recovered fungal species

Percentage colonization frequency (CF%) and A/F ratios were calculated for each fungal species from various samples by using the following formulae:

$$\text{CF (\%)} = \frac{\text{Number of samples colonized by a specific fungi}}{\text{Total number of samples studied}} \text{ x } 100$$

On the basis of percentage colonization frequency, each of the recovered fungal species is distributed among five frequency classes (Raunkiaer,1934) :

Frequency class	Frequency %
A	0 – 20
B	21 - 40
C	41 - 60
D	61 – 80
E	81 – 100

$$\text{A/F ratio} = \frac{\text{Abundance}}{\text{Colonization frequency}}$$

$$\text{where, abundance} = \frac{\text{Total number of colonies of a specific fungi}}{\text{Number of samples colonized by a specific fungi}}$$

A/F ratios describe the distribution pattern of each fungal species into one of the following three categories:
A/F ratio of < 0.025 depicts that the fungal species has regular distribution.
A/F ratio between 0.025 – 0.05 depicts that fungal species has random distribution.
A/F ratio of > 0.05 depicts that the fungal species has contageous distribution.

Results:-

During the period under investigation, 25 fresh samples of fruit of *Phyllanthus emblica* collected from various wholesale and retail shops as well as various orchards of Jammu province were studied for fungal flora. In all, 25 fungal species belonging to 12 genera were recovered (Table 1). The fungal species recovered from the various fresh fruit samples of *Phyllanthus emblica* included members of Zygomycetes, Ascomycetes and Deuteromycetes. Class Zygomycetes was represented by two species of *Mucor* (*M. hiemalis* and *M. microsporus*) and one species of *Rhizopus* (*R. stolonifer*). Ascomycetes was represented by *Emericella nidulans* var. *echinulatus*, whereas Deuteromycetes consisted of maximum representation including nine species of *Aspergillus* (*A. japonicus, A. flavus, A. fumigatus, A. niger, A. nidulans, A. parasiticus, A. ochraceous, A. terreus* and *A. sydowii*); three species of *Penicillium* (*P. chrysogenum, P. funiculosam and P. waksmanii*); two species each of *Fusarium* (*F. verticilloides and F. solani*); *Cladosporium* (*C. cladosporioides and C. oxysporum*) and *Paecilomyces* (*P. liliacinus*) and one species each of *Acremonium* (*Acremonium implicatum)*; *Alternaria (Alternaria alternata)*; *Doratomyces (Doratomyces purpureofuscus)*; *Curvularia (Curvularia lunata)* and *Trichothecium (Trichothecium roseum).*

Their values for abundance, colonization frequency percentage (CF%) and abundance/frequency ratio (A/F ratio) are depicted in table 1. Perusal of data shows *Penicillium funiculosum* with maximum colonization frequency (50), closely followed by *Aspergillus terreus, Cladosporium oxysporum* and *Fusarium verticilloides* with 45 % CF and thus belonging to the Raunkiaer's frequency class C. *Aspergillus ochrac*eous and *Emericella nidulans* var. *echinulatus* represented the rare fungal species and belonged to Raunkiaer's frequency class A, since both these species were present in less than 10 % of the total samples. Percentage colonization frequency (CF%) of micromycetes of *P. emblica* was also represented in figure 1.

Discussion:-

This study clearly indicates that various aspergilli and penicilli including *Aspergillus japonicus, A. flavus, A. fumigatus, A. niger, A. nidulans, A. parasiticus, A. ochraceous, A. terreus, Penicillium chrysogenum, P. funiculosum and P. waksmanii* are commonly associated with the samples of fruit of *Phyllanthus emblica.* In addition, some other mycofungi including *Emericella, Paecilomyces* and *Curvularia* etc. were also recovered from investigated medicinal plants. Realizing the importance of the quality of medicinal plants, a large number of workers have recently engaged themselves in the study of surface mycoflora of various herbal drug plants during storage and marketing (Aziz et al., 1998; Stevic et al., 2012). These workers reported diverse range of fungal species belonging mainly to *Aspergillus, Penicillium, Rhizopus, Chaetomium, Fusarium, Eurotium* and *Cladosporium.*

In the present investigation, fresh fruits of amla were contaminated with a plethora of fungal species. These fungal genera might had reached the fruit surface directly from the orchard or during picking and packing operations or they might had been carried along with the packing leaves, straw and baskets or might had originated within the enclosure of fruit and vegetable shops (Meredith, 1961; Panduranjan and Suryanarayanan, 1985; Sumbali and Badyal, 1990). Another possible reason for the fruit contamination is that it continues to respire after harvesting, the resultant heat accelerates respiration and ageing which in turn makes the fruit even more susceptible to the attack by Mycoflora (Dasgupta and Mandal,1989). This showed that fruit surface is an important habitat, which influences the occurrence and development of rot. Some of the fungal spores present on the fruit surface may colonise the host on getting a suitable microhabitat while some get killed due to unfavorable conditions or due to antagonistic effect of other microorganisms present on the surface. Similar observations have also been made by Kalafatoglue and Karapinar (1989) while working on the surface mycoflora of other fruits.

Conclusion:-

In the present investigation, detection of large number of mycopathogens from the fresh fruit samples of *Phyllanthus emblica* clearly indicates that its powdered formulations are not completely safe for direct human consumption. However, due to lack of proper knowledge and use of unscientific methods of collection and storage these fresh fruits of amla may become prone to contamination with fungal spores. In view of the detected mycobial contamination, an urgent need for proper collection and storage of the fruit of amla is ardently required.

Table 1:-Percentage Colonization Frequency, abundance and A/F ratios of the recovered micromycetes from fruit of *Phyllanthus emblica*

S. No		Number of samples colonized	Total colonies recovered	CF (%)	Abundance	A/F ratio
1	*Alternaria alternata*	8	18	40	2.25	0.0563
2	*Acremonium implicatum*	5	12	25	2.40	0.0960
3	*Aspergillus niger*	6	9	30	1.50	0.0500
4	*Aspergillus japonicus*	5	9	25	1.80	0.0720
5	*Aspergillus terreus*	9	18	45	2.00	0.0444
6	*Aspergillus ochraceous*	1	3	5	3.00	0.6000
7	*Aspergillus flavus*	3	5	15	1.67	0.1111
8	*Aspergillus parasiticus*	6	11	30	1.83	0.0611
9	*Aspergillus fumigatus*	6	16	30	2.67	0.0889
10	*Aspergillus nidulans*	4	13	20	3.25	0.1625
11	*Aspergillus sydowii*	6	13	30	2.17	0.0722
12	*Cladosporium oxysporum*	9	24	45	2.67	0.0593
13	*Cladosporium cladosporioides*	6	18	30	3.00	0.1000
14	*Doratomyces purpureofuscus*	4	9	20	2.25	0.1125
15	*Emericella nidulans* var. *echinulatus*	1	4	5	4.00	0.8000
16	*Fusarium solani*	3	9	15	3.00	0.2000
17	*Fusarium verticilloides*	9	16	45	1.78	0.0395
18	*Mucor hiemalis*	4	12	20	3.00	0.1500
19	*Mucor microsporus*	3	6	15	2.00	0.1333
20	*Paecilomyces liliacinus*	3	6	15	2.00	0.1333
21	*Penicillium chrysogenum*	7	22	35	3.14	0.0898
22	*Penicillium funiculosum*	10	31	50	3.10	0.0620
23	*Penicillium waksmanii*	3	6	15	2.00	0.1333
24	*Rhizopus stolonifer*	3	10	15	3.33	0.2222
25	*Trichothecium roseum*	7	20	35	2.86	0.0816

CF(%) – Colonization frequency percentage
A/F ratio – Abundance / Frequency ratio

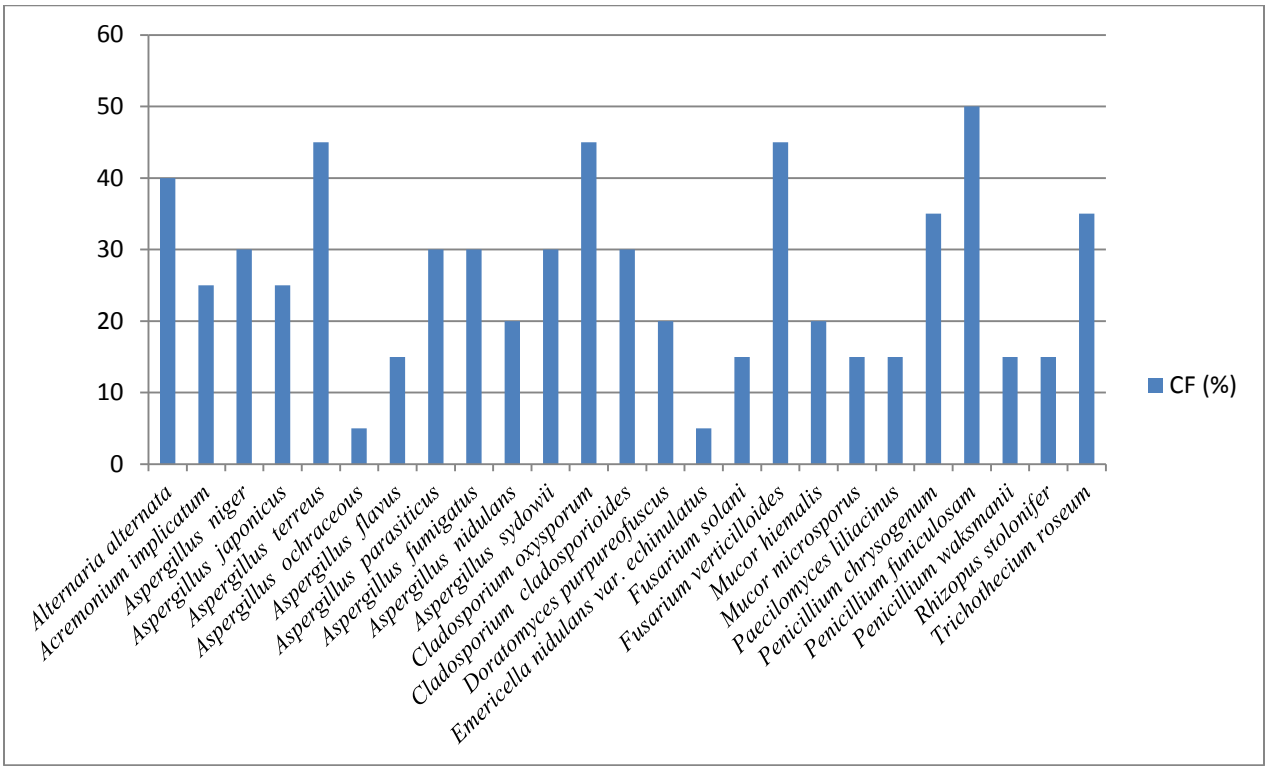

Fig. 1:- Percentage colonization frequency (CF%) of recovered mycoflora from *P. emblica*

Fig. 1:-Sample of fresh fruit of amla from markets and orchard of Jammu region

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