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Seasonal variation of leaf surface mycoflora of some ornamental plants from Shendra Midc Area, Aurangabad, Maharashtra

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Abstract
The present investigation was carried out during the months October 2015 to September 2016 to observe the leaf surface mycoflora of ornamental plants from Shendra MIDC area, Aurangabad (MS). Leaf surface mycoflora were studied season wise that is winter, summer and rainy. Three plants were selected viz. *Hibiscus rosa-sinesis* L., *Acalypha wilkesiana-kona* Gold and *Caesalpinia pulcherima* (red). Leaf samples were collected in different seasons and mycoflora were isolated and pure cultures were maintained on Potato dextrose agar. Identification of mycoflora was done by morphological characters and microscopic observations. During this investigation 18 fungal species were recorded. Average percentage frequency of *A. niger* were higher in all three plants and lower average percentage frequency were observed in *R. bataticola* from *Hibiscus rosa-sinesis* L., *Trichoderma* from *Acalypha wilkesiana-kona* Gold and *R. macrophomina* from *Caesalpinia pulcherima* (red). Higest mycoflora were recorded during rainy season followed by winter and summer seasons in decreasing order.

Keywords: Seasonal variation, Mycoflora, *A. niger, R. bataticola, Trichoderma*, ornamental plants

1. Introduction
Leaf surface mycoflora of ornamental plant from industrial area have been poorly studied as compared to endophytes, saprobes and pathogenic fungi. Numerous investigation carried out on the fungus flora of leaf surface of several plants growing in garden or cultivated in many parts of the world by several researchers' (A. Eicker 1976, A.H.M. EI-Said. 2001, H. M. Abdel-Fattah *et al.* 1977, Nagaraja T. G. 1991, Sharma P. D. 1974, S. I. I. Abdel-Hafez 1985) [1, 2, 5, 7, 8, 9]. They were reported the fungi from leaf surfaces and observed that they are basically similar on the two types of media and the most common fungi were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Gibberella, Memnoniella, Mycosphaerella, Setosphaeria and Stachybotrys.*
The spores present in air are inhaled by various living beings like human beings, animals, birds, and also they settled on the plant surfaces, where they germinate and produce mycelia or they may produce toxins which are allergic in nature. Sometimes they cause diseases of incurable forms to living beings. These fungal spores act as allergens to human beings and also pollute the air. Many physical, chemical and biological factors bring out causative changes in composition of aero-mycoflora of an area and different fungal species are restricted to that of particular area with specific environmental conditions (Verma K. S. 1990).The airborne fungal spores and their concentration vary from place to place. To view this type of study in an industrial area, the present investigation was undertaken.
To view such causal organism deposited on leaf surfaces, the experimental work was undertaken to identify the mycoflora from leaf surface of an industrial area.

2. Materials and Methods
2.1 sample collection
The leaves of *Hibiscus rosa-sinesis, Acalypha wilkesiana-kona Gold and Caesalpinia pulcherima.* were collected in three different seasons in sterile zip- lock bags from Shendra MIDC area Aurangabad (MS).

2.2 isolation of leaf surface mycoflora
Leaf wash and leaf print method was used for isolation of mycoflora. Dorsal and ventral leaf impressions were taken on PDA medium. Collected leaves samples were cuts and about 10 gm of sample stir into 100 ml distilled water in a conical flask. This liquid sample was used for isolation of fungus. All the Petri dishes were incubated at room temperature 27^0 C ± 3^0C for one week. The fungi growing out from the sample were sub cultured on fresh PDA medium to get pure culture and stored in slants.

2.3 Identification of leaf surface mycoflora
The leaf surface mycoflora were identified on the basis of morphological and microscopic observations.

3. Result and Discussion.
During the present investigation *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus carbonarious, Aspergillus nidulus, Cladosporium oxysporum, Rhizopus, Fusarium roseum, Fusarium oxysporum, Rhizoctonia bataticola, Rhizoctonia macrophomina, Curvularia lunata, Penicillium, Torula, Phytophthora rubra, Trichoderma and Mycogone* mycoflora were recorded on leaves of three ornamental plants from Shendra MIDC, Aurangabad (MS) in different seasons. In *Hibiscus rosa-sinesis L*. percentage frequency of *A. niger* was maximum i.e. 19.84 % in leaf wash and 19.30 % in leaf print

followed by *Cladosporium fulvum (*11.34 % LW and 14.37 % LP), *Curvularia* (10.70 % LW and 11.30 % LP), *Alternaria alternata* (9.76 % LW and 9.70 % LP), *Aspergillus nidulans* (6.66 % LW and 4.73 % LP), *Fusarium oxysporium* (6.55 % LW and 4.61 % LP), *Aspergillus flavus* (5.91 % LW and 4.83 % LP), *Rhizopus* (5.75 % LW and 6.78 % LP), *Trichoderma* (5.29 % LW and 4.76 % LP), *Penicillium* (4.16 % LW and 4.80 % LP), *Fusarium roseum* (3.99 % LW and 3.61 % LP), *Aspergillus fumigatus* (2.84 % LW and 2.44 % LP), *Torula (2*.01 % LW and 2.49 % LP), *Mycogone* (1.57 % LW and 1.15 % LP), *Aspergillus carbonarious* (1.46 % LW and 3.07 % LP), *Phytophthora rubra* (1.21 % LW and 1.13 % LP) and minimum percentage frequency were observed in *R. bataticola* i.e. 0.88% in leaf wash and 0.88% in leaf print (Table 1).

Table 1: Percentage frequency of leaf surface mycoflora of *Hibiscus rosa-sinesis* L.

Percentage frequency of leaf surface mycoflora of *Hibiscus rosa-sinesis* during different months of 2015-2016.

Sr. No.	Mycoflora species	Winter								Summer								Rainy								Average		Average %	
		Oct		Nov		Dec		Jan		Feb		Mar		April		May		June		July		Aug		Sept					
		LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP
1	*A.alternata*	1	2.1	11.1	9.2	6.2	12	7.4	3.2	1.2	2.3	0	0	0	0	0	0	0	0	0	0	16	14	12	14	4.6	4.8	9.769	9.706
2	*A.niger*	6.7	7.2	5	5.5	7.1	7.8	14	16	8	8.1	13	11	8.9	8	6.2	5.8	10	12.4	12	14	11	9.8	10	8	9.4	9.5	**19.84**	**19.3**
3	*A. flavus*	0	0	4.1	3	6.2	4.1	4	1	1	0	8	9.3	7.1	6	3.1	5.1	0	0	0	0	0	0	0	0	2.8	2.4	5.918	4.836
4	*A. fumigatus*	0	0	6	4.1	6	7	0	0	0	0	4.1	3.3	0	0	0	0	0	0	0	0	0	0	0	0	1.3	1.2	2.844	2.444
5	*A.carbonarious*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0	6.1	0	3.7	5.1	8.3	0.7	1.5	1.466	3.071
6	*A. nidulans*	0	0	0	0	0	0	5	5.2	5.1	1.9	8.1	7.1	0	0	0	0	0	0	9.4	4.8	0	0	10	8.9	3.1	2.3	6.66	4.734
7	*C. fulvum*	10	10	4.2	6.1	0	0	0	0	0	0	0	0	0	0	0	0	13	15.1	0	13	12	14	25	26	5.4	7.1	11.34	14.37
8	*curvularia sp.*	3.4	3.2	0	0	0	0	0	0	0	0	3	2.3	0	0	0	0	29	30.9	0	0	0	0	25	30	5.1	5.6	10.7	11.3
9	*F. oxysporum*	0	0	5.9	2.1	3.3	2.3	5.2	4.1	0	0	0	0	3.2	4.1	3.1	4.2	12	8.1	0	0	0	0	4.1	2.3	3.1	2.3	6.554	4.616
10	*F. roseum*	2.1	3	2.3	2.4	3.1	2.4	0	0	0	0	0	0	0	0	0	0	2.1	5.1	7.1	4.3	0	0	5.9	4.1	1.9	1.8	3.992	3.614
11	*Mycogone*	2.3	2.1	3.1	2.3	1.5	1.4	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0.7	0.6	1.572	1.154
12	*Penicillium*	1.9	3.2	3	2	4.5	4.2	4	3	4.1	5.9	6.1	7.9	0	0	0	0	0	2.1	0	0	0	0	0	0	2	2.4	4.169	4.802
13	*P. rubra*	0	0	3.7	1.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	2.2	3.5	0	0	0.6	0.6	1.219	1.137
14	*R. bataticola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3.1	0	2.1	4	0	0.4	0.4	**0.883**	**0.88**
15	*Torula*	6	6.1	0	0	0	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	3.6	1.4	0	1	1.2	2.014	2.494
16	*Trichoderma*	4	1.5	4	2	6	8	0	0	5.3	6.7	4.1	3.7	0	0	0	0	3.2	2.1	0	1	3.4	3.1	0	0	2.5	2.3	5.299	4.768
17	*Rhizopus*	7	6.1	3.2	6	6.2	7.4	6	6.2	0	0	0	0	0	0	0	0	7.1	12.1	3.1	0	0	2.2	0	0	2.7	3.3	5.759	6.788
18	Total	45	45	55.6	46	50	57	46	39	29	30	46	44.6	19	18	12	15	84	87.9	32	48	45	58	103	102	47	49	100	100

LW = Leaf wash, LP = Leaf Print.

In *Acalypha wilkesiana-kona Gold* percentage frequency of *A. niger* was maximum i.e. 29.56 % in leaf wash and 28.30 % in leaf print, followed by *Aspergillus flavus (12*.17 % LW and 9.23 % LP), *Alternaria alternata* (12.01 % LW and 11.67 % LP), *Cladosporium fulvum* (10.41 % LW and 12.81 % LP), *Fusarium roseum* (*6*.86 % LW and 7.56 % LP), *Penicillium* (*5*.81 % LW and 4.58 % LP), *Torula (4*.86 % LW and 4.91 % LP), *Fusarium oxysporium* (*3*.86 % LW and 5.80 % LP), *Rhizopus* (*3*.50 % LW and 2.22 % LP), *Rhizoctonia bataticola* (*2*.44 % LW and 1.94 % LP), *Rhizoctonia macrophomina* (*2*.20 % LW and 0.95 % LP), *Aspergillus fumigatus* (*1*.95 % LW and 2.33 % LP), *Phytophthora rubra* (*1*.91 % LW and 2.84 % LP), *Aspergillus nidulance* (1.48 % LW and 4.27 % LP) and minimum percentage frequency were observed in *Trichoderma* i.e. 0.92 % in leaf wash and 0.51% in leaf print (Table 2).

Table 2: Percentage frequency of leaf surface mycoflora of *Acalypha wilkesiana-Kona Gold*.

Percentage frequency of leaf surface mycoflora of *Acalypha willkesiana-Kona Gold* during different months of 2015-2016.

Sr. No.	Mycoflora species	Winter								Summer								Rainy								Average		Average %	
		Oct		Nov		Dec		Jan		Feb		Mar		April		May		June		July		Aug		Sept					
		LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP
1	*A.alternata*	11	8.1	11.2	9.4	8.1	10	0	0	3.4	2.7	6.3	2.9	3.1	2.9	0	0	2.2	3.6	6.3	6.7	11	9.1	7.8	8.1	5.9	5.3	12.01	11.67
2	*A.niger*	21	20	20	21	17	21	16	14	20	21	15	16.2	4.1	3.2	7.1	3.9	14	13.1	12	10	9.8	6.2	17	4.3	14	13	**29.56**	**28.3**
3	*A. flavus*	11	4.2	5.1	3.2	9.1	12	0	0	4.4	2.1	3.9	4.1	2.1	3.8	3.7	2.1	0	0	30	16	0	0	2.1	3.1	5.9	4.2	12.17	9.233
4	*A. fumigatus*	2.3	1.9	0	0	0	0	9.1	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.1	1.951	2.331
5	*A. nidulans*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.7	7.1	0	9.1	0	7.1	0.7	1.9	1.489	4.277
6	*C. fulvum*	2.6	2.8	3.2	4.1	0	0	0	0	0	0	0	0	0	0	0	0	18	14.4	0	12	11	12	26	24	5.1	5.8	10.41	12.81
7	*F.oxysporum*	5.1	6.2	0	2.1	0	0	0	0	0	4.1	0	0	2.1	3.9	0	0	0	0	3.2	6.1	8.1	9.2	4.1	0	1.9	2.6	3.868	5.8
8	*F. roseum*	4	3.4	7.1	6.2	15	11	0	0	3.3	4.3	0	2	2	2.1	0	0	5.2	7.1	0	0	0	5.3	3.4	0	3.3	3.4	6.863	7.562
9	*Penicillium*	3.4	2.8	3.6	4.8	4.9	3.1	0	0	11	4.4	8.1	7.1	0	0	0	0	0	0	0	0	3.2	2.8	0	0	2.8	2.1	5.819	4.589
10	*P. rubra*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.1	2.1	3.1	0	6.1	9.1	3.2	0.9	1.3	1.917	2.845
11	*R. bataticola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.3	4.1	0	3.1	4.2	7.1	4.1	1.2	0.9	2.447	1.946
12	*R.macrophomi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.8	3.1	6.1	2.1	0	0	0	0	1.1	0.4	2.208	0.954
13	*Rhizopus*	4.1	3.1	7.1	2.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.8	3.2	2.1	6.1	0	1.7	1	3.508	2.221
14	*Torula*	3.2	2.6	6.1	4.1	0	0	1.9	2.1	0	0	0	0	0	0	0	0	10	8.2	7.1	9.8	0	0	0	0	2.4	2.2	4.861	4.919
15	*Trichoderma*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.4	2.8	0	0	0.5	0.2	**0.924**	**0.51**
16	Total	67	55	63.4	57	54	57	27	27	42	39	33	32.3	13	16	11	6	56	54.9	80	78	55	69	83	54	49	45	100	100

LW = Leaf wash, LP = Leaf Print.

In *Caesalpinia pulcherima (red)* percentage frequency of *A. niger* was maximum i.e. 19.27 % in leaf wash and 19.20% in leaf print, followed by *Alternaria alternata (*17.65 % LW and 15.98 % LP),
Cladosporium fulvum (12.54 % LW and 17.41% LP), *Fusarium roseum* (12.48 % LW and 7.47 % LP), *Aspergillus carbonarious* (7.79 % LW and 4.38 % LP), *Aspergillus flavus* (6.21 % LW and 5.98 % LP), *Trichoderma* (5.70 % LW and 5.39 % LP), *Penicillium* (5.33 % LW and 5.93 % LP), *Aspergillus fumigatus* (3.18 % LW and 3.55 % LP), *Rhizopus* (3.08 % LW and 3.75 % LP), *Curvularia* (2.28 % LW and 3.40 % LP), *Aspergillus nidulans* (1.28 % LW and 3.06 % LP), *Rhizoctonia bataticola* (*1*.26 % LW and 2.52 % LP), *Torula* (1.11 % LW and 1.28 % LP) and minimum percentage frequency were observed in *R. macrophomina* i.e. 0.80 % in leaf wash and 0.60% in leaf print. Higest mycoflora were recorded during rainy season followed by winter and summer seasons in decreasing order (Table 3). These results were confirmed by many authors and found that *Aspergillus niger* was the most common phyllosphere fungus found on the leaf surface of different plants (Bhuyan P. M. *et al.* 2013, Dalal 2014, K. L.Tiwari and P.K. Saluja. 2010, Waill A. Elkhateeb *et al.* 2016) [3, 4, 6, 10].

Table 3: Percentage frequency of leaf surface mycoflora of *Caesalpinia pulcherima (red)*.

Percentage frequency of leaf surface mycoflora of *Caesalpinia pulcherima(red)* during different months of 2015-2016.

Sr. No.	Mycoflora species	Winter								Summer								Rainy								Average		Average %	
		Oct		Nov		Dec		Jan		Feb		Mar		April		May		June		July		Aug		Sept					
		LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP
1	*A.alternata*	3.2	2.6	4.1	6.2	0	0	7.1	2.1	3.1	1.1	6.1	3.1	2.1	2.3	1.1	2.3	2.4	1.6	21	19	15	14	25	32	7.5	7.2	17.65	15.98
2	*A.niger*	17	15	10.1	15	0	0	9.1	10	11	8.7	4.1	2.8	6.3	7.8	17	19	3.2	4.8	7.3	8.4	9.3	8.4	4.2	3.1	8.2	8.6	**19.27**	**19.2**
3	*A. flavus*	0	0	3.6	6.1	0	0	0	2.6	4.7	5.2	6.7	5.9	0	0	4.7	2.2	0	2.4	0	7.8	12	0	0	0	2.7	2.7	6.21	5.988
4	*A. fumigatus*	3.4	2.9	2.1	4.2	0	0	0	7.2	0	0	0	0	0	0	0	0	4.4	0	3.3	2.6	0	2.2	3.1	0	1.4	1.6	3.183	3.552
5	*A. nidulans*	0	0	3.2	2.1	1.2	0	2.2	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.6	1.4	1.289	3.069
6	*A.carbonarious*	0	0	0	0	20	5.1	6.1	4.3	0	0	14	14.2	0	0	0	0	0	0	0	0	0	0	0	0	3.3	2	7.791	4.389
7	*C. fulvum*	0	0	0	0	0	0	5.9	14	0	0	0	0	0	0	0	0	0	16.1	15	14	17	25	26	24	5.4	7.8	12.54	17.41
8	*curvularia sp.*	0	0	0	0	4.1	3.2	2.1	3.1	0	0	0	0	0	0	0	0	0	3.1	2.1	3.4	2.3	2.4	1.1	3.1	1	1.5	2.285	3.403
9	*F. roseum*	3.1	2.9	6.1	4.2	0	0	15	0	0	6.1	8.9	0	2.1	3.1	0	3.4	4	0	10	3.1	4.1	5.2	10	12	5.3	3.4	12.48	7.476
10	*Penicillium*	0	4.2	3.2	4.1	0	0	2.4	0	3.2	4.3	2.2	4.1	0	0	0	0	4.1	2.1	12	11	0	0	0	2.4	2.3	2.7	5.331	5.933
11	*R. bataticola*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2	3.6	0	0	4.3	5.1	0	4.9	0.5	1.1	1.269	2.529
12	*R. macrophom*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1	3.2	0.5	0	1.5	0	0.3	0.3	**0.801**	**0.6**
13	*Rhizopus*	0	0	0	0	2.1	3.4	0	0	4.1	2.3	0	0	0	0	0	0	0	3.7	5.5	7.4	0	3.4	4.1	0	1.3	1.7	3.085	3.757
14	*Torula*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	0.7	1.6	0	0.1	3.2	0.5	0.6	1.113	1.283
15	*Trichoderma*	5.5	7.1	8.9	3.7	0	0	0	0	0	0	4.1	0	0	3.7	0	0	0	0	0	2.3	4.6	12	6.1	0	2.4	2.4	5.702	5.393
16	Total	32	35	41.3	46	28	12	50	58	26	28	46	30.1	11	17	23	27	20	40.4	82	82	71	78	82	85	43	45	100	100

LW = leaf wash, LP = Leaf Print.

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