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Biodiversity in phyllosphere mycoflora of *Duranta erecta* L. from polluted area

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Abstract
The phyllosphere mycoflora of *Duranta erecta* L. was studied during the different seasons of the year from MIDC Shendra (polluted area), Aurangabad (MS). The mycoflora were isolated and pure cultures were maintained on Potato dextrose agar. The phyllosphere mycoflora shows seasonal variation as well as quantitative and qualitative variation. A total of 20 fungal species were recorded by leaf wash and leaf print method in phyllosphere of *Duranta erecta L.* during different seasons. In the present study *Alternaria alternata* shows maximum percent frequency (27.4 % and 21 %), where as minimum percent frequency was experienced for *Aspergillus carboniferous* (0.46 % and 0.89 %) by leaf wash and leaf print method respectively. There was statistically significant variation in number of colonies among the fungal species as well as in various seasons (p=0.01). Higher number of colonies was recorded during winter (68.1 in LW, 69.2 in LP) followed by rainy (59.8 in LW, 66.5 in LP) and summer (14.3 in LW, 14.8 in LP) seasons in decreasing order due to favourable temperature, rainfall and relative humidity. The average number of colonies was 15.80 and 16.72, when measured by leaf wash and leaf print method respectively. The difference in the number of colonies was statistically significant (p=0.01) by both methods.

Keywords: phyllosphere mycoflora, *Duranta erecta*, statistically significant, biodiversity

1. Introduction
Fungi which deposited on leaf surfaces and the study of such leaf surface environment is called the phyllosphere. The term phyllosphere was first coined by Last, (1955) [18] to denote the leaf surface environment. The report of the intensive investigations on leaf surface mycoflora has been reported by (Last and Deighton, 1965) [3, 19]. The existence of active mycelium on the phyllosphere was studied by (Kerling, 1958; Last & Dcighton, 1965; Dickinson, 1965; Fokkema, 1968, Warnock 1973) [3, 19, 9].

The bio-diversity and density of phyllosphere fungi were influenced by various factors such as humidity, temperature, incidence of sunlight, nutrient availability, leaf age and type, presence of inhibitors and arrival and settlement of viable propagules (Pugh and Mulder, 1971; Vorholt, 2012; Bulgarelli, 2013) [24, 28, 2]. The plant exudates secreted and thin nutrient films deposited from the atmosphere on the leaf surface further facilitates the microbial colonization (Kinkel, 1997) [15]. When plants grow, a new surface became available for fungi. Depending on the susceptibility of host plant, some fungi became phytopathogenic. All these observations motivated mycologists and plant pathologists to look at the phyllosphere as a distinct micro-habitat for study of leaf surface micro-flora and their dynamics.

Fungal diversity of several parts of the world was studied by Kirk, 2001 [16]. This includes floristic information of fungi from soil, plant parts and litter, herbivore dung, entomogenous, freshwater and marine, animal and human (Barron, 1968; Ellis, 1971, 1976; Matsushima, 1971, 1975; Subramanian, 1971, 1983; Ingold, 1975; Kohlmeyer and Kohlmeyer,1979;DixandWebster,1995)[1, 6, 7, 20, 21, 25, 26, 13, 17, 4]. Phyllosphere mycoflora from industrial area have been poorly studied as compared to endophytes, saprobes and pathogenic fungi. Some of the investigation have been carried out on the phyllosphere flora of some plants by some researchers (Nagaraja, 1991, EI-Said. 2001, Florin-Daniel, 2015, Undugoda, 2016) [23, 5, 8, 27] reported the most common fungi were *Alternaria, Aspergillus, Cladosporium, Curvularia, Gibberella, Memnoniella, Mycosphaerella, Setosphaeria* and *Stachybotrys.* To view this type of study in polluted area, the present investigation was undertaken.

2. Materials and Methods
2.1 Collection of sample
The mature and green leaves of *Duranta erecta* L. was collected randomly with a sterile scissor, in three different seasons, in sterile zip- lock bags from MIDC Shendra, Aurangabad (MS).

2.2 Isolation of phyllosphere mycoflora
For isolation of phyllosphere mycoflora, leaf wash and leaf print method was used. In leaf print method dorsal and ventral leaf impressions were taken on PDA medium, while in leaf wash method 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 phllosphere fungi. All the Petri dishes were incubated at room temperature 26^0 C ± 3^0C for seven days. The fungi growing out from the sample were sub cultured on fresh PDA medium to get pure culture and stored on slants.

The percent frequency was calculated by using formula,

Percent frequency = [(Number of colonies of fungal species) / (Total number of fungal colonies)] x 100

2.3 Identification of phyllosphere mycoflora

The Phyllosphere Mycoflora was identified on the basis of morphological and microscopic observations as well as by slide culture (Gilman, 1957; Mukadam, 2006) [11, 27]. Some mycoflora were identified by Agharkar Research Institute, Pune.

3. Result and Discussion

In the present study, total of 20 fungal species viz. *Alternaria alternata, Alternaria citri, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus carboniferous, Aspergillus nidulus, Cladosporium fulvum, Curvularia lunata, Fusarium oxysporum, Fusarium roseum, Mycogone, Penicillium, Phytophthora rubra, Rhizoctonia bataticola, Rhizoctonia solani, Torula, Nigrospora sacchari, Trichoderma viride* and *Rhizopus stolonifer*, were recorded by leaf wash and leaf print method in phyllosphere of *Duranta erecta L.* from polluted area during different seasons. Percent frequency of fungal species occurred on *Duranta erecta* L. in different months during various seasons is shown in Table 2. There was statistically significant variation in number of colonies, among the 20 fungal species (p=0.05) as well as seasons (p=0.01), when the mycoflora was studied by both leaf wash and leaf print method. *A. fumigates, C. lunata* and *R. solani* were found in the month of November and July, where as *Torula* observed only rainy season. Maximum frequency of occurrene was observed for *A. alternata* (27.4 and 21 % respectively) by leaf wash and leaf print method, which was followed by *A.citri* (11.9 and 9.31 % respectively) with higher number of colonies in winter season. Minimum percent frequency was experienced for *A. carboniferous* as it occurred only in the months of November and December that too more frequently under the leaf print (0.89 %) method.

Quantitative variation in number of fungal colonies in phyllosphere mycoflora of *Duranta erecta* L**.** was given in Table 1. Higher number of colonies was recorded during winter (68.1 in LW, 69.2 in LP) followed by rainy (59.8 in LW, 66.5 in LP) and summer (14.3 in LW, 14.8 in LP) seasons in decreasing order due to favourable temperature, rainfall and relative humidity. The average number of colonies was 15.80 and 16.72, when measured by leaf wash and leaf print method respectively.

These results were confirmed by many authors. Wojciech (2015) [31] studied fungi occurring on the plants of the genus *Amaranthus* L. The most frequently recorded taxa within the associations of fungi isolated from the phyllosphere were *Cladosporium cladosporioides, C. Herbarum, Alternaria alternata* and *Epicoccum nigrum.* Gagrepatil and Vanmare (2016) [16] observed incidence of fungal diseases of *Capsicum* in relation to seasonal variation. Indu Soni (2016) [12] studied fungal diversity with special reference to winter season. In this study 44 fungal species and 31 fungal genera were obtained in which *Aspergillus, Penicillium, Rhizopus, Cladosporium, Alternaria* and *Fusarium* were most dominant. Isolation and identification of endophytic, phylloplane and phyllosphere fungal diversity from different plants cultivated in four reclaimed areas at Assiut Governorate in Egypt were stidied by Waill (2016) [29].

Table 1: Quantitative variation in phyllosphere mycoflora of *Duranta erecta* L.

Sr. No.	Months	No. of colonies				Temp.^{0}C	Relative Humidity (%)	Rainfall (mm)
		L.W		L.P				
1	Nov-15	30.7	68.1	41	69.2	28	46	18.31
2	Dec-15	19.7		20		27	35	0.4
3	Jan-16	17.7		8.15		27	31	0.2
4	Mar-16	4.33	14.3	7.67	14.8	33	21	14.67
5	Apr-16	5.33		3.82		36	19	4.69
6	May-16	4.66		3.34		36	30	22.38
7	Jul-16	27.3	59.8	17	66.5	28	75	230.61
8	Aug-16	13.6		27.8		27	77	181.75
9	Sep-16	18.9		21.7		27	79	310.1
Mean		15.80		16.72		29.88	45.88	87.012
S.D		9.7		12.49		3.73	23.21	113.19
C.V.		61.39		74.72		12.46	50.57	130.09

L.W. = Leaf Wash,
L.P. = Leaf Print,
S. D. = Standard deviation,
C.V. = Coefficient of variation

Table 2: Percent frequency in phyllosphere mycoflora of *Duranta erecta* L.

Sr. No.	Mycoflora species	Winter						Summer						Rainy						Percent Frequency	
		Nov		Dec		Jan		Mar		April		May		July		Aug		Sept			
		LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP	LW	LP
1	***A. alternata***	9	8	5	4.5	13	2.16	0	0.83	0.33	0.33	0.33	1	4	3	4	6	3.33	5.83	**27.4**	**21**
2	*A. citri*	3	4.33	6	4	0	0	0	0	0	0	0	0	2.3	0	2.3	1.67	3.3	4	11.9	9.31
3	*A. niger*	0	0	0.33	0.5	2.33	2.16	1.33	3.5	1	1.83	1.33	1.67	0	0	2	2	1	2	6.56	9.08
4	*A. flavus*	2	1	0.33	0.67	0	0	0	0	0	0	0	0	1	2.66	0	0	0	0	2.34	2.88
5	*A. fumigatus*	4	3.33	0	0	0	0	0	0	0	0	0	0	6.33	2.83	0	0	0	0	7.27	4.09

6	*A. carboniferous*	0.33	0.67	0.33	0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.46	0.89
7	*A. nidulans*	0	0	0	0	0	0	2.33	0	0	0	0	0	0	0	0	0	2.3	3	3.26	1.99
8	*C. fulvum*	0.67	4	0	0	0	0	0	0	0	0	0	0	4	1	0.33	8.83	3.33	1.83	5.86	10.4
9	*Curvularia lunata*	1.67	0	0	0	0	0	0	0	0	0	0	0	0.33	3	0	0	0	0	1.41	1.99
10	*F. oxysporum*	0	0	0	0	0.67	0.5	0	0	0	0	0	0	0	0	0	0	3	3	2.58	2.33
11	*F. roseum*	0	3	0.33	3	0	0	0.67	1.67	2	0.33	3	0.67	0.33	0.83	0	0	0	0	4.45	6.31
12	*Mycogone*	0	2.33	3.67	0	0	0	0	0	0	0	0	0	0	0	1.67	2.33	0	0	3.76	3.1
13	*Penicillium*	3.33	2.83	0	0.5	0	1.33	0	1.67	2	1.33	0	0	0	0	0	0	0	0	3.75	5.09
14	*P. rubra*	0	0	0.33	0.5	1.67	2	0	0	0	0	0	0	0	0	1	2.5	0	0	2.11	3.32
15	*R. bataticola*	0.33	0.83	0	0	0	0	0	0	0	0	0	0	0	0	2.33	0.67	0	0	1.87	1
16	*R. solani*	2	2	0	0	0	0	0	0	0	0	0	0	0.67	0.67	0	0	0	0	1.88	1.77
17	*Torula*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	1.16	1.41	2.77
18	*N. sacchari*	1.33	2.67	1.33	2.67	0	0	0	0	0	0	0	0	5.33	0	0	0	0	0	5.62	3.55
19	*T. viride*	1	2	2	3	0	0	0	0	0	0	0	0	3	3	0	0	0	0	4.22	5.32
20	*R. stolonifer*	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0.83	0.67	0.83	1.88	3.76
Total		30.7	41	19.7	20	17.7	8.15	4.33	7.67	5.33	3.82	4.66	3.34	27.3	17	13.6	27.8	18.9	21.7	15.8	16.7
F value (LW)		Fungal species					2.10 (p = 0.05)					Season					4.66 (p = 0.01)				
F value (LP)		Fungal species					2.34 (p = 0.05)					Season					5.10 (p = 0.01)				

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