Phyllosphere mycoflora of ***Duranta erecta*** L. from polluted and non polluted area

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Abstract-

From MIDC Shendra (polluted) and Dr BAMU (non-polluted) area, Aurangabad, the phyllosphere mycoflora of *Duranta erecta* L. was studied in the selected months. The phyllosphere mycoflora were isolated on Potato Dextrose Agar (PDA) medium by leaf wash and leaf print methods. The phyllosphere mycoflora shows seasonal variation as well as quantitative and qualitative variation. A total of 20 fungal species were recorded in polluted area whereas 24 from non-polluted area. In the present study *Alternaria alternata* shows maximum percent frequency 27.4 % and 21 % from polluted area while 20.1 % and 17.4 % from non-polluted area by leaf wash and leaf print method respectively. Minimum percent frequency was experienced for *Aspergillus carboniferous* (0.46 % and 0.89 %) from polluted area while *Fusarium incarnatum* (1.01 % and 1.08 %) from non-polluted area by both methods. There was statistically significant variation in number of colonies among the fungal species as well as in various seasons (p=0.01) from both areas. Higher number of colonies was recorded during winter season (polluted area) and rainy season (non-polluted area) whereas minimum number of colonies was observed in summer season. The difference in the number of colonies under both areas were statistically significant (p=0.01) by both methods.

Keywords – Phyllosphere mycoflora, *Duranta erecta*, statistically significant, biodiversity.

Introduction-

The association of fungi and plants is an ancient and it involves many different fungi. 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) to denote the leaf surface environment.

Studies on seasonal variations and diversity of fungal communities on leaf surfaces of various plants such as *Calamus* (Girivasan and Suryanarayanan, 2004), *Eucalyptus viminalis* (Cabral, 1985), mangroves (Sivakumar and Kathiresan, 1990), *Carrisa congesta* and *Ficus benghalensis* (Jacob, 2000), *Liquidambar styraciflua*, *Quercus germana* and *Q. sartorri* (Heredia, 1993) have been made. Vardavakis (1988) estimated phylloplane fungal flora of *Arbutus unedo*, *Cistus incanus* and *Quercus coccifera*. Jager et al. (2001) studied the fungal diversity of mango phylloplane. Osono et al. (2004) studied the successional pattern of phyllosphere fungi on living and decomposing leaves of *Swida controversa.* Pusz and Pląskowska

(2012) studied fungal communities inhabiting phyllosphere, roots, rhizoplane and rhizosphere of symptomless ornamental foxtail A*maranthus paniculatus*.

The biodiversity 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 (Vorholt, 2012; Bulgarelli, 2013). The plant exudates secreted and thin nutrient films deposited from the atmosphere on the leaf surface further facilitates the microbial colonization (Kinkel, 1997). When plants grow, a new surface became available for fungi. 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 *et al*., 2001. This includes floristic information of fungi from soil, plant parts and litter, herbivore dung, entomogenous, freshwater and marine, animal and human (Barron, 1968; Ellis, 1976; Matsushima, 1975; Subramanian, 1983; Dix and Webster, 1995).

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) 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 leaves of *Duranta erecta* L. was collected in three different seasons in sterile zip- lock bags from MIDC Shendra (polluted) and Dr BAMU (non-polluted) area, Aurangabad, (MS).

2.2 Isolation of phyllosphere mycoflora: - The phyllosphere mycoflora were isolated on Potato Dextrose Agar (PDA) medium by leaf wash and leaf print methods. 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). Some mycoflora were identified by Agharkar Research Institute, Pune.

3. Result and Discussion.

a) Qualitative variation: Percent frequency of fungal species occurred on *Duranta erecta* L. in different months during various seasons is shown in Table 2. In polluted area, 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 occurrence 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.

The variation in the number of fungal colonies was produced by different fungal species (24) as well as those in various season (summer, winter and rainy) were statistically significant (p=0.01) under non-polluted area (Table 3). This indicated that the nature of mycoflora differed in different seasons. In addition, there was significant variation in the number of colonies formed by various fungi identified in the mycoflora by both methods. The average percent frequency of occurrence was found to be higher in case of *A.alternata (*20.1 % in LW and 17.4 % in LP), which was followed by *A. niger* (15.6 % and 15.1 % respectively) with higher number of colonies in rainy season. Minimum percent frequency was observed by *F. incarnatum* (1.01 and 1.08 % LW and LP).

b) Quantitative variation: Quantitative variation in number of fungal colonies in phyllosphere mycoflora under polluted and non-polluted areas was given in Table 1 (fig. 1). Higher number of colonies was recorded during winter season followed by rainy and summer in decreasing order under polluted area. The number of colonies was more in rainy season than winter and summer seasons under non-polluted area. Maximum number of colonies was found by leaf wash method in non-polluted area. The average number of colonies was 15.80 and 16.72 in polluted areas, where as 69.3 and 66.9 under non-polluted areas, when measured by leaf wash and leaf print method respectively. The difference in the number of colonies under polluted and non-polluted areas was statistically significant (p=0.01) under both the methods.

These results were confirmed by many authors. Phyllosphere microbial community may differ by seasons observed by Redford and Fierer (2009) and also differ between urban and non-urban locations found by Jumpponen and Jones (2009). The differences could be caused by climatic variation studied by Finkel (2011) or due to the limited dispersal of the colonizing taxa observed by Finkel (2012). Microbial compositions within plant species may different due to geographic locations studied by Rastogi (2012).

Wojciech (2015) 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.* Indu Soni (2016) 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).

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

Sr. No.	Months	No. of colonies(Polluted Area)				No. of colonies (Non-Polluted area)			
		L.W		L.P		L.W		L.P	
1	Nov-15	30.7	68.1	41	69.2	95.5	233	90.1	213
2	Dec-15	19.7		20		83.2		82.3	
3	Jan-16	17.7		8.15		53.9		41	
4	Mar-16	4.33	14.3	7.67	14.8	65.9	112	63.5	104
5	Apr-16	5.33		3.82		26.9		22	
6	May-16	4.66		3.34		19.6		18.3	
7	Jul-16	27.3	59.8	17	66.5	95.5	279	108	285
8	Aug-16	13.6		27.8		91		94.4	
9	Sep-16	18.9		21.7		92.2		82.5	
Mean		15.80		16.72		69.3		66.9	
S.D.		9.7		12.49		29.74		32.64	
C.V.		61.39		74.72		42.92		48.79	
t value		LW = 4.19 (p = 0.01)				LP = 3.52 (p = 0.01)			

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

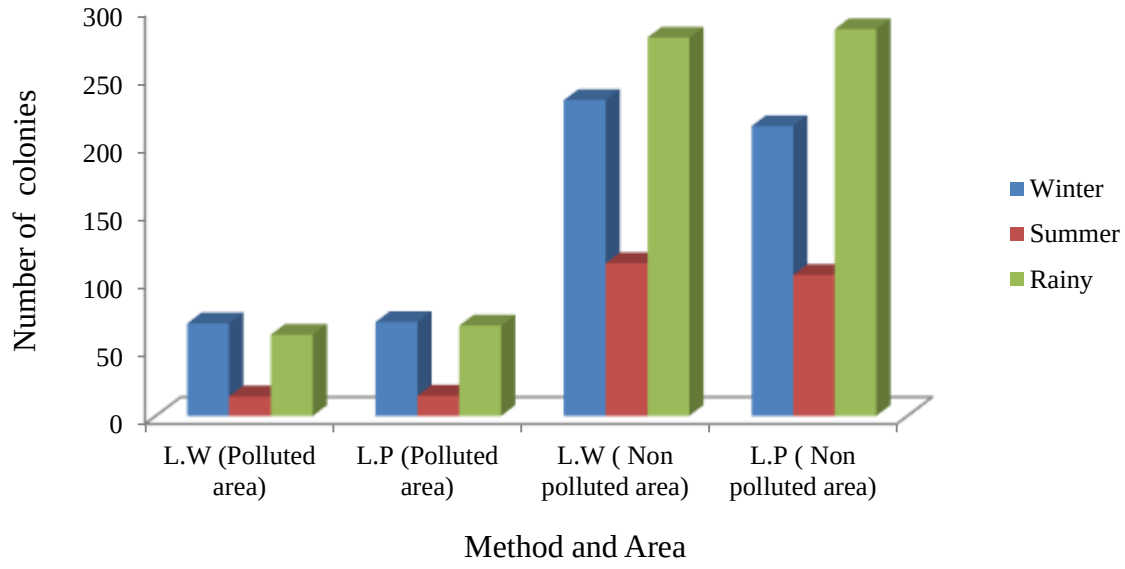

Table 2: Percent frequency in phyllosphere mycoflora of *Duranta erecta* L. from polluted area

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	*C. 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)

Table 3: Percent frequency in phyllosphere mycoflora of *Duranta erecta* L. from non polluted area

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***	14	15	9	7.67	12	6	11	13	2	1	5.3	1	31	33.3	30	13	11.3	15	**20.1**	**17.4**
2	*A. citri*	1	4.33	0	0	0	0	0	0	0	0	0	0	2.3	3.67	2.3	1.67	3.3	4	1.43	2.27
3	*A. niger*	10	12	11	7,33	14	16	13	11	9	9	6.3	6	12	14	11	11	11	12	15.6	15.1
4	*A. flavus*	4	3	6.3	4	4	0	8	9.3	7	6	3	5	0	0	0	3.3	2	1.3	5.5	5.3
5	*A. fumigatus*	6	4.67	6	7	0	0	4	3.3	0	0	0	0	3	4.3	0	4.3	0	2.67	3.05	4.36
6	*A. carboniferous*	3.3	1.33	2.3	3	3	0	0	0	0	0	0	0	0	6.67	0	3.67	5	8.3	2.18	3.82
7	*A. nidulans*	3	3.3	2.3	4	0	0	0	0	0	0	0	0	1	4.3	2.3	3	3	3.3	1.86	2.97
8	*Colletotrichum* sp.	0	0	3.3	2	4.3	2	0	0	0	0	0	0	0	1.3	3	3	4	1	2.34	1.55
9	*C. fulvum*	1.3	3	6.7	7.83	0	0	0	0	0	0	0	0	3.3	2.3	2	3	1.3	0	2.34	2.68
10	*C. lunata*	3	4	3.3	2.5	0	0	3	2.3	0	0	0	0	0	2	3.3	2	4.7	2	2.77	2.46
11	*C. musae*	1.3	4	4	0	0	0	0	0	0	0	0	0	6	0	0	4	3.3	0	2.34	1.33
12	*F. oxysporum*	6.7	3	3.3	6	5.3	5	0	0	4.3	6	5	6.33	3	3.3	0	0	4.3	2.3	5.11	5.31
13	*F. roseum*	2.3	3	3	6	5	6	0	0	0	0	0	0	8	9.3	7.3	0	6	4	5.07	4.7
14	***F. incarnatum***	1	1.33	2	3.33	2.3	0	0	0	0	0	0	0	0	0	0.33	1.33	0.67	0.5	**1.01**	**1.08**

Table 3: Contd.

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
15	*Helminthosporium* sp.	4	2.3	2	4.33	0	0	2.3	3.3	0	0	0	0	3	2.3	2.3	2.3	5	3.5	2.98	3
16	*Penicillium*	3	2	4.7	4.67	4	3	6	7.67	0	0	0	0	0	0	3.3	3.3	2	2	3.69	3.76
17	*P. rubra*	2	1.83	0	0	0	0	0	0	0	0	0	0	2.3	4.3	2.3	3.5	5	4	1.86	2.27
18	*R. bataticola*	1,3	2	3	0	0	0	0	0	0	0	0	0	2.3	3.3	2	2	4	3	1.81	1.71
19	*R. solani*	7.7	0	0	0	0	3	5.3	0	0	0	0	0	2	0	0	4	1.3	0	2.61	1.16
20	*N. gregarium*	3.3	2	0	0	0	0	2.3	3.33	1.3	0	0	0	0	0	3	3	0	0	1.59	1.38
21	*Torula*	8	5	5.7	9.33	0	0	0	0	0	0	0	0	2	1	0	4	1.3	0	2.73	3.21
22	*N. sacchari*	1.33	3	1.33	2.67	0	0	0	1.33	3.3	0	0	0	11	7.67	11	13	8.7	8.3	5.88	5.98
23	*T. viride*	5	4	4	8	0	0	11	9	0	0	0	0	3.3	3.3	3.3	3	3.7	3.3	4.86	5.09
24	*P. archeri*	4.3	6	0	0	0	0	0	0	0	0	0	0	0	1.3	2.3	3	1.3	2	1.27	2.04
Total		95.5	90.1	83.2	82.3	53.9	41	65.9	63.5	26.9	22	19.6	18.3	95.5	108	91	94.4	92.2	82.5	69.3	66.9
F value (LW)		Fungal species						4.99 (p = 0.01)				Season				5.89 (p = 0.01)					
F value (LP)		Fungal species						4.84 (p = 0.01)				Season				8.07(p = 0.01)					

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