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Qualitative and quantitative variation in phyllosphere mycoflora of *Hamelia patens* Jacq.

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Abstract
The Phyllosphere mycoflora of *Hamelia patens* Jacq. was studied during the different seasons of the year 2015-16 from MIDC Shendra area, Aurangabad (MS). The mycoflora were isolated and pure cultures were maintained on Potato dextrose agar. Identification of mycoflora was done by morphological characters and microscopic observations. The phyllosphere mycoflora shows seasonal variation as well as qualitative and quantitative variation. In the present study, total of 21 fungal species were recorded by leaf wash and leaf print method. The average percent frequency of occurrence was found to be in case of *Cladosporium fulvum* (19.2% LW and 27.3% LP), which was followed by *Gibberella avenacea* (14.4% and 13.2%). Minimum percent frequency was experienced by *Fusarium oxysporum* (0.1% and 0.28%) by both methods. There was statistically significant variation in number of colonies among the fungal species. However the variation due to season was statistically significant (p=0.01) in leaf wash method, while non-significant in leaf print method. Higher number of colonies was recorded during rainy season whereas minimum number of colonies was observed in summer season. The average number of colonies was 36.98 by leaf wash and 39.91 by leaf print method.

Keywords: Phyllosphere mycoflora, *Hamelia patens*, statistically significant, *Cladosporium fulvum*

1. Introduction
The term phyllosphere was first coined by Last, (1955) to denote the leaf surface environment. 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) [8, 4, 5, 11] reported the most common fungi were *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Gibberella*, *Memnoniella*, *Mycosphaerella*, *Setosphaeria* and *Stachybotrys.*
The qualitative and quantitative variation in phyllosphere fungi were affected by temperature, humidity, rainfall, nutrient availability, leaf age and type. They were also affected by presence of inhibitors, arrival and settlement of viable propagules (Vorholt, 2012; Bulgarelli, 2013) [12, 2]. 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, 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.

2. Materials and Methods
2.1 Collection of sample: The leaves of *Hamelia patens* Jacq. was collected in three different seasons in sterile zip- lock bags from MIDC Shendra area 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 ^{0}C 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 were identified on the basis of morphological and microscopic observations as well as by slide culture (Mukadam, 2006).

3. Result and Discussion

In the present study, total of 21 fungal species were recorded by leaf wash and leaf print method on phyllosphere of *Hamelia patens* Jacq., viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus citri, Aspergillus nidulus, Cladosporium fulvum, Fusarium oxysporum, Rhizopus stolonifer, Fusarium roseum, Rhizoctonia bataticola, Curvularia lunata, Penicillium, Torula, Phytophthora rubra, Trichoderma viride, Gibberella avenacea, Colletotrichum musae, Fusarium solani* and *Mycogone* from Shendra MIDC, Aurangabad (MS) in different seasons. The maximum percent frequency was observed for *Cladosporium fulvum* (19.2% LW and 27.3% LP), which was followed by *Gibberella avenacea* (14.4% and 13.2%). Minimum percent frequency was experienced by *Fusarium oxysporum* (0.1% and 0.28%) in both methods. There was statistically significant variation in number of colonies among the fungal species. However the variation due to season was statistically significant (p=0.01) in leaf wash method, while non-significant in leaf print method (Table 2). The quantitative variation shows higher number of colonies during rainy (143) followed by winter (133) and summer (56.6) in decreasing order in leaf wash method whereas, higher number of colonies was recorded during winter (153) followed by rainy (144) and summer (62) in decreasing order in leaf print method due to favourable temperature, rainfall and relative humidity. The average number of colonies was 36.98 by leaf wash and 39.91 by leaf print method (Table 1).

These results were confirmed by many authors and found that *Cladosporium fulvum, Aspergillus niger* was the most common phyllosphere fungus found on the phyllosphere of different plants (Tiwari and Saluja, 2010; Bhuyan, 2013; Seema Verma, 2013; Dalal, 2014; Waill, 2016) [9, 1, 3, 10, 13].

Table 1: Quantitative variation of *Phyllosphere mycoflora* of *Hamelia patens* Jacq

S. No	Months	No. of colonies				Temp.^{0}C	Relative Humidity (%)	Rainfall (mm)
		L.W		L.P				
1	Nov-15	40.8	133	74.7	153	28	46	18.31
2	Dec-15	48.3		36.2		27	35	0.4
3	Jan-16	44		42.2		27	31	0.2
4	Mar-16	30.3	56.6	27	62	33	21	14.67
5	Apr-16	18.3		24.3		36	19	4.69
6	May-16	7.99		10.7		36	30	22.38
7	Jul-16	47	143	40.5	144	28	75	230.61
8	Aug-16	56.8		59.8		27	77	181.75
9	Sep-16	39.3		43.8		27	79	310.1
Mean		36.98		39.91		29.88	45.88	87.012
S.D		15.51		19.05		3.73	23.21	113.19
C.V.		41.95		47.73		12.46	50.57	130.09

Table 2: Percent frequency of phyllosphere mycoflora of *Hamelia patens* Jacq. during different months of 2015-2016

S. 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*	0	0.83	0	0	0	0	0	0	0	0	0	0.33	0	0	6	15.5	15	20	6.31	10.2
2	*A. citri*	3.67	2	3.33	0	0	0	0	0	0	0	0	0	3	5	11	9	5	6.33	7.81	6.22
3	*A. niger*	10	16.8	4	2	9	11.7	8	4.17	2	1.17	0.33	0.5	0	1.33	2	3	0	0	10.6	11.3
4	*A. flavus*	4	3.33	0.67	0.67	0	0	3	3.17	1	0.33	0.33	0.33	4	5.67	0	0	0	0	3.9	3.76
5	*A. fumigatus*	0	3.17	0	0	0	0	0	0	0	0	0	0	4.2	0.5	2.5	0	0	0	2.01	1.02
6	*Nigrospora*	0	0	6.33	5.83	6	0	0	0	0	0	0	0	4	2	5	3	1.33	0	6.81	3.02
7	*A. nidulans*	0	0	0	0	0	0	0.33	0.83	0	0	0	0	1	1	0	0	1	1	0.7	0.79
8	*C. musae*	1.33	4	4	0	0	0	0	0	0	0	0	0	8	0	0	4	4	3.33	5.21	3.16
9	*C. fulvum*	9	28.2	10	8	12	19	9	10	12	15	7	9	3	6	1.67	2.5	0.33	0.5	19.2	27.3
10	*C. lunata.*	2	1	2	1	0	0	0	0	0	0	0	0	0	0	2	2	0.33	1.17	1.9	1.44
11	*F. oxysporum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	1	0.1	0.28
12	*F. roseum*	1.33	0.33	0	3.33	0	0	0.67	0.83	0.67	1	0	0	0	0.5	0.67	1.17	0	0	1	1.99
13	*F. solani*	2	3	3	1.17	0	0	0	2	0	0	0	0	6.33	7.67	0.33	1.33	0.67	0.5	3.7	4.36
14	*G. avenacea*	3.67	4.83	5	5.67	4	0	6	4	2.67	4.83	0	0	7.67	8.33	10	11.8	9	8	14.4	13.2
15	*Mycogone*	0	0	2	3	2	0.33	0	0	0	0	0	0	0	0	0	0	0	0	1.2	0.93
16	*Penicillium*	1	3	0.67	2.5	0		1.33	1	0	2	0.33	0.5	0	0	0	0	0	0	1	2.51
17	*P. rubra*	0	0	3	3	0	0.5	2	1	0	0	0	0	3	2	12	0	0	0	6.01	1.81
18	*R. bataticola*	1.5	1	3.3	0	0		0	0	0	0	0	0	2.8	0.5	0.3	3.3	0	0	2.37	1.34
19	*Torula*	0	0	0	0	8	7.67	0	0	0	0	0	0	0	0	3	2	0.33	1	3.4	2.97
20	*T. viride*	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	2	1	1.2	1.11
21	*Rp. stolonifer*	1.33	3.17	1	0	1	0	0	0	0	0	0	0	0	0	0.33	1.17	0	0	1.1	1.21
Total		40.8	74.7	48.3	36.2	44	42.2	30.3	27	18.3	24.3	7.99	10.7	47	40.5	56.8	59.8	39.3	43.8	37	39.9
F. value (LW)		Fungal species						2.43 (p= 0.01)				Season						2.89 (p= 0.01)			
F. value (LP)		Fungal species						2.52 (p= 0.01)				Season						1.76 NS			

LW = Leaf wash, LP = Leaf Print

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