STUDIES ON PHYLLOPLANE MICROFLORA OF DHAK (*Butea monosperma* (Lamk.) Taub)

Deepika Chauhan[1], Abhishek Swami[2] and Navneet[3]

[1,3]*Department of Botany and Microbiology*, Gurukul Kangri Vishwavidyalaya, Haridwar-U.K, India

[2]*Department of Applied Sciences, Dronacharya Group of Institutions*[2], *Greater Noida-U.P, India*

Abstract*-The leaves of dhak (*Butea monosperma* (Lamk.) Taub) are used in the preparation of platters (dona and pattals). Phylloplane microflora of mature and young leaves of dhak was studied by dilution plate method using Martin's rose bengal agar medium for fungi and Nutrient agar medium for bacteria in the two successive years. The qualitative and quantitative estimation of fungi and quantitative estimation of bacteria was carried out. Quantitatively the density of fungi of mature and young leaves varied between 257.56 and 68.18 (mature leaf) 43.27 and 11.88 (young leaf) propagules/100cm^2 while that of bacteria was 205.61 and 37.85 (mature leaf) and 60.58 and 9.74 (young leaf) propagules/100cm^2. The maximum density of fungi on mature leaves was found in the month of February while on young leaves it was found in the month of August. Qualitatively a total of 17 fungal forms were isolated during the period of investigation. The dominant fungal species on leaves were *Alternaria alternata, C. cladosporioides, T. koningii, Aspergillus niger, Curvularia lunata, A. flavus, A. fumigatus and A.terreus

***Index Terms:* Phylloplane, *Butea monosperma,* microflora and *Aspergillus fumigatus*.**

I. INTRODUCTION

Leaves are the organs of limited growth, which arise laterally and exogenously at the stem apex. Leaf surface provides a suitable habitat for the growth, reproduction and multiplication of microorganisms because the surface medium of leaf comprises of exudates, chemical compounds resulting from biological activity of various microbes inducing nitrogen fixers and components resulting from atmospheric pollution. The age and position of a leaf is an important factor for microbial colonization of its surface. Moreover, the physical factors like temperature, relative humidity, light and wind velocity also influences the leaf surface colonizers. In the present study qualitative and quantitative estimation of phylloplane microflora of dhak (*Butea monosperma* (Lamk.) Taub) (mature and young leaf) in relation it environmental factors were studied by dilution plate method.

II. MATERIAL AND METHODS

The young and mature leaves of dhak were collected from Raja Ji National Park in the year 2006-2007 and 2007-2008 in sterilized plastic boxes of 17x17x 6 cms, dimension and processed the same day. The discs of 7mm diameter were cut from each leaf with the help of sterilized cork borer. Forty discs of each variety were shaken separately in 100 ml of sterilized water for half an hour. One ml aliquots of the resulting suspension were poured out in six sterilized petri dishes, three for fungi and three for bacteria. Martin's agar medium for fungi and nutrient agar medium for bacteria were over poured in the petri dishes in warm and melted form with the dilutions. The plates were incubated at 30^0 C.

Observations were taken after two days for bacteria and after five days for fungi. A complete record of data of the number of colonies of each species was kept. The density, percentage of abundance, and percentage contributions of each species were calculated.

II. RESULTS AND DISCUSSION

The phylloplane microflora of young and mature leaves of dhak was analysed quantitatively as well as qualitatively by dilution plate technique in 2006-2007 and 2007-2008 and presented in table 1 to 5. A total number of 12 samplings each of fungi and bacteria were observed at two months interval. In the first year and second year a total of 933 and 589 fungal colonies were observed on mature leaves while on the young leaves 172 and 226 fungal colonies were observed respectively. The quantitative analysis of phylloplane mycoflora (table -1) at two months interval in the first year on mature leaves revealed that the density was maximum in the month of February and minimum in June and in second year it was maximum in November and minimum in July. In the first year the fungal density (propagules/cm^2) on young leaves was maximum in the month of August and minimum in the month of December and in second year it was maximum in October and minimum in March.

The bacterial density on mature leaves in the first year showed in **Table-2,** was maximum in the month of February and minimum in October and in second year it was maximum in November and minimum in January while on the young leaves the maximum density was recoded in the month of February and minimum in the month of April and in the second year maximum in November and minimum in January. The dominant fungi on mature leaves in the first year was *Alternaria alternata* (7.50%) and in second year *C. herbarum* (7.80%) to total phylloplane mycoflora and on the young leaves in first year was *Alternatia tenuissima* (11.04%) and second year *Trichderma koningii* (9.29%) was dominant (table-5). *Alternaria alternata, Cladosporium* spp. *and Aspergillus* spp. were recoded in all the twelve samplings on mature leaves while *Alternaria alternata, A. tenuissima, A.flavus, A. fumigatus, A. terreus. Cladosporium cladosporioides, C. herbarum, Curvularia lunata, Fusarium equseti* and *P. purpurogenum* and white sterile form were recoded on mature leaves.

In the present investigation *Aspergillus* (5species) and *Penicillium* (3species) represented by highest number of species and is due to the presence of high humidity (80-85%). *Aspergillus* spp. belongs to the class Deuteromycetes, Kurup et al.[12]. Due to small spores size of *Penicillium* and *Aspergillus spp* (2-3µm) and they easily travel through the airways. Several species of *Aspergillus* have been associated with leaf surface (phylloplane) and cause skin diseases and respiratory problems Burge,[3] . *Aspergillus spp*. isolated from contaminated fruits, vegetable, plants materials and indoor environment *A. fumigatus*, Lander et al., [15].

The microflora was higher on mature leaves than young ones as also repoted by various workers. In *Juniperus procera.* Bacterial densities increased throughout spring and early summer, decreased in late summer and then increased again in late autumn as was also reported by Pennycook and Newhook [18] on apple leaves. Sharma and Mukherji [19] also found that moderate temperatures of October favored the appearance of a higher fungal population on cotton leaves, but very low temperatures of January and December resulted in a decline in the total fungal population. *Aspergillus niger* was sensitive to low temperatures of winter months. In addition, temperature variations are also known to bring about changes in growth pattern of *Pythium* and *Rhizopus stolonifer* , Pierson [17]

In the present investigation in the month of February and November recorded 80% and 82% relative humidity and highest fungal propagules/cm^2 were recoded in February and November (first year and second year on mature and young leaves) and in the month of June, July, October December and March and were recoded very low spore concentration in this month only 54% and 52% humidity was recoded. Dickinson [7] while studying the fungal colonization of *Pisum* leaves found the lower relative humidity earlier in the season delayed or altered the course of colonization while the slight increase in rainfall and relative humidity is sufficient to increase the growth of leaf saprophytes significantly.

The highest fungal concentration was found in winter seasons. The phylloplane microflora show a distinct seasonal pattern, with maximum in autumn, winter and minimum in summer and directly correlated with humidity and inversely with temprature as also reported by Vardavakis [23]. The micro-climatic factors were always correlated while studying the presence of microorganisms on the leaves of *Triticum aestivum* Burrage, [4] of *Solanum tuberosum* , Kumar and Gupta, [13] of *Poa flabellate,* Hurst *et al.,[10]* and *Brassica compestris* var. sarson and *Ercica sativa*, Sharma *et al*., [21]. Airborne spores impact on leaf surfaces and may adhere due to structural or chemical features of the epidermis and the spore , Andrews and Buck, [1]. Spore release from many fungi inhabiting the phylloplane is passive through the action of wind or rain splash; however, other spores are actively propelled into the atmosphere by various mechanisms, Aylor, [2].

Phylloplane microflora varies in size and diversity depending on the influence of numerous biotic and abiotic factors, which affect their growth and survival, Bakker [5]. These factors include leaf age, external nutrients, interactions between populations of different microorganisms, Blakeman, [6], temperature, humidity, light intensity, wind speed and the presence of air pollutants , Dix and Webster, [8].

Cladosporium, Alternaria, Penicillium, Aspergillus, and *Mucor* were reported to be the commonest allergenic fungi, Malling [16] *Cladosporium* is believed to be the most common one causing mold allergy, Malling [16]. However, the most prevalent airborne fungi are not necessarily the most potent allergens, at least as determined by prick testing, Terracina and Rogers [12]. Spores of *Alternaria alternata* and those of the closely related genera *Stemphylium* and *Ulocladium* are considered to be the most important mold allergens in the United State, Hoffman [9]. *Penicillium* exposure was a risk factor for asthma, while *Aspergillus* exposure was a risk factor for atopy (a genetic trait of increased allergen sensitivity), Sakamoto [20]. *Aspergillus species* and in particular *Aspergillus fumigatus* appeared to be the etiological agents in various lung diseases and allergens. Inhalation of low doses of *Aspergillus* spores may induce sensitization and asthma in sensitive patients, while inhalation of high doses may trigger alveolitis and farmer's lung by Wallenbeck [24]. *Curvularia lunata* was found to be a cause of allergic bronchopulmonary disease, Halwig [11] and a common inhabitant of leaf surface, Joshi *et al.*, [12].

Since dhak leaves are mostly used as a raw material for the preparation of platters (dona and pattals), information on fungi that live on the leaves of these plants surface is very much necessary because these fungi may cause infection which sometimes could be poisonous and harmful for man.

Table 1: Variation in the density (propagules/cm$^{2)}$ of phylloplane mycoflora of dhak as obtained by dilution plate method (mature leaf and young leaf).

Plant	Types of leaf	Date 16/10/06	Date 12/12/06	Date 12/02/07	Date 17/04/07	Date 22/06/07	Date 22/08/07	Date 10/11/07	Date 20/01/08	Date 20/03/08	Date 20/05/08	Date 20/07/08	Date 16/10/08
1.Dhak	Mature leaf	145.00	211.03	257.56	212.11	68.18	119.02	135.25	123.37	99.54	106.03	71.42	91.98
	Young leaf	31.36	11.88	31.36	34.61	37.85	43.27	34.61	37.85	30.29	33.53	31.36	38.81

Table 2: Variation in the density (propagules/cm$^{2)}$ of phylloplane bacteria of dhak as obtained by dilution plate method (mature leaf and young leaf).

Plant	Types of leaf	Date 16/10/06	Date 12/12/06	Date 12/02/07	Date 17/04/07	Date 22/06/07	Date 22/08/07	Date 10/11/07	Date 20/01/08	Date 20/03/08	Date 20/05/08	Date 20/07/08	Date 16/10/08
1.Dhak	Mature leaf	37.85	87.66	205.61	133.11	60.58	140.68	163.40	76.81	122.27	89.80	84.44	106.03
	Young leaf	36.78	22.72	54.09	9.74	30.8	38.96	60.58	23.79	43.27	42.20	49.77	51.94

Table: - 3 Percentage of abundance of phylloplane mycoflora of dhak by dilution plate method (mature leaf).

YEAR		FIRST YEAR						SECOND YEAR					
S.No.	Microorganism	Date 10/11/07	Date 12/12/06	Date 12/02/07	Date 17/04/07	Date 22/06/07	Date 22/08/07	Date 10/11/07	Date 20/01/08	Date 20/03/08	Date 20/05/08	Date 20/07/08	Date 16/10/08
		Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak
1.	*Alternaria alternata*	4.47	5.12	8.40	918	7.93	10.0	4.8	6.14	1.08	4.08	7.57	5.88
2.	*Alternaria tenuissima*	3.73	6.15	6.30	7.14	4.76	6.36	5.6	3.50	2.1	4.08	9.09	7.05
3.	*Aspergillus candidus*	5.22	4.10	5.46	7.14	4.76	5.45	7.2	6.14	2.1	3.06	4.54	5.88
4.	*A.flavus*	6.71	8.71	5.46	7.14	6.34	3.63	9.6	1.75	4.34	5.10	7.57	4.70
5.	*A.fumigatus*	5.97	6.15	6.72	6.12	7.93	10.90	5.6	3.50	4.34	6.12	6.06	4.70
6.	*A.niger*	5.97	5.64	6.72	6.53	15.87	8.18	4.00	10.52	8.69	8.16	7.57	4.70
7.	*A.terreus*	5.22	5.64	7.56	7.53	7.93	6.36	4.8	8.77	7.60	8.16	4.54	7.05
8.	*Cladosporium cladosporioides*	5.22	8.20	5.88	7.14	12.69	7.27	3.2	4.38	8.69	2.04	4.54	7.05
9.	*C.herbarum*	5.22	5.64	7.98	8.54	4.76	3.63	8.00	7.89	11.95	9.18	4.54	4.70
10	*Curvularia lunata*	3.73	7.17	7.14	7.53	3.17	7.27	7.2	13.15	5.43	3.06	4.54	5.88
11.	*Fusarium equiseti*	5.22	5.64	6.30	6.12	7.93	6.36	7.2	6.14	5.43	3.06	4.54	4.70
12.	*Penicillium citrinum*	5.22	5.12	4.62	4.08	------	1.81	8.8	3.50	10.86	9.18	4.54	7.70
13.	*P.cyclopium*	6.71	4.61	5.04	4.08	6.34	2.72	6.4	3.50	8.69	11.22	6.06	5.88
14.	*P.purpurogenum*	6.71	6.15	3.36	4.59	3.17	5.45	3.2	4.38	3.26	6.12	7.57	5.88
15.	*Rhizopus oryzae*	8.95	4.10	-----	1.53	3.17	7.27	4.8	7.89	3.26	7.14	4.54	8.23
16.	*Trichoderma koningii*	7.46	5.12	5.88	-----	3.17	1.1	6.4	7.89	6.52	6.12	9.09	8.23
17.	*White sterile form*	-----	4.61	7.14	5.10	-------	6.36	3.2	9.64	5.43	4.08	3.03	4.70

Table: - 4 Percentage of abundance of phylloplane mycoflora of dhak by dilution plate method (young leaf).

YEAR		FIRST YEAR						SECOND YEAR					
S.No.	Microorganism	Date 10/11/07	Date 12/12/06	Date 12/02/07	Date 17/04/07	Date 22/06/07	Date 22/08/07	Date 10/11/07	Date 20/01/08	Date 20/03/08	Date 20/05/08	Date 20/07/08	Date 16/10/08
		Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak	Dhak
1.	*Alternaria alternata*	10.34	9.09	13.79	6.25	14.28	10.0	6.06	5.71	-----	3.22	----	5.63
2.	*Alternaria tenuissima*	13.79	9.09	6.89	12.5	11.42	10.0	3.03	5.71	3.57	6.45	----	5.63
3.	*Aspergillus candidus*	6.89	------	6.89	6.25	14.28	5.00	----	11.42	3.57	3.22	----	5.63
4.	*A.flavus*	3.44	------	10.34	6.25	5.71	5.00	9.09	2.85	17.85	3.22	----	8.45
5.	*A.fumigatus*	6.89	-----	6.89	3.12	5.71	10.0	----	2.85	-----	3.22	----	8.45
6.	*A.niger*	6.89	-------	6.89	3.12	8.57	7.5	3.03	5.71	7.14	9.67	-----	7.04
7.	*A.terreus*	10.34	-----	3.44	3.12	2.85	15.0	6.06	----	3.57	-----	6.89	5.63
8.	*Cladosporium cladosporioides*	6.84	------	----	21.87	2.85	----	3.03	-----	-----	3.22	10.34	4.22
9.	*C.herbarum*	3.44	------	10.34	-----	8.57	10.0	6.06	8.57	7.14	16.12	6.89	5.63
10	*Curvularia lunata*	3.44	18.18	3.44	3.12	2.85	-----	3.03	2.85	7.14	3.22	6.89	4.22
11.	*Fusarium equiseti*	10.34	9.09	3.44	6.25	2.85	----	3.03	2.85	3.57	3.22	13.79	4.22
12.	*Penicillium citrinum*	6.84	9.09	6.89	-----	2.85	----	9.09	8.57	-----	6.45	6.89	7.04
13.	*P.cyclopium*	6.84	9.09	3.44	------	5.71	----	9.09	5.71	7.14	9.67	3.44	9.85
14.	*P.purpurogenum*	6.84	9.09	3.44	6.25	----	7.5	6.06	8.57	3.57	6.45	3.44	4.22
15.	*Rhizopus oryzae*	----	9.09	3.44	6.25	----	5.00	6.06	14.28	3.57	9.67	-----	2.81
16.	*Trichoderma koningii*	-----	------	13.79	9.37	5.71	2.5	12.12	14.28	7.14	6.45	3.44	8.45
17.	*White sterile form*	-----	18.18	10.34	6.25	5.71	12.5	12.12	-----	21.42	19.33	3.44	2.81

Table –5 Percentage contribution of phylloplane fungi of dhak leaves as obtained by dilution plate method (mature & young leaf).

S.No.	Microorganism	Year 2006-2007	Year 2007-2008	Year 2006-2007	Year 2007-2008
		Mature	Mature	Young	Young
1.	*Alternaria alternate*	*7.50*	4.58	10.46	3.98
2.	*Alternaria longipes*	6.00	4.92	----	----
3.	*Alternaria tenuissima*	----	-----	11.04	4.42
4.	*Aspergillus candidus*	5.46	4.92	7.55	4.42
5.	*A.flavus*	6.53	5.43	5.81	7.07
6.	*A.fumigatus*	6.96	4.92	6.39	3.53
7.	*A.niger*	7.39	7.13	6.39	5.75
8.	*A.terreus*	6.84	6.79	6.97	3.98
9.	*Cladosporium cladosporioides*	7.18	4.75	5.81	3.53
10	*C. herbarum*	6.53	7.80	6.39	7.96
11.	*Curvularia lunata*	6.43	6.79	3.48	4.86
12.	*Fusarium equiseti*	6.10	5.26	4.65	5.30
13.	*Penicillium citrinum*	3.96	6.96	3.48	8.40
14.	*P.cyclopium*	4.82	6.79	3.48	8.84
15.	*P. purpurogenum*	4.93	4.75	5.23	5.30
16.	*Rhizopus oryzae*	3.53	5.94	3.48	5.30
17.	*Trichoderma koningii*	*4.07*	7.13	3.48	9.29
18.	Black Sterile form	5.68	5.09	------	----
19.	White sterile form	-------	-----	5.81	7.96

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