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Research Article

DIVERSITY OF SURFACE MYCOFLORA ON *TINOSPORA CORDIFOLIA*

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ABSTRACT
The leaf surface in open atmosphere is exposed to a continuous air current which carries numerous fungal spores, along with various other microscopic objects both living and non living. Apart of this spores is occasionally trapped by leaves through various devices viz. hairs and sticky surface etc. Once the spore happens to fall upon a leaf surface some of them finding a suitable micro habitat and try to form colonies. The complicated processes occurring at the surface of the leaves have attracted the attention of physiologist and pathologist. In present study leaves of *Tinospora cordifolia* were collected randomly from the plants monthly. Five petriplates were used in each sampling period. Then the petriplates were incubated at 25+ 1^0 C in incubation chamber for 6 days. After incubation the fungi were recorded and identified. 23 species of leaf surface fungi obtained, where 2 species of Zygomycotina, 8 species of Ascomycotina, 11 species of Deuteromycotia and 2 from mycelia sterilia reported. In leaf surface 172 colonies reported in rainy season, 101 and 62 colonies from winter and summer season respectively, total 335 colonies recorder reported.

Keywords: *Aerobiology, Fungal Spores, Microhabitats, Air Spora*

INTRODUCTION

Aerobiology is the multidisciplinary environmental science which deals information from plant science, science of weather forecasting, palynology, air pollution, geography, chemistry, plant pathology, remote sensing etc. The term aerobiology came in to the use during 1930s as collective term for studies of air spores like air borne fungal spores, pollen grains and other microorganism. Gregory 1952 suggested the term "Air spora" to describe the fungal and pollen flora. The leaf surface in open atmosphere is exposed to a continuous air current which carries numerous fungal spores, along with various other microscopic objects both living and non living.

Aerial parts of the plant serve as the microhabitats for a variety of micro organism present in the air spora. The leaf surface accommodates micro organism by providing a complete ecological niche where the exudates from leaves provide nutrition, moisture, pH and temperature.

The green leaves exhibit a phenomenon of removing some of the microbes normally carried by wind. Once the leaves unfold a series of complex events follow due it interest between leaf surface exudates and microbes whose spore happen to colonize the leaf (Sharma and Mukharji, 1974: Tiwari, 1977). Generally pathogenic fungi are more common on the ageing parts than young parts (Sahu, 1992, 1995; Tiwari, 1977).

Geogray and Stedeman (1953) studied that deposition of airborne spores on various freely exposed surfaces was much influenced by the type of spores, by the wind spread and orientation and stickiness of the trap surface. Spores reach the leaf in three ways (1) by dry wind (2) by rain drops (3) by rain splash droplets.

Last, in 1955 gave the term "phyllosphere" to denote the external leaf surfaces of plants, at term analogous to rhizosphere was coined by Ruinen (1956). Prasad and Bilgrami (1969) state that phyllosphere term in used to designate all those organisms (including fungi, bacteria, actinomycetes) which are in some way are the other under the influenced of the exposed part of the plant, like leaf, stem, bud, flower and fruit etc.

Bessems (1973) considered that the phyllosphere is the environment of microorganisms on the wet surface and in the space between leaf sheath and stem. It is now accepted that phyllosphere is the

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environment and phyllosphere should be restricted to the zone near leaves and "phyloplane" used when referring to actual leaf surface (Kerling, 1958) Kendrick and Burgas (1962), Dickinson (1965), Kapooriya and Singh (1969), Last and Warren (1972), Sharma (1973) and Mukharjee (1974).

It is well known that an active population of fungi exists on the surface of physiologically active green leaves and these have been termed as phylloplane by Kerling (1958), Sharma (1973) states that phylloplane may be define as the leaf surface medium, where a heterogeneity of microorganisms grow, reproduce and multiply in dynamic equilibrium with the interacting micro and macro environment of the leaf itself.

The phylloplane is a natural habitat on the leaf surface which represents a heterogeneous population comprising of both pathogen and non pathogen (Laben, 1965; Mukerjee, 1973; Mishra and Tiwari, 1976). Much of the interest in phyllosphere microbiology has been driven by the need to better understand the behavior and control of the pathogen that are prominent member of this community.

Plant productivity can be effected by microbes A) where as other produce phytohormones that have the potential to effect plant development and productivity. B) Much less is understood about the identity or properties of numerous non pathogenic microbes that inhabit the phyllosphere. Kerling (1964) studied phylloplane of the leaves of Rye and Strawberry.

Last and Deighton (1965) studied non parasitic microbes colonizing green leaves. Sinha (1965) studied microbial complex of phyllosphere and disease control, he also studied the microflora on leaves of *Capsicum annum* and some other plants.

MATERIALS AND METHODS

Tinospora cordifolia is economically and medically important crop in India. Leaf surface mycoflora of the test plant was studied during July 2009 to June 2010. For the study of leaf surface mycoflora leaves were collected randomly from the plants monthly. The leaf samples were collected in sterilized polythene bags.

Than the collected leaves were brought in to the laboratory for the isolation of leaf surface mycoflora. Than the collected sample leaves were placed in 150 ml. of conical flask containing 75 ml. of sterilized distilled water.

For the homogenous suspension of fungal organisms attached to the leaf surface the conical flask was hand shaken for 30 minutes.

This suspension was used for the study, and then 1 ml. of the suspension was poured in to the PDA media containing each petriplates. Five petriplates were used in each sampling period. Then the petriplates were incubated at 25$\pm$ 1^0 C in incubation chamber for 6 days. After incubation the fungi were recorded and identified.

Potato-Dextrose-Agar (Riker and Riker, 1936) media-

Potato (peeled and sliced)	200 Grams
Dextrose	20 Grams
Agar-Agar	20 Grams
Water	1000 ml.
pH	6.0 -6.5

For the ecological study the percentage frequency and percentage abundance of the fungal species is calculated with the help of following formulae.

$$\text{Percentage frequency} = \frac{\text{Number of observation in which a species appeared}}{\text{Total Number of observation}} \times 100$$

$$\text{Percentage abundance} = \frac{\text{Total No. of colonies of a species in all the observation}}{\text{Total Number of colonies}} \times 100$$

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RESULTS AND DISCUSSION

23 species of leaf surface obtained, where 2 species of Zygomycotina, 8 species of Ascomycotina, 11 species of Deuteromycotia and 2 from mycelia sterilia reported (table 1). In leaf surface 172 colonies reported in rainy season, 101 and 62 colonies from winter and summer season respectively, total 335 colonies recorder reported.

Month August is the dominant contributor during the study period (table 1). In the study most frequently observed fungal species from leaf surface were *Aspergillus fumigates* (13.13 %), *A. nidulans* (11.04 %), *Alternaria alternate* (9.55 %), *Aspergillus niger* (7.46 %) and *Cladosporium cladosporiodes* (5.67 %) (Table 5, figure 3).

In rainy season % contribution of Zygomycotina 7.55, Ascomycotina 48.83, Deuteromycotina 39.53 and Mycelia sterilia 4.06 has been reported (table 4, figure 2A). In winter season % contribution of Zygomycotina 0.99, Ascomycotina 31.68, Deuteromycotina 50.49 and Mycelia sterilia 6.93 has been reported (table 4, figure 2B).

In summer season % contribution of Zygomycotina 00, Ascomycotina 54.83, Deuteromycotina 33.87 and Mycelia sterilia 11.29 has been reported (table 4, figure 2C). The annual contribution of aeromycoflora is Zygomycotina 7.16, Ascomycotina 44.77, Deuteromycotina 41.79 and Mycelia sterilia 6.26 has been reported (table 4, figure 2).

Months wise percentage contribution of each fungal group to the total leaf surface mycoflora was observed. In July, Zygomycotina 7.40, Ascomycotina 77.77 and Deuteromycotina 11.11 were observed mycelia sterilia was absent in the month.

In August, Zygomycotina 13.43, Ascomycotina 55.22, Deuteromycotina 28.35 and mycelia sterilia 2.98 were reported. In September, Zygomycotina 3.84, Ascomycotina 38.46, Deuteromycotina 51.92 and mycelia sterilia 5.76 were reported.

In October, Zygomycotina was absent, Ascomycotina 23.07, Deuteromycotina 69.23 and mycelia sterilia 7.69 were reported.

In November, Zygomycotina 15.62, Ascomycotina 34.37, Deuteromycotina 37.5 and mycelia sterilia 12.5 were observed. In December, Zygomycotina 17.24, Ascomycotina 31.0, Deuteromycotina 41.37 and mycelia sterilia 10.34 were reported.

In January, Zygomycotina 4.00, Ascomycotina 36.00, Deuteromycotina 60.00 was reported and mycelia sterilia was absent. In February, Zygomycotina and mycelia sterilia were absent, Ascomycotina 20.00, Deuteromycotina 80.00 were reported.

In March, Zygomycotina was absent, Ascomycotina 42.85, Deuteromycotina 47.61 and mycelia sterilia 9.52 were reported.

In April, Zygomycotina and mycelia sterilia were absent, Ascomycotina 61.11 and Deuteromycotina 38.88 was reported. In May, Zygomycotina was absent, Ascomycotina 41.66, Deuteromycotina 16.66 and mycelia sterilia 41.66 were reported.

In June, Zygomycotina and mycelia sterilia were absent, Ascomycotina 81.81 and Deuteromycotina 18.18 were reported respectively (table 1).

Prasad and Bilgrami (1969) reported 28 fungi from the leaf surface of Litchi. Mishra and Shrivastava (1970) 23 and 22 fungi from the yellow and green leaves of *Oryza sativa*. Tiwari (1977) reported 30 and 21 fungal species from the leaf surface mycoflora of Pea and Linium.

Sahu (1995) reported 16 fungal species which were isolated from the leaf surface mycoflora of Onion. Sharma (2006) reported 35 fungal species belonging to 23 genera of fungi from the leaf surface mycoflora and 32 species belonging to 18 genera from aeromycoflora of the *Curcuma longa*. Kumar and Sinha (1996) reported 46 fungi from the leaf surface of Spinach. Sharma (2001) also reported 33 fungal species from the leaf surface of *Ocimum sanctum*.

Conclusion

Environmental factor like temperature and relative humidity play a important role in determining the increase and decrease of fungal population on leaf surface. Dickinson (1967) reported that environmental

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factors are the most important physical factors which affect the total number of fungal population present on the leaf surface.

The fungal population is not homogenous throughout the year and shows seasonal variations. During the investigation it was observed that maximum fungal population was observed in rainy season due to favorable condition i.e. humidity, moderate in winter seasons and minimum number of fungal population was reported in summer season, possibly due to high temperature and low humidity. Varma and Khare (1987b), who observed that maximum fungal population was in winter.

Tiwari (1977) reported that minimum fungal species were observed in summer season. Minimum number of fungal population was also reported by Gour and Kasana (1981), Tiwari and Sahu (1995b), Sahu (1995).

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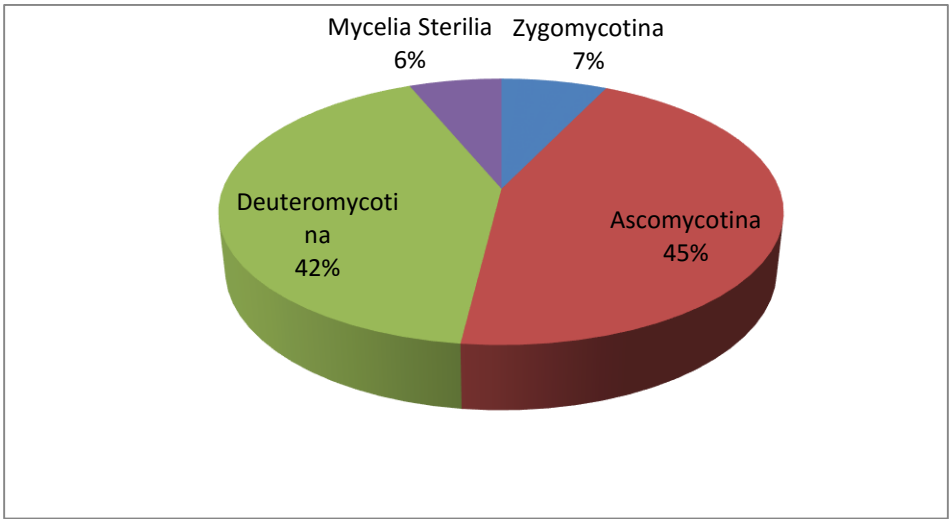
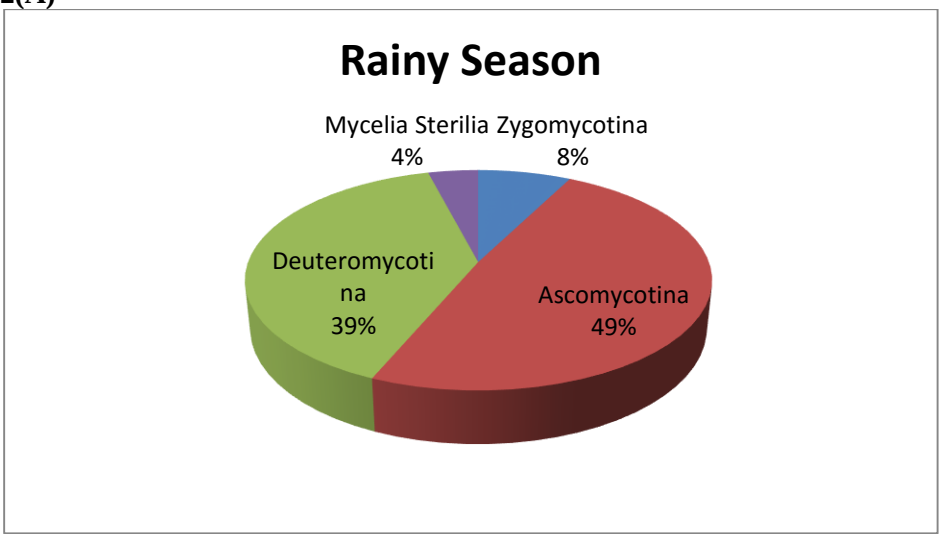


Figure 1: The Annual Percentage Contribution of Each Group to the Total Leaf Surface Mycoflora of *Tinospora cordifolia* Plant

2(A)



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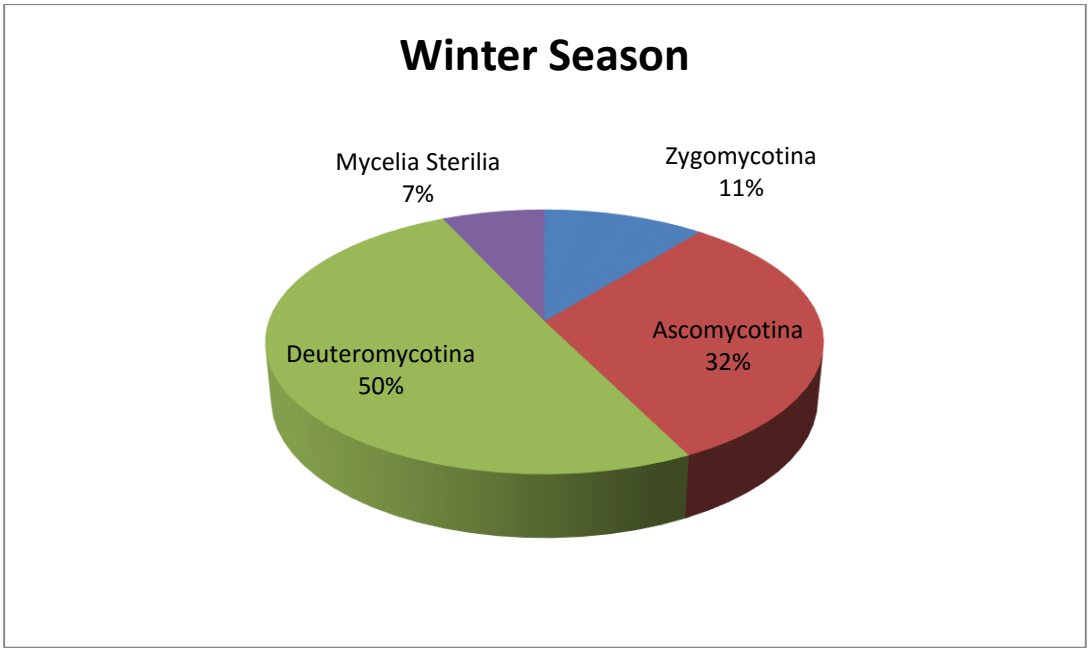

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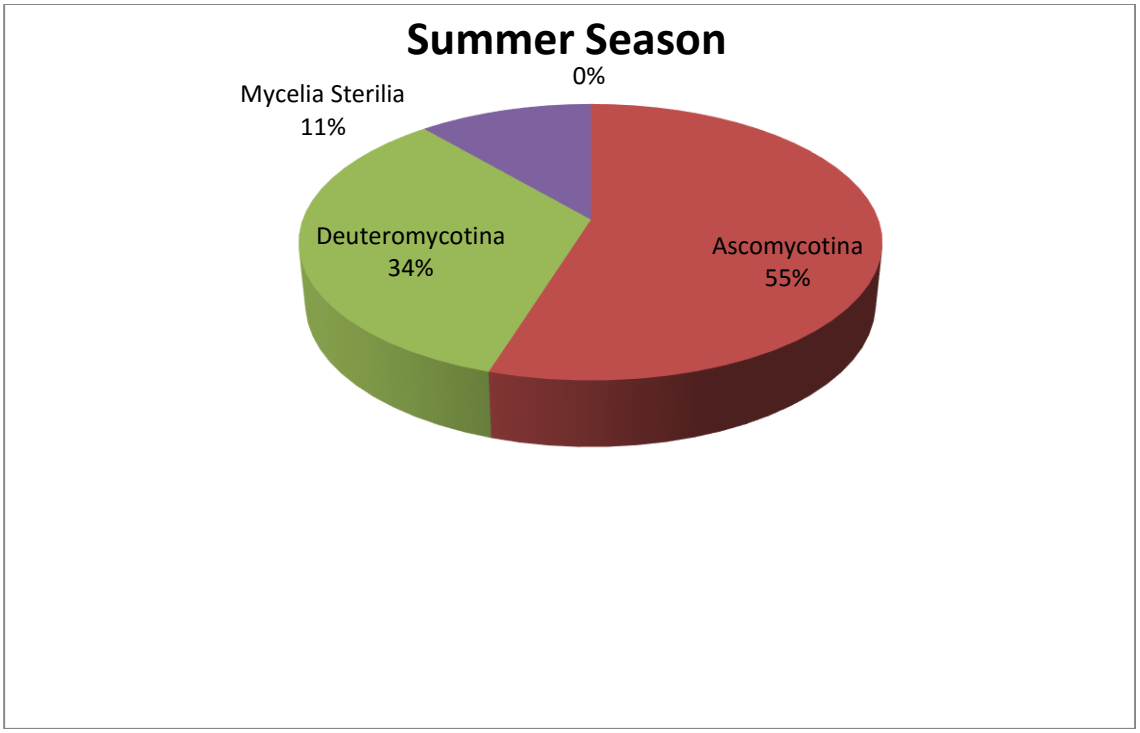

Figure 2: The Seasonal Percentage Contribution of Each Group to the Total Leaf Surface Mycoflora of *Tinospora cordifolia* Plant

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Table 1: Showing Number of Colonies on Leaf Surface of *Tinospora cordifolia*

S.N.	Name of Fungi	Rainy Season					Winter Season					Summer Season					Total Colonies of sps.
		Jul	August	Sept	Oct	**Total**	Nov	Dec	Jan	Feb	**Total**	Mar	Apr	May	June	**Total**	
	CLASS-ZYGOMYCOTINA																
1	*Mucor recemosus* Fresen.	2	6	-	-	**8**	3	4	-	-	**7**	-	-	-	-	**-**	**15**
2	*Rhizopus stolonifer* Lind.	-	3	2	-	**5**	2	1	1	-	**4**	-	-	-	-	**-**	**09**
	CLASS-ASCOMYCOTINA																
3	*Aspergillus flavus* Link Ex Fries	3	3	2	-	**8**	-	2	-	-	**2**	-	-	-	3	**3**	**13**
4	*A. Fumigates* Fresenius	9	10	5	-	**24**	2	4	3	3	**12**	3	3	2	-	**8**	**44**
5	*A.nidulans* Eidan	5	9	6	2	**22**	-	-	2	-	**2**	4	5	-	4	**13**	**37**
6	*A. niger* Van. Tieghem	4	4	-	1	**9**	3	1	2	-	**6**	2	3	3	2	**10**	**25**
7	*A. terreus* Thom.	-	6	3	3	**12**	2	1	-	-	**3**	-	-	-	-	**-**	**15**
8	*Drechslera* sps.	-	-	-	-	**-**	-	-	2	-	**2**	-	-	-	-	**-**	**02**
9	*Penicillium nigricans*. Bainier	-	3	2	-	**5**	3	1	-	-	**4**	-	-	-	-	**-**	**09**
10	*P. rubrum*	-	2	2	-	**4**	1	-	-	-	**1**	-	-	-	-	**-**	**05**
	CLASS-DEUTEROMYCOTINA																
11	*Alternaria alternate* Fr. Keissler	-	3	5	2	**10**	2	3	3	7	**15**	2	3	-	2	**7**	**32**
12	*A. tenuis* Fries	-	-	-	-	**-**	-	-	2	1	**3**	-	-	-	-	**-**	**03**
13	*Cladosporium cladosporiodes* Fr.	-	-	-	2	**2**	2	5	3	2	**12**	3	2	-	-	**5**	**19**
14	*C. herbarum* (Pers.) Link.	-	3	6	4	**13**	-	-	-	-	**-**	4	-	-	-	**4**	**17**
15	*Curvularia clavata* Jain	-	3	-	-	**3**	-	-	-	-	**-**	-	-	-	-	**-**	**03**
16	*C. lunata* Wakker	1	3	3	2	**9**	-	-	-	-	**-**	-	2	2	-	**4**	**13**
17	*Fusarium oxysporium*	-	-	5	2	**7**	2	-	-	-	**2**	-	-	-	-	**-**	**09**

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	Schl. Ex. Fries																
18	*Helminthosporium australienae* Bu.	-	-	2	3	**5**	3	-	-	-	**3**	-	-	-	-	-	**08**
19	*Nigrospora oryzae* Berk. Br.	3	3	-	-	**6**	-	-	-	-	**-**	-	-	-	-	-	**06**
20	*Paecilomyces varioti* Bainier	-	-	-	-	**-**	-	-	3	2	**5**	-	-	-	-	-	**05**
21	*Trichoderma harbarum*	-	4	6	3	**13**	3	4	4	-	**11**	1	-	-	-	**1**	**25**
	CLASS- MYCELIA STERILIA																
22	*Mycelia sterilia* Black	-	-	-	2	**2**	2	2	-	-	**4**	2	-	2	-	**4**	**10**
23	*Mycelia sterilia* White	-	2	3	-	**5**	2	1	-	-	**3**	-	-	3	-	**3**	**11**
	Total colonies in the month	**27**	**67**	**52**	**26**	**172**	**32**	**29**	**25**	**15**	**101**	**21**	**18**	**12**	**11**	**62**	**335**

Table 2: Showing Percentage Frequency and Percentage Abundance of Leaf Surface Mycoflora

S. N.	Name of Fungi	Rainy Season								Winter Season								Summer Season							
		Jul		Augst		Sept		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		June	
		%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A	%F	%A
1	*Mucor recemosus* Fresen.	40	7.40	40	8.95	-	-	-	-	20	9.37	40	13.79	-	-	-	-	-	-	-	-	-	-	-	-
2	*Rhizopus stolonifer* Lind.	-	-	20	4.47	20	3.84	-	-	20	6.25	20	3.44	20	4.00	-	-	-	-	-	-	-	-	-	-
3	*Aspergillus flavus* Link Ex Fries	40	11.11	20	4.47	20	3.84	-	-	-	-	20	6.89	-	-	-	-	-	-	-	-	-	-	40	27.27
4	*A.*	10	33.	10	14.	8	9.6	-	-	2	6.	6	13.	4	12.	2	20.	2	14.	2	16.	2	16.	-	-

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	Fumigates Fresenius	0	33	0	92	0	1			0	25	0	79	0	00	0	00	0	28	0	66	0	66		
5	*A.nidulans* Eidan	80	18.51	100	13.43	80	11.53	40	7.69	-	-	-	-	20	8.00	-	-	20	19.04	40	27.27	-	-	20	36.36
6	*A. niger* Van. Tieghem	40	14.81	60	5.97	-	-	20	3.84	20	9.37	20	3.44	40	8.00	-	-	20	9.52	40	16.66	20	25.00	20	18.18
7	*A. terreus* Thom.	-	-	100	8.95	60	5.76	40	11.53	20	6.25	20	3.44	-	-	-	-	-	-	-	-	-	-	-	-
8	*Drechslera* sps.	-	-	-	-	-	-	-	-	-	-	-	-	20	8.00	-	-	-	-	-	-	-	-	-	-
9	*Penicillium nigricans*. Bainier	-	-	60	4.47	20	3.84	-	-	40	9.37	20	3.44	-	-	-	-	-	-	-	-	-	-	-	-
10	*P. rubrum*	-	-	20	2.98	20	3.84	-	-	20	3.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	*Alternaria alternate* Fr. Keissler	-	-	40	4.47	80	9.61	40	7.69	20	6.25	40	10.34	40	12.00	80	41.66	20	9.52	40	16.66	-	-	20	18.78
12	*A. tenuis* Fries	-	-	-	-	-	-	-	-	-	-	-	-	20	8.00	20	6.66	-	-	-	-	-	-	-	-
13	*Cladosporium cladosporioides* Fr.	-	-	-	-	-	-	20	7.69	20	6.25	60	17.24	40	12.00	20	13.33	40	14.28	20	11.11	-	-	-	-
14	*C. herbarum* (Pers.) Link.	-	-	40	4.68	80	11.53	40	15.38	-	-	-	-	-	-	-	-	40	19.04	-	-	-	-	-	-
15	*Curvularia clavata* Jain	-	-	20	4.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	*C. lunata*	20	3.7	40	4.4	4	5.7	2	7.6	-	-	-	-	-	-	-	-	-	-	2	11.	2	16.	-	-

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	Wakker		0		7	0	6	0	9											0	11	0	66		
17	*Fusarium oxysporium* Schl. Ex. Fries	-	-	-	-	80	9.61	20	7.69	20	6.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	*Helminthosporium australienae* Bu.	-	-	-	-	20	3.84	40	11.53	40	9.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	*Nigrospora oryzae* Berk. Br.	40	11.11	40	4.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	*Paecilomyces varioti* Bainier	-	-	-	-	-	-	-	-	-	-	-	-	40	13.63	20	13.33	-	-	-	-	-	-	-	-
21	*Trichoderma harbarum*	-	-	60	5.97	80	11.53	40	11.53	40	9.37	40	13.79	40	16.00	-	-	20	4.76	-	-	-	-	-	-
22	*Mycelia sterilia* Black	-	-	-	-	-	-	20	7.69	20	6.25	20	6.89	-	-	-	-	20	9.52	-	-	20	16.66	-	-
23	*Mycelia sterilia* White	-	-	20	2.98	40	5.76	-	-	20	6.25	20	3.44	-	-	-	-	-	-	-	-	40	25.00	-	-

Table 3: The Month Wise Percentage Abundance (Contribution) of Each Group to the Total Leaf Surface Mycoflora of *Tinospora cordifolia* Plant

S.N.	Group	July	Agst	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
1	ZYGOMYCOTINA	7.40	13.43	3.84	-	15.62	17.24	4.00	-	-	-	-	-
2	ASCOMYCOTINA	77.77	55.22	38.46	23.07	34.37	31.0.	36.0	20.0	42.85	61.11	41.66	81.81
3	DEUTEROMYCOTINA	11.11	28.35	51.92	69.23	37.5	41.37	60.0	80.0	47.61	38.88	16.66	18.18
4	MYCELIA STERILIA	-	2.98	5.76	7.69	12.5	10.34	-	-	9.52	-	41.66	-

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Table 4: The Seasonal Percentage Abundance (Contribution) of Each Group to the Total Leaf Surface Mycoflora of *Tinospora Cordifolia* Plant

S.N.	Group	Rainy	Winter	Summer	Annual
1	ZYGOMYCOTINA	7.55	10.89	-	7.16
2	ASCOMYCOTINA	48.83	31.68	54.83	44.77
3	DEUTEROMYCOTINA	39.53	50.49	33.87	41.79
4	MYCELIA STERILIA	4.06	6.93	11.29	6.26

Figure 3: Percentage Contribution of Leaf Surface Mycoflora during the Study i.e. July 2009 to June 2010

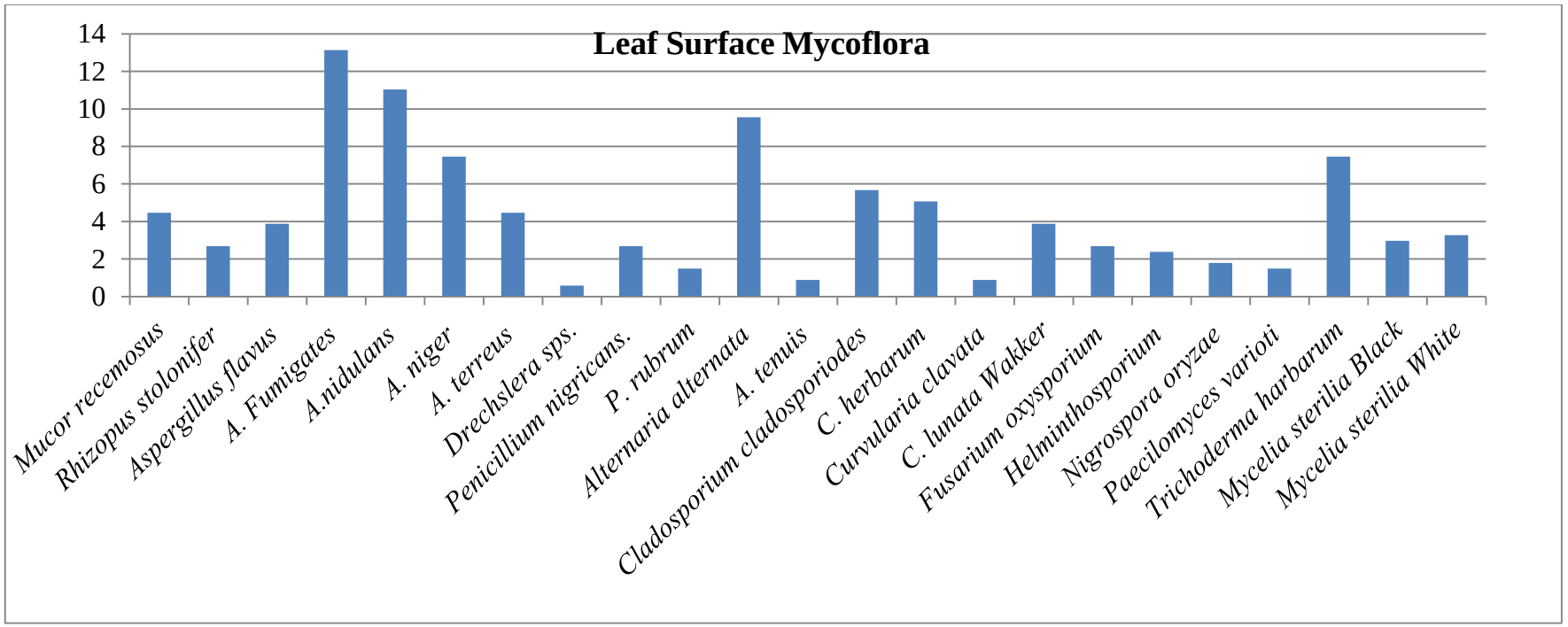

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Table 5: Showing Percentage Contribution of Leaf Surface Mycoflora

S.N.	Name of Fungi	Leaf Surface Mycoflora
1	*Mucor recemosus* Fresen.	4.47
2	*Rhizopus stolonifer* Lind.	2.68
3	*Aspergillus flavus* Link Ex Fries	3.88
4	*A. Fumigates* Fresenius	13.13
5	*A. nidulans* Eidan	11.04
6	*A. niger* Van. Tieghem	7.46
7	*A. terreus* Thom.	4.47
8	*Drechslera* sps.	0.59
9	*Penicillium nigricans*. Bainier	2.68
10	*P. rubrum*	1.49
11	*Alternaria alternata* Fr. Keissler	9.55
12	*A. tenuis* Fries	0.89
13	*Cladosporium cladosporiodes* Fr.	5.67
14	*C. herbarum* (Pers.) Link.	5.07
15	*Curvularia clavata* Jain	0.89
16	*C. lunata* Wakker	3.88
17	*Fusarium oxysporium* Schl. Ex. Fries	2.68
18	*Helminthosporium australienae* Bu.	2.38
19	*Nigrospora oryzae* Berk. Br.	1.79
20	*Paecilomyces varioti* Bainier	1.49
21	*Trichoderma harbarum*	7.46
22	*Mycelia sterilia* Black	2.98
23	*Mycelia sterilia* White	3.28
	Total	**100 %**

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