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# Circadian And Seasonal Variation In The Incidence Of Thermotolerant Fungi In The Ambient Air Of Jabalpur

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#### **Abstract**

The present paper deals with mean annual circadian and seasonal variations in the concentration of airborne thermotolerant mycoflora of Jabalpur during May, 2009 to April, 2010. Air sampling was done at 5 distant sites of Jabalpur during morning, evening and noon by using Anderson 2-stage viable particle air sampler at 10 day interval. Thermotolerant fungi were isolated by using PDA, YpSs, GYP media and by incubating at 45±1 °C for a period of 3 to 5 days. A total of 27 thermotolerant fungi was identified morphologically by using standard literature. The maximum concentration of these fungi was observed during the evening (876/m³ air) followed by noon (804.8/m³ air) and morning (699 /m³ air). The highest concentration of thermotolerant fungi was observed during autumn (864.8/m³ air), followed by winter (748.4/m³ air), rainy (653.1/m³ air), spring (564.8/m³ air) and summer (458.9/m³ air). The autumn season was having favorable temperature of 28 °C, relative humidity 67.2% and average total rainfall 4.1mm. On the contrary, summer season had hot and dry climate (34.1°C and RH 22.6%) unfavorable for fungal growth. The analysis of variance for CFU number/m³ air of different seasons indicates that there was a significant difference in the fungal content (no/m³ air) of ambient air of different seasons at P≥0.05.

**Keywords:** Thermotolerant fungi, circadian, season

# Introduction

Among the eukaryotes, only thermophilic and thermotolerant fungi have the exceptional ability to grow at high temperature of 50-60°C [1]. Thermophilic fungi are defined as those that are capable of growing optimally at or above 50°C [2]. The thermotolerant fungi are those that are growing optimally at a temperature range of 28 to 40°C and are capable of growing at higher temperature (beyond 40°C). The has summarized certain concepts related to thermophilism and thermophilic fungi [3]. However, a definite explanation has not yet been established.

Some species of fungi are able to grow up to 120°C in thermal springs and hydrothermal vents [4]. Thermophilic fungi are chief component of the micro flora that develop in heaped masses of plant material, piles of agricultural and forestry products and other organic material which provide a suitable environment for their growth and development.

Cooney and Emerson (1964) provided a taxonomic description of 13 species of thermophilic fungi along with their habitats and general biology. Presently, a large number of thermophilic fungal species have been described as thermotolerant which constitute a heterogeneous physiological group of various genera of Zygomycetes, Ascomycetes. Basidiomycetes and Deuteromycetes[5].

Concentration of airborne fungal spores has been linked to wind velocity, relative humidity (%), temperature (°C) and rainfall (mm) [6]. The ambient air temperature and relative humidity play an important role in increasing fungal population in the environment [7].

Circadian variation refers to changes in the concentration and composition of the concerned mycoflora in different periods of the day. Seasonal variation in climatic parameters affects aeromycoflora of the area. Fungal spores are not equally distributed in the environment. Their distribution varies according to geographical location and meteorological condition. The airborne fungi are responsible for biodeterioration of storage material, archeological monuments, wall painting, electronic equipments, library materials and other important material.

#### Material and methods

The aeromycological survey was conducted at five distant sites viz., Site I: Rani Durgavati University area, Site II: Cattle Field, Adhartal, Site III: Ranital Stadium area, Site IV: Shaw Wallace Gelatin Factory area and Site V: Tilahari, Mandla Road of Jabalpur city. The sources of thermotolerant fungi like garbage dumping sites, compost pits and industrial waste heaps were monitored during May, 2009 to April, 2010 for the incidence of airborne thermotolerant fungi.

Air sampling at each site was done at 10 days interval by using aluminium Anderson 2-stage viable particle air sampler. Air samples were taken by operating the sampler for one minute, one feet above the surface of the compost pits/garbage dumpings. The air sampling of all the five sites was completed between 7.00 hrs. to 11.00 hrs. The additional air sampling was carried at noon (between 12.00 to 13.00 hrs.) and at evening (17.00 to 18.00 hrs.) at Site I: R.D.University area for studying the circadian variation in the incidence of airborne thermotolerant fungi. The concentration of the thermotolerant fungi (no./m³ air) was calculated by multiplying the CFU count with conservesion factor (35.3) of the Anderson sampler.

The isolation of thermotolerant fungi was done by using PDA (potato extract- 200 g, dextrose- 20 g, agar- 20 g. distilled water- 1000 ml), YpSs (yeast extract- 4.0,  $K_2HPO_4$ - 1.0 g, MgSO<sub>4</sub>.7H<sub>2</sub>O- 0.5 g, soluble starch- 15.0 g, agar- 20.0 g. distilled water- 1000 ml) and GYP (glucose-5.0 g. yeast extract- 3.0 g. peptone-5.0 g, agar- 20 g, distilled water- 1000 ml) media. The antibacterial antibiotic i.e. gentamycine sulphate (50 mg/L) + Penicillin G (100 mg/L) were added in the medium after sterilization. The PDA medium containing petridish was put into stage I. whereas YpSs medium containing petridish into stage II of the Anderson air sampler. The date and time of sampling, type of medium and stage was recorded on the cover of the petriplates. The incubation temperature was  $28\pm1$  °C for 24 hours and thereafter 45+1 °C for a period from 3 to 5 days. The isolated airborne thermotolerant fungi were transferred to GYP slants and identified on the basis of morphological characteristics compared with standard literature.

The data on meteorological parameters such as temperature, relative humidity and rainfall were procured from the Department of Meteorology, Government of India. Adhartal, Jabalpur (M.P.). The average concentration of CFU of each sampling time was used to analyzed the significant different in the circadian changes by using the ANOVA technique.

The assessment of the seasonal changes in the species diversity and concentration of airborne thermotolerant fungi was done by similarity index and by performing ANOVA test. The total number /average number of fungi in 6 sampling dates of each of the following seasons was analyzed in this test viz., Spring (February-March), Summer (April-15<sup>th</sup> June), Rainy (16<sup>th</sup>June-15<sup>th</sup> September). Autumn (16th September-November) and Winter (December-January). Each sampling date had 5 air samples (one from each sites). The six air samples at a site per season were analyzed to assess the seasonal variation in fungal incidence.

The assessment of circadian and seasonal changes in the species diversity and concentration of airborne thermotolerant fungi was done by Sorenson's similarity index and by applying ANOVA test respectively. For assessing the circadian changes in the incidence of airborne thermotolerant fungi, 36 samples each of morning, noon and evening were analyzed by performing ANOVA through SPSS software. The six air samples at a site per seasons (spring, summer, rainy, autumn, and winter) were analyzed by using ANOVA to assess significance seasonal changes in the fungal incidence.

## **Results and discussion**

The data on mean annual concentration occurrence of airborne thermotolerant fungi in morning, noon and evening (circadian changes) are presented in Table 1. The maximum mean number of CFU was observed during the evening (876/m³ air) followed by noon. (804.8/m³ air) and morning (699/m³ air). Mishra concluded that circadian changes in the concentration of airborne fungi show seasonal variation. Their results indicate that fungal flora of summer peaked at 8:00 hrs. spring at 12:00 hrs, whereas rainy, autumn and winter peaked at 12:00 hrs[8].

The seasonal mean concentration of airborne thermotolerant fungi in relation to climatic parameters is presented in Table 2. The analysis of variance for CFU number/m³ air of different seasons is presented in Table 3. The table indicates that there was significant difference in the fungal content (no/m³ air) of different seasons at P>0.05. The highest concentration of thermotolerant fungi was observed during autumn (864.8/m³ air) followed by winter (748.4/m³ air), rainy (653.1/m³ air), spring (564.8/m³ air) and summer (458.9/m³ air). The autumn which supported maximum concentration of airborne thermotolerant fungi was characterized by having an average temperature of 23.7 °C, relative humidity (67.2 %) and total rainfall of 65.1 mm. The winter season ranked second in term of favourable climatic conditions. The other scientists also reported that warm and moist conditions favor the fungal growth and sporulation[9,10].

The summer season which had minimum concentration of thermotolerant fungi was having hot and dry climate (mean temperature 34.1 °C and 22.6 % RH) that was unfavourable for fungal growth and spore liberation mechanism. The rainy season is also suitable for fungal growth and for active spore discharge mechanism. However, the heavy rains decrease the atmospheric spore load through the washing off.

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**Table 1** Annual mean concentration (n=36 samples) of airborne thermotolerant fungi in three different periods of a day

S.No.	Fungi	Mean concentration (CFU no./m³ air)				
		Morning	Noon	Evening		
1	Acremonium alabamensis	17.7	28.2	17.7		
2	Alternaria alternate	35.3	45.9	38.8		
3	Aspergillus flavus	42.4	52.9	31.8		
4	A. fumigates	28.2	21.2	42.4		
5	A. niger	28.2	56.5	67.1		
6	A. sparsus	105.9	67.1	45.9		
7	A. terreus	49.4	77.7	56.5		
8	A. versicolor	74.1	63.5	35.3		
9	Chaetomium globosum	17.7	31.8	38.8		
10	C. thermophilum	21.2	28.2	21.2		
11	Cladosporium cladosporiodes	42.4	49.4	17.7		
12	C. herbarum	28.2	14.1	28.2		
13	Curv <mark>ularia lu</mark> nata	35.3	14.1	14.1		
14	Fusarium oxysporum	35.3	24.7	31.8		
15	F. soloni	31.8	17.7	35.5		
16	Helminthosporium	10.6	14.1	21.2		
	oryzac Humicola grisea					
17	Mucor globosus	3.50	21.2	31.8		
18	Paecilomyces variotii	10.6	31.8	49.4		
19	Penicillium expansum	7.10	24.7	17.7		
20	P. nigaricans	10.6	14.1	21.7		
21	Rhizomucor michei	28.2	24.1	24.7		
22	R. pusillus	3.50	14.1	35.3		
23	Rhizopus microsporus	7.10	14.1	31.8		
24		0.0	10.6	31.8		

	R. oryzae			
25		3.5	7.10	21.2
26	Thermomyces lanuginosus	10.6	14 .1	21.2
27	Trichoderma viridie	10.6	21.0 2	45.9
Total		699	804 .8	876

Sampling period: Morning (7:00 to 11:00 AM), Noon (12:00 to 1:00PM), Evening (5:00 to 6:00)

**Table 2** Seasonal mean concentration (6 samples at a site/season) of airborne thermotolerant fungi and climatic parameters during May, 2009 to April, 2010

Sites/climatic	Mean number of CFU & respective concentrations/mean valus of parameters									
paratmers	Spring		Summer		Rainy		Autumn		Winter	
	No.	Conc.	No.	Conc.	No.	Conc.	No.	Conc.	No.	Conc.
Site I	17	600.1	15.5	547.2	19.5	688.4	22.5	794.3	21.5	758.9
Site II	15	529.5	11.5	405.9	17	600.1	24	847.2	18.5	653.1
Site III	15.5	547.2	13.5	476.6	17.5	617.8	24.5	864.9	23.5	829.6
Site IV	16.5	582.5	13	458.9	19.5	688.4	25.5	900.2	20.5	723.7
Site V	16	564.8	11.5	405.9	19	670.7	26	917.8	22	776.6
Total	80	2824	65	2294.5	92.5	3265.3	122.5	4324.3	106	3741.8
Mear	16	564.8	13	458.9	18.5	653.1	24.5	864.8	21.2	748.4
Temperature (°C)	24.6		34.1		24.1		23.7		17.8	
Relative Humidity (%)	45.4		22.6		75.4		67.2		67.5	
Rainfall(mm) Avervage of Monthly total	7.9		4.1		367.1		65.1		15.1	

Sampling period: Spring (February-March), Summer (May-April), Rainy (July-August), Autumn (October-November) and Winter (December-January)

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**Table 3** The analysis of variance for CFU number/m<sup>3</sup> air of different seasons at  $P \ge 0.05$ 

0.	Seasons	F-value	Sig.	$H_0: \mu_1 = \mu_2$
1.	Spring Vs. Summer	13.3	0.006	Rejected
2.	Spring Vs. Rainy	15.6	0.004	Rejected
3.	Spring Vs. Autumn	144.5	0.000	Rejected
4.	Spring Vs. Winter	33.2	0.000	Rejected
5.	Summer Vs. Rainy	36.6	0.000	Rejected
6.	Summer Vs. Autumn	142.9	0.000	Rejected
7.	Summer Vs. Winter	54.2	0.000	Rejected
8.	Rainy Vs. Autumn	55.4	0.000	Rejected
9.	Rainy Vs. Winter	7.6	0.025	Rejected
10.	Autumn Vs. Winter	10.2	0.013	Rejected

 $H_A: \mu_1 \neq \mu_2$  Accepted