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**RESEARCH ARTICLE**

# AEROMYCOLOGICAL STUDIES OVER SOME PADDY FIELDS IN THE RATNAGIRI DISTRICT OF MAHARASHTRA STATE

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**ABSTRACT**

Airborne microbial analysis revealed in 50 components of fungal spore types of which 2 belongs to Zygomycotina; 3 belongs to Basidiomycotina; 17 belongs to Ascomycotina, 28 belong to Deuteromycotina, and three of other types in 2009 and 2010. Among all these type of spore groups, Deuteromycotina contributed highest percentage and lowest percentage contribution was found to be that of Zygomycotina to the total airspora. The order of dominance of airborne microbial groups was Deuteromycotina contributed (61.02% and 60.84%) followed by Ascomycotina (23.27% and 24.92%), Basidiomycotina (9.44% and 7.74%), Zygomycotina (0.66% and 0.80%) and other types (5.62% and 5.69%) in 2009 and 2010 respectively. Throughout the period of investigation the concentration of Cladosporium (6.98 &7.31 %), Alternaria (3.79 & 2.88 %), Helminthosporium (3.81& 3.96 %), Nigrospora (3.26 & 3.3 %) & Cercospora was found to be more as compare to other types of spores and concentration of Meliola (0.20 & 0.24 %), Cunninghamella (0.21 & 0.25 %) was found to be minimum as compare to other types of spores. During the investigation it was found that the fungal spores occurred were mostly parasitic on paddy crop, which lead to disease formations. Eventually pathogenic types like Curvularia, Helminthosporium, Alternaria, Nigrospora and phoma species were recorded. The weather parameters like daily minimum and maximum temperature, minimum and maximum Relative humidity, rain fall and wind speed were compared. It has clearly brought out the correlation between the spore concentration in the atmosphere, meteorological data and disease incidence in paddy crop.



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## INTRODUCTION

The air surrounding us forms a major part of the global ecosystem and, depending upon its constituents, it regulates the quality of the environment. It has long been known that there are different types of particles present in the atmosphere. These particles may be biological (for example, pollen grains, fungal spores, viruses, actinomycetes and other bacteria, fern and moss spores, algal colonies, plant fragments, small seeds, protozoa, mites and insect fragments) or non-biological (for example, soot, diesel exhaust particles ashes, sand and mineral fragments such as silicate minerals). Aerobiology is a scientific discipline that deals with the transport of organisms and biologically significant materials through the atmosphere (Isard and Gage, 2001). Aerobiology also encompasses the generation, uptake, translocation, dispersion, viability, deposition and infection/infestation of seeds, viruses, fungi, bacteria and other agents, including insects such as aphids and mosquitoes, which act as virus vectors. Finally, this discipline deals with agriculturally significant insects such as locusts, bush flies and moths. Aerobiology is mainly an experimental science and it is interdisciplinary, with applied aspects. It involves the interests of allergists, plant pathologists, microbiologists, entomologists, palynologists, mycologists, air pollution specialists and biometeorologists. Aerobiology is basically concerned with the study of airborne organisms, with their sources; take off, dispersal, deposition and their effects on other organisms, a sequence termed the aerobiological pathway (Edmonds, 1979) and the effect of environmental factors on each of these stages. Broadly Aerobiology is classified into two categories I. e. Indoor or Intramural Aerobiology and Outdoor or Extramural Aerobiology. Intramural Aerobiology deals with the problems of contagious allergens and storage pathogens in a rather closed atmosphere. Extramural Aerobiology concerns with dissemination, dispersion and consequences of microbial components in the outside air. The airborne particles released from its substrate or environment in different ways are transported up in the atmosphere due to turbulence and air currents. The concentration of particles in a volume of air above the ground depends on the amount of particles release from the source per unit time, on the

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meteorological conditions. It has been found that intramural aerosols have a large range that is smaller than in outdoor air. In many work environments very high total counts have been found which are much higher than usually found in outdoor environments. The composition and concentration of the airborne flora shows great variations depending on geographical locality, meteorological situation, time of day and sampling techniques used (Gregory, 1973). When a micro-organism becomes airborne the immediate fate of the particle largely depends on the local meteorological conditions. The turbulence is dependent on the ground topography, the temperature in the air mass and the wind speed. Deposition mechanisms can be either dry or wet. Most wet deposition occurs as a result of washout by rain. The efficiency of raindrops to capture spores depends on the size of the spores and the raindrops, the rate and duration of rainfall, as well as the depth of the precipitation and spore layers. Wet and dry depositions are closer in number than has been suggested by their relative deposition rates because there are many more dry hours than wet hours. Spores delivered during rain will be more likely to initiate disease because leaves will be wet and infection can begin immediately. The uncertainty in estimating the rate of wet deposition is large and it is difficult to ascribe to this mechanism a representative role (Smith, 1981). The fungal spores and hyphal fragments are commonly recorded in the air and are important for the survival and subsequent continuation of generations. Many of the fungal spores are endowed with unique structures and capacity to survive under unfavourable environmental conditions and this probably accounts for the predominance in the air. Numerous airborne organisms, fragments as well as particles of biological origin passively float in the atmosphere. Small insects, bacteria, viruses, plant pollen, Diasporas fragment of tissue and such organic compounds mycotoxins or allergens can be found in the air. Along with temperature and relative humidity, the UV component of solar radiation, which is the most lethal, controls survival of spores in the atmosphere. Most spores, which will be transported through the atmosphere and deposited within a few hundred kilometres of the source, remain with the mixed layer of the atmosphere (Clarke *et al*., 1983)

The Italian botanist Micheli (1729) was the first scientist who in studied fungi and their spores under the microscope. He also observed that dust particles taken from fungus and placed on fleshy cut slices of melon generally reproduced the same kind of fungus. He concluded that the dust particles were taken from the Seeds (spores) of fungus and are occasional different fungi appeared were produced from spores carried through the air. A comprehensive knowledge of the biological and weather interactions which govern the movement of organisms in the atmosphere is essential for the development of successful management strategies for controlling their harmful effects on terrestrial ecosystems. Emigration and immigration of organisms to and from habitats can significantly regulate the dynamics of populations (Isard and Gage, 2001). The interest in the nature and behaviour of organism present in the air was created during the middle of last century, when Louis Pasteur (1861) proved in his classical experiment in the combating theory of spontaneous generation of life and developed germ theory of disease, that air is the carrier of many common germs. Since then several group of investigators will varied objective have gathered a large amount of information of atmosphere now commonly designated as air-spore. The existing knowledge on composition of the air spore can be said to have started from 1830, when Ehrenburge first published information on the microorganism which he had found in the atmospheric dust. In the earlier years the activities of Aerobiologists were limited without reference of meteorology. Aerobiologists were mainly interested in the problems of entrapment, identification and enumeration of transported biological materials in the atmosphere, since it served as an important medium of dispersal of microbes. The meteorologist, on the other hand, had shown little interest in the result of biological investigations though it would have possibly help to know more about the mixing process in the atmosphere and the movement of air masses.

## MATERIALS AND METHODS

In the present investigation spore trapping was done by using TIlak‘s continuous air sampler and Petri-plate exposure method. The apparatus run on electric power supply (AC-220V) and provides a continuous sampling of air for eight days. It consist of a cubical tin box of 10.4” x 10.4” x 8” dimension. The electric clock fitted in an instrument and was synchronized with the drum. Air was sucked through the orifice of the projecting tube at the rate of five litres per minute and it impinges on transparent cellotape which was of 1.5 cm in breadth and stacked on the slowly rotating drum. The drum completes one circle in eight days. The tape was slightly coated with petroleum jelly and faces the orifice of the outward projecting tube 0.5 cm away from it. Before the tape was mounted on glass slide at the end of seven days, it was divided into eight equal parts, measuring 8.4 cm in length, which was again subdivided into two parts, measuring 4.2 cm in length and then cut. Each piece, thus obtained, represent the 12 hours sampling area for a day or night accordingly. The tape for 12 hours was mounted on slide with glycerine jelly & made permanent. Scanning was done by dividing this tape into six equal parts, each part representing two hours trace area. The air was sucked through the tube with help of small fan having three prongs and fixed in the circular opening in the cover of the sampler. An exhaust hole measuring 6 x 2.7 cm is kept in a lid of apparatus. An instrument is modified form the spore clock model of Panzers *et al*. (1957) 24 hours slide spore collector. When it was compared with other spore traps, it was found that the Rotorod sampler (Perkins, 1957) is useful only for spot sampling, although its collection efficiency is 85%. The Hirst trap (Hirst, 1952) with minimum 45% collection efficiency has the disadvantages of the capital cost, power requirement and unsuitability both for culture and trapping splash dispersed spores. The Panzer’s slide spore collector (Panzer, 1957) with 70% collection efficiency has less retention capacity and requires attention after every twenty four hours, where as the present air sampler has 75% collection efficiency, greater retention capacity and is also economical besides it provides data for 7 days i. e. of one week. Air sampling was started before sowing and continued up until harvesting. from 17th June to 30th October, 2009 (140 days) and from 5th June to 22nd October, 2010 (136 days). The sampler was installed in the centre of the crop field in both the Kharif season at a height of 1 meter from the ground. Sampling was carried out by operating continuously above described air sampler with its orifice kept at constant height of one meter above the ground level in the paddy field before plantation. The sampler was well protected from rain by a polythene cover, keeping orifice tube exposed outside, which does not impair the sampling efficiency. Air was sampled at the rate of 5litres/min. and the transparent cello tape coated with petroleum jelly was changed weekly. The exposed cello tape

was cut into fourteen equal parts, each part represent twelve hours trace area of day or night accordingly. The piece of cello tape was mounted on glass slides using glycerine jelly as mountant. Glycerine jelly as a mountant has the best optical properties for the visual examinations. Measured amount of glycerine and distilled water was mixed in a beaker and heated in a water bath for 2-3 hours. During heating this mixture, gelatine was added slowly by stirring to avoid the clumping. After complete dissolution of gelatine, phenol crystals were added as preservative and metabolic inhibitor. This glycerine jelly was used for the preparation of permanent slide. Scanning was done regularly, areas 9600 square microns of total area as the trace obtained in a day, was scanned under 10x X 45x eyepiece- objective combination of binocular research microscope. Assuming the trapping efficiency to be 75% and the counted were converted in to number per cubic square meter of air. The identification of spores caught was based on (I) Microscopic characters, (ii) Comparison with parasitic and saprophytic fungal material collected in and around the field, (iii) Comparison with cultural characters. In all possible cases, generic counts were made which are based on colour, shape, size and other diagnostic features of the spores. In general, climatic conditions at this place are favourable for agriculture growth, similarly favourable rain and humidity during most of the days indirectly favours the growth of the diseases.

## RESULTS

The present investigation 50 components of fungal spore were observed, out of which 2 belongs to Zygomycotina; 3 belongs to Basidiomycotina; 17 to Ascomycotina, 28 belong to Deuteromycotina, and three of other types. The total concentration was 422884 spores/m3 and 384370 spores/m3 in the first (2009) and second (2010) kharif season respectively. The order of dominance of airborne microbial groups was as under : Deuteromycotina contributed (61.02% and 60.84%) followed by Ascomycotina (23.27% and 24.92%), Basidiomycotina (9.44% and 7.74%), Zygomycotina (0.66% and 0.80%) and other types (5.62% and 5.69%) in the first (2009) and second (2010) kharif season respectively. Out of 50 spore type 2 of them were belonging to Zygomycotina group which includes Albugo and Cunninghamella. The genus Albugo contributed (0.45% and 0.55%) and Cunninghamella contributed (0.21% and 0.25%) to the total air-spora in the first (2009) and second (2010) kharif season respectively. The group Ascomycotina is represented 17 spore types belonging to order Sphaeriales, Pleosporales, Hysteriales and Dothidiales etc. Occurrence of many spore types in air-spora revealed incidence of many saprophytic and parasitic ascomycetes in paddy field. Climatic factors play important role in release of ascospores. Month wise concentration showed considerable variation as (7.44% and 7.31%) in June, (17.45% and 18.05%) in July, (34.05% and 30.63%) in August, (28.86% and 30.61%) in September and (12.19% and 13.4%) in October to the total catches of respective type during first (2009) and second (2010) kharif season respectively. Basidiomycotina group was represented by smut spores, Uredospores and Teleutospoes. Among all the types smut spores showed highest contribution 4.06% & 4.36% as compared to Teleutospore 2.9% & 1.89% and Uredospore 2.48% & 1.49% found in Kharif season 2009 and 2010 respectively. It is observed that temperature, humidity, growth and age of plant favour the incidence and spread of disease. Deuteromycotina was represented 28 different spore type. The order Melanconiales and Moniliales showed considerable domination as compare to other orders. Throughout the period of investigation the concentration of Cladosporium (6.98% & 7.31%), Curvularia (4.09%) in both the season, Alternaria (3.79% & 2.88%), Helminthosporium (3.81% & 3.96%), Nigrospora (3.26% & 3.3 %) and Aspergillus 2.28% and 3.05% found to be contributed in maximum percentage to the total air-spora. Meliola (0.20% & 0.24 %), Cunninghamella (0.21% & 0.25 %) was found to be minimum as compare to other types of spores. Phoma (2% & 1.72%), Fusariella (1.01% & 0.91%), and Fusarium (0.76% & 0.99%) species were recorded. During the investigation it was found that the fungal spores occurred were mostly parasitic on paddy crops, which leads to disease formations. The present investigation also includes presence of other types such as fungal fragments, unidentified spore and insect parts. Fungal fragment contributed 2.76% and 3.41%, unidentified spores contributed 2.32% and 1.87% whereas insect parts contributed 0.54% & 0.41% to the total air-spora in Kharif season 2009 and 2010 respectively. Abundant presence of fungal fragments was found to be of great importance because their viability was recognised to produce colonies in order to cause infection in pathogenic fungi. Their percentage found to be increase in high wind velocity. Air-borne fungal fragments are primarily conidiophores of sexual forms, culturing of these fungal fragments revealed that they mostly belong to Cladosporium, Alternaria, Aspergillus or Penicillium. Due to their viability, also serves as mean of propagation. The significant contribution of fungal fragments was noted during day time.

## DISCUSSION

The present aeromycological studies would be a step foreword in devising an efficient disease forecasting system for paddy crop of this region and alternate measures may be taken to stop the disease incidence and prevent the loss of production. The obtained results, during the present investigation, have been satisfactory analyzed and presented in thesis in the form of plotted pie charts, bar graphs and line graphs. Besides, the thesis has been well supported by incorporating photomicrographs of some airborne fungal spore types. Thus present investigation will help to understand to various components of air and their occurrence over paddy crop. It was observed that, the occurrence of various types of spores was in relation with weather changes, field operation, crop growth and disease incidence on paddy crop during the period of investigation. The result obtained and conclusion drawn would definitely help in knowing the incidence of diseases and enable to serve as a basis for devising disease forecasting system leading to efficient control of diseases. The incidence of pathogenic spores noted in the present studies is also of considerable help to farmers and cultivators as it would serve the purpose of alarming them for likely occurrence of fungal diseases so that they can plan for the proper preventive measures before hand.

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