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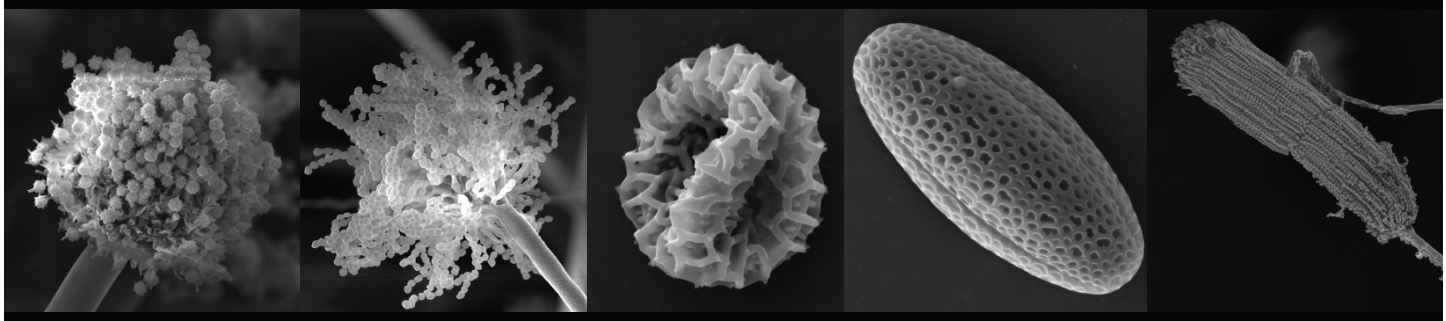

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Review Article

A REVIEW ON AIRSPORA STUDIES IN INDIA IN RELATION TO ALLERGY

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The atmosphere contains a mass of bio aerosols such as viruses, bacteria, fungal spores, pollen grains, dander's and dust mites etc. Among them, the fungal spores and pollen grains have special significance due to the fact that they are the main cause of some forms of human allergic disorders, such as allergic rhinitis, bronchial asthma, atopic dermatitis and some eczema. Aerobiology, an atmospheric biology is a scientific discipline focused on the transport of organisms and a biologically significant materials by the atmosphere. Because of the practical application in the diagnosis and treatment of respiratory disorders, atmospheric biology studies have assumed new dimensions. The susceptibility of people to certain airborne fungal spores of fungal allergens changes with their seasonal liberation time and also on weather parameters. A heavy load of airborne fungal spores cause hypersensitivity to sensitive persons.

Atmosphere contains an array of bioparticulate materials such as viruses, bacteria, fungi spores, pollen, dander's and dust mites etc.. However, among them, airborne pollen and fungal spores have special significance to bring about the incidence of allergic disorders. In India the first authentic aerobiological work was carried out at Calcutta, who recorded the daily content of the air by using "Aerconioscope". For about 100 years, there was a conspiracy of silence on aerobiological researches in India. Sreeramulu had reviewed the work on the ever-changing airspora of India. This is, in spite of the fact that magnitude of the problem of allergic diseases is alarming because 10–15 per cent of the population suffer from such diseases. This reflects the magnitude of the work to be done on aerobiological aspects with respect to allergic patients in Indian subcontinent. Being located between 7' N to 36'N and 67'E to 98'E this region is given with a wide range of different biomes (desert to alpine tundra). Such diversity in the vegetation would contribute enormous variations in the quantity and quality of Airspora. Subsequently, the symptomatology of suffering from respiratory disorders is extremely variable among the inhabitants of the different ecozones. This reflected the magnitude of work to be done on aerobiological aspects in Indian Territory. For about more than 100 years, there was a intrigue of silence on aerobiological researches in India. Work resumed late in 1950s to reveal array of atmospheric microflora content in different parts of the country. This is in spite of the fact that magnitude of the problem of allergic diseases was alarming due to more than 15 per cent of the population was suffering from allergic diseases. Such an enormous variation in vegetation, contributed variations in quantity and quality of array of bioparticles in the atmosphere. Consequently the symptomatology of patients suffering from respiratory disorders was seen extremely variable among the inhabitants residing in different ecozones .This might have reflected the magnitude of the work to be done on aerobiological aspects with respect to allergy patients in India.

Key Words: Aerobiology, airspora, allergy. India

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AIRBORNE POLLEN ALLERGENS

The term aerobiology was coined by Meirs (USA) in 1930s. A systematic work on airborne pollen was conducted in some of the metropolitan and some other cities revealed that term, covers the transport of airborne microorganism and also biologically significant materials by the atmosphere[1-7]. Due to practical application in the diagnosis and treatment of the respiratory allergic disorders, the airspora studies were assumed with new dimensions. As it is evident that the first aerobiological work from Kolkata[6] (West Bengal) was done by operating the "Aeroconioscope "and by collecting the daily airborn count[7-16].

The most systematic work on airborne pollen grains conducted in some of the metropolitan and other cities, reveal the following essential meaningful features as under:

1. Amaranthaceae, Asteraceae, Moraceae, Pinaceae, Casuarinaceae, Leguminosae, Gramineae, Meliaceae, etc., are the chief constituents of the pollen airspora;
2. The pollen of the different species show diurnal, and seasonal variations and also annual variations;
3. Two pollen seasons were observed i.e., from February to April for trees, whereas from August to October dominated by herbaceous and weedy taxa.

Based on the locality where the plant taxa grow and occur of different seasons is given in the Table 1.1. Clinical investigations have been mostly carried out in

Table 1.1: Common allergenic plants and their seasonal periods in India

	Spring	Autumn	Winter
	February – April	September – October	November – January
Grass flora			
	Cynodon dactylon	*Bothriochloa pratunsa*	*Cynodon datylon*
	Diacanthium annulatum	*Cenchrus ciliaris*	*Eragostrossis tenella*
	Imperata cylindrical	*Heteropogon contortius*	*Phalaris minor*
	Paspalum distichum	*Pennisetum typhoides*	*Poa annua*
	Poa annua	*Sorghum vulgare*	---
	Polypogon mon-spliensis	*Lemon grass*	---
	Lemon grass	----	*Lemon grass*
Weeds Flora			
	Cannabis sativum	*Amaranthus spinosus*	*Ageratum conyzoides*
	Chenopodium sp.	*Artemisia scoparia*	*Argemone mexicana*
	Parthenium hysterophorus	*Cassia occidentalis*	*Chenopodium album*
	Plantago major	*Ricinus communis*	*Asphodelus tenuifolius*
	Suaeda fruticosa	*Xanthium strumarium*	*Euphorbia hirta*
Tree Flora			
	Ailanthus excelsa	*Anogeissus pendula*	*Cassia siamea*
	Holoptelea integrifolia	*Eucalyptus* spp.	*Salix tetrasperma*
	Prosopis juliflora	*Prosopis* spp.	*Salvadora persica*
	Spathodia campanulata	*Melia azadirach*	*Citrus medica*

and around Delhi, Lucknow and earlier at Jaipur[17-21]. In addition to these surveys, characterization, diagnosis and treatment of inhalant allergens have been carried out at several other centers in the country[22-25].

Some of the major allergenic pollen offenders from India were recorded from different parts of India are *Acalypha* (September - October), *Amaranthus spinosus* (August - October), *Chenopodium album* (July - October), *Parthenium hysterophorus* (round the year), *Eucalyptus* sp., (September - October), *Xanthium strumarium* (round the year), Grass pollen (round the year), *Morus alba*, *Prosopis julifera, Holoptelea integrifolia, Salvadora persica, Cynodon dactylon, Lantana camara, Tephrosia* sp., *Bougainvillea* sp. The pollen of herbaceous flora contributed in highest percentage, followed by tree pollen and shrub pollen. While deciding the pollen allergy in a patient, it is desirable for the physician to consider this aspect. Keeping this in view, the seasonal prevalence and the distribution of pollen grains have to be determined and tabulated. This will help the physicians in treating the patients of the allergy.

The common tests employed for diagnosis are intradermal/skin prick test, bronchial provocation test, and Ophthalmic. Very commonly, now a days total and specific IgE estimations have also been initiated at certain places where clinical investigation are being carried out.

Clinical test comprise three parts, (i) *Preparation of pollen up to 90% purity*, (ii) *Preparation of antigenic extract of pollen grains*, (iii) *Intradermal skin test*.

Several diagnostic tests, such as patch tests, oral, nasal and bronchial challenge tests, scrap tests have been in use at various allergy clinics. Attempts are being made to isolate and characterize allergenic factors from some of the important pollen allergens like *Amaranthus spinosus, Prosopis julifera, Parthenium hysterophorus*[26-28].

Pollen grains may initiate the allergic response in susceptible human, resulting several types of allergic disorders like asthma (allergic lung fever), hay fever (allergic rhinitis), upper tract respiratory troubles, conjunctivitis, urticaria, etc. Reaction of pollen to cause allergic symptoms depends on some factors. The essential requirements for a species to cause reaction in man include widely distributed plants that must produce large quantity of pollen, in addition, pollen should contain hay fever allergen[29].

In India, Tilak[30,31] brought out the information on palynological research in the form of books. Some survey of airborne pollen and fungal spores were made from Eastern part of India[32-35]. Singh reported some allergenic pollen from Shillong and Manipur[36-37]. Review work on airborne entomophilous allergenic pollen grains was done by Tilak[38]. Vishnu mitre and Khandelwal[39] reported the incidence of 48 pollen types from Lucknow, while Singh and Babu[40] studied the bio-allergens of Delhi with their seasonal periodicity. Agashe and Anand[41] recorded airborne pollen and spores from Bangalore. Dominance of *Parthenium* sp. was also reported from the air of Visakhapatnam[42].

Tripathi et al.[43] surveyed the airborne pollen at Bhopal. Jain and Dutta[44] reported 41 allergenic pollen from Gwalior, Kalkar and Patil[45] have contributed to pollen flora of Bhupal. Marathe and Reddy[46] suggested that one has to aim for four way correlation between flowering, pollen count, weather parameters and allergenic manifestations. Kundu et al.[47] clinically tested 16 pollen types and found all of them to be allergenic and more or less significant.

AIRBORNE FUNGAL ALLERGENS

When compared to the aerobiological information on the pollen, data on air borne fungal spores are scanty. Much of the knowledge on fungal spore content of the atmosphere is concerned with the epidemiology of pathogens that infect crop plants. Nevertheless the data gathered from the aerial surveys conducted at Delhi, Mumbai, Lucknow, Kolkata, Chennai, Visakhapatnam, Mysore, Bangalore, Aurangabad etc.[48-54] revealed the following features: (a) the major components of the airspora are *Alternaria, Cladosporium, Drechslera, Aspergillus, Penicillium, Curvularia, Nigrospora, Epicoccum, Mucor* etc.; (b) Two dormant spores throughout the country are *Alternaria* in North and *Cladosporium* in south; (c) fungal spores do not have specific peak season and are found through the year, and (d) they also exhibit diurnal, seasonal and annual variations.

Fungal spores originate predominantly from the environment, especially where soils become dry and wind-blown[55]. Germinated spores typically form mycelia and sometimes fragment of mycelia may produce allergenic hazards. Certain fungal spores produce toxins that can be deadly even in non-susceptible individuals[56]. Fungal spores can cause respiratory infection, allergies and toxic reaction, but not contagious diseases[57].

Various methods of aeromycological technique have been described. The spore calendar made on the basis of air monitoring helps to predict the outdoor fungal spore level which in turn influences the indoor air levels. To obtain a picture of the personal exposure of patients to allergenic bio particles, Bohem's individual pollen collector has proved to be useful[58-60]. Table 1.2 and 1.3 revealed lists of the spores of fungi in Marathwada region, Maharashtra.

Table 1.2: Some aeroallergenic fungal spores from Marathwada region, Maharashtra

Alternaia sp.	*Paecilomyces* sp.
Arthrinium sp.	*Penicillium* sp.
Aspergillus sp.	*Phoma* sp.
Botrytis sp.	*Puccinia* sp.
Candida sp.	*Rhizopus* sp.
Drechslera sp.	*Saccharomyces* sp.
Epicoccum sp.	*Stachybotris* sp.
Epidermophyton sp.	*Stemphylium* sp.
Fusarium sp.	*Trichoderma* sp.
Ecliocladium sp.	*Trichophyton* sp.
Helminthosporium sp.	*Tricothecium* sp.
Humicola sp.	*Ustilago* sp.
Microspora sp.	*Verticillium* sp.
Mucor sp	

Formulation of regional and national pollination calendar as well as Fungal spores calendar is a prerequisite for allergen research. The pollen/spore wall protects them against the desiccation and often pigmented wall make them the less vulnerable to radiation damage from ultraviolet light in the atmosphere. Liberated and transported spores in the air get sedimented and subsequently germinated. The spores can be deposited on mucous and thus exhibit symptoms.

Table 1.3: Results of intradermal tests showing number of positive cases against fungal antigens

Sl. No.	Type of Antigens	+	++	+++	++++	Total positivity positivity	Average percentage of
1.	*Candida albicans*	9	6	–	–	15	16.30
2.	*Trichoderma* sp.	10	3	–	–	13	14.13
3.	*Curvularia* sp.	6	4	1	–	11	11.96
4.	*Alternaria* sp.	6	2	–	1	9	9.78
5.	*Aspergillus tamarii*	3	4	2	–	9	9.78
6.	*Mucor* sp.	6	2	–	1	9	9.78
7.	*Aspergillus flavus*	4	4	–	–	8	8.70
8.	*A. niger*	3	3	–	–	6	6.53
9.	*A. fumigatus*	3	2	–	–	5	5.43
10.	*Rhizopus* sp.	3	2	–	–	5	5.43
11.	*Helminthosporium* sp.	2	–	–	–	2	2.18
Total						**92**	**100.00**

ALGAE

Changes in the aero algal community are also due to the effect of climatic conditions on the growth of individual algal groups at the source. Algae are widely distributed in salt waters and fresh water around the world. Algae were first identified[62] as possible airborne allergens for allergies e.g. *Anabaena, Chlamydomonas, Chlorella, Chlorococcum*, etc. Particles from the algae may become aerosolized on fragmentation. They may also be ingested by those exposed to them.

Some algae can produce the toxins that may be involved in causing respiratory problems or toxic reactions. Algae toxins can have varying effects like central nervous system damage, neurotoxic effects and paralysis.

In India, there are many reports on algal aerobiology, as the climate is most favorable for algal spores and filamentous airborne algae[61]. Using vertical cylinder spore trap at Mysore, highest catches of airborne algae was observed in February[63]. Several authors[64,65] conducted preliminary survey of aeroallergenic algae in the atmosphere of Delhi and Nagpur.

DUST MITES ALLERGENS

Dust mites are always identified as allergens, by generally only where there is considerable dust disturbance[66]. Dust mites thrive in warm and damp environments. The primary species responsible for most allergies are *Dermatophagoides ferinea* and *D. pteronyssinua*. Allergy to dust mites is far more prevalent due to their constant presence in indoor environment and the inevitable exposure to occupants. Dust mites can be found in high concentrations in upholstered furniture, mattresses, pillows, carpets, etc. (Table 1.4). Dust mites are not truly capable of airborne transport for a long distance, but in shortest distances allergic reactions to dust mites are usually noticed due to having their much smaller size.

There is a valuable literature of Jogdand[67] on aerial dispersal of insects, insect parts, mites, plant materials, and algal fragments. However, elaborate studies on house dust mites (HDM) remained unattended. Research on HDM was carried out through aerobiological studies on Jowar crop in Marathwada region[68]. Subsequently, home dust mites, flour mites, bird mites, animal mites, phytophagoid mites , coconut mites, groundnut pest mites were also recorded from Marathwada regions and that too from Pune which were found to be responsible for causation of health disorders such as respiratory and skin allergy, asthma etc.[69,70]. In addition, the dust mites

also cause allergies[71]. The work on HDM in relation to allergy stated that the population of the mites (allergen load) in bed and floor dust of patients found to be proportional to the severity of allergic attacks in two typical cases[72]. They also recorded highest percentage of contribution of home dust mites during rainy seasons in the month of September[73]. Dust mites are acarida that thrive in warm and damp environments. The majority of asthmatic children are sensitized to dust mite allergy.

Table 1.4: List of common allergenic mites in India

Sl. No.	Category (types)	Zoological name
1.	Dust mites	*Dermatophagoides ferinae* *Dermatophagoides pteronyssinus* *Dermatophagoides microceras* *Blomia tropicalis*
2.	Storage mites	*Leidoglyphus destructor* *Acarus siro* *Tyrophagus* spp. *Glycyphagus urticae*
3.	Spider mites	*Panonych citri* *Panonychus citri*

Allergy to dust mites is far more prevalent due to their constant presence in indoor environment and the inevitable exposure of occupants. Dust could be found in high concentrations in upholstered furniture, mattresses, pillows, and carpets. Storage mites pose problems for individuals only in certain environment, such as where cereal based foods or grains are stored. Storage mites that have been implicated in allergies include *Lepidoglyphagus destructor, Achrus siro* and *Tyrophagous putrescentior.*

FURTHER PLANS OF PRIORITIES

On the basis of the above account of the work carried out in aerobiology in India in comparison to the magnitude of allergic problems in the Indian Territory and the vast diversity in the flora and climate, the following priorities are needed to be recognized:

(i) Extensive intra-mural and extra-mural aerial surveys in different eco-geographical regions in India covering rural and urban areas,

(ii) Quantitative and qualitative analysis of airspora and storage of retrieval of data,

(iii) Systematic morbidity surveys about the incidence of allergic disorders in all over the country,

(iv) Identification and characterization of airborne pollen and fungal aeroallergens from different regions,

(v) Preparation of regional and national pollination calendar and atlas of pollen and fungal spores,

(vi) Recommendation for planting of non-allergenic plants of appropriate species in gardens, road sides,

(vii) Development of computerized forecasting models.

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Research Article

MINERAL FORTIFICATION OF *CALOCYBE INDICA* (P&C) AND ITS IMPACT ON COLONY MORPHOLOGY AND METABOLITES PRODUCTION

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Minerals are essential for the proper growth and development of the human body. The deficiency of these minerals is the cause of various abnormalities and diseases in human health. Fortification is an alternative approach for enhancing nutrients and minerals in food crops. Cultivating edible mushrooms on a mineral-enriched submerged cultivation medium can be an effective way to produce mineral-fortified food. In this study, *Calocybe indica*, known as the Milky white mushroom, was cultivated in static batch submerged cultivation conditions using different concentrations of Iron (Fe) and Zinc (Zn) mineral-supplemented mediums and successfully recorded microscopic and morphologic colony variation on mediums. The results showed that mineral supplementation influenced mycelium growth and metabolite production. In Fe supplementation, the highest biomass (31.76±1.75 g/l) was obtained in a 400 mg/l Fe-supplemented medium, and similarly, in Zn supplementation, achieved the maximum biomass (19.67±0.50 g/l) at 400 mg/l. The present results indicate that Fe supplementation gives higher productivity than Zn supplementation. Furthermore, the principal component was analyzed, and the different nutritional elements and their interrelation were interpreted. PC1 and PC2 accounted for 68.92% of the overall variation in the observed variables. The results showed that the mineral supplementation influenced and enhanced the mycelium growth and metabolites production. Thus, fortified *C. indica* mycelium is an excellent alternative for developing functional food with highly enriched nutraceutical materials and will prospect for approaching the treatment of mineral deficiency-related diseases in humans.

Key Words: Bioaccumulation, Bioactive compounds, Submerge cultivation, Functional food, and Nutraceutical.

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INTRODUCTION

Minerals play an important role in the biological system and are essential for the proper growth and development of the human body. Minerals such as Iron (Fe) and Zinc (Zn) are essential trace elements for human health[1]. The Fe is involved in DNA synthesis and hemoglobin, maintaining healthy connective tissue and muscle metabolism. Additionally, Fe is required for cellular activity, hormone synthesis, neurological development, and physical growth[2]. Similarly, Zn is a component of several enzymes that participate in biochemical reactions like synthesizing and degrading different biomolecules[3]. Zn is present in Zn-metallothionein and Cu-Zn superoxide dismutase and has antioxidant properties[4]. The deficiency of these minerals is the cause of various abnormalities and diseases in human beings. About 75% of the world's population lacks micronutrients such as Zn, Copper (Cu), and Fe[5]. According to the World Health Organization's (WHO)[2] most recent study, 1.62 billion individuals worldwide, mostly children and women, have Fe deficiency anemia, an epidemic-level global public health issue. Other abnormalities related to Fe deficit are parasite infections, chronic inflammation, hereditary disorders, etc[6].

A deficiency of Zn in the diet can lead to several adverse effects like oral ulceration, night blindness, pneumonia, the severity of diarrhea, delayed growth, loss of appetite, prolonged labor, bleeding, and reduced testosterone production (NIH, 2019)[7]. These consequences demand strategies for making mineral-containing balanced diets or supplements for humans; therefore, research on fortified food is getting much attention from worldwide

researchers. Mineral fortification is a natural way of enrichment in which minerals are provided to food crops during crop growth and development and used to increase dietary mineral intake[8-10]. Compared to the conventional method, Fe and Zn food fortification seems to be a favorable risk-benefit ratio to promote health and avoid several human diseases. Mushrooms are well known for their bioaccumulation potential, accumulating mineral elements from the substrate that make them a good source of minerals and nutrients[11]. Because of that, they are excellent candidates for mineral fortification[12]. Mushroom cultivation is divided into two types: solid-state and submerged liquid. In solid-state cultivation, mushrooms were mainly grown from agro-industrial residues[13]. It is labor-intensive and time-consuming. Thus, it is making unsuitable for producing metabolites at a commercial scale[14]. On the other hand, in submerged mushroom cultivation, the opportunity to grow in a liquid medium where nutrients are dissolved and the oxygen supply is boosted by agitation[15].

As a result, a higher rate of mycelium is produced, so interested metabolites are easily acquired[16]. It is more suitable than solid-state cultivation because it requires less time and space, is easy to maintain physiological conditions, and is less prone to contamination. Therefore, numerous edible mushroom strains may grow in submerged liquid cultures for biomass production and are targeted to a wide range of bioactive substances, including proteins, enzymes, lipids, and carbohydrates[14,17]. *Calocybe indica* (P&C), the milky white mushroom, is an edible mushroom that originated in India[18,19]; it was first reported by Purkayastha and Chandra (1996) from West Bengal, India. It is a grassland species and saprophytic by nature. It also has various metabolites, mainly proteins, and carbohydrates, less content of fats, and a rich amount of dietary fiber sources. However, these nutritional properties make it the perfect choice for nutraceutical development[20,21]. The primary objective of this study is to enhance the biomass and nutritional value of *C. indica* mycelium by Fe and Zn mineral supplementation at batch static submerged cultivation condition. Thus, fortified mushrooms can be a good option as a functional food and other nutraceuticals development. It will be an alternative food source for the fight against various mineral deficiency-related diseases in human health.

MATERIALS AND METHODS

Microorganism and culture condition

The *C. indica* pure culture was obtained from the Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). This culture was routinely maintained on potato dextrose agar (PDA) medium[22] and used for further experiments.

Microscopic observations of spores

The mature mycelium was stained with lactophenol cotton blue and observed under the inverted microscope.

Study of morphological characters of mycelium

The mature culture of *C. indica* was punched with the help of a cork borer (05 mm) and aseptically transferred on PDA medium with various concentrations of mineral supplemented with Zinc and Iron (200, 400, 800, 1600, and 3200 mg/l). Radial growth was observed every three days until the full-grown colony was obtained.

Submerge cultivation on supplemented medium

Initially grown ten-day-old mycelium of C. indica on a petriplate was taken; it was aseptically punched with the help of a cork borer (5 mm), and then after transferred to a 250 ml flask containing 100 ml of potato dextrose broth (PDB) medium. All prepared medium was supplemented with Zn and Fe minerals in the form of Zinc sulfate ($ZnSO_4$) and Ferrous sulfate ($FeSO_4$), with increasing concentrations (200, 400, 800, 1600, and 3200 mg/l). These inocula were incubated for 27 days at 30ºC; over the incubated periods, the mycelium-containing mediums were filtrated with Whatman filter paper no. 1 and biomasses were collected, which were used for further study and simultaneously weighed the wet and followed dried biomass. The wet mycelium was dried in an oven at 40ºC overnight; after that, it was crushed with the help of a mortar-pastle to convert to a fine powder.

Extract preparation

The collected mycelium powder was immersed with methanol in a ratio of 1:3 for 24 hours and then filtered with the help of Whatman filter paper 1. Filtered extracts were saved at 4ºC for future analysis.

Proximate analysis of mycelium and fruiting bodies

Total moisture content - Initially, wet biomass was weighed, dried in an oven at 100°C, and further cooled in a desiccator. The following formula was used to calculate the moisture content:

$$\text{Moisture (\%)} = \frac{\text{(Initial weight of the sample - Final weight of the sample) X 100}}{\text{The initial weight of the sample}}$$

Determination of total ash - The total ash content was estimated according to Alam[23]. In brief, 0.1 g of mushroom sample was taken in the crucible on a clay pipe triangle, heated over low flame until all the samples burnt entirely and turned into white or grayish-white colored powder. After that, it was cooled at room temperature in a desiccator and finally weighed. The below formula determined the total ash content:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of the mushroom sample taken}}$$

Estimation of total protein content - The total protein content was determined following the methodology of Thetsrimuang (2011)[24]. In brief, 0.1 g of mycelium was taken in a glass beaker containing 10 ml of 1N NaOH solution (w/v) and boiled for 30 minutes. Further, the solution was cooled to room temperature and centrifuged at 4000 rpm for 10 min; the supernatant was collected and used for protein estimation. The Bradford method was used to determine total protein content with minor modifications. 1 ml of supernatant and 5 ml of Bradford reagent were combined in a test tube. After a 5 minute dark incubation period at room temperature, the absorbance was measured at 595 nm, and the protein content was determined using bovine serum albumin (BSA) as a standard.

Determination of total lipid content - The total lipid estimated according to the protocol followed by Folch[25] with slight modification. Particularly, 0.1 g of crushed mycelium was taken in a glass container, suspended in 25 ml of chloroform: methanol (2:1 v/v), mixed thoroughly, and finally, let stand for three days. After that, the solution was filtered from the mixture, and the filtrate was centrifuged at 4000 rpm for 10 minutes. After centrifugation, the upper layer of methanol was discarded, and the remaining filtrate was kept in a hot air oven at 50°C to evaporate the chloroform. Finally, the residue was collected and analyzed to determine the total lipid. The following formula calculated the total lipid content:

$$\text{Total lipid (\%)} = \frac{\text{Weight of the container with extract - Weight of the empty container} \times 100}{\text{Weight of the mushroom sample}}$$

Determination of total crude fiber - The total crude fibers of cultivated mushrooms was determined according to the procedure of Alam[23]. In brief, 1 g of dried ground mycelium was rinsed in 20 ml of 0.25 NH_2SO_4 (w/v), followed by boiling for 30 minutes, and the mixture was filtered through a muslin cloth. Finally, the remaining residues were washed with hot water and transferred into the fresh beaker. Furthermore, 20 ml of 0.313 N NaOH (w/v) was added to the residue and boiled for 30 minutes. The mixture was filtered through a muslin cloth, and once more, the precipitates were washed with hot water, followed by absolute alcohol. After that, residues were dried overnight at 50°C and weighed (W).

Crude fiber (%) = [100 - (Moisture + Lipid)] × [(W)/ Weight of the mushroom]

Estimation of the total carbohydrate - The given formula evaluated the total carbohydrate content:

Carbohydrate (%) = 100 - [Moisture + Ash + Crude fiber + Protein + Lipid)g/100g].

Statistical analysis

All statistical analysis was performed using SPSS 20. The observed and recorded data were analyzed and expressed as mean ± standard error of three replicates (n=3). Different letters were significantly expressed differently from each other at $p<0.05$ (DMRT) compared to using analysis of variance (ANOVA).

RESULTS

Microscopic observation of spores

The primary mother culture of mycelium was successfully stained and investigated on an inverted microscope. The microscopic study revealed that spores containing mycelia appeared, shown in Fig. 2.1. The

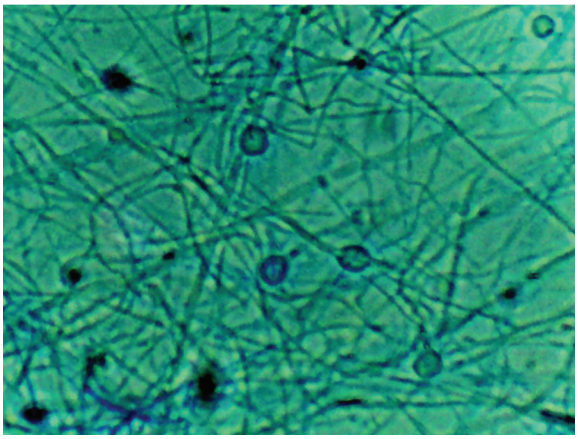


Fig. 2.1: Microscopic observation of *Calocybe indica* mycelium (400x)

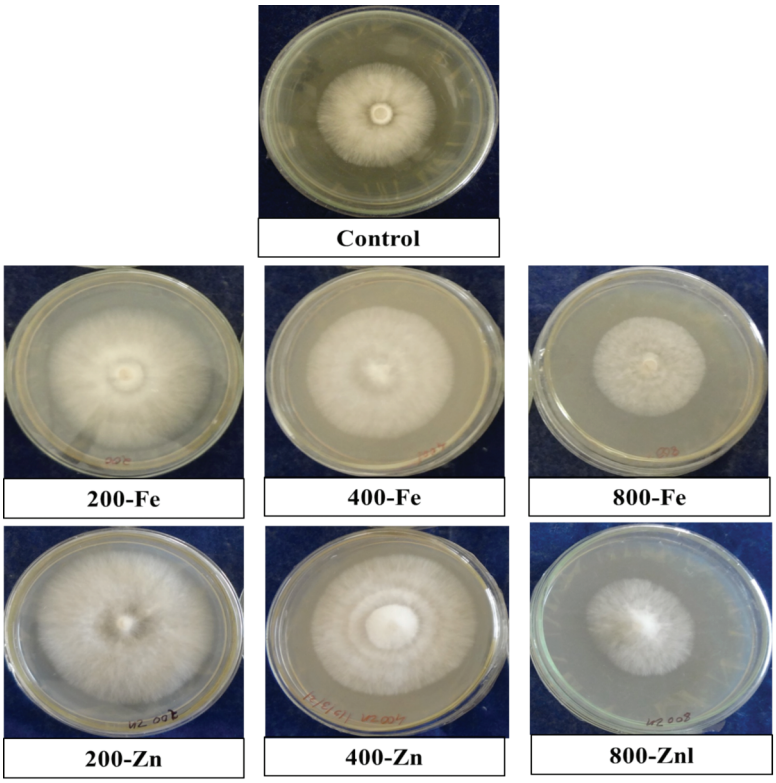


Fig. 2.2: Morphological observation of the colony

spore morpho-logy is oval, smooth, and cylindrical shaped. The presence of mature spore at the proper condition with the static condition has entirely grown on the medium.

Morphological behaviors of *C. indica* colony on minerals-supplemented medium

The in *vitro growth* and characteristic features of *C. indica* were recorded on PDA medium. Mycelium is successively transferred with various mineral-supplemented PDA medium concentrations (Fig. 2.2).

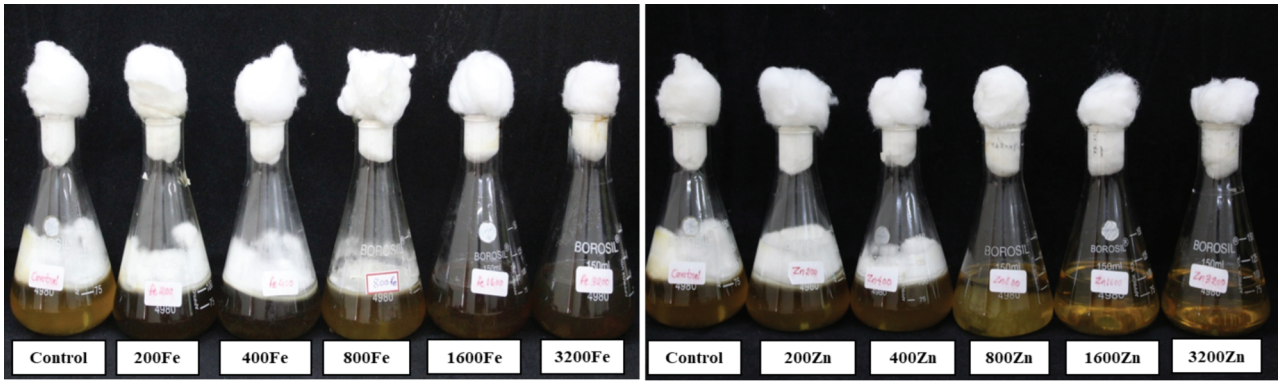
Morphological features of cultivated mushroom's mycelial colonies on a PDA medium have recorded mycelium running pattern, growth, and morphology. Among all the supplementation, 200 mg/l mineral concentration for both minerals (Fe and Zn) were found to be fast-growing mycelium and full growth (Fig. 2.2) was found on the 13th day at a 90 mm petridish. The morphological features of mycelium are displayed in Table 2.1 and Fig. 2.1.

Effect of minerals biomass

The mineral fortifications in C. indica, the most edible attractive and test favor mushroom on static submerge experimentation cultivation successfully executed after incubation, mycelium biomass was collected. The

Table 2.1: *In vitro* assessment of colony morphology of *Calocybe indica*

Treatment	3rd day	6th day	9th day	Colony morphology on PDA
Control	9.33± 0.66b	29.00± 0.57ad	40.00± 0.57bc	Radiating colony growth, wavy margin
200-Fe	9.66 ± 0.33b	31.66± 0.88e	44.00± 2.08c	Dull white radiating colony growth with a wavy margin
400-Fe	9.66± 0.66b	27.00± 1.00c	33.00± 1.15c	Thin cottony growth with a smooth margin
800-Fe	5.00± 00a	11.00± 0.57b	17.00± 1.15b	Thick cottony upwards smooth margin
200-Zn	10.00±0.06b	31.66±1.20e	48.66±0.88d	Radiating white colony with wavy mycelia
400- Zn	9.33±0.58b	34.33±0.33f	46.00±0.58d	Thick cottony growth with dense mycelia
800- Zn	11.00±0.39c	32.66±0.54e	42.33±0.33c	Thick cottony center, thin, smooth margin



(A) Fe (B) Zn

Fig. 2.2: Effect of mineral (Fe in A and Zn in B) supplementation on biomass production

Table 2.2: Effect of mineral treatment on biomass production

Mineral conc. (mg/l)	Fe		Zn	
	Fresh weight (g/l)	Dry weight (g/l)	Fresh weight (g/l)	Dry weight (g/l)
Control	7.55 ± 0.24b	2.26 ± 0.12b	7.55 ± 0.24b	2.26 ± 0.12b
200	15.71 ± 0.45c	4.65 ± 0.07c	15.60 ± 1.69c	4.82 ± 0.41c
400	31.76 ± 1.75d	9.34 ± 0.34d	19.67 ± 0.52d	5.83 ± 0.16d
800	4.50 ± 0.31a	1.25 ± 0.16a	3.35 ± 0.20a	1.07 ± 0.07a
1600	nd	nd	nd	nd
3200	nd	nd	nd	nd

nd: Not detected

mycelium biomass yields in the mineral-enriched medium were distinguishable from those in the control group (Fig. 2.3).

Fe and Zn supplementation increased biomass production at 200 and 400 mg/l concentrations, respectively. In Fe-supplemented medium (400 mg/l), the highest biomass weight was (31.76 ± 1.75 g/l) for fresh mycelium and dried mycelium (9.33 ± 0.34 g/l). Similarly, Zn-supplemented medium (400 mg/l) gave the highest biomass weight (19.67 ± 0.52 g/l) forfresh mycelium and 5.82 ± 0.15g/l for dry weight. The flask containing 800 mg/l of Fe and Zn-supplemented medium gave the least biomass. No growth or biomass production was observed in the 1600 and 3200 mg/l supplementation mediums, respectively (Table 2.2).

Effect of minerals on proximate composition

A proximate composition (%), under which protein, carbohydrate, crude fiber, ash, and lipid content were analyzed and presented in Table 3. Lipid content did not vary among all the supplemented and control treatments; it ranged between 5.87 ± 1.04) and 7.33 ± 0.67. Crude fiber content increased (13.64 ± 0.14) in 200 mg/l Zn supplemented, followed by 13.43 ± 0.23 in 800 mg/l Zn and 12.50 ± 0.15 in the control treatment. The minimum crude fiber contents were obtained in Fe mineral-supplemented medium and found to be between 6.41 ± 0.31 and 8.19 ± 0.22. Ash content was found in the range of 16.80 ± 5.60 to 31.33 ± 0.13, and among all the treatments, mycelium with 800 mg/l Fe supplemented gave the highest ash content was found 31.33 ± 0.13. Similarly, protein content increased with 200 to 400 mg/l supplemented for both minerals; the maximum protein was 35.65 ± 0.28 and 31.44 ± 0.21 for Fe and Zn supplemented, respectively. Carbohydrate was high among all the supplemented mediums, data presented in Table 2.3.

Table 2.3. Proximate composition (%) of the mineral-fortified mycelium of Calocybe indica

Mineral conc. (mg/l)	Protein	Carbohydrate	Crude fiber	Ash	Lipid
Control	19.86 ± 0.17b	41.20 ±1.06e	12.50 ± 0.15c	19.07 ± 0.48ab	7.33 ± 0.67
200 - Fe	22.10 ± 0.44d	40.32 ± 0.30de	8.19 ± 0.22b	16.80 ± 5.60a	6.67 ± 0.67
400 - Fe	35.65 ± 0.28f	26.89 ± 0.74a	6.49 ± 0.27a	23.87 ± 0.58abc	7.33 ± 0.67
800 - Fe	21.35 ± 0.15cd	34.29 ± 0.36b	6.41 ± 0.31a	31.33 ± 0.13c	6.67 ± 0.67
200 - Zn	20.95 ± 0.30c	36.79 ±1.38bc	13.64 ± 0.14d	22.40 ± 0.23abc	6.13 ± 1.04
400 - Zn	31.44 ± 0.21e	25.99 ± 1.11a	13.43 ± 0.23d	23.20 ± 0.46abc	6.00 ± 1.15
800 - Zn	16.67 ± 0.38a	38.01 ± 0.73cd	12.40 ± 0.20c	26.80 ± 0.23bc	5.87 ± 1.04

Each value is expressed as mean ± standard deviation (n=3). Means with different letters within a column are significantly different ($p < 0.05$).

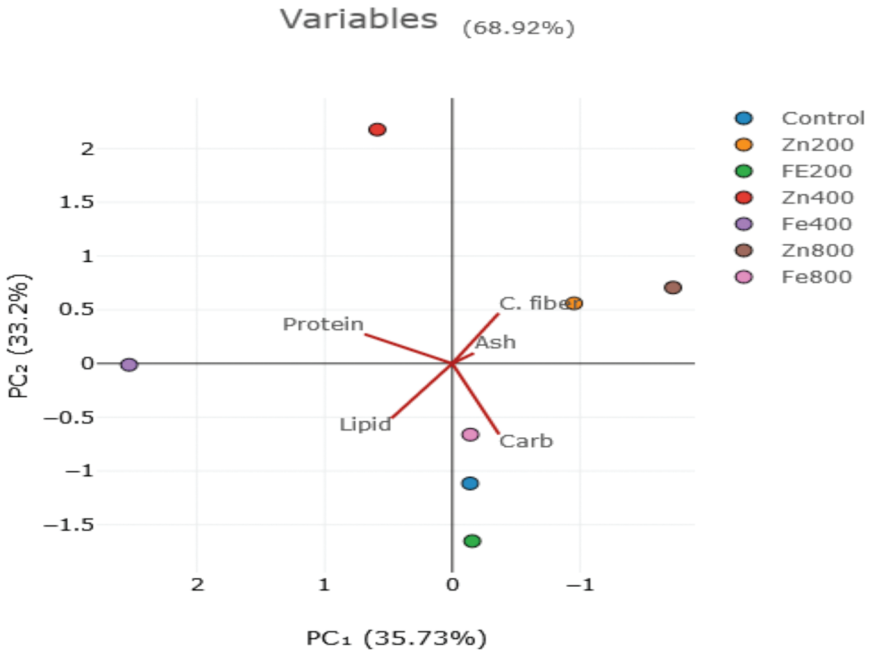


Fig. 2.4: Principal component analysis of proximate compositions

Principal component analysis (PCA)

The PCA was successfully analyzed with all the proximate compositions and analyzed findings' loading plots for PC1 and PC2 based on the nutritional metrics percentage. According to biplot data in Fig. 2.4, principal component 1 described 35.73% of the original information, while principal component 2 accounts for 33.2%.

Table 2.4: Correlation matrix of proximate component (%) of mineral-fortified mycelia

		Correlation Matrix			
Variables	**Protein**	**Carbohydrate**	**Crude fiber**	**Ash**	**Lipid**
Protein	1.000	-.728	-.270	-.196	.302
Carbohydrate	-.728	1.000	-.235	-.137	.220
Crude fiber	-.270	-.235	1.000	-.336	-.511
Ash	-.196	-.137	-.336	1.000	-.361
Lipid	.302	.220	-.511	-.361	1.000

The nutritional loading in PC1 and PC2 displayed the correlation between various parameters, which affected the fundamental component. The values of crude fibers and ashes grouped with favorable loading on the right lower side of the biplot, thus defining their positive connection. At the same time, crude protein illustrated a robust negative relationship with carbohydrates, followed by fats, as shown in Table 2.4.

DISCUSSION

Calocybe indica is a highly valued and nutritious mushroom, generally cultivated in solid-state medium, but there are enormous limitations for producing huge biomass and specific bioactive components. The alternative approach is static submerge cultivation, which can facilitate submerged cultivation of mushrooms and successfully achieve biologically important constituents in short periods with industrial scale-up levels. The mushrooms' mature fruiting body or mycelium produces spores. Mature sporophores, the mycelium of *C. indica*, release spores, spores transmitted by air and water[26], when increasing a load of spores in the atmosphere that causes immunologic lung sickness like hay fever, and farmers or workers are affected with lung disease[27]. The spore's walls contain antigens that cause allergy symptoms[28]. Therefore, a previous mycologist researcher stated the fungal spore causes air-borne respiratory allergies[29]. Thus, the recent research attention on cultivating mushroom biomass by static and non-static-based bioreactors for acquiring potentially bioactive compounds. The present study focused on eradicating mineral deficiency with the fascinating edible mushroom *C. indica* and effectively executed a novel and natural method of supplying food with Zn and Fe supplements. The present experimentation showed that treatment of various inorganic mineral concentrations via cultivation medium dramatically accelerated growth, produced more biomass, and enhanced nutritional contents compared to untreated mycelia. Numerous researchers conducted similar investigations for mineral supplementation in plants. They came to the same conclusion, finding that supplements directly impact the physiology of plants, including biochemical parameters, photosynthetic characteristics, shoot length, root length, and size. It's also reported in mushrooms that the mineral supplement caused different morphological and physiological changes in cultivated mushroom mycelia[30,31]. Consequently, researchers are trying hard to enhance the efficiency of mycelia and crops by modulating their biochemical and physiological trials. The submerged culturing of mycelium in shaken flasks or fermenters has been widely used for producing high amounts of fungal mycelium[32,33]. Furthermore, supplementation improves the mushroom's overall nutritional composition; conversely, biofortification was performed for particular nutrients, minerals, or vitamins. In the series, Wlodarczyk[34] reported the bioavailability of Mg and Zn from the enriched biomass in mushrooms; they found both minerals responsible for increased biomass production due to the property of easy bioaccumulation. Similarly, Scheid[30] reported that regardless of the culture medium or the ability of the fungus to bioaccumulation Fe in the mycelial biomass efficiently, the availability of Fe in mycelium varies depending on the species. The excellent choice for producing mycelia biomass has been Fe-enriched *S. communes* mycelial biomass, followed by *G. lucidum, P. ostreatus*, and *P. eryngii*. Das[35] worked on the wild mushroom species *L. squarrosulus* collected from the gardens of Tripura, India. They used different carbon and nitrogen sources enriched in basal medium and achieved high mycelium biomass. Manjunathan and Kaviyarasan[36] reported on the mycelium culture of *L. tuberregium* in other carbon, nitrogen, and vitamin sources to enhance mycelial growth. Zhong and Tang[37] said the submerged culturing of mushroom mycelium in enriched growth mediums for obtaining mycelium with higher bioactive compounds could be utilized for therapeutic purposes. So, submerged mycelium cultivation has been widely used for producing high amounts of fungal mycelium[38].

CONCLUSIONS

Submerged cultivation of mushroom mycelium is very prominent since it produces a lot of biomass and a variety of metabolites. Fortified mycelium with minerals can be a good option as a functional food, also the development of nutraceuticals, and be utilized to treat mineral deficiency-related diseases. *Calocybe indica*, already having numerous nutritional compounds, was fortified with Zn and Fe minerals using submerged cultivation conditions, and its biomass production and metabolite contents were estimated. The results showed that mineral supplementation influenced mycelium growth and metabolite production, and fortified

C. indica mycelium could be a good source for nutraceutical production. Further optimization is required to scale up the process of targeted bioactive compounds and add another substrate for *C. indica* cultivation for limited metabolites compound production at the industrial level. In contrast, additional clinical studies are necessary to assess the value of fortified *C. indica* mycelium for medical applications.

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Research Article

AEROMYCOFLORA ASSOCIATED WITH MUSEUM AND HERBARIUM OF A DEGREE COLLEGE AT KAMPTEE (NAGPUR), MAHARASHTRA

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In academic institutions, the museum and herbarium are dedicated to preserving and interpreting the primary tangible pieces of evidence. Museum and herbarium are mainly unique and constitute the raw material of study and research. The herbarium is like a library, but the information is stored in a biological form—as pressed, dried, and annotated plant specimens. Herbarium is a reference material for nomenclature, identification and classification. In the present study, the museum reveals remarkable diversity in form, content and even function. Fungal spores are the chief component of airspora. Still, their composition and concentration depend on the source of contamination in the environment, location, types of vegetation and meteorological parameters of that region. In the aeromycological survey of the museum and herbarium of the Department of Botany of S. K. Porwal College, Kamptee of Nagpur city, sampling was done by gravity Petriplate method with potato dextrose agar (PDA) medium. At the end of the incubation period, fungal colonies were counted, isolated and identified with the help of available standard literatures and reference slides. The maximum number of fungal species belonging to group Deuteromycotina i.e., anamorphic fungi followed by Ascomycetes, and Zygomycetes and the lowest contribution was by Sterile Mycelia. Altogether 1050 CFUs were recorded from the sampling period, i.e., from January 2017 to December 2017. A total of 27 species belonging to 18 genera were observed. Aspergillus niger contributed the highest with 99 CFUs. *Rhizopus nigricans* contributed 2nd highest contribution followed by *Penicillium chrysogenum* and *Cladosporium cladosporioides*. A maximum number of fungal species was observed in January which coincides with optimum temperature and relative humidity favorable for fungal growth. At the same time, the minimum number of 11 fungal species was recorded in May which is the hottest and dry month in Kamptee city.

Key Words: Aeromycospora, biodeterioration, museum, herbarium, fungal spores, Maharashtra.

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INTRODUCTION

Air is the mixture of different gases, inorganic particles, water vapour, bioparticles (bacteria, fungi, protozoa) and non-living materials. Fungi omnipresent in nature. The hazardous effect of fungi on the health of humans, animals and plants can be minimized by monitoring the quality of air for knowing the diversity, abundance and variations of fungi in it, according to seasonal changes[1].

The word museum in its Greek form, 'mouseion' meant, 'Seat of Muses' and designated a philosophical institution or a place of contemplation. In the institute, the museum is dedicated to in preserving and interpreting the primary tangible pieces of evidence. Museums are mainly unique and constitute the raw material of study and research. Fungal spores are the major component of the bioaerosol; they usually transport ample viable spores by means of air. The unique distinguishing quality of fungi that are grown on the minimal substrate has many adverse effects such as biodegradation or biodeterioration of valuable things[2]. Fungal growth is the biggest threat to precious items kept in museums. The movement of audiences and air cannot be stopped inside any museum. The only safeguard from fungal attacks is the eco-management of the indoor atmosphere of the museum. Eco-management allows the control of unwanted organisms inside a closed environment while keeping the ecology of that place intact. Eco-management is necessary and needed in the present hour. It helps in maintaining a balance between control practices and the ecology of the place. It also helps to remove unwanted microorganisms that have gained access to the indoor environment without damaging the ecology and the health of the individuals inside the museum[3].

The air of the departmental museum and herbarium carries many microorganisms which are harmful to articles. Microorganisms enter the indoor environment through air currents which contaminate the museum environment. In the museum, precious articles are preserved, which cannot be found again or try hard to have them again. Microorganisms attack these articles for their nutrients, required for growth and development.

Museum articles are the main source of food for fungi. Herbarium specimen is a pressed plant sample with collection data deposited for future reference. It supports research work and may be examined to verify the identity of the specific plant used in a study. A voucher specimen must be deposited in a recognized herbarium committed to long-term maintenance. Herbarium is a critical resource for biodiversity, ecological and evolutionary research studies. It is a primary data source of dried and labelled plant specimens that are arranged to allow for easy retrieval access and archival storage.[4] Herbarium and museum collections comprise the basic materials for obtaining information about the local biodiversity which is chiefly made up of organic matter which is associated with microorganisms which deteriorates the organic matter. Herbarium is like a library, but the information is stored in a biological form—as pressed, dried, and annotated plant specimens. Herbarium is actually a reference material for nomenclature (the naming of specimens to avoid the problem of ambiguity), identification (how they are recognized) and classification (grouping of plants with similar properties) of the plants and a critical resource for biodiversity, ecological and evolutionary research studies, also.

Biodeterioration can be defined as "any undesirable change in a material brought about by the vital activities of organisms".[5] The Biodeterioration phenomena observed on materials of cultural heritage are determined by several factors as following:

(i) The chemical composition and nature of the material itself,

(ii) The climate and exposure of the object,

(iii) The manner and frequency of surface cleaning and housekeeping in museums.[6]

In museums and collections, as well as herbarium, fungi play the most important role in biodeterioration. Infections are mostly airborne with significant seasonal variations and high numbers of spores can accumulate in dust layers[7]. Poor ventilation and non-homogeneous surface temperature can produce water condensation points and local micro-climates with higher water availability than in the rest of an indoor environment. These circumstances are favourable to some fungal species; as a result, these can proliferate in places where the overall environmental conditions would otherwise appear to be hostile to microbial life.

In museum and herbarium racks, fungi play the most important role in biodeterioration, since, in comparison with bacteria, they can grow in environments with lower temperatures and relative humidity. Typical fungal infections colonizing kinds of organic artefacts are often caused by species of slow-growing Ascomycetes such as genera *Aspergillus, Penicillium, Cladosporium, Alternaria, Chaetomium, Eurotium* etc.[6] Bacteria rarely exist on museum objects and their number increases significantly only when the museum or herbarium rack is damp, flooded or when the drying process of this type of material is too slow. Microbial contamination in environments depends not only on species-related properties but also on climatic conditions, such as temperature, humidity and ventilation.[8]

MATERIALS AND METHODS

Nagpur is the second largest city of the state of Maharashtra and Kamptee is a suburban area of Nagpur, at a distance of 15 km. Kamptee (21.2243 N, 79.1894 E) was founded in 1821 when the British established a military cantonment on the bank of the Kanhan river. It is previously known as Camp-T due to its geographical state.

The climatic condition of Kamptee city is divided into rainy seasons (July-October) winter (November-February) and summer seasons (March-June). The study area was a specimen containing museum and herbarium of the department of Botany of S. K. Porwal College, Kamptee. The detection and identification of fungal species associated with biodeterioration are the first necessary step for understanding the effects. The culture plate exposure method was employed for the isolation of fungal species. Indoor aeromycoflora were isolated on PDA (Potato Dextrose Agar) medium with 0.1% streptomycin. Potato dextrose agar media is primarily exercised for the growth and isolation of different yeasts and moulds. PDA media serves as an ordinary growth medium for yeasts and moulds. The potato infused in the PDA medium supplies the nutrient base for the development of yeasts and moulds. Dextrose is a fermentable carbohydrate that supplies a sole C source to promote fungal growth.[9] Sampling was done in fourth nightly intervals with Petriplates having a PDA medium of 10 cm diameter from January 2017 to December 2017. Ten Petriplates were exposed for 10 minutes in the museum and in the herbarium rack where many

specimens in dry format were preserved and some forms were stored in preserving material. The exposed Petriplates brought out were incubated at 28 ± 1°C for 7 days. After the 3rd, 5th and 7th days of incubation, the fungal colonies were counted. At the end of the 7th day of incubation, the fungus was isolated and pure culture was maintained. The viable microbial concentrations were calculated as colony-forming units/m^3 air (CFU/m^3). For identification and microphotography, slides were prepared with lactophenol cotton blue as the standard stain. Isolates were identified with the help of available standard literatures. Fungal genera were identified based on morphological characters of mycelia, conidia and conidiophores identified using standard literature and then classified[10-16]. Micro-photography was performed by digital microscope in the laboratory of the Department of Botany, S. K Porwal College, Kamptee.

For statistical analysis the Spearman correlation test (r) between total CFU count and meteorological factors (average temperature, relative humidity and total rainfall) was calculated by using Microsoft Excel-10. Meteorological data were collected from the Regional Meteorological Centre, Nagpur (Table 3.1).

Percentage contributions of individual species were calculated as per the standard formula:

$$\% \text{ Contribution} = \frac{\text{Total no. of colonies of one species}}{\text{Total no. of colonies of all species}} \times 100$$

RESULTS

Table 3.2 and Table 3.3 depict the result of the statistical analyses and 2-tailed Spearman's correlation significance test with meteorological parameters. Statistical analyses of the data showed a positive correlation between the concentration of fungal colonies and air humidity. A close observation throughout the year and statistical analysis of paired t-tests leads us to significant results. It was seen that average temperature above 30°C, relative humidity at 65% and total rainfall above 115 mm and below 85 mm impacted significantly fungal diversity and count. No sign was denoted as positive correlation, while (–) denotes a negative correlation.

Table 3.4 was about the concentration, diversity and count of aero fungal flora observed in the museum and herbarium rack combined sampling period from January 2017 to December 2017. A total of 27 species belonging

Table 3.1. Meteorological parameter data* of study area during January-December 2017.

Sl. No.	Months	Maximum Temp. (°C)	Minimum Temp. (°C)	Average Temp. (°C)	Average Relative Humidity (%)	Average Rain fall (mm)
1.	January	28.6	12.4	20.5	59	10.2
2.	February	32.1	15.0	23.5	58	12.3
3.	March	36.3	19.0	27.6	55	17.8
4.	April	40.2	23.9	32.5	48	13.5
5.	May	42.6	27.9	35.2	51	16.3
6.	June	37.8	26.3	32.0	77	172.2
7.	July	32.0	25.0	28.5	67	271.0
8.	August	30.4	23.6	27.0	80	291.6
9.	September	35.0	29.0	32.0	53	176.9
10.	October	35.0	30.0	32.5	68	58.3
11.	November	33.0	28.0	30.5	67	19.6
12.	December	29.0	12.0	20.5	59	9.0

*Source: Regional Meteorological Department, Nagpur.

Table 3.2: Statistical analyses of meteorological parameters

	N	Mean	SD	Sum	Min	Max
Maximum Temperature	12	34.25	4.47325	411	28	42.6
Minimum Temperature	12	22.675	6.48188	272.1	12	30
Average Temperature	12	28.4625	4.9242	341.55	20	35.25
Reltive Humidity	12	61.67333	10.24207	740.08	48	80
Rainfall	12	89.78333	107.7896	1077.4	9	291.6

to 18 genera were observed (Table 3.4; Fig. 3.1 & 3.2). Together 1050 CFU's recorded during the study period. *Aspergillus* genus is represented by five species namely, *A. flavus, A. fumigatus, A. niger, A. terrus* and *A. nidulans*. *A. niger* formed maximum number of CFU, i.e., 99, during the study and occurred all the months. *A. flavus, A. fumigatus* and *A. niger* incidence occurred only during monsoon and winter seasons. *Cladosporium* is represented by three species. *Alternaria, Rhizopus* and *Penicillium* are represented by 2 species. *Rhizopus nigericans* colonies formed all the year round (Fig. 3.2). However, *R. soloni* incidence occurred in March, April, August and October and only a few colonies were recorded. *Sporotrichum* and *Trichoderma* are recorded only in winter months.

The least contribution was shown by Mycelia sterilia and *Phoma* spp. with 0.76% contribution. During the period of March - June, minimum spore count was recorded. This was probably because of the fact that, during this period average temperature was on the highest side and the relative humidity percentage was on the lower side. Hence, the parameters showed a direct correlation with the fungal count. July and November-February periods were noticed as favourable months for the enhancement of fungal aerospora. Statistical analysis of paired t-test and Pearson's correlation test leads us to significant results that showed a strong correlation between detailed meteorological parameters and the total number of colony-forming units during the study period. The fungal isolates including *Penicillium* spp. *Aspergillus* spp., *Chaetomium* spp., and *Fusarium* sp. members may be not directly responsible for the deterioration, but these strains are known to degrade organic materials and must be regarded as a threat to the museum and herbarium objects. Maximum percentage contributions of fungal species (49.90%) are observed in the winter season, moderate percentage contribution (32.95%) in the rainy season, while minimum percentage contributions (17.14%) are reported in the summer season.

Table 3.3: Two-tailed Spearman correlation significance test for meteorological parameters

		Max. Temp.	Min. Temp.	Ave. Temp.	R. Humidity	Rainfall
Max. Temp.	Spearman Corr.	1	0.54991	0.85614	-0.5289	-0.11208
Max. Temp.	2 tailed Significance test	.—	0.06398	3.79E-04	0.07705	0.72874
Min. Temp.	Spearman Corr.	0.54991	1	0.84063	0.0979	0.46154
Min. Temp.	2 tailed Significance test	0.06398	.—	6.16E-04	0.76212	0.13095
Ave. Temp.	Spearman Corr.	0.85614	0.84063	1	-0.20666	0.17513
Ave. Temp.	2 tailed Significance test	3.79E-04	6.16E-04	.—	0.5193	0.58616
R Humidity	Spearman Corr.	-0.5289	0.0979	-0.20666	1	0.58042
R Humidity	2 tailed Significance test	0.07705	0.76212	0.5193	.—	0.04786
Rainfall	Spearman Corr.	-0.11208	0.46154	0.17513	0.58042	1
Rainfall	2 tailed Significance test	0.72874	0.13095	0.58616	0.04786	.—

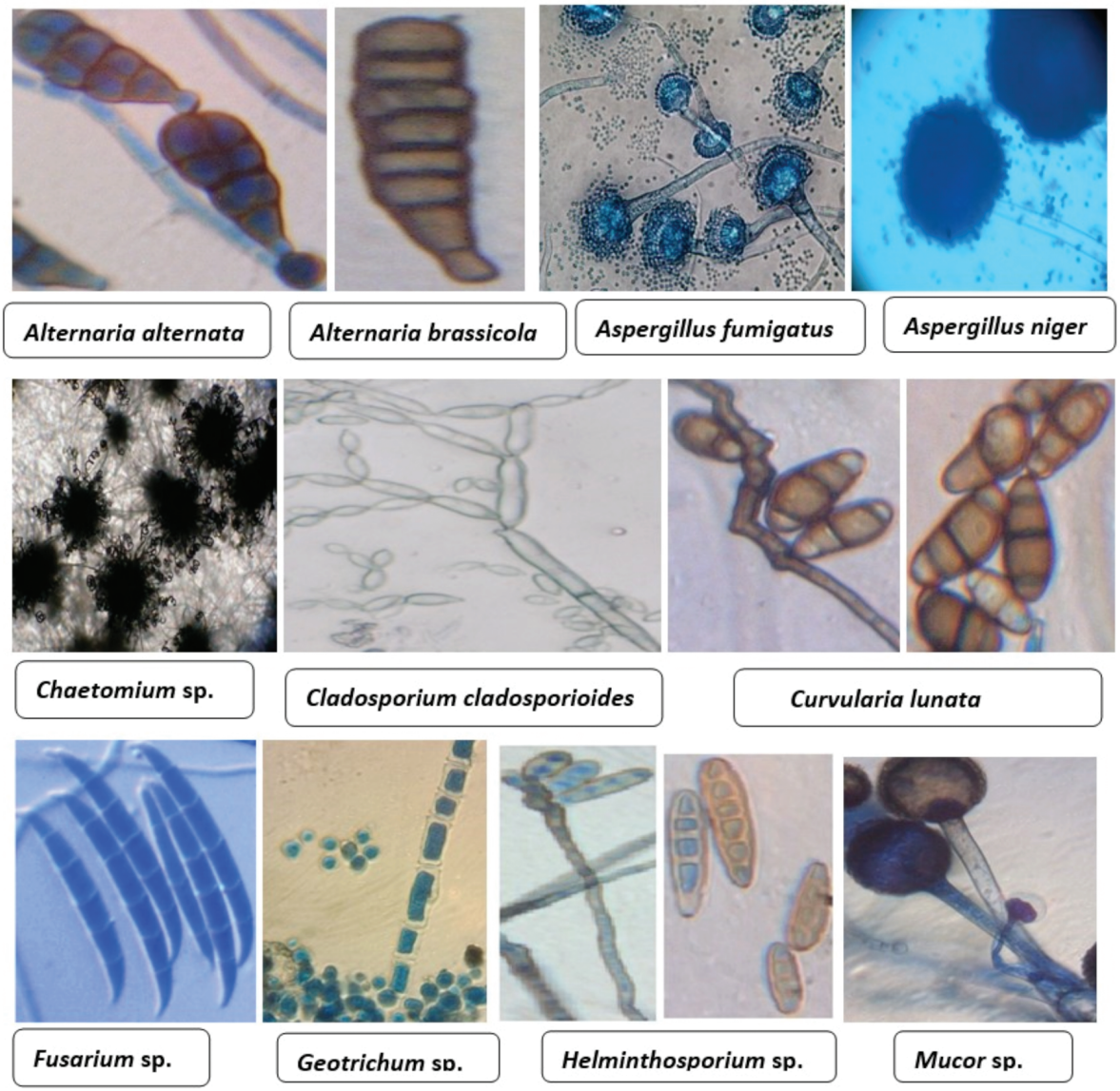

Fig. 3.1: Light microphotographs of some fungal spores recorded during study period

DISCUSSION

In museum, fungi play the most important role in biodeterioration, since, in comparison with bacteria, they can grow in environments with lower temperature and relative humidity.[17] There was no spore-free season amid the investigation time frame[17-18]. In the present examination, it is accounted that the fungal population is firmly connected with the season and climatic conditions.

Fungal organisms are responsible agents for the weakening and degradation of natural material, and diseases in plants, creatures, and individuals. In India *Rhizopus, Helminthosporium, Aspergillus* and *Curvularia* are allergic fungi and cause various diseases in plants as well as in human[15]. The spores of *Alternaria, Aspergillus, Cladosporium, Curvularia, Helminthosporium, Mucor, Penicillium* and *Rhizopus* were the major component in the air inside the college and university library[19]. Indoor aeromycoflora from laboratory museum is known to be significant in respect of allergic as well as airborne diseases and is also involved in the deterioration of cellulosic and non-cellulosic materials. The present study reveals that there are diverse mycoflora in the indoor environment of the laboratory. A further detailed study based on the atmospheric parameters could provide data for a better understanding of the correlation between abiotic and biotic factors. The effect of airborne fungal spores on human health is also a point of concern, which could be studied by using preliminary data provided in this report. Exposure to indoor airborne inhalant mould allergens develops respiratory symptoms, airway disorders and allergies and may impose a direct impact on students and other human populations inside and outside the laboratory.[20]

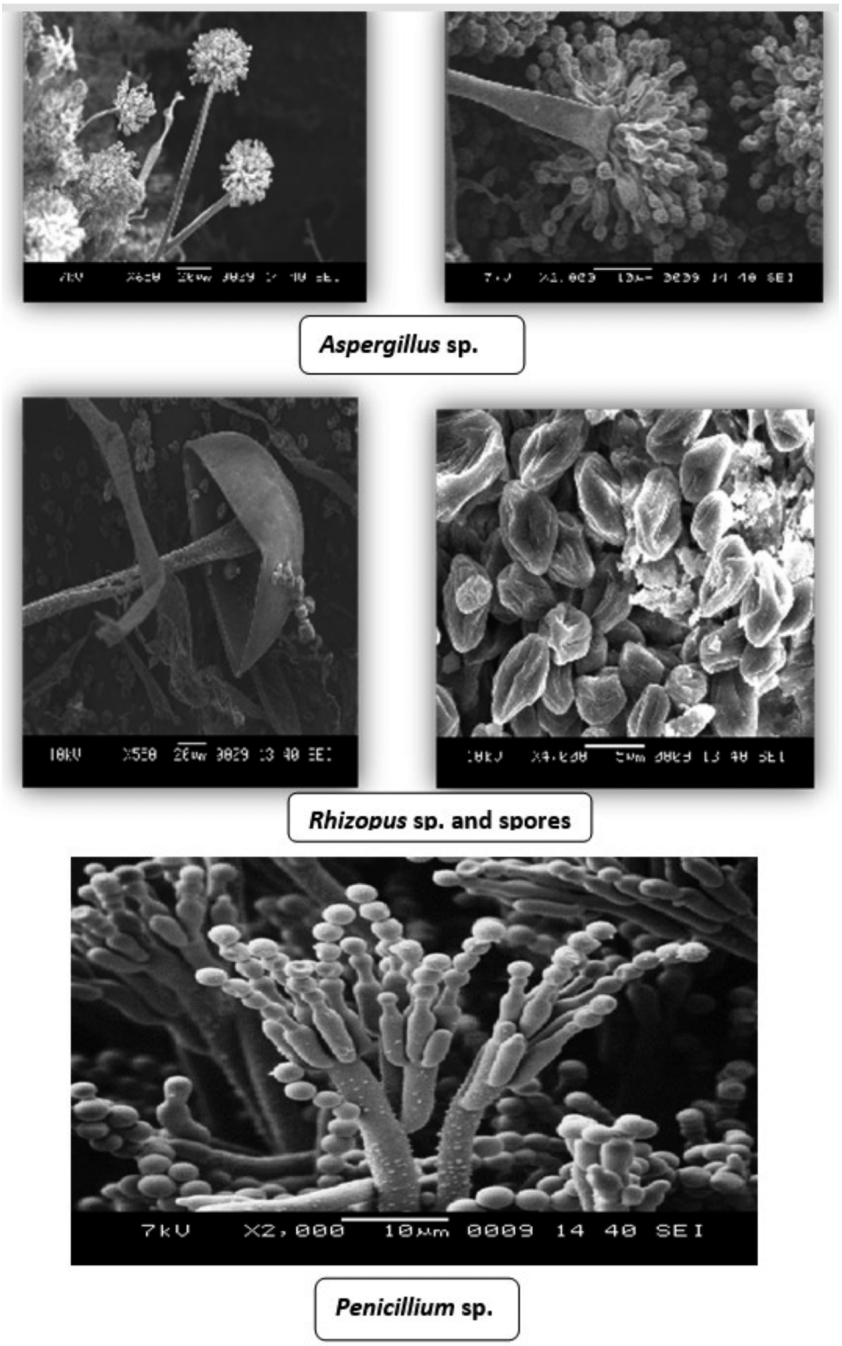

Fig. 3.2: Scanning Electron Micrographs of some Fungi and fungal spores

Some niches in museums were often colonized by fungus. These niches are characterized by scarce ventilation and the presence of a water vapour gradient after a sudden drop in temperature or night–day Thermo hygrometric cycles. These peculiar, often very local, conditions in usually dry environments seemed to promote the development of xerophilic and osmophilic fungal species.[21-25]

The importance of simple cleaning is still underestimated, even though it is well known that dust layers on objects carry high numbers of fungal spores and bacteria, serving as a nutrient source for those organisms as well. Microbiologists must increase the awareness of these preventative measures by consulting with and instructing restorers, preservationists and museum curators. More emphasis must be focused on simple prevention measures such as the cleaning of dust layers and frequent observation of objects. In restoration and conservation, exceptional rules are necessary for the application of efficient toxic substances or preserving materials. More effort is necessary for the development of alternative decontamination methods, and modification of light and micro-climates. Monitoring methods must be optimized to be able to assess the effects of conservation treatments, climate change or other applications.[26-28]

Table 3.4: Percentage contribution of each species recorded in the museum and herbarium rack areas of S. K. Porwal College, Kamptee during January-December, 2017

Sl. No.	Name of Fungi	Rainy season					Winter season					Summer season					Grand total no. of fungal colonies formed	Percentage contribution
		Jul	Aug	Sept	Oct	Total	Nov	Dec	Jan	Feb	Total	Mar	Apr	May	Jun	Total		
	Zygomycotina																	
1.	Mucor sp.	3	-	2	1	06	6	7	9	6	28	-	-	-	3	03	37	3.52%
2.	Rhizopus nigricans	5	3	4	5	17	8	5	12	7	32	4	3	5	8	20	69	6.57%
3.	Rhizopus solani	-	7	-	8	15	-	-	-	-	-	5	5	-	-	10	25	2.38%
4.	Cunninghamella spp.	3	-	-	-	03	8	13	-	7	28	-	-	-	-	-	31	2.95%
	Total. no. of CFU's formed	**11**	**10**	**06**	**14**	**41**	**22**	**25**	**21**	**20**	**88**	**9**	**8**	**5**	**11**	**33**	**162**	**15.42%**
	Ascomycotina																	
1.	Chaetomium globasum	7	3	5	4	19	5	4	8	7	24	3	6	6	3	18	61	5.80%
2.	Curvularia lunata	2	2	1	3	8	3	4	4	3	14	1	1	3	2	7	29	2.76%
3.	Geotrichum candidum (budding yeast)	-	-	-	-	-	5	6	6	6	23	-	-	-	-	-	23	2.19%
4.	Nigrospora spp.	6	-	-	-	06	-	-	8	7	15	-	-	-	-	-	21	2%
5.	Phoma spp.	-	-	-	-	-	-	-	5	3	08	-	-	-	-	-	08	0.76%
6.	Trichoderma spp.	-	-	-	-	-	2	4	5	5	16	-	2	4	5	11	27	2.57%
	Total. no. of CFU's formed Anamorphic fungi or	**15**	**5**	**6**	**7**	**33**	**15**	**18**	**36**	**31**	**100**	**4**	**9**	**13**	**10**	**36**	**169**	**16.09%**
	Fungi imperfecti																	
1.	Alternaria alternata	4	4	3	2	13	4	4	3	2	13	5	6	4	2	17	43	4.99%
2.	Alternaria brassicicola	3	3	6	2	14	4	3	3	2	12	2	-	-	-	02	28	2.66%
3.	Aspergillus flavus	6	3	2	-	11	-	3	8	4	15	-	-	-	-	-	26	2.47%

Contd.

Contd. Table 3.4

		Jul	Aug	Sept	Oct	Total	Nov	Dec	Jan	Feb	Total	Mar	Apr	May	Jun	Total		
4.	Aspergillus fumigatus	6	8	7	2	23	8	10	6	6	30	-	-	-	-	-	53	5.04%
5.	Aspergillus niger	8	9	8	11	36	7	12	9	10	38	4	5	7	9	25	99	9.42%
6.	Aspergillus terreus	5	-	-	-	05	8	7	6	5	26	-	-	-	-	-	31	2.95%
7.	Aspergillus nidulans	5	4	5	6	20	-	-	-	6	06	8	-	-	-	08	34	3.23%
8.	Cladosporium cladosporioides	7	4	5	4	20	5	3	5	8	21	3	3	4	6	16	57	5.42%
9.	Cladosporium herbarum	-	8	-	9	17	5	7	8	2	22	-	-	-	2	02	41	3.90%
10.	Cladosporium oxysporum	7	8	-	-	15	9	10	2	-	21	-	-	-	-	-	36	3.42%
11.	Fusarium oxysporum	5	-	-	-	05	-	8	7	10	25	-	-	-	-	-	30	2.85%
12.	Helminthosporium solani	9	7	8	6	30	-	8	7	8	23	7	3	-	-	10	63	6%
13.	Penicillum chrysogenum	8	9	-	-	17	7	8	9	8	32	-	8	2	7	17	66	6.28%
14.	Penicillium digitatum	6	-	5	9	20	-	-	6	5	11	8	4	-	-	12	43	4.09%
15.	Sporotrichum spp.	5	-	-	-	05	-	7	9	8	24	-	-	-	-	-	29	2.76%
16.	Torula spp.	6	4	5	3	18	-	-	-	3	03	-	-	-	-	-	21	2%
	Total. no. of CFU's formed	**90**	**71**	**54**	**54**	**269**	**57**	**90**	**88**	**87**	**322**	**37**	**29**	**17**	**26**	**109**	**700**	**66.66%**
	Mycelia sterilia																	
1.	Mycelia sterilia (white)	-	-	-	-	-	2	2	1	1	06	-	-	-	-	-	06	0.57%
2.	Mycelia sterilia (Black)	-	2	-	1	03	-	4	1	-	05	-	1	-	1	02	10	0.95%
3.	Mycelia sterilia (Red)	-	-	-	-	-	-	2	-	1	03	-	-	-	-	-	03	0.28%
	Total. no. of CFU's formed	**-**	**02**	**-**	**01**	**03**	**02**	**08**	**02**	**02**	**14**	**-**	**01**	**-**	**01**	**02**	**19**	**1.80%**
	Grand total of Fungal colonies formed in each month	**116**	**83**	**66**	**76**	**346**	**96**	**141**	**147**	**140**	**524**	**50**	**47**	**35**	**48**	**180**	**1050**	
	Grand total of Fungal species recorded in each month	**21**	**17**	**14**	**16**		**17**	**23**	**24**	**26**		**11**	**12**	**08**	**11**			

CONCLUSION

Control of microorganisms in the laboratory can be done by maintaining a low humid temperature environment. The doors should be equipped with air curtains to disallow the entry of microorganisms and to remove the entrants of the surface mycoflora. The restoration work should be done by using such chemicals which disallow the growth of microorganisms. The windows and other ventilators should be sieved so that minimal entry to microbial should be allowed.

Hygiene, maintenance, cleanliness and proper ventilation of the laboratory museum and herbarium racks are the key factors for the immediate or progressive recovery from the damage to the organic contents of the museum and herbarium.

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Research Article

RHIZOSPHERIC FUNGAL DIVERSITY OF STRYCHNOS POTATORUM L. F. : A THREATENED AND ETHNOMEDICINALLY VALUABLE TAXA OF INDIAN SUBCONTINENT

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'Symbiotic association' is a key component of ecosystem interactions which plays significant role in nutrient recycling, soil fertility, plant growth promotion and maintains shealth and integrity of microbiome. Rhizospheric region is an integral functional zone of soil adjacent to the roots of vascular plants that are continuously interacted with soil microflora. The soil microflora, in turn, can come to air and become airborne as dried suspended particle. Rhizospheric fungi are predominant soil microflora which takes part in minerals and nutrient solubilisation, mobilization and uptake from the soil. Rhizospheric fungi also plays an essential role in various biochemical reactions that improves plants growth and development. Rhizospheric fungi have mutualistic relationship with higher vascular plants, due to which many threatened, endangered and endemic medicinal plant species can sustain the stress in various climatic regions and soil types.

In view of above context, a study was conducted with the aim to isolate, identify and characterise the seasonal diversity of rhizospheric fungal community of *Strychnos potatorum* L.f., a threatened taxa from Central India. Rhizospheric soil Samples of *S. potatorum* were collected from 3 natural dominant population habitats, viz.: Shankarpur forest site, Banjadi forest site and Korambhi forest site of Chandrapur District (MS), India. Physico-chemical properties of these selected soil samples were analysed such as organic C, total N, P, CEC, WHC, texture etc. The rhizosphere spores were isolated by using the pouring method, followed by wet filtration and same were identified by observing morphological characteristics on the basis of composition, colour, shape and size of spore. In this study, total 18 fungal isolates were observed and identified belonging to fungal class Ascomycotina, Basidiomycotina and Deuteromycotina. Further research is going on for development of protocol to promote plant establishment for conservation and ecorestoration of selected taxa in the natural habitat of selected sites.

Key Words: Rhizospheric fungi, seasonal diversity, spore density, *Strychnos potatorum* L.f.

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INTRODUCTION

The rhizosphere is an interface of soil and root, where the microbial community converts complex material into accessible plant nutrients. It harbours diverse microorganisms such as fungi, bacteria, nematodes, viruses, protists and others. In 1904 the German agronomist and plant physiologist Lorenz Hiltner first coined the term "rhizosphere" to describe the plant-root crossing point, a word originating in part from the Greek word "rhiza", meaning root[1,2]. Soil fungi play an important role in the biogeochemical cycling of the elements, maintaining soil fertility, humus formation by decomposing organic materials[3,4] and beneficial for biodiversity especially for endemic plant species. Phosphate solubilization, nutrient mobilization, metal solubilization, and production of organic acid and extracellular enzymes by fungi have been reported from different soil niches[5,3] in various ecosystems. The rhizosphere is a nutrient-rich zone for microorganisms and contains diverse microbiota with high population density as compare to bulk soil. Extensive research data have been documented about the mutualistic interaction of mycorrhizal fungi for enhancing plant growth, crop yield[6,7,8], and plant health. Soil fungi possess an extraordinary ability to degrade complex natural and synthetic organic compounds by producing a wide range of extracellular enzymes[9,10,11] and perform crucial role in nutrient recycling in the soil and promoting plant growth.

Plants influence the rhizosphere microbiome by releasing root exudates into the soil and producing root litter[12,13]. One of the most important and abundant microbial communities in the rhizosphere are the fungi,

which play a major role in carbon and nutrient cycling in ecosystems. Some of the soil fungi may create a symbiotic connection with plant roots, in which the fungi subsist on organic carbon transferred by the plant to its underground parts. In return, the plant receives nutrients and the alleviation of various forms of stress[14]. Rhizo-spheric microbes affect the plant physiology by imparting several useful effects such as nitrogen fixation, nutrient uptake, and production of secondary metabolites in the medicinal and aromatic plants[15]. Other fungi populations act as decomposers (saprotrophs), playing a major role in nutrient cycling, thus providing nutrients for plants[16]. There are also plant pathogenic fungi, which feed off the plant and provide nothing in return, thus harming the plant[17]. As a result of their continuous effort, the scientific community has determined the function of many fungal taxa and has developed tools to analyse the function of the fungi in a community[18]. Medicinal plants produce secondary plant metabolites in one or more of their parts and affect a wide range of microorganisms[19]. These plants have been used as medicine for humans[20] for at least 5000 years [21], and even today their importance is recognized globally[22]. Many of the drugs developed over the last several decades are either defined mixtures of botanical products, unaltered natural products, or natural product derivatives; many derived from, or are, plant parts. Interestingly, some of the medicinal compounds in plants are not synthesized by the plants themselves but rather by their microbiome[23, 24, 25].

MATERIALS AND METHODS

Study site

The present study was conducted in Shankarpur forest, Banjadi forest, Korambhi forest areas located at 20.6228204N 79.5517046E, 20.6790816N 79.637943E, and 20.6037608N 79.5873244E respectively forming a large, prestigious and ecologically valuable region of Chandrapur district (MS), India. The common basic climate of the study site is characterized by rainy (July–October), winter (November–January) and prolonged dry Summer (March–May). The plant-growing season commences soon after the first rains, between July and September. Average multi-annual rainfall is 275-400 mm, and the mean multi-annual temperature is 27-39°C. Vegetation is dominated by seasonal grasses, herb, bushy shrubs and deciduous trees.

Ecophysiology and Medicinal Use of Researched Plant

The *Strychnos* L. is a pantropical genus, and species are split across four geographical regions, namely American, African, Asian and Australian regions[26]. It includes approximately 200 species ranging from climber to shrubs or lianas, sub-shrubs or shrubs to trees[27], with some species presenting more than one growth habit depending with the surrounding environment[26,28]. *Strychnos potatorum* L.f. (Loganiaceae) is a much branched and moderate sized tree found especially in Peninsular India, Sri Lanka, Myanmar, Madagascar, and Rwanda to Limpopo (Fig. 4.4). It grows primarily in the seasonally dry tropical to deciduous biome[29,30,31]. Indian Forest Department, MOEF circular dt. 04.10.200032, and Alliance for Natural Health International, has been recognized *S. potatorum* L. as an endangered and vulnerable plant species. All parts of this tree have a significant medical value; hence, it is widely used as a multipurpose ethnomedicine in the Indian traditional system for the treatment of ailments such as eye and urinary tract infections, gonorrhea, leukeorrhea, gastropathy, bronchitis, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, ulcers and many others. The ripe seeds are used for clearing muddy water. The clarification is due to the combined action of colloids and alkaloids in the seeds[33,34,35,36]. The medicinal properties of plant parts such as leaf, stem, bark and seed could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals present in them[37,38].

Method for rhizosphere soil collection, processing and fungi identification

During the winter, spring, summer and rainy season, about 100 g soil samples (0–10 cm, n=3) along with the roots were collected from the rhizosphere approximately 15-30 cm depth from three different plants of the selected plant species at the study region. Control samples were taken from an open inter-plant space with a minimal distance of 3m from the researched plants. The soil and the plant root samples were transported to the laboratory in the pre-sterilized polythene bags and stored at 60C (±2) after sieving (2 mm mesh) in order to remove other organic debris, stones, and root particles for isolation of mycorrhizal spores and root colonization.

Processing of Rhizospheric Soil Samples for Assessing the Colonization

The rhizospheric fungi were isolated on potato dextrose agar (PDA). Serial dilution technique was performed up to 10^{-5} dilution. A serial dilution technique[39] is best suited for rhizospheric mycoflora. Add 10 g of dry rhizosphere soil in 100 ml sterile water and mix thoroughly using magnetic stirrer for 15 min. Prepare the dilutions by transferring 1ml aliquot of the suspension aseptically into additional dilutions blanks having 9 ml sterile water in test tubes or flasks respectively. Likewise, the serial dilutions for 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 should be made. Finally, add 1ml of aliquot of various dilutions to sterile petriplates in triplicate. Then add sterile, cool and molten (approx. 45°C) PDA medium.

The petriplates should be swirled in clockwise or anticlockwise direction to disperse the diluted soil suspension in agar. Incubate the petriplates in an incubator for 7 days at 26°C. Record the observations and calculate the colony forming unit, percent occurrence of fungi and rhizosphere effect from the serial dilutions made. The cultures were then incubated for 3 and 5 days at 28°C to observe the fungal growth (Fig. 4.6). Fungal structures were selected based on their morphological characteristics, picked and re-cultured in fresh media for purification to generate pure cultures. The pure cultures were maintained at 4°C.

Isolation and identification of Rhizosphere spores from the selected soil samples

Soil samples (100 g) were collected from the field and examined for spore density analysis. Spores were extracted from the soil by the wet filter pour method[40] followed by centrifugation of the supernatant with 50% added sugar solution[41]. The extracted spores were observed and counted under a dissecting microscope with 35x magnification. Identification of rhizospheric spores was carried out by observing morphological characteristics (composition, color, shape, size etc.)[42]. The taxonomic identification of spores (Fig. 4.5) follows the latest nomenclature according to Schussler and Walker[43] and Redecker *et al.*[44].

Table 4.1: Physicochemical analysis of rhizospheric soil samples collected from different study sites

Sl. No.	Soil Properties	Sampled Rhizosphere		
		Shankarpur forest Soil-1	Banjadi forest Soil-2	Korambhi forest Soil-3
1.	pH	5.70	6.50	5.98
2.	Electrical Conductivity (µs/cm)	244.79	126.41	183.5
3.	Water Holding Capacity (%)	31.68	26.89	29.37
4.	Clay (%)	47.67	31.13	39.24
5.	Silt (%)	21.97	8.72	15.19
6.	Sand (%)	30.36	60.15	43.11
7.	Colour	Brown	Grey	Grey
8.	Moisture (%)	2.74	0.235	1.47
9.	Bulk Density g/cc	1.080	1.022	1.063
10.	Chloride as Cl (%)	0.059	0.636	0.254
11.	TKN as N(%)	0.149	0.338	0.239
12.	Organic Carbon(%)	0.056	0.170	0.113
13.	Cation Exchange Capacity meq/100g soil	7.93	7.83	7.87
14.	P_2O_5 (%)	0.056	0.081	0.063

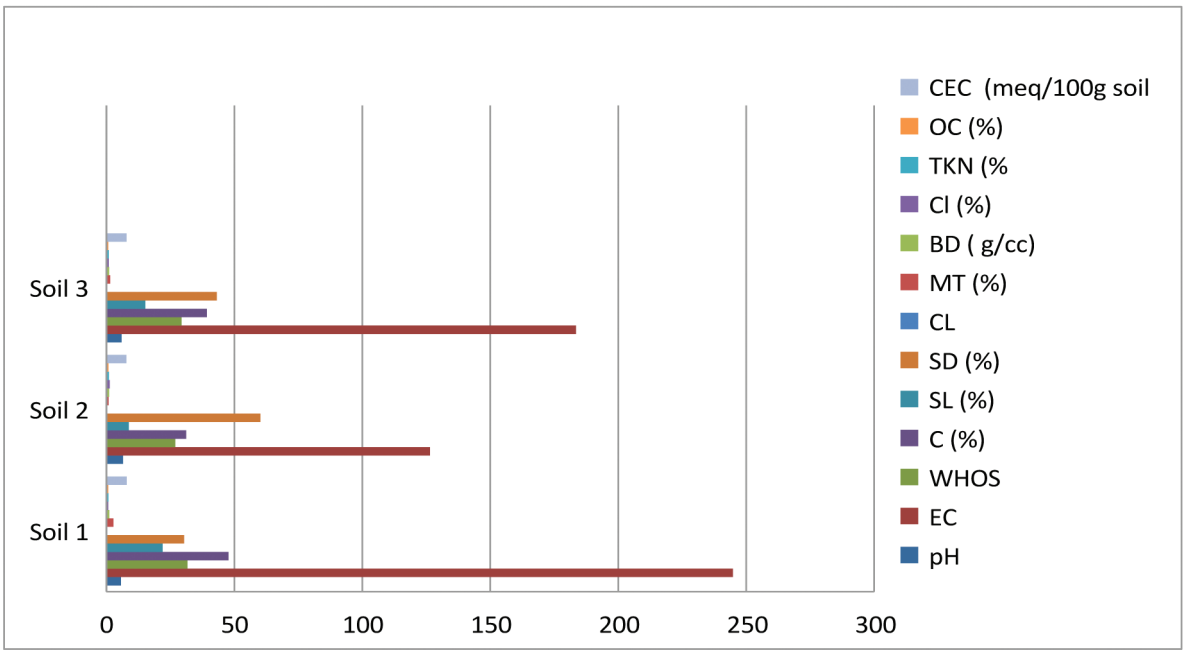

Fig. 4.1: Physicochemical analysis of rhizospheric soil samples collected from (1) Shankarpur forest, (2) Banjadi forest and (3) Korambhi forest area respectively.

Characterization of Fungal Isolates

All the fungal isolates were morphologically identified according microscopic observation of spores and spore bearing structures. It was performed by the use of lactophenol cotton blue mounts and the isolates were identified at the Genus level according to the illustrations by Barnett and Hunter,[45], Schenck and Perez[42], Morton *et al*.,[46], Nagamani *et al*.,[47]. The nomenclature of fungal spores follows the latest nomenclature according to Schussler and Walker[43] and Redecker *et al*.[44].

RESULTS

Physico-chemical Properties of soil

The chemical and physical properties of the soil are presented in Table 4.1, showing that the soil pH at the Banjadi forest location was highest (6.50) and significantly different from other locations. This site also had higher organic C (0.170%) and total N (0.338%) which were significantly different from other locations. The highest available P was shown in Banjadi forest site (0.081) where values were significantly different from that in other two locations. In general, soil texture in the growing habitat of *S. potatorum* required combinations of sand+clay+slit. At the Shankarpur forest, clay fraction is higher and Banjadi forest site, the sand fraction was dominant. Larger the cation exchange capacity determine capacity of soil to supply nutrient cation to soil solution for plant uptake. Shankarpur forest and Korambhi forest area CEC were detected highest (7.93 meq and 7.87 meq per 100 g soil).

However, several studies have reported that the concentration of available P affects spore density.[48] High concentration of available P decreases the diversity of AMF[49,50]. Chaudhary *et al*.,[51] identified the same biotic and abiotic factors driving diversity of free-living rhizospheric fungi composition in tropical and temperate regions. Several factors have been identified that may influence distributions, including abiotic (e.g., soil physico-chemical properties, latitude, climate)[52,53] and biotic (e.g., host plant) factors[54,55] and intrinsic properties of species (e.g., dispersal ability). Organic C, total N and pH affect the diversity of fungal community[56]. The relative contribution of environmental factors showed that components such as elevation, relative air humidity, soil pH, and soil available P, K, and Mg influenced spore production and root colonization of *Picconia azorica*. Organic C, N, P and pH were positively correlated with species richness.[53]

Rhizosphere study

During rhizospheric fungal study, 18 species of fungi were isolated from the soil samples collected from the vicinity of roots of the selected plant (Table 4.2, Fig. 4.2). These isolates are *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Arthrinium* sp., *Mucor* sp., *Rhizopus* sp., *Chlamydospores*, *Glomus* sp.,

Table 4.2: Rhizospheric Soil Samples Analysis Collected from different Sites

Sl. No.	Name of Rhizospheric Fungal Isolate	Class	Spore density		
			Shankarpur forest Soil-1	Banjadi forest Soil-2	Korambhi forest Soil-3
1.	*Aspergillus* sp.	Ascomycotina	99	44	35
2.	*Penicillium* sp.	Ascomycotina	33	23	31
3.	*Trichoderma* sp.	Ascomycotina	9	11	13
4.	*Cladosporium* sp.	Ascomycotina	27	12	13
5.	*Curvularia* sp.	Ascomycotina	12	13	17
6.	*Chaetomium* sp.	Ascomycotina	93	77	65
7.	*Drechslera* sp.	Ascomycotina	56	41	34
8.	*Arthrinium* sp.	Ascomycotina	23	-	18
9.	*Mucor* sp.	Zygomycotina	43	34	31
10.	*Rhizopus* sp.	Zygomycotina	49	42	19
11.	*Chlamydospores*	Zygomycotina	23	13	14
12.	*Glomus* sp.	Glomeromycotina	431	323	578
13.	*Nigrospora* sp.	Hyphomycotina	67	43	21
14.	*Clavulina spores*	Basidiomycota	32	26	19
15.	*Helminthsprium* sp.	Deuteromycotina	5	9	14
16.	*Fusarium* sp.	Deuteromycotina	15	23	29
17.	*Alternaria* sp.	Deuteromycotina	109	96	77
18.	*Pithomyces* sp.	Deuteromycotina	9	11	15

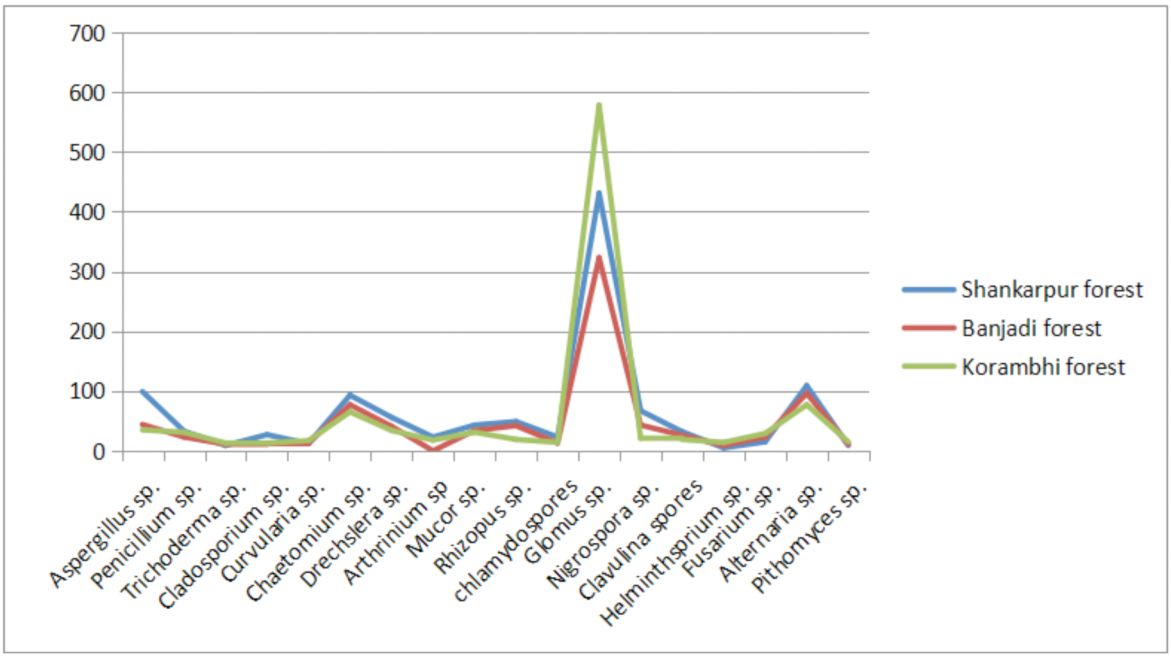

Fig. 4.2: Graphical representation of Fungal spore density in Rhizospheric Soil samples collected from different Sites.

Table 4.3: Comparative seasonal analysis of rhizospheric fungi of soil samples collected from different sites

Sl. No.	Name of Fungus Isolate	Class	Winter	Spring	Summer	Rainy
1.	*Aspergillus* sp.	Ascomycotina	+	-	-	+
2.	*Penicillium* sp.	Ascomycotina	-	+	+	+
3.	*Trichoderma* sp.	Ascomycotina	-	+	-	+
4.	*Cladosporium* sp.	Ascomycotina	+	-	-	+
5.	*Curvularia* sp.	Ascomycotina	+	-	-	+
6.	*Chaetomium* sp.	Ascomycotina	-	-	-	+
7.	*Drechslera* sp.	Ascomycotina	-	-	-	+
8.	*Arthrinium* sp.	Ascomycotina	+	-	-	+
9.	*Mucor* sp.	Zygomycotina	-	+	+	+
10.	*Rhizopus* sp.	Zygomycotina	-	+	+	-
11.	*Chlamydospores*	Zygomycotina	-	+	+	+
12.	*Glomus* sp.	Glomeromycotina	+	+	-	+
13.	*Nigrospora* sp.	Hyphomycotina	-	-	+	-
14.	*Clavulina spores*	Basidiomycota	-	-	+	-
15.	*Helminthosprium* sp.	Deuteromycotina	-	+	-	+
16.	*Fusarium* sp.	Deuteromycotina	+	+	+	+
17.	*Alternaria* sp.	Deuteromycotina	+	-	+	+
18.	*Pithomyces* sp.	Deuteromycotina	-	-	+	+

Nigrospora sp., *Clavulina* spores, *Helminthosporium* sp., *Fusarium* sp., *Alternaria* sp. and *Pithomyces* sp. A comparison of seasonal distribution of these isolates revealed that maximum number of fungi was recorded (15 species each) during rainy season (83%), 8 species in spring season (50%), 7 species in winter season (39%) followed by 9 species (50%) in summer season. The results in the present investigation are also in conformity with the work of Sule and Oyeyiola[57] with different fungal isolates in the rhizosphere of *Cassava*

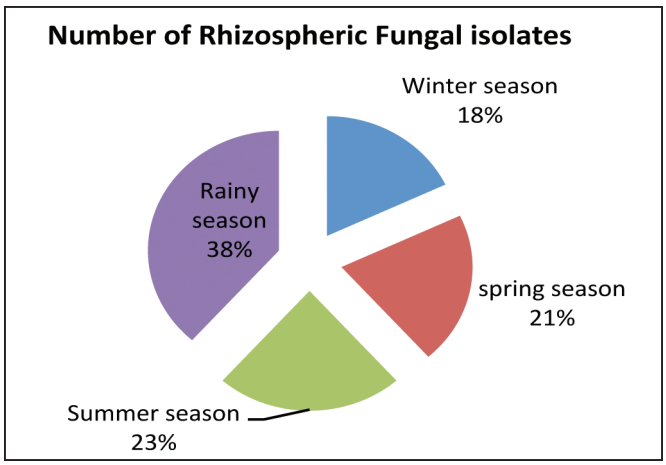

Fig. 4.3: Diagrammatic representation seasonal diversity of Rhizospheric fungal community

cultivar. Various workers have reported similar fungal genera from the rhizosphere of different plants.[58-60] *Amygdalus scoparia*,[48] *Ulmus chenmoui*,[61] *Coccothrinax crinite*,[62] *Ferula sinkiangensis*,[63] *Carissa edulis*[64] and several other species are threatened with extinction in the Himalayas.[65] High fungal community diversity was reported in roots of *H. jongheana, C. caulescens* and *C. cinnabarina* orchids.[66] Furthermore, AMF is essential for plant growth and health.[67] As a result, it is critical to investigate the diversity of these fungi in the rhizosphere community structure of *P. verticillatum*. Several academics are working on identifying certain mycorrhizal fungus and their role in phytochemical production.[68]

Further these rhizospheric fungi isolated from the root adhering soil samples of selected plant species, belong to subdivisions Ascomycotina (*Asperigillus, Penicillium, Trichoderma, Cladosporium, Curvularia, Chaetomium, Drechslera* and *Arthrinium*), Zygomycotina (*Mucor, Rhizopus* and chlamydospores), Glomeromycotina (*Glomus*), Hyphomycotina (*Nigrospora*) Basidiomycota (*Clavulina* spores) and Deuteromycotina (*Cladosporium, Helminthosporium, Fusarium* and *Alternaria*). The dominant class was ascomycotina (8 species) followed by Deuteromycotina (3 species), Zygomycotina (3 species), Glomeromycotina (1 species), Hyphomycotina (1 species) and Basidiomycota (1 species).

Visser and Parkinson,[69] stated that for a given community only a few fungal species were dominant which may strongly affect the environmental conditions for other species. The most abundant fungal phylum in all samples was Ascomycotina and second most abundant was Basidiomycotina in soils globally,[70] Ascomycotina is not only the most abundant fungal phylum, but also the largest in terms of number of species.[71]

It was observed that maximum isolated genera belong to subdivision Ascomycotina, and Deuteromycotina which can tolerate wider environmental conditions as compared to the other fungal populations.[72] Shaikh and Nadaf[73] screened various rhizospheric fungal isolates from different soil samples of rice and found that hyphomycetes were predominant in most of the tested rice varieties. Gadgil[74] observed the colonization of Hypomycetes fungi in the root region. Similarly,

Fig. 4.4: Habitat and habit of *Strychnos potatorum* in the selected study areas.

fungal diversity in rhizosphere soil of several crops has also been well documented by Gopal and Kurein.[75] A comparison of seasonal distribution of these isolates revealed that maximum number of fungi was recorded (15 species each) during rainy season (83%), 8 species in spring season (50%), 7 species in winter season (39%) followed by 9 species (50%) in summer season (Fig. 4.3, Table 4.3). Maximum number of fungi was recorded during the rainy season. Manoharachary[76] reported a direct correlation of moisture and fungal members of various soils. It was also observed from the present study that mycoflora isolated from the rhizo-

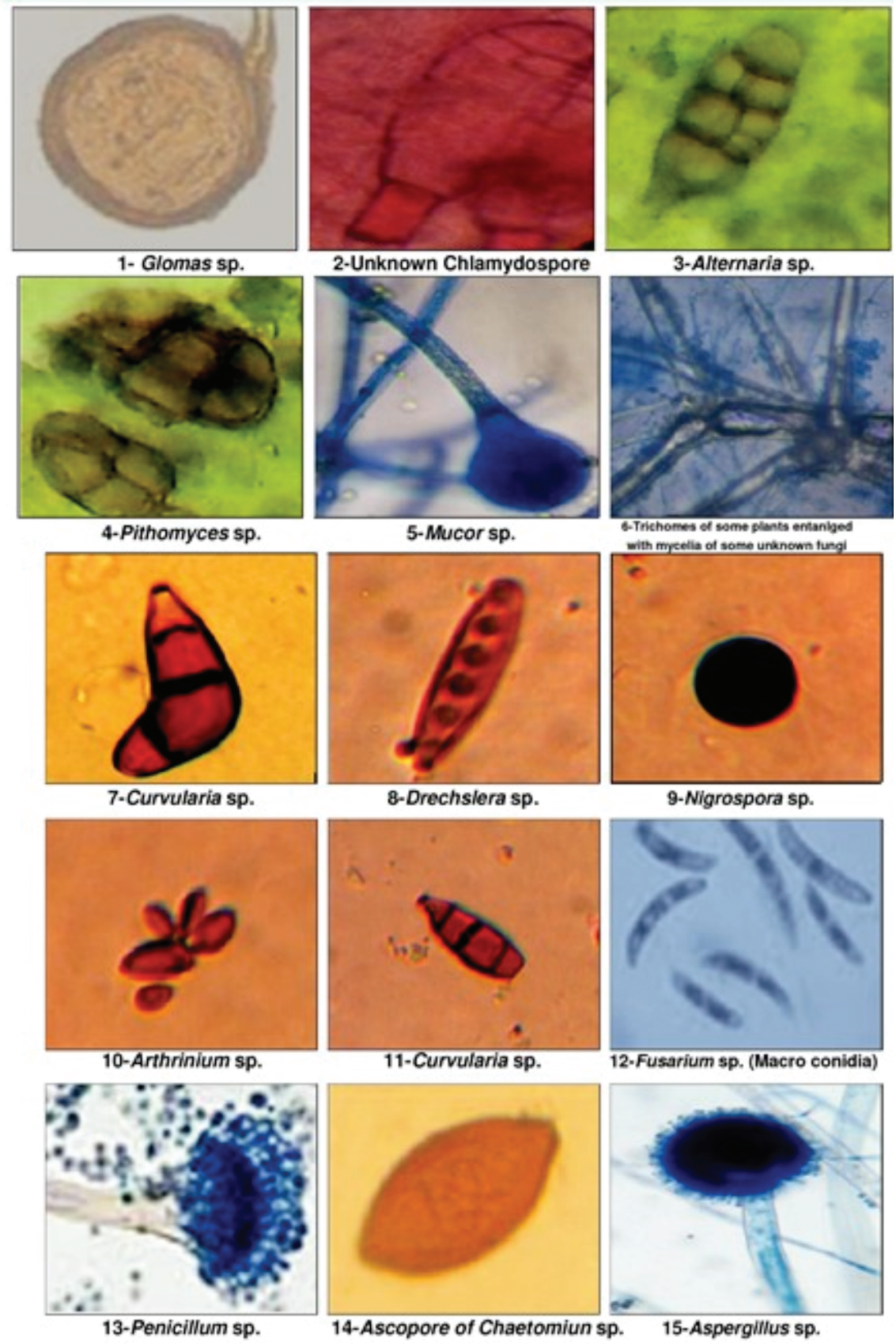

Fig. 4.5: Rhizospheric fungal spores observed on *S. poattorum* root zone.

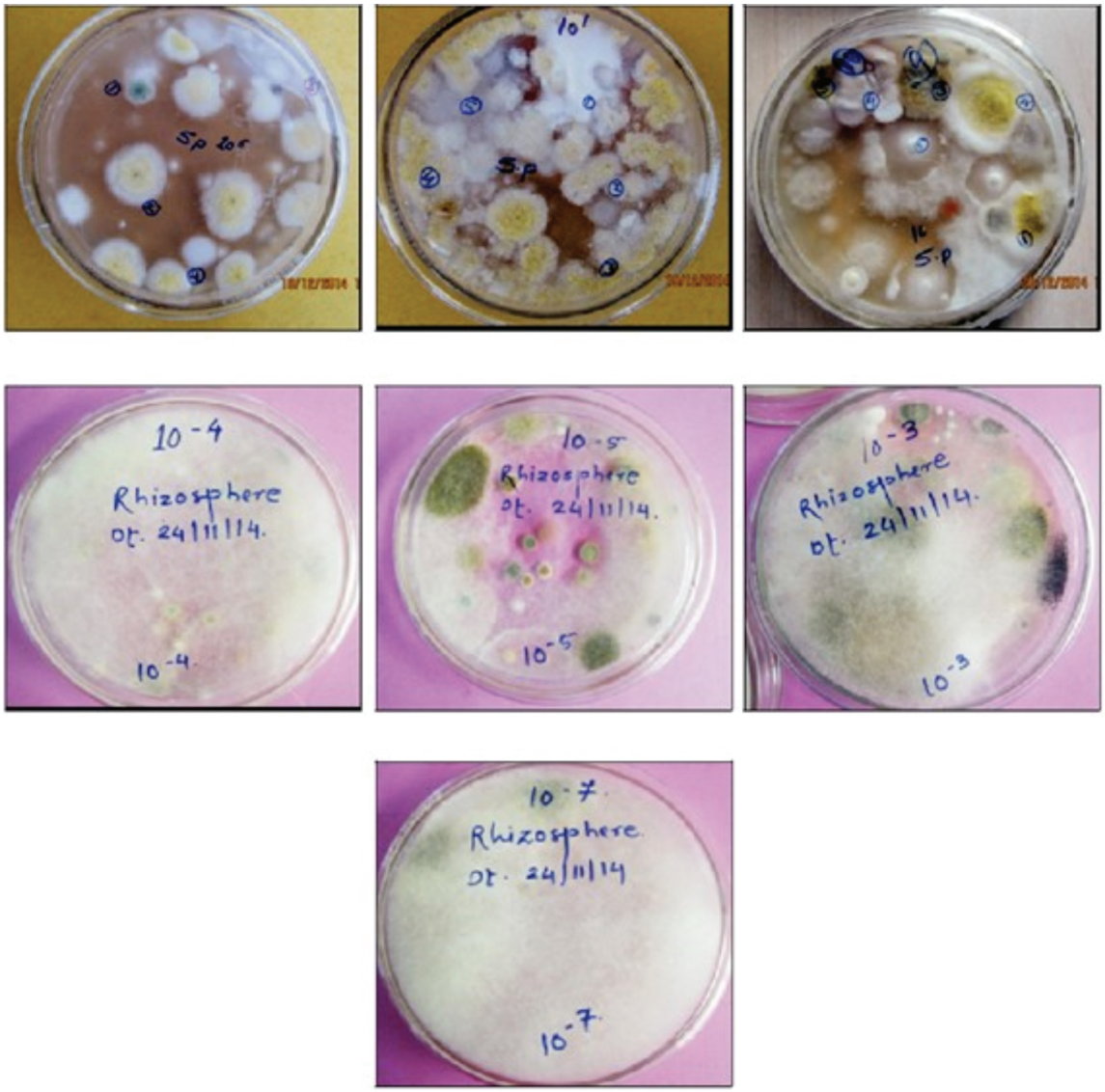

Fig. 4.6: Rhizospheric fungal colonies on petriplates using Richard's media

sphere soil did not show any uniform pattern of appearance and distribution.

DISCUSSION

The role of the mycorrhizal fungi has been described as that of a fundamental link between plant and soil.[77,78] Rhizosphere microflora in natural ecosystems play significant role in the re-establishment of nutrient cycling.[79] They can modify the structure and function of plant communities[80] and may be useful indicators of ecosystem change.[81] Although AM fungi have not been used specifically to increase the production of medicinal compounds in plants, their ability to enhance the plant growth and root health has been demonstrated earlier in many crop species.[82, 83]

During rhizospheric fungal study, 18 species of fungi were isolated from the soil samples collected from the vicinity of roots of the selected plant (Table 4.2, Fig. 4.2). These isolates were *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp., *Curvularia* sp., *Chaetomium* sp., *Drechslera* sp., *Arthrinium* sp., *Mucor* sp., *Rhizopus* sp., Chlamydospores, *Glomus* sp., *Nigrospora* sp., *Clavulina spores, Helminthosporium* sp., *Fusarium* sp., *Alternaria* sp. and *Pithomyces* sp. The dominance of Glomus species in alkaline soil was reported by many workers[84,85], while the present study claimed that the *Glomus* species also found in slightly acidic soil. The level of AM fungal association depends on root morphology, metabolism and rate of plant growth[86] balance between root and fungal activity, as well as on specific soil plant system in term of chemical nature of root exudates.[87] In natural system[88] observed that seasonal fluctuations of mycorrhizal associations were closely related to plant phenology.

These findings were in accordance with the study conducted by other researcher.[89] Association of arbus-

cular mycorrhizal fungi with the roots of medicinal and aromatic plants has also been observed.[90] Tejavathi *et al*.[91] reported the positive correlation between percent mycorrhizal colonization and plant growth in *Andrographis paniculata*. The maximum spore population during rainy season might be correlated with the fact that during this period most photosynthetase is allocated to roots and rhizomes, which helps fungal symbiont to produce more spores.[92]

Some minerals such as phosphorus, iron, zinc and copper are of very limited mobility in the soil and are only found in extremely low concentrations in soil solution. The mycorrhizal medicinal plants have higher nutrient uptake capacity and growth than non-mycorrhizal plants.[93-94] AM fungi-colonized plants have greater tolerance capacity over non-mycorrhizal plants to several biotic and abiotic stresses such as toxic metals, root pathogens, drought, high soil temperature, saline soils,[95-96] adverse soil pH and transplanting shock.[97-98]

CONCLUSION AND FUTURE PROSPECTS

The processes occurring in the rhizosphere controls and mechanism of elemental cycling and recycling help to sustain the plant life at variety of ecosystem level.[99-101] This study provides information on the status of rhizospheric fungal community and diversity of *S. potatorum* species. Mycorrhizal study helps to select appropriate microbial strains as plant inoculants that could be of use in conservation and management of threatened taxa in specific ecological niches. Also improved understanding on soil biochemistry and microbial diversity across different soil types and locations are essential for production of food, fibre and fuel for sustainable ecosystem management, conservation of endemic and engendered plant biodiversity, expansion of agricultural activities sustainable management of wastelands and future environmental safety.

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Research Article

AEROMYCOLOGICAL SURVEY OF FUNGAL DIVERSITY AND INVESTIGATION OF BIODEGRADABILITY AND ENZYMATIC ACTIVITY OF FUNGI FROM KORADI REGION OF NAGPUR DISTRICT

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Intramural and extramural aeromycological survey was conducted for various locations of Koradi area of Nagpur district in post winter season. Koradi region is infamous for its polluted air due to soot released from Koradi thermal power station. The Hi-Media air sampler was used for sampling. Total 9 type of fungal spores were identified and rest others were classified as unidentified. *Aspergillus* sp. was found to be prominent one. Ascomycota contributed 75.13%, Zygomycota contributed 8.11%, and other fungi contributed 16.75%. Ascomycota was recorded slightly higher in month of February. *Trichoderma viridae* showed largest radial growth rate as compared to other fungi. Enzymatic activity was also recorded by using different enzymes. Cellulase activity was recorded by using endoglucanase and exoglucanase. Maximum cellulolytic activity was shown by *Fusarium oxysporium*. *Aspergillus flavus* showed maximum amylase activity. Maximum extracellular protease activity was observed in *A. niger* and *F. oxysporium*. Biodegrading ability of various fungi were recorded by weight loss test of sugarcane bagasse. Determination of weight loss gives general idea on performance of fungus. *T. viridae* and *R. stolonifer* gave the highest weight loss. government or the respective authorities must check the quality of street food from time to time.

Key Words: Aeromycology, Biodegradability, Enzymatic activity, Koradi, Nagpur.

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INTRODUCTION

About 80,000 to 1,20,000 species of fungi have been described to date, although the total number of species is estimated at around 1.5 million[1]. This would render fungi one of the least explored biodiversity resources of our planet. Various species of fungi are present in all kinds of environment. There is essentially no fungus-free environment in our daily lives. Fungi prosper in conditions within the human comfort range and certain fungi can survive not only at low or high temperatures but also at limited water activities, low pH or high pH and very low oxygen content[2]. Along with bacteria, they are important decomposers and disease-causing organisms. Fungi are found everywhere from the tropics to the tundra and in both terrestrial and aquatic environments[3].

Fungi form major component of aeroflora and are responsible for causing various diseases in plants and animals. These fungal killers are particularly problematic because fungi are animals' closest relatives. Drugs that can kill fungi often have toxic effects on animals, including humans[4]. Saprophytic fungi are also important in industrial fermentation, for example, the brewing of beer, the making of wine, etc., parasitic fungi cause diseases in plants, humans, and other animals[5,6]. Spores of most fungi do not survive significant periods in air because of the absence of food and energy and those who survive have quite specific mechanisms to prevent damage from desiccation and irradiation[7]. Pollen grains and fungal spores and some bacteria are among the most abundant airborne bioparticles[8]. In air, prevalence of fungal spores is dominant and has been estimated ten times than the pollen grains[9]. The aeromycoflora of a particular place depends of parameters such as rainfall, humidity, temperature, light intensity, wind speed, sand storm and organic matter available[10,11].

The airborne fungal spores show great variation in composition, concentration and may vary from place to place[12]. The types and concentration of fungal spores is determined by time, day, weather parameters and seasons[13]. Different study and reports show that fungal

spores are main cause of seasonal and other type of allergic reaction[14]. Allergic disorder has grown tremendously in recent years making scanning of aerobiota not only desirable but imperative as over one million people suffer from asthma alone. This need is compounded further by allergic disorderness, hay fever, urticaria, rhinitis, seasonal sneezing, dermal problems, gastro-intestinal allergies[15].

Cellulose, the major constituent of all plant materials, forms about half to one-third plant tissues and is constantly replenished by photosynthesis. One of the largest cellulosic agro industrial by-products is sugarcane bagasse, it is a ligno-cellulosic residue (by product) of the sugar industry and is almost completely used by the factories themselves as fuel for the broilers[16]. Truly cellulolytic fungi, while growing on cellulosic articles, attack the fibres and degrade the cellulose, thereby causing a loss in weight of the material. Therefore, loss in weight is considered as an important criterion for determining cellulolytic activity of test isolates[2].

Literature survey indicates that till now there is miniscule information about aeromycoflora of this area, hence it seems to be important to conduct extensive and systematic aeromycological survey from Koradi region, of Nagpur district.

METERIALS AND METHODS

The present aeromycological study was carried out in one indoor stadium, one outdoor stadium, Cinema hall, one Food storage warehouse and a Market place in Koradi region of Nagpur district. Koradi is a small town situated in Kamptee tehsil north of Nagpur city in the state of Maharashtra. It is situated between 210 14'16.0944" North Latitude and 7906'45.0396" East Longitude. Koradi region was chosen for study as it is constantly in news for its deteriorating air quality due to smoke released form Koradi thermal power station (KTPS).

The present aeromycological study was carried out for the period of two months viz. January 2023 to February 2023 using Air Sampler (Make-Hi-Media) in both indoor and outdoor environments during afternoon (12:30 P.M. -2:30 P.M.). Sterile air sampler media strip was filled with Potato dextrose agar nutrient jelly and was fitted in Hi-Media air sampler. After collection these stripes were scanned and spores were observed qualitatively.

Calculation of colony forming units

After three days of incubation, the fungal colonies were counted for individual species and the total number CFUs were calculated. The percentage contribution of spores was calculated taking the average of the two readings of each experimental site.

Radial growth rate and Mycelial biomass

Petridishes were inoculated in the centre of the plate using inoculum (4 cm of diameter) from the periphery of each fungal strain grown on potato dextrose agar. The radius of the mycelial growth was measured every 12 hours until full invasion of the plates. The radial growth rate was calculated as the slope of the radius of mycelial growth versus time plots, analysed by linear regression17. The mycelial biomass was evaluated in each plate after full invasion of the culture media. The mycelia were separated from the culture media using a boiling-water bath in a pre-weighed watch glass. This was weighed, and then oven-dried at 60°C for 24 h, then weighed again[17].

ENZYMATIC ACTIVITY

Fungi cultivation

For inoculation, five fungi *Aspergillus niger. Aspergillus flavus, Trichoderma viride, Rhizopus* sp., *Fusarium* sp., were grown in PDA slants at 280 C for 7 days, after which the asexual spores are normally produced. Fungal spores at the concentration of 10^7 spores/ml. were prepared in sterilized distilled water for use in pre-treatment.

Biomass pretreatment

The collected bagasse was chipped into small particles using a grinder, dried to less than 10 % moisture content and screened to even size. The chips were powdered using grinder. A total of 1g of the powdered bagasse was then transferred into a conical flask. Ten millilitres of synthetic medium with concentration per litre of 0.01 g $MgCl_2$, 0.002 g KH_2PO_4 and 0.0005 g of $CaCl_2$, were taken using pipette and spread evenly on the powder surface, Conical flasks were closed with aluminium foil to avoid moisture loss and contamination and then autoclaved at 121°C for 20 min. A total of 10 mL of inoculum was added into the bagasse medium and left at room temperature for 2 weeks. Ten conical flasks were prepared, each species had two replicates and the treatment was repeated thrice at different times.

Filtrate preparation

After two weeks, the mycelium covering the samples was first washed away by adding 30 mL of sodium acetate buffer, pH 5.0. Conical flasks were shaken gently for 20 min at room temperature. The sample was filtered using a filter paper (Whatman, No. 1). The filtrate was centrifuged at 8500 rpm, 4°C, for 20 min. The supernatants were stored at 4°C for further analysis.

Enzyme analysis

Endoglucanase: It's activity was assayed using 50 mM KOH-KH_2PO4(pH6.2), 2% carboxymethyl cellulose (CMC) and 0.5 ml culture supernatant. The mixture was maintained at 50°C for 30 min and the reaction was terminated by adding 3 mL of dinitrosalicyclic acid (DNS) reagent and heated in boiling water bath for 15 min. The absorbance was recorded at 565 nm One unit of endoglucanase was expressed by 1μ mol of glucose liberated per ml. enzyme per min.

Exoglucanase: Determination of exoglucanase was done by measuring the amount of reducing sugar released in reaction mixtures which consisted of 50 mM KOH-KH_2PO_4. (pH6.2), 1% Avicel and 0.5 mL culture supernatant. Mixture was maintained at 50°C for 30 min[18]. Three milliliters of DNS reagent were added and mixtures were heated in boiling water bath for 15 min. Absorbance was read at 565 nm and 1 unit of exo-glucanase was expressed by μ mol of glucose liberated per mL enzyme per min[19].

Amylolytic activity: A reaction mixture containing 1μ ml of enzyme broth (filtrate) and 0.5 ml of 1% starch was incubated for 30 minutes at 37°C. The reaction was stopped by using DNS as colour reagent. The reaction mixture was diluted to 5 ml with distilled water. The reddish brown colour was observed which was measured spectrometrically at 540 nm. Amount of sugars produced were read off from a standard solution. One unit of amylase was defined as the amount of enzyme that releases 1μ mol of reducing sugar as maltose per min under the assay conditions[20].

Cellulolytic activity: The reaction mixture made up of 1ml filtrate, 1ml of citrate acetate buffer 0.5M (pH 5) and 2.5 ml of 1% CMC as a substrate was incubated at 1 hr. at 37°C. This reaction mixture served as sample for determining the presence of glucose by DNS method. One unit of cellulose was defined as 0.1 mg of glucose released from 1% CMC at 37°C and pH 5[20].

Proteolytic activity: Protease activity was measured by degradation of casein, 1 ml of filtrate was added to 1 ml of 1% (w/v) casein (pH 7.5) and incubated for 1 hr at 45°C. The reaction stopped by protein precipitation agent, 3 ml of 0.5 M Trichloroacetic acid (TCA). Solutions were centrifuged at speed of 5000 rpm for 30 mns and absorption of filtrate was measured at 275 nm. One enzymatic unit represents the quantity of enzyme which liberates 1 µg of tyrosine under enzyme assay condition[20].

BIODEGRADING ABILITY

Weight loss test method

The bagasse biomass filtered from the above method was oven dried at 90°C for 3 days and weighed to determine the weight loss.

The isolates were grown individually on PDA media, with bagasse as the sole source of carbon. The experiment was carried out in petriplates of 90 mm diameter. Petri-plates contained bagasse of known weight along with 10 ml of PDA media without sucrose. Duplicates were maintained for monitoring the results on 10th and 20th day. Similar sets maintained in identical conditions were served as the control. All plates prepared in this manner were autoclaved at 121°C for 20 min.

The inoculum was prepared in the form of a uniform suspension of spores (10^7 spores/ml) from 15d old cultures of the respective isolates grown on PDA medium by adding 10 ml of sterile distilled water, followed by shaking on a vortex mixer. One millilitre of the suspension was added to each of the respective plates as inoculum. In the control set, sterile distilled water was added instead of spore suspension. The plates were incubated at 28° for 10 and 20 days respectively.

At the end of the respective incubation periods bagasse powder were oven dried at 80°C, allowed to cool down to ambient temperature in a desiccator and then weighed on a balance. The difference in weight of bagasse was computed by comparing it with the original dry weight and also of the control set. The net loss in weight was attributed to cellulose degradation. The percent loss in weight brought about by each isolate was calculated using the formula, % loss in weight = (difference in weight/initial weight) ×100.

RESULTS AND DISCUSSION

The results of the two months data on aeromycofloral survey showed that the atmosphere of Koradi region of Nagpur district, Maharashtra was never free of fungal spores. A total of 9 type of fungi spores were identified and rest all spore types which are not identified were grouped under unidentified spore types (Table 5.1). Out of the isolated fungal species, *Aspergillus* was recorded as prominent moulds[21]. A number of *Aspergillus* sp. such as *A. flavus, A. niger* were reported to be of high incidence similar to findings of Singh *et al*.[22-24]. Among the isolated fungal taxa *Aspergillus niger, Rhizhopus stolonifer* were predominant aeroallergens that may cause different types of respiratory diseases in atopic human beings. *Aspergillus flavus* a mycotoxin producing fungus was abundantly recorded from three out of five sites (Table 5.1). The fungal isolates were found to be allergic and causing cold, irritation of eyes nose, ears, itching skin eruptions, fever, headache, vaginal irritation, nervous disorder, mussels slackening, lung infections[25].

Table 5.1: Report on Fungal Aerospora from different sampling sites of Koradi region of Nagpur district, Maharashtra

Fungal types	Sites	09.01.23	20.01.23	09.02.23	20.02.23	Total Colonies
Aspergillus niger	A	03	05	04	06	18
	B	02	07	03	04	16
	C	06	04	03	01	14
	D	01	03	04	02	10
	E	07	08	05	03	23
Aspergillus flavus	A	-	-	02	02	04
	B	01	05	04	03	13
	C	05	06	04	03	18
	D	03	03	05	02	13
	E	01	-	02	05	08
Aspergillus terreus	A	04	03	-	-	07
	B	-	02	-	-	02
	C	-	-	-	-	-
	D	01	-	-	-	01
	E	03	-	-	-	03
Aspergillus sp.	A	03	02	03	05	13
	B	02	03	06	05	16
	C	05	07	01	04	17
	D	01	05	05	03	14
	E	02	08	02	06	18
Trichoderma viridae	A	01	02	02	03	08
	B	02	03	03	04	12
	C	01	02	01	03	07
	D	02	05	02	03	12
	E	02	03	04	02	11

Contd.

Contd. Table 5.1

Fungal types	Sites	09.01.23	20.01.23	09.02.23	20.02.23	Total Colonies
Trichoderma sp.	A	01	02	02	03	08
	B	04	05	03	02	14
	C	01	01	-	-	02
	D	02	01	05	03	11
	E	01	04	05	03	13
Fusarium oxysporium	A	03	01	03	03	10
	B	05	04	05	01	15
	C	-	-	-	-	-
	D	03	02	03	05	13
	E	02	02	01	03	08
Fusarium sp.	A	05	03	04	03	15
	B	02	01	02	03	08
	C	-	-	-	-	-
	D	07	05	05	04	21
	E	01	04	03	02	10
Rhizopus sp.	A	02	01	01	01	05
	B	04	05	03	05	17
	C	-	-	-	-	-
	D	05	04	02	03	14
	E	01	03	02	04	10
Unidentified fungi	A	01	01	02	03	07
	B	04	02	03	01	10
	C	01	-	01	-	02
	D	01	02	01	-	04
	E	05	01	01	02	09
Sterile mycelia	A	07	03	04	02	16
	B	06	-	07	06	19
	C	-	02	-	-	02
	D	05	02	03	03	13
	E	06	03	-	04	13

A-Indoor stadium; B-Outdoor stadium; C-Cinema hall; D-Food storage warehouse; E-Market Place.

Exposure to fungal aerospora has been linked to a range of detrimental health effects in both infants and adults[26]. Conversely, the hygiene hypothesis, which posits that exposure to microbial material early in life can actually be preventive in developing disease later in life, continues to find empirical support[27,28].

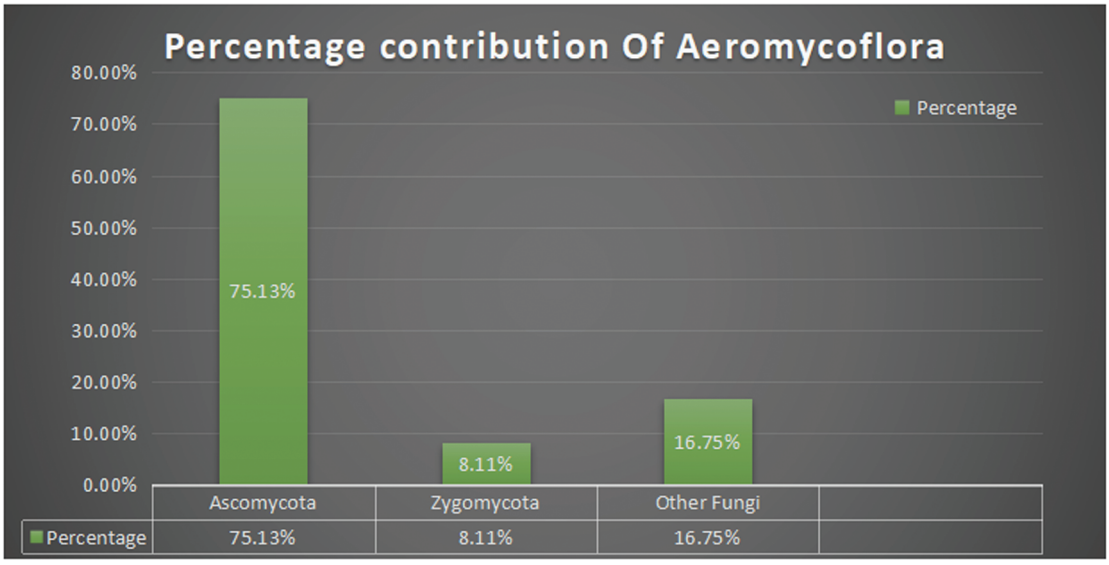

Fig. 5.1: Average percentage contribution of each group found to the total aerospora during study period from January to February 2022.

Percentage contribution of Aeromycoflora in accordance with colony forming units

Airborne fungal spores recorded were representatives of the major groups Ascomycotina with minimum representatives of Zygomycotina and other fungi (Fig. 5.1). Aeromycological study employs a number of sampling methods of which, gravity settling of spores on culture medium is the one widely used by workers both in indoor and outdoor environments[29]. The member of Basidiomycota did not appear on the agar plates. This is in agreement with the findings of Ananna[30-32], who reported the greatest count of fungal isolates as well as higher fungal colony count in indoor aeromycoflora by culture plate exposure test.

Enzyme analysis

Two types of enzyme assays were conducted to determine cellulase activity. The results showed that *T. viride* recorded the highest activity for endoglucanase and it remained low in other four species (Fig. 5.2). *F. oxysporum* recorded the highest activity for exoglucanase follwed by *T. viride*, while *A. niger* recorded the lowest (Fig. 5.2).

A. flavus was found to be potential amylase producer with maximum amylase activity (Fig. 5.3). Other isolates *A. niger, F.oxysporum, T. viride* and *R. stolonifer* showed moderate to low amylase activity.

F. oxysporum produced maximum cellulase activity followed by *T. viride*, while other isolates such as

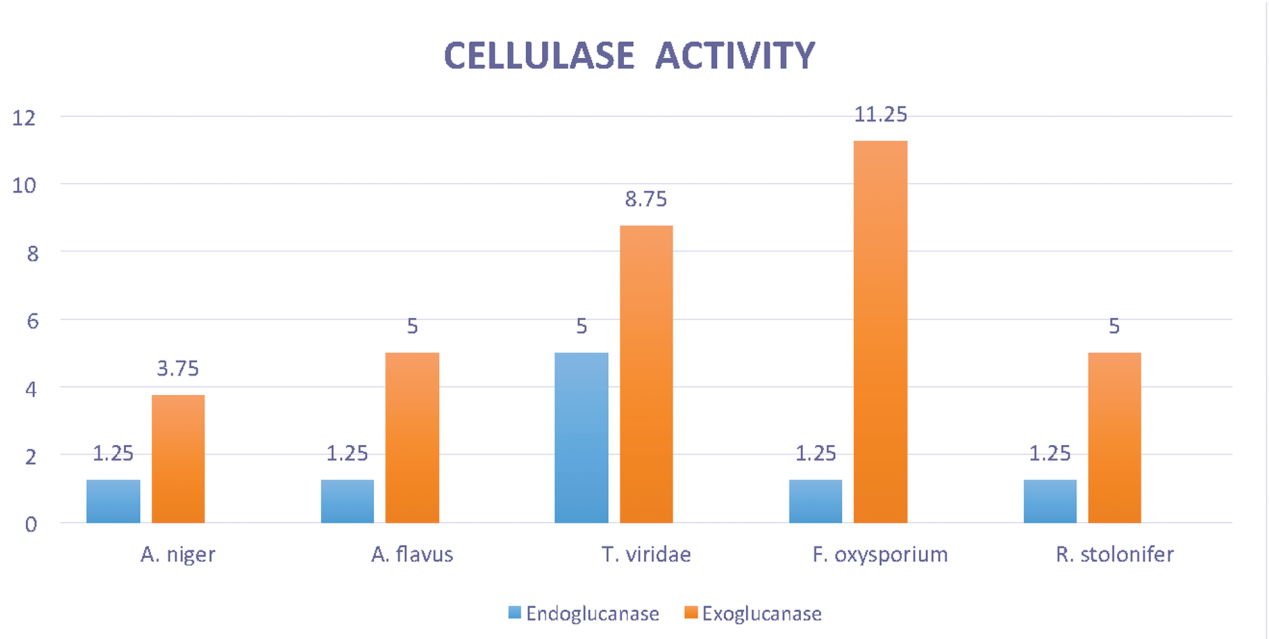

Fig. 5.2: Activities of two cellulase enzymes in fungal filtrates after two weeks of incubation in bagasse medium.

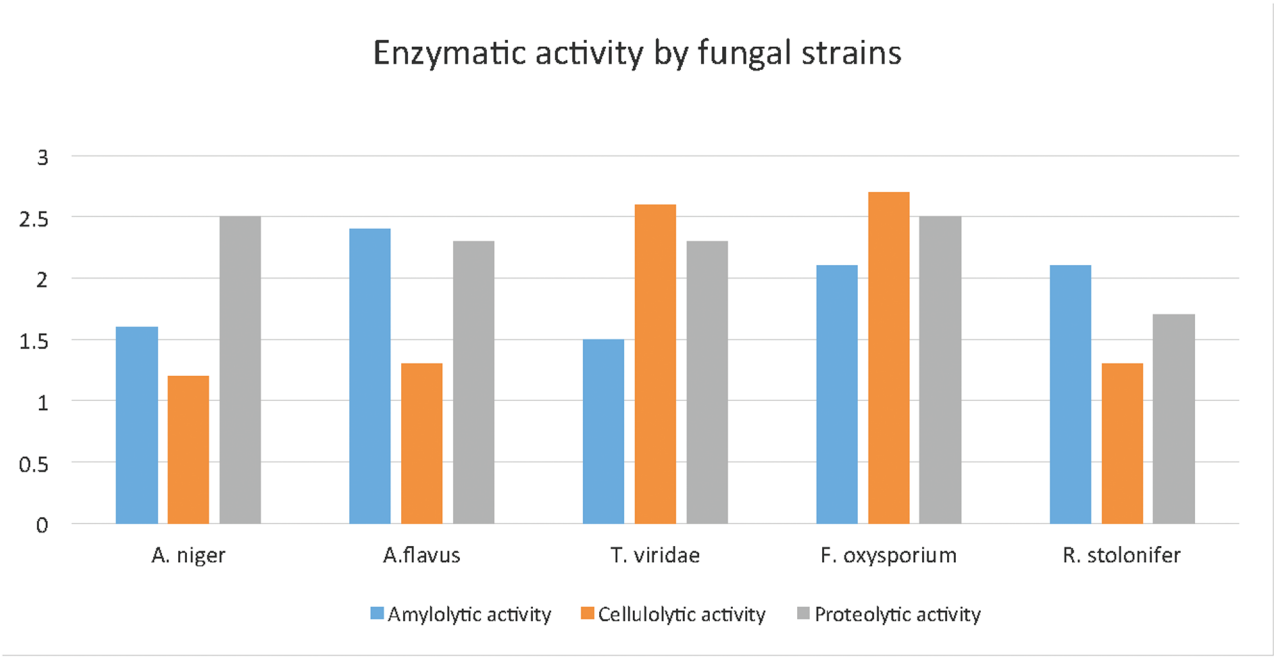

Fig. 5.3: Enzymatic Activity shown by fungal strains.

A. flavus, A. niger and *R. stolonifer* exhibited moderate to low activity (Fig. 5.3). Among the tested organisms, maximum extracellular protease activity was observed in *A. niger* and *F. oxysporum*, other isolates of *A. flavus*, T.viride and R.stolonifer exhibited moderate protease activity (Fig. 5.3).

Biodegrading ability by weight loss test method

Weight loss indicates degradation of biomass caused by the fungi when comparing the five fungal species. Percentage loss in weight of bagasse biomass pretreated by fungal spores in concentration of 10^7 spores/ml in the synthetic medium exceeded more than 94.50% in *T. viridae*, *A. flavus* and *R. stolonifer* (Fig. 5.4), while *A. niger* and *F. oxysporum* showed less weight loss of bagasee biomass (Fig. 5.4). Determination of weight loss gives a general idea on the performance of a fungus.

Percent weight loss in PDA media with bagasse as the sole source of carbon inoculated with fungal spores in concentration of 10^6 spores/ml was also observed (Table 5.2). The maximum percentage loss of substrate was noticed in *T. viridae*, *A. flavus* and *A. niger*, while minimum percentage loss of substrate was demonstrated in *R. stolonifer* (Table 5.2).

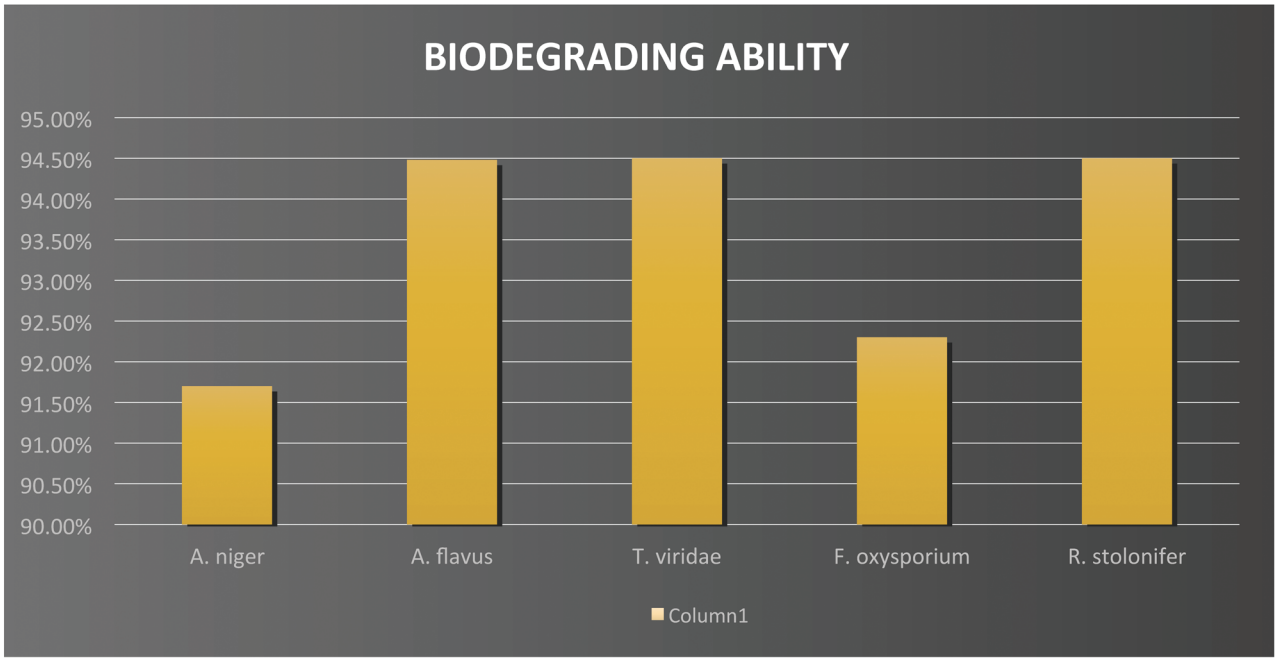

Fig. 5.4: Weight loss of bagasse biomass treated with five species of fungi.

Table 5.2: The percentage loss of substrate by selected species of fungi

Fungi tested	Amount of Substrate (g)	% Loss	
		Period of Incubation	
		Day 10	Day 20
A. niger	0.1	14.37	18.67
	0.5	14.62	19.09
A. flavus	0.1	14.85	19.58
	0.5	14.20	18.80
T. viridae	0.1	14.34	18.62
	0.5	14.68	19.42
F. oxysporium	0.1	15.24	16.55
	0.5	14.29	18.33
R. stolonifer	0.1	13.32	16.63
	0.5	13.38	16.62

CONCLUSION

The impact of airborne fungal spores including their release, dissemination, deposition and effect is of great significance to identify the health hazards and physiological disorders in human beings. The isolated aeromycoflora such as species of *Aspergillus, Trichoderma, Rhizopus, Fusarium* is also known to be involved in deterioration of cellulosic and non-cellulosic materials, hence causing infrastructural damage, which might affect the economy. *Aspergillus* is an aeroallergen which is a ubiquitous fungus, common in the environment with potential to cause a spectrum of diseases in human beings. *Fusarium* and *Rhizopus* are known to cause food spoilage and various plant diseases.

The dominant fungal contribution exhibits a clear picture that it is appalling if the maintenance is inefficient and dismal. Further studies with longer monitoring period on their occurrence in indoor and outdoor air could bring about better understanding of their potential function. There should be proper monitoring of human activities which might increase the moisture content which should be monitored so as to inhibit the growth of fungi especially in intramural environment in rainy and winter seasons. In addition to this, good cross ventilation measures should be taken in order to replace the aeroflora.

Though there is no practical method to abolish these ubiquitous contaminants but precautionary measures like wearing mask, ventilated area, preventing dampness etc., are to be taken in consideration to prevent the outcome of fungal infections contracted to people in these affected environment. There is a greater need to develop the environment through the new innovative technological tools and diagnostic preventive steps.

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Research Article

PREVALENCE OF ALLERGIC DISEASES AMONG THE POPULATION OF A POLLUTED AND A LESS POLLUTED AREAS OF WEST BENGAL

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There is uncertainty regarding the precise causes for the prevalence of allergic diseases. The cumulative effect of climate, meteorological factors, pollutants, patients' lifestyle, occupation, socioeconomic status and immunity, etc., can enhance the sensitivity of patients to a particular allergen. The present study aimed to find out the prevalence of various allergic symptoms among the population of a polluted and a less polluted areas of West Bengal. A total of 3391 subjects (1526 subjects from Durgapur and 1865 subjects from Santiniketan) were studied both at the Durgapur as well as Bolpur sub-divisional hospitals from 2013 to 2016. Out of 3391, a total of 1536 (Male 845 and Female 691) allergic subjects with age group between 6 months to 78 years were studied through a questionnaire both at Bolpur sub-divisional hospital and Durgapur sub-divisional hospital in the presence of the clinicians. Some of the allergic diseases showed higher prevalence in the polluted area like allergic cough (30.5%), allergic asthma (45.39%), rhinosinusitis (9.2%), while allergic rhinitis (38%), allergic conjunctivitis (33.3%) were more prevalent in the less polluted area. In case of allergic skin diseases, 95.3% allergic subjects were reported to have different types of skin related symptoms in Santiniketan (less-polluted area) which was 34% higher than that of polluted area Durgapur (61.3%). The outcome of the present study confirms the highly prevalence of allergic cough and allergic asthma at polluted area (Durgapur). These two diseases are interrelated and highly associated with the heavy atmospheric load of PM2.5 and PM10.

Key Words: Allergic Prevalence, Questionnaire, Hospitalization data, pollution, West Bengal, India

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INTRODUCTION

Allergy is a disease with many faces that can affect different organs like the upper and lower respiratory tracts, eyes, intestinal tract and skin. The various symptoms may be manifested as allergic rhinitis[1], allergic asthma[2], IgE-associated atopic dermatitis[3], food allergy[4], insect venom allergy[5], etc. The common characteristic of allergic diseases is a switch to the production of allergen-specific IgE raised against normally innocuous environmental allergens[6] that, in special cases, might also cross-react with self-antigens[7,8]. At this asymptomatic stage, the person is sensitized to a given allergenic source due to the presence of allergen-specific IgE in serum, a condition also called 'atopy'. In atopic individuals, however, re-exposure to the offending allergen induces cross-linking of the high-affinity receptor FceRI-bound allergen-specific IgE on effector cells and, thus, causes immediate release of anaphylactogenic mediators[9]. To explore the prevalence of allergic diseases among local inhabitants, the hospital survey is a prerequisite that plays an important role. The clinicians often ask the patients to fill up a questionnaire encompassing health status, occupation, food habits, family history, etc., of patients from where he or she gathers ideas about the source and nature of allergens. Therefore, it is essential to know the nature of symptoms and the time of onset of allergy. Case history of patients may also provide information about the relationship between patients' allergy symptoms and environmental and/or genetic factors if any. Personal characteristics of patients like diet, smoking habit, occupation, period of stay in the locality, lifestyle, hobby, family history, etc., may also have a connection with allergic manifestation. Since allergen avoidance is one of the effective measures to cure an allergy, all the probable factors are to be taken into consideration for the correct diagnosis and treatment of allergic patients.

In the present study, an extensive health survey of the two selected polluted and less polluted areas of West Bengal was undertaken to prepare a detailed report on the prevalence of various allergic symptoms among local allergy patients and their probable cause.

METERIALS AND METHODS

Area of the study

Durgapur (polluted area) is located at 23.48°N 87.32°E.[10] with an average elevation of 65 m m.s.l. and it is a tier-II city in Paschim Bardhaman district, in the state of West Bengal, India on the bank of the Damodar River (Fig. 6.1). In the 2011 census, Durgapur had a population of 522,517, out of which 294,255 were males and 272,262 were females. The effective literacy rate for the 7+ population was 87.70[11]. Durgapur is by far the most industrialized city in eastern India and the second-planned city in India.

Santiniketan (less polluted area) is located at 23.68°N 87.68°E (Fig. 6.1)[12]. It has an average elevation of 56 meters above m.s.l. It is a small rural setup near Bolpur town in the Birbhum district of West Bengal, India, approximately 160 km north of Kolkata metropolis. Santiniketan-Bolpur Municipality has a total population of 80, 210 (as of the 2011 census), out of which 40,468 are males and 39,742 are females. The male-female ratio of Santiniketan-Bolpur is 1: 0.982. with an 86.77% literacy rate[13]. Santiniketan was established by Maharshi Devendranath Tagore and later expanded by his illustrious Nobel laureate son, Rabindranath Tagore whose vision became what is now a university town, Visva-Bharati University.

Pollution and Meteorological data

The average of minimum and maximum temperature, relative humidity, average wind speed and rainfall of the study areas were collected from the local Meteorological Centre at Durgapur and Santiniketan. The daily concentrations of SO_2, NO_2, PM10 and PM2.5 of the study areas were collected from the West Bengal Pollution Control Board at Kolkata, India (Table 6.1).

Health survey through hospitalization data

The health survey was conducted in many hospitals and clinics. These include Bolpur sub-divisional hospital and Durgapur sub-divisional hospital, Institute of Child Health (Kolkata), Allergy Satellite Centre (Burdwan). A total of 3391 subjects (1526 from Durgapur and 1865 from Santiniketan) were studied at both the Durgapur and Bolpur sub-divisional hospitals from 2013 to 2016. Out of 3391, a total of 1536 allergic subjects (Male 845 and Female 691) having age group ranged from 6 months to 78 years) were investigated.

Questionnaire study and health data collection

A health survey of local patients was carried out by visiting the outpatient department of the sub-divisional hospital of the respective study areas. The data on the demographic and medical history of the studied allergic patients were collected in the presence of physicians using a standard questionnaire which was prepared

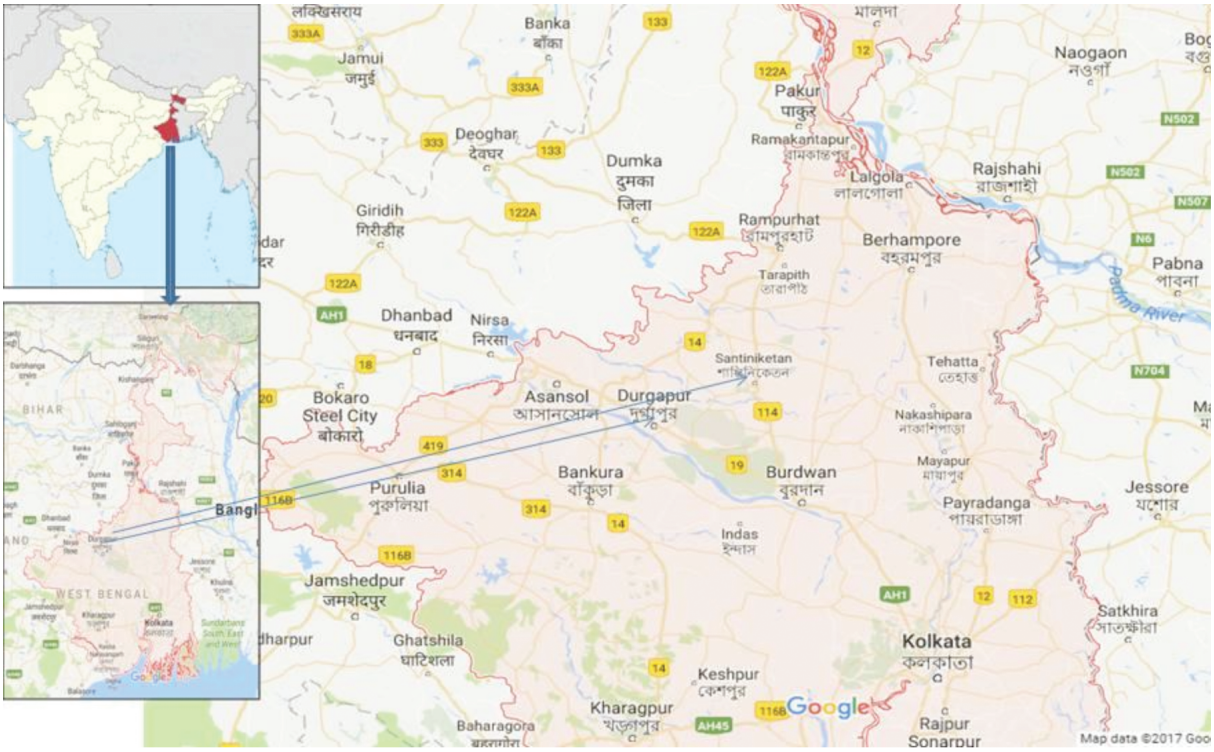

Fig. 6.1: Map of West Bengal showing sampling sites : Durgapur and Santiniketan.

Table: 6.1: Information on pollutants, meteorological factors, fungal spore load in Durgapur and Santiniketan

Pollutants, Meteorological factors, Fungal spore load	Durgapur		Santiniketan	
	Total conc. in 24 Months	Monthly average conc.	Total conc. in 24 Months	Monthly average conc.
NO_2 (µg/m³)	1011	42.13	719	29.98
PM10 (µg/m³)	2838	118.29	2177	90.74
PM2.5 (µg/m³)	1257	52.40	1234	51.44
SO_2 (µg/m³)	335	13.98	129	5.40
Average Max Temp (°C)	770	32.08	768	32.02
Average Min Temp (°C)	494	20.58	493	20.56
Average Rainfall (mm)	64	2.70	1394	58.11
RH (%)	1518	63.26	1761	73.40
Windspeed (km/hr)	34	1.45	1081	45.04

according to WHO (2010) with some modifications based on local socio-economic conditions. The patients were clinically examined by the physician before collecting information from them. The questions concerning allergic symptoms were related to cough, breathlessness, allergic rhinitis, allergic conjunctivitis, allergy-related skin disease and food allergy. Occurrence patterns of symptoms whether seasonal, perennial or irregular, worst month and time of onset of symptoms were also recorded.

RESULTS

The two study fields are different from each other according to their geographical position as well as their pollutant content in the air (Fig. 6.1 & Table 6.1). According to Pollution Control Board data, Durgapur is much more polluted than Santiniketan. So we aimed to find out the prevalence of allergic diseases in both polluted and less polluted areas of West Bengal. A total of 1536 (Male 845 and Female 691: age group 6 months to 78 years) subjects were diagnosed as allergy patients by the enlisted clinicians which was confirmed through the study of the authentic questionnaire.

Some of the allergic diseases showed higher prevalence in the polluted area like allergic cough (30.5%), allergic asthma (45.39%), rhinosinusitis (9.2%), while allergic rhinitis (38%), allergic conjunctivitis (33.3%) were more prevalent in the less polluted area (Table 6.2). In case of allergic skin diseases, 95.3% allergic subjects were reported to have different types of skin related symptoms in Santiniketan which was 34% higher than that of Durgapur (61.3%). Subjects with family genetic allergic record and food allergy were not reported from Durgapur, which may be due to the small sample size, but few subjects were reported to have a genetic allergy (44.1%) and food allergy (16.2%) in Santiniketan (Table 6.2).

DISCUSSION

Air pollution, especially pollution associated with heavy traffic areas and industrial belts, has a significant impact on respiratory-related morbidity and mortality. The prevalence of allergic diseases is now a worldwide concern[14]. In recent decades, the prevalence of allergic disease has noticeably increased globally, particularly in industrialized countries[15]. Among the known causes of allergic diseases, urban air pollution has been attracting attention as an important environmental and extrinsic etiologic agent[16]. Pollutants include gaseous materials such as ozone (O_3) and nitrogen dioxide (NO_2), as well as particulate matter (PM), which are generated by automobile traffic and industry. Strong epidemiological evidence along with experimental studies have elucidated the cellular and molecular events that explain how these pollutants are related to the exacerbation of asthma and other allergic diseases and

Table 6.2: Allergic disease prevalence recorded from polluted (Durgapur) and less polluted (Santiniketan) areas of West Bengal, India.

Prevalence of allergic diseases from polluted and less polluted areas	Durgapur (polluted area)		Santiniketan (less polluted area)	
	Percentage of monthly average patients	Percentage of allergic	Percentage of monthly average patients	Percentage of allergic
Allergy patients admitted at Santiniketan and Durgapur Sub-divisional Hospital OPD	5.88	9.23	13.13	16.89
Allergic Cough (R05)	1.79	30.50	3.71	28.20
Allergic Asthma (J45)	2.66	45.39	3.33	35.08
Allergic Rhinitis (J30.9)	1.58	27.00	3.25	38.00
Chronic allergic conjunctivitis H10.45 (ICD-10-CM) (perennial, seasonal)	0.50	8.50	3.29	33.30
Rhinosinusitis	0.54	9.20	0.38	3.20
The allergic family genetic record	-	-	1.88	44.10
Other allergic symptoms (insect bite allergy, Bed mite allergy etc.)	0.58	9.90	3.13	27.70
Food allergy	-	-	1.63	16.20
Allergic skin diseases	3.71	63.10	9.38	95.30

induced adverse effects in the respiratory system[17,18]. A questionnaire survey of 1536 local allergic patients was obtained by visiting the outpatients' departments of Bolpur and Durgapur Sub-divisional hospitals. The number of Allergy patients admitted at Santiniketan Sub-divisional Hospital OPD is higher than at Durgapur Sub-divisional Hospital (Table 6.2 & Fig. 6.2). Some of the allergic diseases like allergic cough, allergic asthma and rhinosinusitis showed higher prevalence in the polluted area, which may be due to the heavy load of particulate matter in the air as per pollution control board data, while allergic rhinitis, allergic conjunctivitis, and allergic skin diseases were more prevalent in the less polluted area (Fig. 6.2). The local people in various age groups frequently suffer from various allergic incidences which may have a significant relationship with concentration of pollen, fungal spore and pollutants in the air along with other physiological factors. The most common allergic symptom was cough followed by allergic rhinitis, breathlessness, food allergy, allergic conjunctivitis and allergy-related skin diseases (Table 6.2 & Fig. 6.2).

CONCLUSION

The present survey gives a basic idea about the prevalence of different allergic symptoms in Durgapur (A polluted area) and Santiniketan (a Less polluted area) of West Bengal. A wide-scale survey is needed to find out the significant result of allergic prevalence. Allergy can manifest due to several reasons and to find out the reasons we need to study the aeroflora of pollen and fungal spore concentration, various airborne and inorganic pollutants concentration, meteorological factors and socioeconomic status etc. of the study area.

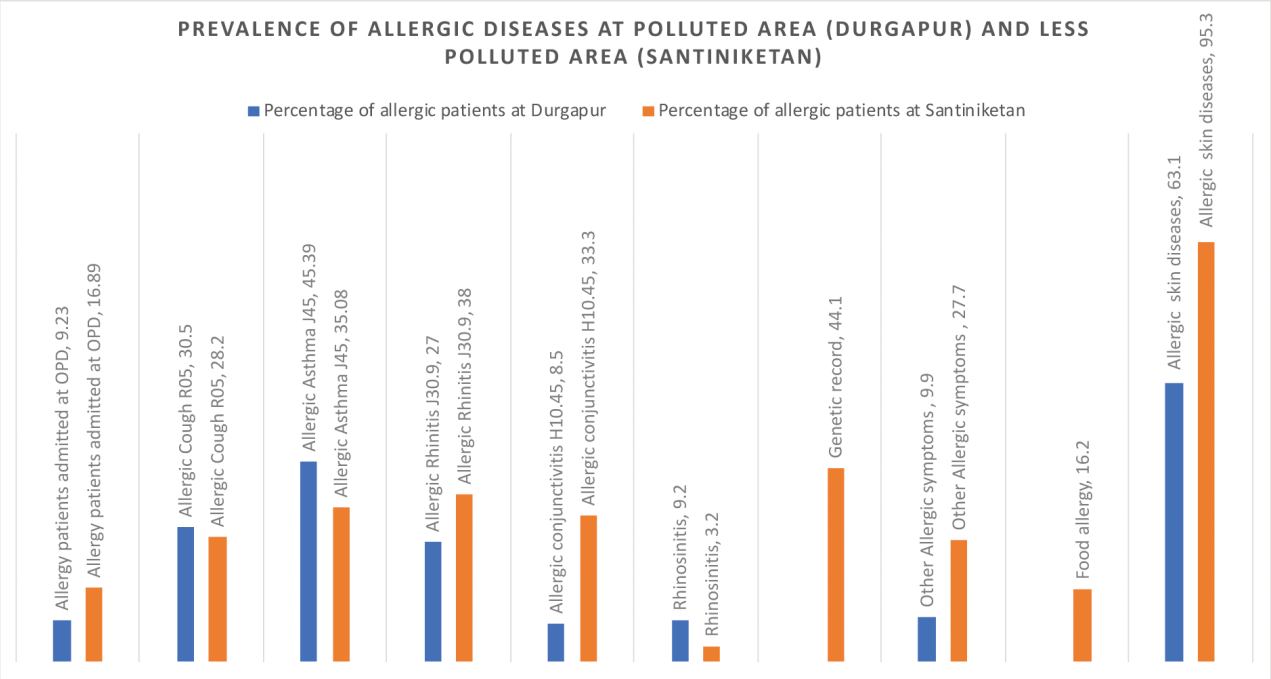

Fig. 6.2: Prevalence of allergic diseases at polluted area (Durgapur) and less polluted area (Santiniketan).

Identification, isolation and characterization of allergic proteins from common airborne fungus and pollen of West Bengal need to explore. The fungal spore and pollen calendar of the study sites may be helpful to the physicians in diagnosis and immunotherapy of allergy sufferers, particularly those residing in these unexplored biozones of eastern India. Considering all the factors, a statistical model with significant correlation can be used to predict the different types of allergic disease prevalence.

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