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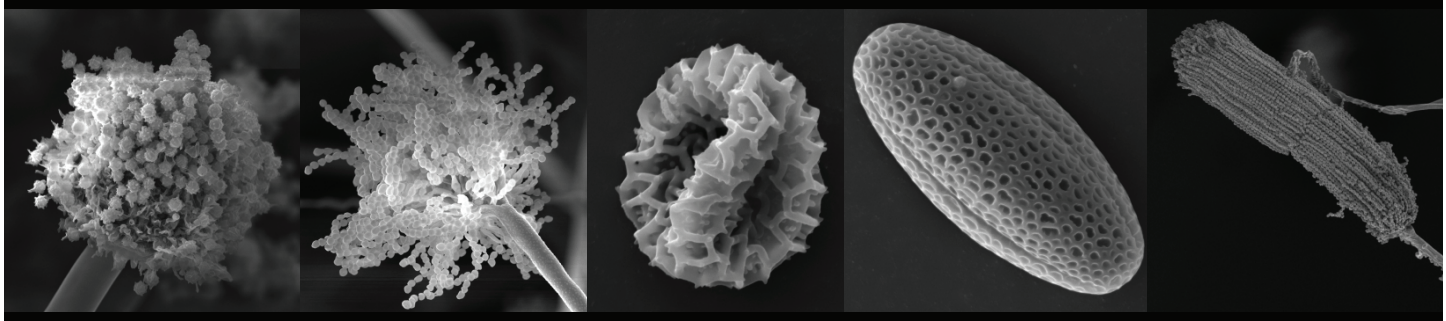

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AEROBIOLOGY OF *PARTHENIUM* WITH A FOCUS ON MANAGEMENT OF *PARTHENIUM* - LINKED DISEASE

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The role of aerobiology as such and linking with *Parthenium* research, in particular, needs more attention. The presence of *Parthenium* pollen grains in air has been reported from Central, Southern, East and Western India by the aerobiologists and aeropalynologists. Therefore, it may be concluded that the air quality is likely to get deteriorated in future unless immediate corrective measures are taken in time, owing to congress grass, keeping in view the climate change. *Parthenium* is an allergic and aggressive exotic weed, growing luxuriantly almost everywhere, due to its wide climatic adaptability. During the last 60 years, it has spread throughout the country except in colder deserts.

Parthenium dermatitis is an immune-inflammatory disease caused by *Parthenium hysterophorus* and is the commonest cause of plant dermatitis in India. In addition to airborne dry plant parts and trichomes, sesquiterpene lactones from *Parthenium* are the most important triggers responsible for contact dermatitis. The combined type IV and type I hypersensitivity to *Parthenium* has been recently postulated. In sensitized individuals, it can cause a spectrum of clinical patterns, such as classical airborne pattern, chronic actinic dermatitis-like presentation and other rare patterns. There is definite trend towards change from airborne pattern to chronic actinic pattern in natural history of *Parthenium* dermatitis. Contact sensitivity to *Parthenium* is everlasting, and hence the disease runs a chronic course with exacerbation during summers. Patch testing with acetone or aqueous plant extract is the simplest way of confirming *Parthenium* contact allergy. Management includes avoiding allergen contact, use of topical corticosteroids/acrolimus and other immuno-supressives. In future, we expect *Parthenium* dermatitis to become less prevalent due to rapid urbanization and possible development of new biological methods to eradicate the weed. Genetic factors associated with susceptibility to *Parthenium* dermatitis need to be studied too.

Key Words: Aerobiology, Pollen allergy, allergen, dermatitis, chronic actinic dermatitis, *Parthenium hysterophorus*, sesquiterpene lactones.

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INTRODUCTION

Some of the plant species of Asteraceae are the commonest causes of plant dermatitis and are the members of the second largest family of flowering plants in the world[1]. Asteraceae dermatitis affects 0.7-1.4% of the general population and 4.5% of the occupationally-exposed groups[2]. *Parthenium hysterophorus*, a member of compositae family, is popularly known as "bitterweed," "feverfew," or "escobaramarga." In India, it is known as "Congress grass" or "Congress weed," which refers to the US congress (who allocated the shipment for Pune, India)[3]. *Parthenium* is a native of tropical America and was introduced into Asia in cereal and grass seed shipments from U.S.A. during the 1950s and has become the "Scourge of India," producing an epidemic of allergic contact dermatitis. Ranade, Lonkar, and Jog were the first to report *Parthenium* dermatitis at Pune, India in 1968. Since then, it has been widely reported across the Indian subcontinent4. Subsequently, many cases were reported from various parts of the country namely, Bangalore, Hyderabad, Madhya Pradesh, Uttar Pradesh, Haryana, and Himachal Pradesh[5-8]. The authors have reported epidemics of *Parthenium* dermatitis in Chandigarh and Delhi and in their adjoining states[2,8]. Presently, *Parthenium hysterophorus* is the commonest cause of plant dermatitis in India and is responsible for nearly 40% of all patients attending contact dermatitis clinics.

Parthenium hysterophorus

Parthenium hysterophorus belongs to the family Asteraceae. It is an erect annual herb with alternate, deeply-dissected leaves, growing up to 2 m tall with

much branched inflorescences bearing white flower heads [Fig. 1.1]. It grows in almost all types of soil, except near the seashore as the saline soil is not conducive to *Parthenium* flowering[9, 10]. New outbreaks of the weed have been linked to movement of earth and transport of stock, fodder, and grain from *Parthenium hysterophorus*-infested areas.

Fig. 1.1: *Parthenium* flowering

PARTHENIUM AEROBIOLOGY MENACE

Several studies have established the presence of pollen, microbes, plant parts, trichomes, spores of certain allergenic fungi (e.g., *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp. etc.) in the air samples, widely dispersed under humid conditions[11-12]. An attempt was made to review the researches on the important pollen/fungal allergens prevalent in the different parts of India, which showed the presence of *Parthenium* pollen in air having potential for causing allergy[13], which was also confirmed in East India[14] as well as in Western India[15]. Now it is presumed that the air quality is likely to deteriorate throughout the country owing to proliferation of *Parthenium* everywhere in India except cold deserts.

Spread of Parthenium in India

Parthenium is an allergic and aggressive weed growing conveniently in wastelands, orchards, forest lands, flood plains, agricultural lands urban areas, overgrazed pastures and also along road sides and railtracks[9]. This has spread across many nations creating serious biohazards. In India, for the first time it was observed growing in Pune during 1956. An introduction of this weed was through PL 480 scheme as food aid from US. A plant can produce more than 100,000 seeds during the completion of life cycle .The dispersal of seeds mainly through animals, the movement of vehicles, livestock, grain, stock feed and to a lesser extent by the wind. Most of the long distance spread is through vehicles, flooding. The scientists all over the Indian subcontinent as per their convenience organized an awareness campaign on occurrence, ill effects and management of the *Parthenium*, wherein the electronic media played a vital role[16,17]. Besides the survey of the weed at various aerobiological centers, studies on the derived bioagents from *Parthenium* plant were also undertaken dealing with ill effects to human, animal, soil and plant health[18] and thus has led to the strengthening of the '*Parthenium* Aerobiology'.

Parthenium: Effect on animal health

In general, animal never graze on *Parthenium*, however in case of intake their milk becomes bitter and is not palatable to livestock. In animals it may cause clinical signs such as salivation diarrhea, pruritus, alopecia and dermatitis. *Parthenium* if consumed also causes loss of hairs and marked depigmentation of skin. A study of the toxicity of the weed to cattle has shown that 10–50% *Parthenium* in diet can kill animals within 30 days. Earlier workers[19] detected taints in meet in group of sheep that were given the diet of 30% *Parthenium*.

Strategies to manage the Parthenium linked health problems

Parthenium sp. is also a major threat to biodiversity as this weed quickly colonizes and leaves very little space to grow due to allelopathic effects on certain plants like pasture and grass land 45.3%, cropped area 12.92% and forests 6.3%[20]. Several medicinal plants have become the victim of the *Parthenium*[21]. Maximum possible efforts are being taken by aerobiologists to monitor the concentration of pollen grains of Parthenium, trichomes etc., suspended in the air which may lead to overall risk assessment. This management can also be done by understanding the background of the patients by allergologists for effective treatment. Hence the overall strategy requires contributions from: (a) *Parthenium* Aerobiologists, (b) Weed Specialists and (c) Dermatologists and drug therapy.

(a) *Parthenium* Aerobiology

Aerobiological studies can help by preparation of country wide calendar for concentration of *Parthenium* pollen and tichomes suspended in the air to understand

the background of patients by allergologist for effective treatment

(b) Weed Specialists

They can guide to make reduction of flower pollen/ trichomes/foliage production of weeds using any integrated weed management strategy. They can create an awareness to spray 10 to 15 % common sally solution under specific situations to destroy *Parthenium*. Introduction of *Parthenium* feeding insect *Zygogramma bicolorata* and use of *Kocheaindica* may also be considered as another way to control the growth of this weed.

(c) Dermatologists and Drug Therapy

Dermatologists can suggest judicious use of barrier creams on the exposed skins after washing the surface with non-medicated soap. The creams trap the antigens and should be disposed off carefully at 3-4 hrs. intervals to avoid their accumulation on the skin. Doctor's prescription may suggest topical steroids, antihistamines for localized *Parthenium* Dermatitis (PD). However, systemic corticosteroids and azathioprine are frequently taken for severe dermatitis as per instruction.

PARTHENIUM POLLEN ALLERGY CASE STUDIES FROM INDIA

Pollen monitoring of the environment has been extensively carried out in Indian subcontinent. Earlier work was essentially oriented towards quantitative and qualitative estimation of the pollen load in the atmosphere, preparation of pollen calendar and pollen spectra. Pollen calendars, thus, are helpful in correct diagnosis and proper timely treatment of allergy cases.

Aurangabad

In the year 1964, Prof. S. T. Tilak of B. A. Marathwada University, Aurangabad, interacted with Dr. T. Sreeramulu of Aerobiology Laboratory, Andhra University. This incidence, kindled his interest in the new field of aerobiology[22] and he received the training in Aerobiology from Prof. P. H. Gregory at Rothamsted Experimental Station, Harpenden, in 1965-1966. Later, he developed a new modified indigenous sampler[23], named 'Tilak Air Sampler'. This sampler was awarded Presidential medal by the Invention Board, New Delhi in 1972.

Aerobiology Laboratory of Marathwada University, Aurangabad, organized the first National Conference in Aerobiology in October, 1981, where famous world-level aerobiologists like Dr. S. Nilsson (Swedan), Dr. Ruth M. Leuschner (Switzerland) were present. Hence, Aurangabad has got emerged as one of the main aerobiological research centers in India[24-25]. Tilak and Patil (1983)[26] reviewed the status of *Parthenium* pollen grains as aeroallergen. Pande[26] detected the pollen of *Parthenium* with highest allergenic sensitivity (in intradermal skin test among 140 patients) and 12.74% contribution to total aeropollen load of Aurangabad leading to the deterioration of air quality.

Manipur

In Imphal, aerobiological survey was carried out under the leadership of Dr. N. I. Singh, of Aerobiology Laboratory, Department of Life Science, Manipur Central University[27-29]. Their study revealed the presence of *P. hysterophorus* pollen as single pollen grain in clump form.

Karnataka

Aerobiological studies at Bangalore were initiated by the research team of Dr. S. N. Agashe and recorded the dominance of *Parthenium* pollen in the atmosphere of Bangalore[30]. They also looked at the aeroallergens and air pollutants from biological and clinical perspectives[31-33] with special emphasis on *Parthenium* pollen. Their study showed that *Parthenium* pollen was present in the atmosphere in significant amounts either as single pollen grain or in the form of clumps during the months from June to August[34].

Delhi

In 1962, Dua and Shivpuri[35] reported atmospheric pollen from the atmosphere of Delhi in details and prepared a pollen calendar. Later, Singh *et al*.[36] studied the diurnal and seasonal periodicities of pollen of Delhi with their productivity. A survey of airborne pollen grains was also conducted during 1988-89 at the human height level[37], where it was reported that *Parthenium hysterophorus* along with *Ricinus, Chinopodium, Ameranthus, Morus, Artumisia, Prosopis* etc. are important pollen contributors to the atmosphere, especially at low lower height.

Investigations on clinical manifestation, diagnosis and treatment have been carried out by various Dermatologists at Ram ManoharLohiya Hospital and AIIMS, New Delhi[38, 39] to establish the treatment procedure as well as to prepare standardized diagnostic kits (Patch

Test etc.)[40]. Among the examined 156 patients, 78 found to be suffered from (CAD) Chronic Actinic Dermatitis), showing 1.9% positive reaction to *Parthenium*.

Kolkata

Pioneering Aerobiological study was carried out in Kolkata[41] in 1873. After a gap of almost a century, Chanda and Nandi[42] reported the occurrence of pollen grains in the atmosphere of Kolkata. Similar reports were obtained[43-46] from various places of West Bengal. The rapid development in the field of Aerobiology has resulted in the understandings of prevalence of different allergens. Gupta-Bhattacharya and Chanda[14] observed that clinically one out of six patients from the heavily *Parthenium* infested area of Kolkata had positive response in IgE-ELISA (Enzyme Linked Immunosorbent Assay).

Nagpur

The pollen survey of Nagpur city was undertaken a pollen calendar was prepared by Chitaley and Deshpande[47-48] in mid 1970s. Inspired by the leadership of Dr. G. V. Patil, the subject Palynology and Aerobiology was established at Nagpur University by A. A. Saoji, who reported the *Parthenium* plants as highly allergenic and hazardous to human, animals and crop plants. She conducted aerial survey of *Parthenium* pollen and carried out and correlated with the patients allergy[49-50]. Airborne components of Nagpur was studied by Kalkar and Patil (1994)[51]. The allergenic potential of pollen grains was also emphasized by Tidke (2018)[52].

PATHOGENASIS

Parthenium allergy varies from person to person. The allergic reaction varies from skin discoloration, thickening of the skin to lesions on the skin. *Parthenium* dermatitis is a disease, where due to contact sensitization by *Parthenium* antigen a cell-mediated hypersensitivity immune response takes place with early sensitization phase and a subsequent elicitation phase[53]. The response is mediated by a series of cellular and molecular mechanisms. Epidermal Langerhans' cells and other cutaneous dendritic cells transport the allergen from the skin to regional lymph nodes, where it is presented to T-lymphocytes, and T-cell proliferation occurs with production of effector and memory cells. The reaction is characterized by the infiltration of T-lymphocytes into re-exposed skin sites and the development of cutaneous inflammation.

Lakshmi and Srinivas (2007)[54] showed that all the patients of *Parthenium* dermatitis showed significantly ($P < 0.001$) elevated levels of TNF-α, IL-6, IL-8, and IL-17 levels as compared to healthy controls and decrease in the anti-inflammatory cytokines IL-4 ($P < 0.217$) and IL-10 ($P = 0.001$), suggesting the involvement of pro-inflammatory cytokines in the pathogenesis of *Parthenium* dermatitis.

Akhtar *et al*. (2010)[53] postulated that a combined immediate (Type I) and delayed hypersensitivity (Type IV) mechanisms may be operational in the initiation and perpetuation of *Parthenium* dermatitis, inducing exacerbation of lesions of sensitized subjects with atopic diathesis.

MODE OF SENSITIZATION

Parthenium dermatitis is mainly caused by dried leaves and trichomes. Pollen grains play role, mainly in respiratory allergy. These may not pass beyond the nasal mucosa and hence more often causes allergic rhinitis rather than bronchial asthma[55]. **Sesquiterpenelactones (SQLs)** have been identified in the leaves, stem, and waxy coat of the pollen, and thus *Parthenium* is capable of producing "dermatitis at a distance" or exacerbating an active outbreak of eczema[56]. Direct or indirect contact sensitization is also possible in view of the rampant growth of the weed[57].

Allergens in *Parthenium hysterophorus*

In addition to glycoproteins[58], most important allergens responsible for allergic contact dermatitis are SQLs[56]. They are lipophilic, and are present mainly in fraction of the plant. It is present in the leaf, stem, flower and pollen, but the highest concentrations of SQLs are present in the small glandular hairs (trichomes) present on the undersurface of the leaves and stem. Over 200 skeletal types and 1350 individual types of SQLs have been described, each having multiple functional groups attached to them.

Among the sesquiterpene lactones, *Parthenium* contains pseudoguinolide class of SQLs, with has an alpha methylene group, exocyclic to gamma lactone. It is present in the leaf, stem, flower and pollen, but the highest concentrations of SQLs are present in the small glandular hairs (trichomes) found on the undersurface of the leaves and stem. Coronophilin, hymenin and parthenin are specific SQLs, present in *Parthenium*[59].

Some Compositae members, like *Xanthium strumarium, Helianthus annuus, Chrysanthemum coronarium* and others (*Magnolia stellata* and *Laurus nobilis*) also contain similar SQLs and hence these plants may have cross sensitivity with *Parthenium* and vice versa. However, there is no recognized pattern on clinical examination in patients co-sensitized to other plants.

CLINICAL FEATURES

Susceptible individuals may suffer from hay fever, asthma or dermatitis, when exposed to dust and debris from the plant as well as from pollen[60]. A random clinical questionnaire survey along with skin tests conducted in Bangalore revealed that 7.1% of the study population was suffering from allergic rhinitis due to exposure to *Parthenium* pollen[61]. In a study from Delhi, 35.7 % of the 70 atopic dermatitis patients were positive to *Parthenium* as found in skin prick test[62]. It suggests that *Parthenium* plays a role as an exposure source of nasobronchial allergy and atopic dermatitis. The severity of dermatitis in India is greater in comparison to other countries because of its abundant growth in India and high level of the sesquiterpene lactone, mamed parthenin[63]. Lightly-clothed middle-aged or elderly persons who are engaged in outdoor activities get more affected (male-to-female ratio 5.5:1).

Patterns of *Parthenium* dermatitis

Table 1.1: Clinical features

- **Airborne Pattern Contact Dermatitis (ABCD)**: Classical Pattern affects eyelid/ neck
- **Chronic Actinic Dermatitis (CAD)**: Lichenfied papules, Plaques, or Nodules over the exposed areas.
- **Mixed Pattern Dermatitis (MPD)**: Universal involvement of skin in the form of scaling, erythema and eduration.
- **Hand and Feet Dermatitis.**

The clinical pattern of *Parthenium* dermatitis can undergo changes after the onset, i.e. progresses from airborne ABCD to mixed pattern or CAD pattern

DIAGNOSIS

In diagnostic clinics, prick test is performed with *Parthenium* antigen extract, which is included in the Indian standard series (ISS), with leaf "as is" or plant materials crushed and diluted with saline buffer. The immediate reaction at 15 minutes and the late phase reaction at 24-48 hours is recorded.

RAST (Radio allergosorbent test) for *Parthenium* specific antibodies is also another diagnostic assay for *Parthenium* allergy.

MANAGEMENT OF *PARTHENIUM HYSTEROPHORUS*

It is necessary to control the menace causing weed *P. hysterophorus* in time before spreading, because of its negative impact on natural and agro eco-systems[21]. There are several methods available for controlling the parthenium weed. The methods are: (1) manual and mechanical control, (2) chemical and (3) biological control and managing the weed by proper utilization.

1. **Manual and mechanical methods**: This is one of the most common methods for management among the rural population of India. Farmers manage this weed within their crop field by uprooting/hoeing the plants out and burning the dried plants. However, they don't care to manage the *Parthenium* weed along the adjoining road side, wasteland, which soon causes re-infestation. Uprooting the weed after seed setting will increase the area of infestation. Hence, the manual removal is usually neither very effective nor economical. In addition, pulling a plant in flower causes the dispersal of pollen grains, resulting in allergic reactions.
2. **Chemical control**: Chemical control of *Parthenium* weed in India is gaining popularity. The plants have to be treated before flowering/seed setting and when other plants, especially grasses, are actively growing and can re-colonize the infested area. Maintaining competition is important for control of *Parthenium* weed, so spraying with a selective herbicide that does not kill other species is recommended. In open wasteland, non-cropped areas and along railway tracks and roadsides, the spraying of a solution of common salt (Sodium chloride) at 15-20% concentration has been found effective. *Parthenium* is found mostly in no man's land. So, use of chemicals in such areas need community efforts. There are many herbicides available in the market for controlling the weed. With the onset of monsoon, emerged seedlings of sufficient height can be controlled by spraying of glyphosate at 1% solution. This appli-

cation should be made at any cost before blooming. The list of some common herbicides for controlling the weeds is mentioned in Table 1.2.

Table 1.2: Herbicides used for successful control of *Parthenium hysterophorus*

	Herbicides		Dose (kg a.i.ha-1)*
A.	Non Cropped area	Paraquat	0.5
		Glyphosate	2.5
		Mon 8793	2.7
		Common salts	20%
B.	Land Commercial, Industrial, Pastures, Rights of way	Metribuzin	0.7
		Atrazin	6.0
		2-4-D	1.0
		Metsulfuron methyl	0.007

*(kg a.i. ha-1) = Kilogram active ingredient/hector.

3. **Biological control**: Even if the various chemicals control the weeds effectively in time, the continuous use of the same causes the pollution hazards in the eco-systems. Therefore, weed management strategy needs to be shifted towards non-chemical methods. Managing weeds using biological means is less expensive, permanent and pollution free. Biological control is the intentional manipulation of natural enemies by man for the purpose of controlling harmful weeds. Biological control seldom means complete eradication of the unwanted organism, but rather maintaining its population at lower than average that would occur in the absence of the bio-control agent. *Parthenium* is mainly a weed of waste and fallow land. Hence bio-control is the most economical and practical way to keep the weed under check. This is the most cost-effective, environmentally safe and ecologically viable method. Several insects and pathogens have been tried from time to time for controlling the weed. The leaf-feeding beetle *Zygogramma bicolorata* and the stem-galling moth *Epiblema strenuana* are widely used in several countries, including India, to manage Parthenium[64-65]. The moth significantly reduces flower and seed production of the weed, especially at a young age. Other major biocontrol agents used include *Bucculatrix parthenica* (leaf-mining moth)[66] and others[64].

Vermicomposting: It involves the cutting of the *Parthenium* weed into small pieces and spreading the material on the ground to a thickness of 10 cm layer, over which *Trichoderma viridi* and 0.5% urea solution. This sequence of layers is repeated up to a meter high and finally it is plastered with mud or clay soil (moisture level 50-60%). After two weeks, the thoroughly mixed compost becomes ready for field application. It is a good source of nutrient and helps to maintain soil properties through aggregate formation[67]. The 'Parthenin' content of the mixrure reduces growth of other invasive weeds[68] and enhance the growth of the next crop.

CONCLUSION

Since *Parthenium hysterophorus* is ubiquitous, a change of residence or job is not a suitable which would also lead to social and economic consequences. Hence, prevention is aimed for the reduction of exposure by following ways:

1. Removal of the *Parthenium* plants from the immediate environment by applying eco-friendly methods.
2. To cover the skin as much as possible by clothing.
3. Frequent washing of the exposed skin areas.
4. Overall exposure avoidance.

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DIVERSITY OF SOIL AND LEAF SURFACE MYCOFLORA: A SOURCE OF AEROMYCOFLORA

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Microorganisms are introduced into the air from various sources. The important sources of these microorganisms are soil and vegetation of that area. Microorganisms, which are found on plants' surface either as pathogens or as saprophytes, also get suspended in the air. Man-made actions like digging or ploughing the soil may also release soil-borne microbes into the air. The surrounding atmosphere plays an important role as the sources of organisms in the experimental area. The studies were carried out from February 2006 to March 2007. In the present study, aeromycoflora, mycoflora were observed from soil and plant near the experimental sites as their sources. The Potato Dextrose Agar medium containing plates were used for the isolation of mycoflora from their sources around the Panabaras of Rajnandgaon district. During the present study, a total of 22 fungal species of 120 fungal colonies belonging to 14 genera were reported from the soil. While 24 fungal species of 166 fungal colonies belonging to 16 genera were isolated from the leaf surface. *Aspergillus fumigatus* (10.00%) showed the maximum percentage contribution, followed by *Fusarium oxysporium* and *Khuskia oryzae* (8.33%), *Aspergillus japonicas and Paecilomyces variotii* (7.5%) and *Alternaria radicina, Penicillium notatum* (5.83%) in the soil mycolfora. It is also shown that *Cladosporium cladosporioides* (11.44%) followed by *Aspergillus niger* (9.63%), A. fumigates (6.62%), *Monodictys fluctuata* (6.02%), *Curvularia lunata* (5.42%) and *Aspergillus fumigatus* (4.81%) were the most contributed to leaf surface mycoflora.

Key Words: Fungal diversity, aeromycoflora, sources, soil, leaf surface.

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INTRODUCTION

Fungi are very successful inhabitants of soil due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavorable conditions[1]. The diversity and activity of fungi are regulated by multiple biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) factors[2,3]. Fungi can be found in almost every environment and can live in a wide range of pH and temperature[4]. Fungal populations are strongly influenced by the diversity and composition of the plant community and in return, affect plant growth through mutualism, pathogenicity, and their effect on nutrient availability and cycling[5-7]. The contribution of soil organisms is very significant in many soil functions such as supporting the growth of plants, absorbing, neutralizing and transforming compounds that might otherwise become pollutants in the environment. Soil is a complex habitat for microbial growth and these microbes generally exist as micro-colonies or biofilms on mineral particles, organic matter, and roots. Currently, microorganisms are exploited to get valuable products that include enzymes, secondary metabolites, therapeutic agents and industrial products. Such potential microorganisms are usually isolated from the soil sample. Among such microbes, filamentous fungi dominate our globe as sources of food, plant and animal pathogens, and other worthy products' bio-synthesis.

The phylloplane, the surface of plant leaves, is a complex terrestrial habitat, characterized by a variety of microorganisms, including bacteria, filamentous fungi and yeast. Pathogens, saprobes and epiphytes occur in

this habitat and numerous studies have described the phylloplane populations from various plant species[8-13]. The non-pathogenic fungi that inhabit the phyllosphere depending on nutrients exuded from the leaf or those deposited from the atmosphere[11,14]. The leaf surface in the open atmosphere is exposed to a continuous air current which carries numerous fungal spores, along with various other microscopic objects, both living and non-living. Aerial parts of the plant serve as the micro-habitats for a variety of microorganisms present in the aerospora. The leaf surface accommodates microorganism by providing a complete ecological niche where the exudates from leaves provide nutrition, moisture, pH and temperature.

The aerospora constitutes both the source of fungi that colonize the leaf surface and the spores are released from the leaf surface by various dispersal mechanisms[15-17]. Spore release from many fungi inhabiting the phylloplane is passive through the action of wind or rain splash; however, other spores are actively propelled into the atmosphere by various mechanisms[16-18]. The most prevalent microorganisms, viruses, bacteria, and fungi, are introduced into the atmosphere from many anthropogenic sources such as agricultural, industrial and urban activities, termed microbial air pollution (MAP), and natural sources. These include soil, vegetation, and ocean surfaces that have been disturbed by atmospheric turbulence. The airborne spore concentrations range from nil to great numbers and change as functions of time of day, season, location, and upwind sources. While airborne spores, they may settle out immediately or be transported great distances. Further, most viable airborne cells can be rendered nonviable due to temperature effects, dehydration or rehydration, UV radiation, and/or air pollution effects[19]. The present study aimed to investigate soil and leaf surface mycoflora of the Panbaras region of Rajnandgaon district.

MATERIALS AND METHODS

Study site

The present study was carried out from Panabaras of Rajnandgaon district of Chhattisgarh state. Panabaras village is located in Manpur Tehsil of Rajnandgaon district in Chhattisgarh, India. It is situated 12 km away from sub-district headquarter Manpur and 87 km away from district headquarter Rajnandgaon. As per 2009 stats, Panabaras village is also a gram panchayat. The total geographical area of Panabaras village is 1267.48 Hectares.

Collection of sample

The soil and leaf sample were randomly collected in the study area for one year with the help of sterile polythene bags, and immediately both the sample were brought into the laboratory for further study.

Isolation of soil and leaf surface mycoflora

Soil plate method[19]: About 0.005 g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45°C) potato dextrose agar medium (PDA) was added, which was then rotated gently to disperse the soil particles in the medium. The inoculated petri plates were then incubated at 28 ± 20°C for 4-5 days.

Leaf surface mycoflora were enumerated by the spore suspension method. A leaf sample was shaken for 30 minutes with 250 ml of sterile distilled water containing a drop of Twin 80 for proper homogenizations. The spore suspension was then inoculated on the Petri plates containing potato dextrose agar medium. All the plates of both samples were incubated at 28 ± 1°C for 4-6 days of the incubation period. After the incubation, fungal colonies of both the sample were counted and pure cultured was then examined microscopically. The identification was done with the help of available standard literature[20-24]. Percentage frequency and percentage contribution was assessed with the help of the following formula:

$$\text{Percentage Frequency} = \frac{\text{No. of samples in which a particular fungal species occurred}}{\text{Total no. of samples examined}} \times 100$$

$$\text{Percentage Contribution} = \frac{\text{Total no. of fungal colonies of an individual species}}{\text{Total no. of fungal colonies of all species}} \times 100$$

RESULTS

Diversity of Soil mycoflora

In the present study, a total of 22 species of fungi belonged to 14 genera were identified (Table 2.1). Among the total isolates, 18 species belonged to anamorphic fungi, 2 species/2 genera belonged to Zygomycotina and 01 species of Ascomycotina and mycelia sterilia (Fig. 2.1). During the study period, the highest concentration of fungi was found in the winter

Table 2.1: Diversity of soil mycoflora of Chhattisgarh

S.N.	Name of fungi	Summer season	Rainy season	Winter season	Total no. of fungal colonies	Percentage contribution
1.	*Absidia sp.*	-	2	-	2	1.66
2.	*Rhizopus oryzae*	2	-	1	3	2.5
3.	*Cheatomium globosum*	-	2	4	6	5
4.	*Alternaria alternata*	2	2	2	6	5
5.	*A. radicina*	-	3	4	7	5.83
6.	*Aspergillus flavus*	-	-	1	1	0.83
7.	*A. japonicus*	2	4	3	9	7.5
8.	*A. niger*	1	-	3	4	3.33
9.	*A. fumigatus*	6	2	4	12	10
10.	*Cladosporium cladosporioides*	-	-	2	2	1.66
11.	*Curvularia lunata*	2	2	1	5	4.16
12.	*C. pallescens*	-	1	1	2	1.66
13.	*Drechslera* sp.	3	1	2	6	5
14.	*Fusarium oxysporum*	4	2	4	10	8.33
15.	*F. moniliforme*	1	1	-	2	1.66
16.	*Khuskia oryzae*	5	4	1	10	8.33
17.	*Paecilomyces variotii*	3	3	3	9	7.5
18.	*Penicillium notatum*	2	1	4	7	5.83
19.	*P. verruculosum*	1	-	2	3	2.5
20.	*Torula caligans*	-	-	1	1	0.83
21.	*Trichoderma* sp.	1	2	2	5	4.16
22.	*Mycelia sterilia*	3	1	4	8	6.66

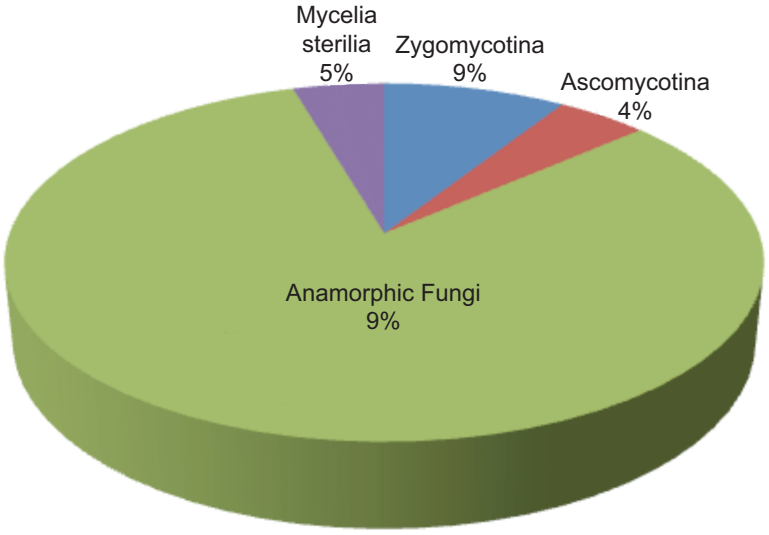

Fig. 2.1: Class-wise fungal diversity of soil mycoflora in Chhattisgarh

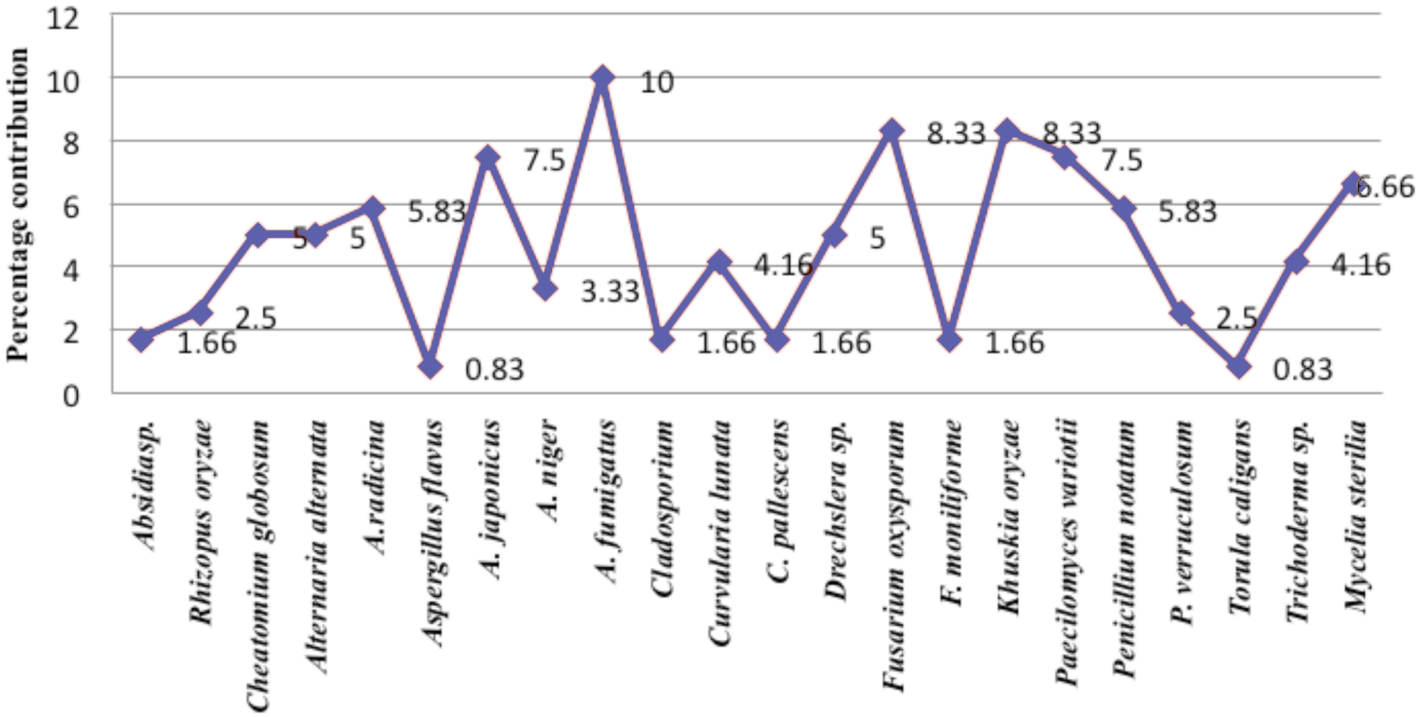

Fig. 2.2: Percentage contribution of soil mycoflora in Chhattisgarh

season. About 20 species were present during the winter period, it might be due to favorable environmental conditions, whereas 16 fungal species in the rainy season and 15 fungal species in the summer season.

In the study, *Aspergillus japonicus, A. fumigatus* and *Fusarium oxysporium* were the most frequent fungi. *Alternaria alternata, A. radicina, Paecilomyces variotii, Khuskia oryzae* and *Penicillium notatum* was the moderate, while *Torula caligans, Curvularia pallescens, C. lunata* and *Absidia* species were observed as least frequent fungi.

The percentage contribution of soil mycoflora was also assessed. *Aspergillus fumigatus* (10%) was found as the maximum contributors followed by *Fusarium oxysporum* and *Khuskia oryzae* (8.33%), *Aspergillus japonicus* and *Paecilomyces variotii* both having the species contribution of 7.5%, In comparison, *Aspergillus flavus* and *Torula caligans* (0.83%) were minimum contributors in the total mycoflora (Fig. 2.2).

Diversity of Leaf Surface Mycoflora

In the leaf surface mycoflora, a total of 16 fungal genera of 24 species were isolated (Table 2.2). Out of total isolates, 19 fungal species belonged to anamorphic fungi, 03 species belonged to ascomycotina, zygomycotina and mycelia sterilia. Both the group shared 01 species each (Fig. 2.3). Seasonal variation of mycoflora plays a significant role in the distribution of fungi. 20 fungal species were isolated in the winter season, 16 and 15

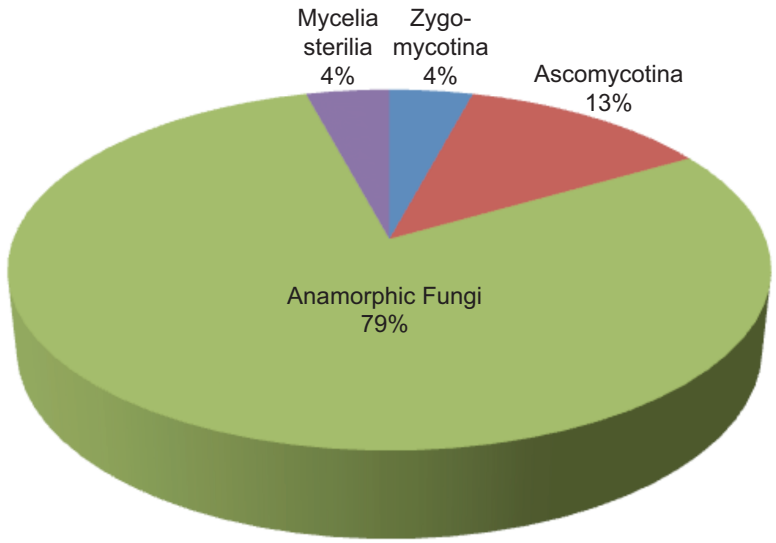

Fig. 2.3: Class-wise fungal diversity of leaf surface mycoflora in Chhattisgarh

Table 2.2: Showing fungal diversity of leaf surface mycoflora in Chhattisgarh

S.N.	Name of fungi	Summer season	Rainy season	Winter season	Total no. of fungal colonies	Percentage contribution
1.	*Absidia* sp.	-	4	3	7	4.21
2.	*Ascochyta* sp.	-	4	1	5	3.01
3.	*Emericella nidulans*	5	-	2	7	4.21
4.	*Khuskia oryzae*	-	2	3	5	3.01
5.	*Alternaria alternata*	1	5	4	10	6.02
6.	*A. brassicicola*	-	3	-	3	1.8
7.	*Aspergillus aculeatus*	1	-	1	2	1.2
8.	*A. fumigatus*	8	-	3	11	6.62
9.	*A. flavus*	-	7	1	8	4.81
10.	*A. japonicus*	-	5	2	7	4.21
11.	*A. niger*	4	6	6	16	9.63
12.	*A. terreus*	2	2	3	7	4.21
13.	*Cladosporium cladosporioides*	4	6	9	19	11.44
14.	*Curvularia clavata*	-	5	2	7	4.21
15.	*C. lunata*	-	3	6	9	5.42
16.	*Drechslera australiensis*	1	2	2	5	3.01
17.	*Fusarium oxysporum*	-	-	6	6	3.61
18.	*Monodictys fluctuata*	4	2	4	10	6.02
19.	*Penicillium funiculosum*	1	-	5	6	3.61
20.	*P. turbatum*	-	3	-	3	1.8
21.	*Pestalotiopsis glandicola*	-	-	2	2	1.2
22.	*Stemphylium* sp.	-	-	2	2	1.2
23.	*Trichoderma* sp.	2	-	1	3	1.8
24.	Mycelia sterilia	2	3	1	6	3.61

fungal species were isolated in rainy and summer season, respectively. The winter season favored the highest concentration of fungi due to favorable environmental conditions. During the present investigation period, *Aspergillus niger* and *Cladosporium cladosporioides* were the most frequent, followed by *Alternaria alternata, Aspergillus fumigatus, A. terreus* and *Penicillium funiculosum*, which were moderate. *Stemphylium* species, *Pestalotiopsis glandicola, Aspergillus aculeatus* was the least frequent in the leaf surface mycoflora. Among the total isolates, *Cladosporium cladosporioides* (11.44%) showed the maximum percentage contribution followed by *Aspergillus niger* (9.63%), *A. fumigatus* (6.62%), *Alternaria alternata* and *Monodictys fluctuata* (6.02%) and *Curvularia lunata* (5.42%). *Aspergillus aculeatus, Pestalotiopsis glandicola* and *Stemphylium* species (1.2%) was the least contributors (Fig. 2.4).

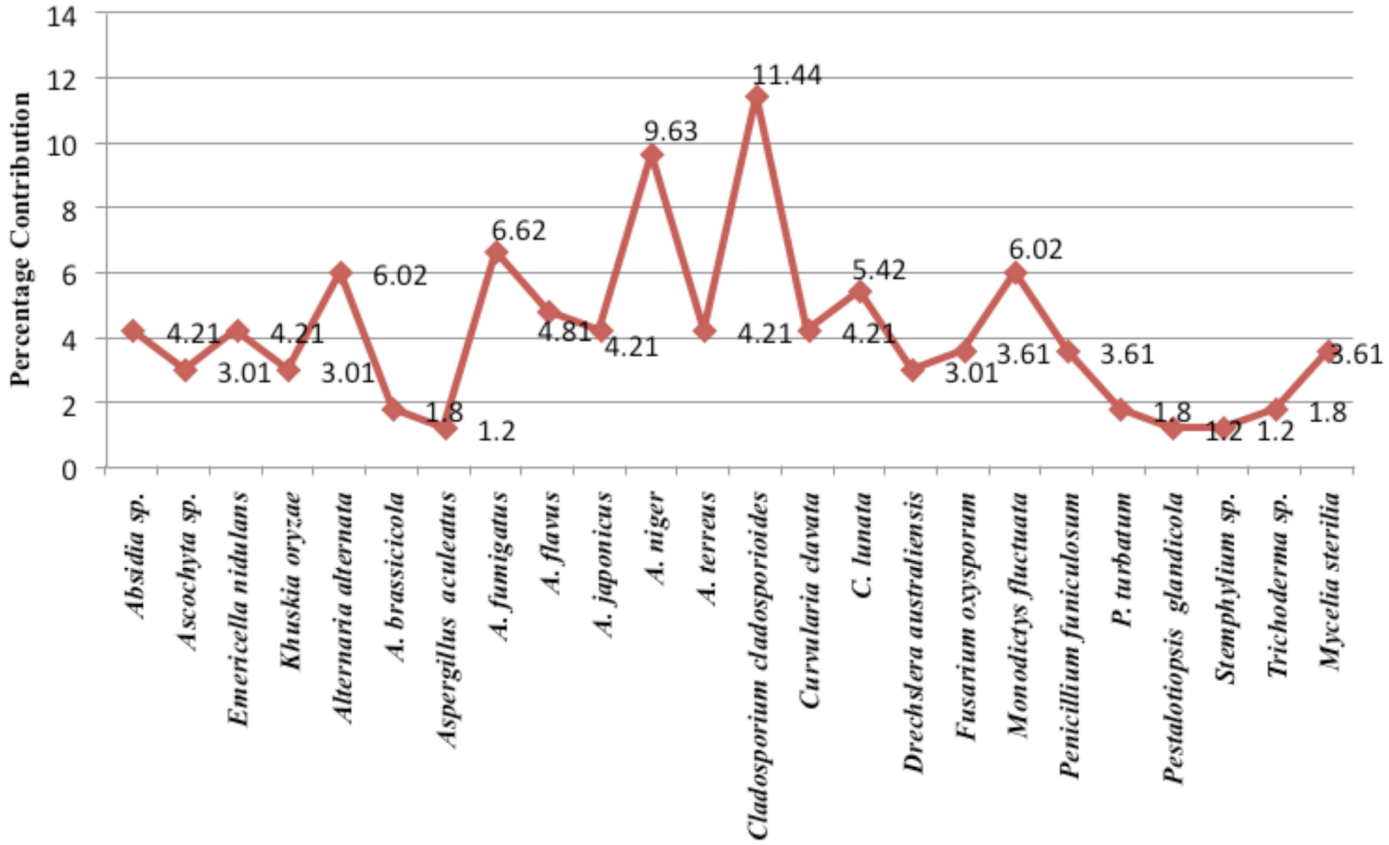

Fig. 2.4: Percentage contribution of fungal species from leaf surface mycoflora in Chhattisgarh

DISCUSSION

Diversity of soil-borne fungi in the study area was dominant. They belonged to anamorphic fungi, and, which are ubiquitous soil-borne fungi. They have also been reported in various agricultural fields and forest soil in Thailand[25-26]. During the study period, anamorphic fungi were reported as the most dominant group in soil and leaf surface mycoflora. Similar findings have also been revealed by the different researchers in many study areas from other places of the world[27-30]. *Aspergillus niger, A. fumigatus* was also most dominant in both the study area. Jalander and Gachande[31] reported a similar result in phylloplane mycoflora of some medicinal plants from Maharashtra. *Cladosporium, Aspergillus niger,* and *A. fumigatus* were reported the most dominant on different plants' leaf surface from many places[32,33]. ELkhateeb *et al*.[34] showed that Cladosporium *cladosporioides, C. herbarum* and *Alternaria alternata* were the most frequent from the phyllosphere of the genus Amaranthus. Cherkupally *et al*.[35] and Ibrahim and Shehu36 reported that *Aspergillus niger* was the most dominant fungi in the rhizosphere and non-rhizosphere soil from Brinjal crop field and Cassava growing fields of Sokoto, Nigeria. Similarly, *A. niger* as dominant fungi was isolated from the soil samples from Obafemi Awolowo University Ife[37]. Ishaq and Khann[38] also reported that *Aspergillus niger* and *A. fumigatus* were dominant among fungal species, isolated from soil samples from Katao and agricultural fields at Ramgahn, India.

CONCLUSION

In this investigation, isolated fungi showed great diversity with soil and leaf surface habitats. The diversity of fungi may depend on environmental factors and vegetation in the area. Anamorphic fungi was found as the highest contributor during the winter season and *Aspergillus niger, A. fumigatus and Cladosporium cladosporioides* were the most dominant fungi, found in both the study areas. Thus this present study indicates that the vegetation and soil are considered as the main sources of fungal spores for aeromycoflora.

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EFFECT OF AIRBORNE GRASS POLLEN AND $PM_{2.5}$ LEVEL ON ANTI-ALLERGIC RESPIRATORY MEDICATION SALE IN THE CITY OF HOWRAH, INDIA

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Airborne grass pollen grains and particulate matters with ≥2.5 μm aerodynamic diameter ($PM_{2.5}$) are the most important environmental allergen and pollutant respectively, affecting respiratory health in world. Anti-allergic respiratory medicine (AARM) consumption is a primary indicator of respiratory disorders in a population, which is often influenced by the allergenic pollen and pollutant levels in air. Based on this view, a survey was conducted in the city of Howrah, West Bengal, India. Regular data of airborne grass pollen grains (Burkard 7-day volumetric trap), $PM_{2.5}$ concentration (West Bengal Pollution Control Board) and AARM sale in local medical stores were collected during January-December, 2018. The effect of ambient grass pollen and $PM_{2.5}$ on AARM sales was assessed by linear regression. Grass pollen was perennial with major peak at late January (62 grains/d/m^3 air). $PM_{2.5}$ concentration was consistently unhealthy (90-250 μg/m^3 air, according to Indian National Standards) during the periods between January to first week of April and again mid-September to December. In the medical stores, an average of 3544 pieces/store AARM were sold throughout the year. Among these, 52.62% was sold on the basis of prescriptions, and rest 47.38% was directly over-the-counter sale. The highest AARM sale was recorded in mid-January (38 pieces/day) and end of March (36 pieces/day). However, the lowest sale was recorded in mid-July (2 pieces/day). Ambient grass pollen and $PM_{2.5}$ level depicted positive correlations with AARM sale and the correlation was somehow weak for the grass pollen level and moderate ($p<0.05$) for $PM^{2.5}$.

Key Words: Airborne grass pollen, PM2.5, anti-allergic respiratory medicine sale, correlation.

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INTRODUCTION

Respiratory allergy in human is caused by both genetic predisposition and environmental exposure to allergenic particles[1,2]. A number of epidemiological studies revealed that respiratory allergies are often simultaneously influenced by airborne allergen and atmospheric pollutants[3]. Airborne pollen grains are important triggers of respiratory allergic problems like allergic rhinitis (AR), allergic asthma (AA), allergic rhino-conjunctivitis (ARC) in susceptible population[4,5]. Among the airborne allergenic pollen, grass pollen grains are reported to be in the leading position throughout the world[6].

On the other hand, among the atmospheric pollutants, particulate matters with ≥2.5 μm aerodynamic diameter ($PM_{2.5}$) are reported to be most harmful for respiratory health, as they can reach up to the lowest level of respiratory tract, causing impairment of lung function[7]. The ambient pollen and pollutant concentration always differs regionally all over the world[8,9]. Hence, in a particular region, the concentration of airborne grass pollen and $PM_{2.5}$ in the atmosphere may be marked as two important indicators of air quality in respect to respiratory health of the allergic population[10,11]. Comprehensive analyses and understanding of the relation between the prevalence of airborne grass pollen and $PM_{2.5}$ with their contribution behind the complaints of airway symptoms are useful for prediction and preparedness integrating the grass pollen and $PM_{2.5}$ exposure.

The increase of the respiratory allergic symptoms like frequent sneezing, nasal congestion, running nose and bronchial distress subsequently cause the rise of the use of anti-allergic drugs[12]. Hence, anti-allergy respiratory medication use may be associated with the allergenic bioparticles and respirable particulate matters in air. There are a number of anti-allergic respiratory medicines (AARM), which are taken by common people as over-the-counter (OTC) in addition to prescribed medicines for the relief from respiratory allergy[13]. In this way, the overall selling data of AARM for respiratory disorders provides indirect reflection of the occurrence of respiratory allergic episodes in a particular population.

So, the present study was conducted to investigate the effect of atmospheric grass pollen and $PM_{2.5}$ level in the industrial city of Howrah of eastern India, on the sale of AARM from local medical stores.

MATERIALS AND METHODS

Study area

The study was conducted in Howrah (22.5959°N 88.2636° E), the second largest city of West Bengal. It is an important industrial city of eastern India (Fig. 3.1), situated at the western bank of river Ganges (Hooghly), providing the gateway to its twin city Kolkata. The city has a population of 1,077,075, according to 2011 Census of India.

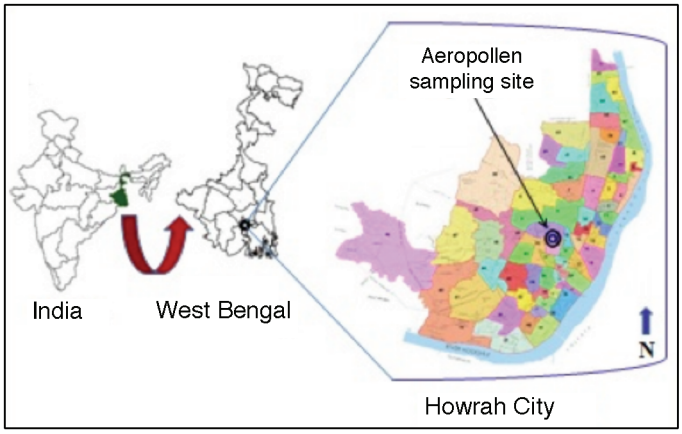

Fig. 3.1: Location map of the sampling site for recording the atmospheric concentration of grass pollen in Howrah city during 2018.

Atmospheric grass pollen monitoring

The aerobiological monitoring of grass pollen grain was recorded at Narasinha Dutt College, situated at Belilious road, crossing between 21 and 22 number ward of Howrah Municipal Corporation for the year 2018. The sampling was conducted with Burkard 7-day spore trap (Burkard Manufacturing Co., UK), which was placed at the rooftop of the 4-storeyed building at the west block of the college. Daily pollen data was collected from the exposed strips of the sampler. The counting was carried out following the protocol of the British Aerobiology Federation (1995)[14], resulting into daily number of pollen per cubic meter of air (pollen/d/m^3 air).

Collection of the data for atmospheric $PM_{2.5}$ concentration

The data of the $PM_{2.5}$ concentration were recorded for two sites of the city (Howrah Municipal Corporation and Bator, with $PM_{2.5}$ sampling system) from the Air Quality Information System of West Bengal Pollution Control Board (http://emis.wbpcb.gov.in/airquality/filter_for_aqi.jsp). We obtained daily $PM_{2.5}$ data for the months of January and February, whereas from March-December, it was for 18-19 days per month.

Recording of the anti-allergic respiratory medicine (AARM) sale in local medical stores

The day-wise data of Over-the-Counter (OTC) and prescription-based AARM sale were collected from four medical stores of Belilious Road, Howrah. The medicines were categorized in seven major classes, including first and second generation antihistamine, leukotriene receptor antagonist, nasal corticosteroid spray, a-adrenergic receptor agonist, inhalants (Short acting β_2 agonist and long acting β_2 agonist as described in Table 3.1.

Table 3.1: The frequent categories of respiratory allergic medications (prescribed and over-the-counter) recorded in the study

Sl. No.	Respiratory allergy medication category	Chemical name with example of trade name
1.	First generation antihistamine	Chlorpheniramine (Chlor-trimeton), Diphenhydramine (Benadryl)
2.	Second generation antihistamine	Cetirizine (Zyrtec), Loratadine (Claritin), Levocetirizine (Xyzal),
		Desloratadine (Clarinex), Bilastine (Bilazest), fexofenadine (Allegra)
3.	Leukotriene receptor antagonist (LTRA)	Montelukast (Singulair)
4.	Nasal corticosteroid spray	Budesonide (Budecort, Foracort), Fluticasone (Flohale, Floease)
5.	α adrenergic receptor agonist spray	Xylometazoline (Otrivine)
6.	Short acting β_2 agonist (SABA) inhalant	Salbutamol (Asthalin)
7.	Long acting β_2 agonist and Inhaled Corticosteroid (LABA+ICS)	Salmeterol/Fluticasone propionate

Statistical analyses

Linear regression was observed for AARM sale, as the dependent variable upon the ambient level of grass pollen and $PM_{2.5}$ of the city, to get the line of best fit for prediction. Spearman's correlation was determined to observe the level of correlation of ambient grass pollen and $PM_{2.5}$ level on the consumption of the AARM, as an outcome of the occurrence of respiratory allergic problems in study area. The analyses were performed using SPSS 2.0 software.

RESULTS AND DISCUSSION

In the atmosphere of Howrah city, grass pollen grains were found to be present round the year (Fig. 3.2). In the first half of the year, the pollen concentration level increased from mid January, reached the peak at the end of this month (62 grains/d/m^3 air), then gradually came down. In the latter half of the year, the pollen grains showed higher level of concentration like 21 grains/d/m^3 in 19th Sept. and reached a peak with 28 grains/d/m^3 concentration at the end of the first week of October. In previous studies[15-17] from other different sites of Gangetic plain, grass pollen was identified as the highest contributor of atmospheric pollen load, contributing upt to 12.32% of total pollen load in air, with peak period during March-April and September. All of these studies were from suburban areas, whereas, the present study reflects the scenario of an urban and industrial zone, where ambient grass pollen level reaches the maxima during dry season, at the last spell of winter. Ghosh *et al*.[3] observed two peaks of grass pollen in March and September in Kolkata, the twin city of Howrah, with a significant association with asthma-related hospital admission.

The level of $PM_{2.5}$, an important fine particle of the atmosphere was found to be consistently unhealthy for a long period of the year, starting from the first week of January till the first week of April and again from the second week of September to the end of December (Table 3.2), except some days associated with heavy precipitation (data not shown). In this way, it is evident that the people of Howrah city are exposed to $PM_{2.5}$ level beyond the permissible limit of national standard[18] for a long period of time. In 2016, it was observed that 63.38% of Howrah district was affected by poor air quality[19].

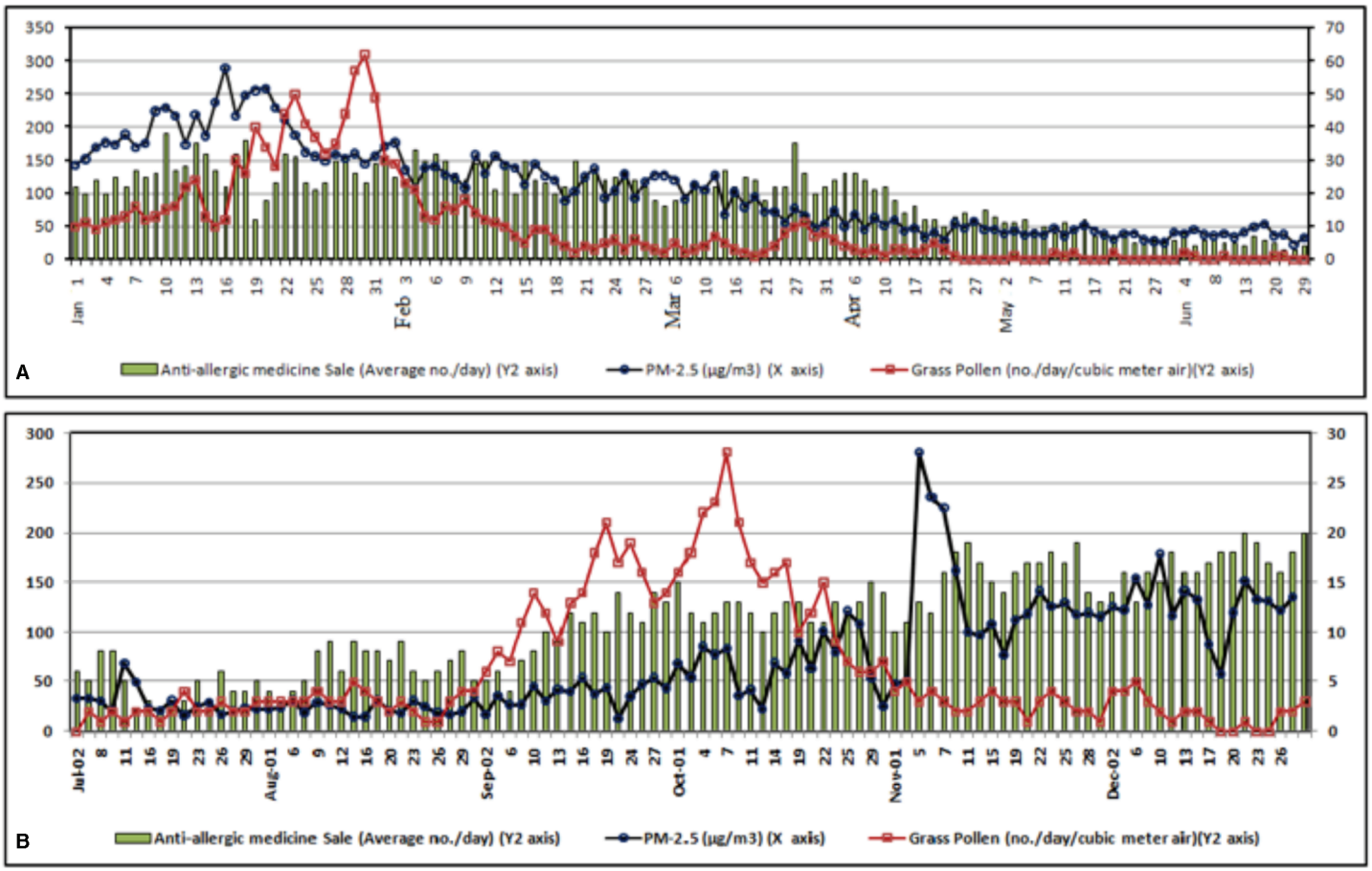

Fig. 3.2: Ambient grass pollen periodicity, $PM_{2.5}$ level variation and anti-allergic respiratory medicine sale record in the city of Howrah, during January-June A and July-December B in 2018.

Table 3.2: Summary of the $PM_{2.5}$ level recorded in the ambient atmosphere of Howrah city in 2018, depicting probable health impacts according to National Ambient Air Quality Standards of India.

Health Impacts for various Air Quality Index(AQI) Categories			Record of the AQI category of $PM_{2.5}$ in study period
AQI Category	**$PM_{2.5}$ ($\mu g/m^3$) for 24 hours**	**Associated Health Impacts**	
Good	0-30	Minimal impact	Only for few days like 21st April (28 $\mu g/m^3$), 24-28th May (25-29 $\mu g/m^3$), 28th June (22 $\mu g/m^3$)
Satisfactory	31-60	May cause minor breathing discomfort to sensitive people.	Last week of March, end of June, (few exceptions are there)
Moderately polluted	60-90	May cause breathing discomfort to people with lung disease such as asthma, and discomfort to people with heart disease, children and older adults.	March 14-31 (few exceptions are there)
Poor	91-120	May cause breathing discomfort to people on prolonged exposure, and discomfort to people with heart disease.	Mid February-Mid March
Very poor	121-250	May cause respiratory illness to the people on prolonged exposure. Effect may be more pronounced in people with lung and heart diseases.	January-Mid February and November-December (without some exceptions)
Severe	>250	May cause respiratory impact even on healthy people, and serious health impacts on people with lung/heart disease. The health impacts may be experienced even during light physical activity.	Three days-in January: 16th (290 $\mu g/m^3$), 19th (256 $\mu g/m^3$) and 20th (259 $\mu g/m^3$) and 5th November (280 $\mu g/m^3$)

The survey of anti-allergic respiratory medicine (AARM) sale in the medical stores of the study area showed that average of 3544 pieces medicines/store were sold throughout the year. Among the total number, 52.62% was sold on the basis of prescription, rest 47.38% sale was over-the-counter (Fig. 3.3). Regarding the respiratory allergy medication category (Table 3.1), the selling percentage of second generation anti-histamine (35.13%) was followed by short acting β_2 agonist (SABA) inhalant category of medicine (24.68%). The consumption data of anti allergic respiratory medicines are the indirect outcome of the occurrence of respiratory allergy in local population. The maximum number of medicine sale was recorded in January–March, with gradual decline in April, which again increased in November-December (Fig. 3.2). The highest average number was recorded in the second week of January (38 pieces/day) and end of March (36 pieces/day), whereas the lowest was observed in the middle of July (2 pieces/day).

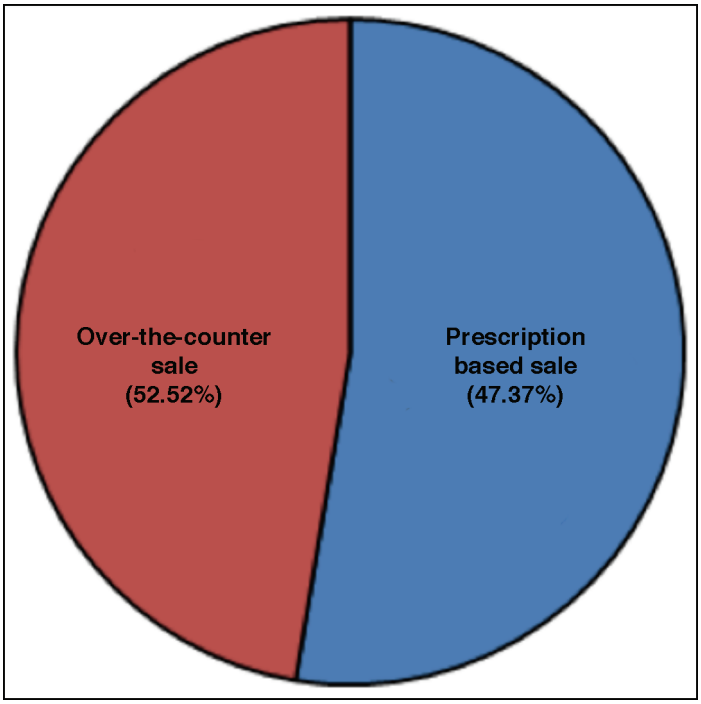

Fig. 3.3: Anti-allergic respiratory medicine sale percentages in the medical stores of the Howrah city during 2018 (January-December).

Allergy medication use is reported to be associated with higher pollen concentrations[12,20-22]. In the present study, there may be some probability of association between two independent atmospheric components - airborne grass pollen and $PM_{2.5}$ level with the consumption of anti-allergic respiratory medicine (AARM) by local people. On the basis of this viewpoint, linear regression was calculated for the local AARM sale with airborne grass pollen and $PM_{2.5}$, respectively. The regression was made to find out the best fit of paired data of AARM sell (dependent) and grass pollen (Fig. 3.4) and $PM_{2.5}$ (Fig. 3.5) respectively.

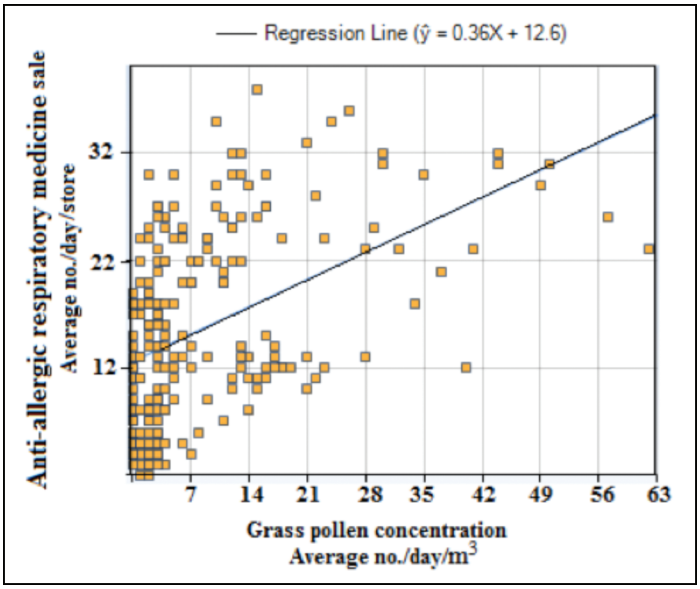

Fig. 3.4: Linear regression of anti-allergic respiratory medicine sale in relation to grass pollen concentration in the ambient air of Howrah city in 2018 (January-December).

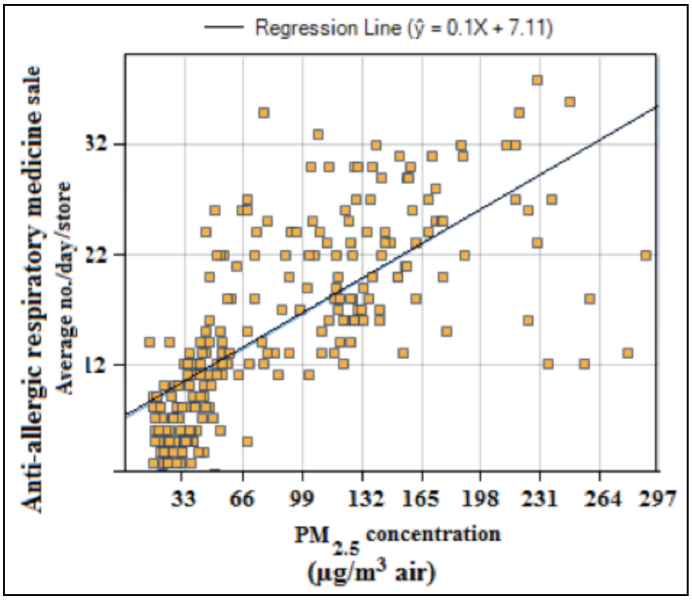

Fig. 3.5: Linear regression of anti-allergic respiratory medicine sale in relation to $PM_{2.5}$ concentration in the ambient air of Howrah city in 2018 (January-December).

In case of grass pollen and AARM sell, the line of best fit is depicted by the equation:

$$Y = 0.36463\ X + 12.59819.$$

In this case, the correlation coefficient R is 0.4674 and R^2, the coefficient of determination is 0.2185. The p value is <0.00001, which is significant at 0.05 level. Although there is a positive correlation, it was found to be a weak one.

In case of $PM_{2.5}$ and AARM, the regression equation appeared as: $Y = 0.09609\ X + 7.10967$.

The value of correlation coefficient R is 0.706 and R^2, the coefficient of determination is 0.4984. The related p value is <0.00001, which is significant at 0.05 level. There was a moderate positive correlation, which means there is a tendency of high X variable score of $PM_{2.5}$, when there is an increased anti-allergic respiratory medicine sale in the study area and vice versa.

There were a large number of studies dealing with association of respiratory allergy/asthma related emergency hospitalization with exposure to allergen and/or air pollutants[3]. However, a limited number of researches dealt with such relation with medicine consumption of anti-allergic drugs, which was first reported comprehensively by Fuhrman *et al.*[20] in 2007. Later, this parameter was studied in detail by Sheffield *et al.*[21](2011), Caillaud *et al.*[22](2015), Wang *et al.*[12](2017), and Grundstrom *et al.*[23](2017). Among them, only Grundstrom *et al.*[23] studied the effect of both the pollen and pollutants in air. He found that air pollution can worsen the symptoms of respiratory allergy, as reflected by the rise of over-the-counter sale of antihistamines in a city of Sweden. Kazuhiko *et al.*[24] observed significant association of mid spring airborne tree pollen with both the medication sale and emergency department (ED) visits in New York city during 2002-2012. However, there was no comprehensive study conducted in respect to consumption of AARM from the Indian subcontinent.

In the present study, positive correlation of ambient grass pollen and $PM_{2.5}$ was observed with medicine sale. The correlation was somehow weak for the grass pollen level and moderate for $PM_{2.5}$. In an industrial urban area, with less percentage of green cover, pollen grains may not be acting as high-level risk factor for respiratory allergy. However, long-term exposure to $PM_{2.5}$ in the study area certainly plays a role to enhance allergic respiratory symptoms. In addition, there may

also be a combined effect of allergic pollen and $PM_{2.5}$ exposure on the respiratory symptoms of susceptible population as described by Baldacci *et al*.[25].

CONCLUSION

Consumption of anti-allergic medicine is an indicator of respiratory allergy for a local population, which is a less focused parameter in environmental allergen research. The first-time information about positive correlation of atmospheric grass pollen and fine particulate pollutant $PM_{2.5}$ exposure with anti-allergic respiratory medicine sale in Howrah city will be helpful in risk assessment and management of respiratory allergy in the tropical eastern India.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ATMOSPHERIC BIOPOLLUTION BY AIRBORNE MICROFUNGI IN RURAL AND URBAN AREAS OF PUDUCHERRY: A CASE STUDY

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Atmospheric bio-pollution deals with the incidence of biological particulates and their behavior, survivability and eventually dispersion in the atmosphere. Airborne fungi are one of the bio-pollutants mostly abundant in their distribution in outdoors and indoors of varied environments. They organize the major part of the floating bio-particulate materials in the atmosphere. So, it is necessary to analyze their incidence and diversity in different environments, since they are concerned with various disorders among atopic human beings as well as plants. In the present study, an aero-mycological case study of rural and urban areas of Puducherry district were conducted with volumetric Burkard's personal sampler on agar plates for two consecutive years from 2014 to 2016. Aerobiological survey was conducted at 15 day intervals for isolating the prevailing fungi from the two study sites at diurnal timings viz., morning, noon and evening. Altogether, 58 fungal species under 39 genera were isolated, among which Aspergilli were recorded as the dominant type, followed by Penicilli. The occurrence of fungal spores was variable in the two localities. Rural area was found to be dominated with larger number (n = 2641) of fungal spores in comparison to urban area (n = 1993. Regarding the overall diurnal pattern, noon time was found to harbor a greater number (upto 500 CFUs) and diversity of fungal spores, compared to morning and evening. The trend of seasonal periodicity of airborne fungal spores showed higher concentration in winter followed by summer and rainy season. In addition to *Aspergillus* and *Penicillium*, fungal spores of *Absidia, Alternaria, Cladosporium, Curvularia, Fusarium, Monascus, Mucor, Paecilomyces, Rhizopus, Trichoderma, Verticillium* and *Wallemia* were also recorded.

Key Words: Atmospheric bio-pollution, airborne micro fungi, Rural and urban areas, Burkard's sampler on agar plates.

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INTRODUCTION

Bioaerosols are the biological particulate materials found naturally within the environment1. These comprise airborne particles particularly from living organisms, such as fungi, bacteria, viruses and parts of living organisms, like pollen grains, fungal hyphae and endotoxins from bacteria or mycotoxins from fungi[2]. The size, density and shape of the bioaerosol influence its survivability, performance and eventually its dispersion in the environment[3,4]. Prevalence of fungi in indoors and outdoors of various environments has been considered as unfavorable, principally because of its affinity to decrease the air quality[5]. Rural environments, particularly the villages with unutilized plant materials and dumped debris very often serve as the reservoirs of saprophytic fungi[6]. These fungi are often found to induce diseases in crops and plants of the vicinity, for their requirements of nutrients[7-8]. Aero-mycological studies on urban and rural environments had reported that fungal spores regularly go from outdoors to indoors[6,9] and their abundance is higher in indoor air, when compared with the outdoors[10]. Atmospheric fungal are well known triggers of respiratory allergic health problems among atopic human beings[11-13]. Especially, various strains of *Aspergillus* and *Penicillium* are found to be main allergenic types causing asthma and allergic alveolitis[14]. In the present study, there is a qualitative and quantitative comparison of airborne fungal spores of a rural (Muthialpet) and urban (Peramabai) environments of Puducherry district, Puducherry, India. The results may be useful in predicting possible risks of allergenic airborne fungi in the village as well as urban people of Puducherry.

MATERIALS AND METHODS

The volumetric aero-mycological study was carried out in two different environments, rural and urban areas viz., Perambai and Muthialpet of Puducherry, India from March 2014 up to February, 2016 (Fig. 4.1).

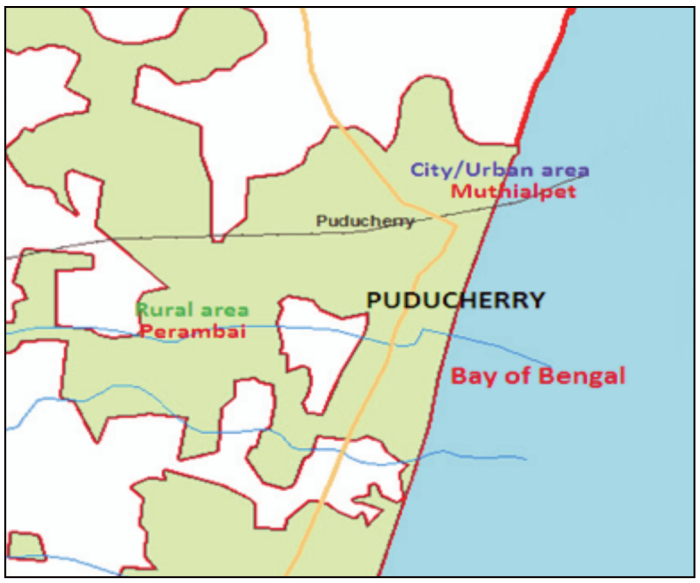

Fig. 4.1: Rural and Urban study sites of Puducherry

Study sites

Two sites were selected for study, which are described as following:

Site 1: Urban locality of Muthialpet, which is situated at the northern part of Puducherry city (11.9416°N and 79.8083°E), the capital of Puducherry state, in the coast of Bay of Bengal. It was ruled by the French colonizers, earlier to its Union Territory status. Its eastern part is restricted by Bay of Bengal and Western & Northern parts are restricted by Tamil Nadu. The locality is characterized by government offices, hotels and modern style residential houses.

Site 2: Perambai, a rural locality, situated at north western parts of Puducherry city, approximately 5 km away from the city area. The surrounding areas of the village localities are covered with agricultural fields and varied vegetations.

Air Samplings

Outdoor air samplings were conducted at monthly intervals, mostly at 6 AM, 12 noon and 6 PM for continuous two years (March 2014 - February 2016), using Burkard's personal volumetric sampler on agar plates at 10 ft height from the ground to avoid dust and other disturbing materials. Sabouraud Dextrose Agar (SDA) was used for the sampler containing with streptomycin (50 mg-l), which was carried to the study sites with sterilized container and kept in the sampler in order to run for five minutes to collect the airborne fungal spores on the media plates. Altogether 48 Petriplates were exposed in two areas for two years. After sampling, exposed plates were studied at the Microbiology Laboratory, Department of Botany, K. M. Govt. Institute for Postgraduate Studies, Puducherry. These were then incubated in BOD incubator at 25 ± 3°C, upside down for 10 days with constant observation, just after three days of incubation. Fungal colonies developed in plates were recorded for individual species and the total number colony forming units (CFUs). Microscopic studies with lactophenol-cotton blue were prepared for each CFU, which were microscopically observed to for identification up to species level. The inoculated plates were sub cultured in PDA/CDA media for identification of the CFUs. The identification of the fungal taxa was based on laboratory experience and relevant taxonomic literature consultation15-20. The total number of fungal CFUs recorded from the two environments was converted into CFUs/m^3 of air, based on the following calculation:

Burkard's sampler suction rate is 10 litres of air per minute, on the media plate.

Suction rate per 10 minutes = 10x10 = 100 litres = 0.1 m^3 of air

If 0.1 m^3 of air has one spore/CFUs, then 1 m^3 of air would have $\frac{1}{0.1}$ = 10 spores/CFUs

For 10 minutes of operation = 10 spores are there and the conversion factor is 10.

For 5 minutes of operation = 10 spores are there and the conversion factor would be, 10 ÷ 2 = 5

No of fungal spores/CFUs m^3 of air was calculated as

$$\frac{\text{Total number of spores}}{\text{CFUs counted from the plates}} \times 5$$

Annual and monthly percentage occurrence of individual fungus was determined with their seasonal variation. Different statistical tests were analyzed to find out the significance of the means, variance of fungal distribution in between the village environments.

RESULTS AND DISCUSSION

During the period, a total number of 4634 fungal CFUs m^3 of air were isolated altogether from rural Perambai village as well as city area of Muthialpet, Puducherry. The rural area contributed 57% of aeromycoflora, whereas it was 43% for city environment (Fig. 4.2). The occurrence of fungal spores was recorded to be higher during 2015-16 in comparison to 2014-15 in both the rural and urban areas of Puducherry. The maximum occurrence of airborne fungal spores in the rural area may be related to the local vegetation, which contributed the fungal spores from phylloplane and the litter of dumped plant materials in the area. However, the pollutant level and lack of plantation in the urban area might have reduced the fungal spores[21]. The dominant airborne fungal species, their CFUs contribution and total occurrence recorded in each sites of the region are described on Table 4.1. Qualitatively, altogether 58 fungal species were isolated comprising of 39 genera from both the sites. Among the isolated fungal genera, Aspergillus was the dominant type, represented by highest number of species (n = 14), followed by Cladosporium, Penicillium, Rhizopus and White sterile mycelia. Aspergillus niger was found as the dominant species followed by Cladosporium herbarum in both the environments (Table 4.1). Monthly and diurnal incidence of dominant airborne fungi isolated from two

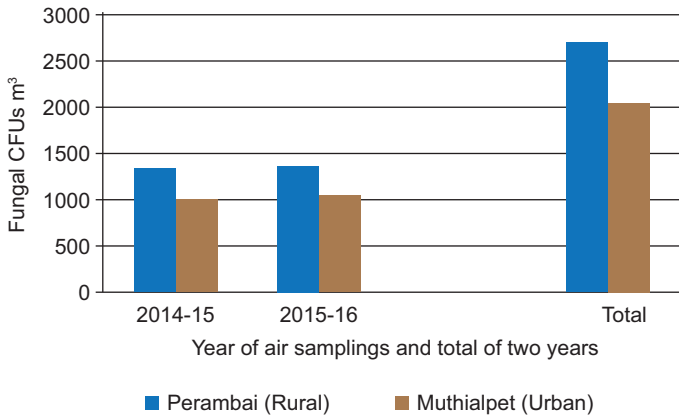

Fig. 4.2: Total fungi/m^3 air isoated from rural and urban areas of Puducherry

localities (Table 4.1) showed that *Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Paecilomyces* sp., *Penicillium citrinum, P. digitatum, Rhizopus stolonifer* and white sterile mycelia were dominant fungi and contributed more than the percentile of other isolated fungi at two sites. Based on the relative and diurnal incidence, noon was found to harbor the maximum number of fungal spores compared to morning and evening in both the urban and rural environments. The months of September and December contributed the maximum spores followed by April, May and June. The period of February-July didn't show any much difference in respect of fungal prevalence in both the areas. In addition to the dominant fungi, other fungal members like *Alternaria, Absidia, Curvularia, Drechslera, Fusa-*

Table 4.1: Relative occurrence of dominant fungi isolated from rural and urban areas of Puducherry

Dominant Fungi	Rural area						Urban/City area					
	Perambai (2014-15)			Perambai (2015-16)			Muthialpet (2014-15)			Muthialpet (2015-16)		
	M	N	E	M	N	E	M	N	E	M	N	E
Aspergillus flavus	**8.5**	**8.9**	**7.2**	**8.1**	**6.8**	**6.8**	**8.1**	**6.6**	**7.7**	**6.5**	**6.5**	**7.58**
Aspergillus fumigatus	7.1	3.2	5.7	6.8	3.2	3.5	6.1	3.04	5.3	5.7	3.1	5.2
Aspergillus niger	**25.7**	**22.5**	**35.9**	**30.4**	**29.8**	**35.2**	**12.4**	**11.3**	**18.9**	**16.4**	**14.4**	**21.0**
Cladosporium herbarum	**20.5**	**26.7**	**20.1**	**21.9**	**23.2**	**23.4**	**27.2**	**27.9**	**22.0**	**15.9**	**32.3**	**20.7**
Paecilomyces sp.	1.9	3.2	1.7	1.1	4.6	4.7	2.0	1.1	2.1	1.9	0.9	2.1
Penicillium citrinum	**2.8**	**3.7**	**2.2**	**1.8**	**3.2**	**1.1**	**-**	**-**	**2.1**	**-**	**-**	**3.8**
Penicillium digitatum	**1.2**	**4.6**	**4.4**	**3.6**	**4.7**	**1.1**	**0.57**	**3.3**	**6.3**	**0.82**	**3.1**	**4.8**
Rhizopus stolonifer	3.3	1.4	4.2	3.2	1.4	4.1	1.16	3.3	4.2	1.1	2.8	1.4
White sterile mycelia	2.6	3.8	2.2	3.6	3.6	2.3	5.2	6.6	4.9	6.0	5.8	15.2

M: Morning, **N**: Noon, **E**: Evening

rium, Mucor, Neurospora, Saccharomyces, Syncephalastrum, Trichoderma and few other mycelia sterlia were also recorded. The composition of ambient fungal members corroborates with the previous reports worldwide[12, 22-25].

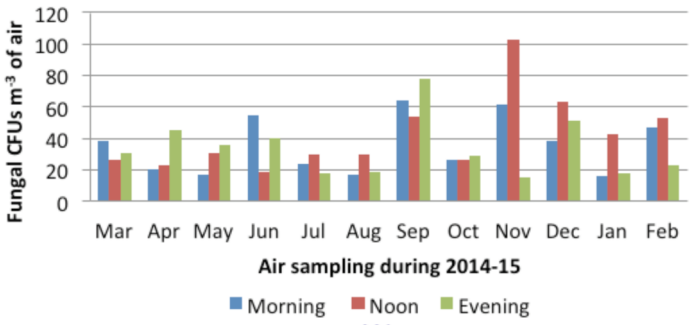

Fig. 4.3: Incidence of fungal spores in Perambai during 2014-15

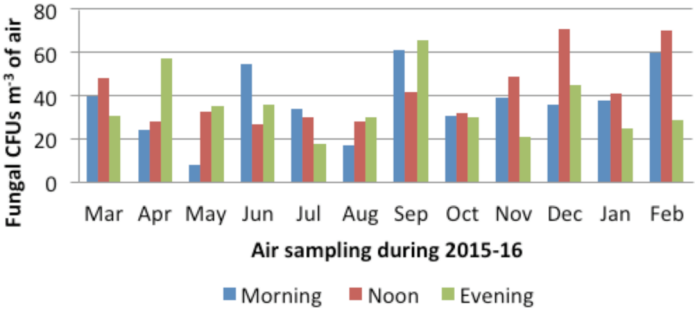

Fig. 4.4: Incidence of fungal spores in Perambai during 2015-16

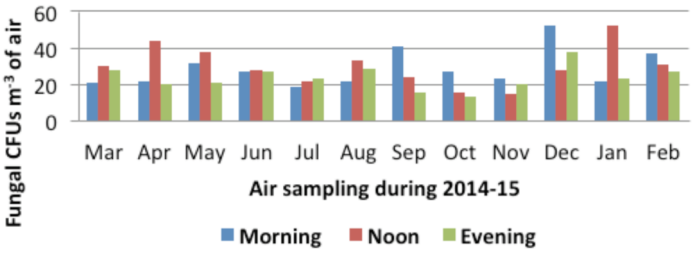

Fig. 4.5: Incidence of fungal spores in Muthialpet during 2014-15

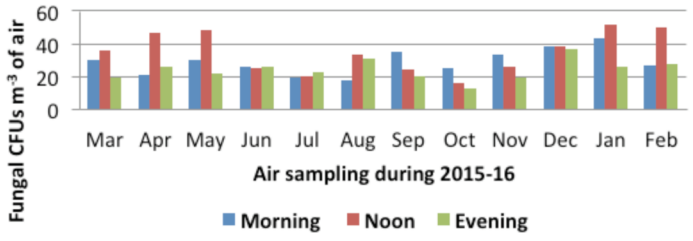

Fig. 4.6: Incidence of fungal spores in Muthialpet during 2015-16

Monthly and diurnal incidence of fungal spores in rural area for 2014-15 and 2015-16 are depicted in Figs. 4.3 and 4.4, whereas Figs. 4.5 and 4.6 described the same for the urban area. Based on monthly record, months of September, December and February were found to hourbor higher number of spores in the rural area, in both the years. However, in city area, December-February period was found to record greater number of fungal spores. There are previous reports[9, 26] showing winter months to be associated with larger number of fungal spores in the atmosphere and the result of the present study agrees with them. It was found that the winter months were followed by rainy and summer months in respect to the level of aeromycoflora.

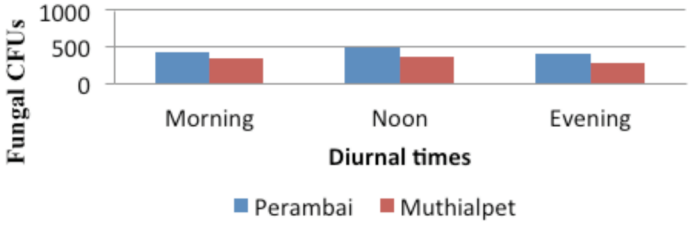

Fig. 4.7: Diurnal distribution of fungal spores in rural and urban areas during 2014-15

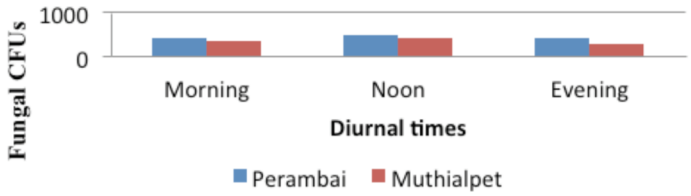

Fig. 4.8: Diurnal distribution of fungal spores in rural and urban areas during 2015-16

The observations on diurnal distribution of fungal spores in rural and urban areas during 2014-15 and 2015-16 (Figs. 4.7 and 4.8) revealed that the noon time was the harbor of the maximum numbers of fungi in the air, which was followed by the levels at morning and evening respectively. A similar trend was reported by Nayak and Behera[27] in 1996 from Berhampur, Orissa.

The correlation among the fungal spore concentration in between rural and urban sites were found significantly positive (in the t test) between two variables at morning, noon and evening (data not shown). The airborne fungal spores in the study sites may be controlled by the meteorological parameters viz., temperature and relative humidity as reported by the previous workers[6, 9].

November was found as the month highest level of aeromycoflora (100 CFUs/m^3 air) in Perambai village during noon. However, in September it was recorded with large number of fungal spores in all the three times viz., morning, Noon and evening during 2014-15 and 2015-16 (Figures 4.3 and 4.4). It was also found that December and February recorded more fungal occurrence during Noon time only.

There were great similarities in the incidence of fungal spore in the city area during 2014-15 and 2015-16 (Figures 4.5 and 4.6), which confirmed that the ambient fungal spore levels are always higher in winter months particularly in December- February.

The diurnal distribution of fungal spores in rural and urban areas was represented with the maximum incidence of fungi in the atmosphere in 2015-16 at both rural and city areas in comparison to 2014-15 (Figures 4.7 and 4.8). Moreover, it was found that among all the diurnal times, noon was predominated by greater number of fungal spores in all the atmospheres. It was also found that the rural area was harboured with a greater number of fungi, compared to the urban area.

CONCLUSION

This is an aeromycological case study of two different environments viz., rural and urban areas of Puducherry district, conducted by volumetric Burkard's personal sampler on agar plates for two consecutive years, 2014 and 2016. Altogether, 58 fungal species of 39 genera were isolated, among which *Aspergillus* members were recorded as the dominant followed by *Cladosporium*. In the present study, rural area dominated in respect to fungal composition as well as concentration compared to urban area. In diurnal variation, noon was found to harbor the maximum spores than morning and evening. In seasonal distribution, the winter months were predominated with the fungal spores in the air and it was followed by summer and rainy season. Rural site was probably dominated with fungal spores due to the abundance of variable crops and large vegetation in comparison to urban site. The reduction of airborne fungi was possibly due to higher level of pollution and lack of vegetation in city area.

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CONFLICT OF INTEREST

The authors declare no conflict of interest among the authors.

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ANALYSIS OF THE RELATIONSHIPS BETWEEN ENVIRONMENTAL FACTORS AND RESPIRATORY PROBLEM RELATED HOSPITAL ADMISSION IN BOLPUR-SANTINIKETAN, WEST BENGAL, INDIA

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Aerobiological study in Bolpur-Santiniketan - a famous tourist and educational place of West Bengal, India - was conducted for one year time period (July 2018 to June 2019) using portable Burkard Personal Volumetric Sampler as well as culture plate method to make pollen-spore calendar of the study site. A total of 40 types of pollen, 18 different spores types and 20 culturable fungal species were recorded. Fungal species like *Alternaria* sp., *Penicillium* sp. and *Aspergillus* sp. were predominant in the area with higher percentage of *Candida* sp., *Fusarium* sp. and sterile mycelia colonies. In both the sites, maximum number of aerospora concentration and CFU number were documented from winter season (November) due to having large number of agricultural accumulation supplemented by conducive meteorological factors like low rain fall, low humidity, low wind speed and low temperature. On the other hand, *Acacia* sp., *Cassia* sp. and Poaceae (grasses) were the major pollen types recorded from the study area. Pollen count was found to be highest in March and lowest in August which was correlated with meteorological factors. It was observed that total airspora and PM10 positively correlated with the respiratory allergy related hospital admission. Maximum number of allergic patients was admitted in hospitals in November, while the minimal value was obtained in August. Other factors like CFU or pollen count, SO_2 had no significant role in respiratory problem related patients' admission, while NO_2 showed some correlation with the aerospora concentration.

Key Words: Aerospora, Pollen/Spore calendar, Meteorological factors, Inorganic pollutants, Respiratory problem related hospital admission, Bolpur-Santiniketan, India

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INTRODUCTION

Aerobiology is a scientific and multidisciplinary approach focuses on the transport of organisms and biologically significant materials[1] which is particularly related to meteorology. Thus the interest of the meteorologists in aerobiological survey is primarily towards the atmospheric process involved in dissemination of such organisms. Meteorologists is interested in such types of spores which would help in forecasting the weather condition. Jecob[2] clearly pointed out the close relationship of aerobiological survey and meteorological condition.

The importance of aerobiological investigation in the pathogenesis of respiratory allergic disease has been recognized since the earliest day of allergology. More than 20-30% of the world population is known to suffer from one or other allergic disease such as bronchial asthma, allergic rhinitis and atopic dermatitis etc.[3]. It also becomes a major concern for the developing countries like India and China where 10 out of 26 megacities fall under the most polluted cities of the world[4,5]. India also has a large population of asthmatic and skin diseases[6] and the number is gradually increasing. Therefore, identifying environmental factors behind asthma-related allergy and their correlation with aerobiology and meteorological factors could be helpful in determining a certain region's public health policy.

Ambient aeroallergens and organic or inorganic air pollutants are known to cause asthma and other allergy exacerbation and subsequent respiratory problem related hospital admissions (RPRHAs)[7-9]. In the present study an attempt has been made to survey RPRHAs and their correlation with airspora, inorganic pollutants and meteorological factors in Santiniketan-Bolpur, a township with a major tourist hub and an educational center (Visva-Bharati – a Central Govt. University) of West Bengal. This place is blessed with rich tropical vegetation and farming fields which are the major sources of atmospheric allergenic spores and pollen[10,11]. Previously some aerobiological survey was conducted from this area[12-14] but no health survey in relation to aerobiology has so far been done.

The objective of the present study was to determine the relationship between respiratory problem related hospital admissions and aerospora, atmospheric inorganic pollutants as well as meteorological factors in Santiniketan-Bolpur township.

METERIALS & METHODS

Sampling Sites

In the present investigation, four study sites namely, Bazar site (Site A), Hospital site (Site B), School site (Site C) and Residential site (Site D) in Bolpur–Santiniketan (23.6776°North, 87.6852°East) area were selected. The study was conducted for one year during July, 2018 to June, 2019.

Sampling Methods

Air sampling was carried out volumetrically using Burkard Personal air sampler (Burkard Manufacturing Co., Rickmansworth, Herts, England) thrice in a month for assessment of both airborne pollen and fungal spores. The sampler has an airflow rate of 10L/min and operated for 5 minutes[15]. Petriplate method using potato dextrose agar (PDA) medium supplemented with streptomycin and Chloramphenicol (concentration = 50 μg/ml) as antibacterial agents was used for trapping culturable fungi. After exposure, petriplates were incubated at 27ºC for 3 days to allow fungal colony formation. The sporulating fungal forms were observed under high resolution light microscope and were identified following the standard manuals[16,17]. The number of colonies recorded from both petriplates were combined and converted into number of colony forming unit (CFU) per cubic meter of air sampled by multiplying with an appropriate conversion factor (12) following the method of the British Aerobiology Federation[18].

Environmental Parameters

The weather report on average temperature, average humidity, average rainfall and average windspeed and data on air pollutants (SO_2, NO_2) and particulate matters (PM10) of Bolpur-Santiniketan was collected from Government of West Bengal website[19-21].

Health Survey

Data on RPRHAs were collected from local hospitals (Bolpur health centre, and Sub-divisional hospital, Sian, Bolpur). A total of 2756 patients' data were collected from health survey. We have excluded smoker (cigarette, Bidi), Tuberculosis patients, and those have a congenial heart disease. Entire study was based on the modified version of the European Academy of Allergy and Clinical Immunology (EAACI) questionnaires for health survey with some modification.

RESULTS & DISCUSSION

Aeropollen study

During the entire sampling period, a total of 40 pollen types were identified from the study sites. Among them 22 were trees, 3 were shrubs, 11 were herb, one climber and the rest were variable (Table 5.1). A total of 25620 pollen per m^3 of air were trapped which covered 40 different plant species and family during whole study period. Among them dominating pollen represented by grass family (16%) followed by *Acacia auriculiformis* (12%) and *Cassia* spp. (10%) (Table 5.1).

It was found that among the 40 different plant groups trees contributes 55% followed by the herbs (30.5%) and shrubs (14.5%). A pollen calendar was made (Fig.1) which shows highest pollen load (3860 m^{-3}) in march followed in degree of prevalence by April (3790 m^{-3}) and May (2980 m^{-3}), while the lowest pollen counts were recorded in July (730 m^{-3}), August (1080 m^{-3}) and December(1200 m^{-3}) (Fig. 5.1). It was also evident that pollen of *Acacia auriculiformis, Cassia* spp. and Poaceae were found round the year.

Aero fungal spore study

A total of 18 different spores types (Table 5.2) and 20 culturable fungal species (Table 5.3) with sterile mycelium and unidentified species were recorded. *Alternaria*, Ascospore, Aspergilli/Penicilli, *Drechslera, Nigrospora*, Rust spore, *Trichoconis*, etc. were found in all the sampling sites. Among these, Ascospore, Aspergilli/Penicilli showed their highest abundance followed by Rust spore, Nigrospora. Basidiospores were reported from three sampling sites in the range of 6.45 to 10.66%. Interestingly, Basidiospores were absent in Bazar area (Sampling site A). *Spegazzinia, Cladosporium, Helminthosporium, Fusarium, Beltrania, Papularia* represented their lowest abundance (Table 5.2).

Based on abundance of spores in the ambient air, a one year spore calendar (July 2018 to June 2019) of Bolpur-

Table 5.1: Percentage contribution of total pollen in Bolpur-Santiniketan (July, 2018 – June, 2019)

Sl. No.	Plant Species/ Family	Plant Habit	Total Pollen (No. m^{-3})	Pollen Percentage
1.	*Eucalyptus* spp.	Tree	870	3.39
2.	*Justicia* spp.	Herb	360	1.40
3.	*Litchi chinensis*	Tree	420	1.63
4.	*Madhuca latifoia*	Tree	840	3.27
5.	*Mimosa pudica*	Herb	720	2.81
6.	*Morus* sp.	Shrub	510	1.99
7.	*Phoenix sylvestris*	Tree	210	0.81
8.	*Phyllanthus emblica*	Tree	960	3.74
9.	Poaceae (Grasses)	Herb	4010	15.65
10.	*Psidium* spp.	Tree	500	1.95
11.	*Ricinus communis*	Shrub	330	1.28
12.	*Shorea robusta*	Tree	480	1.87
13.	*Tectona grandis*	Tree	240	0.93
14.	*Tinospora* sp.	Climber	270	1.05
15.	*Acacia auriculiformis*	Tree	2950	11.51
16.	*Albizia lebbeck*	Tree	360	1.40
17.	*Areca catechu*	Tree	480	1.87
18.	Asteraceae	Herb	570	2.22
19.	*Azadirachta indica*	Tree	330	1.28
20.	*Borassus flabellifer*	Tree	510	1.99
21.	*Carica papaya*	Shrub	240	0.93
22.	*Cassia* spp.	Variable	2500	9.75
23.	Cheno-Amaranthus	Herb	870	3.39
24.	*Clerodendrun* spp.	Herb	240	0.93
25.	*Cocos nucifera*	Tree	510	1.99
26.	Convolvulaceae	Herb	130	0.50
27.	*Croton bonplandianum*	Herb	960	3.74
28.	*Delbergia sissoo*	Tree	300	1.17
29.	Euphorbiaceae	Variable	180	0.70
30.	Malvaceae	Herb, Shrub	1020	3.98
31.	*Mangifera indica*	Tree	300	1.17
32.	*Syzygium* sp.	Tree	250	0.97
33.	Acanthaceae	Herb	330	1.28
34.	*Cassuarina equisitifolia*	Tree	220	0.85
35.	*Delonix regia*	Tree	300	1.17
36.	*Xanthium strumarium*	Herb	420	1.63
37.	Lamiaceae	Herb	270	1.05
38.	*Zizyphus jujuba*	Tree	240	0.93
39.	*Alstonia scholaris*	Tree	60	0.23
40.	*Bombax ceiba*	Tree	360	1.40

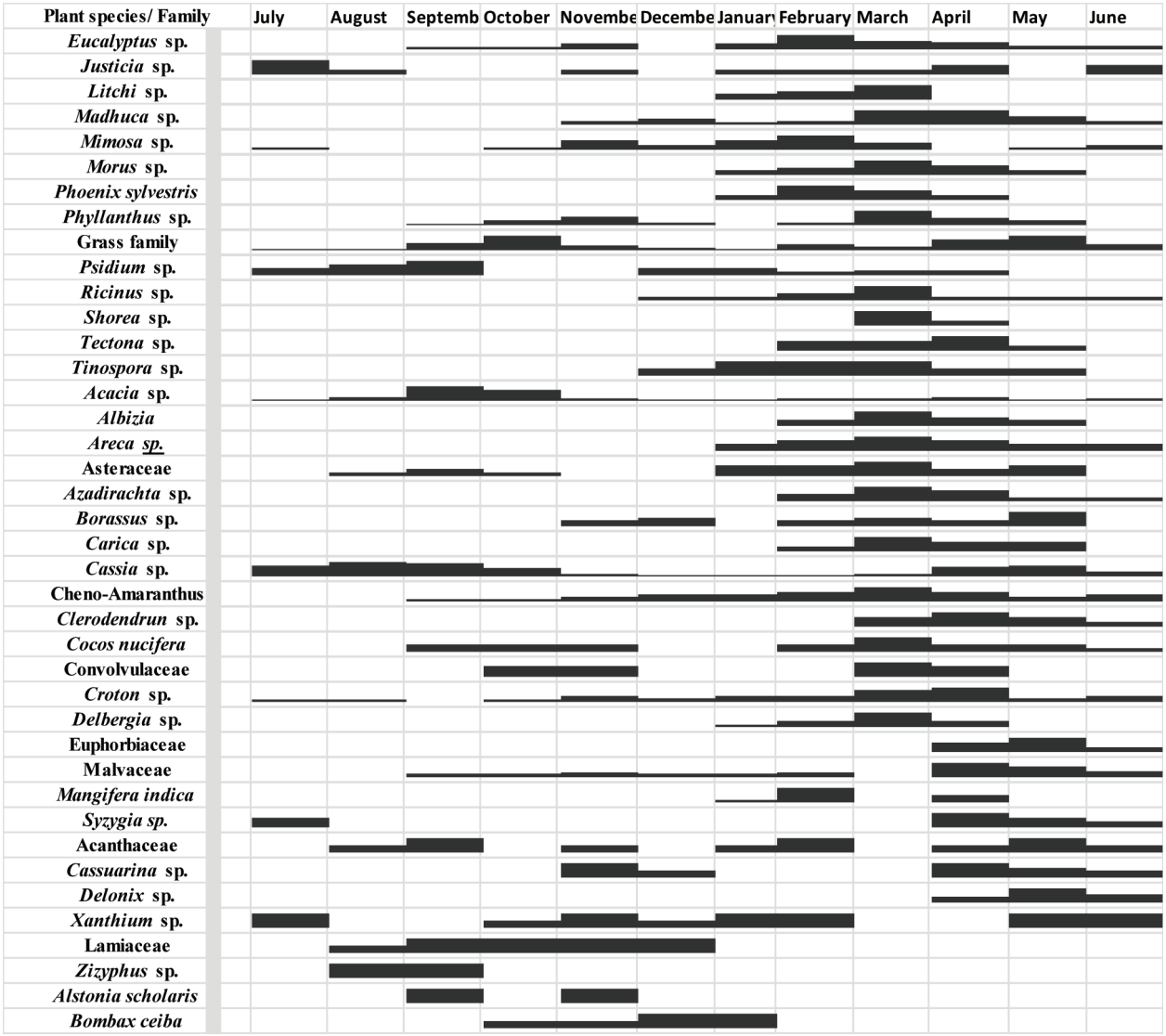

Fig. 5.1: Pollen calendar of Bolpur-Santiniketan (July,2018-June.2019)

Table 5.2: Percentage contribution of total fungal spores in Bolpur-Saniniketan (July, 2018 – June, 2019)

Sl. No.	Fungal Spore	Percentage Contribution (%)			
		Bazar (Site A)	Hospital (Site B)	School (Site C)	Residence (Site D)
1.	*Alternaria*	1.85	2.04	2.61	4.84
2.	Ascospore	33.49	45.05	52.97	34.02
3.	Aspergilli/Penicilli	48.57	30.28	13.96	41.7
4.	Basidiospore	0	9.53	6.45	10.66
5.	*Beltrania*	0	0.26	0	0
6.	*Curvularia*	0	0	0	4.07
7.	*Cercospora*	0	0.26	0.15	0.1
8.	*Cladosporium*	0	0	0	0.1
9.	*Drechslera*	0.3	0.26	0.6	1
10.	*Dwayabeeja*	0	0.13	0.15	0.2
11.	*Helminthosporium*	0	0	0	0.1
12.	*Fusarium*	0	0	0.15	0
13.	*Nigrospora*	6.37	2.67	2.26	4.32
14.	*Papularia*	0	0.13	0	0
15.	*Periconia*	0	2	4.52	0
16.	Rust spore	9.1	7.07	14.33	1.6
17.	*Spegazzinia*	0	0.13	0	0.1
18.	*Trichoconis*	0.3	0.13	1.81	0.8

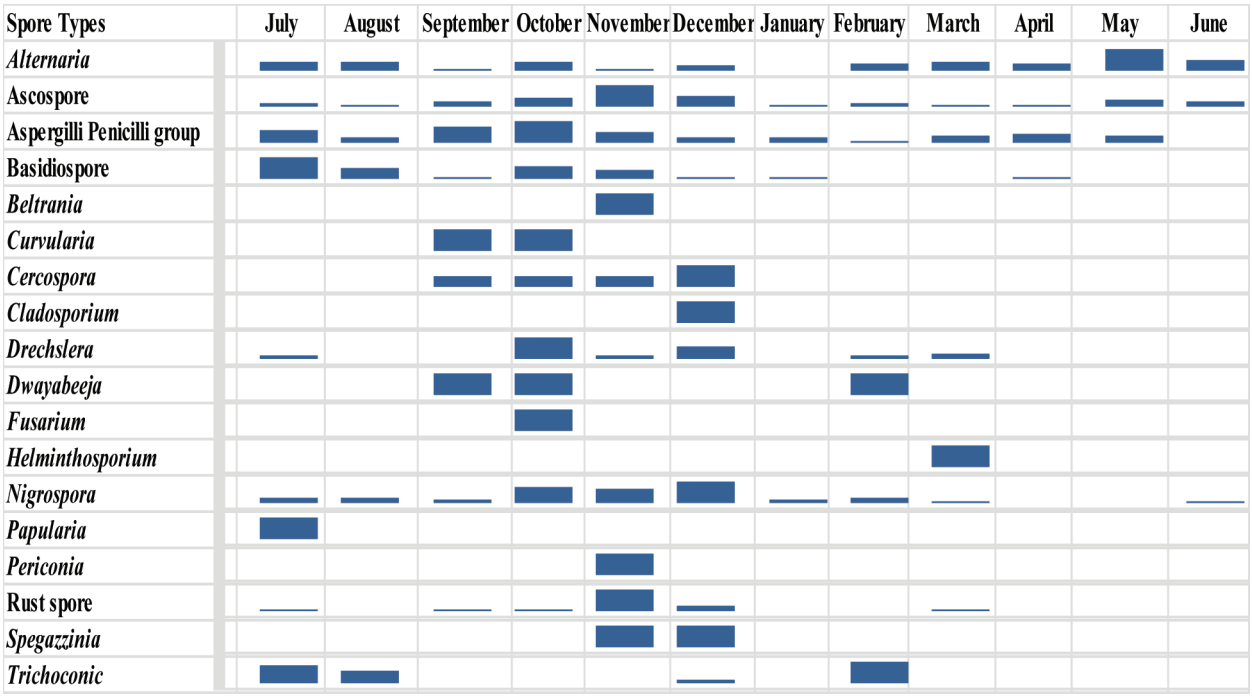

Fig. 5.2: Spore calendar of Bolpur-Santiniketan (July,2018-June 2019)

Table 5.3: Percentage contribution of culturable fungal species to total annual count in Bolpur-Saniniketan (July, 2018 – June, 2019)

Sl. No.	Fungal Spore	Percentage Contribution (%)			
		Bazar (Site A)	Hospital (Site B)	School (Site C)	Residence (Site D)
1.	*Alternaria* sp.	0	0	0	6.13
2	*A. alternate*	0	0	11.83	0.61
3	*A. tennuis*	0	0.65	4.14	4.29
4	*Aspergillus* sp.	1.93	3.94	1.18	3.06
5	*A. flavus*	11.59	0	0	0
6	*A. niger*	7.24	0.65	5.91	5.52
7	*A. ochraceous*	12.07	2.63	2.36	6.74
8	*A. versicolor*	4.83	0	8.87	0
9	*Candida* sp	3.38	1.97	4.14	3.68
10	*Carvularia* sp.	3.38	1.31	2.95	0
11	*C. lunata*	0	0	1.77	1.22
12	*Clodosporium* sp.	0	3.94	0	0
13	*Fusarium* sp.	1.44	0	2.95	1.22
14	*Penicillium* sp.	2.41	23	21.89	31.9
15	*P. brefeldium*	12.56	0	0	0
16	*P. nigricans*	0	0.65	0	0.61
17	*P. oxalicum*	0	0.65	0.59	0
18	*Syncephalastrum* sp.	0	0.65	0	0
19	*Rhizopus* sp.	5.79	1.97	0	0
20	Sterile mycelia	14	51.97	26.03	30.67
21	Unidentified	19.32	5.92	5.32	4.29

Santiniketan area was made (Fig. 5.2). All total 18 different spores were observed in the ambient air. Among them, Ascospore, Aspergilli/Penicilli group (except July), *Alternaria* (except January) and *Nigrospora* (except April and May) were found round the year, while *Beltrania* (Only November), *Fusarium* (Only October), *Helminthosporium* (only March), *Papularia* (Only July), *Periconia* (Only November) and *Cladosporium* (only December) showed their very low concentration in the ambient air (Fig. 5.2).

A total 153460 spores were counted per m^3 of air in the entire study period. The peak spore count was recorded in the month of November, 2018 (33,902/m^3) followed by October, 2018 (23170/m^3) and December, 2018 (16585/m^3). Lowest spore count was recorded in the month of January (4390/m^3) followed by February (5609/m^3) and June (5750/m^3) in 2019 (Fig. 5.3). Among all the fungal spores Ascospores were the dominating fungal spores constitute almost 40% of the total spore count followed by Aspergili-Penicilli group (36%) and Rust spores (8%). *Alternaria* sp. (4%) and Basidiospore (4%) were also contributed a significant amount.

Culturable fungal spores

More than 50% of the total CFUs were constituted by *Aspergillus* and *Penicillium* species. At four sampling sites, each fungal species were found in almost equal percentage, but sterile mycelium showed a prevalent percentage in the entire study (Table 5.3). Mainly four *Penicillium* species [*P. nigricans, P. oxalicum, P. brefeldium* and *Penicillium* sp.(?)] and five *Aspergillus* species [*A. favus, A. niger, A. ochraceous, A. versicolor* and *Aspergillus* sp.(?)] were identified. Highest CFU concentration was demonstrated by *Penicilluim* sp. in the three sampling sites [site D (31.90%), site B (23%), site C (21.89%). *Aspergillus* spp. was the second most dominating fungal species present almost all the sampling sites with highest 12.07% value of *Aspergillus ochraceous* in site A (Table 5.3). In all the sites, a significant percentage of sterile mycelium were found (14% in site A, 51.97% in site B, 26.03% in site C, 30.67% in site D). Other fungal species such as *Candida* sp., *Curvularia* sp., *Carvularia lunata, Cladosporium* sp., *Fusarium* sp. are present in very low concentration.

A total 719 CFU were counted in the entire study period, of which highest count was recorded in January, 2019 (118) followed by December, 2018 (116) and July, 2018 (75). The lowest CFU count were recorded in August, 2018 (24) followed by May, 2019 (37) and April, 2019 (38) (Fig. 5.3).

The present results corroborate with the findings of the previous studies made in Santiniketan and Sriniketan[22,29] and also with the earlier investigation carried out in West Bengal[23, 24]. However, the present study showed slightly difference from the reports of Sreeramulu and Ramalingam[25] and Singh *et al*.[26] done in Visakhaatnam and Dehradoon respectively which may be due to difference in the geographical location of the sampling sites.

Respiratory problem related hospital admission (RPRHA)

From the local hospital survey, a total of 2756 number of respiratory problem related hospital admission (RPRHA) were recorded in Bolpur-Santiniketan area. The admission of highest number of patients was

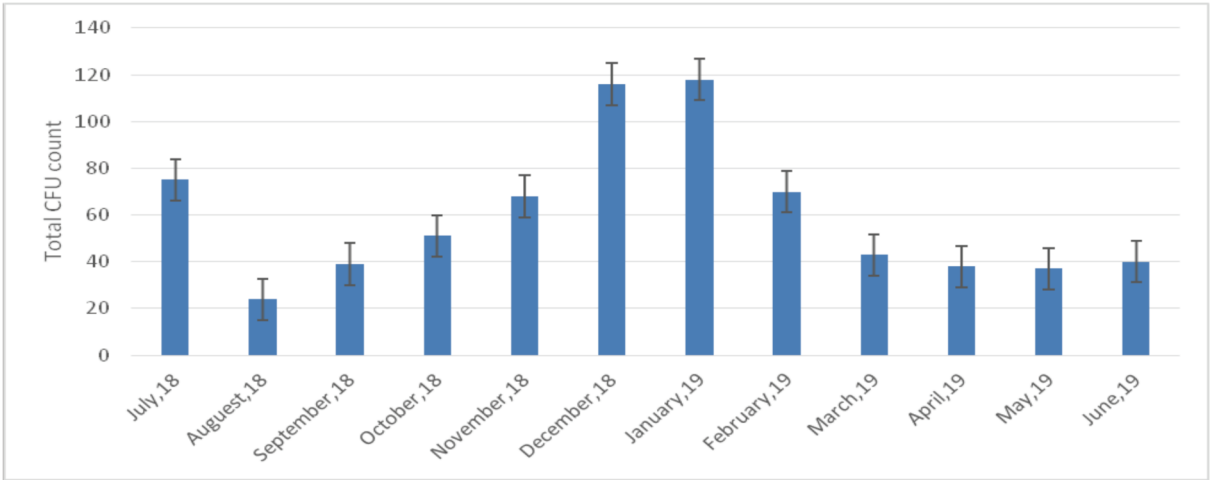

Fig. 5.3: Culturable fungal spore load in the ambient air of Bolpur-Santiniketan (July, 2018-June, 2019).

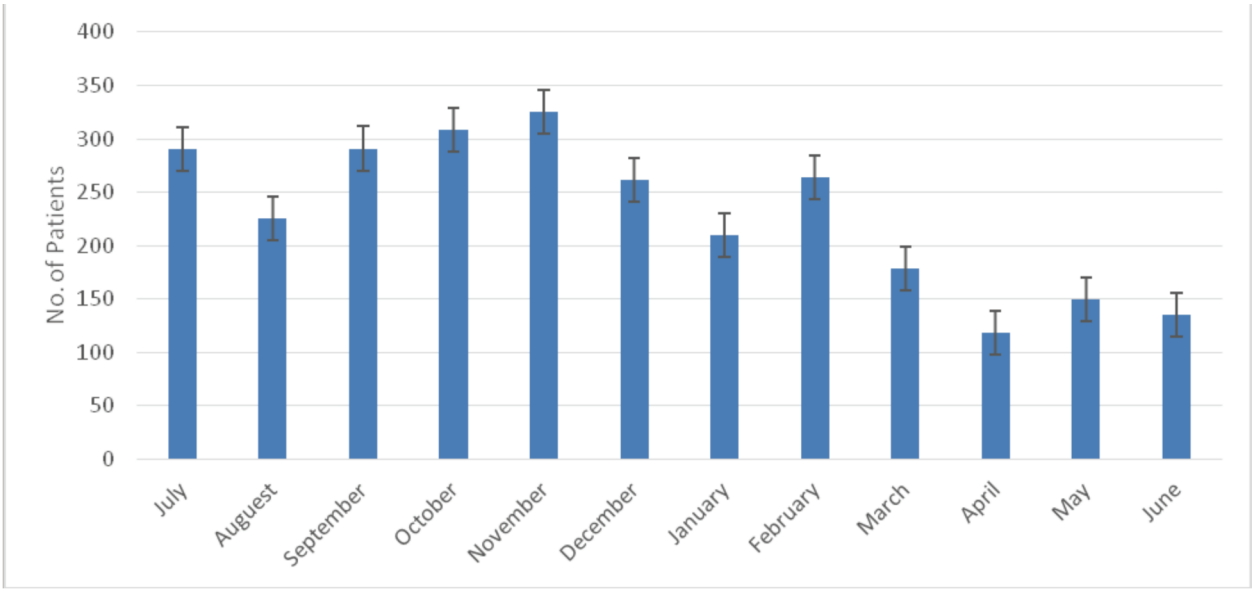

Fig. 5.4: Number of respiratory problem related hospital admission in Bolpur-Santiniketan (July, 2018-June, 2019).

reported in November, 2018 (325) followed by October, 2018 (308) and September (295). However, the lowest hospital admission was recorded in April, 2019 (118) followed by June, 2019 (135) and May, 2019 (150) (Fig. 5.4). From the study it was found that 19% of the total patient (12089) admitted in the hospital suffered from allergy related problems.

Association between RPRHA and different environmental parameters

With regard to meteorological factors the total pollen and spore count showed a negative correlation with the total rainfall (Fig. 5.5). This suggests that when the rain fall is lass the total pollen-spore count in the ambient air would be greater and this happened because the rain washed off the aerospora. In dry season, aerospora have better chances to spread in the air (Fig. 5.5). Similarly, wind speed, average temperature and relative humidity (RH) also showed negative correlation with CFU and total spore count. The CFU count showed its peak in December-January when RH and temperature declined to their lowest points with minimum wind speed. In August-September, when the wind speed, relative humidity and temperature were high the CFU count was found to be lowest (Fig. 5.5).

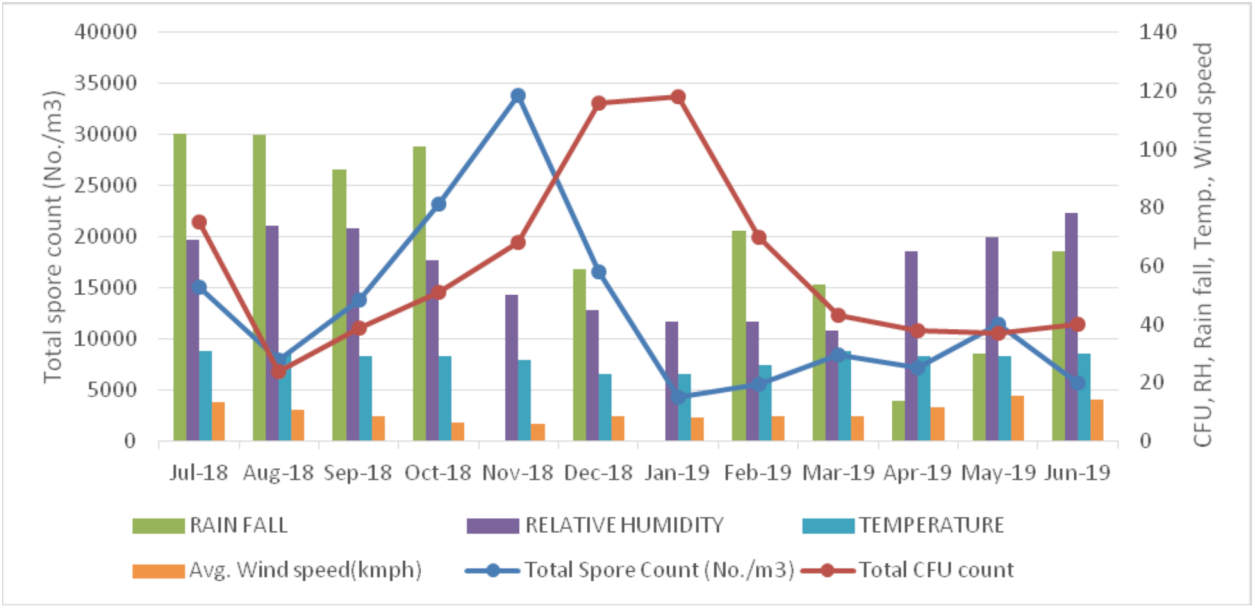

Fig. 5.5: Monthly correlation of CFU and Total spore Count with Average Rainfall, Relative Humidity and Average temperature in Bolpur-Santiniketan (July, 2018-June, 2019).

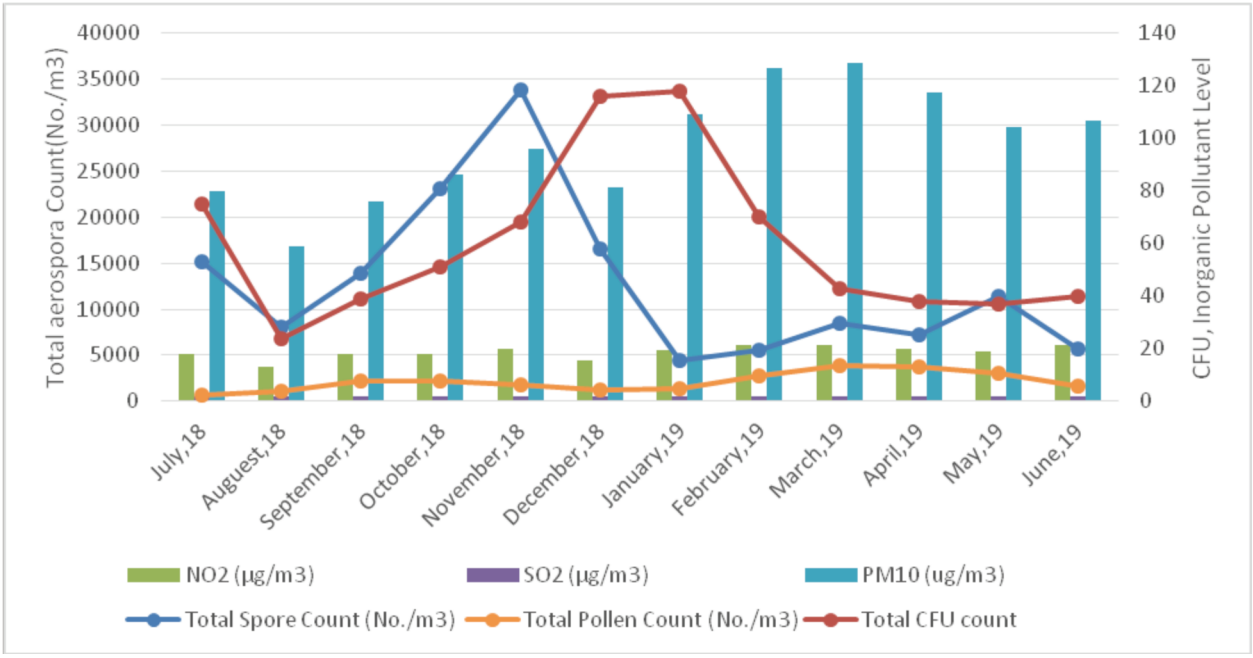

Fig. 5.6: Correlation between Inorganic pollutant level (SO_2, NO_2, PM10), Total aeropora Count and CFU count in Bolpur-Santiniketan (July, 2018-June, 2019).

From environmental pollution (Inorganic Pollutants) data of Bolpur-Santiniketan (Fig. 5.6) it was clear that SO_2 plays no such role in the concentration of total spore as well as CFU count or in pollen load. But NO_2 in the ambient air showed a positive correlation with the total aerospora count and CFU count (Fig. 5.6). It was found that in the months of October, November, December and January the NO_2 level and total spore and CFU count were high. March and April also showed a significant increase in NO_2 amount along with total pollen count, while their level were low in August, NO_2 level also showed a clear positive correlation with PM10. Months like November, January, February, March and June showed a high level of PM10 as well as NO_2 level, while they showed low concentration in August. But association between PM10 and Total spore /CFU count did not demonstrate any clear correlation. Though we recorded PM10 in a significant high amount in November and low amount in August when the total spore count as well as CFU count were high and low respectively (Fig. 5.6), on the contrary PM10 value confirmed a clear positive correlation with total pollen count.

Synergistic associations of pollen, spore, inorganic pollutants, and/or climatic factors have been suggested to affect Asthma Related Hospital Admission by some

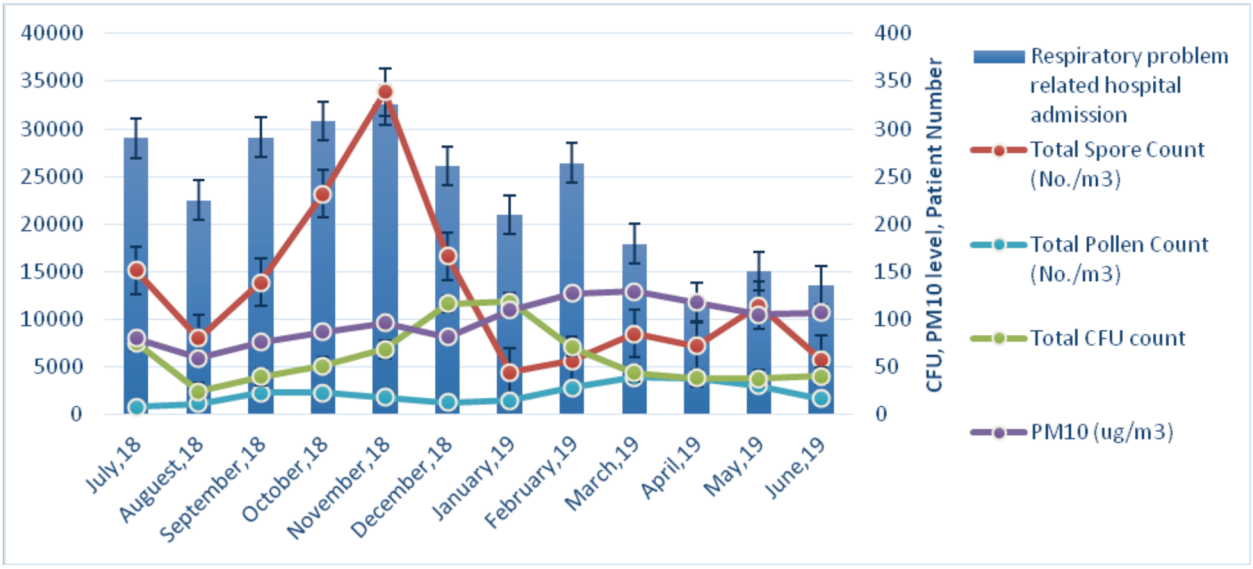

Fig. 5.7: Correlation between Respiratory problem related patients, PM10 level, Total aerospore Count and Culturable fungal spores count in Bolpur-Santiniketan (July, 2018-June, 2019).

earlier workers[27]. In the present study we made an effort to find out the effects of possible aeroallergen – inorganic pollutant interactions in Bolpure-Santiniketan. We found a direct correlation between culturable fungal spore, aerospora level, PM10 level and respiratory problem related hospital admission (Fig. 5.7). A sharp rise in Asthma related hospital admission could be observed during September from countries like Australia and Canada, which is known as the "September epidemic"[28]. In contrast, our study confirms that respiratory problem related hospital admission in Bolpur-Santiniketan (Fig. 5.7) increases, with predictable regularity. A gradual increase can be seen from September to November, which was recorded highest number of respiratory patients admission. Similarly, in PM10 level and ambient environment, total spore count reached its high level in November, and some special types of pollen (*Alstonia* sp., *Eucalyptus* sp., *Borassus* sp. and some grass) accomplished their peak during that period (Fig. 5.7). A positive correlation was observed between these parameters. Again, lowest respiratory problem related patients number recorded in August, aerospora count were low (Fig. 5.7).

Although in the month of February there was a significant increase in the number of respiratory problem related hospital admission (325) regardless the low total pollen count and low value of culturable fungus (70 CFU). However, there was extreme level of total spore count (33,902/m^3) and high value of PM10 (116.99 $\mu g/m^3$) suggesting that increase in the number of patients could be because of the elevated total spore count and PM10 level in the ambient air (Fig. 5.7).

CONCLUSION

The present study demonstrates that respiratory problem related hospital admission in the town like Bolpur-Santiniketan, West Bengal exhibited a nearly regular seasonal pattern that was governed by the allergenic content of the atmosphere, especifically the allergenic fungal spores like Ascospores, Aspergilli/Penicilli group, *Curvularia* sp., *Fusarium* sp., *Cladosporium* sp. and *Alternaria* sp. Respirable particulate matter (PM10) also contribute a significant role in this matter. Pollen grains like, *Alstonia* sp., *Eucalyptus* sp., *Phoenyx* sp., *Borassus* sp., *Cassia* sp., *Acacia* sp., *Madhuca* sp., Poaceae (grasses) also play a moderate role in respiratory problem related hospital admission[29]. The present study is the first of its kind to describe and correlate between the seasonal pattern of respiratory problem related hospital admission and its association with the outdoor spore and pollen content, meteorological factors and pollutants level of Bolpur-Santiniketan - a rapidly developing and a major tourist and educational place of the lateritic zone of Indian subcontinent. The results revealed that total spore content in the ambient air and PM10 plays an important role in the respiratory problem related hospital admission in Bolpur-Santiniketan. The study illustrates an overall picture on the aeroallergens, meteorological factors, atmospheric pollutant level and respiratory problem related hospital admission in the town. The pollen-spore calendar of Bolpur-Santiniketan made in the present study may guide the tourists for the suitable time to the visit this place, doctors for treating the respiratory problem related patients and researcher for their future studies.

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SEASONAL DISTRIBUTION OF AIRBORNE FUNGI AT THE PERIPHERY OF RAIPUR CITY, CHHATTISGARH, INDIA

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Raipur is the capital city of Chhattisgarh state. The city is located centrally in the state of Chhattisgarh. Fungal spores are widely distributed all over the world, which constitute the major component of the air-borne microflora. Various environmental factors affect the distribution of fungi in a particular area. Occurrence and the type of fungal species change with the season and geographical location. Seasonal variation affects the distribution of fungi in a particular area. To investigate this fact, a Survey of air-borne fungi was carried out from March 2018 to February 2019 by using the Gravity petri-plates method containing PDA (Potato Dextrose Agar) medium. The study recorded a total of 35 fungal species belonging to 14 fungal genera. The dominant species noted were *Aspergillus niger, A. flavus, A. fumigatus, A. oryzae, Alternaria alternata, Cladosporium* sp. *Curvularia lunata, Fusarium* sp. and *Phoma pomorum*. It was observed that medical and phytopathological consequences are associated with fungal spores. In that respect, study elucidated the distribution and occurrence of air-borne fungi during the year 2018-2019 at the periphery of Raipur city.

Key Words: Airborne fungi, Seasonal distribution, Phytopathological, Fungal spores.

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INTRODUCTION

Raipur is the capital city of Chhattisgarh state in India. It is situated between 22° 33'N to 21° 14'N Latitude and 82° 6' to 81° 38'E Longitude. The city is located centrally in the state of Chhattisgarh, and now serves as a regional hub for trade and commerce for a variety of local agricultural and forest products. Increased urbanization and industrialization in recent time has made a significant impact on air quality of the area. Seasonal variation affects aero-mycoflora of the area. The microbial population of the atmosphere at any place constitutes its aero-spora. Fungal spores are not equally distributed in the environment; their distribution varies according to geographical location and metrological conditions. The concentration of airborne fungal spores has been linked to wind, humidity, temperature, rainfall, altitude, vegetation and various specific reservoirs of contamination. Also, fungal propagative units may be dispersed in the air by insects[1]. Fungal spores are part of air quality depending on the time of the day, weather, season, climatic conditions, and local source of spores[2]. Based on the microbiological analysis of air samples from inhabited areas, it was reported that airborne fungi are among the most common organisms correlated with the air pollution that have adverse effects on human health as well as causing plant diseases. In light of the above knowledge, the present investigation on airborne fungal flora is essential to understand the deposition and dissemination of fungal spores at the periphery of Raipur city.

MATERIALS AND METHODS

Description of the study site

The study was conducted at the periphery of Raipur city, Chhattisgarh, India. 4 different villages in surrounding of Raipur city, were selected viz. Chandanidih (21° 15'NL and 81° 32'EL), Zora (21°v23'NL and 81° 71'EL), Boriakala (21° 19'NL and 81° 64'EL) and Dhaneli (21° 33'NL and 81° 65'EL). The present study was conducted for a period of one year that is from March 2018 to February 2019.

Sampling and calculation

The culture plate exposure method was adopted for trapping the airborne fungi. PDA (Potato, Dextrose and Agar) was used as a culture medium. 10 ml of sterilized

PDA medium was aseptically poured into petri plates and allowed to solidify. Five petri plates containing potato dextrose agar (PDA) medium were exposed in the air for 5-10 minutes at 1 meter above the ground level in study sites. The study was conducted in a fortnight interval. The exposed petri dishes were sealed with the help of cello tape and brought to the laboratory and incubated for 3 to 6 days at 26 ± 1ºC. After incubation, fungal colonies were counted, isolated and identified with the help of literature[3,4]. Microscopic slides of air borne fungi were prepared using glycerin gel as mounting media and lactophenol and cotton blue stain by following Jadhav and Tiwari[5]. The percentage contribution of the air borne fungi was calculated by using the formula of Jadhav[6]:

$$\% \text{ Contribution} = \frac{\text{Total No. of colonies of species in all the observations taken together}}{\text{Total no. of colonies of all the species}} \times 100$$

RESULTS AND DISCUSSION

In the present study, 3221 fungal colonies, 35 fungal species belonging to 14 fungal genera were recorded (Table 6.1). *Aspergillus niger, Penicillium* sp., *Alternaria alternata, Cladosporium* sp., and *Curvularia* species were dominant in all three seasons. A majority of fungi were members of Anamorphic fungi, while 3 fungal species belonging to Zygomycotina and 6 species to Ascomycotina were recorded.

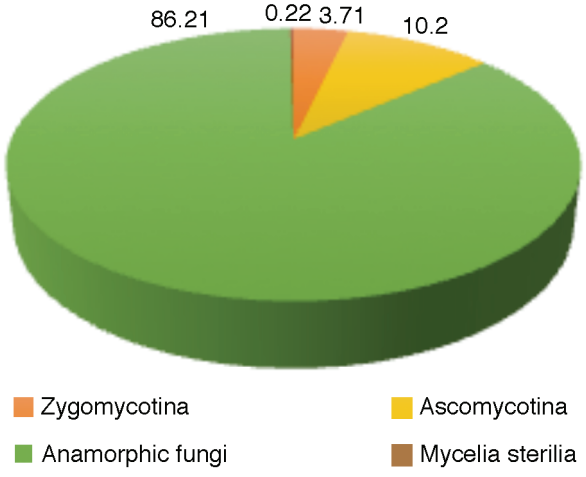

Fig. 6.1: Percentage contribution of fungal species of Zygomycotina, Ascomycotina, Anamorphic fungi and Mycelia sterilia in total fungal class, at the periphery of Raipur city during 2018-2019.

In present studies, seasonal variation of the air borne fungi was also observed. In the Summer season, 9 fungal genera were recorded in Chandanidih, 11 each in Zora and Boriakala, 10 in Dhaneli. In the rainy season maximum of 13 fungal genera and 26 fungal species were recorded in Dhaneli followed by 12 fungal genera and 26 fungal species in Zora, 12 fungal genera and 23 fungal species in Boriakala and 10 fungal genera and 18 species in Chandanidih (Fig. 6.1 and 6.2). Air borne fungi comprised *Aspergillus niger, Penicillium* sp., *Alternaria alternata, Cladosporium* sp., and *Curvularia* as a dominant species.

In the winter season, the number of fungal spores was further increased. 13 fungal genera and 29 species were recorded in Chandanidih followed by 14 fungal genera and 27 species in Dhaneli, 12 fungal genera and 25 species in Zora and 11 fungal genera and 24 species in Boriakala (Fig. 6.1 and 6.2). It was observed that *Aspergillus niger, Alternaria alternata, Cladosporium* sp., *C. oxysporum, Curvularia lunata, Penicillium* sp. were most frequent fungi throughout the year followed by *Khuskia oryzae, Mucor* sp. and *Mucor cymosus*, while; *Alternaria citri, Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigatus, Acremonium strictum, Curvularia clavata, Fusarium* sp. and *Fusarium oxysporum* were moderately found fungi. On the contrary, *Gongronella butleri, Emericella nidulans, Phoma destructive, P. pomorum, Drechslera australiensis*, and *Mycelia sterilia* were less frequent fungi amongst the total air borne fungi. Verma and Khare[7] also reported the *Aspergillus, Cladosporium* and other fungal species as most dominant throughout their study period. Verma and George[8] also noted *Aspergillus niger* as most frequent in the air of Jabalpur. *Aspergillus* was found to be the most predominant in the air of Raipur observed by Tiwari and his group[9-12].

The result also indicated that the maximum percentage contribution was of *Aspergillus niger* (17.52%), *Cladosporium oxysporum* (11.86%), *Alternaria alternata* (9.97%), *Curvularia lunata* (6.71%), *Khuskia oryzae* (5.53%), *Cladosporium* sp. (5.50), *Aspergillus flavus* (5.38%), *Aspergillus fumigatus* (4.17%), *Penicillium* sp. (3.64%). Zygomycotina was represented by *Mucor cymosus* (1.65%), *Mucor* sp. (1.62%), *Gongronella butleri* (0.44%). In Ascomycotina 06 fungal species were recorded and their % contribution was *Khuskia oryzae* (5.53%), *Phoma glomerate* (1.43%), *Phomosis vexans* (1.03%). *Phoma destructive* (0.84%), *Phoma pomorum* (0.69%), *Emericella nidulans* (0.50%). Maximum contribution of *Khuskia oryzae* (2.02%) and *Emericella*

Table 6.1: Seasonal distribution of Air borne fungi at the periphery of Raipur city, Chhattisgarh, investigated during the year 2018-19.

Sl. No.	Name of Aeromycoflora	Chandanidih			Zora			Boriakala			Dhaneli			Total no. of Fungal colonies	%Contribution in total fungal class
		S	R	W	S	R	W	S	R	W	S	R	W		
1.	*Mucor cymosus*	-	05	05	-	07	11	01	05	-	03	06	10	53	1.65
2.	*Mucor* sp.	08	06	05	02	04	00	03	07	06	03	05	-	52	1.62
3.	*Gongronella butleri*	-	-	-	-	-	-	-	-	-	-	02	12	14	0.44
4.	*Emericella nidulans*	-	-	-	-	05	-	-	01	03	-	05	02	16	0.50
5.	*Khuskia oryzae*	-	-	32	25	09	19	11	12	23	-	08	39	178	5.53
6.	*Phoma destructive*	-	14	03	03	-	-	-	-	-	-	03	04	27	0.84
7.	*Phoma glomerate*	-	11	-	-	-	-	-	35	-	-	-	-	46	1.43
8.	*P. pomorum*	-	-	03	-	01	01	13	-	-	02	02	-	22	0.69
9.	*Phomosis vexans*	-	-	05	-	01	14	-	01	08	-	-	04	33	1.03
10.	*Alternaria alternata*	35	28	56	15	17	45	19	23	28	11	20	24	321	9.97
11.	*A. tenuissime*	02	-	-	-	05	09	-	02	-	-	-	02	20	0.63
12.	*A. redicina*	-	-	05	-	-	04	10	10	11	-	-	02	32	0.99
13.	*A. citri*	08	04	05	01	02	06	-	-	03	01	01	01	32	0.99
14.	*Aspergillus niger*	47	55	80	35	45	30	52	37	27	50	56	50	564	17.52
15.	*A. flavus*	13	37	21	09	07	14	-	23	15	13	06	15	173	5.38
16.	*A. oryzae*	-	-	03	08	07	06	09	10	11	01	03	21	79	2.46
17.	*A. fumigatus*	13	18	23	10	14	10	-	10	14	01	04	17	134	4.17
18.	*A. nidulans*	-	-	03	-	04	-	03	01	-	-	03	06	20	0.63
19.	*A. sclerotiorum*	-	-	04	-	01	01	-	06	03	-	-	-	15	0.47
20.	*A. versicolor*	-	-	04	01	04	05	-	-	02	03	23	-	42	1.31
21.	*Acremonium strictum*	-	05	13	07	10	05	-	10	16	06	20	14	106	3.30
22.	*A. implicatium*	-	-	09	-	06	-	-	-	-	01	05	-	21	0.66
23.	*A. terricola*	-	-	03	-	-	-	-	-	-	-	07	25	35	1.09
24.	*Cladosporium* sp.	10	20	10	08	06	15	17	13	23	27	15	13	177	5.50
25.	*C. oxysporum*	55	20	35	23	22	45	14	14	50	30	40	34	382	11.86
26.	*Curvularia lunata*	25	15	26	15	15	05	11	18	13	25	21	27	216	6.71
27.	*C. clavate*	05	18	03	-	02	03	-	07	02	04	10	03	57	1.77
28.	*Drechslera tetramera*	-	-	26	-	-	06	-	-	10	-	-	05	47	1.46
29.	*D. australiensis*	-	-	02	-	-	01	-	-	-	-	-	06	09	0.28
30.	*Fusarium* sp.	02	05	02	02	02	02	-	07	03	05	03	03	36	1.12
31.	*F. oxysporum*	-	10	07	03	06	06	03	02	01	03	08	07	56	1.74
32.	*F. pallidoroseum*	03	09	05	03	07	03	06	-	06	-	-	-	42	1.31
33.	*Monilia fructigena*	16	-	-	05	-	-	07	-	-	08	03	01	40	1.25
34.	*Penicillium* sp.	03	19	18	04	04	05	08	10	13	05	09	19	117	3.64
35.	*Mycelia sterilia*	-	-	05	-	-	-	01	-	01	-	-	-	07	0.22
	Total no. of colony season wise	245	279	419	177	260	271	187	264	292	202	286	366	3221	

S (Summer), R (Rainy), W (Winter), (-) indicate absence of species.

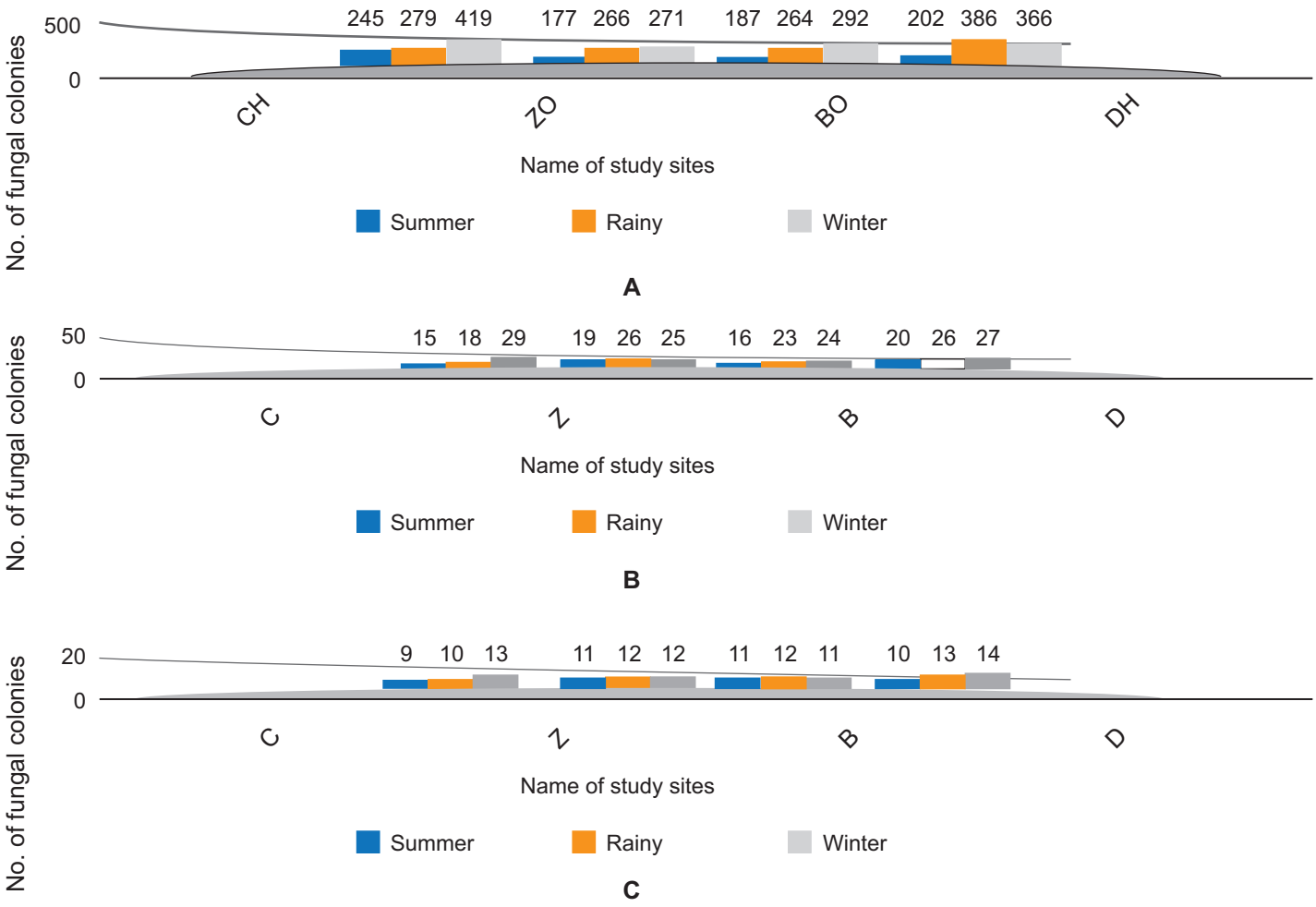

Fig. 6.2: Total no. of fungal colony (A), Total no. of fungal species (B) and Total no. of fungal genera (C) in different season and in different sites at the periphery of Raipur city, investigated during the year 2018 – 2019.

nidulans (0.62%) was reported by Singh[12] in the aeromycoflora of Raipur. However, a comparatively higher % contribution was recorded in the present study. Results also indicated that rainfall had direct or indirect impact on ascospores released. The fact was also noted by Meredith[13]. In Anamorphic fungi, a total of 25 fungal species were recorded and % contribution was highest to the total air borne fungi. *Aspergillus niger* (17.52%), *Cladosporium oxysporum* (11.86%), *Alternaria alternate* (9.97%), *Curvularia lunata* (6.71%), were major contributors in the group of Anamorphic fungi, whereas *Penicillium* sp. (3.64%), *Acremonium strictum* (3.30%), *Aspergillus oryzae* (2.46%), etc. were moderate contributors, while; *Drechslera australiensis* (0.28%) was least contributor to the total air borne fungi. Khan *et al*.[14] recorded maximum contribution of Aspergillus and Cladosporium in the aeromycoflora of Raipur. Similar was noted during the present study. Mycelia sterilia (0.22%) was the least contributor to the total air borne fungi.

Aspergillus sp. was the most common genus found to be the most predominant in the air of Raipur was recorded by Tiwari and Sahu[9], and Sahu[15]. Highest concentration of *Aspergillus* sp. was observed in places, like Aurangabad by Tilak and Srinivasulu[16], Raipur by Sharma[17], Tiwari and Jadhav[18], Tiwari and Tiwari[19], Pondicherry by Nayak and Nanda[20]. Similar results were also noted in the present study. Similarly, *Cladosporium* sp. was predominant and most frequent airspora recorded in present study was also reported Saroja and Bhagyalaxmi[21] at Hyderabad, Mishra *et al*.[22] in Sonbhadra (U.P.), Khan and Shrivastava[23] at Bilaspur.

CONCLUSION

In the tropics, the climatic condition is very favorable to the growth of fungi, resulting in a high concentration of spores in the air. The study revealed the qualitative and quantitative data of the air borne fungi at the periphery of Raipur city. A total of 35 species of fungi were isolated and identified during the year and their occurrence with seasonal variation was noted. In the winter and rainy season, the highest spore concentrations were detected and decrease spore concentrations in the summer season. The temperature and humidity

provide the optimum conditions for the development of fungi in the winter and rainy season and the summer season may be related to the absence of rain and low humidity, which are not optimum conditions for the growth of fungal spores[24]. During the study period, the dominant fungal species were the *Aspergillus niger, Cladosporium oxysporum*, and *Alternaria alternata*.

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GAMMA RAY MEDIATED IMPROVEMENT OF *SCHEFFEROMYCES STIPITIS* FOR ACETIC ACID AND ETHANOL TOLERENCE AND ENHANCED XYLOSE FERMENTATION TO ETHANOL

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Mutational improvement of xylose fermenting yeast, *Schefferomycesstipitis* (NCIM 3507) applying mild gamma ray treatment for better ethanol and acetic acid tolerance suitable for lignocellulosic ethanol fermentation was carried out. Mutagenesis with gamma ray @ 5, 10 and 15 Krad followed by adaptation to acetic acid and ethanol led to the selection of three different mutants; namely, G50, G120 and G200. Patching of cells on agar supplemented adaptation medium was found to give better adapted strain as compared to spreading of cells on the same medium. The mutant G200, isolated from viable cells irradiated with gamma ray of dose 15 Krad followed by adaptation showed better ethanol fermentation rate (13.5 g/l) with initial xylose concentration of 30 g/l and acetic acid (0.2% v/v) as well as ethanol (70 g/l) tolerance. Mild gamma treatment and adaptation involving patching technique on semisolid adaptation medium may, therefore, be important methods for strain improvement of this pentose fermenting yeast for better biotechnological applications.

Key Words: Gamma mutagenesis, *Schefferomyces stipitis*, acetic acid-tolerance, ethanol-tolerance.

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INTRODUCTION

Lignocellulose has been considered to be an attractive raw material for fuel ethanol production[1]. It contains about 10-40 % of xylose in its carbohydrate fraction[2], whose utilization for ethanol conversion is, therefore, important for lignocellulosic-ethanol technology[3]. In order to release free sugar monomers from the hydrolysis of lignocellulose matrix, the latter is required to be subjected to one or more pretreatment steps. However, during the pretreatment of lignocellulosic biomass, a broad range of inhibitory compounds are formed[4], of which more than 35 potential microbial inhibitors have been identified[5]. A number of methods have been suggested for removal of most of these inhibitors[6], acetic acid, however, remains a problem which causes inhibition of yeasts growth[7] and subsequent inhibition of fermentation of hydrolysate[8]. A limited number of naturally occurring xylose-fermenting yeast species, including S. stipitis, *Candida shehatae* and *Pachysolen tannophilus* have been studied[9]. Of which *S. stipitis* has been found to be a preferred one for lignocellulosic-ethanol technology for various reasons[10]. The pentose fermenting yeasts vis-à-vis hexose fermenting *Saccharomyces cerevisiae*, however, suffer from such weaknesses as low specific ethanol productivity[11] and low ethanol tolerance[12]. Strain improvement has, therefore, been regarded as important for the development of appropriate lignocellulosic-technology applying *S. stipitis*[13,14]. The present study provides the first time report of the use of mild dose of gamma ray as mutagen and patching of cells on adaptation medium as a technique. The technique is useful for simultaneous improvement of acetic acid and ethanol tolerance and rate of ethanol production in *S. stipitis* suitable for the production of lignocellulosic ethanol.

MATERIALS AND METHODS

Yeast and growth media

The yeast, *S. stipitis* NCIM 3507 was procured from National Collection of Industrial Microorganism, NCL, Pune (India). All strains used in this study were grown and maintained at 25°C and 4°C respectively on agar slants containing xylose, 20 g l^{-1}; yeast extract, 3 g l^{-1}; malt extract, 3 g l^{-1}; peptone, 5 g l^{-1}; and agar, 20 g l^{-1}.

Mutagenesis

Freshly grown yeast cells in YEPD broth was isolated by centrifugation, washed with sterile distilled water and re-suspended in the same. The suspension was

serially diluted to obtain about 10^5 cells/ml. 10 ml of this diluted cell suspension was taken in the sterile petridish and subjected to gamma irradiation (doses: 5, 10 and 15 Krad) at Jawaharlal Nehru Cancer Institute, Bhopal. The irradiated sample and non-irradiated suspension were further diluted to 10^{-4} and 0.1 ml of thus diluted suspension was used for viable counts.

Primary screening

Suitably diluted irradiated-cells were inoculated on PDA supplemented with ethanol (0.1% v/v) and acetic acid (30 g/l) and incubated at 30°C for two days to test for ethanol and acetic acid tolerance. Altogether, twenty strains showing faster growth from each treatment were selected for further screening.

Secondary screening for fermentation

Fermentation was carried out in large Durham tubes[15]. The tubes were filled with 15 ml yeast extract medium (0.5 % yeast extract with 50 mM xylose) supplemented without or with acetic acid (0.001, 0.01, 0.02, 0.05, 0.1 and 0.5% v/v) added prior to sterilization. Inoculum was prepared by inoculating 24 h culture of yeast cultivated on a slant YPX agar (1% yeast extract, 2% peptone, 2% xylose and 1.2% agar), into 5 ml of yeast extract broth. Each tube was inoculated with 500 μl of fresh inoculum of 0.1 OD and incubated at 25°C for one week. The CO_2 column as a result of fermentation created in the inserted tube was measured as function of time to estimate rate of evolution of CO_2 (= fermentation).

Adaptation to acetic acid and ethanol

The selected mutants were either inoculated in PD broth (Potato extract, Dextrose) or spread or patched onto PDA containing either acetic acid (0.2 or 0.3 % v/v) or ethanol (50 g/l) or in combination of both (acetic acid : ethanol = 0.3% : 40 g/l) (PDAae) and incubated for 30 days at 25oC. The broth was incubated on shaker at 150 rpm. The medium was diluted suitably and 0.1 ml of it was inoculated to PDAae and faster growing colonies were selected. Faster growing colonies semi-solid adaptation media were also diluted to 10^{-4} and spread on PDAae. The colonies showing normal growth on PDAae were selected as adapted strains.

Growth kinetics

The dry weight of biomass was determined by centrifugation of culture for 15 min at 6000 rpm, washing the cells with distilled water and drying the precipitated mass at 100°C for 24 h and weighing the residues, in three replicates[16]. Cell concentration was also determined by measuring its absorbance spectrophotometrically at 620 nm.

Ethanol tolerance assay

The mutants were grown in 50 ml of YFM, supplemented with increasing concentration of ethanol in such as 30, 40, 50, 70 and 100 g/l concentration on shaker at 150 rpm. The culture was incubated at 150 rpm and 30°C for 48 h and growth was determined by taking out medium at 6h-intervals and measuring OD_{620} spectrophotometrically[17]. The concentration of alcohol, at which the growth of the yeast was just inhibited, was recorded[18]. The concentration of ethanol at which half of the highest growth rate obtained was deemed to be E_{50}.

Acetic acid tolerance assay

YEPD medium supplemented with acetic acid to the final concentration of 0.001%, 0.01%, 0.02%, 0.05%, 0.1%, 0.2%, 0.3% and 0.5 % (v/v) were used to test growth of the mutants. The culture was incubated at 150 rpm and 30°C and growth was measured spectrophotometrically at 6 hour intervals.

Sugar Utilization

Total reducing sugar was estimated by DNS method[19] and pentose (xylose) was assayed by means of orcinol reaction[20].

Ethanol Fermentation

Ethanol fermentations were carried out at 30°C in 250 ml Erlenmeyer flasks having 100 ml YFM containing xylose as sole carbon source on a shaker at 150 rpm. Ethanol concentrations were determined through the laboratory scale distillation of fixed volumes of culture fermentation broth[21]. Ethanol in distillate was estimated using standard method[22]. The 20-subculture generations of the mutants were tested for their rate of fermentation in presence d-xylose, every time in triplicate.

Statistical Analysis

All measurements were performed three times (n = 3) using the same sample, and statistical analysis was performed manually. Statistical significance of the means was evaluated using one-way analysis of variance.

Subsequent comparisons were performed using the LSD test. Differences were accepted as significant when $P < 0.05$.

RESULTS AND DISCUSSION

The viability count of γ-irradiated yeast-cells with different doses (viz., 5, 10 and 15 Krad) exhibited about 10%, 15% and 20% mortality, respectively. However, there was no conceivable phenotypic-change exhibited by any of these mutants. Further screening led to selection of twenty vigorous colonies tolerant to 0.1%-acetic acid and 30 g/l-ethanol.

The sixty strains thus isolated were tested for the rate of evolution of CO_2 in presence of xylose as the sole carbon source. They showed different rate of CO_2 evolution (data not shown). One each fastest CO_2 evolving strain viz., G50, G120 and G200 from the population of cells irradiated with 5, 10 and 15 Krad respectively, was finally selected. Growth kinetics of mutants (G50, G120 and G200) vis-à-vis WT showed that in the early phase (6h) WT surpassed all the mutants. After 48 h, however, G120 showed slight edge (by 8%) over WT. Other mutants maintained slower growth, the percentage retardation showed by G50 and G200were 47 and 26 respectively (Fig. 7.1).

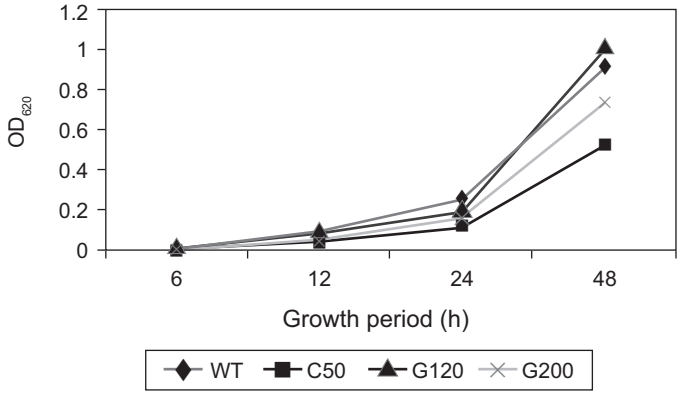

Fig. 7.1: Growth (OD_{620}) pattern of WT (■), G50 (♦), G120 (▲) and G200 (×). (Experimental conditions are described in materials and methods).

Although, the techniques of directed mutation have largely replaced the traditional techniques of spontaneous mutation, the latter has yet not lost relevance completely[13]. Alignocellulosic ethanol technology can only be viable if it takes account of pentose in addition to hexose sugars[3]. Pentose fermenting yeast, therefore, holds the key for a viable lignocellulosic ethanol technology. *S. stipitis* is one of the most promising pentose fermenting yeasts, whose further improvement has been taken up as an area of prime research all over the world[12, 22, 23].

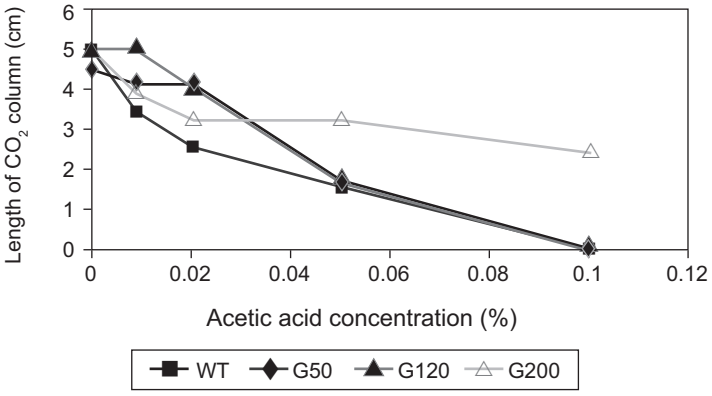

Fig. 7.2: Effect of acetic acid on the rate of evolution of CO_2 as a function of time.

Among the three mutants, G120 showed highest growth rate, higher than even WT. It was concurrently highly tolerant to 0.01% acetic acid in xylose fermentation medium, although above this concentration this mutant showed a sudden retardation. On the other hand, G200 showed intermediate growth rate among all strains and WT (Fig. 7.1), but showed ability to ferment xylose at the highest concentration of acetic acid (Fig. 7.2). The two mutants, therefore, seem to have adopted different strategies to adapt themselves to overcome the effect of acetic acid. As much as 490 of the 650 determinants of tolerance to acetic acid were identified in *S. cerevisiae* involved in tolerance to this weak acid. However, presence of higher concentration of potassium ion was found to improve the expression of maximal tolerance to acetic acid[24]. Acetic acid has been found to affect yeast cell adversely[25] through lowering cytoplasmic pH and inhibiting the activity of some enzymes, especially endolase, phosphoglyceromutase, aldolase and triosephosphate isomerase[8, 26]. These mutants may provide further insight into acetic acid tolerance.

While ethanol tolerance remained unaltered in the mutants, an improvement of E_{50} (half of the maximum growth-rate in presence of ethanol) was showed by G200 (40 g/l). The growth-rate of WT and mutants in presence of different concentration of ethanol was, however, variable. At lower ethanol concentration (30 g/l) it was higher for WT, while at higher concentration (40 g/l onwards) G200 showed higher growth. Moreover, while G50 and G120 showed conceivable growth up to a 50 g/l of ethanol concentration, WT and G200 showed up to 60 and 70 g/l respectively (Table 7.1).

Table 7.1: Growth of WT and mutants at different concentrations of ethanol, grown for 48 hours in YM broth containing D-xylose as major carbon source.

Yeast Growth (OD_{620}) in presence of ethanol (g/l) at different periods (h)

		30			40			50			60			70	
Strain	6h	24h	48h	6h	24h	48h	6h	24h	48h	6h	24h	48h	6h	24h	48h
WT	0.03	0.23	0.02	0.57	0.18	0.32	0.02	0.14	0.14	0.01	0.05	0.05	0.01	0.02	0.02
G50	0.01	0.13	0.30	0.01	0.11	0.12	0.01	0.08	0.08	0.01	0.02	0.02	0.01	0.02	0.02
G120	0.01	0.10	0.23	0.02	0.15	0.29	0.01	0.07	0.07	0.01	0.02	0.02	0.01	0.02	0.02
G200	0.02	0.16	0.44	0.02	0.19	0.44	0.02	0.14	0.15	0.02	0.09	0.10	0.01	0.05	0.05

In YM broth supplemented with various concentration of acetic acid, the growth of G200 was better than WT (Fig. 7.2). In xylose fermentation medium, while all strains were able to tolerate 0.001% acetic acid, only G120 could evolve CO_2 (ferment) in presence of 0.01% acetic acid and the rate was same as in presence of 0.001% acetic acid. Moreover, only G200 could ferment xylose into ethanol after 1 week at higher concentration of acetic acid (0.2%). Other strains, i.e., WT and G50 showed complete inhibition of fermentation above 0.01% acetic acid concentration. In presence of ≤ 0.2% (v/v) concentration of acetic acid in xylose medium, all strains, i.e., WT and its mutants showed complete inhibition of ethanol fermentation (Fig. 7.3).

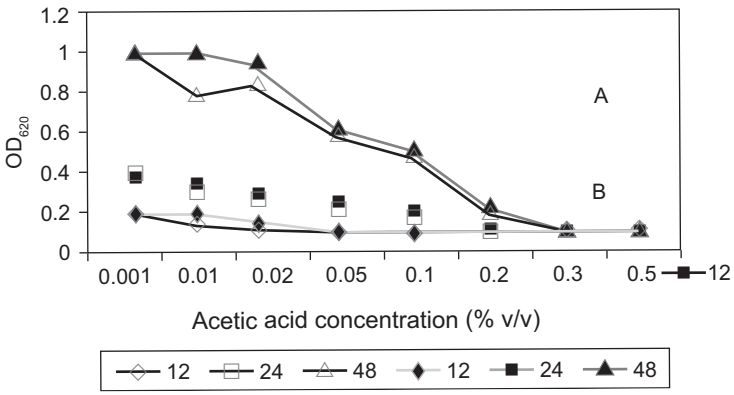

Fig. 7.3: Growth (OD_{620}) of G200 (▲♦■) and WT (Δ◊□) at 12 h(♦◊), 24 h (■□)and 48 h(▲Δ) in presence various concentration of acetic acid. Experimental conditions are described in materials and methods.

All the three selected mutants were able to ferment glucose, mannose, galactose and xylose (data not shown). Ethanol fermentation rate was found higher in case of G120 and G200, it was lower for G50 as compared to that of WT, the highest being for G200, yielding 13.5 g/l in presence of initial xylose concentration of 30 g/l (Table 7.2). The fermentation-phenotype of G200 was found to be stable for 20 subculture generations.

Table 7.2: Xylose utilization and ethanol fermentation by WT and mutants

Sl. No.	Yeast Strain	Xylose (g/l)	Ethanol Conc. (g/l)	
			Initial	Residual
1.	WT	30.0	7.0	11.50
2.	G50	30.0	8.5	10.00
3.	G150	30.0	7.5	11.00
4.	G200	30.0	5.5	13.50

The mutant, G120 was not as efficient in ethanol fermentation or in ethanol tolerance as G200 was (Table 7.1), though former showed higher growth rate indicating that improvement for the rate of growth and fermentation have to be considered separately. It is immature to conclude the linkage between slower growth rate and continuance of residual fermentation until the higher concentration of acetic acid and ethanol is reached.

The ethanol tolerance of *S. stipitis* was found not to exceed 20 g/l 26 and fermentation is completely inhibited at 42 to 45 g/liter of ethanol[10]. G200 showed about 50% fermentation at 40 g/l of ethanol and residual growth and fermentation in presence of 50-60 g/l of ethanol. The ethanol tolerance of budding yeasts has been explained in terms of many factors such as lipid composition of the plasma membrane[28], especially sterols and hopanoids (analogous to sterols)[29] and factors such as the activity of plasma membrane ATPase and superoxide dismutase, capacity of a strain to produce trehalose[30-31] or heat-shock protein Hsp104[33], and mitochondrial stability[34]. The slower growth and higher

ethanol fermentation of G200 seem to be associated with membrane. Further analysis is required to understand exact mechanism of mutation in G200.

Adaptation as a method of improvement of the tolerance to inhibitors has earlier been applied successfully[13, 23, 34] in case of *S. stipitis*. Recently, UV treatment has been found to improve the ethanol production and tolerance in the mutants[14]. Acetic acid tolerance is very important for the production of lignocellulosic ethanol production as this is one of the major inhibitors present in acid hydrolysate of wood[35].

CONCLUSION

In the present study, since G200 gave stable phenotype for 20 subculture-generations. This fact is possibly associated with its haploid-nature[35]. Hence, it may be an important strain for application in wood hydrolysate-fermentation.

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MONITORING OF ALLERGENIC FUNGAL SPORES IN SOME INTRAMURAL SITES OF AURANGABAD CITY (MS)

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Intramural environment provides congenial atmosphere for allergens as well as microorganisms. Their contributions are more than outside due to low temperature and moist humid climate. In the present study, air monitoring was carried out from 1st January 2016 to 31st December 2016 with the help of samplers and petriplate exposure method at five places of Aurangabad city. Altogether 26 fungal types were recorded, of which 08 are allergenic, 08 are deteriorating and the remaining are saprophytic fungi. The 16 species under 08 genera are potentially allergenic in which *Alternaria alternata, A. tennis, Aspergillus fumigatus, A. flavus, A. niger, Cladosporium tenuissimum, C. cladosporioids* and *Chaetomium globosum, C. cellulolytic* are found to be dominant allergens.

Key Words: Allergenic fungi, Intramural environment, Petriplate, Aurangabad

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INTRODUCTION

Atmosphere contains many kinds of particles, of which pollen and fungal spores have special importance as they are main cause of allergic diseases[1]. Air monitoring of intramural sites are necessary to study the allergenic fungi. A regular monitoring network has to be established to predict the changes in environment induced by bio-particles along with meteorological factor.

Intramural environment are amiable for allergens and microorganisms. The incidence of allergens and microbes are more in inside as compare to outside due to having low temperature and moist humid climate. In the present study, five intramural sites of Aurangabad city were selected which include vegetable market, warehouse, cattle shed, Government library and Government hospital. Human exposure to airborne microbes results in a variety of adverse health effects including infectious diseases like skin irritation, reddening of eyes, sneezing, etc. The concentration of fungal spores is directly related to the sensitization of individual's immunity. Hence present study was undertaken to monitor allergenic fungal spores in indoor environment and to detect their concentration in various intramural sites of Aurangabad city.

MATERIAL AND METHOD

The present investigations were carried out by using air samplers (Rotorod sampler and Tilak sampler) as well as by exposing Petriplates containing "Potato dextrose agar" medium at fortnightly interval for a period of one year (1st January 2016 to 31st December 2016). The selected indoor sites include (i) Municipal vegetable market, (ii) Cattle shade (Osmanpura), (iii) Warehouse at Waluj, (iv) Government Library, (v) Government Hospital (Ghati).

The identification and description of fungal spore types trapped on sampling slide were based on their morphology, nature of colony, colour, etc., and also by comparing with the reference slides. Identification was confirmed by using manual of Barnett[2] and Tilak and Kulkarni[3]. DNA Barcoding data were also considered for species identification[4, 5]. The meteorological data were collected from WALMI research center.

RESULTS

The result of the experiments obtained during the period of investigation revealed a wide range of airborne fungal components. Altogether 26 fungal types were recorded on slides, out of which 8 are allergenic and 8 are deteriorating, while remaining are saprophytic in nature. List of the catches identified from the slides are categorized below:

Zygomycotina: *Rhizopus*;

Ascomycotina: *Melanospora, Chaetomium*;

Basidiomycotina: *Basidiospores*;

Deuteromycotina: *Alternaria, Aspergillus, Blastomyces, Brachysporium, Carvularia, Cercospora, Cladosporium, Collectotrium, Epicoccum, Exosporium, Fusarium, Drechslera, Heterosporium, Memnoniella, Monillia, Monochetia, Myrothecium, Niqrospora, Pithomyces, Spegazinia, Torula, Tetraploa*, and other types include hyphal fragment and unclassified spores.

For Basidiomycotina all the basidiospores were kept under one group. It was observed that spore concentration of the Deuteromycotina was dominant among all the groups and their percent contribution was 76.82% to the total airspora (Table 8.1). This was followed by Zygomycotina (12.87), Ascomycotina (7.18%), Basidiomycotina (2.13%) and other types (1.0%).

Table 8.1: Contribution of Fungal group

Sl. No.	Group of Fungi	%Contribution
1.	Deuteromycotina	76.82%
2.	Zygomycotina	12.87%
3.	Ascomycotina	7.18%
4.	Basidiomycotina	2.13%
5.	Other types	1.0%

The maximum spore concentration was noticed in three months namely, September, October and December. Total number of fungal spores and their percent contribution to the total airspora is portrayed in Table 8.2, while the percent contribution of each spore in different study sites is presented in Table 8.3. The percent contribution of each spore depends on the meteorological conditions as well as the intramural activity of people. Month wise meteorological conditions are shown in the Figure 8.1. Monthly incidence of airborne fungi on exposed petridish using PDA medium and their percentage contribution to the total airspora are represented in the Figure 8.2. Allergenic fungal spores were present in all the five indoor sites.

Table 8.2: Contribution of total number and Total percentage of airspora in different Indoor sites

Sl. No.	Spores	Total No.	Percentage
1.	*Alternaria*	90690	25.31
2.	*Curvularia*	26880	7.50
3.	*Rhizopus*	46120	12.87
4.	*Nigrospora*	22810	6.37
5.	*Cladosporium*	7625	2.13
6.	*Aspergillus*	62260	17.38
7.	*Fusarium*	30920	8.63
8.	*Colletotrichum*	1993	0.56
9.	*Brachysporium*	1390	0.39
10.	*Drechslera*	1170	0.33
11.	*Pithomyces*	1090	0.30
12.	*Torula*	1570	0.44
13.	*Blastomyces*	2980	0.83
14.	*Memnoniella*	130	0.04
15.	*Melamospora*	1926	0.54
16.	*Monillia*	2618	0.73
17.	*Epicoccum*	1778	0.50
18.	*Basidiospores*	2407	0.67
19.	*Speguzinia*	2845	0.79
20.	*Heterosporium*	4248	1.19
21.	*Monochetia*	730	0.20
22.	*Myrothecium*	4924	1.37
23.	*Tetraploa*	5746	1.60
24.	*Exosporium*	5047	1.41
25.	*Cercospora*	4556	1.27
26.	*Chetomium*	23800	6.64
	Total	358253	100.00

Some of the salient features of commonly observed spores in indoor environment are given below:

Rhizopus: It is a rapidly growing filamentous fungi. Spores were observed almost in all the sites. It causes

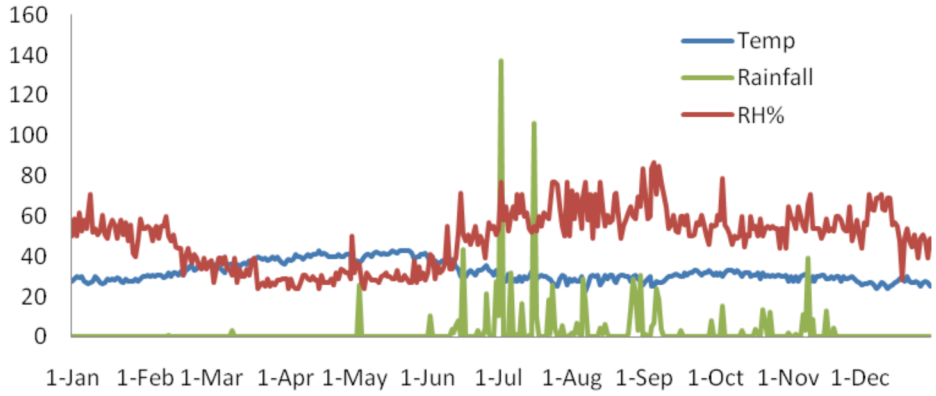

Fig. 8.1: Meteorological data of Aurangabad city

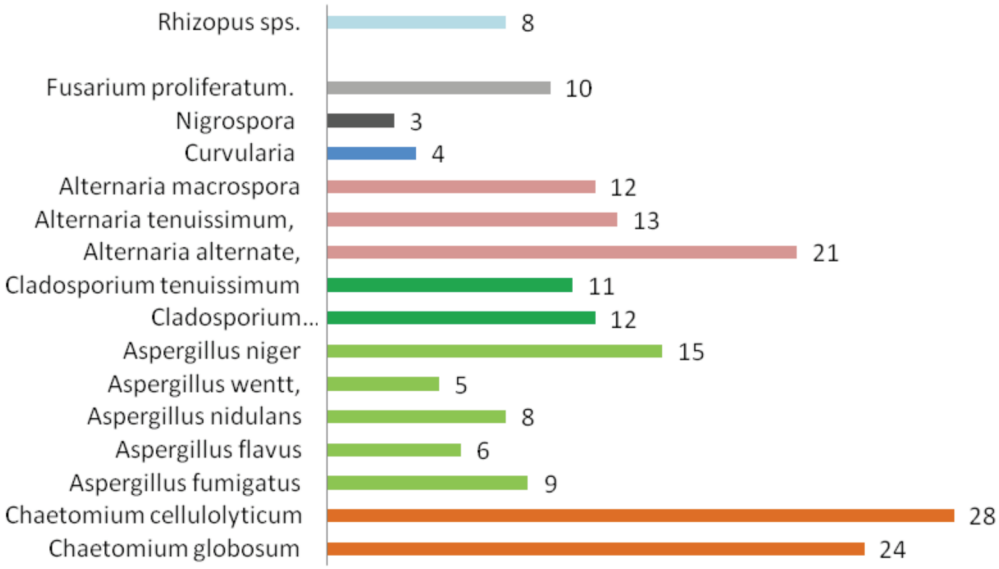

Fig. 8.2: Allergenic fungi and their number of colonies in petriplate culture

the soft root and black mold rot of various vegetables in vegetable market. Percentage of *Rhizopus* was highest (24.22%) in the warehouse followed by vegetable market (15.18%) and cattle shed (11.96%) (Table 8.3). Percentage of Rhizopus increased in warehouse perhaps due to having moisture content in rainy season which was even observed on some seeds collected from the warehouse. A total 8 colonies were recorded by petriplate culture technique. Kramer *et al*.[6] reported *Rhizopus* spore from Kansas, USA. In India, *Rhizopus* spores were recorded by Tilak and Bhalke[7] from Aurangabad. *Rhizopus* contains 31 allergenic proteins, which may produce respiratory and nasal symptoms. Food-handling workers are particularly at risk if they are allergic to mold[8].

Basidiospores: The percent contribution of basidiospores to the total airspora was very low (0.67%), though they are recognized as potential causes of respiratory allergies[9].

Ascomycotina: Two spore types of this group were recorded which contributed 7.18 % to the total airspora. Release of ascospores in the atmosphere depends on the rainfall and humidity. *Melanospora* and *Chaetomium* were recorded from library as well as vegetable market.

Melamospora contributed only 0.54% to the total airospora in January, February and March in all sites. Percentage of *Chaetomium* was high (21.88%) in the library followed by vegetable market (6.84%) (Table 8.3). The cellulolytic fungus, *Chaetomium globosum* was collected from damp paper materials in the library and also from vegetable waste in vegetable market. *Chaetomium globosum* was reported to be the most common species of the *Chaetomiaceae* in the indoor environment[10]. Several species of *Chaetomium* are associated with hay fever, asthma, and allergic sinusitis. In some cases, it can produce mycotoxins that are hazardous if ingested in considerable amounts[11]. In petriplate culture, 52 colonies of *Chetomium* were recorded in library, vegetable market and warehouse, of which *Chaetomium globosum* contributed 24 colonies and *Chaetomium cellulolyticum* 28 colonies.

Table 8.3: Contribution of total number and Total percentage of airspora in different Indoor sites

Sl. No.	Spores/Sites	MVM	CSO	WHW	GL	Gh
1.	*Alternaria*	22.78	29.87	24.87	20.71	29.83
2.	*Curvularia*	8.99	5.11	8.56	9.82	4.12
3.	*Rhizopus*	15.18	11.96	24.22	6.30	8.48
4.	*Nigrospora*	4.04	6.64	0.20	7.77	14.36
5.	*Cladosporium*	1.54	5.65	0.96	0.87	0.63
6.	*Aspergillus*	12.68	19.03	24.39	12.50	22.00
7.	*Fusarium*	11.59	9.73	7.79	7.45	5.04
8.	*Colletotrichum*	1.03	0.27	0.36	0.37	0.81
9.	*Brachysporium*	1.21	0.50	0.00	0.00	0.00
10.	*Drechslera*	0.29	0.35	0.36	0.25	0.43
11.	*Pithomyces*	0.19	0.31	0.53	0.22	0.37
12.	*Torula*	0.71	0.33	0.70	0.25	0.20
13.	*Blastomyces*	1.98	1.31	0.29	0.13	0.00
14.	*Memnoniella*	0.16	0.00	0.00	0.00	0.00
15.	*Melamospora*	0.77	0.84	0.26	0.28	0.42
16.	*Monillia*	0.92	0.46	0.99	0.51	0.96
17.	*Epicoccum*	0.12	0.29	0.48	0.99	0.63
18.	*Basidiospores*	0.35	0.21	0.37	2.07	0.00
19.	*Spegazinia*	0.29	0.25	0.20	0.96	2.90
20.	*Heterosporium*	0.68	0.38	0.81	1.52	3.20
21.	*Monochetia*	0.19	0.00	0.00	0.38	0.51
22.	*Myrothecium*	2.39	1.96	1.21	0.76	0.00
23.	*Tetraploa*	1.01	0.94	1.51	2.19	2.79
24.	*Exosporium*	1.89	2.03	0.70	0.90	1.27
25.	*Cercospora*	2.19	1.58	0.24	0.92	1.07
26.	*Chetomium*	6.84	0.00	0.00	21.88	0.00
	Total	100.00	100.00	100.00	100.00	100.00

MVM-Municipals Vegetable Market, CSO - Cattle Shade Osmanpura, WHW-Warehouse at Waluj, GL-Government library, GH-Government hospital.

Deuteromycotina: It is a heterogeneous group of unrelated species which contributed to a major portion of the airspora. *Alternaria* spores were recorded all over the study period, contributed 25.31% to the total airspora (Table 8.2).The highest percent contribution was recorded in August, 2015. Spores number increased with rain followed by dry weather. It clearly indicates that rainy season favours the growth and reproduction of fungi whereas dry weather helped the spore liberation. *Alternaria* constituted the bulk of component with the highest number of colonies (46). During the petriplate study three species of *Alternaria* were recorded which are *Altenaria macrospora* (12 colonies); *Altenaria tenuissimum* (13 colonies) and *Altenaria*

alternata (21 colonies). It was found to be dominant in all sites (Table 8.3). All the three recorded species are known to be allergenic[12]. Contribution of *Aspergillus* to the total airspora was 17.38 % (Table 8.2). The highest concentration was recorded in August where average temperature was 28°C and average RH was 80 %. These favoured the sporulation and development of *Aspergillus* spp. In petriplate 43 colonies belonging to five species were recorded in various indoor sites. The most prominent species include *Aspergillus fumigatus* (9 colonies), *Aspergillus niger* (15 colonies), Aspergillus flavus (6 colonies), *Aspergillus wentti* (5 colonies), *Aspergillus nidulans* (8 colonies) (Fig. 8.2). Spores of *Aspergillus fumigatus, Aspergillus flavus, Aspergillus nigerare* are known to be causative agent of respiratory diseases in human[13,14]. Maximum concentration of *Cladosporium* was recorded between 14th to 19th January 2015. It was observed that moderate temperature, high humidity and wind velocity helped this saprophytic fungi for its development and subsequent release of spores in the atmosphere. In the cattle shed, percentage of spores was 5.65%, followed by municipal vegetable market (1.54%). *Cladosporium tenuissimum* (11 colonies) and *Cladosporium cladosporioides* (12 colonies) were recorded on petriplate culture. Most species of *Cladosporium* do not cause disease in humans but long-term exposure to this mold can cause adverse health effects, including allergies and asthma symptoms[15]. *Cercospora* spores were present round the year except in May 2015, though its average percent contribution was less i.e. (1.27%). The conidia show its higher incidence in vegetable market (2.58%) and cattle shade (1.58%). *Curvalaria* spores contributed 7.50 % to the total airspora and were present in the all sites throughout the year with high concentration except in the month of May. Number of spores increased in January due to favourable climatic conditions like 300C temperature, 60 % RH and low rainfall. Petriplate exposure showed 4 colonies on PDA. Most of the species of *Curvularia* were recorded as saprophytic fungi, but *Curvularia lunata* was reported as allergenic to human being[16]. *Fusarium* spores belong to 'Wet Spora' group. They were caught during the present investigation and their contribution to the total airspora was 8.63%. Total 10 colonies were recorded by petriplate culture from all the five sites. The highest catches were recorded (11.59%) in vegetable market and in cattle shed (9.73%). *Nigrospora* contributed 6.37% to the total airspora. Maximum percentage was recorded in Government hospital (14.36%). Colonies of *Nigrospora* were also recorded in petriplate culture. Colletotricum spores occurred in the air from 1 august 2015 and contributed 0.56% to the total airspora. *Epicoccum* spores contributed 0.50% to the total airspora. The highest catches were recorded in July 2015 from Government library (Table 8.3). *Drechslera* spores occurred in the entire study period with very low percentage (0.33%) to the total airospora. *Heterosporium* (1.19 %) conidia usually occurred singly. *Memnoniella* (0.04%) was also collected from dead and decaying parts of the vegetables and seeds of warehouse (Table 8.3). *Pithomyces* (0.30%) conidia were also recorded in very low percentage.

DISCUSSION

In the present study, spores of *Alternaria alternata, A. tennis, Aspergillus fumigatus, A. flavus, A. niger, Cladosporium tenuissimum, C. cladosporioides* and *Chaetomium globosum, C. cellulolyticare* were found to be the dominant potential allergens causing sneezing and redness of eyes, swelling and irritation of skin, and asthma to human beings. It was observed that some workers and people stayed in intramural environment were suffering from respiratory problems (personal communication and questionnaire). Meteorological factors like temperature, rain fall, RH and wind speed play an important role in the dispersion, dissemination and concentration of spores in the environment. Hence airspora are continuously fluctuating due to change in season and locality. It was also perceived that concentration of allergic fungal spores were higher in municipal vegetable market, cattle shed and in Government hospital as compare to Government library and warehouse.

Species of deteriorating fungi like *Blastomyces, Brachysporium, Collectotrium, Monochetia, Myrothecium* and *Rhizopus* were frequently found on waste dumped sites. Book deteriorating fungi like *Chetomium* were generally grown on cellulose materials due to having its cellulolytic enzymes. So deterioration of paper strips, cotton, clothes are the common incidence in the study area (personal observation through questionnaire). Remaining fungi were the common saprophytic fungi recorded from almost all the study sites. Health survey was carried out (personal communication through questionnaire) to combat respiratory disorders among local inhabitants. The following outcomes have been predicted: (1) reduce garbage, paper waste, etc. (2) proper sanitation of indoor environment, (3) maintenance of personal hygiene.

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AEROMYCOFLORA INSIDE A LIBRARY OF COLLEGE CAMPUS IN BHAGALPUR, BIHAR

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Aeromycoflora inside a Library of Sunderwati Mahila Mahavidyalaya (a renowned women's college situated in Bhagalpur, Bihar) was investigated for a period of one year from February 2016 to January 2017 by using the Culture plate method. The culture plate method was preferred as it is most suited for organisms that thrive on a solid or semisolid surface. Altogether 17 genera of fungal colonies were identified, of which maximum genera belonged to Deuteromycetes followed by Zygomycetes and Ascomycetes. *Cladosporium* (18.93%) was most dominant inside the library of college campus followed by *Aspergillus* (11.53%), *Drechslera* (7.57%), *Alternaria* (7.06%), *Chaetomium* (6.54%), *Curvularia* (5.33%), *Fusarium* (5.16%), *Epicoccum* (4.30%), etc. The least percentage is shown by *Nigrospora* (1.72%) and *Geotrichum* (1.55%). Among the 17 fungal genera identified, 10 are reported to cause damage and deterioration of books and papers. These are *Cladosporium, Aspergillus, Curvularia, Alternaria, Penicillium, Fusarium, Rhizopus, Chaetomium, Epicoccum,* and *Trichothecium*. The concentration of fungal colonies were maximum in February and least in June.

Key Words: Aeromycoflora, Library, Indoor, Fungal colony, Deterioration.

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INTRODUCTION

Aerobiology can be defined as a discipline of science which focuses on the transportation of organisms and biologically significant materials by the atmosphere. Soil and water definitely contain higher number of micro-organisms, but air suspension provides them better opportunity to travel distant places with the help of the wind. Fungi are ubiquitous, it pervades everywhere where life can be found and are very important part of bio-aerosol. The biological components of the atmosphere follow a definite aerobiological pathway starting from the source of origin, release, and deposition. Environmental factors are also known to contribute to each of these stages. Fungal spores predominate the other bioparticles in the airspora. Due to their extreme adaptability, the fungal spores are encountered more or less throughout the year. The importance of fungus as a major indoor pollutant and a potential source of health hazard has long been recognized. The level of these indoor contaminants is of particular concern since it is estimated that most people spend about 90% of their time indoors, and the library is one of them to draw our immediate attention.

Library is an organised treasure house of knowledge and collection of source of information which may be maintained by individual, private or public authorities and can accessible to a defined few or to public in general for reference or borrowing. The knowledge books contain inherited from generation to generation. Writers devote decades and then what achieved, books contain. Man's life is short comparatively hence had the books not been in existence, progress of mankind could be imagined. All would have been lost in course of time had the books not been there and therefore arises the question of preservation of books. Knowledge of the past which is indispensable to the present and future generations is preserved in the books in the libraries. These thoughts, ideas and facts from all ages, areas and directions make the library repository of wisdom.

The study of aeromycoflora of the library is especially important as the old books with bindery glues and fabrics support the growth of fungi. In the favourable condition, they proliferate and damage the books by staining and foxing. They can destroy cellulose and decompose binding materials, leather, and plastics.

The fungal spores are present in both the outdoor and indoor environments. The abundance of fungal spores in the indoor environment results from their capacity to grow in or on practically any substrate including paper, leather, glass, textiles, electrical pieces of equipment and other building materials, and to produce large quantities of spores in a short period, thus initiating deteriorative processes[1,2].

The importance of micro-fungi in biodeterioration of books cannot be overlooked, especially, when wood cellulose forms the basic constituent of paper, the glue in bounded books and leather as a binding material, all forming an ideal substrate for growth, sporulation, and proliferation of fungi[3,4].

The primary source of these contaminants in the ambient air of a library is *de novo* or they may be carried from the outdoor source environment[5,6]. During the past three decades extensive work on the aeromycoflora of the outdoor environment has been done in various parts of the country but little attention has been paid to aeromycoflora of indoor environments. Indoor environments, though small in area, are important as they provide microbes with an environment different from the outdoor environment[7]. Since the aeromycological surveys of the libraries are scant, the present investigation was undertaken to study the aeromycoflora of the library of Sunderwati Mahila Mahavidyalaya.

Most of the library materials like paper, leather, and textiles are likely to be affected by these agents. On the other hand adverse environment condition refers to considerable fluctuation in day and night temperature, exposure to light and excessive humidity. When paper is exposed to high temperature with low humidity, dehydration of cellulose fibres take place resulting in brittleness of the paper. If the humidity is high along with the temperature, it provides a suitable breeding ground to the fungus. Fungi are hazardous inside the library; their attack is harmful for both, the material that is kept in the library as well as for the person who looks after them. They possess the capacity to stay dormant for a long time and sprout when they get favourable atmosphere. The favourable temperature for growth of fungi is between 15 - 35°C. Air spores have been studied widely and the scope for study is both the outdoor and indoor environment .The development and proliferation of fungi in library is to a great extent determined by indoor climate, availability of nutrients not in the materials available in the library but from atmosphere as well. The second factor is physical factor like mishandling of books, passing of books from one reader to another in normal course it is inevitable and a big cause of damage to the books in the long run. Sometimes the reader twists the corner of the pages, some others remove the important pages, underlining the important matters is also harmful. This physical damage can't be stopped, but proper handling can definitely reduce this type of damage and extends the life of the book. Acts like tearing the pages, marking are unpardonable and must be avoided.

MATERIAL AND METHODS

Petriplate Exposure Method

Culture Plate Technique is one of the oldest settle plate methods of testing the air for microbial contamination. This method is used to assess the likely numbers of microorganisms depositing on to the exposed surface in a given time. The method involves opening and exposing petridishes containing Czapek's Dox Agar (CDA) media suitable for the growth of microorganisms available in the air. Plates are supplemented with antibiotics to suppress bacterial growth to avoid bacterial contamination.

Collection of Fungal Spores

At the experimental site (i.e. library) the sampling was carried in a regular interval of 15 days, manually by exposing petriplates without the use of a sampler for a period of one year (February 2016 – January 2017). The petriplates of 9 cm diameter were exposed horizontally in 15 days. After several trial exposures the period of 40 min. was found to be optimum for culture plate exposure for the isolation and analysis of airborne fungi. The exposed petriplates containing Czapek's Dox Agar Media were exposed and were incubated at 25°C ± 2°C for 7 days.

Identification

Identification was made with the help of published keys, monographs, and relevant literature[27,28,29]. In all possible cases, specific and generic counts were made which are based on the colour, shape, and other diagnostic features of the spore types.

RESULTS AND DISCUSSION

During the period of investigation, a total of 581 fungal colonies belonging to 17 genera were identified from the indoor atmosphere of the library. Maximum belonged to Deuteromycetes which were followed by Zygomycetes and Ascomycetes. The most dominant

Table 9.1: Total No. of airborne fungal colonies and their percentage contribution in the library during (Feb. 2016-Jan. 2017)

Sl. No.	Fungal colonies	Feb. 2016	Mar. 2016	Apr. 2016	May 2016	June 2016	July 2016	Aug. 2016	Sep. 2016	Oct. 2016	Nov. 2016	Dec. 2016	Jan. 2017	Total	%
1.	*Cladosporium*	32.0	15.0	5.0	2.0	3.0	2.0	2.0	15.0	14.0	12.0	5.0	3.0	110.0	18.93
2.	*Aspergillus*	10.0	9.0	7.0	5.0	2.0	5.0	6.0	2.0	5.0	12.0	2.0	2.0	67.0	11.53
3.	*Alternaria*	9.0	7.0	8.0	1.0	1.0	1.0	1.0	1.0	4.0	7.0	0.0	1.0	41.0	7.06
4.	*Curvularia*	4.0	2.0	5.0	2.0	1.0	1.0	1.0	1.0	5.0	6.0	1.0	2.0	31.0	5.33
5.	*Drechslera*	7.0	9.0	6.0	2.0	2.0	3.0	2.0	2.0	2.0	5.0	2.0	2.0	44.0	7.57
6.	*Epicoccum*	5.0	3.0	3.0	2.0	1.0	1.0	4.0	3.0	1.0	2.0	0.0	0.0	25.0	4.30
7.	*Fusarium*	6.0	5.0	3.0	0.0	2.0	3.0	5.0	4.0	1.0	0.0	0.0	1.0	30.0	5.16
8.	*Helminthosporium*	3.0	2.0	0.0	0.0	0.0	2.0	3.0	2.0	2.0	3.0	2.0	2.0	21.0	3.61
9.	*Chaetomium*	4.0	3.0	2.0	1.0	1.0	5.0	5.0	5.0	5.0	5.0	1.0	1.0	38.0	6.54
10.	*Geotrichum*	0.0	1.0	1.0	2.0	1.0	0.0	1.0	1.0	2.0	0.0	0.0	0.0	9.0	1.55
11.	*Penicillium*	3.0	1.0	3.0	2.0	1.0	3.0	2.0	2.0	3.0	2.0	0.0	2.0	24.0	4.13
12.	*Nigrospora*	2.0	0.0	0.0	0.0	1.0	2.0	1.0	1.0	3.0	0.0	0.0	0.0	10.0	1.72
13.	*Mucor*	1.0	1.0	0.0	0.0	1.0	0.0	2.0	1.0	0.0	7.0	1.0	1.0	15.0	2.58
14.	*Stachybotrys*	3.0	2.0	1.0	1.0	0.0	1.0	2.0	1.0	7.0	6.0	2.0	1.0	27.0	4.65
15.	*Trichoderma*	1.0	1.0	0.0	1.0	0.0	3.0	2.0	3.0	7.0	2.0	1.0	0.0	21.0	3.61
16.	*Rhizopus*	1.0	0.0	1.0	1.0	0.0	1.0	1.0	1.0	6.0	1.0	0.0	0.0	13.0	2.24
17.	*Trichothecium*	1.0	2.0	6.0	1.0	0.0	0.0	2.0	1.0	2.0	0.0	0.0	0.0	15.0	2.58
	Unidentified	4.0	9.0	10.0	1.0	1.0	7.0	1.0	1.0	1.0	2.0	2.0	1.0	40.0	6.88
	Total	96.0	72.0	61.0	24.0	18.0	40.0	43.0	47.0	70.0	72.0	19.0	19.0	581.0	100.0
	%	16.5	12.3	10.4	4.1	3.0	6.8	7.4	8.0	12.0	12.3	3.2	3.2		100.0

fungus was *Cladosporium* (18.93%) followed by *Aspergillus* (11.53%), *Drechslera* (7.57%), *Alternaria* (7.06%), *Chaetomium* (6.54%), *Curvularia* (5.33%), *Fusarium* (5.16%), *Epicoccum* (4.30%), etc. The least percentage is shown by *Nigrospora* (1.72%) and *Geotrichum* (1.55%) (Table 9.1).Two types of fungal genera were isolated from Phycomycetes (*Mucor* and *Rhizopus*). The group Ascomycetes was represented by *Chaetomium* only. Rest 14 types of recorded fungal genera were from Deuteromycetes. *Cladosporium* formed the first major component in the total mycoflora (18.93%). *Aspergillus* accounted for 11.53% to the total mycoflora and ranked 2nd position in our findings. The other genera which made a significant contribution to the total mycoflora were *Drechslera* (7.57%), *Alternaria* (7.06%), *Chaetomium* (6.54%), *Curvularia* (5.33%), *Fusarium* (5.16%), *Epicoccum* (4.30%), etc.

The month of February recorded a maximum number of colonies (96 colonies) followed by March and November (72 colonies), while the minimum number of colonies were recorded in June (18 colonies) due to hot weather. Monthly variations in the total number of fungal colonies trapped are illustrated in Fig. 9.1 and the percent contribution of dominant fungal genera is shown in Fig. 9.2.

The predominance of *Cladosporium* and *Aspergillus* in the library is also reported[8,9]. Singh *et al*.[10] also reported the dominance of *Cladosporium* followed by *Aspergillus, Penicillium*, and *Alternaria*. Paradkar and Munshi[11] stated the highest percentage of *Cladosporium* in the aerospora of the library. Out of the 17 types of fungal genera identified, 10 types are reported to cause paper deterioration. These include *Cladosporium, Aspergillus, Alternaria, Curvularia, Penicillium, Fusa-*

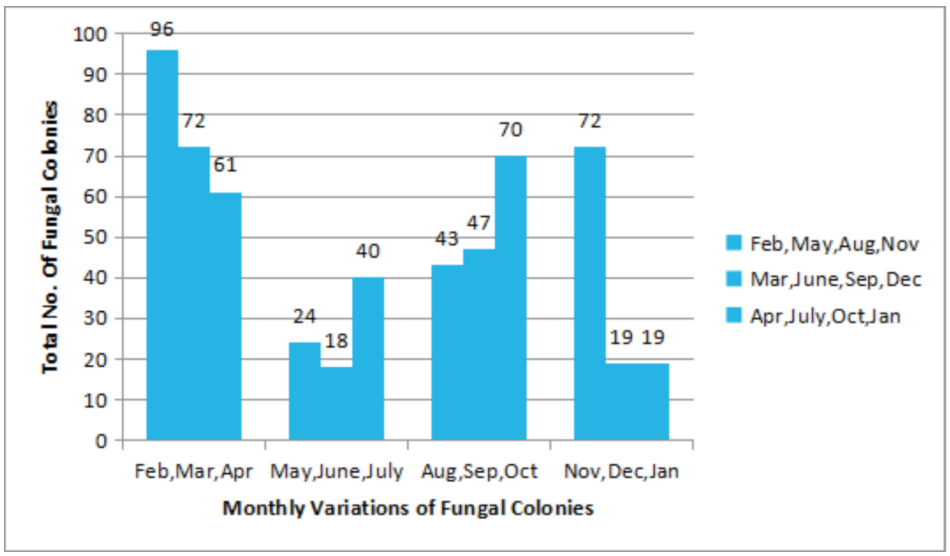

Fig. 9.1: Monthly variation in the number of fungal spore colonies in the library during 2016-17

rium, Rhizopus, Epicoccum, Trichoderma, and Trichothecium[12]. The dominance of *Cladosporium*, *Aspergillus, Penicillium*, and *Alternaria* in the library also corroborates the findings from the other libraries of the country, like Allahabad[13], Aurangabad[14], Bangalore[15], Chenai[16], Delhi[17], Gorakhpur[18], Nagpur[19], and Visakhapatnam[20]. High incidence of airborne *Cladosporium* spores are also reported from Chennai[25]. The high concentration of *Cladosporium* spores in the Indoor air is known to cause allergic disease in a number of people[26].

The spore concentration was higher in winter than during rainy and summer months. These variations are suspected due to the fluctuation in relative humidity, rainfall, and temperature in the atmosphere. The distribution of aeromycoflora in the environment changes from place to place associated with variation in the meteorological factors, vegetation and flora combination[21]. The concentration of airborne fungal spores have been correlated with wind velocity, R.H, temperature and rainfall[22].

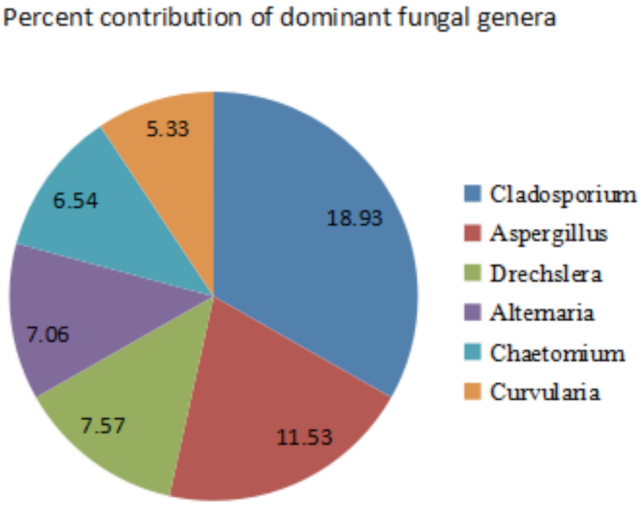

Fig. 9.2: Percent contribution of dominant fungal genera

CONCLUSION

Aeromycoflora inside a library, besides damaging the books, may also cause allergic diseases to library users especially the workers of the library who spend most of their time in the indoor environment of the library. The entire library should be cleaned from time to time with a vacuum cleaner which will help to reduce the concentration of aerospora as well as dust microorganisms.

It is established that indoor molds can have an effect on human health and in the study sites there were no aeroallergenic fungal spore- free phases[23]. Further students and especially staff members associated with the maintenance of the library are being constantly exposed to these spores, of which a good number are known for their hypersensitive reactions leading to respiratory illness like asthma, rhinitis and hypersensitivity pneumonitis or extrinsic allergic alveolitis plus mold specific conditions such as bronchopulmonary aspergillosis. Thus, a clinical investigation of the staff members is essential who frequently complain of respiratory allergy. It is equally true that the libraries cannot be free of micro-fungi, but certain corrective measures[24] can reduce their frequency of occurrence, like, installation of an exhaust fan to remove spores from the indoor, before they have a chance to settle and begin new growth or pose a health hazard; well ventilation of room with plenty of light; use of vacuum cleaner to remove dust, paying special attention to backs of shelves, floor, and furniture; disinfecting the shelves with fungicides; discarding the damaged books; installation of a window fan where air conditioning is not possible; and start planning for regular cleaning and preventive process.

Most of the works mentioned here are from previous time as it is obvious from the previous literature that the

data on microbial concentration and their long term impacts on health in the damaged and moist buildings of indoor environment have been barely studied. Even in the previous time the work has been not done extensively. Recent works done by few scholars has been mostly based upon the principles and aspect of the previous works even though we have tried to include some of the recent works.

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