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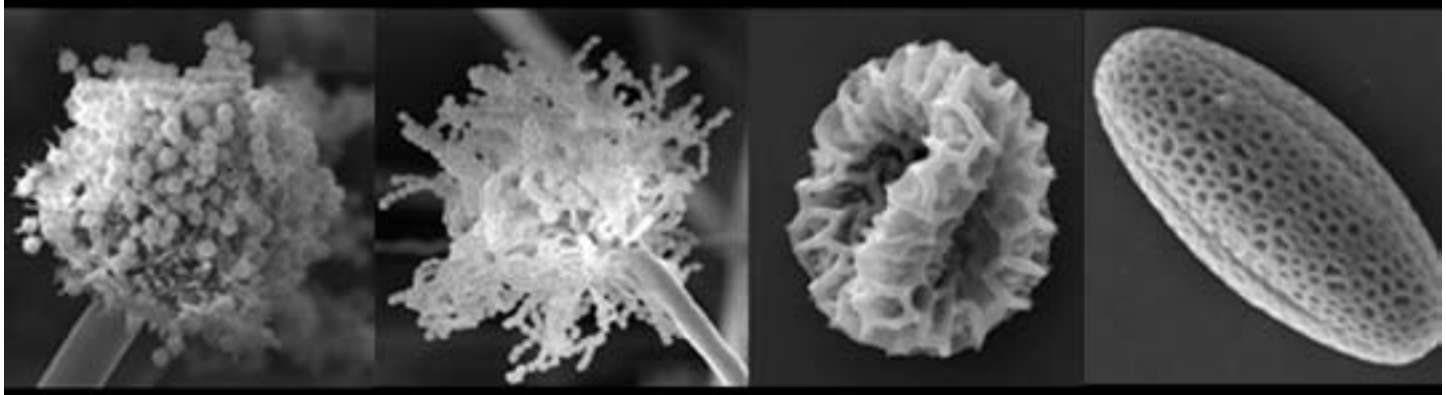

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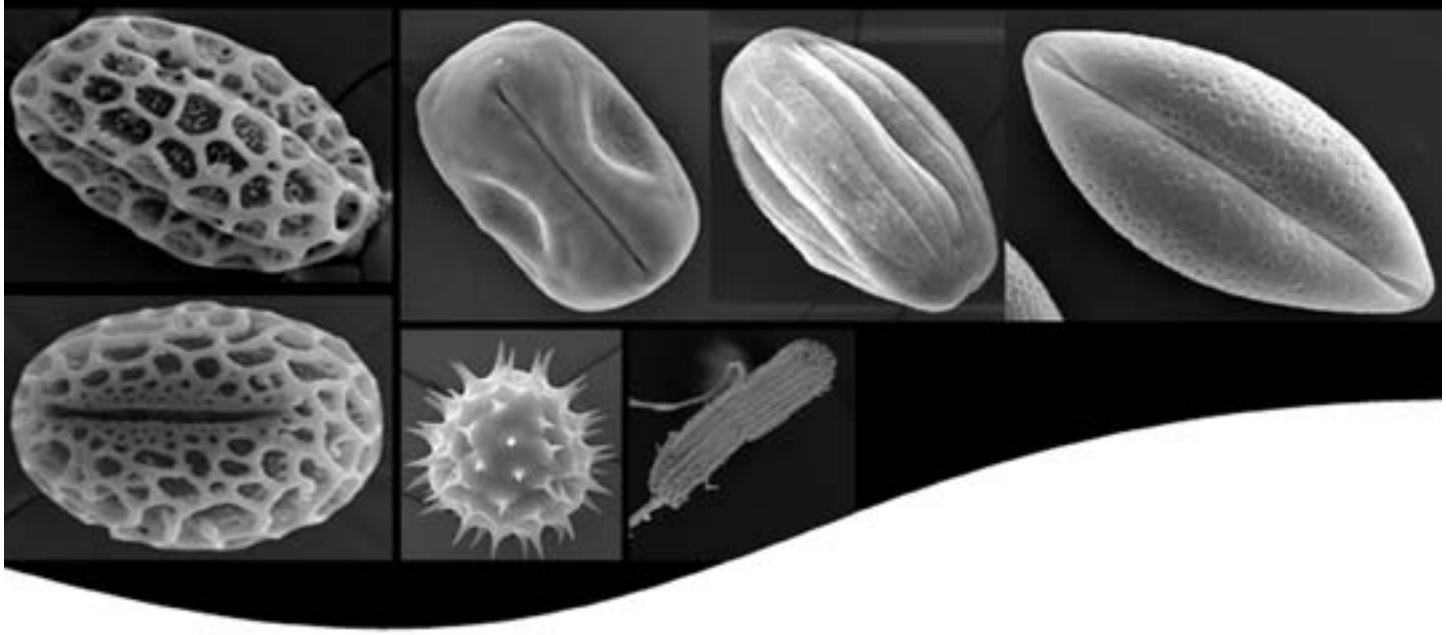

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Indian J. Aerobiol., Vol. 29, No. 1 & 2, pp 5-11 [2016]

COMPUTATIONAL ANALYSIS OF L-ASCORBATE OXIDASE HOMOLOG PROTEIN FROM NICOTIANA TABACUM L.

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In the present investigations, **L-ascorbate oxidase homolog** protein from *Nicotiana tabacum* was screened in-silico for its allergic and antigenic characters. **L-ascorbate oxidase homolog** having 554 amino acids residues, which shows 19 antigenic determinants. The in-silico studies have shown 62033.672 average molecular weight of the amino acids comprising the protein. It contains 50.00 % and 50.00% hydrophilic and hydrophobic amino acids respectively. The protein contains 54 basic amino acids and 45 acidic amino acids. Motif map shows more receptors on T-cell than on B-cell. MHC-class 1 receptors are also found in motif map. Protein statistics is also carried out with respect to several parameters with the help of Peptool 2.0. Predicted results are support to elicit, the **L-ascorbate oxidase homolog** is allergic.

Keywords: Pollen protein, Pollen allergy, Antigenicity, Nicotiana tabacum.

INTRODUCTION

Many plants release prodigious quantities of wind-dispersed pollen that trigger hay fever, seasonal asthma and related immune reactions in humans. The release of pollen into the air is a normal part of the sexual cycle in many wind-pollinated plants. Unfortunately, however, certain pollen grains contain specific proteins or glycoproteins that can result in the familiar debilitating symptoms of hay fever and asthma in humans. This, together with the dramatic increase in the incidence of allergic disease in recent years, has led to increasing public concern about allergenic pollen. Up to 25% of adults suffer these allergic responses as a result of inhaling pollen-laden air (Knox and Suphioglu, 1996).

Thus looking towards the significant role of the allergenic patients from pollen antigens in the diagnosis and therapy of allergic patients there is need of identification and characterization of potential pollen protein allergens for estimation of relative potency as a first step towards risk assessment [Gomase, et al, 2008, Rokade, et al, 2010a and 2010b]. Therefore it has been proposed to undertake such an important aspect of pollen allergenic studies

MATERIALS AND METHODS

Prediction of hydrophobicity: The **L-ascorbate oxidase homolog** protein was scanned for Hydrophobicity. Hydrophobicity [or hydrophilicity] plots are designed to display the distribution of polar and apolar residues along a protein sequence. Most commonly, this analysis has the goal of predicting membrane-spanning segments [highly hydrophobic] or regions that are likely exposed on the surface of proteins [hydrophilic domains] and therefore found to useful to identify potentially antigenic segments. Scale of hydrophobicity have been developed, which were derived from experimental studies on partitioning of peptides in apolar and polar solvents.

Prediction of antigenic sites: L-ascorbate oxidase homolog protein sequence processed for the

prediction of antigenicity and MHC class peptide binding, which allows potential drug targets to identify active sites against allergenic reactions. Antigenic epitopes are determined by using the method of Kolaskar and Tongaonkar [Kolaskar and Tongaonkar, 1990]. Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. Prediction of antigenicity program predicts those segments from within the **L-ascorbate oxidase homolog** protein sequence that are likely to be antigenic by eliciting an antibody response. A method given by Saha and Raghava [Saha and Raghava, 2006] used for prediction of B-cell epitopes.

Secondary alignment: Chou and Fasman (1978) and Garnier (1978) method used to predict secondary structure. These methods are based on information theory. The outputs of these programs are alpha-helix, beta-sheet and coil and gives probable value for each secondary structure. The predicted structure is one of the highest probably compatible with experimental structure.

Protein statistics: In-silico protein statistics was carried out with respect to several parameters such as atomic weight, average molecular weight, hydrophobic and hydrophilic amino acids percentage, number of basic and acidic amino acids, linear charge density and solubility of protein, amino acid frequency and hydrophobicity

OBSERVATIONS AND RESULTS

The **L-ascorbate oxidase homolog** protein contains 554 amino acids and its total atomic weight is 62033.672 daltons. The in-silico studies have shown 111.974 average molecular of the amino acids comprising the protein. It contains 50.00% hydrophilic amino acids and 50.00% hydrophobic amino acids. The protein contains 54 basic amino acids and 45 acidic amino acids. The total linear charge density of the protein is 0.18231. The solubility of protein is 1.40912. Structural characters of the protein also observed in silico [Table No. 1].

Hydrophobicity information is useful in identifying coil regions, exposed loops, interior domains, B-cell antigenic determinants and membrane spanning regions with in the sequence. The hydrophobicity of **L-ascorbate oxidase homolog** protein is also determined [Fig. No.1]. The *antigenic* peptide prediction program found the antigenic determinants by finding the area of greatest local hydrophilicity. The Kolaskar-Tongaonkar antigenicity scale shows that **L-ascorbate oxidase homolog** protein is highly antigenic nature and has nineteen antigenic determinants [Fig. 2 and Table 2].

Motif map of **L-ascorbate oxidase homolog** protein shows 345 motifs out of which 135 are MHC class 1 related, 165 motifs have functional relationship with B-cell membrane and others have functional relationship with T-cell membrane [Fig. 3].

The **L-ascorbate oxidase homolog** protein structure is predicted by using Chau-Fasman [1978] and Garnier [1978]. Each residue is assigned values for α-helix, β-sheet and extended coil using window size seven residues. [Fig. 4].

Amino acid frequency and percentage amino acid weights are also determined in-silico by using peptool [Fig. 5]. Glycine is 9%, each in total residue of amino acid in this protein, followed by leucine and asparagine. Cystein is represents 1% amino acid frequency. Glycine is 9%, each in total residue of amino acid in this protein, followed by leucine and asparagine. Cystein is represents 1% amino acid frequency [Fig. 6].

CONCLUSION

Human health hazard such as allergic dermatitis, hay-fever and respiratory problems are due to allergenic reactions caused by the pollen protein of *Nicotiana tabacum*. Small peptide fragments from *Nicotiana tabacum* pollen involve multiple antigenic components to direct and empower the immune system to protect the host from

Table 1 : Protein Statistics of L-ascorbate oxidase homolog protein

Sr. No.	Parameter	Value
1.	Molecular weight [*Daltons*]	62033.672
2.	Number of amino acids	554
3.	Mean amino acid weight [Daltans]	111.974
4.	Average hydrophobicity	-0.329422
5.	Ratio of hydrophilicity to hydrophobicity	1.28799
6.	Percentage of hydrophilic amino acids	50.0
7.	Percentage of hydrophobic amino acids	50.0
8.	Ratio of percentage hydrophilic to Percentage hydrophobic	1.0
9.	Mean beta hydrophobic moment	0.205387
10.	Mean helix hydrophobic moment	0.161095
11.	Number of basic amino acids	54
12.	Number of acidic amino acids	45
13.	Estimated pH for protein	9.2
14.	Total linear charge density	0.18231
15.	Polar area of extended chain [Angs2]	35844.3
16.	Non-polar area of extended chain [Angs2]	62204.2
17.	Total area of extended chain [Angs2]	98048.5
18.	Polar ASA of folded protein [Angs2]	7797.63
19.	Non-polar ASA of folded protein [Angs2]	11830.8
20.	ASA of folded protein [Angs2]	19628.4
21.	Ratio of folded to extended area	0.213808
22.	Buried polar area of folded protein [Angs2]	25182.3
23.	Buried non-polar area of folded protein [Angs2]	43889.1
24.	Buried charge area of folded protein [Angs2]	2877.97
25.	Total buried surface [Angs2]	71949.3
26.	Number of buried amino acids	239
27.	Packing volume [est] [Angs3]	75447.4
28.	Packing volume [act] [Angs3]	73963.3
29.	Interior volume of protein [Angs3]	53955.0
30.	Exterior volume of protein [Angs3]	2008.3
31.	Partial specific volume [ml/g]	0.72508
32.	Fisher volume ratio [act]	0.370833
33.	Fisher volume ratio [idealized]	0.496225
34.	*Protein solubility*	1.40912
35.	Estimated radius of folded protein [Angs]	31.8255
36.	RMS end to end distance of extended chain [Angs]	246.86
37.	Radius of gyration of extended chain [Angs]	100.78
38.	Solv. Free energy folding [Kcal/mol]	-532.44

allergic infections. The nonamers shows high antigenic response because of presence of beta sheets regions. The small peptide fragments of antigen can induce immune response against whole antigen. This theme is may be implemented in designing subunit and synthetic peptide vaccines. The antigenicity analysis method allows potential drug targets to identify active sites, which forms antibodies against *Nicotiana tabacum* pollen allergy. The results are further confirmed by studying the protein statistics [Table 1] and antigenic motifs of **L-ascorbate oxidase homolog** protein, which are found to contain antigenic sites. Antigenic epitopes of **L-ascorbate oxidase homolog** protein are important antigenic determinants against the allergic reactions.

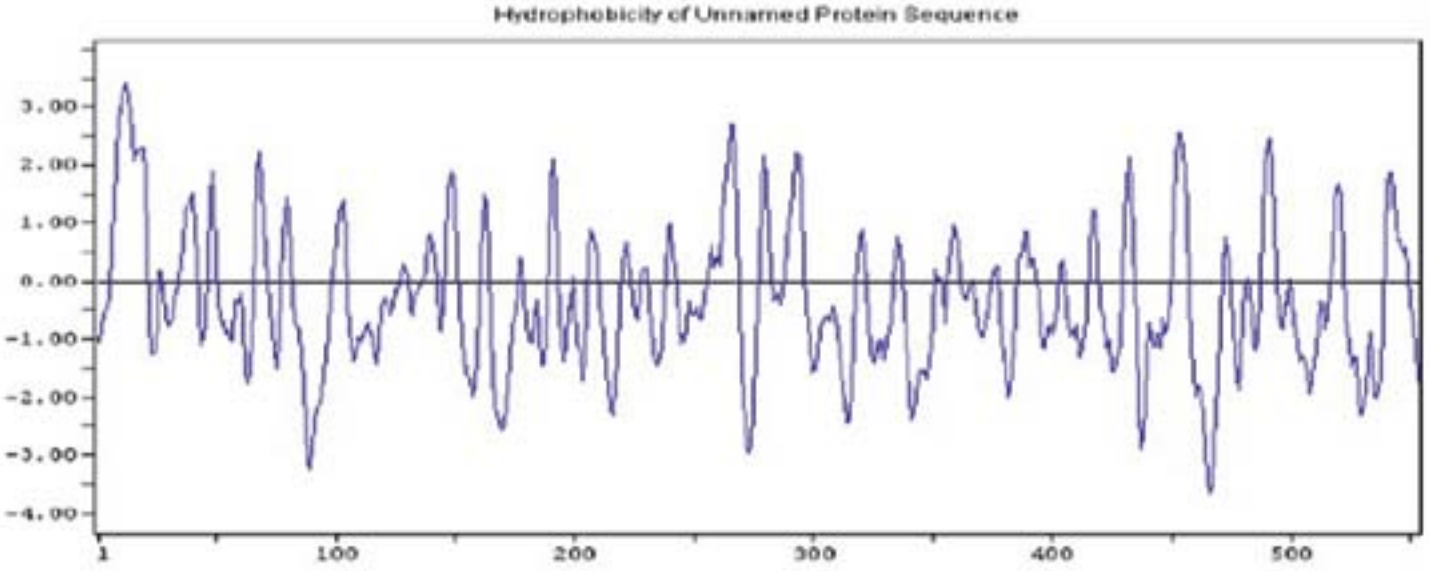

Fig. 1 : Hydrophobicity of L-ascorbate oxidase homolog protein

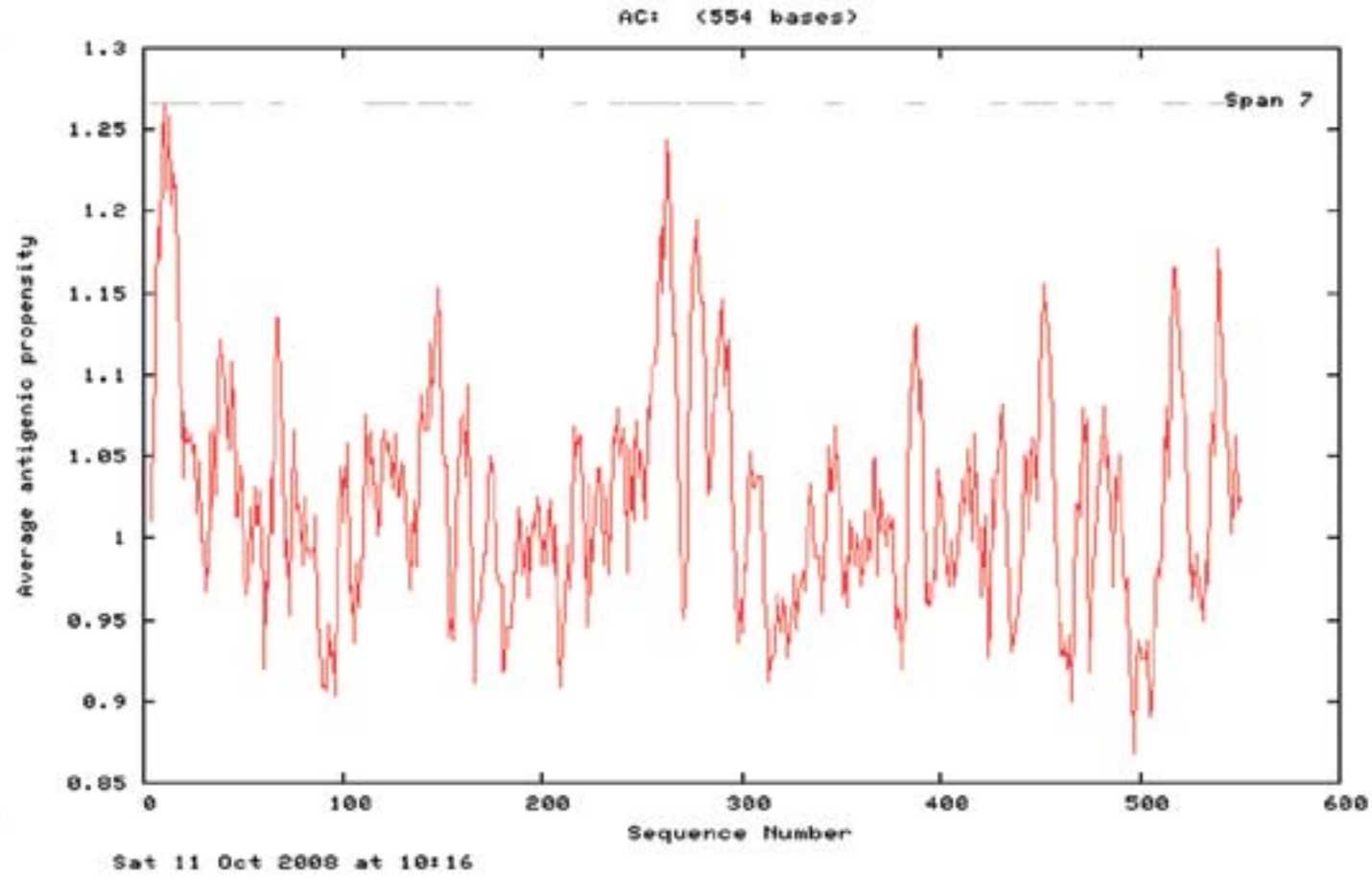

Fig. 2 : Antigenic plot for sequence of L-ascorbate oxidase homolog protein

Table 2 : Antigenic determinants in L-ascorbate oxidase homolog protein

Sr. No.	Start Position	Sequence	End Position
1	4	GKVTFVALLLCLSVGVIAEDPYLYF	28
2	33	TYGTIAPLGVPQQGILIN	50
3	64	NNIVVNV	70
4	111	YRFQVKDQIGSYSYFPTTALHRA	133
5	138	GALNVHSRALIPVPF	152
6	157	DEYNVFVG	164
7	216	RYRFCNL	222
8	235	HPMKLVEL	242
9	244	GSHTVQNIYDSLDLHVGQCLSVLVTA	269
10	273	PKDYYLVVSSRFLKQALSSVAIIR	296
11	304	ASPELPT	310
12	343	QGSYHYGQ	350
13	384	TPLKLVEY	391
14	426	YRNFVEI	432
15	441	IRTYHLDGYSFFAVAVE	457
16	468	NYNLVDG	474
17	479	NIQVYPNS	486
18	512	LGEQLYFSVLSPS	524
19	535	DNHPLCGIVKGLSMPA	550

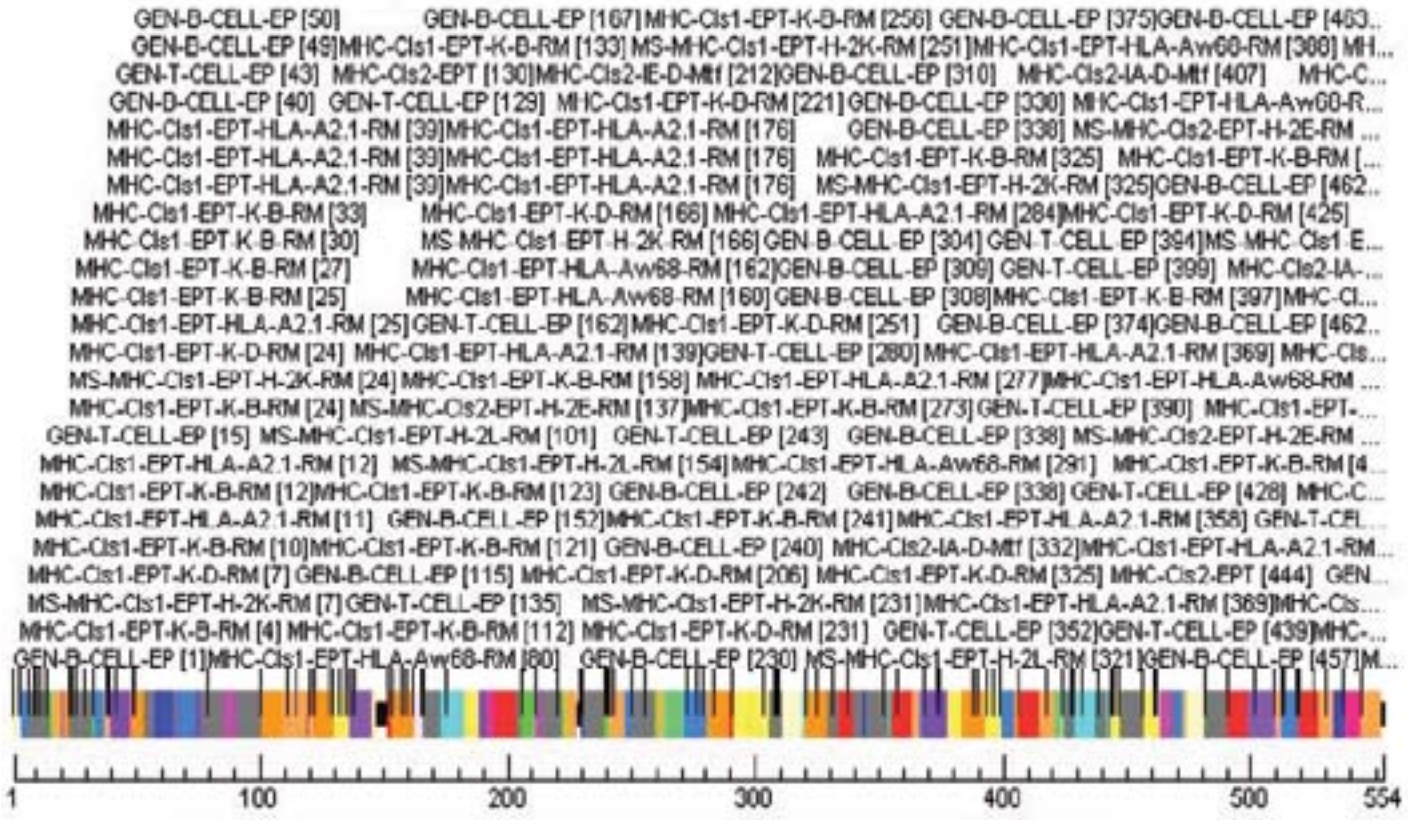

Fig. 3 : Motif map of L-ascorbate oxidase homolog protein

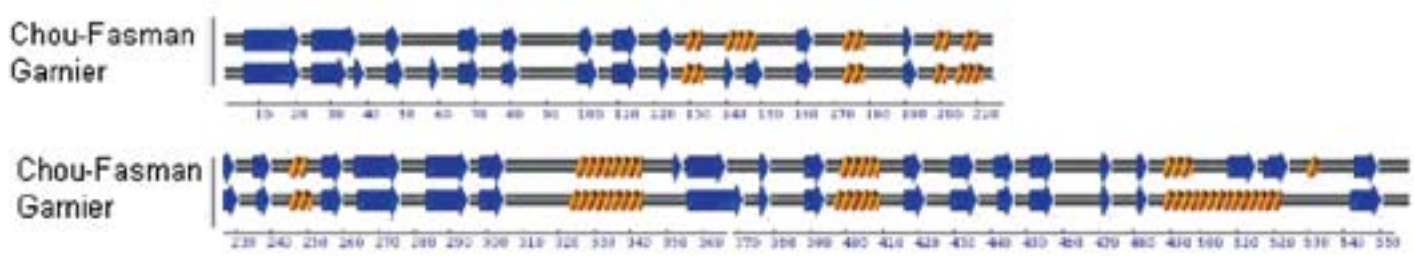

Fig. 4 : Structure prediction of L-ascorbate oxidase homolog protein

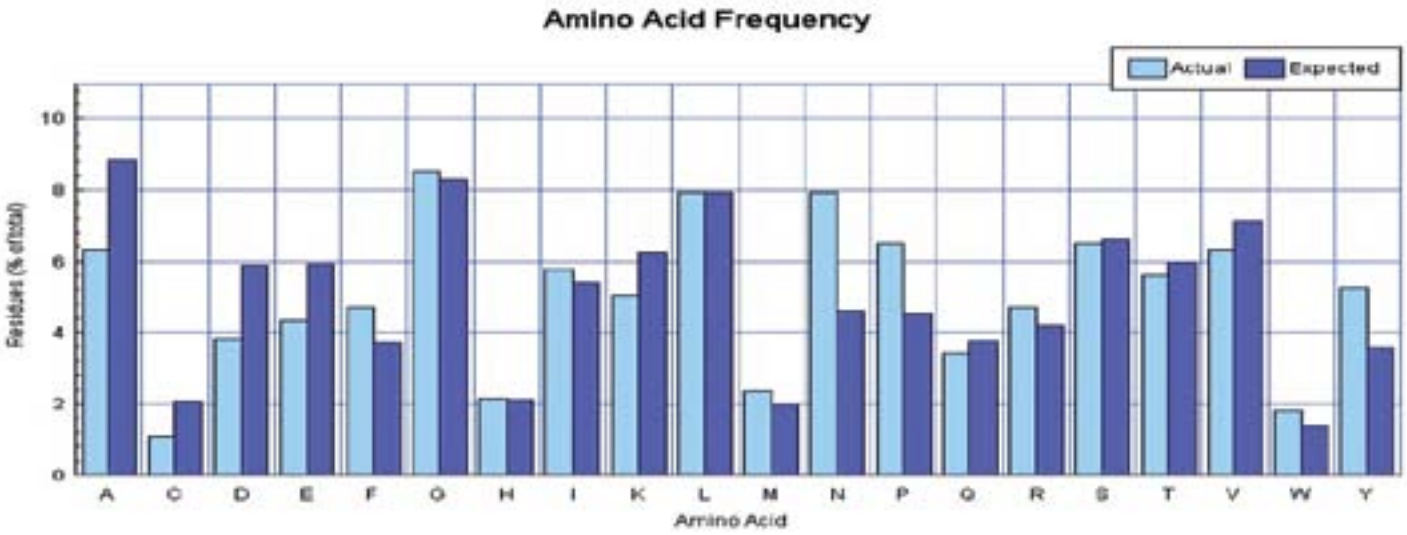

Fig. 5 : Actual and expected amino acid frequency in L-ascorbate oxidase homolog protein

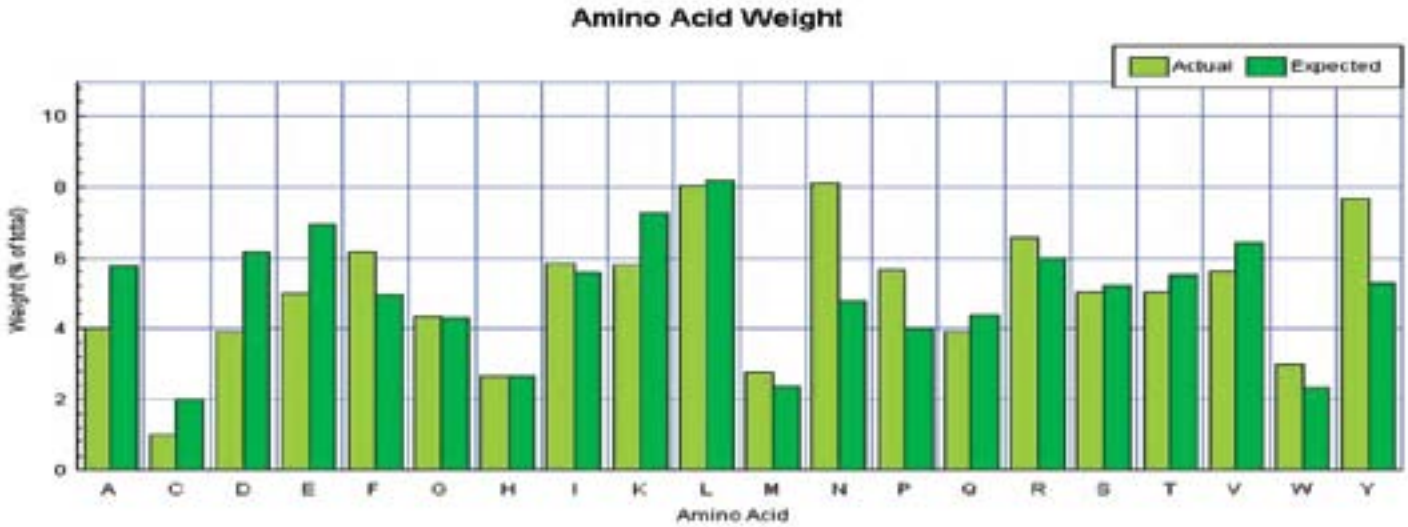

Fig. 6 : Actual and expected percent amino acid weight in L-ascorbate oxidase homolog protein

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Indian J. Aerobiol., Vol. 29, No. 1 & 2, pp 12-18 [2016]

BIO -MONITORING OF AIRBORNE FUNGAL SPORES OF INDOOR AND OUTDOOR ENVIRONMENTS FROM A SUBURBAN AREA NEAR INDO-BANGLADESH BORDER

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Airborne fungi, a significant constituent of atmospheric bio-aerosol, are well known source of *allergens* and can cause allergic rhinitis and bronchial asthma in sensitive objects. Aerobiological studies, which provide qualitative and quantitative information about fungal spores of a given region and also complete biochemical characterization of fungal allergens is required for adequate medication of fungal spore allergy and immunotherapy. The objective of the present study is to monitor fungal aerosol in the air of indoor [college library] and outdoor [pottery belt] environment of Habra, a suburban area near Indo-Bangladesh border, for a period of one year [September' 2016 to August '2017] and to study their seasonal periodicity and to determine the effect of different meteorological factors on their availability and their impact on human health. The samples were collected by the Burkard Personal Sampler and the Andersen two stage viable sampler. A quantitative analysis was done by measuring the airborne spore concentration, correlation of spore concentration with different meteorological parameters and identification of dominant fungal species in both the environments. The study revealed rich biodiversity of fungi in the library and pottery belt. The comparative study between these two environments showed that the spore concentration in the air of library was higher than the pottery belt. It may be because of the paper, glue and leather of books in library, which forms an ideal substrate for growth , sporulation and proliferation of fungi. More than 20 non viable and 7 viable fungal spore types were recorded. Among viable fungal spores, *Aspergillus, Penicillium, Rhizopus Cladosporium* and *Curvularia* and *Cladosporium* were the most dominant. Among non viable fungal spores, Ascospore, Basidiosore, Aspergilli/Penicilli,,*Curvularia* and *Cladosporium* were the most dominant. Among viable fungal spores concentration of *Cladosporium* was higher in pottery belt than library. The local meteorological factors showed significant effects on their availability. So, data generated from this study may help to develop an aeromycobiota of the library and pottery belt and its possible relation to human health

Key Words: Viable and non viable spores, Bio-aerosol, Meteorological parameters, Aero-mycobiota.

INTRODUCTION

Fungal spores are one of the most prevalent components of airspora and are known to play an important role in respiratory allergies, such as bronchial asthma and allergic rhinitis as well as other diseases, affecting the lungs and alveoli [1-2]. Allergy affects nearly 25% of total world population [3]. An increase of 30% patients suffering from allergy has been observed in the last 40 years [4]. More than 25% Indians suffer from allergic problems [5]. Airborne fungal spores are an important source of aero-allergens in both indoor as well as the outdoor environments. So, aerobiological studies are essential to get qualitative and quantitative information about airborne fungal spores of a given region and also the complete biochemical characterization of fungal allergens is required to help the respiratory allergic and asthmatic patients and clinicians to know about the environmental allergen exposures and avoidance policy.

Habra, a suburban area of N-24 Parganas district of West Bengal, is situated 29 km away from Indo-Bangladesh border. Basically surrounded by rural areas with rich tropical and moist vegetation, this town was affected by two remarkable population influx in 1947 [during the partition of India, after independence] and 1971 [during the freedom of Bangladesh from Pakistan]. Habra is probably the fastest growing town in the eastern

part of India and is an important hub for import-export between India and Bangladesh. There has been an enormous change in the vegetation and ecological environment of this area due to the incursion of immigrants from Bangladesh, Burma and other parts of the country [6]. Habra has also undergone rapid urbanization, due to which the existing heterogeneous plant community has undergone large-scale destruction, and new taxa have been introduced, which would have been of allergenic potential. It has also been reported that airborne fungal spores could cause occupational diseases such as respiratory tract allergy, allergic rhinitis, bronchial asthma and other lung diseases [7]. So an aeromycological study was carried out in different working environments of Habra to study the atmospheric diversity of fungal spores, to explore the influence of the meteorological parameters on the occurrence of fungal spores. Main objective of the present study was to monitor and compare both viable and non-viable fungal spores of indoor environment of a college library and outdoor environment of pottery belt of Habra and also to determine and correlate fungal spore concentration with meteorological parameters.

MATERIAL AND METHODS

The prevalence of fungal spores in the atmosphere changes from place to place, season to season and year to year, depending upon the changes of ecological and climatic changes. So extensive aerobiological studies have been done in different environments like an agricultural farm, potato cold store house, cowshed, milk dairy, bakery, rice mill, library, etc. [8-14] for constructing the spore calendar of that particular environment.

A systematic quantification of the indoor fungal spores of the college library and outdoor fungal spores of pottery belt of Habra was carried out for Sept'2016 to August'2017. The library is nearly 50 years old, with a single entrance and lacks proper ventilation as most of the windows are closed. It has many old books and journals. Damaged books are either kept in the racks or they are stacked in one corner for repair. The paper, glue and leather in books, forms an ideal substrate for growth, sporulation and proliferation of fungi. On the other hand, potters of pottery belt are also exposed to many hazards like metals, silica, heat, soil and airborne fungi. Air sampling was done at monthly intervals by two types of air samplers-1] Andersen two stage air sampler [for collecting viable fungal spores] and 2] Burkard Personal Sampler [for collecting non-viable fungal spores]. After air sampling, the exposed petriplates of Andersen sampler were stored and incubated at 37^{o} C for 3-5 days until sporulation takes place. Ultimately fungal colonies were identified to the genus level under microscopy [Leitz, Diaplan, Germany], using lactophenol blue stain [Sigma-Aldrich, Australia]. Non-viable spores trapped on the glass slides of Burkard Personal Sampler were mounted by cover slip with DPX followed by the scanning under microscope and finally they were identified using specialized references [Ellis, 1971; Onions et. al. 1981] and counted according to the guidelines given in the British Aerobiological Federation [The British Aerobiology Federation, 1995]. Meteorological parameters have a direct effect on fungal sporulation and dispersal. Temperature [^{o}C] and Humidity [%] were recorded with the help of a wet and dry bulb hygrometer [G. H. Zeal Ltd., London, UK]. But two other parameters, Rainfall [mm] and Wind speed [km/hr] were collected from the Dumdum regional office of the Indian Meteorological Centre. The entire sampling period covered four seasons viz. pre-monsoon [Mar-Jun], monsoon [Jul-Aug], post-monsoon [Sept-Oct], and winter [Nov-Feb].

RESULTS AND DISCUSSION

The study revealed rich biodiversity of fungi in the indoor environment of library and outdoor environment of pottery belt. More than 20 non-viable and 7 viable fungal spore types were recorded from the sampling sites. The local me-

teorological factors showed significant effects on the spore concentration.

a. In **library** [**indoor environment**], a total of 7680.56 CFU/m^3 [2320.66 CFU/m^3 in pre-mosoon, 1931.92 CFU/m^3 in monsoon, 1507.84 CFU/m^3 in post-monsoon, 1920.14 CFU/m^3 in winter] viable spores were collected. Similarly, a total of 11,290 spores/m^3 [3130 spores/ m3 in pre-monsoon, 2300 spores/ m^3 in monsoon, 3080 spores/m^3 in post-monsoon, 2780 spores/m^3 in winter] non viable spores were sampled from library. This result clearly indicates that the total non viable spore concentrations were dominated over the total viable spore concentrations.

[i] Maximum **viable fungal spore** concentrations were recorded mainly during March to April [pre-monsoon], while September to October [post-monsoon] showed lowest spore concentration. [Fig.1]

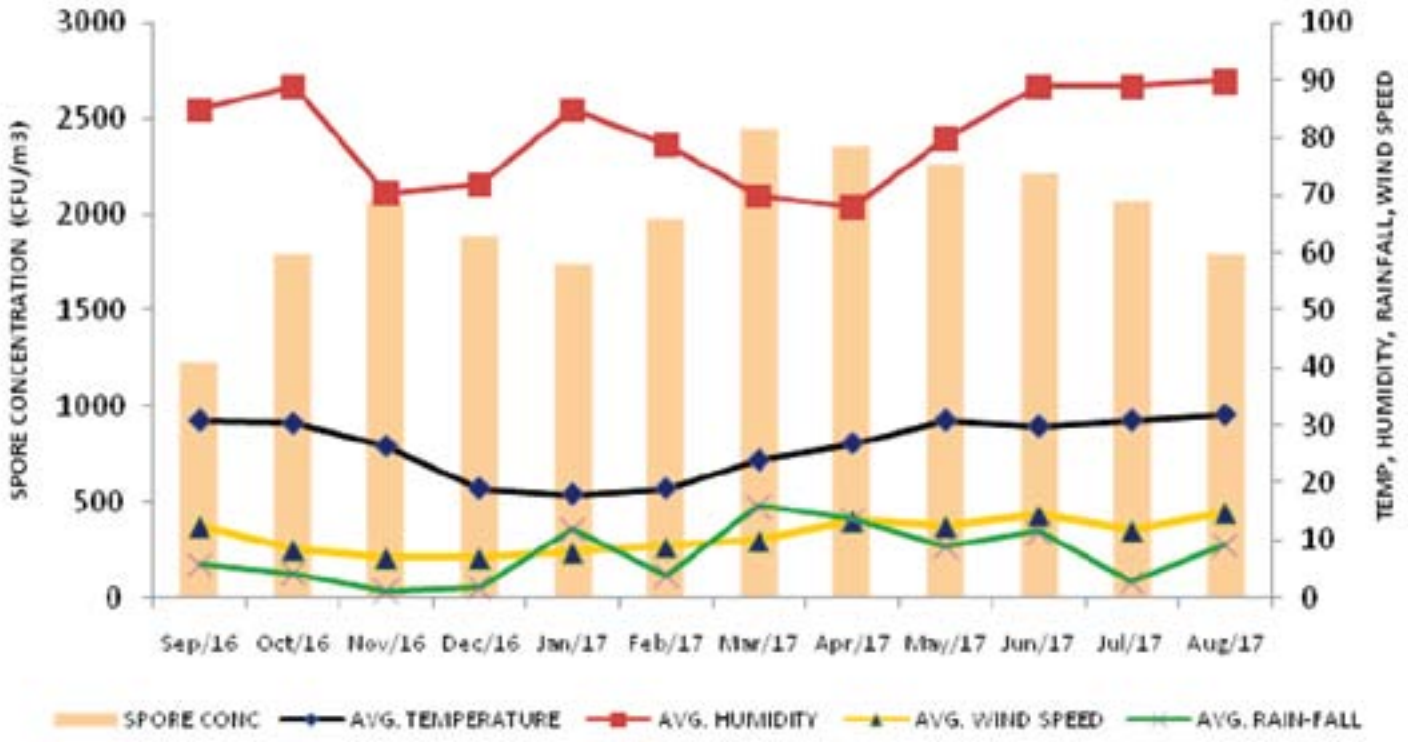

Fig. 1 : **Correlation of viable fungal spore concentration with different meteorological parameters in library** [2016-17].

Temperature, humidity and rainfall showed mixed impact on fungal spore concentration, probably due to the day-to-day differences in optimum conditions for sporulation size, release take-off and flight mechanism for different spore types.

Among viable fungal spores Aspergillus, *Penicillium*, *Curvularia*, *Fusarium* and *Rhizopus* were the most dominant. [Fig. 2]

[ii] Maximum **non viable fungal spore** concentrations were recorded mainly during October and April to May, while lowest spore concentrations were recorded during June to July. [Fig-3]

Among non viable fungal spores Ascospore, Basidiospore, Aspergilli / Penicilli, *Curvularia*, *Cladosporium*, *Periconia* were the most prevalent [Fig.4].

b. In **pottery belt** [**outdoor environment**], a total of 5991.2 CFU/m^3 [1713.41 CFU/m^3 in pre- monsoon, 1775.28 CFU/m^3 in monsoon, 1252.84 CFU/m3 in post-monsoon, 1250.39 CFU/m^3 in winter] viable spores were collected. Similarly, a total of 8,020.25 spores/m3 [2064.25 spores/ m^3 in pre-monsoon, 2270 spores/ m3 in monsoon, 2020.5 spores/m^3 in post- monsoon, 1665.5 spores/m^3 in winter] non viable spores were sampled.

[i] Maximum **viable spore concentrations** were recorded during July, while October showed lowest spore concentrations [Fig.5]

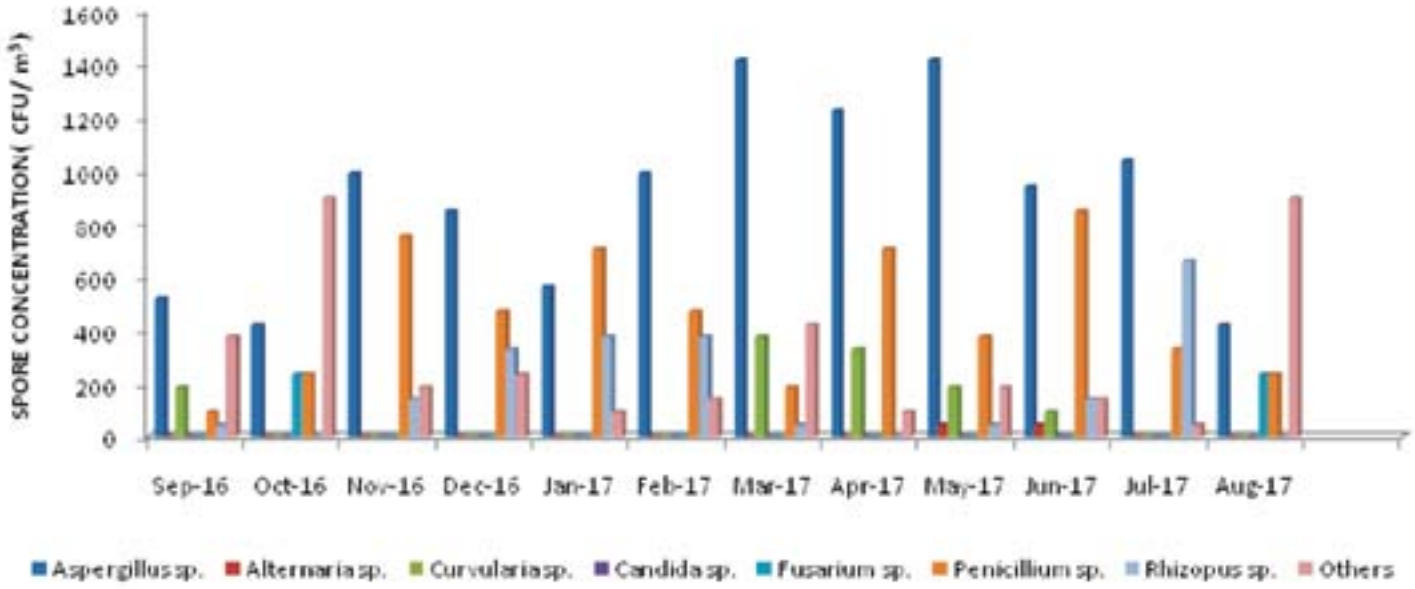

Fig. 2 : Spore concentrations of different dominant viable fungal species in library [2016-17]

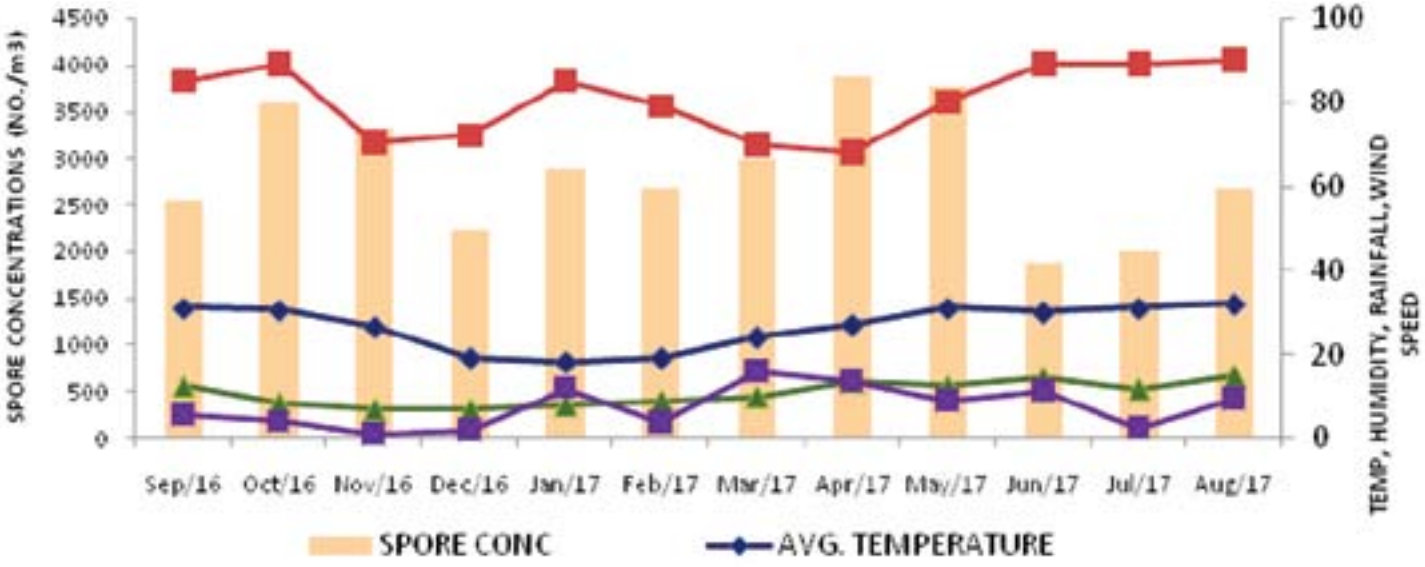

Fig. 3 : Correlation of non viable fungal spore concentration with different meteorological parameters in library [2016-17]

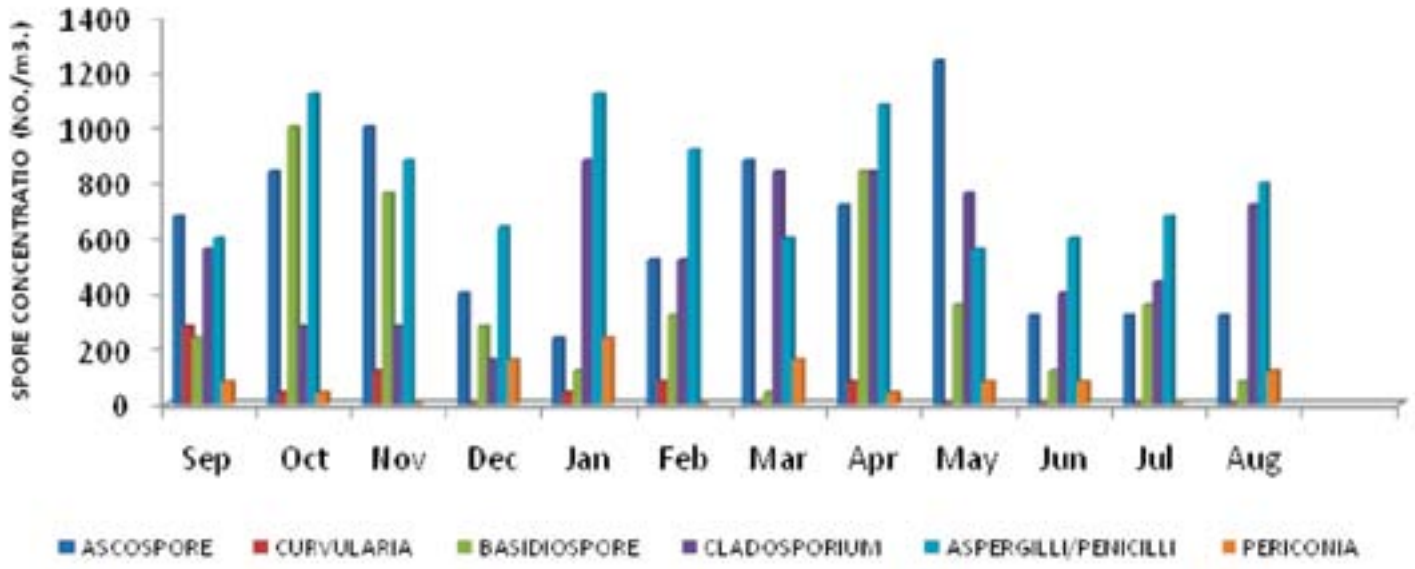

Fig. 4 : Spore concentration of different non viable fungal species in library (2016-17).

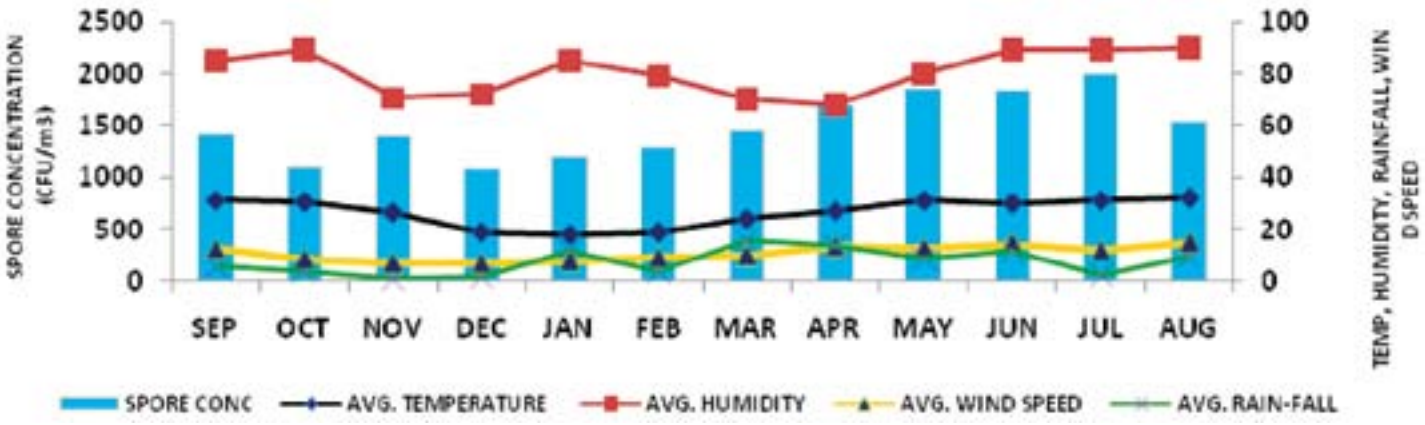

Fig. 5 : Correlation of viable fungal spore concentration with different meteorological parameters in pottery belt [2016-17].

Fig.6 : Correlation of non viable fungal spore concentration with different meteorological parameters in pottery belt [2016-17].

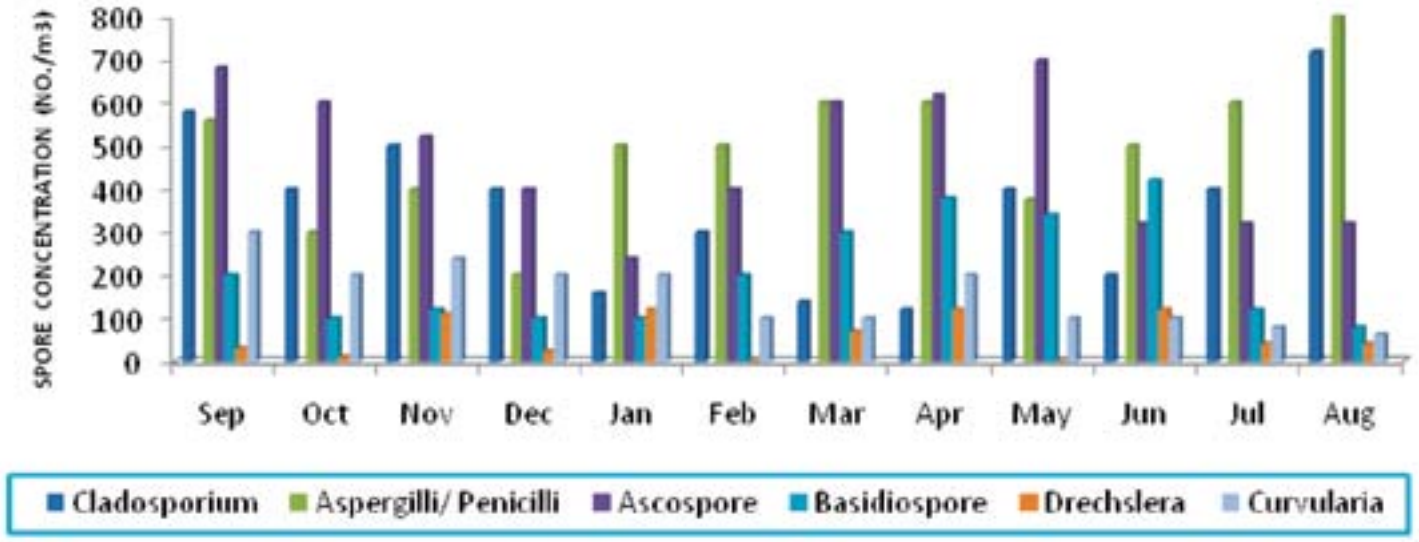

Fig. 7 : Spore concentration of different dominant non viable fungal species

Among viable fungal spores *Aspergillus, Penicillium, Curvularia, Rhizopus, Cladosporium* and *Fusarium* were most prevalent. Concentration of *Cladosporium* was higher in pottery belt than library.

[ii] Maximum **non viable fungal spore concentrations** were recorded mainly during August to September, while lowest spore concentrations were recorded during December [Fig. 6].

Among non viable fungal spores *Aspergillus*, *Penicillium, Curvularia, Rhizopus,* and *Fusarium* were most prevalent. [Fig. 7]

CONCLUSION

The study revealed rich biodiversity of fungi, with highest contribution of asexual spores. More than 20 non viable and 7 viable fungal spore types were recorded from the sampling sites. *Aspergillus*, one of the most allergy causing species was found in the highest number in both the sites. The result shows that the total non-viable spore concentrations were dominated over the viable spore concentrations. The local meteorological factors showed significant effects on the spore concentration. In college library, highest viable spore concentration was observed in pre- monsoon and lowest spore concentration was observed in post-monsoon [because college remain closed during post monsoon i.e. during puja vacation], whereas highest non -viable spore concentration was found in pre-monsoon and lowest non -viable spore concentrations was found in monsoon [because rainfall washes the spores from the air]. On the other hand, in pottery belt, highest viable spore concentration was observed in pre-monsoon and lowest was found in winter, whereas the highest non viable spore concentration was observed in post monsoon and winter showed lower concentration of fungal spores [comparatively drier season, unfavourable for the growth of fungi].

This comparative study shows that fungal spore concentration was higher in library than pottery belt. It may be because of the favorable conditions in library for the growth of fungi like stored books and the dust present on the books, deficiency of cleanliness and presence of humidity [Fig.8].

Besides, it has also been found that concentration of *Cladosporium* was higher in pottery belt than library. Certain preventive measures like installation of exhaust fans, adequate ventilation, use of mask, and proper work practices may reduce the risk of allergy and asthma in different working environments. Regular monitoring of fungal spores may give better knowledge on fungal diversity and for identification of specific group of fungi which cause various types of health problems in human being, which can help in medication of fungal spore allergy and immunotherapy. This study will provide valuable information about the diversity and periodicities of airborne fungal spores in the atmosphere of indoor and outdoor environments. The data will also help clinicians, basic scientists, allergic and asthmatic patients for the disease management

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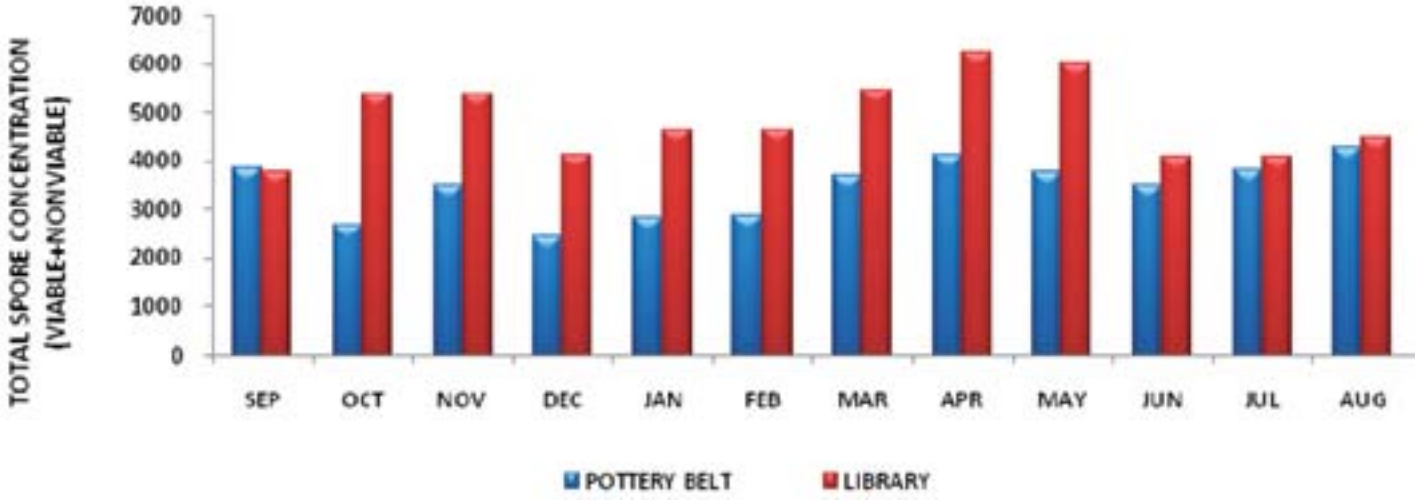

Fig. 8 : Comparative study of total spore concentration between Library and Pottery belt [2016-17].

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ATMOSPHERIC FUNGAL SPORES OF ASPERGILLUS: ITS IMPACT ON RESPIRATORY ALLERGY AND ASTHMA-RELATED HOSPITALIZATION IN SUBURBAN WEST BENGAL, INDIA

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Aspergillus is a ubiquitous fungal genus liberating huge number spores in the air, which cannot be identified up to specific level in standard aerobiological survey, together with *Penicillium*, and categorized under Aspergilli-Penicilli group in microscopic study. In the present study, the diversity and seasonal patterns of airborne viable/cultivable spores of *Aspergillus* were monitored for the year 2012 using Andersen volumetric sampler, in a suburban area of West Bengal, near India-Bangladesh border. Eight atmospheric *Aspergillus* species were identified. Among these, *A. fumigatus* [23.9%], *A. niger* [18.6%] and *A. terreus* [15.6%] were most frequent and perennial types showing concentration up to 88.0 Colony Forming Unit/ m^3 air in the peak period. In skin reaction on fungal sensitized respiratory allergic patients [n = 124] with allergenic spore extract, *A. fumigatus* showed optimum sensitivity [43.63%] in general, whereas *A. terreus* elicited the highest percentage [30.43%] of +2/more level skin reaction. The result was confirmed by IgE-ELISA. Airborne *A. terreus* spore showed significant *association* [$p < 0.05$] with asthma related hospitalization record in local hospital. In IgE immunoblotting of soluble protein extract of *A. terreus* spore, three IgE reactive components of 21, 30 and 35 kDa *molecular* weight was detected with sensitized pooled patient sera. The result indicated *Aspergillus terreus* spore as a probable source of aeroallergen and risk factor associated with asthma related hospitalization in the study area.

Key words : Airborne *Aspergillus* spore, allergenic potential, *Aspergillus terreus*, asthma related hospitalization, IgE-reactive protein, West Bengal.

INTRODUCTION

The systematic study of airborne bioparticulate matters in respect of their origin, dispersal and impact, is called Aerobiology. Fungal spores are important contributor of the atmospheric bio-particles1. Due to the adverse effect to induce human respiratory diseases upon inhalation, aerobiological monitoring of fungal spores is very important. It is estimated that 10% of global population are sensitive to fungal allergens[2].

The members of the ubiquitous fungal genera *Aspergillus*, are well known to produce numerous airborne spores in the atmosphere with diameter small enough to reach the alveoli in the human lungs upon inhalation[3]. Hence airborne spores of the genus *Aspergillus* in particular are considered as a risk factor for allergic asthma and allergic bronchopulmonary aspergillosis in human[4,5].

In all kinds of standard aerobiological monitoring by volumetric samplers, the spore/conidia members of *Aspergillus* and *Penicillium* cannot be identified separately under microscope, hence these are classified under Aspergilli-Penicilli group. For this reason, proper identification up to specific level and quantifications of viable/cultivable *Aspergillus* spores are carried out in suitable culture media with Andersen volumetric sampler[6]. The quantitative data of the viable/cultivable *Aspergillus* spore occurrence in terms of CFU [colony forming unit]/m3 air highlight the specific exposure level of a particular species of *Aspergillus* for inducing respiratory problem in susceptible individuals.

The objective of the present studywas: [1] to present the diversity and seasonal pattern of airborne cultivable spores of different species of *Aspergillus*

and [2] to evaluate their allergenic potential and association with asthma-related hospitalization [ARH] of local population in a suburban area of West Bengal, near India-Bangladesh border

MATERIALS AND METHODS

Aerobiological survey

Monitoring of viable/culturable airborne Aspergillus spores was conducted in the suburban area of Habra [22.83º N 88.63º E], 24 Parganas district, West Bengal, situated near India-Bangladesh border. Sampling was conducted with Andersen Two-Stage Viable Sampler [5 minutes at 14 days interval@28.3 litre/minute] at approximate human height [1.5 m] at 14.00 pm. The duration and sampling time of the day were determined after standardization trials in pilot studies. Each stage of Andersen 2-stage Sampler contains 200 pores [1.5 mm diameter at the first stage and 0.4 mm in the second stage]. The sampler drew air [28.3 L/min] through an orifice at the top and impinged airborne fungal spores successively onto Petri plates containing 2% Malt extract agar and 2% Potato Dextrose Agar medium, both supplemented with ampicilin-100, kanamycin-100 and chloramphenicol-34 to check the bacterial growth [50 µg/ml]. After the exposures, Petri plates are incubated at 27 ± 2º C for 3 days for growth of sporulating fungal colonies. From these plates, individual colony was taken and sub-cultured on fresh plates to make isolated pure cultures. The pure cultures were then sent to Agharkar Research Institute, Pune, for authenticated identification. Fungal colonies were identified to the lowest taxonomic rank possible based on morphological features like colour, size, shape, mycelia type and spores. Different guidelines and published literatures were used for authentic identification[7-9]. *Aspergillus* colonies were further sub-cultured on Zapek-Dox Agar medium containing streptomycin sulphate [50 µg/ml] to facilitate their identification up to species level. The numbers of colonies recorded from both the Petri plates were combined and converted into Colony Forming Unit per cubic meter of sampled air [CFU/m^3].

Preparation of Antigenic Extract from Fungal Spore

Soluble protein was extracted [overnight with constant shaking] in 0.1M phosphate buffer [pH 7.2] from the isolated *Aspergillus* spores [> 95% purity] at 4ºC. A clear extract was obtained after centrifugation at 12,500 rpm at 4°C. The supernatant was then was filtered through a 0.22 µm filter membrane [Millipore, USA] and used for Skin Reaction study after protein estimation[10]. To remove interfering fungal pigments and other non-protein contaminants, the extract was further passed through Sephadex G 25 column. The extracts were stored in sterile vials at -20º C

Skin Prick Test and IgE-Enzyme Linked Immunosorbent Assay [IgE-ELISA]

Skin prick tests [SPT] were carried out with the extract according the case history11 [allergic rhinitis and/or bronchial asthma, eye problems, like angioedema and/or conjunctivitis, etc., either alone or in different combination] of adult respiratory allergic patients of study area attending the allergy clinic at Institute of Child Health, Kolkata. Positive response was defined as the skin prick test with a wheal diameter of 3 mm or greater[12] according to international guidelines. Histamine diphosphate [1mg/ml] and PBS were used as positive and negative controls respectively. The wheal response was measured after 15 minutes and graded +1 to + 3 level13. A total of 124 cases of fungal sensitization were included [M:F = 73:51; age range 19-62 years]. Sera/blood samples were collected from allergic subjects with + 2/ + 3 level skin reaction, not receiving immunotherapy. Control sera were collected from non-atopic healthy volunteers [confirmed by negative skin reaction] and having no history of allergic/systemic diseases. The consent of the patient was obtained prior to sera collection. The whole programme of work was approved by the Ethics Committee of the hospital. IgE -ELISA was performed to measure specific IgE levels with the spore extract using patients' sera[11].

Survey on the Asthma-related Hospital Admission in Study Area

The record of emergency hospitalization due to attack of asthma were collected from Habra State General Hospital. The baseline study was conducted in the year 2011 and the data of the year 2012 were used for statistical analyses, which included the patients [n = 897] of age range 25-65 years [mean age = [49 ± 0.87] years] with principal diagnosis of asthma, According to International Classification of Diseases, 9th revision, Clinical Modification [ICD-9-CM] code for asthma [493. xx] ICD -1022 code J45/4d] as mentioned in the first discharge diagnosis [symptom, medication and case history][14].

Statistical Analysis

The relationship between viable spore count of different species of *Aspergillus* [CFUs/m^3] and asthma-related hospitalization were correlated. The statistical studies were performed using Spearman nonparametric correlation analyses wising SPSS version 20.0.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis [SDS-PAGE] and IgE-immunoblotting of *A. terreus* fungal spore extract

SDS-PAGE [11%] was performed with soluble *A. terreus* spore extract, and the protein components were electrophoretically transferred on to PVDF membrane [0.8 mA/cm^2] in a semi dry system for IgE-immunoblotting following standard protocol11 using pooled *A. terreus* sensitive serum samples with P/N value [patient and normal/control optical density ratio] > 4.0 in 1:10 dilution and antihuman IgE-horseradish peroxidise [Sigma, USA, 1:500 v/v].

RESULTS AND DISCUSSION

Viable/cultivable spore types originating from 20 fungal genera were recorded to be present in the ambient air of the study area. Spores of the genus *Aspergillus* dominated the airspora by exhibiting the highest concentration [37.91%] followed by *Cladosporium* [18.5%], *Alternaria* [9.55%], Curvularia [9.02%], *Nigrospora* [8.14%], and others [Figure 1]. The airborne spores of *Aspergillus and*

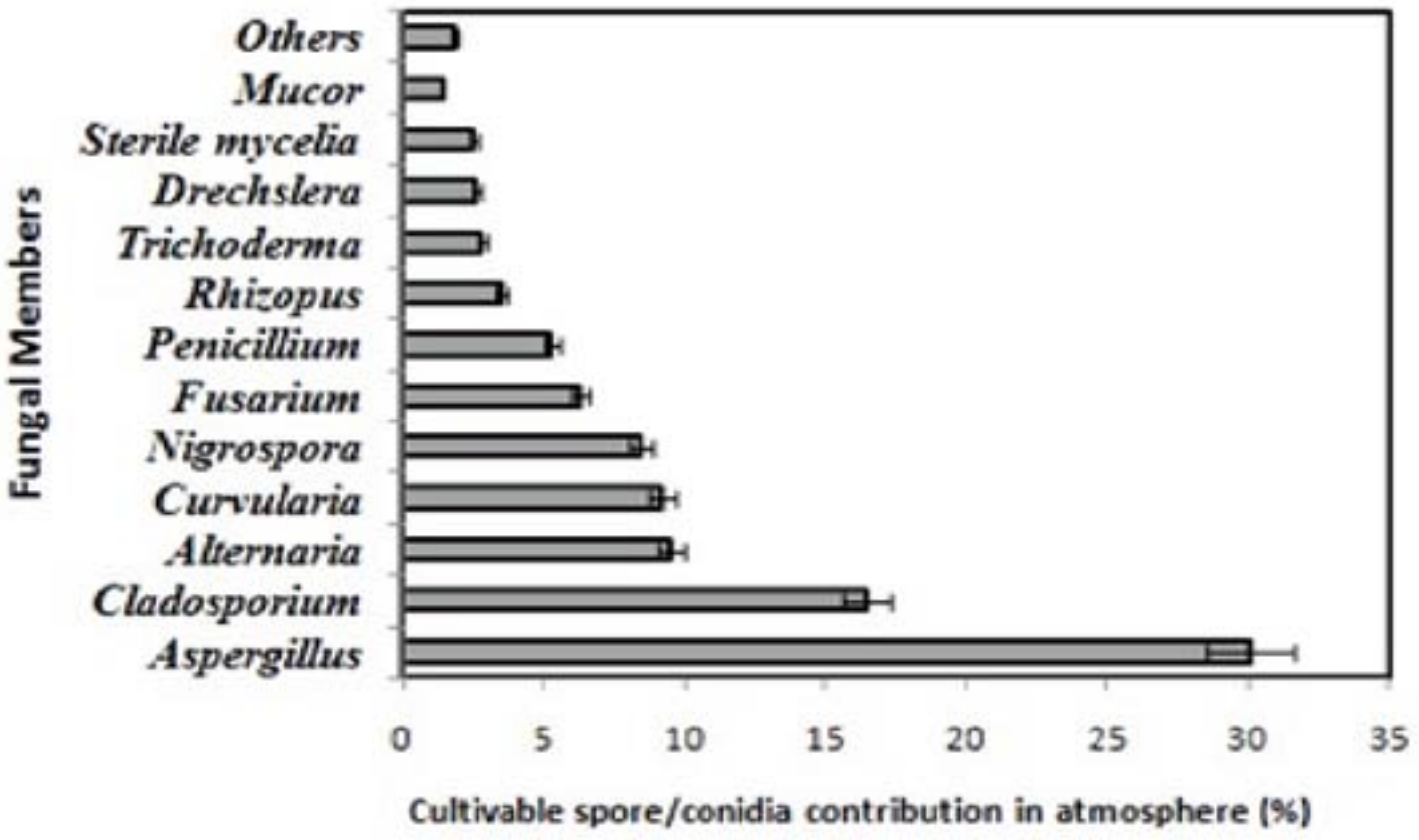

Fig. 1 : Average percentage contribution of fungal spore types in the atmosphere of the study area during 2012.

Penicillium can be identified up to specific level when exposed in nutrient media containing Petri plates and form identifiable colonies. Then the viable spore counts are expressed as Colony Forming Units [CFU].

The study had a focus on the seasonal periodicity of dominant airborne spores originating from the genus *Aspergillus*, which are the producers of one of the smallest sized group of fungal spores [< 3 μm size][3]. Hence, *Aspergillus* spores has the potential to penetrate easily in the lower airways of lung and eventually to induce allergic asthma in susceptible individuals, in addition to allergic bronchopulmonary aspergillosis [ABPA] and upper respiratory allergic disease [e.g., allergic rhinitis][15]. Altogether, eight species of Aspergillus were identified and quantitatively studied for the year 2012 [Fig. 2]. The highest contribution was made by *Aspergillus fumigatus* [23.9%], followed by *A. niger* [18.6%], *A. flavus* [15.6%], *A. terreus* [13.8%], *A. nidulans* [11.6%], *A. oryzae* [6%], *A. sydowii* [5.6%] and *A. ustus* [4.9%] *respectively*.

In comparison, an airspora study from a nearby suburban area recorded viable/cultivable *members* from seven *Aspergillus*[16] species just a decade back [2002], where interestingly *A. tereus* spore was not recorded. Another contemporary report from the urban atmosphere of Kolkata metropolis indicated the presence of same number of Aspergillus species [eight], but *A. ochraceous* was present there and *A. terreus* was not found[17]. In case of Delhi metropolis, cultivable spores from six species of *Aspergillus* were recorded by Singh et al.[18], where *A. niger*, *A. terreus*, *A. oryzae* and *A. ustas* was absent with additional presence of *A. ochraceous* and *A. versicolor* in the indoor and outdoor air of the residence of allergic patients. Usha et al.19, reported the diversity of airborne viable *Aspergillus* spores, represented by 12 species using Petriplate exposure in indoors and outdoors of a rural agricultural area of Pondicherry, indicating the additional incidence of *A. awamori*, *A. candidus*, *A. flaviceps*, *A. tamarii* and *A. versicolor*, whereas *A. oryzae* was not found there.

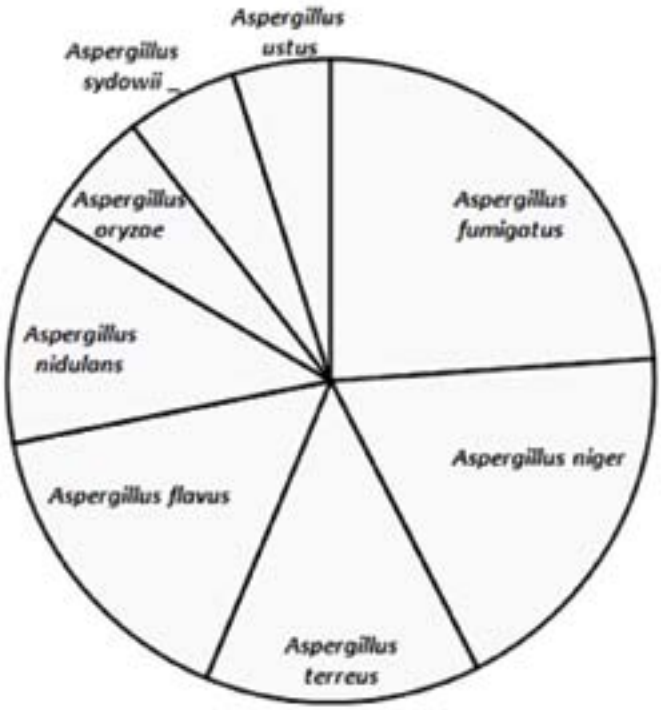

Fig. 2 **:** Percentage contribution of the airborne cultivable/viable spores arising from different species of *Aspergillus*

Among the recorded species of *Aspergillus*, [Fig. 3], *A. fumigatus*, *A, niger* and *A. terreus* were throughout perennial, where *A. flavus* was absent only in April. *A. nidulans* was not found in April-June, *A. oryzae* & *A. sydowii* in January-February & May-June and *A. ustus* in January-May and December. During peak period in April and October, viable spore concentration of *A. fumigatus* reached up to 88 CFU/m^3. *A.niger* had the peak in February & October [up to 63.5 CFU/m^3], *A. flavus*, *A.nidulans*, *A oryzae* and *A. sydowii* in October [14.3-59 CFU/m^3] and *A. terreus* in September [64.7 CFU/m^3]. Corroborative to this result, there was previous report indicating the perennial pattern of airborne *Aspergillus* spores in the atmosphere[17], but details of the seasonal patterns of the particular species was not reported. However, none of the twelve *Aspergillus* species from rural Pondicherry region[19] was found to be perennial.

In skin reaction test among respiratory allergic patients of the locality [n = 124], maximum sensitivity was elicited by *Aspergillus fumigatus* [43.63%], followed by *A. flavus* [43.13%], *A. terreus* [42.59%], *A. nidulans* [39.62%], *A. niger* [36%] and others

[Table 1]. Regarding +2/more level reaction, highest response was showed by *A. terreus* [30.43%], followed by *A. fumigatus* [25%], *A. niger* [22.22%] and others. Specific IgE-ELISA was performed with the serum samples of +2/more level sensitive patient serum, where ratio of patient and normal [control serum from healthy volunteers] sera [P/N value] ranged within 3.7-6.3 [Table 1]. *Previous* researches on skin sensitivity of *Aspergillus* showed *A. japonicas* to be the most reactive [n = 48 and 112 with 50.92% and 54.8% positivity respectively] 16,20 and *A. fumigatus* [73.6%][21] from the nearby suburban area. In any of the studies from this area, *A. terreus* was never included for in vivo [skin reaction] and in vitro [IgE ELISA] clinico-immunological study.

Regarding the association of airborne *Aspergillus* spores and asthma related hospitalization [ARH] of the study area [Fig. 4], significant positive correlation was depicted by *Aspergillus terreus* [$r = 0.6525$, $p < 0.05$] [Table 2].

A. terreus is already known to cause allergic bronchopulmonary aspergillosis[22]. In 2000, Dales et al. reported the association of ambient fungal spores of *Aspergillus-Penicillium* with emergency

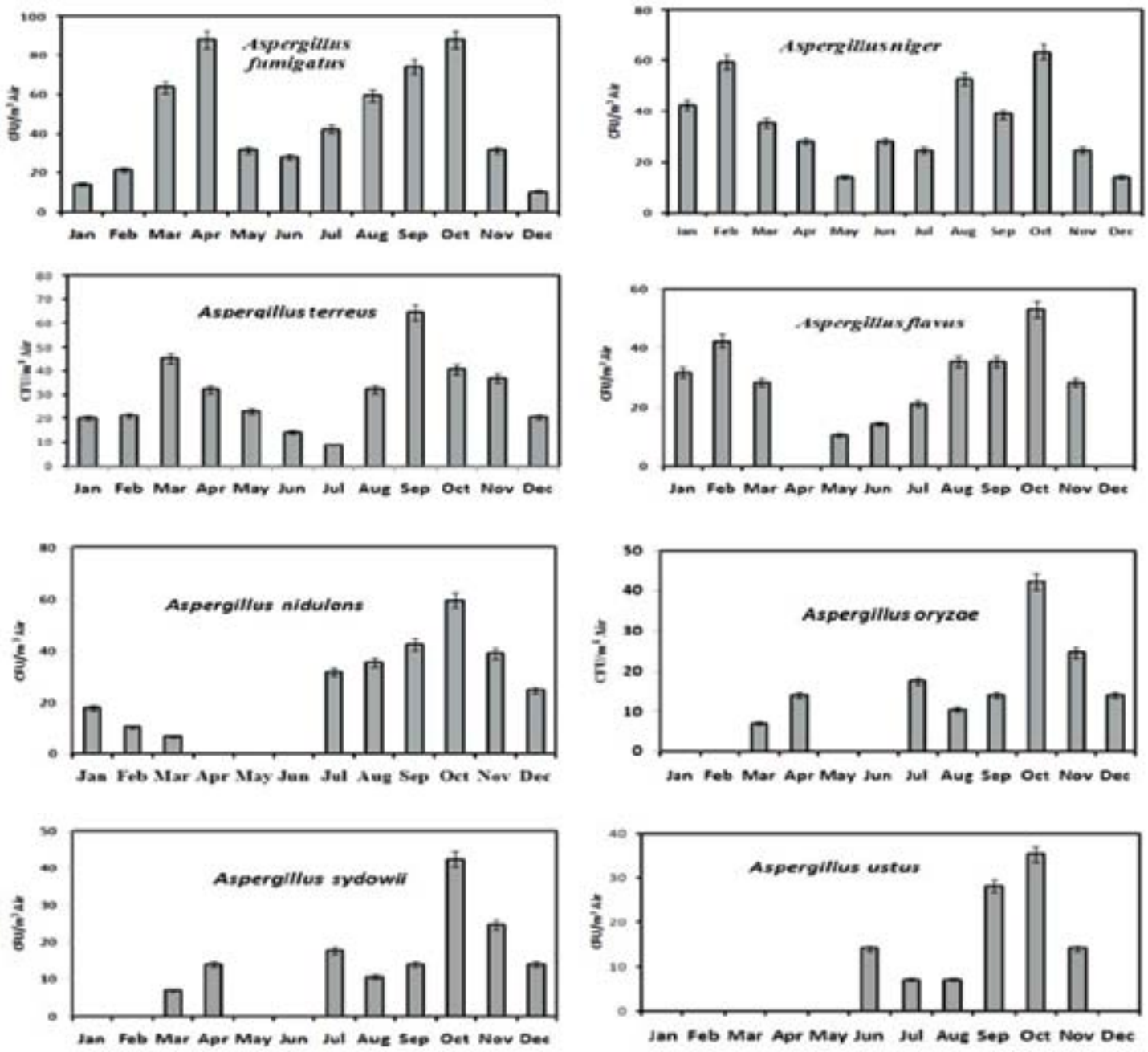

Fig. 3 : Seasonal periodicities of the atmospheric fungal spores of different *Aspergillus* species, during 2012, in the study area. Bars indicate standard deviation.

Table 1 : Result of skin reaction tests and specific IgE ELISA with the fungal sensitized respiratory allergic subjects. [n = 124] for the spore extract of different Aspergillus species.

Fungal spore extracts	Subjects in skin test	Overall positive reaction & percentage	+2/above level reaction & percentage	P/N range in IgE ELISA for +2/more level serum sample
Aspergillus fumigatus	55	24 (43.63%)	6 (25%)	4-5.6
Aspergillus terreus	54	23 (42.59%)	7 (30.43%)	4.2-6.3
Aspergillus niger	50	18 (36%)	4 (22.22%)	3.8-6.0
Aspergillus flavus	51	22 (43.13%)	3 (13.63%)	4.2-6.0
Aspergillus nidulans	53	21 (39.62%)	2 (9.52%)	3.7-5.5
Aspergillus sydowi	51	14 (27.45%)	0	_
Aspergillus ustus	52	12 (23.07%)	0	_

P/N = Ratio of optical density at 492 nm in IgE ELISA for patient and normal (control) sera.

asthma hospitalization in Canada[23]. Regarding Indian subcontinent, an association of airborne *Aspergillus* with asthma exacerbation [n=391] was reported from Pakistan[24]. However, association of viable spores arising from individual *Aspergillus* species with evidence of skin reaction and specific IgE-level was not reported from any of these studies. Based upon the association with

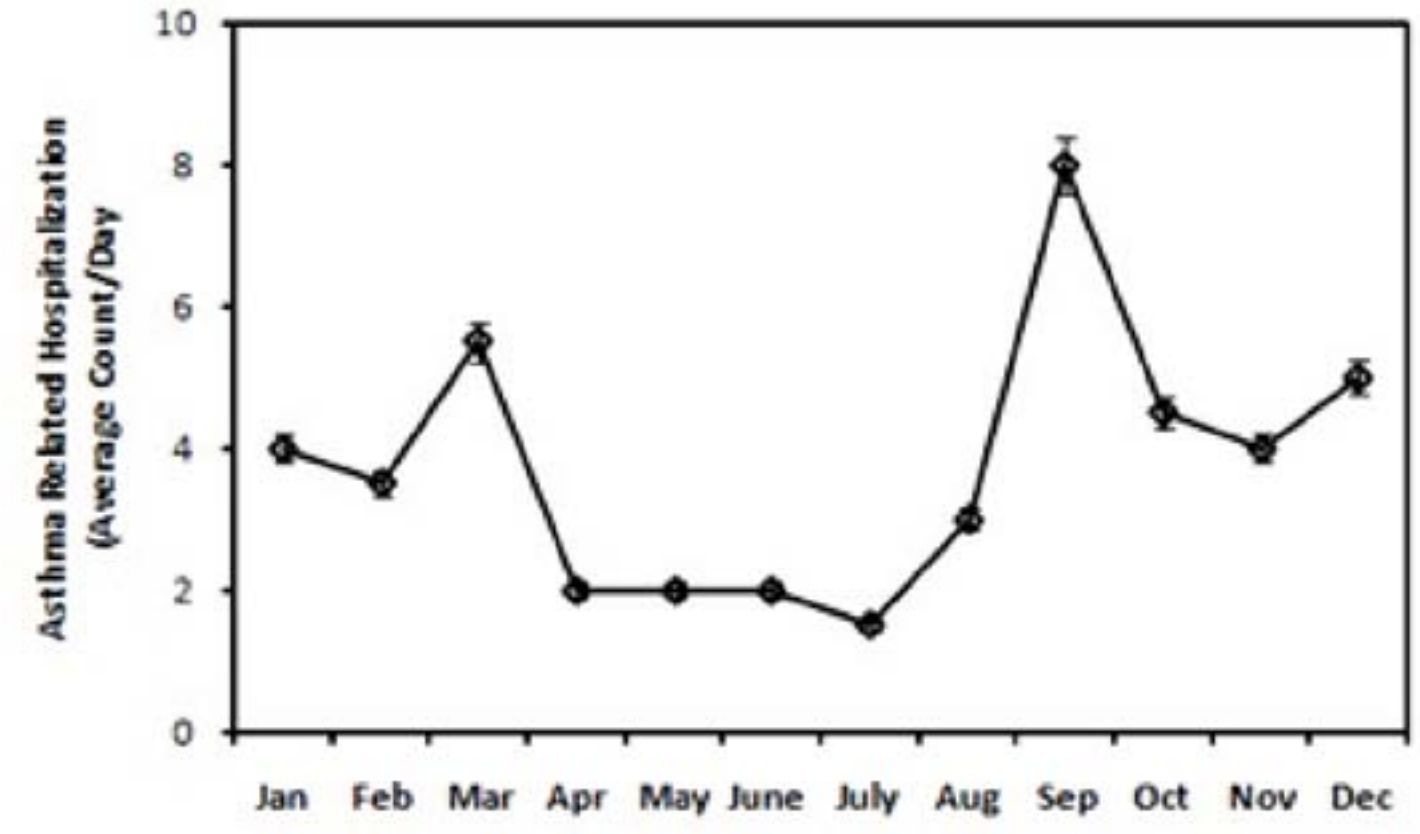

Fig. 4 : Seasonal variation of asthma related hospitalization [monthly average] in the local hospital of study area. Bars indicate standard deviation.

Table 2 : Correlation study for the association of airborne cultivable/viable spores of different *Aspergillus species* and asthma related hospitalization of study area.

Name of Species	Correlation Co-efficient (R)
Aspergillus fumigatus	0.0690
Aspergillus niger	0.2819
Aspergillus terreus	0.6525*
Aspergillus flavus	0.2168
Aspergillus nidulans	0.3847
Aspergillus oryzae	0.4160
Aspergillus sydowii	0.0769

*p Value <0.05 level

ARH, and percentage of +2/more level skin sensitivity, fungal spores of *A. terreus* was further selected for basic immuno-chemical studies to observe its IgE reactive soluble components. When the soluble protein from the pure bulk spore of *A. terreus* was studied by 11% SDS-PAGE, approximately 12 protein components [15-116 kDa molecular weight range] were found by Coomasie Brilliant Blue staining. Among these components, three distinctly visible IgE-reactive components of 35, 30 and 21 kDa molecular weight were observed in the IgE-specific immunoblotting with *A. terreus* sensitized pooled sera [Figure 5].

Atmospheric fungal spores of *Aspergillus:* its impact on respiratory allergy and asthma-related hospitalization in suburban West Bengal, India

Till date, no allergens from *A. terreus* spore have been characterized directly. Some heat shock proteins [e.g. Hsp70][25] have been found to be IgE reactive in other species of *Aspergillus*, was also isolated from *A. terreus*. Alkaline proteases secreted by *A. terreus* are also probable components to be IgE reactive like the alkaline proteases in other fungal spores[26]. Proteomic research with these IgE reactive components will help in their further characterization

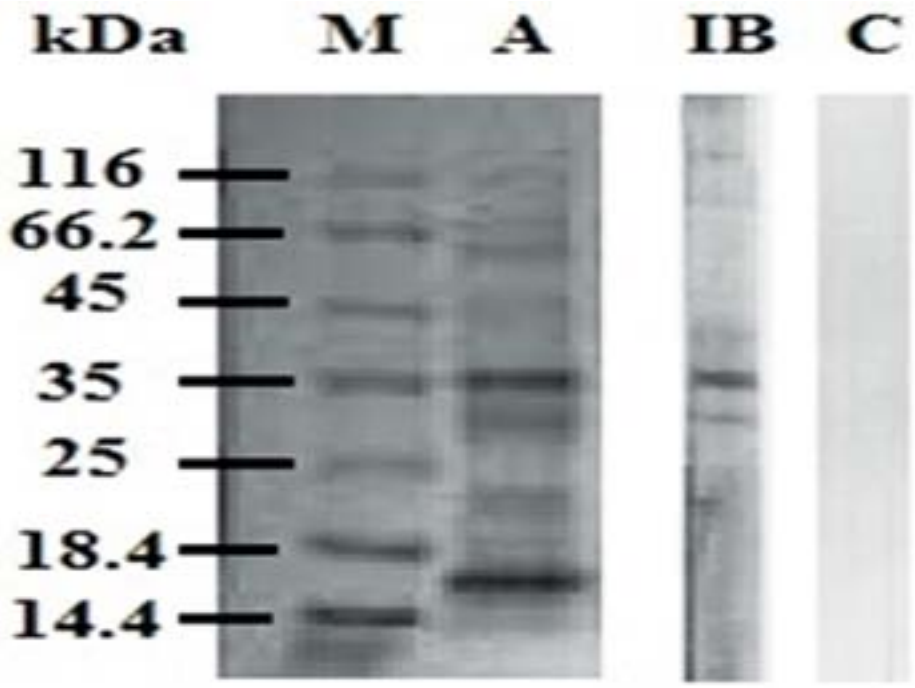

Fig. 5 : SDS-PAGE (11%) profile of Aspergillus terreus spore soluble protein extract [A] and its IgE-reactive protein components [IB] detected by IgE-immunoblotting using sensitized pooled sera. M = Marker, C = immunoblotting with control sera sensitized.

CONCLUSION

In the present study, for the first time a result of comprehensive study on the association of airborne cultivable spores from diverse species of *Aspergillus* and asthma related hospitalization [ARH] from eastern India was reported. Among the eight species, the viable spores of *A. terreus* were found to be perennial, contributing 13.8% of *Aspergillus airspora*, with 42.59% positive skin reactivity, elevated specific IgE level in sensitized patients and significantly associated with ARH of local people. Though other *Aspergillus* spore members did not show significant association with ARH, further long-term and more in-depth study on larger population is necessary to study their exact respiratory health impact. In immunochemical studies, soluble protein of *A. terreus* spore extract elicited the presence of three IgE reactive protein components of approximately 35, 30 and 21 kDa molecular weight. These proteins may be the source of aeroallergens from *A. terreus* spores. Detail study in required to isolate and characterize the IgE reactive proteins for future use in respiratory allergy diagnostics and therapy of local people.

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IMPACT OF MONSOON ON AIRBORNE FUNGAL SPORES OVER *PHASEOLUS VULGARIS* L. CROP FIELD AT PUNE, INDIA

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Airborne fungal spores over *Phaseolus vulgaris* L. crop field at Ganeshkhind, Pune [M.S] was studied during monsoon season [June-September] using volumetric "Tilak air sampler". The meteorological parameters during monsoon have found to play significant role in determining the spore load in the air. Incidence of Myxomycotina and Zygomycotina has been recorded less in comparison with higher fungi like Ascomycotina, Basidiomycotina, Deuteromycotina and Other types. Deuteromycotina have been recorded highest during all the months as compared to all groups of this kharif season i.e. June 61.14%, July 72.51%, August 57.04% and September 56.79% where *Cladosporium* has been found to be highest contributor among all the fungal spores. Date wise investigations of airborne total fungal spore load in comparison with Ascomycotina and other spore types [except *ascospores*] in relevance with meteorological parameters during monsoon 2012 revealed interesting fluctuations and rhythmic dynamics and recorded typical correlations with meteorological parameters in general and rainfall in particular. Because usually fluctuations in relative humidity and temperature have been found to be *determined* and regulated to a maximum extend by rainfall dynamics.

Aim of this investigation was to find out relationship between meteorological parameters and month wise incidence of different groups of fungal spore types, their percentage contribution and fluctuations in their total load in the air in order to understand rhythmic dynamics of the aerospora governed by rainfall, relative humidity and temperature during this season.

Key words: Aerospora, Fungal spores, Meteorological parameters, *Phaseolus vulgaris* L. Kharif season, Pune

INTRODUCTION

Fungal spores are an omnipresent component of the air with concentrations and compositions known to fluctuate according to interaction between biological and meteorological parameters. Meteorological parameters have been found to play significant role in the incidence and load of the fungal spores in the air. The composition and concentration of fungal spores is determined by time, day, night, season, source and locality[1]. Jacobs [1951] pointed out the close relationship of aerobiological survey and its relevance to meteorological parameters[2].Effect of meteorological factors causing impact on the spore composition and load in the air have been studied by many authors for many geographical domains [3-8].

In the dominant groups like Deuteromycotina spores are directly exposed and superficial while in Ascomycotina spores are inside a sac and covered in various fruiting bodies. Ascomycotina fruiting bodies absorbs water in the periderm wall and swell in high relative humidity. So generally after rainfall fruiting bodies break to release ascospores. Due to high wind velocity during rainy season asci and ascospores become airborne and observed in large numbers in airspora showed fluctuations and rhythmic dynamics. For preliminary investigating such ascospores rhythms, study was carried out for one Kharif [rainy] season. Such ascospores recorded typical correlations with meteorological parameters in general and rainfall in particular. So the study was categorized in two major headings i.e. ascospores and other than ascospores [other spore types]. Hence the aim of this Aerobiological investigation was to find out relationships between meteorological parameters and composition of airborne ascospores and other than ascospores [other spore types]

over *Phaseolus vulgaris* L. [Rajma] crop in order to study the dynamics of spore load. The aerobiological study enable us to ascertain the concentration of the fungal spores of different groups present in the atmosphere and give better understanding of the relationship between their concentrations and the weather parameters.

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MATERIALS AND METHODS

The Rajma [*Phaseolus vulgaris* L.] crop field is air monitored for one kharif season from 18th June 2012 to 10th September 2012 at farms of Mahatma Phule Krishi Vidyapeeth Zonal Agricultural Research Station [Western Maharashtra, Plain Zone] located at Ganeshkhind Pune-7. Pune lies between 18° 28′25′′ north latitude, 73° 47′52′′ East longitude and 560 meters altitude exhibiting tropical wet and dry atmosphere. The air sampling was carried out using volumetric continuous '*Tilak* air sampler' installed at a constant height of 1 meter above ground level, in the field[9].Slides have been prepared for identification and examined microscopically. Scanning and detailed calculations were performed according to the standard method[10]. Spore concentration was expressed as number of spores/m^3 of air. The slides so prepared had been scanned under 10x X 40x magnification using binocular research microscope [Lawrence and Mayo].

Daily records of meteorological parameters have been obtained from Indian Meteorology Department [IMD], Shivaginagar, Pune [M.S.] nearest place from the crop field. Identification of fungal spore was based on morphological characters, visual identification by comparing with reference slides prepared from fungal collection from surrounding area and by exposing petriplates with sterile PDA medium. The reference slide were prepared from fungal colonies and identified up to generic level by referring authentic literature[11-14]. Statistical analysis was done for qualitative and quantitative estimation of spores. Correlation coefficient was computed by Pearson Correlation and its significance was tested at 0.05 and 0.01 level of significance. Value of coefficient of correlation was computed for total spore load, ascospores and otherspore types in relation to various meteorological parameters. SPSS software [version 16] and Windows Office 2007 [Excel] was used for the statistical analysis.

RESULTS

Airborne fungal spores over Rajma [*Phaseolus vulgaris* L.] crop field using Tilak air sampler during Kharif 2012, from 18thJune to 10thSeptember, revealed month wise fluctuation in the different groups of fungal spores, in accordance with rainfall and other meteorological parameters. Meteorological parameters have found to play significant role in determining the spore load under different groups like Myxomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, Deuteromycotina and Other types. Incidence of Myxomycotina and Zygomycotina has been recorded least in comparison with higher fungi like Ascomycotina, Basidiomycotina, and Deuteromycotina [Table 1].

Deuteromycotina have been recorded highest during all the months of this kharif season i.e.

June 61.14%, July 72.51%, August 57.04% and September 56.79% where *Cladosporium* alone contributed31.12%, 19.56%, 15.21% and 15.67% respectively. So *Cladosporium* has been found to be highest contributor [Dominant spore type] among all the fungal spores from different groups of fungi and also Deuteromycotina group so this type was emphasized. Load of *Cladosporium* has been found to fluctuate in the inverse proportion of rainfall e.g. in June 2012 when rainfall was less [20.6 mm] *Cladosporium* contributed the highest spore load [31.12%] and when rainfall was highest [204.6 mm] during August 2012, *Cladosporium* has been found lowest [15.21%] may be probably due to wash off of *Cladosporium* by rains. In July 2012 at 72.2 mm rainfall it was 19.56% and September recorded 42.4 mm rainfall when *Cladosporium* contributed 15.67% only [Table 1].

These comparative findings thus indicated clearly that during August 2012 due to high rainfall there was highest wash-off of *Cladosporium*, recording the lowest percentage contribution of the season in the air. Similar is the case with entire group Deuteromycotina, which also contributed low [57.04%] when there was highest rainfall of the season [204.6 mm] during August 2012 [Table 1].

On the other hand observations with respect to Ascomycotina in this Kharif have been found to reveal direct proportions or correlation with fluctuations in the rainfall; e.g. when there was highest rainfall during August 2012 [204.6 mm] the percentage contribution of Ascomycotina also has been recorded high i.e. 19.63% and this increase has been continued further even with less rain [42.4 mm] during September 2012 contributing 25% [Table 1, Fig. 1.a, b]. Although rain fall is scanty in September [42.4 mm] there is prolonged atmospheric effect of humidity due to occurrence of very heavy rainfall in August [204.6 mm]. So the temperature is also decreased during September [24.8° C] and showed rising humidity [84.5%] [Fig.1.b]. Comparatively Ascomycotina was less in June 15.07% at 20.6 mm rainfall and lowest [11.12%] during July 2012 when rainfall was 72.2 mm [Table 1, Fig. 1.a, b].

Basidiomycotina components have been recorded increasing during August [10.09%] and September [12.14%] not because of rains but because of blooming of the cereal when they express symptoms and release smut spores in the air as compared to June-July when there is no dough and blooming of cereals, so no question of release of smut spores. This is clear from the percentage contribution of basidiomycotina group including smut spores from June to September 2012 i.e. 8.60%, 6.49%, 10.09% and 12.14% respectively [Table 1].

The Other types, Myxomycotina and Zygomycotina did not record pronounced month-wise fluctuation at the different extent of rains and other meteorological parameters [Table 1].

Date wise investigations of airborne total fungal spore load in comparison with Ascomycotina and other spore types in relevance with rainfall and other meteorological parameters over Rajma crop field at Pune during Kharif 2012 i.e. from June to September 2012 revealed interesting fluctuations and rhythmic dynamics. Ascospores recorded typical correlations with meteorological parameters in general and rainfall in particular. So ascospore load is so much emphasized. Usually fluctuations in relative humidity and temperature have been found to be determined and regulated to a maximum extend by rainfall dynamics.

It is well known that the rainfall has a definite effect on release of ascospores. The relationship of ascospores in the airspora and its relevance to rainfall was studied during entire investigation period from 18th June 2012 to 10th September 2012. Ascospores data from 18th June to 30th July showed less fluctuation due to low rainfall hence the date wise study was presented from 30th July and onwards [Table 2]. The fluctuation in ascospores was confirmed by considering two prior days i.e. 30th and 31st July before maximum

rainfall [84 mm on 1st August] of the entire Kharif season and in the following days of rainfall and the days after the rainfall had stopped [Table 2].

Observations pertinent to 30th and 31st July revealed 2408 total load comprising 476 ascospore load and 1932 other spore types and *2870* total load comprising 686 ascospores and 2184 other spore types at 5mm and 18mm rainfall respectively, whereas more rainfall [18 mm] on second day recorded increased total load and also the load of ascospores. This proves the direct proportional role of rainfall responsible for the increase of spore load [Table 2].

This progressive increase has been found continuously from 1st August to 8th August 2012 as there is daily rainfall during this week [112.8 mm], hence regulating the relative humidity and temperature and making it favourable for release and dispersal of all fungal spore types in general. High rainfall also favoured the dehiscence of ascocarps and asci, hence leading to release and dispersal of ascospores in particular. On 1st August total load has been increased to 7994 [as compared to previous day i.e. 2870], ascospore load increased to 1596 from 686 and other spore types increased to 6398 from 2184. This increase may be mainly due to 84 mm rainfall on this day. This progressive increase reached to total 12656, out of which number of ascospore was 4438 and other spore types recorded was 8218 on 6th August 2012. The spore load attained peak on 7th and 8th August 2012 as total 16324, ascospore 3976 and other spore types 12348 and total 17430, ascospore 3836 and other spore types 13594 respectively. This clearly indicates the impact of daily rainfall and other related meteorological parameter upon spore release, dispersal and subsequent deposition in the sampler on these pertinent days. Other days of this week also recorded similar dynamics of total spore load, ascospore load and load of other spore types [Table 2].

A pronounced decline in total spore load, load of ascospores and other spore types has been recorded on 16th August [total 11004, ascospores 1428 and other spore types 9576] and 17th August 2012 [total 11970, ascospores 1638, other spore types10332], which has been mainly found due to lack of rains on these days [Table 2].

A rhythmic fluctuation dynamics in the spore load has been recorded with peak started from 29th August [total 18732, Ascospore 3598, other spore types 15134] after only 0.3 mm rainfall regulating temperature and relative humidity and reached highest peak on 1st September 2012 [total 28476, ascospore *4858* and other spore types 23618] with 17mm rainfall. But rhythmically this revealed pronounced decreased on the very next day i.e. 2nd September 2012 [total 20482, ascospores 4158, other spore types 16324] on a rainless day, which may be due to wash off of all other spore types in aerospora and some ascospores due to 17 mm rainfall on previous day [Table 2].

During this period on 30th August 2012 [total 18074, ascospores 3444, and other spore types 14630] comparatively total load was more which declined on 31st August 2012 [total 15946, ascospores 4368, other spore types 11578]. Although total load had declined [14630 to 11578] which may be due to effect of washing off of all other spore types at 38 mm rainfall. In turn the load of ascospores was increasedfrom 3444 to 4368 which may be due to dehiscence of ascocarps and release of ascospores by the rains [Table 2].

Similar rhythmic increase in ascospore load, has been recorded from 5th September [2044] to 10th September [4662] due to release of these ascospores merely by the rainfall. During this period total rainfall was 25.4 mm, temperature range from 23.8° C to 25.5° C and relative humidity range 76.5% to 93% [Table 2].

DISCUSSION

Study of airborne fungal spores over Rajma [*Phaseolus vulgaris* L.] crop field during Kharif 2012, revealed that meteorological parameters like rainfall have found to play significant role in deter-

Table 1 : Month wise fluctuations of percentage contribution of different groups of airborne fungal spores and Cladosporium in relevance to rainfall and other meteorological parameters over Phaseolus vulgaris L. crop field at Pune during Kharif 2012.

Sr. no.	Contents	June	July	August	September
1	Total aerospora	24248	133060	322728	158130
	Fungal spore groups				
2	Myxomycotina	3.06	1.09	2.21	0.54
3	Zygomycotina	3.06	1.85	4.10	0.82
4	Ascomycotina	15.07	11.12	19.63	25.00
5	Basidiomycotina	8.60	6.49	10.09	12.14
6	Deuteromycotina	61.14	72.51	57.06	56.79
7	Other types	9.06	6.92	6.90	4.71
8	*Cladosporium* Link.	31.12	19.56	15.21	15.67
9	Total rainfall (mm)	20.6	72.2	204.6	42.4
10	Avg. temperature (°C)	27.8	26.5	26.3	24.8
11	Avg. Relative humidity (%)	66.07	77.03	80.35	84.5

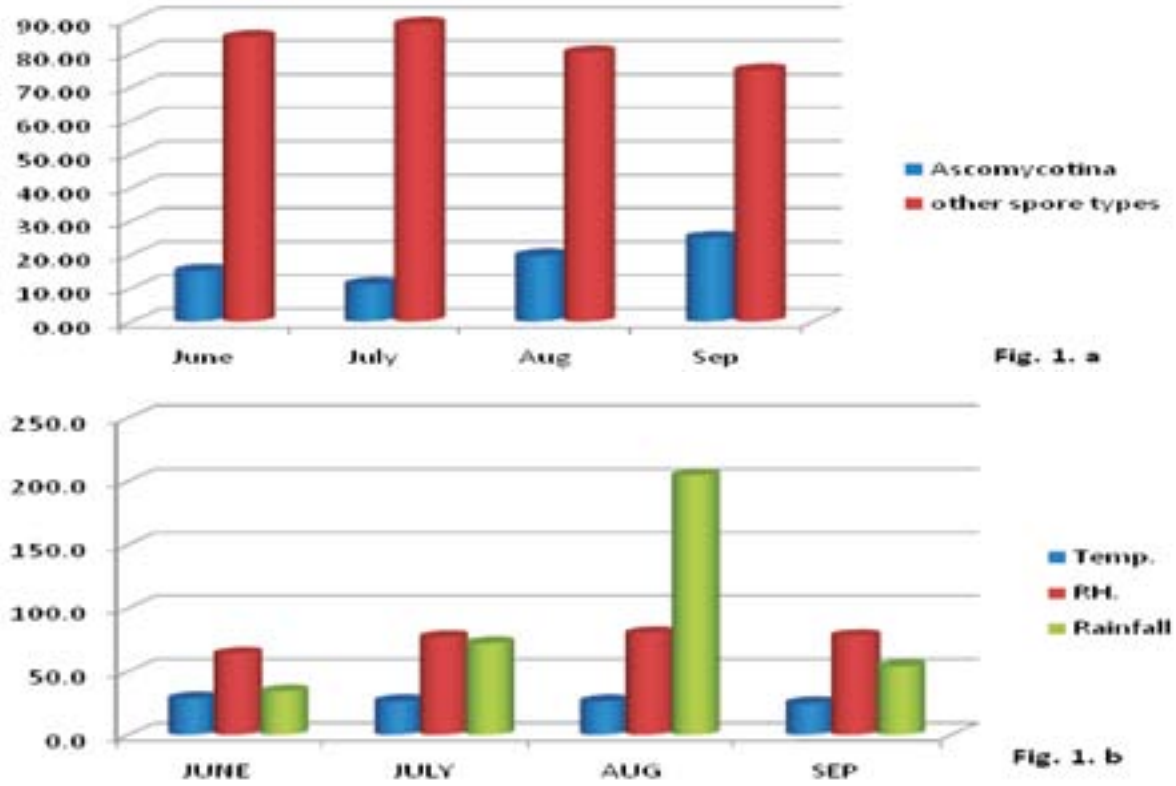

Fig. 1.a : Month wise fluctuations of Ascomycotina and other spore types [except ascospore] b: Month wise meteorological parameters [Temperature, Relative humidity and Rainfall] over *Phaseolus vulgaris* L. crop field at Pune during Kharif 2012.

mining the spore load. For e.g. some of the members of Deuteromycotina have been washed off by the rains, decreasing their load whereas ascospore load has been increased after the rains. Even a small amount of rainfall leads to the release of ascospores. Following the rainfall, the ascospores are released in the first three hours[14]. Due to rainfall dry ascocarp, asci, ascospores absorb rain water and swell to release the ascospores. Rainfall is essential factor in the release of the ascomycetous spores[15, 16]. In rainy days ascospores occurred in maximum numbers during night time[17]. A close relationship between the rainfall and release of ascospores has been studied by Tilak revealed that

Table 2 **:** Date wise fluctuations and rhythmic dynamics of spore load of total aerospora, Ascospores and other spore types [except ascospores) over *Phaseolus vulgaris* L. crop field in Kharif 2012, at Pune in relevance to rainfall and other meteorological parameters.

Sr. No.	Date	Total spore load	Ascospores	Other spore types [except ascospores]	Rain [mm]	Temperature [°C]	Average RH [%]
1	30/07/2012	2408	476	1932	5	24.8	91
2	31/07/2012	2870	686	2184	18	24.3	85.5
3	01/08/2012	7994	1596	6398	84	21.7	91.5
4	02/08/2012	8456	1638	6818	7.6	24	90.5
5	03/08/2012	7812	1862	5950	4	25.1	86.5
6	04/08/2012	7644	1680	5964	5	24.8	85
7	05/08/2012	9604	1414	8190	3	25.4	82
8	06/08/2012	12656	4438	8218	0.2	24.5	88
9	07/08/2012	16324	3976	12348	4	25.3	86.5
10	08/08/2012	17430	3836	13594	5	25.4	85
11	09/08/2012	10234	2254	7980	6	24.6	81
12	10/08/2012	6972	952	6020	19	24.9	83
13	11/08/2012	6188	1092	5096	2	24.6	82
14	12/08/2012	6608	1154	5454	0.7	25.1	79
15	13/08/2012	6426	1036	5390	4	25.4	75.5
16	14/08/2012	9702	2464	7238	0.1	25.4	74.5
17	15/08/2012	15736	1498	14238	0	25.3	74
18	16/08/2012	11004	1428	9576	0	25.3	70.5
19	17/08/2012	11970	1638	10332	0	24.5	68.5
20	18/08/2012	13664	2450	11214	0.7	24.7	76
21	19/08/2012	8092	2086	6006	9	24.1	89
22	20/08/2012	9142	2226	6916	9	25.2	75.5
23	21/08/2012	9198	1204	7994	0	24.6	78
24	22/08/2012	7910	1246	6664	0	25.1	74.5
25	23/08/2012	10150	1596	8554	0.1	25.8	75
26	24/08/2012	9996	1372	8624	0	26.3	69.5
27	25/08/2012	9338	812	8526	0	25.9	71.5
28	26/08/2012	4046	966	3080	0	25.2	75
29	27/08/2012	6580	2800	3780	0.9	23.7	84
30	28/08/2012	9100	1246	7854	2	24.7	86
31	29/08/2012	18732	3598	15134	0.3	25.9	85.5
32	30/08/2012	18074	3444	14630	34	26.6	82
33	31/08/2012	15946	4368	11578	4	25.9	86.5
34	01/09/2012	28476	4858	23618	17	26	78.5
35	02/09/2012	20482	4158	16324	0	26	83.5
37	03/09/2012	12880	2856	10024	1	24.5	86.5
38	04/12/2012	14462	4536	9926	4	23.5	86
39	05/09/2012	10374	2044	8330	9	23.8	89.5
40	06/09/2012	14196	4340	9856	8	24.9	88
41	07/09/2012	12810	3780	9030	0.3	24.1	76.5
42	08/09/2012	13818	3612	10206	0.5	25.3	82
43	09/09/2012	17108	4690	12418	0.6	25.4	81.5
44	10/09/2012	13524	4662	8862	2	24.5	93

gradual increase in ascospore concentration in July-August and September [rainy months] with the rainfall[18]. Jogdand reported higher concentration of ascospores as compared to other spore load during rainy season while investigating aerospora over Jowar crop[19].Findings coincide with above investigators.

In the present study increasing load of ascospores during August [19.63%] followed by maximum load during September [25%] have been recorded, while Ghatge et al. stated that generally concentration of ascospores gradually increases from June and reaches maximum in August[20].Regular abundance of ascospores has been observed in the air during and after rainfall with high humidity was evidenced by the observations of Grinn-Gofron and Bosiacka; Allitt, [8, 21] which coincides with our observations.

Cladosporium [16.84%] has been recorded as dominant spore type during this kharif season. *Cladosporium* has been found to fluctuate in the inverse proportion of rainfall due to wash off by rains. This observation partially coincides with other investigator like Cammack who recorded predominant occurrence of *Cladosporium* during wet and dry period[22]. *Cladosporium* was found to be most common component of aerospora throughout the world1, [19, 23-27]. According to *Shaheen* the abundance of *Cladosporium* in the atmosphere throughout the year may be due to structural features of the spores such as small size, thin exine and smooth wall, this favour and facilitate the easy transport of airborne spores[23]. Prevalence of Cladosporium has been also reported by many researchers over different crop fields[28-30].

Karl Pearson correlation coefficient [r] was computed and its significance was tested at 0.05 and 0.01 level of significance. Pearson correlation coefficient between total spore load, ascospores and other spore types in relation to various meteorological parameters is as follows.

Total spores significantly positively correlated with temperature [r = 0.394**] means Total spores increased with increasing temperature. Ascospores showed significant positive correlation with relative humidity [r = 0.322*] means ascospores increased with increasing relative humidity, as the rain fall occurs relative humidity also increased. All other spore types [except ascospores] significantly positively correlated with temperature [r = 0.450**] means all other spores increased with increasing temperature. Significant correlation between rainfall and some of the ascospore types was also recorded by Trejo et al. in Merida [SW Spain][31]

CONCLUSION

In Kharif season high rainfall and relative humidity promotes release of fungal spores over *Phaseolus vulgaris* L. crop field. Rainfall has been found to be important meteorological parameter responsible for ascospore release. It also regulates and determines the temperature and atmospheric humidity. Thus quantity of rainfall in the month of August influenced ascospore release and rhythmic dynamics. As a result meteorological parameters show profound influence on spore release. Some fungal spores would indicate the prevailing climatic conditions or future meteorological conditions to follow. Thus spores might act as "markers" or "biological indicators". The meteorologist will obviously be interested in such markers this would help in formulation of disease forecasting of crops. Aerobiological studies enable us to determine the relationship between weather parameters and concentration of the fungal spores present in the atmosphere.

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EFFECT OF CARBON AND NITROGEN SOURCE α-AMYLASE ENZYME PRODUCTION FROM *BACILLUS SUBTILIS* MB6

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Amylase enzyme is one of the basic component for lather, backing, textiles and paper industries. It hydrolyzes starch into simple productive sugar by which cost of the product became reduce. In this work, different carbon and nitrogen sources were added at different concentration in production medium, to analyze its effect on amylase enzyme production. It was found that by adding, weight/volume [w/v] of starch 1.5%, yeast extract 1%, ammonium nitrate of 0.1% in the production medium can increase the production of enzyme maximum 456.47± 0.02 U/ml. In this study, it was clearly showed that carbon and nitrogen are rudiment compounds, their presence in production medium played key role for the enhancement of enzyme production.

Keywords : - α-amylase; Enzyme production; *Bacillus subtilis*; Media optimization.

INTRODUCTION

Amylase enzymes are the key for many industries like textile, leather, backing, pharmaceuticals, paper, detergent, etc. It plays an important role, it reduce the production cost and increase the quality of the product. Amylase enzyme can obtained from, plants, microbes and from animal. Among them microbial amylases have enormous applications in starch based industries [Anupama and Jayaraman 2011; Akcan 2011; Sen et al.; 2014, Roohi and Kuddus 2014]. α-Amylase enzyme hydrolyze starch and convert into simple sugars. α-Amylase enzyme act on α-1,4 glycosidic bonds of starch to form reducing sugar. Presently the world market for enzymes is about US$ 2.7 Billion and increases with the rate of 4% annually [Abd-Elhalem et al., 2015; Lall et al., 2015]. Now a day's mostly amylase enzymes are produced from Bacillus bacterial species. Large production of enzyme can be achieved by media engineering and supplementation of different nutrients which provides good growth for microbes and they produce better enzyme [Akcan 2011]. So, proper optimized medium for the enzyme production is very important for the bacterial culture to produce large amount of enzymes [Abdel-Fattah et al., 2013; Kumar et al., 2013]. Different carbon and nitrogen sources like organic and inorganic nitrogen sources are basic need for the enzyme production. With these nutrients, its proper amount is also important for better enzyme production [Anto et al., 2006; Deb et al., 2013]. In this work, medium supplements, Carbon sources and Nitrogen sources [organic nitrogen and inorganic nitrogen) at different concentration was checked and optimized for the large and better amylase enzyme production.

MATERIALS AND METHODS

Microorganism

Bacillus subtilis MB6 bacterial culture was used in this study for the production of α-amylase enzyme. Procured from School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India.

Culture condition and fermentation

Amylase enzyme production was firstly performed in basic modified medium [g/l], Starch - 5 g, Yeast extract - 5 g, K_2HPO_4 - 0.5 g, $MgSO_4$. $7H_2O$

- 0.2 g, $CaCl_2$. $2H_2O$ - 0.1g, Peptone - 5g [Gogoi et al., 1987; Lall et al., 2015]. This basic medium was used to optimize the culture condition [pH, temperature and incubation period], at pH 6, temperature 37°C and incubation period of 48 hours, which was found optimum for amylase production. This optimum condition and basic medium was used as control. At this optimum condition, different carbon and nitrogen sources were added at different concentration Weight/Volume [w/v] to optimize the medium for enzyme production.

Optimization of carbon source

Different carbon sources were added at different concentrations to check the suitable carbon source and its concentration, which increases the production of amylase enzyme. Different carbon sources were- Starch, Rice starch, Potato starch, Sucrose, Maltose and Glucose, which were added to basic medium at different concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% individually. Then bacterial culture was inoculated for production of enzyme.

Optimization of organic nitrogen source

At optimum carbon source and its concentration, different organic nitrogen source was analyzed and checked for suitable organic nitrogen source and its concentration. Yeast extract, Peptone, Urea and Soya peptone at different concentration of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% were checked for higher amylase enzyme production

Optimization of inorganic nitrogen source

Different inorganic nitrogen sources were- Ammonium nitrate, Ammonium chloride, Ammonium sulphate, Ammonium dihydrogen phosphate and Ammonium per sulphate [at different concentrations of 0.1%, 0.2%, 0.3%, 0.4% and 0.5%], which was supplemented to the medium individually after optimizing of carbon and organic nitrogen source, to check the suitable source of inorganic nitrogen, which increases the enzyme production.

α-Amylase assay

Amylase was assayed to determine through reducing sugar by Dinitrosalicylic [DNS] method adding 0.2 ml of crude extract to 1 ml of 1% soluble starch and was incubated for 30 min at 37°C. By adding 1 ml of 3, 5 dinitrosalicylic acid, reaction was stopped and then boiled for 10 min. The absorbance was measured at 540 nm. One unit [U] of amylase was defined as the amount of enzyme that liberates 1µg of reducing sugars, per minute under the assay conditions [Miller 1959; Aiyer 2004].

Statistical analysis

α-amylase production and enzymatic activity was statistically analyzed through SPSS 16 software to a test for significant performed by analysis of variance [ANOVA]. All the experiments were performed in triplicates followed by Mean ± SE.

RESULTS AND DISCUSSION

Optimization of medium composition and addition of nutrient supplements can increase the production of amylase enzyme. In this work, basic medium different nutrient substrates were added at different concentrations to check their effect on the enzyme production. Production of enzyme was performed at optimized incubation condition of pH- 6, temperature 37° C and incubation period of 48 hours.

Optimization of carbon source

In the basic medium, different carbon sources are added individually at different concentrations. Production of enzyme was found maximum 340.48± 0.04 U/ml by the addition of Starch at concentration of 1.5%. It shows higher enzyme production compared to other carbon sources [Fig. 1].

Optimization of organic nitrogen source

After optimization of carbon source, different organic nitrogen source and its concentration were optimized. By the addition of Yeast extract at concentration of 1% maximum 410.58± 0.03 U/ml of enzyme was produced compared to other organic nitrogen sources [Fig. 2].

Optimization of inorganic nitrogen source

At optimized carbon and organic nitrogen source in medium, different inorganic nitrogen source

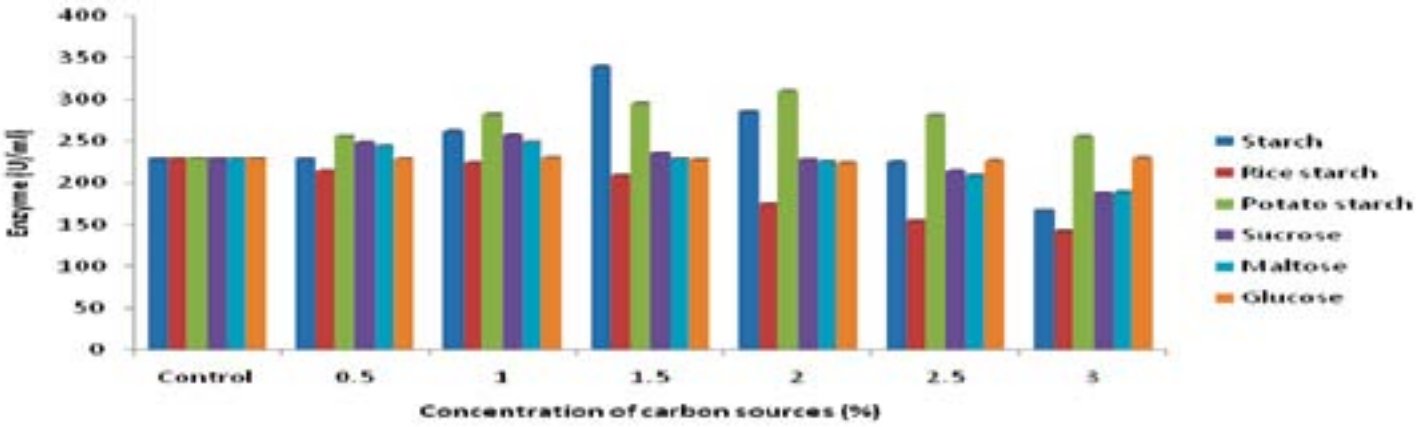

Fig. 1 **:** Effect of different concentration of carbon sources on production of enzyme
ANOVA of different carbon sources: Starch-F= 1.48, $p < 0.000$; Rice starch- F= 1.52, $p < 0.000$; Potato starch- F= 2.81, $p < 0.000$; Sucrose- F= 3.85, $p < 0.000$; Maltose- F = 6.37, $p < 0.000$; Glucose-F= 1.88, $p < 0.000$. Means of each carbon sources checked for significant at 5% level.

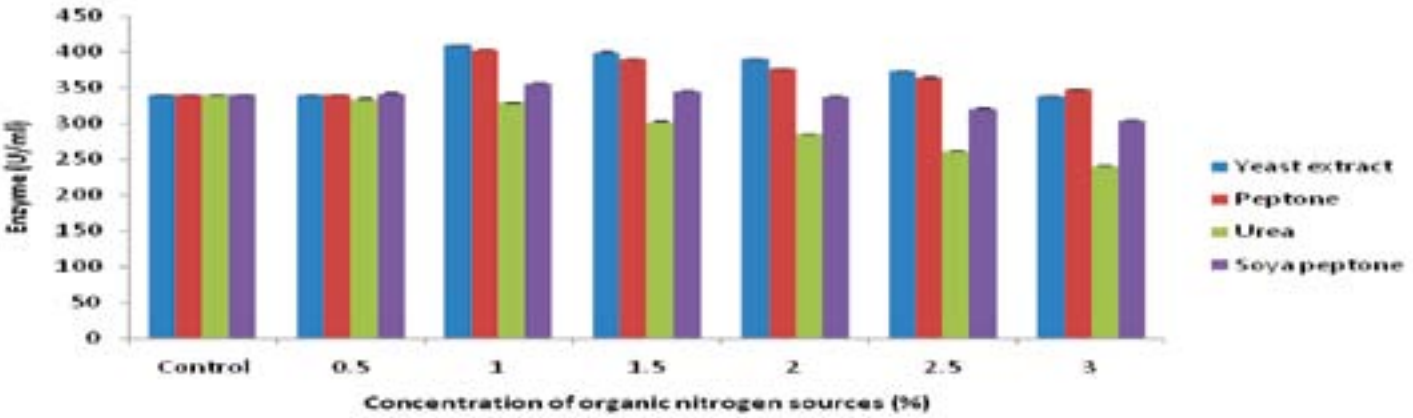

Fig. 2 **:** Effect of different concentration of organic nitrogen sources on production of enzyme
ANOVA of different organic nitrogen sources: Yeast extract- F = 6.33, $p < 0.000$; Peptone- F = 6.28, $p < 0.000$; Urea- F = 1.17, $p < 0.000$; Soya peptone- F = 2.22, $p < 0.000$.
Means of each organic nitrogen sources checked for significant at 5% level.

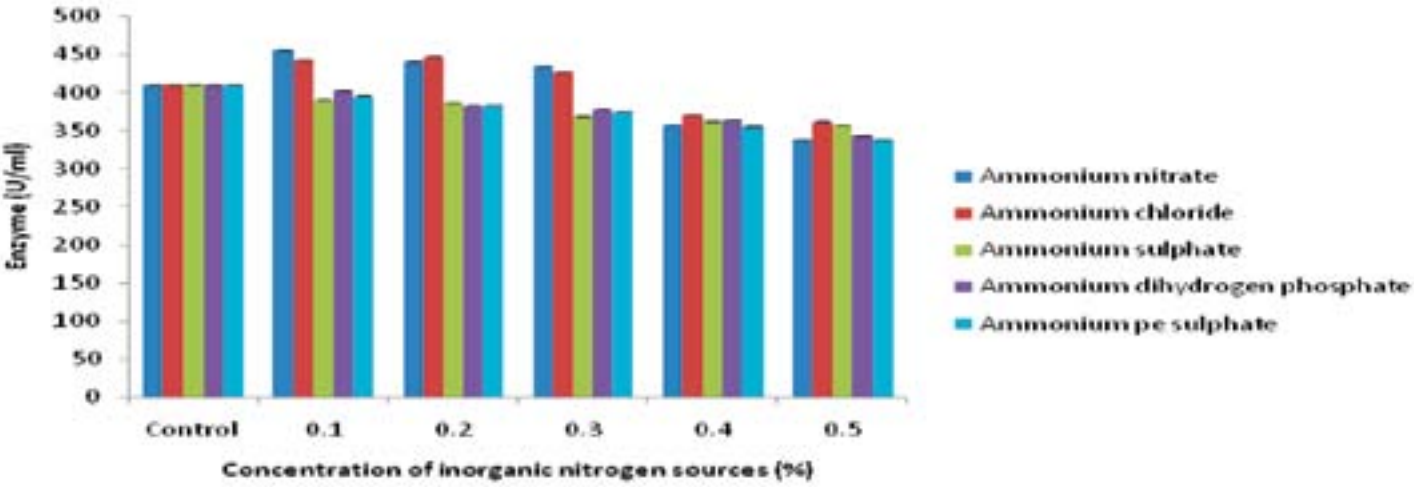

Fig. 3 **:** Effect of different concentration of inorganic nitrogen sources on production of enzyme
ANOVA of different inorganic nitrogen sources; Ammonium nitrate- F= 1.74, $p < 0.000$; Ammonium chloride- F= 1.34, $p < 0.000$; Ammonium sulphate- F= 3.56, $p < 0.000$; Ammonium dihydrogen phosphate- F= 6.47, $p < 0.000$; Ammonium pe sulphate- F= 6.05, $p < 0.000$.

Means of each inorganic nitrogen sources checked for significant at 5% level.

was supplemented at different concentration individually. At 0.1% of Ammonium nitrate, maximum enzyme was produced upto 456.47± 0.02 U/ml, which was higher than other inorganic nitrogen sources [Fig. 3].

Findings of Bekler and Guven [2014] corroborates to our findings, who also found maximum production of enzyme in the presence of starch. They found maximum enzyme production in 2% soluble starch, which is nearly similar to our results. Deb et al., [2013] suggested starch is a good carbon source in the culture medium to produce high amount of amylase enzyme. They also suggested that inorganic source ammonium nitrate was suitable to produce high amount of enzyme and it also increases enzymatic activity. Ravindar and Elangovan [2013] reported Yeast extract should increase the production of amylase enzyme. Aiyar [2004] observed that 1% peptone gave highest amylase yields. It was nearly similar to our finding, where peptone showed second highest in organic nitrogen source.

CONCLUSION

Supplement of nutrients and its optimum concentration is important for the higher alpha amylase enzyme production. In this study, different carbon, organic nitrogen, inorganic nitrogen, ions and some other compounds sources are supplemented to the production medium for increase the production of enzyme. After analyzed it was found that starch, yeast extract and ammonium nitrate was suitable carbon, organic nitrogen and inorganic nitrogen source respectively which enhance the production of enzyme at particular concentration. After this investigation it is clearly showed that, optimization of production medium was important for increase the enzyme production.

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BEE-FLOWER INTERACTION, POLLEN DISPERSAL AND POLLINATION OF *FERONIA LIMONIA* (L.) SWING.

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Present paper deals with flower morphology, flower-visitor interaction, pollen dispersal and pollination of *Feronia limonia* [*L.*] Swing., commonly known as wood-apple or elephant apple which is a medicinally important plant of Rutaceae. The large deciduous tree flowers during February-April and bears numerous nectariferous whitish yellow flowers. Flowers open in between 07:30-09:00 hrs. and all the 10 anthers dehisce sequentially after flower opening. A single flower produces an average of 32,000 powdery, 4-5 colporate pollen grains. Nectar along with pollen grains are the main floral rewards for the flower visitors. After flower opening, different insects like, *Apis cerena indica, Apis dorsata, Apis florea, Helictus* sp. *Trigona* sp. *Megachile* sp., *Syrphid fly*, and Butterfly etc., were found to visit flowers for their forage. During visit, they carry a considerable amount of pollen grains through their body parts and help in pollen dispersal and successful pollination. Among them, the bees especially *Apis florea* were found as the most dominant and frequent visitor. Though the flowers are visited by different insects, a considerable amount of pollen grains were recorded from the ambient air. Maximum 9% pollen grains were trapped from the ambient air by rotorod sampler during peak visiting times of insects [12:00 hrs.]. The effective role of air in successful pollination was confirmed after observing 30% fruit set in netted flowers. Maximum 56% fruit set in natural open condition highlights the role of insects for effective pollen dispersal and pollination.

Key words : Flower-visitors, bees, pollen dispersal, pollination, *Apis* spp., *Feronia limonia*

INTRODUCTION

Pollination, a basic force for gene recombination in flowering plants, plays a key role in plant breeding programme. Flower visitors are essential for pollen dispersal as well as for successful pollination and plant sexual reproduction. Presence of atmospheric pollen grains including several bio-particles may have the allergic properties. The airborne pollen grains also play vital role in effective pollination. The atmospheric pollen incidence depends on floral characteristics, environmental factors and flower-visitors interactions. Distribution of entomophilous pollen grains in air was recorded from various parts of India[1-7]. Effective pollen dispersal also plays an emergent role to maintain genetic diversity. 90% of plant species get pollinated by the help of animals[8]. Insects play an important role in successful pollen dispersal and pollination[9-13]. There are about 19,000 described species of bees in the world and the majority of solitary bees are from the family of Andrenidae, Anthophoridae, Colletidae, Helictidae, Megachilidae and Xylocopidae. The family Apidae comprises of mostly social bees like *Apis*, *Bombus*, *Melipona* and *Trigona*, while only about 1% of bee species are reported from India[14,15]. Bees are important pollen vector and help in pollen dispersal in agricultural crops, home gardens, orchards and also in wild habitats. During visit they also help in atmospheric pollen release. Effective pollen dispersal is very important to maintain the genetic diversity of plant population[16, 17]. There are reports on insect mediated pollen release in the ambient air in different plant taxa[18-21]. There is a scanty report [22] regarding the pollination of *Feronia limonia* [*L.*] Swing. [Rutaceae], hence in the present investigation a detailed study on pollination mechanism of *F. limonia* including pollen dispersal mechanism and flower-visitor interaction have been performed. In addition, different parts of the plant are used as antiscorbutic, carminative, astringent [23,24]

MATERIALS AND METHODS

The study was conducted with the plants growing in and around our University campus at Santiniketan [87º41′ East and 23º42′ North]. *Feronia limonia* [L.] Swing. is an ethnomedicinally important plant of Rutaceae, commonly found in Santiniketan. Flowering twigs were tagged to observe different phenological events following the standard methodologies[25-28]. Pollen grains per anther and per flower were quantified following the method of Mandal and Chanda[1]. Undehisced anthers were collected from flower buds and taken on a glass slide with a drop of 10% glycerine and was crushed and covered with a cover glass [50 mm × 22 mm]. Then it was observed thoroughly under microscope and the pollen grains were counted. Pollen–ovule ratio was calculated following the method of Cruden[29]. Apparent sugar concentration of nectar was measured by ATAGO NAR-1T lab Refractometer[30]. Role of flower visitors in fruit setting was quantified by comparing netting and bagging experiment where bagging and netting of flowers were done at bud stage for ten different inflorescences per plant from same locality. Flower visitors were collected and identified from ZSI, Kolkata and preserved in our laboratory. Collected insects were rinsed with alcohol for the determination of pollen load, then extracted pollen grains were taken on a glass slide with drop of 10% glycerine and covered with a cover glass [50 × 22 mm] and observed under microscope. The hourly atmospheric pollen incidence was determined by operating 'Rotorod' sampler[31]. The sampler was operated for 10 days for 15 minutes at every 2 hrs. interval for each day. Frequency of trapped pollen grains was calculated using the following standard methods [6,32].

$$\text{Percentage of pollen grains of } F.\ limonia = \frac{\text{No of pollen grains in } F.\ limonia}{\text{Total no. of pollen grains trapped}} \times 100$$

RESULTS AND DISCUSSION

Feronia limonia is a medium to large sized tree which starts to flower in February and continued upto April. Clusters of 15- 20 regular, actinomorphic, hypogynous, bisexual greenish white coloured flowers were found in axilary and terminal panicles [Table-1]. Flowers start to open at 07:30 hrs. and continued up to 09:00 hrs. Each flower contains 10 anthers which are yellowish pink at young stage and turned into dull red during maturity. After flower opening anther dehisced sequentially by longitudinal slit. A single flower produces an average of 32,000 powdery, 4-5 colporate pollen grains [Table-1]. Capitate stigma is wet type with papillae. Nectariferous disc situated at the base secrets copious amount of nectar [27 Brix]. During peak blooming period an array of insects like bees [*Apis cerena indica*, *A. dorsata*, *A. florea*, *Trigona* sp., *Megachile* sp., *Helictus* sp.] along with syrphid fly and butterfly were found to visit flowers for their forage. Maximum insect visitation was recorded during 08:00 hrs.-16:00 hrs. when pollen availability was maximum. Though *A. dorsata* and *Megachile* sp. have the highest pollen load but *A. florea* was the most active and frequent visitor followed by *A. cerena indica*, and *A. dorsata* during peak blooming [Table-2; Fig. 1]. Syrphid fly and butterfly were merely the visitors as less amount of pollen grains were found from their body parts. While bees moved from flowers to flowers, the pollen grains deposited over the stigmatic head resulting successful pollination. The role of insects for successful pollination and fruit formation was confirmed after performing the breeding experiment. In natural open condition 56% fruits were formed, while 30% fruit formation were found in netted condition, moreover, no fruit formation was observed in bagged condition. 30% fruit set in netted condition is due to the presence of dispersed airborne pollen grains. Pollen ovule ratio [348:1] also suggested that the xenogamous nature[29] of the bees either landed over petals and move towards the anthers or di-

rectly on the anthers. They also gyrate throughout the anthers and simultaneously crawled through anther whorl to reach at the corolla base for collection of nectar. However, syrphid fly and butterfly landed over the petals and moved slowly to collect nectar from the base in such way that it hardly touches the essential organs. This frequent movement through the clusters of flowers not only increase the chance of the pollen adherent throughout their body parts but also accelerate the dislodging of pollen from flowers to come in the ambient air. Thus insect activities enrich the atmospheric pollen frequency. As a result maximum 9% pollen grains were observed at 12:00 hrs. during peak insect activity [Table-3]. The atmospheric pollen frequency also depends upon the atmospheric temperature and relative humidity. The circadian rhythmicity of the atmospheric pollen incidence showed low pollen incidence at the morning when the flowers were not fully opened and gradually the frequency increased after flower opening and reaches maximum at 12:00 hrs. when air became hot as well as pollen grains dried up to become light. The frequency of pollen gradually decreased at the evening [Table- 3]. Circadian rhythmicity of grass pollen was previously reported[27,33]. From exposed anthers, light, powdery pollen grains are easily liberated out and became afloat in the atmosphere. Anther dehiscence took place usually due to the 'desiccation' of anther tissues[34].The effective role of insects for the atmospheric pollen incidence from entomophillous plants has been reported from different parts of the world [18-20,35-37]. Insect facilitated air pollination has been reported in *Crateva adansonii* [21] .The distribution pattern of entomophillous pollen in air was also observed by previous workers [2,5,7,38] . *A. cerena indica* and *Lassioglossum* were reported as the pollinators of *F. Limonia,* but present observation revealed eight insects species as flower visitors, among them *A. florea* [Table-2; Fig.1C] is most dominant and frequent visitor and plays a major role in pollination. Present observation also highlights about the effective role of airborne pollen grains in pollination and fruit formation. So, the investigated taxon showed ambophilous nature which was also previously reported in different taxa [19,39-43]

Table 1 **:** Floral characters of *Feronia limonia*

Floral characters	Observation
Flowering period	February- Appril
Flower/Inflorescence	15 - 20
Shape	Actinomormhic
Size	2.5 - 3.0 cm in across
Colour	greenish white
Nectar amount/ flower	180 - 210 µl.
Sugar concentration of nectar	24 (brix)
Number of Anther/Flower	10
Pollen production/Flower	32,000 [Average]
Pollen aperture type	4-5 colporate
Mode of anther dehiscence	Longitudinal
Number of Ovule/Flower	92
Pollen ovule ratio	348:1

Table 2 : Foraging activity of flower visitors in *Feronia limonia*

Flower-visitors	Visiting Time	Mean Abundance	Pollen load	Duration of visit/ sec	Flowers visited / minute
Apis cerena indica	Day	22%	300- 1500	5-10	7-10
Apis dorsata	Day	14%	450- 1700	6-9	6-12
Apis florea	Day	52%	290-1550	6-12	10-14
Megachile sp.	Day	5%	400-1600	4-6	9-16
Trigona sp.	Day	1%	50-80	3-8	6-15
Helictus sp.	Day	3%	250-800	3-5	4-10
Syrphid fly	Day	2%	150-200	5-7	3-8
Butterfly	Day	1%	5-8	3-7	5-8

Table 3 : Atmospheric pollen frequency of *Feronia limonia*

Time [hrs.]	06:00	08:00	10:00	12:00	14 :00	16 :00	18:00
Percentage of pollen grains [%]	2	5	6	9	7	5	3

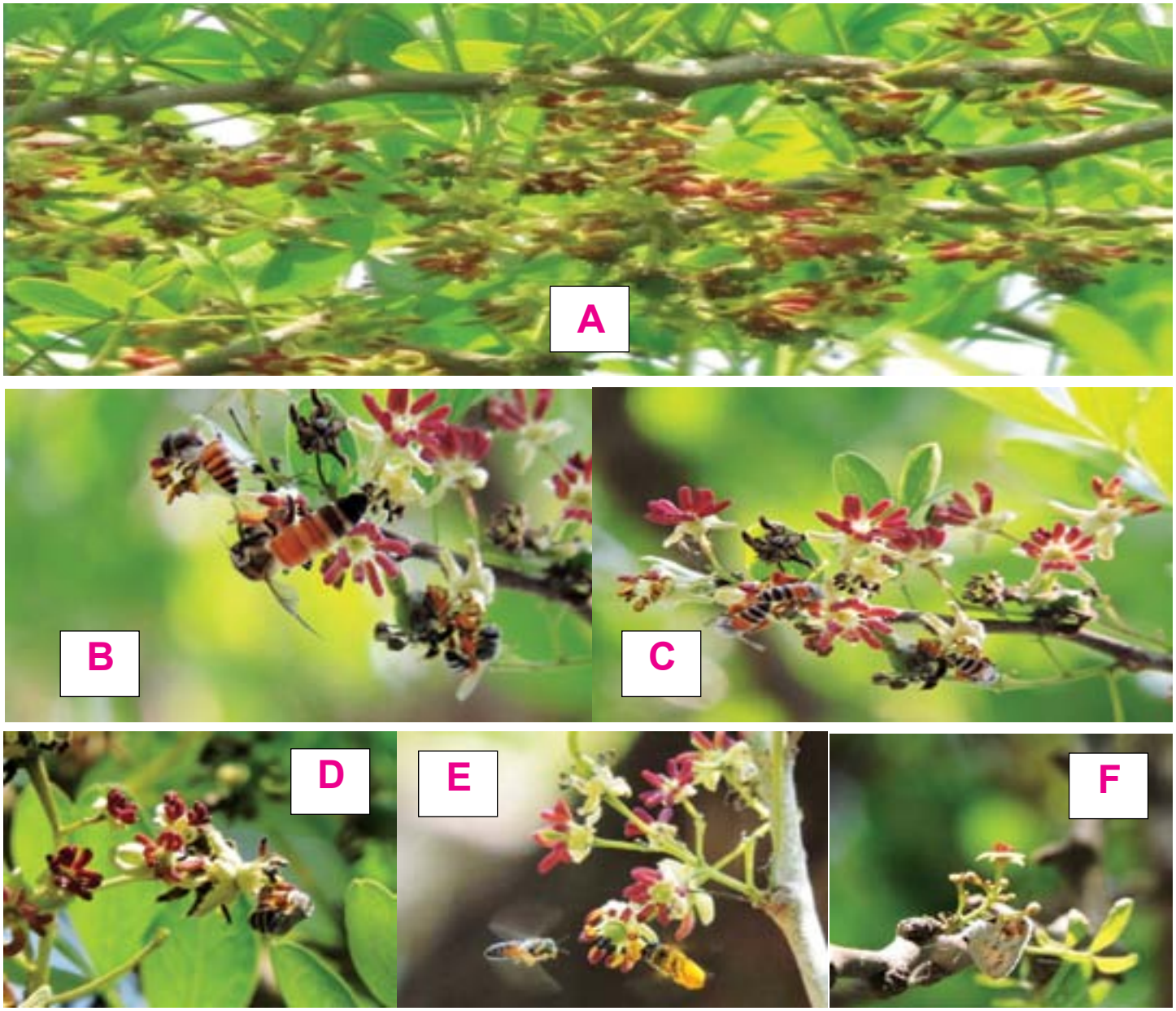

Fig. 1 : A-Flowering twig of *Feronia limonia*; B- *Apis dorsata, A. cerana indica* and *A. florea* visiting flowers; C. Population of *A. Florae*; D. *Helictus* sp. ;E. *Megachile* sp.; F. Butterfly

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PRESIDENTIAL LECTURE, 19th NCIAS-2016

ORIGIN AND EVOLUTION OF LAND PLANTS

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Life was first originated on our planet at the bottom of ocean to escape lethal effect of uv radiations. At a later stage, life migrated from bottom to floating [planktonic] form and ultimately was transmigrated to land habit. The current hypothesis based on morphological and molecular analyses suggests that there was early divergence of two discrete clades - the Chlorophyta, and the Streptophyta comprises the charophytes and their descendants, the land plants – from an common ancestral green flagellate. The several descendants evolved through primary, secondary and/or tertiary endosymbiosis, are characterised by a number of distinct features, many of which are essential for land habits. The eukaryotic evolution took place through horizontal gene transfer across distinct lineages which is regarded as an important force in the evolution of eukaryotes and their genomes. The origin of land plants from a charophyte ancestor was a most important event in the history of life that influenced the creation of entire terrestrial ecosystem and had extensive outcome on atmospheric environment. *Coleochaete* is an excellent model for the green algal ancestor of land plants and may be a modern representative of the green algal group that gave rise to the land plants. Further biochemical characteristics such as presence of sporopollenin, phragmoplast cell division, tendency to retain the zygote in the oogonium until after germination indicate origin of land plants from *Coleochaete*. The land plants acquired their eco-physiological adaptations through enhanced osmoregulation and osmoprotection, desiccation and freezing tolerance, heat resistance, enhancement of spore viability, protection of spores and their dispersal mechanism which helped them for their successful colonization on land habit. Several morphological, biochemical and molecular innovations have been identified in land plants that have allowed successful adaptation to life on land. The further origin and diversification of land plants have been associated with gene family expansion resulting from large-scale gene duplication or whole-genome duplication. Some of the striking examples of expansions of gene families are MADS box genes (pattern formation), glutaredoxin genes [oxidative stress response], several genes act as signalling molecules, etc.

PROF. S. CHANDA ENDOWMENT LECTURE, 19th NCIAS-2016

TRICHODERMA AS BIOCONTROL FUNGUS : BIOLOGY, ECOLOGY AND APPLICATIONS

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Management of crop diseases employing chemical methods are not desirable because of environmental concerns and chances of pathogens attaining resistance to chemicals. The other feasible alternative is to employ biological methods of disease management. The plants or their active ingredients and microbes are used in biological disease control. The commonly used bio control micro-organisms are-

Trichoderma sps., *Coniothyrium* sps., *Talaromyces* sps., *Gliocladium* sps., *Pseudomonas* sps., *Mycorhizae*, *PGPR* etc. Among these organisms, *Trichoderma* sps. are extensively used to manage plant diseases. There are about more than 100 species in the genius *Trichoderma*, amongst them, *Trichoderma asperellum*, *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma koningiopsis*, *Trichoderma reesei* and *Trichoderma virideare* more important.

The species of *Trichoderma* are widely distributed both in tropical and sub-tropical regions of the Globe, more frequently found in the soil, root zones, aerial plant parts, stem bark, seeds etc. They are easily cultivable on PDA/PDB media. They sporulate and colonise the substrate with ease producing enormous soil borne and air borne inocula. The anamorphic stage of the fungus is referred to as Trichoderms *species* while the telomarphic stage is referred to as *Hypocrea* sps. *Trichoderma* produces branched septate mycelium with solitary chlamydospores. Hyprocrea produces ascospores, the sexual spores.

Trichoderma species produce several anti-fungal compounds, enzymes, hormones, plant growth promoting substances, antibiotics etc. Because of this reason they are extensively used as bio control agents to manage soil borne and seed borne plant diseases. They are also used to recline alkaline soils and to support plant growth under saline or drought stress conditions.

PROF. T. SREERAMULU ORATION LECTURE, 19th NCIAS-2016

A JOURNEY FROM AEROBIOLOGY TO AEROACAROLOGY

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Initially aerobiological investigations revealed general aerospora with reports of larger components like pollen grains etc. It was then followed by studies on smaller bioaerosols like fungal spores, bacteria etc. and instead of general aerospora specific studies like crop aerobiology for crop protection, *veterinary* aerobiology for animal protection etc. started with a particular goal.

On these lines I started aerobiological studies on Jowar Crop and worked on 9 air borne diseases for epidemiology and forecasting of jowar diseases with a report of 106 aerobiocomponents and new report of nematodes and phytophagoid mites over the air of jowar crop, exhibiting seasonal dynamics during 1983 to 1987, which yielded in the development of seasonal and annual spore calendars for farmers & allergy patients [Jogdand, 1987]. Thus I started my journey of my research in Aerobiology under the worthy guidance of respected Prof. S. T. Tilak, Father of Indian Aerobiology, in the "School of Aerobiology" at Aurangabad. Underneath the soverginity of Sun anything may be an allergen not in all but only for sensitive victims.

Pioneering work of Storm Van Leeuwen, [1920] a Dutch investigator has been prominent in the search for the source of house dust allergen. Moulds, danders of domestic pets etc. were suspects, but the reactions they elicit do not correspond with house dust allergy. A far harder question was posed by human dander giving skin test reactions in high proportions of atopic subjects [Storm Van Leewen, 1922]. Ancona [1923] reported "Epidemics" of asthma in grain workers to the mite *Pediculodes ventricosus* causing allergic lung disease produced by gross exposure to mites, mite products and faecal pellets. In 1925 Von Leeuwen suggested that mites may be an important allergenic excitant present in the house dust but it was neglected. Kern [1921] was the first to discover a mite *Dermatophagoides pteronyssinus*

and molds to be prominent allergens from the house dust in USA. Hence mites were focussed as an important potential allergen from the house dust.

The work on allergy particularly on house dust mite [HDM] allergy remained sporadic and was restricted to individuals. In India Krishna Rao et al [1973] from Bangluru, Shivpuri et al. [1977] from Delhi, Kashi Ram Maurya et al. [1983] from Kanpur, Modak et al. [1991] from Kolkata, carried some work and Spieksma et al. [1997] from Netherland carried prominent work on mite allergy.

In most of the states of India and Maharashtra the work on HDM remained unattended for a long time even by most of the zoologists. They simply referred "Ticks & Mites" in a small chapter explaining only Ticks but not mites. The possible reason for negligence on mites may be due to the assumption that the mites are typically occurring in the temperate regions of the World. This assumption was *discarded* due to the reports of incidence of mites from tropical regions of the World including India.

Hence this long pending problem had been started for the first time in recent era 1987-2016 by the "School of Aerobiology at Aurangabad". The main worker from Botany, Dr. Jogdand explored important HDM from Marathwada region as important potential allergens initially found in the air over Jowar crop field as Phytophagoid mites followed by HDM studies among 205 allergy patients and 20 controls, reported 16 HDM types in bed & floor dusts of these patients from rural and urban areas in the different indoor environments like *Dermatophagoides pteronyssinus*, *D. farinae*, *Blomia tropicalis*, *Acarus siro*, *Dermanyssus gallinae*, *Cheyletus eruditus*, *Caloglyphus oudemensi*, *Campylochirrus sp.*, *Glycyphagus sp.*, *Ghorea fusca*, *Diplaegidia columbae*, *Megnenia ginglymura*, *Pterolichus obtusus*, *Pterophagus strictus*, *Austroglycyphagus orientalis*, etc. [Tilak & Jogdand, (1989)].

Our observations on a fairly large number of mites, initiating allergic disorders in India has to be given significant considerations. Our studies have clearly brought out the importance of undertaking extensive & intensive work on mites in India and their role causing allergic disorders.

Simultaneously along with aerospora studies, since 1983 and onwards, after undergoing training in the Acarology division of GKVK Agriculture University, Hebbal, Bengalore [Karnataka], under the guidance of Prof. Subba Rao, elaborate studies began on "House Dust Mites" and subsequently on other mites, carrying collection, isolation, qualitative & quantitative analysis of "House Dust Mites" [HDM] leading to their identification, classification, quantification, seasonal & annual or patient wise variation in HDM load, management and control of HDM load, which subsequently yielded in the development of seasonal and annual Mite Calendars, useful to physicians for correct diagnosis of allergens & type of allergy of that particular patient and for patients to avoid exposure to these HDM allergens followed by correct treatment.

Clinical studies of 101 allergy patients using 23 pollen antigens,10 insects, 11 fungi, 4 dusts and 1 house dust mite i.e. *D*. farinae Hughes revealed maximum positive cases for house dust mite antigens [44%] followed by house dust [22.46%], cotton dust [14.49%], paper dust [14.49%] and wheat dust [13.78%]. One patient [p.no. 35] gave late reaction or type III reaction out of 101 allergy patients tested thus accounting 1% to mite antigen tests and 1% to fungal antigen tests. 1% cases showed urticaria and 1% sensitivity to drugs like insulin and unfortunately that patient was a diabetic. Thus house dust mites have been proved to be most potential allergens. [Tilak and Jogdand, 1989].

Subsequent management of their allergy problems was carried by immunotherapy in 10 allergy cases giving full relief [Jogdand, 1994], in co-ordination with Dr. S. H. Talib, Associate Prof. of Medicine, in Govt. Medical College at Aurangabad [M. S]. This centre of medical college was initiated with the joint

efforts of University Botany Department & Medical College, Aurangabad, which has helped in *undertaking* joint work in Aerobiology.

Tilak and Jogdand [1989] observed seasonal variation in percentage contribution of mites among 10 patients and recorded moderate temperature around 25°c(1-2° ±), high relative humidity between 75-85% with rains acted as most congenial environment for the maximum contribution of mites both in bed and floor dust of bed rooms of patients at Aurangabad . On the other hand lower R. H. [less than 55%] and lower temperature [below 20° C] or higher temperature [above 35° C] were found unfavourable for the survival of mites as was revealed during cold dry winters and hot summers during 1984 and 1985, both in rural and urban areas. They also recorded more prevalence of mites in old damp, ill ventilated houses, huts and slum areas.

Jogdand [1996] found some outdoor mites like pig mites, poultry mites, pigeon mites, fowl mites, transported, permanently inhabited and adapted to the indoor environment & varieties of nutrients found in the intramural detritus ecosystem & show prolific environmental and maximum nutritional adaptability to survive in extreme conditions. e. g. one patient strongly +ve [4+] to mite antigen test had pigs' residence around his home [outdoor] wherefrom pig mites [*Campylochirrus sp*] migrated to indoor, inhabited and caused allergy. Similarly another patient had a small poultry behind the bedroom and abundant [over 400 mites/gm dust] poultry mites [*Dermanyssus gallinae*] entered to bedroom and caused severe allergy. The patient was advised to remove the poultry [Source of inoculum] and surprisingly the patient got rid of allergy symptoms.

Jogdand [1998] carried out vertical profile studies of house dust mite fauna at Calicut in Kerala in a four storey hotel and reported incidence of mite population varied along the height and more mite density was found associated with human inhabitation on the II floor, which was over crowded.

Jogdand [2005] also reviewed the recent development in the exploration of house dust mites in India and considered mites as a bio-resource of India. Can we consider these house dust mites as a tool in genetic engineering? By exploring their genome or gene chips which may be used in desensitization of asthmatic patients by transplanting genes for immunity replacing the susceptibility genes? This innovative concept has also been discussed. Incidence of mites has been reported in over 75% of the house dust samples both from bed dust and floor dusts of bed room irrespective of sex, age, type of source. Entire mite body, debris, skin, scales, setae or parts of mite body and guanine rich faecal matter have been found to be allergenic.

Jogdand [2007] reported that House dust mites , poultry mites, grocery mites, flour mites, bird mites, animal mites, phytophagoid mites like coconut mites, ground nut pod mites, house fly mites found in Drosophilla cultures, contaminant mites found in fungal cultures, honey bee mites, human eyelid mites, follicular & human ear mites, garbage mites, rat mites, cat mites, pigeon mites, pig mites, aquatic fresh water or marine water mites, Oribatid mites, forest litter mites etc. have been found to contribute significant allergenic material from different parts of the World, in the intramural environment like house dust, as an independent detritus ecosystem. House dust mites and other mites act as potential intramural allergens causing allergy among the sensitive individuals only.

These intensive studies of HDM at Aurangabad resulted in recording 16 sp. of mites [Jogdand, 1987] subsequently 20 species (Jogdand, 2012) have been recorded from Maharashtra which subsequently yielded in a valuable publication of "House Dust Mite Fauna of Maharashtra" in a special volume no. I [Invertibrates] published by Govt. of India through Zoological survey of India from Pune, Maharashtra.

Role of environment on the dynamics of HDM at Pune revealed interesting findings. Jogdand and Ingole [2013] worked on role of Environment on dynamics of house dust mites [HDM] at Pune, Jogdand [2015] worked on eco-friendly environmental dynamics of House Dust Mites and their role in manifestation of allergy, published in the Proceedings of the 102nd Indian Science Congress 3-7 January 2015, University of Mumbai. Pawar et al. [2016] reported the impact of climate change on the dynamics of rat house mites. Sushama Pawar et al. [2016] recorded the effect of environment on seasonal dynamics of rat house mites at Pune. MS, India, in collaboration with Jogdand.

Thus environment plays detrimental role in percentage contribution, incidence, load and seasonal dynamics of different mite groups. Rat mites have been newly reported and recorded continuously throughout the year in abundance without seasonal variation. Totally 10 genera & 12 species have been reported at Pune in different dust samples. The study is significant from allergy point of view for human health conservation affording healthy joy to one & all.

The Prominent HDM Reported at Pune: 1. *Blomia tropicalis* Bronswijk Cock & Oshima, 2. *Caloglyphus oudemansi* Zachvatkin, 3. *Dermanyssus gallinae* De Geer, 4. *Cheyletus eruditus* Schrank, 5. *Cheyletus malaccensis* Oudemans, 6. *Acarus* siro, 7. *Tyrophagus putrescentiae* Schrank, 8. *Haemolaelaps glasgowi* Ewing, 9. *Echinolaelaps echidninus* Berlese, 10. *Tetranychus hypogeal* Gupta, 11. *Dermatophagoides pteronyssinus* Trouessart, 12. *Dermatophagoides farinae* Hughes [Jogdand and Ingole, 2013].

It was then followed by studies on some new species of house dust mites from slums of Padmavati, Pune and rat mites from rat house husk & dust of Poona college of Pharmacy, Bharati Vidyapeeth, Pune [Jogdand & Ingole, 2013, Jogdand, 2015 & Pawar et al., 2016].

Newly recorded HDM in rat house as Rat mites like 1. *Haemolaelaps glasgowi* [Ewing, 1925) and 2. *Echinolaelaps echidninus* [Berlese, 1887], Laelaptidae, have been found as parasites on the rats, very big in size usually more than 01 mm [1000µm], tough in texture and faintly to deeply coloured brown, with different shades. Irrespective of environmental fluctuations they have been reported throughout the year in good numbers and do not exhibit notable seasonal *variations* as in HDM but slight variation has been recorded. 3. *Glycyphagus geniculatus* [Vitzthum, 1931] male & female specimens and 4. *Saproglyphus negiectus* [Berlese, 1890] male & female Specimens have been recorded in the house dust of Sai Clinic in slum areas of Padmavati as new report from Pune, affecting the sensitive indivusals, causing allergic manifestations. Similarly a new mite genus & species from the poultry dust, 5. *Fuscuropoda agitans* [Banks], a contaminant mite from fungal cultures, 6. *Tyrophagus putrescentiae* [Schrank] & 7. *Macrocheles muscaedomesticae* of family: Macrochelidae [Vitzthum, 1930], Mesostigmata, a housefly mite has been reported as new record from Pune and India for the first time as a contaminant mite from Drosophila culture.

World record of HDM species is '36', comprising Indian record of '29'in Kolkata, '22' in Karnataka, '20' in Maharashtra,'17'in Kerala and now added report of '07' species from Pune, which indicates biodiversity of HDM in the intramural environment at Pune & over the globe, thus changing the previous World record from '36' to '43' species, Indian record from '29' to '36' and record of Maharashtra from '20' to '27' of dust mites. In addition 4 new species which are totally new to science are being identified and described from Maharashtra separately. It will be a new record. Thus Seven genera & 07 species of House Dust Mites have been reported newly in the Intramural Environment at Pune making good addition to the biodiversity of intramural dust mite fauna, which may also be responsible for causation of health disorders such as respiratory or skin allergy, asthma etc. among the people at Pune [Jogdand, 2015].

During August & September 2016, a TY BSc student had itching sensation in her ear, she removed wax from her ear, out of curiosity she mounted it under the microscope and surprisingly she found some live objects in it, which were subsequently identified as a follicular mite i.e. Demodex. Owen [1843], Demodex folliculorum. Tulk [1842] under the family Demodicidae Nicolet, [1855]. So far we have obtained 22 specimens, the work is in progress and will be presented [Jogdand & Meghna, October, *unpublished*, 2016].

Management of Allergy Problem

At present allergy problems are handled after correct identification of allergenic components by intradermal antigen testing by desensitization of the patient using Immunotherapy [Jogdand, 1987, Tilak & Jogdand, 1989]. However, Jogdand added further that regularly observing Naturopathy rules together with practicing Yoga & Pranayam daily, gives full relief to the ailing patients. It is his own observation & personal experience since last two decades published during 2007.

Management of HDM Population in the Patients' Dwellings

Maunsell et al. [1970] developed method for the management of HDM population in the patients' dwellings, which is usually carried on two lines i.e. [i] by starvation of HDM through elimination of human dander from the bed of patient and [ii] thermal destruction. The first one may be achieved by keeping the patient's room regularly most clean and the second by thermal heating or by burning the wood in the centre of the patient's bed room. [iii] Jogdand [2007] proved and suggested that covering the mud floor & wall with cow dung & white soil-mud respectively brings down the total load of HDM in rural and urban patients.

[iv] Then Chemical Method arose as listed here. For chemical control of density of mite population, different pesticides and acaricides have been used through confirmatory studies. Important acaricides and pesticides studied to standardize the doses for killing the mites were 1. Phosphomider [Trade name Dimecron] [concentration worked out in laboratory 0.05–0.25%]. 2. Dicofol [Kelthane – 0.05]. 3. Formothion [anthio-0.05]. 4. Quinalfos [Ekaluxo-05]. 5. Carboryl [Sevin–0.05]. 6. Sulphur [0.26]. 7. Malathion [0.1%]. 8. Endosulfan [thiodon - 0.07]. 9. Oxydemetonmethyl [metasystox 0.05]. 10. Dimetheate [0.05%]. Mortality is recorded after 48 hours of application. Type used for these tests were monotypic culture of '*Typhlodromps tetranchivorus mite*'.

v. In a preliminary study, Joshi et al. [2015] established the prominent Role of burning Dhupa as fumigation therapy, in intramural environmental cleansing and its sustainable conservation. They proved beyond doubt that burning of Dhupa containing many plant-powders with antimicrobial activity, for one hour, has been found to minimize the microbial load prominently in the indoor environment at Pune [from15 to 3/m3 of air]. Jogdand worked as main investigator.

Prospects and New Avenues in Aeroacarology

- Development of a new branch of Aerobiology as Aeroacarology. Proposed to compile and publish literature on Indian House Dust Mites as a new Monograph.
- Collection and identification of new mites in Maharashtra and subsequently in India in relevance to environmental variations.
- Proposed to conduct a coordinated major research project on HDM & allergy in India covering biology, life cycles, behavior and different systems functioning in the mite body in relevance to environmental dynamics.

- Implementation of use of modern technology like database of allergy and asthma biomarker technology in allergen identification of HDM such as MALDI TOF/TOF, LCESIqTOF/TOFMS/MS, IUIS, etc.
- Preparation of Pictorial Keys for the identification of mites in India.
- Is there any possibility of a single vaccine for all allergens from HDM and other groups like fungi, pollen etc. like that of polio, triple, etc.?
- Management programme for the control of HDM population load.
- Conservation of environmental balance & human health.

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INVITED TALKS

DIVERSITY OF POLLEN AS AEROALLERGENS, POLLINATORS AND CONSERVATION

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Pollen, the male partner in the fertilization process of flowering plants, because of its unique structure and haploid nature, provides an excellent material for several physiological and biochemical studies of fundamental and applied nature, which can give interesting clues regarding storage, longevity, viability, different phases of pollen development, etc. Pollen is a major source of morbidity for atopic patients, leading to various allergic disorders. Pollen allergy is one of the most important problems of human pathology all over the world. About 15-20% of the world's population are suffering from allergic disorders i.e. allergic rhinitis, bronchial asthma, atopic dermatitis and urticaria. Pollen allergy is caused by proteins, glycoproteins or even a single peptide which are present in the pollen wall and cytoplasm. Thus, the detection of the site of origin, isolation and characterization of allergy causing proteins or glycoproteins is now a very challenging task for aerobiologists. A study of pollen biochemistry can provide basic data to aerobiologists and allergologists, which can help them understand the role played by the various chemical constituents of pollen in the allergic manifestations.

Pollen has also proved to be an excellent tool in taxonomic studies. The application of pollen characters in solving controversial taxonomical and phylogenetic problems, has now been widely recognized all over the world. Application of pollen characters and constituents in understanding plant affinity and phylogeny is also well documented. However, these studies have been largely confined to major *morphological* characters of pollen grains, including apertural form, number, distribution and position, exine ornamentation and stratification patterns and pollen association and pollen nuclear number, all of which have provided the best taxonomic criteria, being least variable.

Pollination is a basic force for gene recombination in flowering plants, plays a key role in plant breeding programmes. In angiosperms the pollination mechanism is typically developed in three phases: [a] release of pollen from anther, [b] transfer of pollen from anther to stigma, and [c] finally successful placement of the pollen on the receptive stigma surface, followed by germination of pollen grains which begins the next phase of fertilization. Each of the three phases shows great diversity [Faegri & van der Pijl, 1980]. Pollination, pollen germination and stigma receptivity must be analysed critically on a species by species basis, as it reflects the basic criteria for *breeding* programmes. Besides, *pollen* viability and nutrient requirements differ from species to species. Structural, physiological and cytochemical features of the stigma is of prime importance in the biology of sexual reproduction and seed formation, but the pollination biology of most tropical plant species is still unknown. As such, a detailed knowledge about pollination, pollen germination and stigma receptivity will be helpful to produce genetically superior stocks.

Globally, of the estimated 1330 crop plants grown for food, beverages, fibers, spices and medicines, approximately 1000 [75%] are pollinated by animals. It has been calculated that pollinators deliver one out of every three mouthfuls of food we eat, and beverages we drink. Pollinators are essential components of the habitats and ecosystems that many wild animals rely on for food and shelter. Approximately 25 percent of birds include fruit or seeds as a major part of their diet. Plants provide egg laying and nesting sites for many insects. More than 218,000 of the world's 250,000 flowering plants,

including 80% of the world's species of food plants, rely on pollinators for reproduction. For over a decade, biologists have been concerned about apparent declines in pollinators, especially those that migrate between regions, and the concomitant declines in seed production of flowering plants. This concern over plant-pollinator interactions has contributed to a paradigm shift from protecting individual species to protecting inter-specific relationships and landscape-level ecological processes.

Despite the importance of pollinators, the ever-expanding conversion of landscapes to human uses adversely affects their habitats. A growing body of evidence indicates that these beneficial creatures are in serious decline, due to loss, modification, and fragmentation of habitat, and the excessive use of pesticides. The risk of losing the essential role of pollinators required for the successful propagation of plant communities and wildlife habitats is real. As plantings have grown larger, the need for concentrated pollinators at bloom time has grown. At the same time populations of many pollinators has been declining, and this decline has become a major environmental issue today. The study of *pollinator* decline is also interesting to some scientists, as pollinators have the potential to become a keystone indicator of environmental degradation. The ultimate goal of the pollinator conservation system is to ensure healthy and self-sustaining populations of pollinator resources in botanic gardens.

The present paper will highlight the recent problems and future prospects of pollen allergens and pollinator resources in the context of environmental degradation and global warming for biodiversity conservation.

SIGNIFICANCE OF POLLINATION ECOLOGY IN UNDERSTANDING THE PHYLOGENY OF FLOWERING PLANTS

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The primary intension of any species should be to proliferate through space as well as to sustain through time. Proliferation through space at a given time can be achieved by sexual or even asexual means. However, perpetuation through time in changing environment demands new traits which can be acquired in nature only by genetic recombination through sexual reproduction.

Flowers are especially adapted for sexual reproduction which finally leads to the development of viable seeds. An angiosperm plant releases twice two different types of disseminules at the mercy of nature without any control of the mother plant. Those are the pollen grains and the seeds. The success of sexual reproduction of a plant depends solely on whether or not the two distinct kinds of entities can reach their respective destinations successfully.

The successful destination of an angiospermous pollen grain is the receptive surface of a compatible stigma. The process, referred to as pollination, is fundamentally critical for sexual reproduction. Seed-set of a plant is totally dependent on the occurrence of successful pollination. Pollination necessitates the action of biotic or abiotic agents and involves various intricate morphological and physiological devices in attaining the goal. Pollen transfer by animals requires pollinator attraction by various attractants and rewards offered by the flowers.

To unravel the mysteries of pollination ecology of a plant, details of its flowering phenology, floral biology, pollination mode, pollen vectors, floral attractants and rewards, pollinator incidence and breeding system are investigated. Perfection in pollination mechanism makes a species better adapted in nature.

Pollination strategies of a number of species, viz., *Abroma augusta*, *Barleria prionitis*, *Bauhinia racemosa*, *Clerodendrum indicum*, *Hygrophila schulli*, *Solanum surattense*, *Solanum indicum* and *Swietenia mahagoni*, with respect to pollinator specificity, pollen theft, resource allocation, degree of xenogamy and existence of fail-safe mechanism to overcome pollinator crisis have been analyzed thoroughly. The comparative data reveals that pollination mechanism can be utilized successfully as a tool for phylogenetic evaluation of flowering plants. Indiscriminate visitation, pollen robbing, predominance of autogamy, high resource allocation and absence of fail-safe mechanism are indicative of primitiveness. On the other hand, visitor restriction to avoid pollen theft by monophily or oligophily, higher degree of xenogamy, minimum resource allocation and presence of a fail-safe mechanism in case of pollinator crisis reflect advancement of a species.

FLOWERING PHENOLOGY, POLLEN DISPERSAL AND POLLINATION OF TWO ALLERGENIC PLANTS OF EUPHORBIACEAE

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The source, release, dispersion, deposition and impact are the key factors in aerobiological study. The present investigation deals with flower morphology, anthesis, pollen production, flower-visitor interaction and pollen dispersal of. *Gelonium multiflorum* A. Juss. and *Ricinus communis* L. of Euphorbiaceae. *G. multiflorum* is generally dioecious small tree but andromonoecious and gynomonoecious plants are of rare occurrence while *R. communis* L. is a monoecious small tree. The small odoriferous flowers of *G. multiflorum* are in axillary clusters which generally open at early morning [06.30 hrs.- 07.30 hrs.] during February to May while maximum floral density was observed during June-October in *Ricinus communis* L. which generally opens in between 09:00-11:00 hrs. Anther dehiscence takes place simultaneously with the flower opening in both the plants. A single flower produces an average of 22920 and 383160 pollen grains in case of *G. multiflorum* and *R. communis* respectively. High pollen-ovule ratios [*G. multiflorum*: 7,640:1; R. communis: 1, 27,720:1] also indicate the xenogamous nature of both the taxa. The different flower visitors like *Apis*, *Ceratina*, *Trigona*, *Vespa*, ants, members of Lepidoptera, Diptera [Eristalinus and *Chrysomya*] and thrips were found to visit the flowers of *G. multiflorum* while *Apis*, *Ceratina*, *Trigona*, *Vespa*, members of Diptera and Lepidoptera were found in *R. communis* for their forage and help in pollen release, pollen dispersal and pollination. Although the plants were visited by different insects, about 8% [*G. multiflorum*] and 18.18% [*R. communis*] pollen grains were trapped at 11.00 hrs. and 12:00 hrs. respectively from the ambient atmosphere using "Rotorod" sampler. Fruit set in open and netted condition suggest the role of both insects and air for success pollination.

EFFECT OF HEAVY METALS ON POLLEN

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Pollen is carrier of source of inherent genetic information. The biological performance of pollen from development till fertilization is stress up by environmental contaminants. As pollination purpose

pollen grains are freely suspended in the atmosphere. Pollen grains last in the air from few hours to the few days and even can travels from few meters to few kilometers. During this period pollen interact with air pollutant which consist of heavy metals along with other particulate matters. Amongst the air pollutant heavy metals [HMs] are most toxic as it is freely suspended in air and easily gets deposited. The interaction of pollen with heavy metals occured at three different stages- during the development as they exert the stress [growth stress] on the plants, the contamination of anthers or dehisced anthers [if pollen are transmitted by biotic pollinator] and later during the flight of pollen grains via air. Air contaminant present in air get settel in soil which are translocated to the aerial parts of the plant from root system. Movements of heavy metals from soil to plant, plant to pollen are responsible for the accumulation. On *other* hand mature pollen is partially dehydrated when release from anther, and possibly absorbed air pollutant that combined with water. The presence of elements has been reported in pollen but not all elements in pollen has recognized role in growth, function and reproduction. Among the elements Calcium and Boron are essential for pollen germination and tube growth.

The interaction of heavy metals with pollen at any stage of development may induce abnormalities. Heavy metals have impact on reproduction, of which pollen is acutely affected. Therefore interaction of heavy metals with pollen at any stage of development, transfer or germination results in to malfunction of the pollen. Effect of heavy metals on pollen resulted in alteration of pollen development and microsporogenesis, deposition on surface, viability, germination and tube growth, and protein. Accumulation of heavy metal over certain limit pertain the developmental stages of pollen in plants. Many invetsigation worked out different effect on anther and pollen development. The pollen surface, exine contain ornamenting elements which term as sculpture or sculpturing. This ornamentation provides more surface area for the deposition of air contaminants [particulate matters]. Additionally pollen coating consist of a sticky, heterogeneous material called pollen kit, keeping pollen grains together during transport and Lipophilic nature of pollen wall provides the site for accumulation of air born suspended particulate matters. Such accumulation of air contaminants is responsible for alteration in pollen morphology as irregularity, shrinkage, thinning and breakage of the exine. The consequences of pollen and heavy metals interaction not only affect pollen viability, germination, tube growth but also responsible for fertilization and post fertilization changes. Morphology of tube, callose pattern, shape in pollen, were found to be strongly altered after treatment with heavy metals. The areas with presence of heavy metals content in air along with particulate matters reduce the pollen viability in contrast to non polluted areas also brings changes in protein and increased release of cellular material.

The inhibition of pollen functioning depend on the plant species as well as pollutant and its concentration. Pollen is considered to be most sensitive system for analyzing environmental changes than vegetative plant part. The suitability of pollen for the monitoring of heavy metal is based on accumulation which is result of morphological modification of pollen. Hence bioaccumulation of HM in pollen can be effectively used as biomonitor to assess HM pollution.

SEASONAL PERIODICITY OF BETULA POLLEN IN THE ATMOSPHERE OF KASHMIR HIMALAYA

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Betula utilis [Himalayan birch, bhojpatra, Sanskrit: bhūrja] is a birch tree native to the Himalayas, growing at elevations up to 4,500 m [14,800 ft]. The specific epithet, utilis, refers to the many uses of the different parts of the tree. The white, paper-like bark of the tree was used in ancient times for writing

Sanskrit scriptures and texts. It is still used as paper for the writing of sacred mantras, with the bark placed in an amulet and worn for protection. Selected varieties are used for landscaping throughout the world, even while some areas of its native habitat are being lost due to overuse of the tree for firewood.

In Kashmir Himalaya's utilis is commonly growing from 9000ft to the tree lines which 11500ft. In recent years different opinions have been documented about the accurate nomenclature of the species and number of varieties have been assigned to this species [Munshi 2014]

Pollen produced by Birch species are reported to cause severe pollinosis and cause the same types of allergies. Pollen allergenicity due to Birch pollen has been well documented in Europe and have been reported to rank second to the oaks in importance for causing pollinosis among tree types. Birch pollen allergenicity has been reported to be more frequent in Rocky Mountains. Because pollen are small and fine and can be carried for great distances when wind velocity increases. It has been observed that the pollen of birch can be easily inhaled when it comes in contact with your nose, mouth and nasal passages.

Presently the author is working on monitoring the birch pollen count essentially needed to compile the information on birch pollen count net working based on data compiled on pollen prevalence, predominance being important for developing the forecasting model which will be helpful to hay fever sufferers due to birch pollen

If you have a pollen allergy, you may be affected by pollen in different allergy seasons from different type of plants. Spring blooming plants include oak, birch, hickory, pecan, and even some grasses produce pollen. If you suffer from pollen allergies in the late summer and fall, then most likely you are affected by ragweed. Allergenic pollens that cause asthma [Seasonal or perinianal] rhinoconjuctivitis are those from trees or plants which are mostly anemophily Although pollen grains would seem to be too large to easily reach the intrapulmonary airways, the relationship between pollen counts and the presence of asthmatic symptoms is only too evident. This is probably because the allergens inducing seasonal asthma are not only found within pollen grains but also outside the grains in particles of less than 10 mm that are freely found in the atmosphere. The most important pollens producing pollinosis in Spain are those from cypress trees from January-March, birch trees in April [macizo galaico], Platanus *hispanica* [March-April], grasses and olive trees from April-June, *Parietaria* from April-July and *Chenopodium* and/or *Salsola* from July-September. By geographical areas, the main cause of pollinosis are grasses in the center and north of the peninsula, olive trees in the south [Jaén, Sevilla, Granada, Córdoba] and *Parietaria* in the Mediterranean coast (Barcelona, Murcia, Valencia). PMID: 15120027 [PubMed - indexed for MEDLINE]

Aerosampling using Rotorod samplers was conducted in the Institute for Medical Research, Kuala Lumpur, Malaysia, from December 1991 to November 1993. Samples were collected twice a week between 10.00 hours to 12.00 hours. Rods were stained and examined microscopically. A total of 8 and 20 types of pollens and mold spores were collected, respectively. More mold spores were collected than pollens. Grass pollen constituted more than 40 percent of total pollen counts. Gramineae pollen counts peaked in March and September. The most abundant mold spore was *Cladosporium* followed by Rust, *Nigrospora*, *Curvularia* and Smut. *Cladosporium* counts peaked in February and August. Rust counts peaked in June and December whereas counts for *Nigrospora* peaked in February and October. Highest counts of Smut were recorded in March and October. *Curvularia* counts peaked in January, June and September.

MITES IN HOUSE DUST AS ROOT CAUSE OF ALLERGIC ASTHMA IN KOLKATA METROPOLIS

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Background: The prevalence of allergic diseases has increased in an unprecedented manner over the last few decades not only in developed countries but also in developing countries too. The trend in the prevalence of asthma, in particular, is increasing at a rapid pace in India, particularly in metropolitan areas including Kolkata. In India, about 10 million people suffer from asthma and another 15 million from other recurrent allergic disorders. Although house dust has long been associated with sneezing and wheezing in sensitive individuals, the exact nature of allergens within it was unknown for quite a long time. It is now well established that the mites of the genus Dermatophagoides are the most potent allergens in house dust responsible for allergic manifestations.

Methods: During last more than 25 years, studies on different aspects of house dust mite allergy have been carried out on Kolkata population including entomological, clinical and immunological parameter through identification of allergenic mites, allergy skin tests and estimation of total IgE level, and identification of allergen specific IgE antibodies.

Results: An inventory of house dust mite fauna of Kolkata, India has been prepared. A total of 53 species belonging to 34 genera, 12 families and 3 orders have been identified, of which 18 species have been recorded for the first time from India and 7 species were identified as new to science. The genus Dermatophagoides alone constituted 60% of the total acarine fauna, predominated by *D. pteronyssinus* [47%] followed by *Blomia tropicalis*, *D. farinae* and *Austroglyciphagous geniculatus*. Both *D. pterinyssinus* [DP] and *D. farina* [DF] coexisted in the same habitat and maximum number of mites isolated from an individual dust sample was 13750/gm of dust. Seasonal trend indicates mite count was higher in premonsoon and minimum during winter. They are found in all seasons but the population increase when the atmospheric humidity is high [80%] and the temperature is around 25^{o}C. House dust mites are free-living creatures, seem to thrive best in a high protein diet, preferably feed on human dander and are abundant in beds [pillows, blankets and mattresses], padded furniture and carpeted floors etc. The present study indicates that the house dust sample contains an allergen, secreted and excreted by the house dust mites of which some peoples are sensitive and inhalation of this allergen along with dust particles is the root cause for different allergic manifestations. In some cases the entire body of the mites, dead or alive may be the source of allergen while in others the peritrophic membrane, the faecal pellets act as the sites for the origin of allergens. It is also to be noted that the allergenecity of the dust sample depend on the number of mites especially *Dermatophagoides* contained in the dust. Preliminary screening through Skin Prick Test showed 83% patients reacted positively towards allergens of mites [either DP or DF], 92% patients had elevated levels of serum IgE and the mean value higher than control sera [$p << 0.001$]. 85% patients of the study group showed allergen specific IgE antibodies against house dust and *Dermatophagoides* mites. The study indicates that more than 80 % allergic asthma patients residing in Kolkata metropolitan areas are sensitive to dust mites as evident from skin prick test and detection of allergen specific IgE antibodies by Immuno Cap system.

Conclusions : The study confirms that mite belonging to the genus *Dermatophagoides* are the main source of allergen in house dust of Kolkata Metropolis. However, the importance of *B. tropicalis* can not

be ignored. Increase use of heavy curtains, blankets, padded furniture, sofa sets, soft toys and use of foam mattress instead of conventional cotton mattress favour the growth and multiplication of house dust mites, which ultimately increases the chances and duration of exposure to those indoor allergens. Metamorphic changes in life style including diet and dietary habits, acquisition of Western lifestyle, low standard of indoor environment, increasing air pollution and over all intolerable psychological stress are blamed for such an increased occurrence and frequent recurrences of allergic manifestations in Kolkata metropolitan areas. No single satisfactory method has yet been evolved to cure allergic diseases. But the attack may be minimized by some preventive as well as some curative measures. Proper hygiene and a general cleanliness of the bed and bedroom are recommended. In conclusion, it may be stated that the proper identification of offending allergens and their subsequent reduction from the patients' environment may be helpful for the prophylactic management of these dreadful diseases.

SIGNIFICANCE OF AEROBIOLOGICAL INVESTIGATIONS IN SULTANATE OF OMAN AND GCC COUNTRIES

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Aerobiology deals with study of the sources, dispersion, and effects of airborne biological materials, such as pollen, spores, and microorganisms. Over the years, aerobiology has also taken into its fold the particles of inanimate origin which are freely suspended in air, and called as atmospheric particulate matter or simply particulate matter [PM]. These particulates [PM] are microscopic solid or liquid particles [aerosols] which are freely suspended in the air and are of considerable significance to the health and disease of organisms, particularly human beings. Sources of release of these materials may be natural or manmade.

Aerobiological investigation conducted anywhere in the world, be it northern hemisphere or southern; be it temperate or tropical climate countries; be it water bodies or desert environment; be it indoor or outdoor area, yield very useful and important information on the distribution of the invisible particles of biological or abiological origin which impact the plants, animals and human beings.

The Gulf Cooperation Council [GCC] comprise of 6 countries. They are Saudi Arabia, Bahrain, Kuwait, Qatar, Oman, and UAE, which are known for their desert climate and exposure to a water body from at least one side. The general climate in this region is high temperatures during the day with cooler nights, particularly in the sandy/desert areas. During the winter months, some of these countries experience more rain and cold than the others, such as Saudi Arabia and Oman, with areas that could experience rain up to 400mm, and temperatures that could as low as 2°C, whereas the remaining have comfortable temperatures ranging from 16-28°C at the peak of the day. During the summer months, however, many of these countries experience major heat waves and humidity levels that could reach 90%. The humidity is particularly uncomfortable due to the large bodies of salt water around a few of these countries, causing the air to be sticky and suffocating, and prompting the resident to remain indoors. [http://www.intelligentpartners.com/gcc-geography-climate].

Even though research investigations with reference to outdoor and indoor aerobiology have been conducted in Gulf consortium [GCC] countries, the available data indicate that more such reports are available from Kuwait, Saudi Arabia in comparison to Oman and other countries in the council. Long term monitoring programmes are routinely used to provide allergologists and allergic patients with necessary information as well as plant pathologists and food microbiologists, with a range of infor-

mation related to the spore concentration [Halwagy, 1994]. Fungal airspora studies over Kuwait city were conducted for a period of 12 years from 1975 to 1987 in relation to the climatic factors [Halwagy, 1994]. Earlier, Davies [1969] reported spore concentrations of various fungi in the indoor environment, in addition to trapping the pollen grains in the atmosphere of Ahmadi, a new city in Kuwait. Perhaps the first available information on the airspora studies in Saudi Arabia was the results of the investigations conducted by Al-Frayh et al [1988] in the city of Riyadh. In the experiments in Riyadh, Burkard Volumetric Spore Trap for the outdoor experiments and gravity-settle plate technique for indoor *experiments*. *Alternaria, Aspergillus, Cladosporium, Penicillium* and *Rhizopus* have been isolated *frequently* by using culture plate method. Hasnain et al [1989] continued the studies by using Burkard Volumetric Spore Trap to collect pollen and fungal spores in the atmosphere of Riyadh.Hasnain et al [1998] analyzed the concentration of *Alternaria* spores in the atmosphere of three different cities of Saudi Arabia [Riyadh, Jeddah and Al Khobar] and correlated it with the allergic incidence in the patients from various cities. In a recent work on the pollen and fungal allergens causing allergy and asthma in Saudi Arabia, Hasnain et al [2016] have reported the most common pollen and fungal spores involved in respiratory problems.

As per a recent review study [Tsiouri et al, 2014] rapid rate of urbanisation and unprecedented scale of construction in the Middle East Area [MEA] have given rise to health concerns related to the exposure to ambient particulate matter [PM] pollution. Nanoparticle [Ultrafine particle] interaction with the particles of biological origin in the air has also become an important area of research in the recent years.

Studies conducted in Muscat have thrown light on the predominant fungi both indoors and outdoors. Most commonly observed fungal genera were *Aspergillus, Penicillium, Cladosporium*, *Alternaria* and *Helminthosporium*.Present paper will review the work carried out in GCC [Gulf Cooperation Council] countries and significance of such investigations.

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AEROMYCOFLORA OF A SUGAR FACTORY IN RELATION TO RESPIRATORY AND OTHER ALLERGIC AILMENTS IN FACTORY WORKERS

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Aeromycoflora of a sugar factory was investigated for one year from November 2014 to October 2015 in Pune District of Maharashtra where no work as such is reported earlier. The role of fungi as a causative agent in allergic disorders is well established. The present investigation is aimed to find out the concentration of fungi during the operational and non-operational period of sugar factory and also to

detect the workers therein afflicted with respiratory and other ailments based on medical questionnaire.

62 fungal types, both culturable and non-culturable were identified from the indoor and outdoor environment of the factory. The dominant allergic fungal types were *Aspergillus fumigatus, Alternaria, Curvularia, Aspergillus niger, Perigonia, Nigrospora, Rhizopus, Cladosporium spp, Helminthosporium spp, Aspergillus-Penicilli*, basidiospores, smut spores and rust spores. Fungi like *Cladosporium*, *Aspergillus*, *Epicoccum*, smut, rust and basidiospores were contributed more during operational period as compared to non-operational period of the factory. The results of 180 factory workers surveyed for respiratory and other disorders revealed that about 40% of workers were symptomatic. Of these only 4.1% had atopic family history. Of the 73 symptomatic workers, 40.0% were in the age group of 21-40 years. 68.4% were smokers and the symptoms of 45.2% were associated with their work and showed increased symptoms at specific work sites inside the factory.

Keeping above aspects in view, a skin test of symptomatic workers with fungal extracts has been initiated and the involvement of fungi in the allergy of sensitive persons will be worked out soon using various clinico-immunological tests.

ENVIRONMENTAL POLLUTION, HEALTH HAZARDS AND ROLE OF POLLEN IN MANAGEMENT OF AIR POLLUTION

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Pollution is the process whereby various harmful substances are added to the environment [land, water, air etc.] by human and natural activities. Pollution is of various types, viz. air, water, land or soil and noise.

Air pollution refers to the discharge of harmful gases and dust along with microbes, heavy metals etc into the atmosphere. When these harmful substances enter the air around us, they may cause irreversible damage to humans and to our environment. Similarly polluted soil and water also adds pollutants to atmosphere.

Pollution effects are very dreadful. It causes headache, fatigue, respiratory illness, cardiovascular ailments, cancer risk, gastroenteritis, nausea, skin irritation, aging effects and even stress. All lead to affect human health.

This lecture will particularly focus on the role of pollen grains in management of air pollution. Pollen grains are male reproductive unit of plants and thus important in reproduction of plants. Pollen grains are released in air and remain part of the bioaerosol for longer time. During this course they get attacked by hazardous pollutants in the atmosphere. Various aspects of pollen viz. as bioaccumulator of pollutants, as bioindicator of pollution and use of pollen in treatment of various abovementioned diseases will be discussed.

AEROBIOLOGY – A MODEL FOR CROP DISEASE FORECASTING

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Aerobiology relating to crop disease forecasting presents an interdisciplinary approach to the properties and airborne movement of biota that are significant to plant diseases. A focus is on the devel-

opment and implementation of programmes for progressive improvement in the estimation of crop losses due to diseases and pests utilizing the appropriate methods from aerobiology and aerobiological models

The five aerobiological components used by Aylor [1986] in a spore transport model are production of spores, escape of spores from the canopy, turbulent transport and dilution, survival of spores, and deposition of spores on to the plants. Spore trapping techniques for acquisition of biological data for consecutive forecasting models are important. This helps in developing models on dispersal of pathogens or on epidemiology of the disease and to formulate methods of management. A study of the *modeling* of disease epidemics [WMO, 1989] mentions strategic methods, such as host resistance, crop rotation and fertilizer practices, along with tactical methods, such as the application of pesticides or fungicides, in response to model indications of infections or epidemics. The aim of these methods is to achieve prevention rather than containment of damage.

The introduction of a new crop and its susceptibility to disease infection or pest infestation can be tested using a simulation model [Waggoner and Horsfall, 1969]. The Soybean Rust Aerobiology Prediction System [SRAPS], an aerobiological process model for soybean rust, was developed using the Integrated Aerobiology Modeling System [IAMS].

INTERACTION OF AIRBORNE MICRO-ORGANISMS IN THE PHYLLO PLANE AND THEIR EFFECT ON DISEASE DEVELOPMENT.

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The leaves of different vegetable crops were studied to find out the level of interaction of different phyllosphere organisms. These organisms fall on the leaf surface accidentally and may cause infection after entering the leaf epidermis.Every plant has a unique phyllosphere association. These interactions are studied in different host plant and microbial combination, which were isolated from the infected lesions of the leaves. There was marked difference in pH, electrical conductance and SPM between the infected and the non-infected leaves. Of course the leaf surface was surface sterilized before isolation. From the snake gourd [*Trichsanthes cucumerina*] one such bacteria similar to *Bacillus* and pathogenic strain of *Cladosporium spp* were isolated. Slide Bioassay experiments showed no noticeable interaction between the two. Protein estimation showed that the protein concentration of the infected leaf [1.23mg/ml] was double the protein concentration of the normal leaf i.e 0.64mg/ml. SDS-PAGE was performed and on comparing the bands with the protein ladder a 55Kd protein was close to β-1,4-glucanase. This was more in case of co-inoculation between the bacteria and the fungi.

The second host selected was the common leafy vegetable *Amaranthus viridis*, here also the fungi *isolated* was *Curvularia sp*. Along with a bacterial culture obtained was found to be Gram negative short rods. In order to establish their pathogenic nature, Koch's postulates were performed on fresh leaf sections with cultures of *Curvularia* and the bacterium. Both the organisms displayed pathogenicity. Massive impregnation of *Curvularia* spore was also observed within the mesophyll tissue. The bacteria were identified to be *Xanthomon asamarinthicola*. The mechanical penetration of the fungi was more effective than the bacterial infection. In vitro study indicated bacterial inoculum caused 36.7% decrease in spore germination, indicating a fairly negative effect of the *bacteria* on the fungi, but in presence of sugar it

was reduced to 17.46%, which implies the reduced effectiveness of growth control by the bacteria. In presence of peptone , the condition was again reversed.

The third set was between lemon [*Citrus limon*] and *Elsinoe* spp.After citric acid titration, the citric acid content of the leaf was reduced by around 46%, by Elsinoe spp. The in vitro studies of fungal and bacterial interaction showed that the bacteria could reduce the fungal germination to almost about 100% in a sugar medium, and in peptone medium the germination reduction percentage was nearly 96%, thereby indicating its importance as a biological control agent. After 16s rDNA identification, the bacteria was characterized to be *Enterobacter* sp 3242. Thus the phyllosphere bacterial species can reducethe dreadful fungal scab disease.

The fourth plant specimen selected was chilli or capsicum frutescence, three pathogens were isolated from the infected chilli leaves- *Cercospora spp*, *Curvularia spp*, *Colletotrichum spp*. The pathogenic nature of these pathogens was confirmed by performing Koch's postulate experiment. The loss in the dry weight of the infected chilli fruit after 5 days from inoculation confirmed the pathogenic nature of the pathogen, affecting the fruit as well.

The antagonistic nature of *Curvularia* and *Colletotrichum* species were studied by performing a slide bioassay. The result showed an increase in the germination as well as increase in the germ tube length of *Curvularia* as compared to *Colletotrichum*. Thus, the infectivity of one of the pathogens is dominated by the other. *Cercospora* infection did not cross 10% of the total lamina surface, but capsaicin content was inhibitory to all these leaf pathogenic fungi.

Thus it can concluded that vegetable plants are not only protected by their intrinsic resistant mechanism but also there are microbial consortia of the phyllosphere that actually controls the impregnation of the pathogenic organism.

ASSESSMENT OF PHYTODIVERSITY THROUGH PEOPLE'S BIODIVERSITY REGISTER AND MELISSOPALYNOLOGICAL STUDIES

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Biodiversity today is one of the most important subjects and the loss of biodiversity is a matter of grave concern worldwide. To enact the Biological Diversity Act it is essential to document the biodiversity and related people's knowledge at the local level involving the inhabitants of that area because they have firsthand knowledge about the ecological history and present scenario including the prospect, use and status of biodiversity. These documents are capable of bringing together important locality specific information on bio resources and the ecological processes affecting them. As this document is prepared on the basis of people's knowledge it is named as People's Biodiversity Register [PBR]. The PBR includes [i] the diversity of different landscapes; [ii] the diversity of life capes [iii] the diversity of people. The complete PBR document would be very much useful for local biodiversity management like restoration of threatened habitats/species/breeds, entrepreneurship development of commercially potential species and others. The PBR process acts as a catalyst which makes an impetus in generating mass awareness towards biodiversity conservation through the active participation of the local people.

It accelerates the flow of the traditional knowledge from one generation to another which would help its conservation for the prosperity of the nation. Thus PBR is an important driver for the protection, conservation and sustainable use of the biological heritage of the country.

The variety of plants and animals and their speciation has a great impact on man and his environment at large. Phytodiversity, the diversity of plant species in a certain place, is gaining increasing importance considering the present status of planet earth, related to global warming, deforestation and various other issues. Honey is a natural product that helps us to study and understand the diversity of plant species in a particular place and also its neighbouring areas. Honey and its uses have been known to man since time immemorial. Honey is used all over the world for various purposes; its uses in the food and cosmetic industry are now of prime importance. It is the most widely used natural medicines till date and has also been used as medicine in the middle ages as traditional medicine called apitherapy. Honey, a major bee and plant product, made from the nectar of plants, is a natural sweet substance composed mainly of easily digestible simple sugars, monosaccharides, disaccharides and trisaccharides, water; the qualitative and quantitative composition of which is peculiar to the product. It has been observed that bees select grains that are rich in nutrient content for superior honey production. Earlier studies have proven beyond doubt that bees are highly discriminative about selecting the plants for nectar collection. The study of honey samples specifically indicates the vegetational content of the region. Honey also acts as a bio-pollution indicator that can be determined by the presence of heavy metals in the honey samples.

In this study, the samples have been collected from different locations in West Bengal, including Kolkata, parts of North Bengal and South Bengal and the Sunderban Delta. The behaviour of the foraging honey bees also indicates the environmental and vegetational condition of the region. Bees select grains that are rich in nutrient content for superior honey production as well the flora of a particular bio-zone can be noticed.

THE APOCALYPSE OF P-VALUE?

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P-values are widely used in science to test null hypotheses. But P values are slippery, and sometimes, significant P values vanish when experiments and statistical analyses are repeated. In light of misuses of and misconceptions concerning p-values, in this lecture, other approaches like confidence, credibility, or prediction intervals; Bayesian methods; alternative measures of evidence such as likelihood ratios or Bayes factors; and other approaches such as decision-theoretic modeling and false discovery rates will be discussed.

BIOMARKERS IN ASTHMA: FROM BENCH TO BEDSIDE

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Biomarkers are characteristics that are objectively measured and evaluated as indicators of biological or pathogenic processes, or responses to therapeutic interventions, and may provide information on the prognosis or progression of the disease and response to treatment. They are likely to be helpful in the

management of asthma because of the heterogeneity of its pathobiology. Currently available biomarkers have been developed and evaluated to assess the airway inflammation [or bronchitis] associated with airway diseases. However, most such markers are either invasive [markers in bronchoalveolar lavage and bronchial biopsies] or lack specificity [fraction of exhaled breath nitric oxide and markers in peripheral blood]. Sputum quantitative assay provides specific, sensitive, responsive, reliable and noninvasive estimates of airway inflammation. In addition, promising novel biomarkers are being researched in the field of breath metabolomics [volatile organic compounds] and pharmacogenomics. Biomarker research in asthma is increasingly shifting from a single biomarker assessment to multidimensional approaches in which the clinical value of a combination of various biomarkers is studied. This review provides a brief description of these biomarkers with a particular emphasis on how eosinophil and neutrophil counts in sputum could be used to manage airway diseases such as asthma.

IDENTIFYING NOVEL BIOMARKERS FOR ATOPIC ASTHMA USING DAAB

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Asthma is still one of the most under diagnosed and under treated disease in the world. The phenotypic symptoms of atopic, non-atopic asthma and COPD tend to overlap, making the disease diagnosis difficult. Specific biomarkers for accurate disease diagnosis could be of tremendous importance for tackling atopic asthma and other allergies. We have developed a Database of Allergy and Asthma Biomarkers [DAAB] which is a repository of genes/proteins that are differentially expressed in different allergic diseases including food allergy, allergic rhinitis, and atopic asthma. This manually curated repository include entries from genomics [1022], proteomics [419], epigenetics [16] and other low throughput studies [210][1]. DAAB is freely accessible from http://bicresource.jcbose.ac.in/ssaha4/daab.The current version of DAAB lacks detailed information of biomarker molecules including their structure and relevant mutations. We are updating DAAB by incorporating information on [i] protein-protein interaction, [ii] mutation and Single Nucleotide Polymorphisms [SNPs], [iii] protein structure, and [iv] drugs available for each gene. The four relevant data base entity tables with important attributes shall constitute the relational database of updated DAAB. For example, the Protein interaction entity table includes important attributes like Interactor name, Interactor type, UniProt Accession ID.

We selected two genes namely, Myeloid cell Nuclear Differentiation Antigen [MNDA] and Tumor necrosis factor [ligand] superfamily, member 4 [TNFSF4] based on their role in innate and adaptive immunity respectively for experimental validation of DAAB high throughput data.The blood samples of asthmatic patients [before and after treatment] and healthy controls were collected, and expression of these genes along with Single Nucleotide Polymorphisms [SNPs] were studied using RT-PCR. We observed MNDA and TNFSF4 to be 8 and 2 fold high respectively in before treatment asthma patients as compared to control samples. Interestingly, MNDA may be used as a novel biomarker candidate for asthma patient, which plays an important role in the granulocyte/monocyte cell-specific response to interferon. However, we have notidentified any SNPs in these genes in the patient samples. The limitation of the present validation study of putative biomarkers was the low number of asthma patient samples.In summary, DAAB may aid in identifying novel biomarkers for atopic asthma. Besides, the

updated version shall enable better understanding of etiology and patho-physiology of the disease with deeper insights into the interaction pathways, possible mutations, and structural organization. It may also help the clinicians with novel disease management ideas and treatment approaches to resist the allergic march towards asthma.

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PENICILLIUM: A CURSE OR BOON.

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Aspergillus and Penicillium spores represent some of the most abundant aeroallergens in different parts of the world. The qualitative and quantitative reports have pointed out that Penicillium predominates in most regions though it is relegated from its most prevalent position by *Aspergillus* in humid tropics.

The conidia of *Penicillium* like those of *Aspergillus*, are everywhere in the air and in the soil. In the biological laboratories, they are as frequent contaminants. *Penicillium* species are seemingly always involved when human foods and animal feeds, raw materials, or finished products, in fields, storage bins, warehouses and homes, are undergoing deterioration and spoilage. Non-food items, such as bathrooms baseboards or clothing stored in a damp cellar, are subject to attack by species of *Penicillium.*

The presence of *Penicillium* in the air has many implications. *Penicillium* is reported to be one of the common aeroallergens. Various mycotoxins produced by different species of *Penicillium* like ochratoxin A, patulin, citrinin etc. are harmful to the humans, plants and animals.Mycotoxin like patulin produced mainly by *Pencillium expansum* that commonly contaminates apples. It has a broad spectrum of toxicity, including carcinogenicity. In human beings, it has caused nausea, vomiting and gastrointestinal disturbance.

Post harvest decay of citrus fruits viz. oranges, lemons, grapefruits, apples, figs, some dry fruits, etc. are caused by some species of *Penicillium* particularly *P. expansum*, *P.digitatum* and *P. italicum*. It is the so called green mold and blue mold which we so frequently find on citrus and other fruits, on jellies and preserves and on other foodstuffs that have become contaminated with the spores. Penicillia are also responsible for damage to the stored grains. This damage to the fruits and grains in storage and transit causes great economic losses. *Pencillium* is also one of the dominant fungi responsible for biodeterioration in leather and paper industry.

Penicillium species are seemingly always involved when human foods and animal feeds, raw materials, or finished products, in fields, storage bins, warehouses and homes are undergoing deterioration and spoilages The presence of *Penicillium* has many implications and the genus is useful to mankind in many ways as well as harmful in many other ways.

Pencillia are important not only for their harmful effects. Allergenicity of the *Pencillium* is reported and it is quoted as one of the common aeroallergens. The number of fungi that existed in human respiratory parenchyma was dominated with genera like *Aspergillus*, *Candida* and *Pencillium*. Even the healthy respiratory parenchyma of human lung was not normality aerated respiratory parenchyma.

Some species of *Pencillium* are opportunistic pathogens, causing diseases in immunocompromised subjects. Penicillosis, a respiratory disease is caused by the *Pencillium*. Penicilliosismarneffei is a dissemi-

nated and progressive infection caused by *Penicillium marneffei*, a facultative intracellular pathogen and the only thermally dimorphic fungus of the genus *Penicillium*. It is the third most common opportunistic infection in HIV infected patients following extra pulmonary tuberculosis. There is recent report of pulmonary infection with *P. digitatum* a human infection with *P. digitatum* is considered rare as it was the plant pathogen causing post-harvest fungal disease of citrus called green mold.

The best known of antibiotic agents, *Penicillium* in produced by *Penicillium notatum*, *Penicillium chrysogenum* and certain other species. The discovery of Penicillin by Flemming and the success which have attended its use in bacterial infections originally by Florey and later by many workers are very well known. Many antifungal antibiotics like greseofulvin , albidin , cyclopaldic acid, frequentin ,viridian ,tardin ,gladiodic acid , etc. are also produced by different species of *Penicillium*. Even many of the third generation. *Penicillium rockforti* and *Penicillium camemberti* are used in the ripening of cheese, which cause fermentation in cheese and lend it characteristic aroma and consistency. *Penicillicum ochrochloron* is reported to be an extremely tolerant species to copper and can be used for the clean-up operations or for recovery of metal from wastewater. *Penicillium nigricans* is found to be tolerant to relatively high concentration of salts of iron, nickel, cobalt and chromium and suggests its potential in purification of industrial wastes.

Penicillin [PCN or pen] is a group of antibiotics which include Penicillin G, Penicillin V, procain Penicillin, and benzathinePenicillin. Penicillin antibiotics were among the first medications to be effective against many bacterial infections caused by Staphylococci and streptococci. Penicillins are still widely used today. Significant improvements in modern production methods have increased their production and decreased cost. *Penicillium crysogenum* is widely used for penicillin production.Worldwide sales of Penicillin and other beta-lactam antibiotics is now greater than $15 billion [US dollars] per year. These sales numbers exists despite the fact that cost is now at an all-time low. Penicillin now costs $10 per kilogram versus $300 per kilogram in 1953.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF AIRBORNE FUNGAL SPORES IN DIFFERENT RURAL VILLAGES OF PUDUCHERRY REGION

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There is a range of particles that occur in outdoor environments, which may give rise to different allergic response to humans i.e., dust, fibers and grits, pollens and spores of fungi and filamentous actinomycetes. The most abundant spores associated with allergenicity occur in the air arise from fungi commonly found on the leaf surfaces. In our study, an aeromycological survey of different village areas of Puducherry region was carried out by employing volumetric Burkard's personal sampler on agar plates using PDA/SDA media plates during 2015-16. Air samplings were collected for one year at 15 days interval for isolating the prevalent fungi from the study areas at three times of the day viz., morning noon and evening. During the study period, a total of 75 fungal species under 52 genera were recorded, among which *Aspergillus* spp were observed as the dominant followed by *Penicillium* spp. The incidence of fungi was found variable from village to village due to the variability of vegetation available in the rural areas. Moreover, few specific fungi were found dominant in the concerned area based on their substrate and host specificity. In diurnal occurrence, noon was found to harbor more

number of spores in composition and concentration than morning and evening in the sampling areas. Seasonal incidence of fungal spores was quite regular in our finding and showed the trend more in winter followed by summer and rainy. Fungi like *Absidia*, *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Monascus*, *Mucor*, *Paecilomyces*, *Rhizopus*, *Trichoderma*, *Verticillium* and *Wallemia* were also recorded in addition to aspergilli and penicilli. The allergenic fungal spores of aspergilli and penicilli were found to be predominant may be due to their wide host range, substrate adaptability and opportunistic nature. The diversity of local vegetation and climatic alteration had positive/negative influence on occurrence of airborne fungal spores in the studied village environments in the Pondicherry region.

SYNERGISTIC ANTIBIOTIC EFFECT OF VANCOMYCIN AND TETRACYCLINEDRUGS IMPREGNATED WITH BIOSYNTHESIZED NANOPARTICLESAGAINST MDR PATHOGENS

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Throughout the modern days, bacterial pathogens are established to be resistance to most of the antimicrobial agents existing in the bazaar, which ended with more health concerned to the overall public. Furthermore, nanobiotechnology modifies metals into bionanoparticles by the advantage of noble microorganisms, which is found as latent antimicrobials. Silver is used in the practice of metallic silver to treat burns, wounds and several bacterial infections since age-old. In ourcurrentstudy, extracellular biosynthesis of silver and gold nanoparticles was made by the use of airborne mould fungi i.e., *Penicillium* spp. The nanoparticles designed by the fungi showed the extreme absorbance between 420-440nm on UV-spectrophotometer. In toting to Uv-vis spectrometry, the characterisation of both nanoparticles was made by FESEM and XRD analysis, which confirmed the metallic nature of the nanoparticles. Silver nanoparticles displayed good antimicrobial action against the tested bacterial pathogens but combined formulation with antibiotics, viz.Vancomycin and Tetracycline; the biosynthesized nanoparticlesfrom Penicillimagnified the antimicrobial potency of the antibiotics at higher rates against MDR pathogenic bacteria. It was perceived that the antibacterial properties of antibiotics were improved in the presence of silver nanoparticlesand was different from drugs to drugs in order to show their efficiency against the bacterial pathogens.Gold nanoparticles were found to be less active in contrast to silver nanoparticles. The manufactured nanoparticles in the present studywas found simple, pretty fast, biocompatible, and was found free from everylethalcompounds.

EFFECT OF SELECTED CHEMICAL AND BIOLOGICAL FUNGICIDES ON THE POLLEN GERMINATION OF FOUR VEGETABLE CROPS

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Chemical fungicides are widely used today to mitigate the fungal diseases of crops. It is well known that these chemicals not only repels and harms the friendly pollinators, contaminate water bodies but also responsible for human health hazards. The present investigation was carried out to observe

the role of chemical and biological fungicides on the pollen physiology of four vegetable crops viz. *Solanum melongena* [Brinjal], *Lagenaria siceraria* [Bottle gourd], *Vigna unguiculata* [Vegetable cowpea], *Abelmoschus esculentus* [Ladies finger]. It is revealed through in vitro studies that the fungicides like, Vitavax [Carboxin, 75% W.P.] and Hilzim [Carbendazim, 50% W.P.] reduces the rate of in vitro pollens germination of these crops. It is also observed that the growth of the pollen tubes were arrested or deformed when exposed to such chemicals. On the other hand, interestingly, the bio-fungicide like Trichostar [*Trichoderma viride* - 1% WP] and Sudobact [*Pseudomonas fluroscens* - 2 X 109 CFU cells bacteria/ml] does not affect the pollen germination and tube elongation of these crops. This study naturally proves that more research and production of ecofriendly bio-fungicides and their wide application is immediately required for a sustainable future.

AEROBIOLOGICAL, CLINICAL, IMMUNOCHEMICAL, MOLECULAR BIOLOGICAL AND EPIDEMIOLOGICAL STUDIES ON AIRBORNE ALLERGENIC POLLEN AND FUNGAL SPORES OF WEST BENGAL IN LAST TWO DECADES

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Background : Pollen grains, the male gametophyte of the flowering plants and fungal spores, the reproductive unit of fungi often become airborne and inhaled by human. These pollen gains and fungal spores are important triggers of IgE-mediated respiratory and allergic asthma in susceptible individuals. However, their diversity, seasonal variation, allergic potential and long-time health effect varies according geographic location and climatic condition. Hence, regular monitoring of airborne pollen and spore is necessary for evaluation of aeroallergen exposure for sensitive people of a particular locality. The soluble proteins of pollen and spore act as IgE-reactive allergenic protein/allergen, which is required to be identified, characterized, isolated and purified for use in immunotherapy for hypo-sensitization of allergic patients.

Objectives : The study highlights the diversity and periodic pattern of atmospheric pollen and spores of West Bengal, a state of Eastern India as reported in last two decades. The objectives also include the study of airborne pollen and spores as source of aeroallergen, their allergenic potential to cause respiratory allergy in susceptible individual, biochemical nature of allergenic proteins present in them along with cross reactivity, molecular biology, their effect on asthma-related emergency hospitalization and management of respiratory allergy by allergen specific immunotherapy.

Observations : Regarding seasonal periodic pattern, different studies with Burkard 7-day volumetric sampler from West Bengal depicted > 46 pollen types and > 26 fungal spore types to be frequent in the atmosphere, among which some are seasonal and others are perennial. Viable fungal spores were studied using Andersen sampler, which enabled the identification of fungal spores up to specific level and express as colony forming unit per unit volume of air. Among the recorded types, some pollen-spore members are allergenic as evidenced by skin reaction test of allergic patients, serum IgE-ELISA, etc. Grass pollen grains and Aspergilli-Penicilli spore members were found to be most allergenic [> 40% positive reaction in skin test]. In immunochemical studies by IgE immunoblotting, a number of moderate to high molecular weight IgE-reactive proteins were detected in pollen-spore extracts of the specific types. A detailed study of the allergenic protein revealed their characters in case of pollen grains

[*Carica papaya, Catharanthus roseus, Cocos nucifera, Helianthus annus, Lantana camara*, etc.] and spores [Rhizopus oryzae, and others]. In chemiluminescent immunoblotting of the exposed Burkard sampler tapes, presence of atmospheric pollen aeroallergens was also confirmed. Some of the pollen and spore allergens showed cross-reactivity with other allergens in IgE ELISA inhibition and immunoblot inhibition. Among fungal allergens, Rhi o 1 is a recently discovered aspartic protease allergen [MW 44 kDa] from *Rhizopus oryzae*. The full length cDNA of Rhi o 1 was isolated, cloned and expressed in E coli to produce recombinant Rhi o 1.

In an epidemiological survey of suburban population of Kolkata and school-children of the same city, strong correlations of asthma related hospitalization was found with airborne pollen grains of grass, *Areca catechu, Bombax ceiba, Mangifera indica*, etc. and fungal spores like *Alternaria* respectively.

Management of aeroallergen induced respiratory allergy included avoidance of exposure, pharmacotherapy and allergen-specific immunotherapy [AIT]. AIT is mentioned as most effective measure against pollen-spore allergy, without any side effect. In this regard, in a placebo controlled immunotherapy for 2-year with partially purified pollen extract of Phoenix sylvestris, showed significant improvement of symptom-medication score as well as immunological parameters [specific IgE, IgG4, etc.] recorded in a group of susceptible respiratory allergic patients.

Conclusion: A long-time and detail aerobiological, clinical, immunochemical, molecular biological and epidemiological studies and therapeutic trial of airborne pollen grains and/or fungal spores of the state of West Bengal have important implication in the diagnosis, prevention, treatment and overall management of aeroallergen induced respiratory allergy. Such studies will be helpful in the improvement of the life style of susceptible local population and awareness development in society.

P. H. GREGORY AWARDED ABSTRACT

[**Mr.Partha Karak** of Santiniketan (Gold Medal), **Miss Monalisa Mehrolia** of Nagpur (Silver Medal) and **Miss Koyel Sengupta** of Kolkata (Bronze Medal)]

DIVERSITY OF MITES AT POULTRY ENVIRONMENT IN NAGPUR REGION.

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Different mites have been found to cause allergy in human being particularly in sensitive victim .Hence study of mites and preferably poultry dust mites is important and significant. A study on poultry mites has been carried out from July, 2015 to June 2016 in two poultry farms of seminary hills, Nagpur district, Maharashtra. Fortnightly mites were picked up from intramural dust of poultry farm. The main purpose of the study was collection, observation and identification of mites. Hand pick technique was used for the isolation of mites from dust sample. The qualitative and quantitative analysis of poultry mites from poultry farms were investigated separately for their seasonal distribution in relation to meteorological parameters. Seasonal occurrence of poultry mites were highly influence by climatic conditions. Maximum population of mites was observed in rainy season when relative humidity is 80%-90% and temperature ranges from 21°- 24°while minimum in summer season, as they cannot tolerate high temperature .From this investigation it can be interpreted that different environmental parameters like temperature, relative humidity and rainfall play determinative role in maintaining mite population. *Dermanyssus gallinae* [Dg-469-21.30%], *Dermatophagoides* spp [Ds-414-18.8%] urodiaspis tecta [Ut-358-16.26%], *Caloglyphus oudemansi* [Co-358-16.26%] *Caloglphus hypopus* [Ch-283-12.85%], *Dermatophagoides*

pteronyssinus [Dt-148-6.72%], unidentified [U_1-171-7.76%] were among predominant forms recorded from poultry farm from the total [2201] screened specimens. *Dermanyssus gallinae* has been found to be dominant species.

EVALUATION OF ATMOSPHERIC FUNGAL SPORE COUNTS IN KOLKATA AND INVESTIGATION OF ALLERGENIC POTENCY OF *ASPERGILLUS ORYZAE*

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Objectives : Aeromycological monitoring was conducted in an urban megacity Kolkata to identify and quantify the airborne fungal spores, and to detect the allergenicity of one of the most predominant fungi *Aspergillus oryzae*.

Methodology : Two-year long biomonitoring of fungal aerospores was performed by using Andersen sampler and Burkard personal sampler and spore calendar was prepared. Spore concentrations were correlated with different meteorological parameters and hospitalization data. Allergenic potential of *Aspergillus oryzae* was tested by Skin Prick Test and ELISA. Both extracellular and intracellular proteins were analyzed by immuno-proteomic approach. After one and two dimensional gel electrophoresis, IgE reactive proteins were detected from 1D and 2D Immunoblot. For intracellular proteins, presence of glycoprotein in the allergen profile and the role of sugar moiety in IgE-binding were assessed by Periodic Acid Schiff's staining followed by meta-periodate modification. Mass spectrometry based identification of allergens was done by MALDI-TOF-TOF. Major allergen was partially purified by anion exchange chromatography. Results and Discussion: From biomonitoring, maximum spore concentration was observed in outdoor environments and was greatly influenced by meteorological parameters. Hospitalization records were positively associated with spore load. Aspergillus sp. was found to be the most predominant fungi. About 32% of total patient were found allergic to *Aspergillus oryzae*. 18 intracellular and 10 extracellular allergens were detected from 2D immunoblot. For intracellular proteins, three allergens were found having glycol moiety as a major contributor of IgE-epitope. The major allergen was glucan 1, 3-beta-glucosidase A [45.434 kDa], identified by MALDI-TOF-TOF. After anion exchange chromatography, the major allergen [pI 4.63] showed its IgE reactivity by 1D immunoblot confirming successful partial purification of this allergen. Identification and purification of allergens are essential to develop recombinant hypoallergenic variety that can be helpful to develop vaccines to improve existing immunotherapeutic techniques.

PREVALENCE OF ALLERGIC SYMPTOMS AMONG THE POPULATION OF POLLUTED AND LESS POLLUTED AREAS OF WEST BENGAL, INDIA: A HOSPITAL-BASED CLINICAL STUDY

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Background: Several earlier surveys revealed the prevalence of atopic dermatitis, asthma, and allergic symptoms among population of West Bengal which have amplified to a great extent. Several studies have shown that socio-economic status, age of the people and their surrounding environment have a profound role behind allergic diseases. Based on the hospitalisation survey we sought to establish the

relationship between incidence of allergic symptoms and the environmental pollutant status of the two selected polluted [Durgapur- an industrial township] and less polluted [Bolpur- an rural set up] areas of West Bengal.

Methods: We have collected hospitalization data thrice in a week through the survey with the help of a standard Questionnaire among 1078 allergic subjects [Male 587 and Female 491: age group 2 month to 78 years] in different Government hospitals and private clinics of the locality with the help of trained clinicians. The cross sectional study [symptom relationship] was made on five major allergic symptoms based on ICD 10 Code [WHO Standard]. We have divided the total patients into 10 groups on the basis of their sex, age and socio-economic status.

Results: We have enrolled 1078 patients who were diagnosed with allergic asthma, atopic allergy, cough, atopic cough, atopic dermatitis or a combination of those. There were significant differences in the allergic symptoms. The highest incidence [20%] was recorded in dual combination of cough with asthma symptoms. Females were more susceptible [56%] to most of the symptoms than male. Lower economy class people showed highest symptoms of atopic skin disease [65%] than middle or higher economy class people, while allergic urticaria is more prevalent [35.3%] in higher class people. On the other hand, there were significant level of allergic skin diseases [59.2%] and cough [53.9%] among children [2-17 years age] than the other age groups. In Bolpur, skin related allergic diseases [71.42%], conjunctivitis [25.07%] was higher than in Durgapur [63.12% and 8.51% respectively] which may be due to higher concentration of viable fungal air spores in Bolpur. Statistical study shows significant relationship between total spore count and total number of allergic patients [p-0.0002/r-0.68]. Allergic Rhinitis [J30.9 ICD 10] shows its correlation with spores of *Cladosporium* Sphaerospermum [p-0.03/r-0.75]. The antigenic extracts of four dominant fungal species evoked as much as 73.0% skin reactivity

Conclusion : Our findings are in line with the perception of increase in the occurrence of atopic dermatitis, cough, asthma or conjunctivitis in children. At the age below 10 years, more than half of all the children were affected with at least one of such conditions. In general, females show more sensitivity to one or more types of symptoms. The lower economy class people are more vulnerable to atopic dermatitis perhaps due to their ignorance about sanity and poor nourishment.

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