*Plant Archives* Vol. 21, Supplement 1, 2021 pp. 2428-2432 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.396

# ASSESSMENT OF AIRBORNE FUNGAL ALLERGENS: BIOCHEMICAL AND IMMUNOPROTEOMIC APPROACH : A REVIEW

**Amarjeet Kaur**
Department of Botany, Sree Chaitanya College, Habra, P.O. Habra-Prafullanagar, North 24 Parganas

**ABSTRACT**

Atmospheric air contains a wide variety of components like inorganic gaseous, particulate pollutants and biological particles such as fungal spores, pollen grains, dust mites and animal dander that can affect human health. Among biological particles, airborne fungal spores are an essential source of aeroallergens. In India, allergy prevalence has become a major health concern with symptoms ranging from mild rhinitis to severe asthma and even life threatening anaphylaxis. Aerobiological studies provide qualitative and quantitative information about fungal spores of a given region. The proper identification, purification and molecular characterization of fungal allergens are essential for proper diagnosis and to design immunotherapeutic vaccines. Only a few fungal allergens have been characterized by recombinant technology and structural biology. Detailed analysis of fungal biochemistry can be done by using multiple techonologies including Skin Prick Test (SPT), ELISA, immunonoblot, MALDI TOF, genome and transcriptome sequencing, recombinant technology and bioinformatics. The purpose of this review is to describe the different strategies that have been used to identify, purify and characterize the fungal allergens, which may enrich the database of allergy research and help in the implementation of immunotherapy in future.

***Keywords***: Allergen, Skin Prick Test, ELISA, Immunoblot, MALDI TOF.

## 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 (Burge *et al.,* 2000; Bush *et al.,* 2001; Hasnain *et al.,* 2004). Manifestation of allergic diseases, spanning from mild rhinitis to anaphylaxis is a major health problem, affecting the life of millions of people all over the world. More than 20% of world population is affected by different kinds of allergy caused by naturally occurring as well as synthetically produced compounds and their prevalence is increasing day by day (Gonzalez- Buitrago *et al.,* 2007). About 25% of Indians suffer from allergic problems (Mandal *et al.,* 2012). Aerobiological studies and complete biochemical characterization of fungal allergens is required for adequate medication of fungal spore allergy. So extensive aerobiological studies has been done in different environments like agricultural farm, potato cold store house, bakery, milk dairy, and library etc.(Adhikari *et al.,* 2000; Barui *et al.,*2000; Majumdar *et al.,*2004; Majumder *et al.,* 2007; Das and Gupta Bhattacharya , 2008; & Chakrabarti *et al.,* 2012) for constructing the spore calendar of that particular environment which can help respiratory allergic and asthmatic patients and clinicians to know about the environmental exposures and avoidance policies.

Proper identification and characterization of the fungal allergens are also essential for successful diagnosis and subsequent therapy of patients. More than 80 fungal genera have been reported to induce IgE-mediated hypersensitive reactions in susceptible individuals and about 150 specific fungal allergens have already been identified from 23 different fungal genera (Green *et al.,* 2005; Simon-Nobbe *et al.,* 2008). A cross reactive 45 kD allergenic protein was purified to homogeneity and characterized from *Fusarium solani* (Verma *et al.,* 2003). Mannitol dehydrogenase allergen was identified, purified and cloned from *Cladosporium herbarum* (Simmon-Nobbe *et al.,* 2006). A major recombinant allergen Cytochrome-C was prepared from *Curvularia lunata* and three dimensional structure of the allergen has been determined using bioinformatic tools (Sharma *et al.,* 2009, Nair *et al.,* 2011). 63 IgE reactive cytosolic proteins from germinating conidia of *Aspergillus fumigatus* were also identified which includes two already known (Asp f 12 and Asp f 22) and four predicted allergens (Hsp88, Hsp70, malate dehydrogenase and alcohol dehydrogenase) based on their homology with other known fungal allergens (Singh *et al.,* 2010). Another immunoproteomic investigation has identified 14 IgE reactive proteins from *Rhizopus oryzae* of which a 44 kDa Aspartyl protease is the major one (Sircar *et al.,* 2012, 2015). A recent investigation has identified Glyceraldehyde -3-Phosphate dehydrogenase allergen from *Fusarium lateritium* (Dey *et al.,* 2019). The identified allergen holds the potential to develop immunothearapy of *Fusarium lateritium* sensitized patients.

### Strategies used to identify, purify and characterize the fungal allergens

Different strategies which are used to identify, purify and characterize the fungal allergens are as follows:

**1. Aerobiological Study**

Aerobiology is the study of different kinds of airborne biological particles such as pollen grains and fungal spores, their movement and impact on plant, animal and human health. The term 'Aerobiology' was first coined by American plant pathologist Fred Campbell Meier in 1935. 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 meteorological conditions, fungal substrates and human activities. Studies have shown that there is a variation in fungal spore counts among different working environments (Adhikari *et al.,* 2000; Barui *et al.,* 2000; Majumdar *et al.,* 2004; Majumdar *et al.,* 2007; Das and Gupta Bhattacharya 2008 & Chakrabarti *et al.,* 2012). So, fungal spore diversity study will help to monitor and quantify the presence of different types of fungal spore in a particular environment. Air samplers like Burkard personal slide sampler (for trapping nonviable fungal spores) and Andersen 2-stage sampler (for trapping viable fungal sporer) helps in the study of the fungal spore diversity (Fig.1). Non-viable spores trapped on the glass slides of Burkard Personal Sampler are mounted by cover slip with DPX followed by the scanning under microscope and finally they are identified using specialized references (Ellis, 1971; Onions *et al.,* 1981) and also by comparing with reference slides. Fungal spores are counted according to the guidelines given in the British Aerobiological Federation (The British Aerobiology Federation, 1995). After exposure, Petri plates of Andersen Two-Stage Viable Sampler are stored and incubated at $30^0$C for 5-10 days until sporulation takes place. Ultimately fungal colonies are identified to the genus level under a microscope based on the colony characters, spore morphology, using lactophenol blue stain. Finally, non-viable fungal spore counts are converted to number of spores per cubic meter of air ($m^3$) and the developing viable fungal spore colonies per sample are expressed as colony-forming units (CFU) per cubic meter of air ($m^3$). The duration and sampling time are determined after many standardization trials. Mainly two types of nutrient agar media, viz. Potato dextrose agar (PDA) and malt extract agar (MEA) [20g PD/ME in 1L water and 13g agar in 1L water] are preferred for showing the maximum fungal diversity and for best fungal growth in Andersen Two-Stage Viable Sampler. When airborne spores are trapped on a coated slide of Burkard sampler, mostly it can be identified up to generic level under microscope. The spores are better identified up to species level when exposed in nutrient media containing petriplates within Andersen sampler and produce identifiable colonies. To construct a fungal spore calendar, the individual fungal genus is expressed in monthly mean concentration and presented on a particular scale, each level of which shows a particular concentration of the spores. Pure cultures are prepared by subculturing individual colony on sterile PDA plates. PD broth is prepared, sterilized and inoculated with spores. Inoculated broths are maintained at 30◦C with moderate humidity in an incubator without shaking. A mycelial mat of two weeks old culture weighing about 500 mg is harvested and washed with sterile double distilled water. 1 gm lyophilized spore mycelial mat is crushed in liquid nitrogen and soaked in 5 ml sterile 0.1 M Phosphate buffer (pH 7.2) for 72 hours and centrifuged. The clear fungal allergen extract is obtained by filtering the supernatant through a 0.22 µm membrane (Millipore) and is used for Skin Prick Test (SPT) and for further biochemical and immunoproteomic studies (Sircar *et al.,* 2012, 2015).

**2. Biochemical and Immunoproteomic study:**

**i) Skin prick test (SPT) :** Tests are performed with the suspected allergen extract placed on the ventral side of the forearm and each site is pricked with disposable hypodermic needle. Skin prick test (SPT) helps to confirm the atopic sensitivity of the allergic patients (Fig. 2). It involves controlled exposure to a suspected allergen. Histamine diphosphate (1 mg/ml) and 5% glycerinated phosphate buffer saline (0.01 M, pH 7.2) serves as positive and negative control respectively. The wheal diameter is to be measured after 20 minutes of application of the allergen; and is graded from +1 to +3 level (+1 = erythema, 2 mm in diameter, +2 = wheal and erythema, >2 mm in diameter, +3 = wheal > 3 mm and erythema). Isolated patient sera are stored at −20 °C for further studies. Sera from non-atopic individuals will serve as negative control. The skin tests have many limitations and disadvantages; hence there is a need for more convenient and reliable methods for measurement of IgE level like ELISA (Singh *et al.,* 2011).

**ii) Indirect IgE-specific enzyme-linked immunosorbent assay (Indirect ELISA) :**

The concentration of specific IgE in a patient's sera is measured with the help of Indirect ELISA (Fig. 3). Indirect ELISA is preferred for its increased sensitivity and flexibility. Non-smoker subjects with no record of allergic/respiratory disorder and immunodeficiency diseases in the family are taken as negative control (Mandal *et al.,* 2012). The ELISA values are represented as P/N ratio where P refers to average OD value of the replica for each patient's serum and N refers to the mean of OD values of all the control subjects. For a particular serum, P/N ratio greater than 2.5 is considered as in vitro "positive" with markedly elevated level of specific IgE (Sircar *et al.,* 2012).

**iii) ELISA Inhibition Assay (Competitive ELISA)**

It helps to measure the amount of antigen required to completely block entire IgE content of the patient. Here the patient serum is first incubated at varying known concentration of crude allergenic protein extract, acting as inhibitors. Thereafter antigen-antibody mixture is used to carry out rest of the experiment according to indirect ELISA. This mixture serves as a substitute for the primary antibody (Sircar *et al.,* 2012). Here the patients' pooled sera without inhibitor is considered as positive control. % inhibition can be calculated by the formulae-

$$1 - \frac{OD\ of\ sample\ with\ inhibitor}{OD\ of\ sample\ without\ inhibitor} \times 100$$

**iv) Biochemical Identification of Allergen**:

a) **1D immunoblot** :

SDS-PAGE is an electrophoretic method for separating polypeptides according to their molecular weights (M). In 1D immunoblot (Fig. 4), after the transfer of protein onto PVDF membrane, allergen is identified by consecutive application of primary (in the patient sera) and secondary antibody (directed against primary antibody).

A standardized method of protein extraction like TCA-Acetone, Tris-phenol should be employed to maximize the yield of protein. SDS-PAGE can be done following Laemmli

*et al.,* 2004 on homogenous gels (15%T, 2.6%C) under reducing conditions to view the crude protein's banding profile. This is followed by immunoblotting with allergic patient sera, as a primary antibody, to identify the allergenic protein band. Total protein profile in 12% SDS PAGE revealed more or less forty bands within a mol wt. ranged between 14.3 and 97.4 kD in *Rhizopus oryzae* ( Sircar *et al.,* 2012).

b) **2D immunoblot** :

A particular IgE reactive band in a 1 D immunoblot may contain more than one protein. Hence one band and one allergenic protein do not correlate. Isoelectric Focusing is an electrophoretic method that separates proteins according to their isoelectric points (pI). In a pH gradient and under the influence of an electric field, a protein will move to the position in the gradient where its net charge is zero. Keeping this in mind, the present trend of identifying fungal spore allergens has shifted to 2D immunoblot (Fig. 5). This technique resolves the total proteome on a 2D gel followed by blotting either with pooled sera or individual sera, thereby identifying IgE reactive proteins in protein extracts (Sircar *et al.,* 2012). Matched immuno-reactive spots identified in 2D gels are further excised for mass spectrometric analysis.

**c) MALDI TOF/TOF :**

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) determines the molecular weight by measuring the mass-to-charge ratio of ions in the gas phase; and thus helping to deduce the unknown peptide sequence. The allergenic protein spots are digested with trypsin before proceeding to MALDI TOF/TOF (Shevechenko *et al.,* 2006). Monoisotopic peptide masses are analyzed after exclusion of contaminant ions (known matrix and human keratin peaks or peaks of trypsin added for digestion of proteins) (Peri *et al.,* 2001). MASCOT helps to interrogate MALDI-TOF-MS and MS/MS against different protein databases (NCBI, Uniprot, Swiss-Prot, TrEMBL etc). In the absence of genome database *de novo* sequencing can be done. BLAST will aid in searching public databases and multiple sequence alignment using ClustalW which may help in predicting the similarity score. This will ultimately lead to identification of the peptide sequence of the allergen.

**V) Biochemical Characterization of Allergen**

a) **Basophil Activation Test** :

The allergenic activity of purified protein is determined by Basophil activation test. IgE cross-linking directs the phosphorylation of p38MAPK leading to basophil activation. CD63, Cd203c and CD300a expression are detected in activated basophil through flow cytometry. Higher the activation of basophils, higher is the sensitivity of the patient to that particular allergen.

b) **Stripped Basophil Histamine Release Assay** :

Basophils, the effector cells in IgE-mediated allergic reactions are the major producer of inflammatory mediators like histamine. Basal and FcεRI-stimulated histamine release is found to be higher in basophils from asthmatics than from nonasthmatics. The basophils are to be stripped off their IgE and histamine release of more than 10% is considered to be positive. Higher the amount of histamine release, higher is the sensitivity of the patient to that particular allergen.

c) **Glycoprotein assay** :

Glycosylation is a major secondary modification of proteins. N-glycosylation occurs in the presence of the tripeptide Asn-X-Ser/Thr in the polypeptide chain, where Asn serves as attachment point and X may be any amino acid, except proline or aspartic acid. The binding of IgE antibody to plant N-glycans containing β-(1, 2)-xylose and α-(1,3)-fucose has been reported (Andersson *et al.,* 2003). Periodic Acid Schiff's (PAS) staining detects carbohydrate presence and neutral sugar analysis can analyze the carbohydrate structure of glycoprotein.15 kDa and 44 to 67 kDa immunoreactive bands of *Rhizopus oryzae* is stated to be glycoprotein by PAS staining (Sircar *et al.,* 2012).

d) **Periodate modification** :

Post-translational modification can create a new epitope responsible for the allergenecity. In order to identify whether the carbohydrate part has allergic potential, periodate modification comes into play. 2D-immunoblot has a major role in it. Periodate modification involves oxidation of the adjacent hydroxyl groups in glycans into aldehydes. Carbohydrate is a major part of IgE binding epitope in 53 kDa allergen of *Rhizopus oryzae* as detected by PAS staining (Sharma *et al.,* 2009).

e) **Preparation of recombinant allergens** :

Recombinant allergens are utilized in allergen characterization and understanding the immune mechanism of IgE mediated diseases. Since recombinant allergen preparation is relatively expensive, so the current focus should be to ensure the availability of high quality allergen sources and allergen extracts (Sircar *et al.,* 2018).

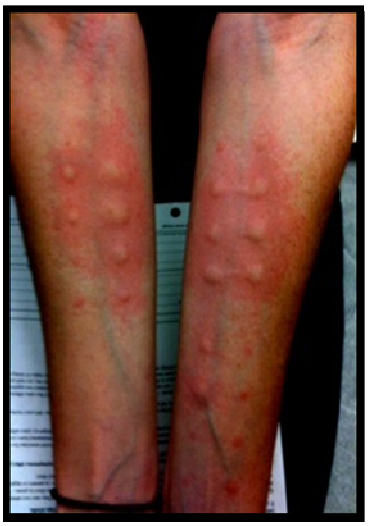
## Conclusion

This review renders a glimpse of possibility in identification of the detected allergen. Unless an allergen can be identified specifically, the future for the cure of allergic patients is at stake. Thus, relying on the guidelines of every possible technique as stated above, one can at least detect the cause of allergy and look into the future for its remediation. This review paves the path for future research in the following areas-

- Structural analysis of the allergen via NMR and X-Ray crystallography.
- Drug designing against a detected allergen.
- Study of Cross-reactivity among different allergic species.
- Implementation of immunotherapy for cure of allergic patients.

A. B.

**Fig.1:** A. Burkard Personal Sampler showing slide with trapped non viable fungal spores B. Andersen 2 stage sampler showing petriplaes with viable fungal colonies .



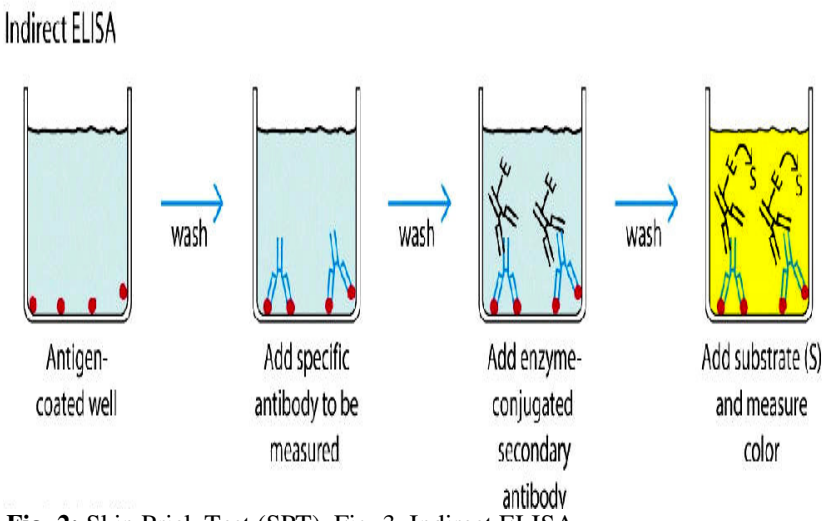
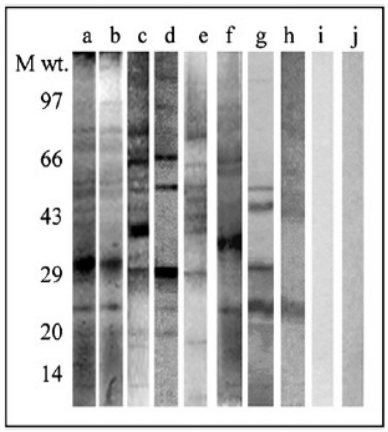



**Fig. 2:** Skin Prick Test (SPT) Fig. 3. Indirect ELISA



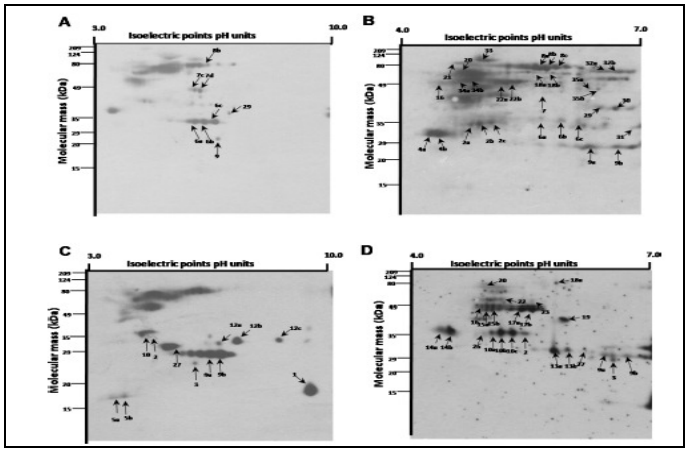




**Fig. 4:** 1D immunoblot Fig. 5: 2D immunonblot

**Acknowledgements**

The authors is thankful to her Supervisor, Prof. Swati Gupta- Bhattacharya of Bose Institute and Principal of Sree Chaitanya College, Habra for continuous support. Sincere thanks are due to Chanchal Chakraborty, Soumyo Subhra Gupta, Mr. Jadab Ghosh, Kaberi Ghosh and Asish Bera of Bose Institute, Kolkata, India, for their technical help.

## References

Adhikari, A.; Sen, M.M.; Gupta-Bhattacharya, S. and Chanda, S. (2000). Incidence of allergenically significant fungal aerosol in a rural bakery of West Bengal, India. Mycopathologia, 149: 35-45.

Andersson, K. and Jonas, L. (2003). Characteristics and immunobiology of grass pollen allergens. International archives of allergy and immunology, 130: 87-107.

Barnett, H.L. and Hunter, B.B. (1999). Illustrated genera of Imperfecti Fungi.4th Edition.St. Pauls, APS.

Barui, N. and Chanda, S. (2000). Aeromycoflora in the central milk dairy of Calcutta, India. Aerobiologia, 16: 367-372.

Burge, H.A. and Rogers, C.A. (2000).Outdoor allergens. Environmental Health Perspectives, 108: 653-659.

Bush, R.K. and Portnoy, J.M. (2001). The role and abatement of fungal allergens in allergic diseases. Journal of Allergy and Clinical Immunology, 107: 430-440.

Chakrabarti, H.S.; Das, S. and Gupta-Bhattacharya, S. (2012). Outdoor airborne fungal spore load in a suburb of Kolkata, India: its variation, meteorological determinants and health impact. International journal of environmental health research, 22: 37-50.

Das, S. and Gupta-Bhattacharya, S. (2008).Enumerating outdoor aeromycota in suburban West Bengal, India with reference to respiratory allergy and meteorological factors. Ann Agric Environ Med, 15: 105-112.

Dey, D. and Gupta- Bhattacharya, S. (2019). Allergenicity assessment of fungal species using immunoclinical and proteomic techniques: a study on *Fusarium lateritium*. Int J Environ Health Res, 1369-1619.

Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Kew.Commonwealth Mycological Institute, London.

Ellis, M.B. and Ellis, J.P. (1998). Microfungi on miscellaneous substrates.An identification handbook. Caroom Helm, London & Timber Press, Portland, Oregon

González-Buitrago, J.M.; Ferreira, L.; Isidoro-García, M.; Sanz, C.; Lorente, F. and Dávila, I. (2007). Proteomic approaches for identifying new allergens and diagnosing allergic diseases. Clinica Chimica Acta, 385: 21-27.

Green, B.J.; Jason, K.S.; and Euan, R.T. (2005). Fungal fragments and undocumented conidia function as new aeroallergen sources. Journal of Allergy and Clinical Immunology, 115, 5: 1043-1048.

Hasnain, S.M. (2004). Presentation of seasonal and diurnal periodicities of airborne pollen and spores. The 6th Asia Pacific Congress of Allergology and Clinical Immunology, 33-34, EX04R9080, Tokyo, Japan.

Laemmli, U.K. (2004). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature1970, 227: 680–685.

Majumdar, M.R. and Bhattacharya, K. (2004). Measurement of indoor fungal contaminants causing allergy among the workers of paper-related industries of West Bengal. India.Indoor Built Environ, 13: 189-122.

Majumdar, M.R. and Barui, N.C. (2007). Aeromycoflora of potato cold store houses in West Bengal (India). Indian J. Aerobiology, 20, 1&2: 56-62.

Mandal, J.; Chakraborty, P.; Roy, I. and Gupta-Bhattacharya, S. (2012). Aerobiological, clinical and immunobiochemical studies on *Lantana camara* pollen and cross-reactivity with other Verbenaceae pollen species. Aerobiologia, 28: 107-119.

Meier, F.C. and Lindenberg, C.A. (1935). Collecting microorganisms from the arctic atmosphere. Science, 40: 5-20.

Nair, S.; Kukreja, N.; Singh, B.P. and Arora, N. (2011). Identification of B cell epitopes of alcohol dehydrogenase allergen of *Curvularia lunata*. PloS one 6, 5: e20020.

Onions, A.H.S.; Allsopp, D.; Eggins, H.O.W. (1981). Smith's Introduction to Industrial Mycology. Edward Arnold.

Peri, S.; Steen, H. and Pandey, A. (2001). GPMAW–a software tool for analyzing proteins and peptides. Trends in biochemical sciences, 26, 11: 687-689.

Sharma, V.; Singh, B.P.; Gaur, S.N.; Pasha, S. and Arora, N. (2009). Bioinformatics and immunologic investigation on B and T cell epitopes of Cur l 3, a major allergen of *Curvularia lunata*. Journal of proteome research, 8, 6: 2650-2655.

Shevchenko, A.; Tomas, H.; Havli, J.; Olsen J.V. and Matthias M. (2006). In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nature protocols 1, 6: 2856-2860.

Simon-Nobbe, B.; Denk, U.; Schneider, P.B.; Radauer, C.; Teige, M. Crameri, R.; Hawranek, T. (2006). NADP-dependent mannitol dehydrogenase, a major allergen of *Cladosporium herbarum*. Journal of Biological Chemistry, 281(24): 16354-16360.

Simon-Nobbe, B.; Denk, U.; Pöll, V.; Rid, R. and Breitenbach, M. (2008). The spectrum of fungal allergy. International archives of allergy and immunology, 145(1): 58-86.

Singh, B.; Sharma, G.L.; Oellerich, M.; Kumar, R.; Singh S.; Bhadoria, D.P.; Katyal, A.; Utz R.; and Asif, A.R. (2010). Novel cytosolic allergens of *Aspergillus fumigatus* identified from germinating conidia. Journal of proteome research, 9: 5530-5541.

Singh, A.B.; Malik, P.; Parkash, D. and Gupta, C.K. (2011). Biological standardization of pollen allergens from India. Asian Pacific Journal of Allergy and Immunology, 10, 2: 103.

Sircar, G.; Chakrabarti, H.S.; Saha, B. and Gupta-Bhattacharya, S. (2012) .Identification of aero-allergens from *Rhizopus oryzae*: An immunoproteomic approach. Journal of proteomics, 77: 455-468.

Sircar, G.; Saha, B.; Mandal, R.S.; Pandey, N.; Saha, S and Gupta-Bhattacharya, S. (2015). Purification, cloning and immune-biochemical characterization of a fungal aspartic protease allergen Rhi o 1 from the airborne mold *Rhizopus oryzae*. Plos one 10, 12: e0144547.

Sircar, G.; Dasgupta, A.; Gupta Bhattacharya, S. and Bhattacharya, K.(2018).Spectrum of allergens and allergen biology in India. International Archives of Allergy and Immunology.

The British Aerobiology Federation. (1995). Pollens and spores – A guide to trapping and counting.1st ed. Kimberley Clark Ltd. Lakefield, Aylesford UK.

Verma, J.; Singh, B.P.; Sridhara, S.; Gaur, S.N. and Arora, N. (2003).Purification and characterization of a cross-reactive 45-kD major allergen of *Fusarium solani*. International archives of allergy and immunology, 130(3): 193-199.