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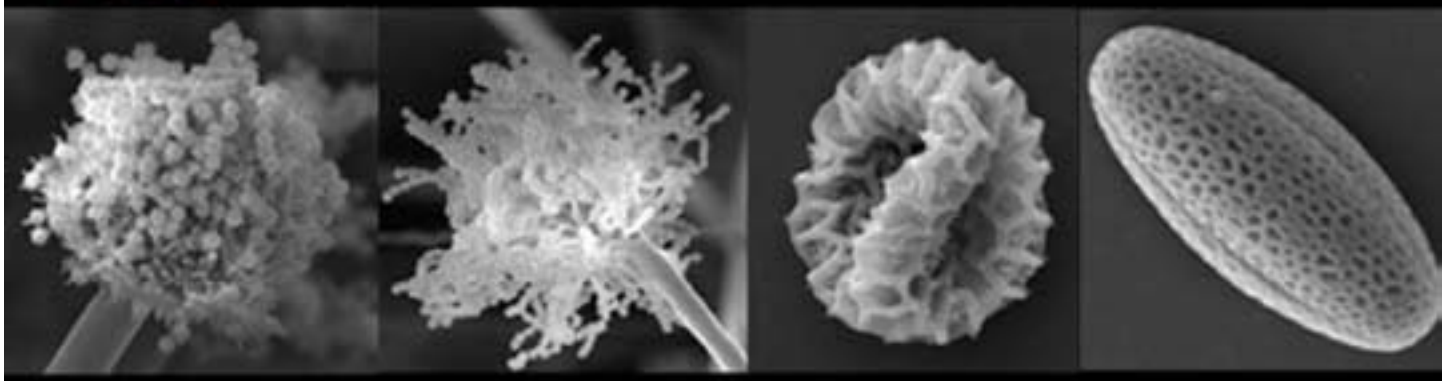
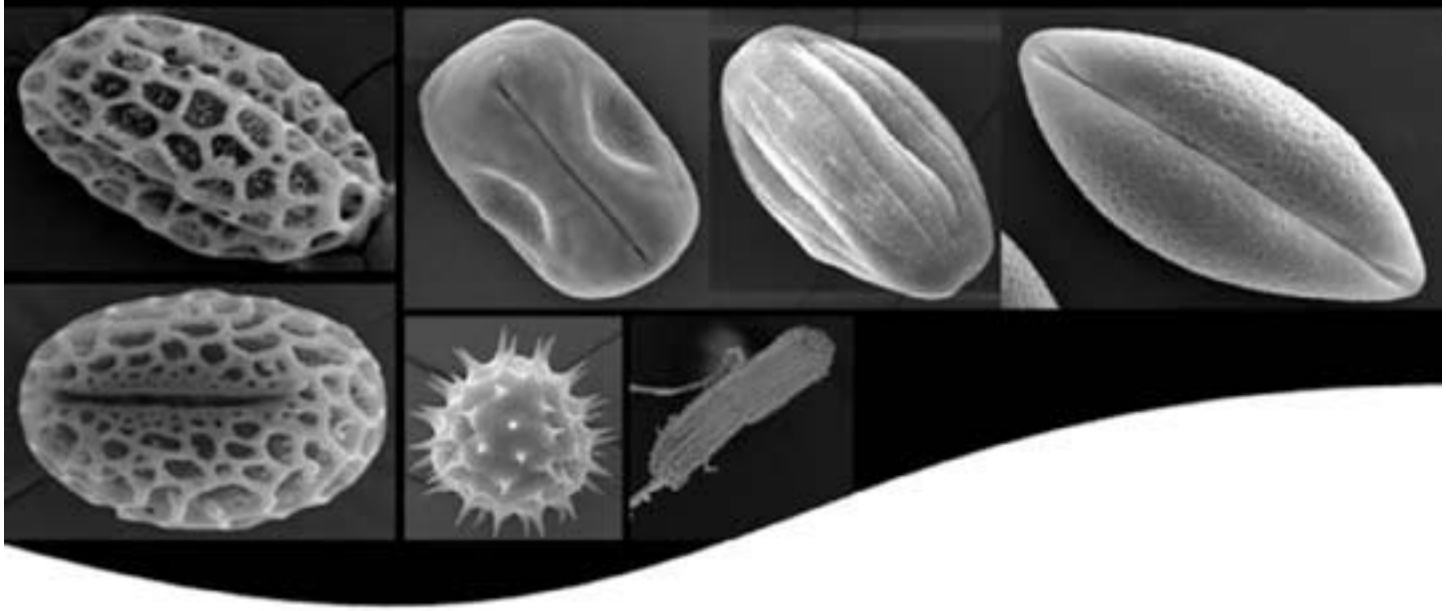


INDIAN JOURNAL OF AEROBIOLOGY

(Prof. Sunirmal Chanda Commemoration Volume)



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Editorial

I am delighted to publish this special commemoration volume of Indian Journal of Aerobiology on our dearest sir, Prof. Sunirmal Chanda who was an internationally acclaimed scientist in the discipline of Palynology and Environmental Biology. Myself and many other Professors of our country and abroad were fortunate to have a guide as well as mentor like Prof. Chanda. We have learned a lot from him especially knowledge-based research, hard working and to keep patience even in most adverse situation. He was the founder president of our society and a fatherly figure to the Indian aerobiologists.

This volume is provided with latest informations about current researches in the field of Aerobiology, applied Immunology and basic Palynology. In this volume you will get review articles as well as original research articles. The volume is also comprised with memorial messages of leading experts in this field and especially with whom Prof. Chanda had a very good relation. We are thankful to all of them (Prof. S.T.Tilak, Prof. A. Saoji, Prof. A.H. Rajasab, Prof. Mahesh Roy and Dr. S.B. Jogdand)

I received immense help from many persons, otherwise it would have not been possible for me to complete this huge task. First of all, I would like to express my appreciation and thanks to Prof. Kashinath Bhattacharya of Visva-Bharati University for his kind help in every aspect in composing this volume. Thanks are also due to my students specially, Pampa Chakraborty, Partha Karak, Gaurab Sircar and Soumyo Subhra Gupta. Special thanks are due to Prof. A.H. Rajasab - President IAS, Prof. Mahesh Roy - Vice President IAS and Prof. J.A. Tidke - Secretary-Treasurer of IAS for their constant support. We are also obliged to mention about the services rendered by the Press.

Above all this volume has got its own merit as it is dedicated to Prof. Sunirmal Chanda, a towering personality who devoted his whole life for the betterment of the subject Palynology and Aerobiology which resulted in the establishment of Aerobiological research in West Bengal and other parts of our country.

We hope that you will find this volume valuable and will consider submitting your own work in the subsequent volumes. The next volume i.e Vol. 31(2018) will be published in November, 2018. We welcome your comments so that we may improve the journal in near future.

Swati Gupta Bhattacharya
Chief Editor
Indian Journal of Aerobiology

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Prof. Sunirmal Chanda (1932- 2015)

BRIEF BIOGRAPHICAL PROFILE OF PROF. SUNIRMAL CHANDA (1932 – 2015)

Name	SUNIRMAL CHANDA
Nationality	Indian
Born	11th January 1932; Mytkiana, Burma(Myanmar)
Died	18th January 2015 (83 years); Kolkata
Father,s name	Late Satyendra Nath Chanda
Mother' name	Late Amiya Rani Chanda
Family	Wife : Late Dipali Chanda Daughter : Sayanti Chanda Son: Sandeepan Chanda
Position held	Former Professor & Chairman Department of Botany Bose Institute, Kolkata- 700009
Academic Qualification	B.Sc.(Hons. in Botany) Calcutta University, 1952 M.Sc. in Botany, Calcutta University, 1954 Dr.rer.nat., University of Gottingen, Germany, 1960
Research work performed in India and abroad at different times	1. With Dr. J. Sen, University of Calcutta, India 2. With Prof. F. Firbas, University of Gottingen, Germany 3. With Prof. G. Erdtman, Palynological Laboratory, Swedish Museum of Natural History, Stockholm, Sweden 4. With Prof. K. Faegri, University of Bergen, Norway 5. Bose Institute, Kolkata, India 6. Royal Botanic Garden, Kew, U.K. 7. British Museum (Natural History), London, U.K. 8. University of Toulouse, France 9. University of Bordeaux, France 10. Rothamsted Experimental Station, Herpenden, Herts, U.K. 11 Wayne State University, Detroit, USA 12. North Carolina Central University, Durham, USA 13. Landesanstalt fur Bienenkunde, University of Hohenheim, Germany 14. Institute of Agricultural Medicine, Lublin, Poland
Recipient of International Fellowship and grants	1. DAAD, German Academic Exchange Service, Germany 2. UNESCO, Paris, France 3. Swedish Natural Science Research Council, Stockholm, Sweden 4. Swedish International Development Agency, Stockholm 5. Norwegian Science Research Council, Oslo, Norway 6. U.S. Atomic Energy Commission, USA 7. French Academy of Sciences, Paris, France 8. Soviet Academy of Sciences, Moscow, USSR 9. The British Council, London, U.K. 10. National Science Foundation, Washington D.C., USA 11. Japanese Science Foundation, Kyoto, Japan 12. Overseas Development Administration, London, U.K.

BRIEF BIOGRAPHICAL PROFILE OF PROF. SUNIRMAL CHANDA (Contd.)

Research Guidance	42 students obtained Ph.D degree under supervision
Publications	336 Research papers, Review articles, Research notes and 8 Edited books
Elected Distinguished Fellows of	1. Indian College of Allergy & Applied Immunology, Delhi 2. The Palaobotanical Society, Lucknow 3. Indian Aerobiological Society, Kolkata 4. Palynological Society of India, Lucknow 5. West Bengal Academy of Science & Technology, Kolkata
Distinctions	1. President, International Association for Aerobiology(1986-90) 2. Founder President, Indian Aerobiological Society(1980-83) 3. President, Indo-German Association, Kolkata (1980-90) 4. President, Indian College of Allergy & Applied Immunology, Delhi (1995-96) 5. President, National Botanical Society, Kolkata(1993-96) 6. Chairman, International Commission on Aerobiology, IUBS (1986-90) 7. Recipient of Prof. Gunnar Erdtman International Award(1983) for excellence in Palynological Resaerch 8. Recipient of First Dr. D.N. Shivpuri Memorial Oration Award instituted by Indian College of Allergy & Applied Immunology, Delhi 9. Member of Executive Council of International Federation of Palynological Society 10. Member of Executive Council of International Society for Palaeolimnolgy 11. Hony Member of COBRA (Centre Oncologiue et Biologique de Recherche Appliqueel), St. Etienne, France 12. Member of Research Advisory Council, Birbal Sahni Institute of Palaeobotany, Lucknow 13. Advisor to the Ministry of Health, Govt. of Kuwait on the matter of Environmental Biopollution 14. Chief Editor, Journal of Palynolgy, Delhi (1987-92); Trans Bose Res Inst, Kolkata (1980 – 1992) 15. Member of Editorial Board of Grana, Aerobiologia, Annals of Agricultural & Environmental Medicine, Science & Culture, World Spore & pollen Flora, Transaction of the Bose Research Institute, Indian Journal of Aerobiology, etc.

MESSAGES

Prof. Sunirmal Chanda – A Friend, Philosopher and Fellow Worker

I am pleased to learn that the members of Indian Aerobiological Society are bringing out a special volume of Indian Journal of Aerobiology to commemorate Prof. Sunirmal Chanda.

The beginning of new year 2015 was not very congenial for Indian Aerobiologists as it brought out a blowing message of the sad demise of our veteran Aerobiologist Prof. Sunirmal Chanda. He breathed his last breath on 18th January 2015 at the age of 83.

Prof. Chanda's early life and education was in Dhaka (Now in Bangla Desh). His research career started when he joined Gottingen University, Germany in 1960. His active scientific pursuit on Palynology started when he joined Swidesh Museum of Natural History at Stockholm (Sweden). Prof. Chanda's contributions were mostly on Palaeopalynology and then he shifted to Palynology followed by Aeropalynology. Many of his earlier publications find a place in Grana, Pollen et Spore, Review of Palaeobotany and Palynology, etc. After his completion of projects abroad, he returned to India to join at Bose Institute, Kolkata and continued the good work till his retirement as Professor & Chairman of Department of Botany. Prof. Chanda widely travelled in Europe and United States. His Contributions were recognized worldwide as is evident from his selection to the post of President of International Aerobiological Association during 1986 to 1990 and Chairman, International Aerobiology Commission (1986-1990).

It would be of great interest to note that I came in close contact with Prof. Chanda in 1970s at New Delhi and our association grew as the years passed on. Myself and Prof. Sunirmal Chanda actively and closely participated to establish Indian Aerobiological Society (1980) and hope you would appreciate with great zeal and enthusiasm our sincere efforts in tracing the steps and stages culminating in the formation of our "Indian Aerobiological Society".

Stages in foundation of Indian Aerobioogical Society

Credit of First workshop involving aerobiologists goes to Dr. D.N. Shivpuri during 27-29 October 1975 at V.P. Chest Institute, Delhi. This was the first step forward to kindle interests of aerobiologists and medical practioners dealing with allergic disorders of human beings. Prof. Chanda, Prof. S.T. Tilak, Dr. M.K. Agarwal, Dr. Sasikumar and Dr. N.I. Singh participated in the programme. The 3 day workshop was focused on Clinical Allergy, Aerobiology, Allergy and Antigens. The orientation of workshop was more on clinical allergy. Another important step forward was during the International Palynological Conference held in Lucknow at Birbal Sahni Institute of Palaeobotany(1977). Dr. P.K.K. Nair, a great palynologist, of repute and President of Indian Palynological Society helped to organize "Active Group of Aerobiologists" which was a forerunner of future Aerobiological Society. The meeting was attended by Prof. S. Nilsson(Sweden), Dr. Ruth Leuschner(Switzerland), Dr. P.K.K. Nair, Dr. A. Khandelwal, Prof. Chanda, Prof. Tilak, Dr. Vishnu Mitre and others. A unanimous decision was taken to form "Aerobiological Society of India" in due course.

This decision was implemented in Bose Institute Kolkata (1980) during an international Workshop on "Modern tends of Aerobiology, with special reference to Plant Pathology and Medicine", was organized jointly sponsored by the Bose Institute, Calcutta, the British Council and the Govt. of Sweden. The Workshop was graced by the presence of almost all aerobiologists researching on aerobiological problems in different parts of India. A team of experts from Great Britain consisted of Dr. John Lacey and Dr. A. Bainbridge (Rothamsted Experimental Station, Harpenden, Herts.), Prof. J. Pepys (Brompton Hospital, London), and Dr. S. Nilsson (Swedish Museum

of Natural History, Stockholm) attended the meeting and delivered specialized lectures and demonstrated the use of instruments needed for aerobiological research. On the concluding day of the Workshop (30th January, 1980) the Indian participants unanimously proposed to form a Society which will act as platform to bring all Indian aerobiologists together. Thus "Indian Aerobiological Society(IAS)" was formed as a scientific body on the same day. Prof. S. Chanda of the Bose Institute, Calcutta and Founder President of IAS was elected President of the International Association for Aerobiology for a term of five years (1985-1990) and presided over the 4th International Aerobiological Conference held in Stockholm, Sweden in 1990. Since its foundation in 1980, the IAS has made, with cumulative effort, a significant progress in the field of aerobiology to bring the Society to the forefront of Indian scientific scenario. The redeeming feature of well over thirty eight years' history consists of a few landmark events:

It would also be of great interest to understand and follow the piece of elderly advice provided for young budding Aerobiologists. Going through the records, we find that in the formative years of the young Chanda's life, there were clear indications that India was going to have a devoted scientist. We have the pleasure to quote a few lines which bear testimony of Prof. Chanda to the acclamations of world's renowned palynologists and aerobiologists. Prof. Gunnar Erdtman, Director, Swedish Natural Science Research Council, Stockholm wrote on 11 October, 1965: "Dr. Sunirmal Chanda working with me under a scheme sponsored by U.S. Atomic Energy Commission has written a paper dealing with pollen morphology of the families Centrolepidaceae, Restionaceae and Flagellariaceae to be published in 'Grana Palynologica'. This paper is of outstanding interest and will possibly be much discussed and referred to in connection with problems in plant taxonomy, pollen morphology and plant geography including the history of the plants in South Africa and Australia".

Prof. Knut Faegri, Director, Botanical Museum and Dean of the faculty of Science, University of Bergen, Norway, with whom Dr. Chanda worked from 1963 - 1965, wrote: "A scientist with a basic training in palynology, especially if that training is as extensive the one that Dr. Chanda has achieved, is therefore capable of participating in a fairly large number of students, scholars and associates, many of these are holding responsible academic positions in India and abroad... I greet him as a friend, as a fellow scientist, and as a scientific administrator and inspirator."

Prof. J.M. Hirst F.R.S., U.K., a pioneer aerobiologist of international fame wrote in a message for Prof. S. Chanda's 60th Birth Anniversary Felicitation volume in 1998: "The history and progress of science are certain to record the vital role that international pioneers such as Professor Chanda have played and which I hope they will long continue passing on to the rising generations of aerobiologists." In the same volume Dr. Stephen Blackmore, Director, The Natural History Museum, London wrote: "I had the opportunity of discussing my work on pollen of tribe Lactuceae with Dr. Chanda. At the end of our discussions Prof. Chanda gave me a short but heartfelt lecture on the origin of the term colporate, explaining that is a compound of colp(us) and orate and not of colpus and porate. At the end of this surprisingly passionate speech he added, 'that's how Erdtman explained it, and that's how it is'. I smile to myself when I consider how many times I have passed on the same message except course that I can say. 'that's how Erdtman explained it to Chanda, and how Chanda explained it to me, and that's how it is". Dr.D.N. Shivpuri, head, Department of Allergy and Applied Immunology, V.P. Chest Institute, Delhi and a very eminent allergologist, wrote on 16th April 1976, in connection with one of Prof. Shivpuri's publications: "This reprint has been exhausted and I have been unable to send it to several earlier requests. But I have such a great regard for you that I take pleasure in sending you one of the 2 personal copies which I had saved for myself." The above anecdotes among many others, testify how Prof. Chanda was regarded by national and international community of scientists. Prof. Sunirmal Chanda followed strong scientific tradition and

ethics in professional life. He leaves behind his daughter (Sayanti) and son (Sayantan), friends and a large circle of students like Prof Kashinath Bhattacharya and Prof. Swati Gupta Bhattacharya. Prof. Sunirmal Chanda, a renowned Palynologist and Aerobiologist of international repute, breathed his last on 18 January, 2015 in his 83rd year. He was active till his last days and even had organized a seminar at Kolkata 5 days before his last breathe. Aerobiologists fell into sorrow due to his sad demise. Prof. Chanda's Parent has named him as "Su nirmal". The exact meaning of Su = "Good" and nirmal = "pure". He has proved and justified this good name "Su nirmal" by his "Thoughts and Deeds" throughout his life for which he will be remembered not only by aerobiologists but by one and all forever.

Prof. S. T. Tilak

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Karve Nagar, Pune-411 052, Maharashtra,
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A Scientist Par Excellence : Dr. Sunirmal Chanda

It is heartening to know that special commemoration volume of Indian Journal of Aerobiology is being published on late Prof. Sunirmal Chanda sir a fatherly figure and an Internationally acclaimed Scientist par Excellence. Really it is unbelievable that Prof. Chanda sir is no more. It was hard to believe in Tumkur conference that a person attending all the conferences so sincerely won't be seen here. Great vacuum created by his absence in Aerobiology family circle won't be filled again.

Prof. Chanda sir was blessed with a towering personality more than 6 ft. in height with strong build up and magnetic smart look. It was delightful experience of watching and feeling special glow of knowledgeable flourish on his face. He was very polite and soft spoken person down to earth, which endears him to all those who came in contact with him.

I recall with gratitude my association of more than five decades with Dr. S. Chanda sir a most sincere and devoted person working till the end of his brightest and most adorable career.

Palynology Special Paper started for post graduation in Botany at the Institute of Science, Nagpur in 1971 under the headship of Dr. Mrs. S.D. Chitaley, who happens to be my Ph.D. guide. In the same year 'Convention in Palynolgy' was held which was a big memorable event as Dr. G.O.W. Kremp of Arizona University, Tuscon, USA received Gunnar Erdtman International Medal for Palynology from Palynological Society of India. Prof. Chanda Sir was closely associated with Dr. Mrs. Chitaley and was invited for the event. First Medal was awarded to Madam Ludmila Kuprianova at Lucknow on 22nd October, 1969 by Padmashri Savitri Sahni . At Nagpur, Madam Kuprianova, Prof. S. Chanda, Prof. P.K.K. Nair, Prof. Kaul and Dr. T.S. Mahabale were present for the Convention. Later Chanda Sir visited Nagpur during All India Palynological Conference in 1987 and then for 13th National Silver Jubliee Aerobiology Conference in 2005. Meanwhile we met in the conferences at Bangalore, Hyderabad, Santiniketan, Raipur, Pune, Davangere, etc. Prof. Chanda sir was examiner for Ph.D. Thesis of my student Dr. Mrs. Rewatkar in Palynology on rice. Instead of asking tedious questions in viva, he himself narrated subject matter so beautifully in a soft manner in a tension free atmosphere that everybody was very happy to be blessed by his precious advice. I would never forget that Chanda sir proposed my name for Fellowship of Aerobiolgy in Bangalore International Conference.

Prof. P.H. Gregory and Prof. Gunner Erdtman (Father of modern Palynology) accorded training to Indian Botanists like Dr. T. Sreeramulu of Andhra University, Dr. P.K.K. Nair from Lucknow, Dr. Bhojraj from Hyderabad and Sunirmal Chanda from Kolkata, Dr. P.K.K. Nair helped to organize 'Active Group of Aerobiologist' which was the fore- runner of future Aerobiological society which included Prof. S Chanda, Prof. S.T.Tilak and Prof. S.N. Agashe . In 1980, Aerobiologist from different parts of India assembled to attend the workshop on "Modern trends in Aerobiology in particular reference to plant pathology and medicine" held at Bose Institute, Calcutta under the Leadership of Prof. Chanda where the Indian Aerobiological Society (IAS) was formed and started functioning from 31st January, 1980. Prof. Chanda was elected founder President, Prof. Tilak as Vice- President and Prof. Agashe as Secretory of the Society. They all are the giants responsible in spreading knowledge and creating interest in aerobiology and due to their sincere efforts, lot of researchers and scientists from different disciplines attracted to aerobiology.

Twenty National Conferences were held at various corners of India regularly, recently 20th conference was held at Amravati University on 29th – 31st January, 2018. The Aerobiology Conferences were great bonanza to young

scientist in presence of all these dignitaries - Prof Chanda, Prof. Tilak, Prof. Agashe, Prof. Vittal, Prof. Subba Reddi , Dr. A.B. Singh. I had a chance of organizing 13th Aerobiology Conference at Nagpur by the blessings of all of them.

Prof. Chanda did his both B.Sc (Hons.) and M.Sc. in Botany from Calcutta university and Dr. rer. Nat. (Environmental Science) from University of Gottingen under the guidance of Prof. F. Firbas in a DAAD fellowship. Later he performed researches with Prof. G. Erdtman of Stockholm, Sweden; with Prof. K. Faegri – Uni of Bergen, Norway; with John Iversen , Geological Survey of Denmark, Copenhagen. He also worked in Royal Botanic Garden, Kew, U.K.; British Museum, London U.K; Wayne State University of Detroit, U.S.A.; North Carolina Central University, Durham, U.S.A. Prof. Chanda transmitted the radiant energy received from various eminent scientists and developed into full brilliance by his lifelong dedicated research especially in Palynology and Aerobiology. He established one of the finest laboratories at the Bose Institute, Calcutta and disseminated his knowledge to a fairly large number of students and scholars, many of whom are holding responsible academic positions in India and abroad. Prof. Sudhendu Mandal and Prof Kashinath Bhattacharya were the past presidents of the Indian Aerobiological Society, Dr. Swati Gupta Bhattacharya continued the hierarchy of Prof. Chanda at Bose institute by her excellent research work and she is now chief editor of Indian Journal of Aerobiology.

Prof. Chanda Supervised Ph.D work of 42 students and published over 350 research papers and edited 8 books. His sustained brilliant research work earned for him many international laurels including Fellowships and Presidentship of several apex national and international bodies. He was honored with distinguished fellowship from Indian College of Allergy and Applied Immunology, Delhi; The Paleobotanical Society, Lucknow; Palynological Society of India, Lucknow, Indian Aerobiological Society, Calcutta; West Bengal Academy of Science and Technology, Calcutta. He was recipient of Dr. D.N. Shivpuri Memorial Oration Award, Prof. Gunnar Erdtman International Gold Medal and Prof A.K. Ghosh Memorial Oration Award.

The scientific contribution of Prof. Chanda were recognized by several learned and professional societies. He was President of International Association of Aerobiology (1986-90), Chairman of International Commission for Aerobiology I.U.B.S. (1986-90), President of Indian College of Allergy and Applied Immunology (1995-96), President of National Botanical Society (1993-96) , President, Indo-German Association, Calcutta (1980-90). He was Executive Council Member of International Federation of Palynological Societies, International Society of Palaeolimnology, Member of International Organizing Committee of INQUA (Birmingham). He was also advisor to the Ministry of Health, Government of Kuwait on the matters of Environmental Biopollution. Apart from this he also received assignments from the British Council, London, U.K., French Academy of Sciences, Paris, France, National Science Foundation, Washington DC, USA, Japanese Science Foundation, Kyoto, Japan and many more. He has received numerous national grants from various Government agencies and attended many Indian and International Scientific conferences all over the world at various times.

His research lab at Bose Institute Calcutta is considered as one of the finest lab of International Repute which produced number of good researchers. A special "Prof. Sunirmal Chanda 60th Birth anniversary felicitation volume" entitled 'Current concepts in Pollen-spore and Biopollution research' edited by N.M. Dutta, Swati Gupta Bhattacharya, S. Mandal and Kashinath Bhattacharya was Published in 1998 by Research Periodicals and Book Publishing house, U.S.A.

Professor Sunirmal Chanda, through his innumerable contributions has brought Palynology in the frontier areas of biological disciplines in India and put a prestigious niche at the national and international level. Indian science had the privilege of seeing him as president, International Aerobiological Congress in Sweden which undoubtedly brought glory to our country.

He started aerobiological work at a time in the country when consciousness about air pollution caused by pollen grains was in its infancy. It was because of him that this area of science could gain strength from one another. His contributions in the field have influenced many people to take the subjects as their research area of interventions.

Prof. Chanda followed strong scientific tradition and ethics in professional life, Chanda sir breathed his last on 18th Jan 2015 in his 83rd year at Kolkata. He was active till his last days and even had organized a seminar at Kolkata 5 days before his last breathe. The news spread like a wildfire throughout scientific community leaving all in agony.

Thought comes in mind, was there any special 'gene' in Prof Chanda, who could manage lot many things at a time at national and international levels, working with people in multiple, managing presidentships of different organizations or must be having the special strength gifted by Almighty and Ma Shakti. Whatever it may be, he was an extraordinary person, he is not only the source of encouragement for the current and emerging scientific community, but will also go on to inspire the generations yet to be born.

I have a great respect for him, his time to time advice and good words for me at the 13th Aerobiological Conference are unforgettable. His last words during the debate of presidential election at Pune 2012 conference 'She is also my daughter' have been carved on my heart forever.

I pay my heartfelt tribute to this great scientist par excellence!

Dr. Mrs Aarti Saoji
Ex Professor and Director,
Institute of Science ,
Nagpur -1, Maharashtra
Mail: aartiasaoji@gmail.com

My memories with Prof. Sunirmal Chanda

I am most grateful for having been given opportunity to participate with a message in connection with Prof. Sunirmal Chanda Commemoration volume of Indian Journal of Aerobiology.

When I hear the name Prof. Sunirmal Chanda, his picture appears in front of my eyes and mind, a picture of a tall attractive person in a standing posture as if he is delivering a lecture. Indeed it is a matter of sorrow and agony that he is no more now, but he left in me a deep impression of a philosophical personality. Although as a research scholar in Mysore University I knew him through his publications, for the first time I got an opportunity to see him during 1980s in Kalyan (of Mumbai) when Prof S T Tilak organized a National conference on Aerobiology there. Prof. Chanda was towering personality, tall good physique and intellect. He used to speak eloquently on all aspects of Aerobiology, Palynology, literature and related subjects. He was seen always surrounded by young and attractive students and scientists from Bengal and various other parts of India. He talked to me with love and affection and encouraged me in all matters of my academic endeavors.

I respect and adore Prof. Sunirmal Chanda for his glittering career and articulated personality. Born in Burma, had school education in Dhaka(now in Bangla Desh), graduated from Calcutta University, Doctorate from University of Gottingen. He worked under the guidance of Prof. Gunner Erdtman in a Fellowship of U.S. Atomic Energy Commission, and he also worked with Prof. Knut Faegri of the University of Bergen, Norway in the field of Quaternary Palynology. It is well known that students of Palynology consider Prof. Gunner Erdtman as father of Palynology. Prof. Sunirmal Chanda by the virtue of his association with Prof. G. Erdtman is matter of great dignity to the Indian Aerobiological Society. He was instrumental in establishing Indian Aerbiological Society with its headquarters in Calcutta. He is also remembered as the First President of Indian Aerobiological Society. I am glad his beloved students, Professors Dr. Kashinath Bhattacharya and Dr. Swati Gupta Bhattacharya are continuing his legacy.

Prof. Chanda's research accomplishments are praiseworthy and invaluable. Besides his research contributions, I liked him for some other reasons. He led very simple life, spent most of time with his students and was very meticulous in remembering even minute details of his interactions with others big or small. I cannot forget the day when I received a postal cover from him, to my surprise inside that cover I found some photographs of his research student with a message from Chanda "Rajasab your good friend is no more and we found some photographs of him along with you. I am sending them to you for your memory". It was a great shock to know that my good friend, a promising research scholar (Mr. Chinmoy Nandi) had embraced an untimely death.

Another occasion with Professor Chanda which I cannot forget was a moment in Visva-Bharati, Santiniketan. We all gathered there for National Aerobiology Conference and on the inaugural evening under the guidance of Prof. Sunirmal Chanda the organizers had arranged Gurudev Rabindranath Tagore's play Dance drama "Shyama" which was enacted in Bengali. A great art all of us witnessed and I was fully engrossed in it. At the end of the drama Prof. Chanda gave brief translation of its summary. The message of the play was "for the sake of one's happiness, he or she has no right to sacrifice the happiness of another". This message penetrated into my

heart and mind and it guarded me throughout my life not to come in the way of other's happiness for the sake of my comfort or happiness. This philosophy has guided me all through my life. But for Tagore's Shyama and the vivid translation by respected Professor Sunirmal Chanda, I would have missed the philosophy of happiness in my life. Thanks to Professor, he lives in me and in all of us.

Prof. A.H Rajasab

President

Indian Aerobiological Society

My Reminiscences about Late Prof Sunirmal Chanda

Though I cannot share the pride of having enjoyed the tutelage from or descend as a research student of Late Prof Sunirmal Chanda (Former Head, Division of Palynology and Environmental Biology, Bose Institute, Kolkata), I can candidly and conscientiously endorse the fact that I was treated by him as endearingly as one of his most deserving students. The intense love and affection, as well as the professional counselling that I most often received from him, are beyond my capacity to capture in words. Truly speaking, the elements of politeness and plainness interweaved in his majestic personality led him to forge a virtual friendship with me in a very short span of few years only, when I happened to meet him in the 17th National Conference of Indian Aerobiological Society (13-15 Dec 2012) at Pune. I was fortunate to have long interactions with him, especially on the talk delivered by Prof. S.N. Agashe. We shared common views on some debatable issues intended to be established by Prof. Agashe in his presentation. We raised some pertinent queries on some of his assumptions, especially regarding common evolutionary descent and inter-continental migration of some angiosperm weeds. Prof. Chanda instantly realized my potentials and appreciated my judgmental tenacity, and constantly interacted on several issues of academic interest during the conference. This laid fertile ground for evolving a sustainable mutual proximity.

I cannot miss recording my experience about the overwhelming influence and acceptability of Prof. Chanda's voice in the IAS fraternity. His commanding and tactful overtures in settling peacefully the hostilely debated issue of voluntary relinquishment of the office of Secretary-Treasurer of IAS by Dr. A.B. Singh mesmerized me, and I was virtually dragged into the auric range of his personality. Further, the apparent depth and dimensions of his remarks as the Chief Guest of the Cultural Programme during 17NCIAS magically enlightened me, and I developed a very high regard for Prof. Chanda.

In the backdrop of the preceding episode, Prof. Chanda's exceptional intellectual genius and creative ambience gradually pulled me closer to him. I sent him the prints of some of his memorable photographs of 17NCIAS with New Years Greetings in January 2013. He reciprocated with a polite and gracious thankfulness, and we gradually entered into an exchange of ideas and experiences through e-mails. He was very impressed with my writings, and editorial proficiency revealed through the International Journal of Mendel. He was kind enough to contribute a review article to the journal [vol.31 (1-2); 41-44, 2014.] which he dedicated to Prof. K.B. Mishra, and sent praiseworthy remarks on the quality and get-up of the publication.

On his invitation, I successively attended 3rd, 4th and 5th National Conference of Asthma and Allergy Research Centre (AARC), Kolkata and also presented my papers. This went to further strengthen the chord of our bilateral relations and prepared the premise for a wider intellectual interface between us.

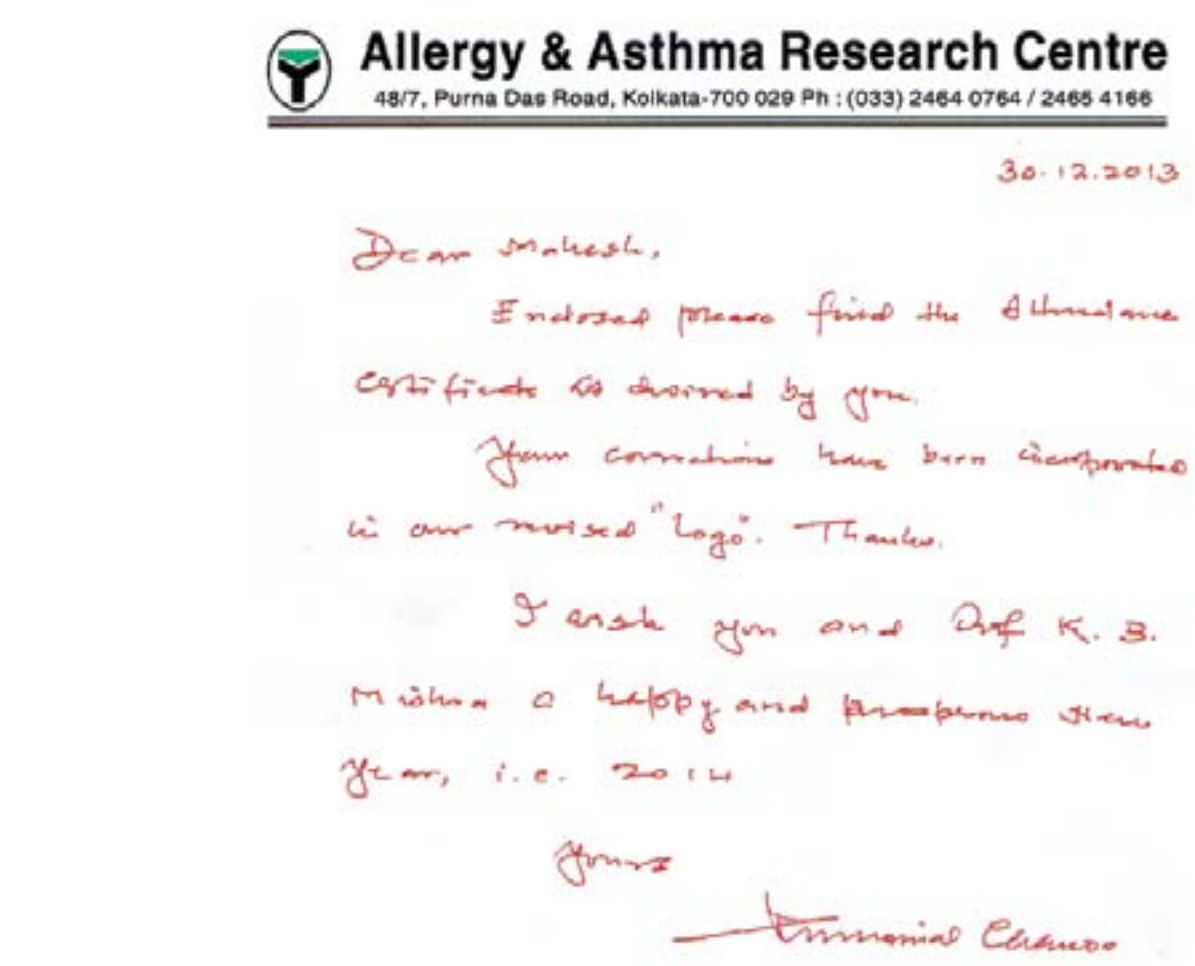
A unique incident manifesting Prof. Chanda's open-mindedness and the boldness of truthful admission of obscure errors committed unknowingly deserves special mention. A cryptic error in the Vedic quote inscribed in the logo of AARC was brought to his kind notice by me after its 4th conference. On my revelation and personal suggestion to get it corrected after consultation with the established orientalists at Kolkata, he got it rectified soon. His alacrity in acknowledging my tangible propensity and brilliance through a letter of commendation was a rare experience for me. Some of the sporadic samples of our written communications or e-mail interactions reproduced on the subsequent pages will add substance to the level of proximity and understanding we could develop so rapidly.

It may be painfully recalled that I had a very brief and inconsistently audible telephonic talk with Prof. Chanda during his hospitalization and just one week before his sad demise. My last e-mail sent to him on January 15, 2015 (06.25 p.m.) unfortunately went unseen and unread by Prof. Chanda as he was critically ill and undergoing treatment in ICU. However, the content of the mail will tell many things which I was apprehensive of. Prof. Kashinath Bhattacharya's mail with the message of the sad demise of Prof. Chanda on Jan. 18, 2015 came like an electric shock to me.

I had profound personal regards for Prof. Chanda not only for his unmatched scholarship and historic research contributions and accomplishments but also for his purity, simplicity, generosity, affection and superb exposition. His lively and illustrious personality has carved an indelible imprint on my mind. The Indian aerobiology fraternity ought to foster his cherished dreams in aerobiological research to pay off the debt it owes to Prof. Chanda.

Mahesh Roy

Aerobiology Laboratory, Department of Botany (P.G.Centre)
R.N. College Hajipur; VAISHALI-844101; BIHAR (INDIA)
[B.R.A. Bihar University, Muzaffarpur]



Allergy & Asthma Research Centre

48/7, Purna Das Road, Kolkata-700 029 Ph : (033) 2464 0764 / 2465 4166

30.12.2013

Dear Mahesh,

Enclosed please find the Attendance Certificate as desired by you.

Your corrections have been incorporated in our revised "Logo". Thanks.

I wish you and Prof K. B. Mishra a happy and prosperous New Year, i.e. 2014

Yours

Sunirmal Chanda

Sunirmal Chanda Feb 2 (2 day ago)

to me

Dear Mahesh,

It was indeed a great pleasure to receive your letter of 28 January 2013 along with a set of photographs which have brought me back a few memorable days when we were attending the 17th NCIAS at MIT Pune. It was so nice and kind of you to have sent the besutiful photographs snapped during the Conference, to cherish my memory. Please accept my warm thanks for your remembering me fondly and affectionately. I really value your friendship, all the more because you happen to have been a distinguished student of my dear friend. Prof. K. B. Mishra.

I hope you do not mind, when I address this letter by your first name, as I feel that I have acquired friendship of a person who bears the name of one of the prominent members of the “Trinity” from our eternally ancient and revered symbol of Indian civilization.

Thank you very much once again for your gift mixed with affection, with a request to convey the same to Prof. K. B. Mishra.

Sincerely

Sunirmal Chanda

mahesh roy < maheshroy54@gmail.com>

Contribution to J. Mandel

2 messages

Sunirmal Chanda <sunirmal2009@gmail.com> Tue, Feb 18, 2014 at 5:14 PM

To: mahesh roy <maheshroy54@gmail.com>

Dear Mahesh,

Thanks for your mail of 14th February 2014. To commemorate the contribution of this great scientist, after whom you have named your journal who made a mark in the early stages of plant science evolution. I shall provide a review article on BIODIVERSITY, highlighting the negative aspect of its ruthless exploitation. There is a systematic effort by man to destroy the treasure of mothe earth, which has become difficult to control owing to uncontrolled population explosion and greed of a section of people.

The article will be dedicated to my dear friend, Prof. K. B. Mishra. When is the next issue of your journal due? I shall try to send the manuscript accordingly.

Affectionately,

Sunirmal Chanda

---

mahesh roy <maheshroy54@gmail.com>

To: Sunirmal Chanda <sunirmal2009@gmail.com>

Dear Sir,

It gave me immense pleasure to find in your mail of Feb 18, 2014 your kind consent to contribute a review article on BIODIVERSITY to my journal. The next vol. of J. Mendel is due in June 2014, but as we are running behind schedule, it may be coming out by September also.

The idea of dedicating your contribution to your endearing friend Prof. K. B. Mishra speaks of your noble gesture, as also your commitment to reciprocate your obligation to scholarship.

Thanking you.

Very Truly Yours,

Mahesh

[Quoted text hidden]

Last E-mail from Prof. S. Chanda
to
Dr. Mahesh Roy, Editor, Int. J. Mendel

J. Mendel, vol. 31 (1-2), 2014

1 message

Sunirmal Chanda <sunirmal2009@gmail.com Fri, Nov 28, 2014 at 5.22 PM

Dear Mahesh,

I am profoundly glad to receive your J. Mendel (current issue) with a number of reprints of my paper on Biodiversity with your letter. Thanks for the same.

I feel, it is an honour for me that my name has been inscribed in your journal as one of the contributors along with other distinguished names under your scholarly editorship.

My paper has come out in a nicely set-rip on art paper. I must pronounce that the journal locks nice and presentable. Please accept my congratulations for bringing out this academically rich series of articles. I shall be grateful if you would kindly make a gift of a complimentary copy of this issue to my friend Prof. K. B. Mishra, to whom my paper was dedicated.

With thanks I have to mention one more thing. The AARC Logo in the last year's invitation card had a few mistakes in the Sanskrit inscription, which you gracefully pointed out. We have taken care that the mistakes were corrected in the current version. You will find it in this year's invitation card (ALLERCON 2014) which is being sent to you separately by Email. Thank you for your scholarly action to turn out our Logo to perfection.

All the best Mahesh.

Affectionately

Sunirmal Chanda

Last E-mail of Dr. Mahesh Roy, Editor
Int. J. Mendel to Prof. S. Chanda

Enquiry about Health

1 message

maheshroy<maheshroy54@gmail.com>

To: sunirmal2009@gmail.com Thu, Jan 15, 2015 at 6.25 PM

Dear Sir,

After a very brief telephonic conversation of few words with me and Prof. K. B. Mishra just after a few days on my return from AARC Conf., I couldn't contact you again through any means. I was when your hospitalisation was reported in the conf. I am quite unware about your latest health position and anxiously awaiting your personal response. I hope you must have recovered, but your advancing age throws a shadow of doubt on my positivism.

Prof. K. B. Mishra is equally anxious about your health. Kindly drop a line in confirmation. More after I hear from you.

Very Truly Yours

Mahesh (Patna)

Nostalgias about Late Prof. Sunirmal Chanda (1932-2015)

Prof. Chanda was born on 11th January, 1932 in Mytkiana of Myanmar where his father, Satyendranath Chanda was a Government employee and he took his early education at his ancestral home - Dhaka (now in Bangladesh). As his family migrated from Dhaka to Calcutta, he did his B.Sc.(Hons) and M.Sc. in Botany from Calcutta University. He obtained his Ph.D. degree as DAAD Fellow from the University of Gottingen, Germany in 1960 under the guidance of Prof. F. Firbas. Later Prof. Chanda joined the Palynological Laboratory, Swedish Museum of Natural History at Stockholm under the guidance of Prof. G. Erdtman in 1965. He also worked with Prof. K. Faegri of the University of Bergen, Norway in the field of Quarternary Palynology. After his successful exposure to the realm of palynology, Prof. Chanda started his career as a Lecturer in the Department of Botany, Bose Institute, Calcutta and became Professor and Chairman in the same Department.

I came in close contact with respected Prof. Sunirmal Chanda since Ist National Conference on Aerobiology held at Aurangabad in 1981, under the leadership of respected Prof. S. T. Tilak. During this conference I heard his lecture on "Aeropalynology". I was very much impressed by his commanding talk which reflected his intensive and extensive study on Palynology & Aeropalynology with deep and vast knowledge of the subject. There after I used to listen him very carefully during all the Aerobiology Conferences and my mind accepted him as one of my beloved "Sadgurus" after respected Prof. Sham Trimbak Tilak as Ist Sadguru". Under the umbrella of these two "Sadgurus" I determined to build my career in Aerobiology and hence I could do so to come to the elevated depth of my knowledge as what I have today. We had several interactions during last three decades about the scientific deliberations, many discussions about the administrative problems of our "Indian Aerbiological Society" and "Indian Journal of Aerobiology" to update the issues from time to time in person or in Executive Council or General Body Meetings and we all tried to solve most of the issues in friendly and healthy ways.

Respected Prof. Sunirmal Chanda was a great Scientist of mountainous height of his caliber, a devoted teacher, a thorough gentleman, with always a helping hand not only to his students but also to one and all who so ever approached him directly or indirectly. He was a worthy Research Guide and a good teacher. Prof. Chanda was highly regarded and recognized by national and international communities of scientists. I express my whole hearted gratitude towards him and expect that His Almighty God may bless His Eternal Soul in Eternal Peace in the Heaven.

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Review Article

SOME CHARACTERISTICS OF AMBIENT AEROALLERGENS, POLLUTANTS AND ASTHMA-RELATED HOSPITAL ADMISSIONS IN KOLKATA, INDIA

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We have previously demonstrated that Asthma-Related Hospital Admissions (ARHA) in the megacity of Kolkata exhibited a very regular pattern. This review summarizes different steps involved in the construction of a regression model of ARHA, and the results of this analysis. We found that ARHA might be associated with specific airborne allergenic pollen load and the concentration of respirable particulate matter in the atmosphere. Future direction of this study has also been outlined with a specific example of advanced statistical approach to gain insight from the data.

Key words: Aeroallergens, pollutants, Asthma-Related Hospital Admission, Correlation, Kolkata

INTRODUCTION

Seasonality in asthma exacerbations has been documented as early as in 400 BC by Hippocrates (Hippocrates. Aphorisms III. 19.22)[1]. Today, asthma accounts for a significant economic burden worldwide, much of which is related to the episodes of exacerbation that often lead to hospitalizations[2]. Several investigators, therefore, studied Asthma-Related Hospital Admissions (ARHA), which is of direct relevance to public health. Seasonal spike in ARHA during September has been reported from countries like Australia and Canada, which is known as the 'September epidemic'[3-5]. Similar seasonal pattern has also been reported from other countries such as UK, Spain and the United States, which has been linked to the levels of ambient aeroallergens and pollutants[6-8]. Airborne allergenic pollen and spores can cause allergenic reactions, irritant responses or oxidative stress leading to asthma exacerbation, with grass/weed pollen often showing the strongest association[9-11]. Furthermore, a recent report on the multicenter European study involving 2637 young adults asthmatics from 15 European countries demonstrated a seasonal variation in asthma symptoms, which was not influenced by the subjects' sensitization to indoor allergens like house dust mite or cat allergen, strongly indicates the role of ambient aeroallergens in precipitating asthma symptoms.[8] Interestingly, in this report, asthmatics sensitized to grass, birch and Alternaria allergens showed distinct seasonal patterns of symptoms compared to unsensitized subjects[8].

In addition to airborne pollen and spores, significant associations between ARHA and levels of air pollutants (particularly NO_2, SO_2, O_3, PM10 and PM2.5) have been reported from several countries[12-15]. However, most of these studies investigating the impacts of air pollutants on asthma exacerbation have ignored the potential confounding effects of aeroallergens.[16] Ko et al studied more than sixty-nine thousand asthma-related hospital admission cases in the city of Hong Kong and identified O_3, among other gaseous pollutants, as a significant predictor in a multi-pollutant model[17]. Other studies have also pointed out the

roles of SO_2, O_3 and respirable particulate matters in multiple European and US cities in relation to seasonal ARHA [14, 15, 18].

Seasonal spikes in asthma attacks have also been studied from the Indian subcontinent in the recent years[19, 20]. The first report came out in 2010 describing ARHA in the megacity of Kolkata in relation to ambient biological and inorganic factors[19]. Such studies brought forward regression models with urban ARHA as the outcome variable, while taking pollen, spore and pollutants as dependent variables[19, 20]. The objective of the current article is to review principal steps of these studies performed in India as well the chief characteristics of ARHA in India in relation to biological factors (ambient airborne pollen, spores) and inorganic pollutants (gaseous and particulate matters). In addition to discussing the methodologies and outcomes of these studies, future needs have been mentioned with specific examples.

METHODS – A BRIEF OUTLINE

Hospital admission data: Records related to Hospital Admission due to physician-diagnosed asthma were obtained from two major hospitals of Kolkata, representing a significant portion of the total ARHA in the city.

Aeroallergen data: Airborne pollen grains and fungal spores were recorded continuously using a Burkard volumetric sampler (Burkard Inc., UK) and a pollen-spore calendar was produced[20].

Sensitization data: Local pollen types were screened for their allergenic properties using skin tests among patients with respiratory allergies/ asthma. Extracts for skin testing were commercially obtained, or prepared in-house following previously published procedures[10, 21, 22].

Data structure and presentation: Pollen and spore loads were expressed as numbers/hour/m^3 of ambient air per day. Gaseous pollutants (NO_2 and SO_2), suspended particulate matter (SPM), and respirable particulate matter (RPM) were expressed in micrograms/m^3. ARHA data was presented in 10-day average time-slots. Three consecutive 10-day slots were generated per month, which came out to be 36 slots in each year. The concentration of allergenic pollens, spores (pollen and spore types showing significant skin reaction in patients) and atmospheric pollutants (NO_2, SO_2, SPM, and RPM) were also presented similarly.

Statistical Analysis: The data was modelled using Poisson generalized linear model (GLM) approach, where ARHA was treated as the outcome variable, and concentrations of pollen, fungal spores, and pollutants were treated as predictor variables. The statistical characteristics and the dispersion parameters of each variable were checked, and over-dispersion was adjusted.

RESULTS

Characteristics of Asthma-Related Hospital Admissions

ARHA outcome: The distribution of ARHA was bimodal, with maximum hospitalization cases occurring during the middle of the months March and September, while the numbers remained low during mid-January and in mid-July. In fact, more than 40% of annual ARHA occurred in just two months i.e. March and September.

Predictor variables: Poaceae pollen reached peak concentrations in March and September, while Cyperaceae pollen concentration peaked during the September to mid-October and Cheno-Amaranthaceae pollen peaked in late March.

On the other hand, total fungal spore load of the atmosphere attained peak values in October (humid harvest season), with Aspergilli, *Cladosporium*, *Alternaria*, and Basidiomycetes as dominant spore types. The concentration of *Alternaria* spores was found to be higher thrice in a year: in February, in May, and in the late October to early November period. SPM, RPM, SO_2, and NO_2 tended to have the highest values at different time-points between November and March.

Results of Regression Analysis: When only aeroallergens (pollen, spore) were used as predictor variables

in the regression model *Areca*, Cheno-Amaranthaceae and Cyperaceae showed significant association with ARHA, while none of the spores were found to be significantly associated. When atmospheric pollutants were included in the model only Cheno-Amaranthaceae, Cyperaceae, SO_2 ($p = 0.0475$), and RPM remained significantly associated with ARHA after correcting for overdispersion, (Table 1).

Figure-2 shows a representative diagram demonstrating the seasonal variations of three most significant pollen types (*Areca*, Cheno-Amaranthaceae and Cyperaceae) and ARHA in the year 2005. Pollen counts are expressed in No./m^3/day, while pollutants are in μg/m^3/day. All data have been presented in 10-day averages, which generated three consecutive datapoints per month (shown in numbered prefixes before the name of the month), and 36 data-points per year.

Unmet Needs and Future Directions

Several studies have been performed around the world to model urban ARHA as an outcome of atmospheric aeroallergen and pollutant concentrations. However, only a few such reports are known from India (from Kolkata). Aerobiological studies conducted by different groups in the country have documented seasonal variations of ambient aeroallergen concentrations and constructed pollen and spore calendars of various parts of India at different time points. The Government-sponsored "All India Coordinated Project on Aeroallergens and Human Health" have documented allergenic aerosols at 18 different centers[23,24]. Although there have been considerable research activities on aerobiology in India, more effort is required to effectively apply aerobiology data to public health. The statistical model of ARHA in relation to aeroallergens and pollutant loads has been reported only from Kolkata. Similar studies are required for other cities of the country. In this regard, some most relevant unmet need and future directions are suggested below, which would greatly enhance further growth of aerobiology research in the country:

Table-1: Result of regression analysis with ARHA was treated as the independent variable and pollen, spore and pollutants were treated as dependent variables. Bold indicates significant comparisons ($p < 0.05$). Table adapted from Ghosh et al (2012)[19,20].

Parameter	Chi-Square	Pr > Chi-Sq
Intercept	25.43	< .0001
POLLENS		
Areca	0.72	0.3975
Carica	0.07	0.7973
Cheno-Ama	**9.08**	**0.0026**
Cocos	0.18	0.6673
Cyperaceae	**15.87**	**< .0001**
Phoenix	2.09	0.1485
Poaceae	3.25	0.0715
SPORES		
Alternaria	0.28	0.5954
Aspergilli	0.34	0.5618
Basidiospore	0.02	0.8877
Cladosporium	1.33	0.2485
Total Spore	0.23	0.6346
POLLUTANTS		
SO_2	**3.93**	**0.0475**
NO_2	0.09	0.7583
RPM	**15.57**	**< .0001**
SPM	0.11	0.7443

Increase in public awareness and funding support: Apart from the above-mentioned All-India project, there has been no report from India on continuing nation-wide coordinated pollen-spore monitoring program. While the importance of aeroallergen monitoring is increasingly recognized by the US and in the EU countries leading to increased funding and organized aeroallergen monitoring efforts, such activities in India are still under-appreciated. For example, multiple operational monitoring stations have recently been established throughout United States to generate uninterrupted aero-pollen data. Data quality is maintained by rigorous training and certification procedures. Data generated by these counting stations are analyzed regularly by participating centers.

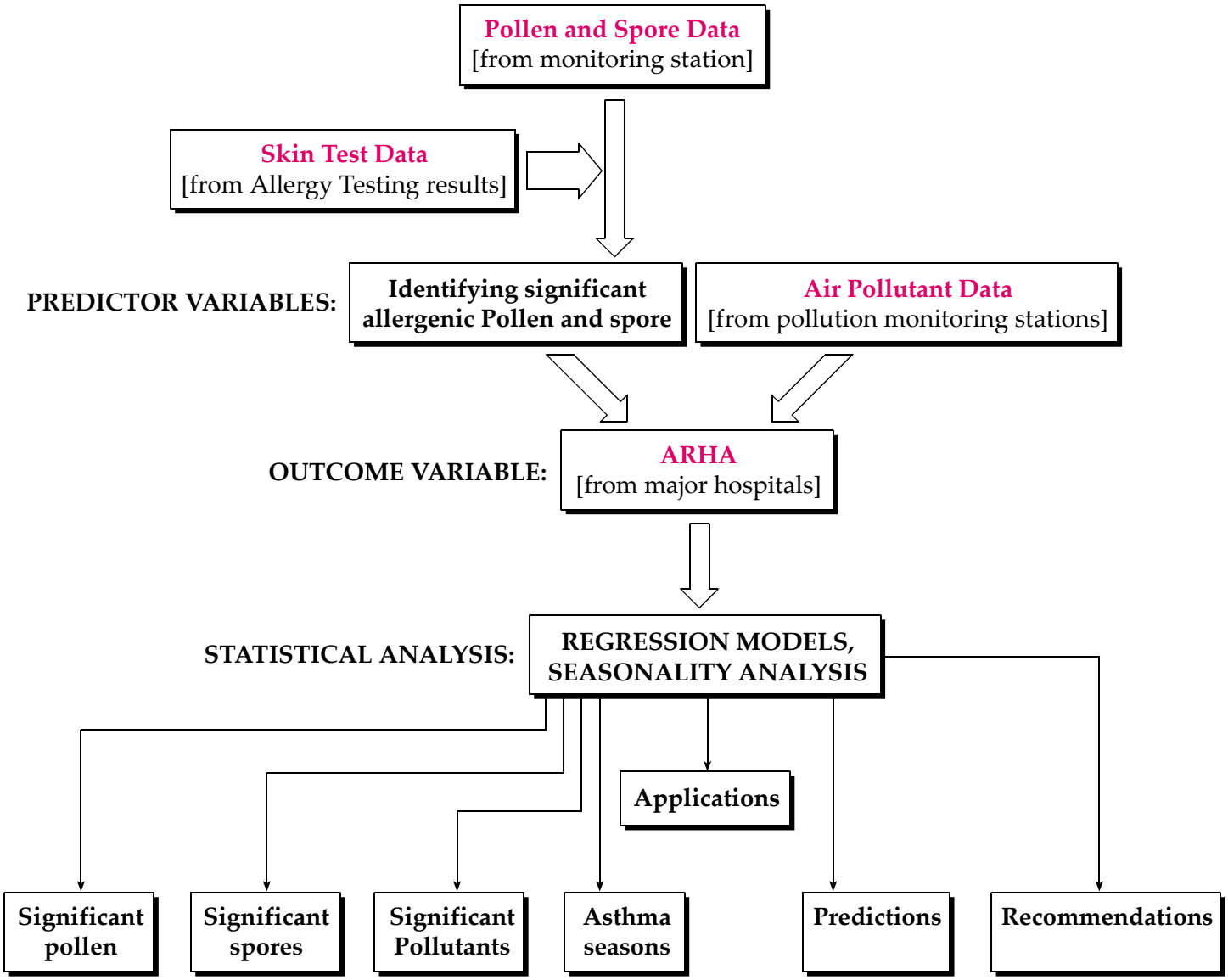

Figure-1: Figure represents a flowchart showing different steps of experimentation, data collection and statistical analyses.

Pollen and spore data were generated by continuous sampling using Burkard 7-day volumetric sampler. Skin test data were obtained from allergy clinics. Most allergenic pollen and spore types were identified. Air pollutant data were obtained from government agencies. Finally, allergenic pollen and spore counts, along with the pollutant data were used as predictor variables, while ARHA data (obtained from apex hospitals) were used as outcome variables to generate hospital admission model. The study can be very helpful for screening out the most significant airborne pollen, spore and pollutants associated with ARHA. Moreover, the model can be used to predict the number of future hospital admission cases, capture seasonality and to make specific recommendations to control hospital admissions due to seasonal allergic asthma.

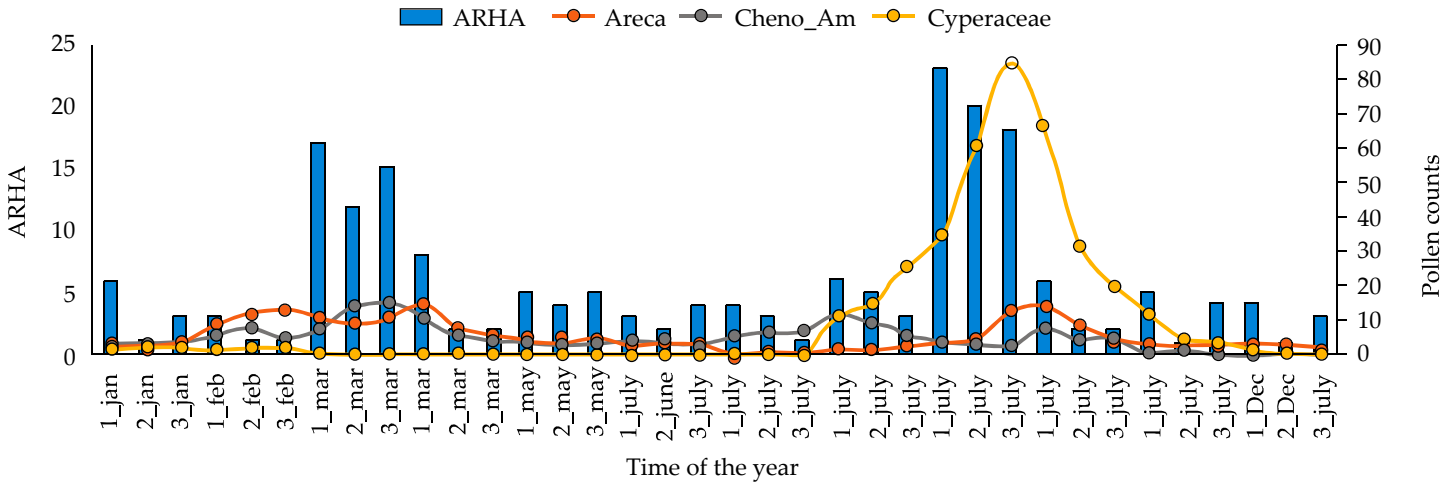

Figure-2: Representative diagram showing the seasonal concentrations of three most significant pollen types (Areca, Cheno-Amaranthaceae and Cyperaceae; in line diagrams) and ARHA (in bar diagram) from Kolkata. Pollen counts are expressed in No./m^3/day, while pollutants are in µg/m^3/day. All data have been presented in 10-day averages, which generated three consecutive data points per month (shown in numbered prefixes before the name of the month), and 36 data-points per year.

Similar efforts have been observed in European countries. More awareness about the importance of aeroallegen monitoring is required. This would be helpful to generate funding support necessary to continue long-term aerobiological studies that are relevant for public health in India.

Increase in collaboration and ease of data exchange and standardization: Aerobiology, being essentially an interdisciplinary field of study, would always benefit from investigators with experiences in various fields such as statistics, public health, medicine, plant biology, biochemistry and Immunology. An increased collaboration between young and experienced investigators is also required. Moreover, exchange of data between different research group should be seamless. While data from genomic, proteomic and transcriptomic experiments are mostly publicly available, data accessibility by researches is still a major bottleneck for aerobiology researchers. Data should be digitized, stored in a standard format and should be made publicly accessible, at least for the data generated using government funds.

Use of advanced statistical tools to gain further insight from data: Previous investigators from India have predominantly used regression studies or ARIMA models to interpret the data or to study seasonality.[19, 20, 25] However, other advanced statistical tools, for example machine learning and pattern recognition programs, could be implemented to gain further insight from data and to predict health outcomes.

Figure-3 represents a specific example of a Regression tree (a pattern recognition approach) constructed using aeroallergen and pollutant data (predictors) and ARHA (outcome) recorded between 2004-2009. In regression modelling, the probability distribution of the outcome variable is modeled in terms of the covariates. However, regression tree, the outcome can be viewed as a pattern recognition problem, to find out the relationship between atmospheric aeroallergen, pollutant exposure and ARHA. In this approach, the root node represents the entire population which gets divided into two or more homogeneous sets, while the leaf represents terminal nodes that do not split. Thus, in figure-2, ARHA in the population has been shown based on the exposure of allergenic pollen, spore and pollutant types. The result of this approach supports our previous Poisson GLM result showing profound roles of Cyperaceae, Cheno-Amaranthaceae followed by other pollen types in the hospital admissions of the area. Ambient fungal spore concentration may not be a significant predictor of over-all ARHA, but may play important roles in case of specific locations

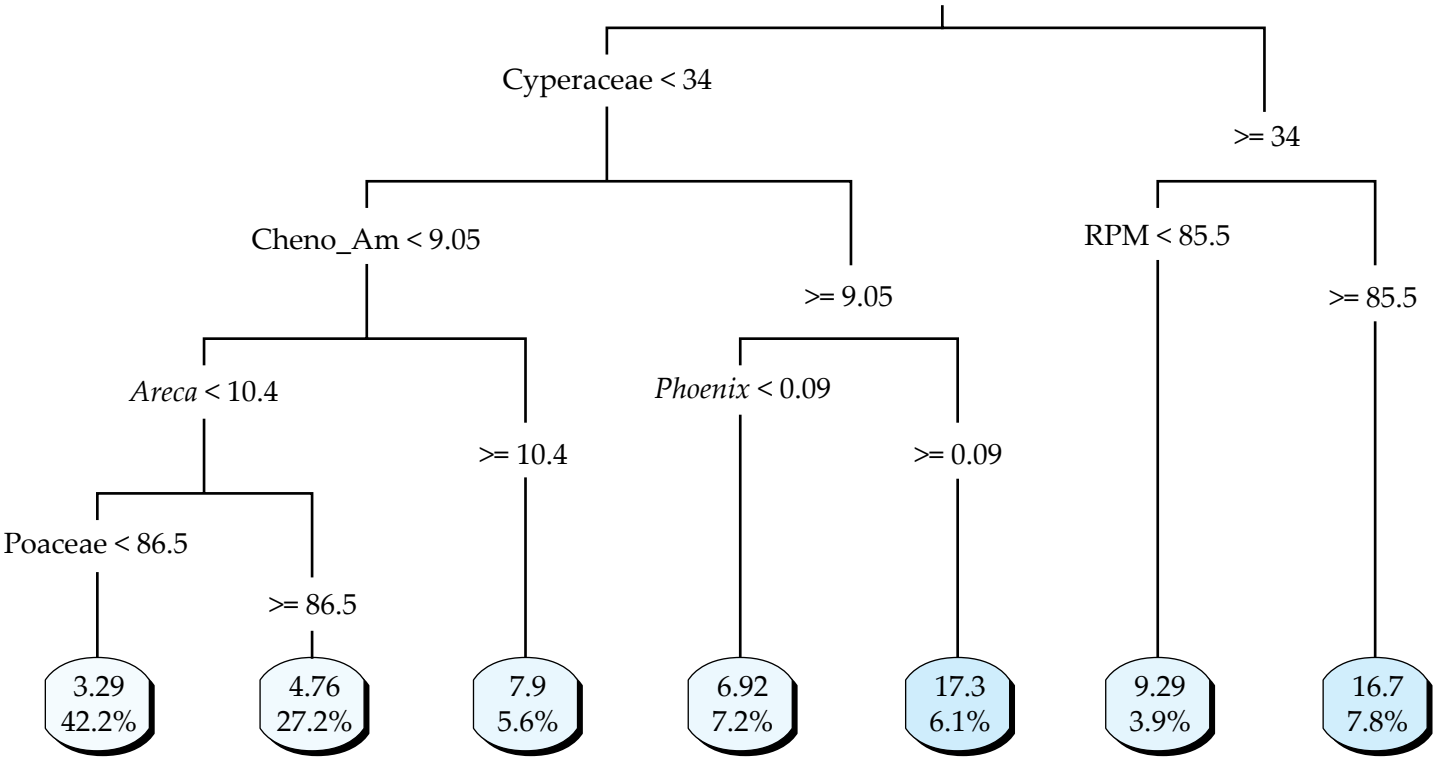

Figure-3: Use of regression tree in interpreting aeroallergen exposure vs. ARHA outcome data: The Regression Tree illustrating the combined effects of Pollen, spore and pollutants for ARHA has been shown. This tree demonstrates the contribution of ambient aeroallergens and pollutants towards ARHA in Kolkata. Pollen and spore loads were expressed as numbers/hour/m^3, while pollutants were expressed in micrograms/m^3. Each internal node in this tree indicates an aeroallergen or pollutant threshold that splits the population into two more homogeneous subpopulations based on whether their levels for the exposure are higher or lower than the threshold. The percentages indicated on the leaves of the tree can be added to make 100% of the hospital admission cases.

and in occupational environments such as market, cow-shades, agricultural farms etc. The percentage values indicated in the terminal nodes could be added up to make 100%. The raw data (pollen, spore, ARHA) poured in the root node, ultimately ends up into one of the indicated outcome patterns. Thus the tree demonstrates the contribution of different aeroallergens and pollutants towards ARHA in Kolkata.

CONCLUSIONS

Asthma patients suffer from periodic exacerbations of the disease, which often lead to hospital admissions imposing a significant economic burden on the society. Ambient aeroallergens and air pollutants can cause asthma exacerbation. An early aerobiological study in India is represented by Douglas Cunningham's systematic and consolidated work "Microscopic examination of air" published in the year 1873 [26, 27]. Since then, several groups have performed air-sampling in different parts of India. In Kolkata, continuous air sampling has been conducted, while the relevant air pollutant data have been obtained form Govt. sources to generate a model of ARHA outcome. This report may serve as the groundwork for more detailed studies and better asthma management in Kolkata, one of the most densely populated megacities of the world.

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Review Article

FATE OF MICROBES IN THE ENVIRONMENT: AN AEROBIOLOGICAL PERSPECTIVE

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Predominant bioaerosols encountered in the outdoor air, which include pollen, fungal spores and airborne bacteria and which chiefly come from plant, soil, water or animal sources, are subjected to a variety of biocidal factors in the environment, viz., sunlight, dehydration (desiccation), thermal heating, freezing, oxygenation, radiations, nutrient deprivation, pressure, aerosolization and anthropogenic pollutants. Edaphogenic airborne microbes may succumb to other factors such as soil pH, water availability and naturally occurring toxins. Studies have shown that airborne microbes in the outdoors often die from various environmental factors before natural death. This fact is largely neglected in aerobiology, and hence needs to be revisited. The present review highlights on the survival of bioaerosols under the constraints of diverse biocidal environmental factors and the consequential impact on their aerobiologically relevant pathogenicity and allergenicity. Any microbial population subjected to a biocidal factor, tends to decay exponentially over time, and a threshold dose is necessary for a measurable effect. Bacterial death occurs due to dry heat and moist heat exposures, desiccation causing water removal, etc. Oxygen toxicity affects microbes only at RH <70% and susceptibility increases with the degree of desiccation. The survival of many species of airborne pathogens is much lower in the outdoor air than in the indoor air. Exposure of microbes to ionizing radiations produces exponential decay in populations. DNA/RNA appears to be the primary site of lethal radiation damage in an exposed virus or bacteria. Non- chromosomal damage can also contribute to irreversible cell death. UV light in the range of 100-400 nm wavelengths has strong biocidal effects. Rapid death of microbes may occur due to nutrient deprivation and building up of toxic wastes, exposure to freezing temperature, high pressure with/without sudden variations, aerosolization, etc. However, the nature of such death is complex and prevents successful plotting of survival curves. Microbes may also recover from a biocidal influence if the latter is not severe enough to destroy the entire population. Some of the factors that can contribute to recovery include RH, visible light and nutrient availability. Reduced populations of airborne microorganisms under the influence of various biocidal factors naturally have a corresponding influence on their overall pathogenicity and allergenicity, which can be purposefully exploited for achieving the desired humanistic goals of aerobiology.

Key words: Microbes, environment, biocidal factors, survival, aerobiological perspective.

INTRODUCTION

Airborne microorganisms, which chiefly include pollen, fungal spores and bacteria, are subjected to a variety of biocidal factors in the natural environment. These biocidal factors comprise sunlight, radiations, dehydration, thermal heating, freezing, oxygenation and a host of anthropogenic pollutants. A good number of extensive studies have shown that these factors result in several kinds of physical, chemical, physiological, genetical and molecular influences on the airborne microbes. The survival potential, pathogenicity and allergenicity of these microbial components are variously affected or even altered by these biocidal factors. The present communication sincerely attempts to delve into the whole gamut of information on the fate of microbes in the environment during their aerial stay and transport. Aerobiological implications of the observed microbial transformations have also been analyzed.

THE GENESIS OF AIRBORNE DISEASES

The speculated genesis of airborne diseases owes primarily to the interacting triad of evolutionary factors: pathogen evolution, human evolution and the naturally accompanying evolution of habitats and technology. The co-evolution of these components is supposed to have brought about the current state of airborne diseases in the world.

Microorganisms in the environment include primary bioaerosols comprising pollen, fungal spores and environmental bacteria, actinomycetes most often encountered in agricultural settings, danders and dust mites. The source of these airborne microbes may be soil, host, organic matter, plants, animals, humans and insects as well. The biogenic components tend to die off in the air under the influence of some biocidal factors of the environment (Open Air Factor-OAF).

GENERAL SCENARIO OF AIRBORNE PATHOGENS AND ALLERGENS

Most of the airborne microbes are pathogenic. Adenovirus, Coxsackie virus, Norwalk virus and Vaccinia virus are never isolated from outdoor air. Environmental bacteria often come from animal or agricultural sources or sewage. Primary outdoor allergens comprising pollen, fern spores and fungal spores/hyphae are more common in the environment while algae and arthropods have small contributions to allergy[1]. The composition of outdoor pathogens and allergens varies geographically, but there are several common species of frequent occurrence. The largest recent study on fungal species was conducted over 12,000 air samples and 1717 buildings in the USA (1996-1998)[2]. Their concentration was usually found lower in indoor air than in the outdoor air.

Airborne microbes are subjected to a variety of biocidal factors of the environment during the course of their aerial stay and transport or migration. These include sunlight, dehydration (desiccation), thermal exposure, freezing, oxygenation, radiations, nutrient deprivation, atmospheric pressure, aerosolization and various pollutants. These factors of the environmental complex are known to influence the bioaerosols in various ways and intensities, sometimes limiting their survival in the atmosphere and occasionally resulting in their recuperative resurgence.

Table 1 : Typical Bioaerosol Levels in Outdoor Air

Bioaerosol	Concentration	Location	Season	Reference
Bacteria cfu/m[3]	327	Agricultural Area	--	Mullins, 2001[3]
	81	Residential Area	--	Mullins, 2001[3]
	146	Business Area	--	Mullins, 2001[3]
	880 - 590,000	Oregon	Summer	Mullins, 2001[3]
	220 - 400	Hong Kong	--	Lee et al.,2002[4]
Fungal Spores cfu/m[3]	85,000	England	--	Mullins, 2001[3]
	449 - 547	Taiwan	--	Mullins, 2001[3]
	20,000	Belgium	Summer	Nolardet *et al.*,2001[5]
	27 - 41,901	IIIinois	All	Chung *et al.*, 1996[6]
Pollen/m[3]	8.4 - 4,105	IIIinois	All	Chung *et al.*, 1996[6]
	26 - 700	IIIinois	Summer	Nelson *et al.*, 1933[7]
	313 - 2,094	Spain	Spring	Carinanos *et al.*, 2004[8]
	7 - 196	Texas	All	Sterling and Lewis, 1998[9]
	110 - 5,000	Philadephia	All	Spiegelman and Friedman, 1968[10]

Allergenically Important Pollen of India[11]

1. *Alnus nitida*
2. *Amaranthus spinosus*
3. *Argemone mexicana*
4. *Cocos nucifera*
5. *Betula utilis*
6. *Borassus flabellifer*
7. *Carica papaya*
8. *Cedrus deodara*
9. *Cassia fistula*
10. *Parthenium hysterophorus*
11. *Chenopodium album*
12. *Dodonaea viscosa*
13. *Mallotus phillipensis*
14. *Plantago ovata*
15. *Prosopis juliflora*
16. *Ricinus communis*
17. *Holoptelea integrifolia*

COMMON BIOCIDAL FACTORS OF THE ENVIRONMENT AFFECTING SURVIVAL OF BIOAEROSOLS

While airborne, microbes are assumed to be in a resting or inactive phase either because they are in the modified state as spores or because of the extrinsic imposition of desiccation and starvation. Several other biocidal factors of the environment may have potential influence. The survival of the aerial plankton is more threatened in the higher airstream (3000-4000m) which is more inhospitable to them. The changes undergone by these microbes during their aerial stay sooner or late lead to their death. Hence, the persistent airborne phase of microbes is often regarded as a retarded decay process[12].

For some definite spheres of interest, survival may be proximately irrelevant, viz., allergenic particles. For Palynology also, in a restricted sense, viability is unimportant. Even then, different levels of degenerative influences of biocidal factors on airborne microbes have remote relations to all areas of aerobiology.

BIOCIDAL EFFECTS OF SUNLIGHT

A broad spectrum of visible light is ranging in wavelength between 400-759.4 nm. Some UV (100-400nm) and infrared (>759nm) components are also present in the sunlight. Exposure of airborne microbes to sunlight has slow biocidal effect while the UV component has a pronounced biocidal influence. The sunlight slowly breaks down any contaminants, including biological agents. Prolonged exposure eventually destroys spores[13-15].The natural rate of die-off is accelerated on exposure to sunlight. It has been found that survival of airborne microbes is a complex species-dependent function.

Microorganisms exposed to any biocidal factor tend to decay exponentially over time as expressed by the following formula:

$$\text{Survival Fraction } S = \frac{Nt}{No}$$

Where Nt = Number of microbes at some time after exposure to a biocidal factor.

No = Original population

A threshold dose is necessary for a measurable effect, else repair mechanisms start[16].

EFFECT OF DESICCATION

Bacterial death may occur during or after desiccation. This is caused primarily due to water removal resulting in cellular dysfunction. The actual death is reported to occur due to a complex interplay of factors[17]. Dried bacteria die from simultaneous stresses mainly related to the oxidation process. Airborne microbes invariably lose water that is replaced upon host infection or when they reach moist environments. The rate of decay of airborne microbes under varying RH's defies analysis with simple rate constants. The conformational stability of cellular macromolecules is affected by RH. As such, microbes may go through a phase transition in which survival is lower in midrange humidities[18].

THERMAL EXPOSURE AND ITS BIOCIDAL INFLUENCE

The death rate of any microbe under heating depends on the temperature of exposure. Under temperatures

ideal for growth, rate constant is zero while under high temperatures; the rate constant has some positive value. Further, the value under dry heat differs from that under moist heat. Many species of microbes show varying degrees of heat resistance; some can withstand prolonged exposure to high temperatures[19]. Most empirical results come from tests done in liquid solution, where pH is known to affect decay rate. The thermal rate constant is a function of temperature, but it is not a linear function[17].

BIOCIDAL EFFECT OF FREEZING

Low temperature can cause cell death; and if microbes are frozen, rapid death occurs. Death from freezing may be due to more than one factor. These include:

[i] Crystallization of cellular water and induced cellular damage at temperatures ranging between -2°C to - 20°C;

[ii] Cooling rate to the freezing point;

[iii] Population density;

[iv] Nutritional status;

[v] Composition of the chilling medium;

[vi] Growth phase

The extent of cell damage due to freezing can be influenced by composition of the chilling medium, salt concentration, pH, etc. Insufficient data are available to quantify the survival curves or characterize the rate constant at various temperatures.

OXYGENATION AS A BIOCIDAL FACTOR

Oxygen at normal levels is not harmful to most of the airborne microbes, while O_2 in combination with desiccation destroys airborne microorganisms. Oxygen toxicity has been observed only at RH's below 70%. Toxic effect of higher concentration of oxygen is found to be maximum at about 30% RH[21,22]. O_2 susceptibility usually increases with the degree of desiccation[18].

The effect of RH on airborne microbes is complex and involves phase changes at the molecular level on account of its influence on the conformational stability of component macromolecules. The microbes may go through a phase transition in which survival is lower in midrange humid ties[18].

O_3 of the outdoor air may also act as a biocide for airborne microorganisms[19, 20]. The survival of many species of pathogens is much lower in the outdoor air than in the indoor air[21]. In general, oxygenation effects may be discarded in comparison to other factors.

INFLUENCE OF IONIZING RADIATIONS

The ionizing radiations include α-rays, β-rays, γ-rays, X-rays, electron beams and emitted protons and neutrons. The exposure of microbes to ionizing radiations causes exponential decay in population[17, 22]. The factors which are known to influence the susceptibility of microorganisms to radiations include growth phase, temperature, water activity, O_2 concentration and the presence of sensitizing agents.

The primary site of radiation damage is DNA/RNA[16]. Cell inactivation occurs due to double-strand breaks in DNA/RNA. Extra chromosomal damage may also cause irreversible cell death[23], while the formation of oxidizing compounds, e.g., OH radicals, may contribute to DNA double-strand breaks[24]. DNA base composition correlates with the susceptibility or resistance of bacteria to radiation[25].

BIOCIDAL IMPACT OF NON IONIZING RADIATION

Visible light (400-700 nm) is strongly biocidal, and UV light (200-300 nm) also destroys microbes. Exposure to UV radiation produces characteristic exponential decay of microbial populations. The maximum bactericidal effect is seen at 265 nm which corresponds to the peak of UV absorption by bacterial DNA[26]. There is a close coincidence of UV light spectrum with a germicidal peak of microbes. The germicidal effectiveness can vary between species and the broader range wavelengths also make a small contribution to inactivation[27].

The UV spectrum comprises four specific ranges: UVA (315-400nm), UVB (280-315nm), UVC (200-280nm) and UVV (100-200nm). All of them, especially UVC, which is absorbed by proteins, RNA and DNA[28, 29] cause photochemical effects.

Germicidal efficiency peak at 260-265 nm corresponds to the peak of UV absorption by bacterial DNA. The

germicidal effectiveness varies with the species[27]. UV radiation inactivates microbes by cross-linking and breaking bonds between nucleic acids. The formation of intra-strand carbonyl pyrimidine dimmers (Thymine dimers) in DNA can lead to mutations or often cell death[30-32]. Lethal effect of UV radiation is primarily due to structural changes in DNA[33].

IMPACTS OF NUTRIENT DEPRIVATION

Reducing nutrient availability literally starves bacteria. Bacteria cultured in a finite medium reaching a critical density of nutrients first enter the stationary phase and then the death phase. The death phase represents the exponential decay of population of microbes. This results due to the combined effect of nutrient deprivation and build up of toxic wastes. After some major reduction in population, a second stage may be enteredextended reflecting survival of a resistant fraction[34]. However, limited data are available befitting a two-stage curve.

Deprivation of certain nutrients causes death linearly over time at rates of about 6-12% per hour[35].

PRESSURE AS A BIOCIDAL FACTOR

Sudden variations in pressure can rupture microbes, and high pressure also can kill microbes. A pressure of 200 atm. can be lethal for many bacteria[36]. Temperature can influence the hydrostatic inactivation pressure[37]. Extreme osmotic pressure (O.P.) existing across cell walls in solution can cause cells to lyse. The sudden heating of bacteria can induce internal pressure spikes that rupture cell walls or lyse the bacterial cell[38].

Low pressure can also damage cells. Bacterial and fungal spores have extraordinary resistance to low pressures and can survive near-vacuum conditions[39,40].

The insufficient date is available to plot characteristic survival curves for bacteria exposed to high/low pressure or to sudden pressure changes.

AEROSOLIZATION EFFECTS

Aerosolization can reduce microbial populations. Airborne microbes tend to die off in the air due to physical stresses[41]. These stresses may be sudden pressure changes or friction involved in high-velocity airstreams. A secondary effect occurs purely as the consequence of being airborne. Die-off in the outside air involves solar radiation, temperature extremes, desiccation and pollutants[17].

Bacteria are known to survive less well in the air than the viruses. The reason for this differential survival is supposed to be the increased susceptibility of bacteria to dehydration.

The nature of death due to aerosolization is, however, complex and no survival curves are available.

EFFECT OF POLLUTANTS

The chemical pollutants may be directly absorbed by airborne microbes during their aerial stay which leads to increased bio-concentration of a host of deleterious components inside the airborne microbes. The accumulation of a chemical species by exposure of bioaerosols to a host of contaminants is called bioaccumulation. All the chemical pollutants tend to bioconcentrate and bioaccumulate in the exposed bioaerosols unless metabolized. The likelihood of a toxicological response is determined by the chemical doze and the duration of exposure. Mercury (Hg) may be retained by the microorganism. Acute and chromic toxicity may lead to the death of the airborne microorganism.

GRADES OF DEGENERATIVE CHANGES

The various forms or grades of degenerative changes undergone by the airborne microbes under the influence of diverse biocidal factors have been found to include the following:

[a] Loss of infectivity before detection of grosser changes.

[b] Loss of susceptibility to bacteriophage replication.

[c] Mutations may occur affecting the growth pattern of the microbe.

[d] Loss of ability to grow and reproduce (Evidence of death).

[e] Loss of allergenicity is often delayed.

[f] Disintegration and vanishing into thin air.

Some bioaerosol components, e.g., anemophilous pollen, spores of ferns, mosses and many fungi, actinomy-

cetes, spore-forming bacteria and encysted protozoans have been identified as Hardy Microbes, while the common inhalant pathogens as the Tender Microbes.

RECOVERY FROM BIOCIDAL INFLUENCE

Microbes may recover from a biocidal influence due to one or more of the following reasons:

[a] The biocidal factor may not be severe enough to destroy the entire population of microbes,

[b] Only the susceptible portion of the airborne microbes may succumb, while the resistant fraction may continue to survive or even grow.

The factors contributing to the above recovery process may include relative humidity (RH), visible light and nutrient availability. Recovery during UV exposure may be due to photoreactivation, wherein visible light can repair thymine dimers and other DNA/RNA damage caused by UV radiation.

Table 2 : Viruses and Bacteria Existing or Surviving Environmentally

Pathogen	Group	Natural source	Survival outside host
Adenovirus	Virus	Sewage	Within sewage for weeks
Coxsackie virus	Virus	Feces, sewage	Survives in stool for weeks
Norwalk Virus	Virus	Environmental waters	Survives in water
Vaccinia Virus	Virus	Agricultural, cattle	Limited
Acinetobacter	Bacteria	Environmental, soil, sewage	Survives outdoors
Actinomyce sisraelii	Bacteria	Cattle	Survives outdoors
Aeromonas	Bacteria	Environmental, water, soil	Survives outdoors
Alcaligenes	Bacteria	Soil, Water	Survives outdoors
Brucella	Bacteria	Goats, cattle, swine, dogs, sheep, camels, etc.	32-135 days
Barkholderia cepacia	Bacteria	Environmental	— -
Burkholderia mallei	Bacteria	Environmental, horses, mules	30 days in water
Burkholderia pseudomallei	″	Environmental, rodents, soil, water	Years in soil and water
Chlamydophila psittaci	″	Bird, fowl	2 -20 days
Clostridium botulinum	″	Environmental	Indefinitely in soil, water
Clostridium perfringens	″	Environmental, animals, soil	Years
Enterobacter cloacae	″	Environmental, soil, water	7 -21 days in food
Enterococcus faecalis	″	Feces	—
Francisellatul arensis	″	Wild animals, natural waters	31 – 133 days
Klebsiellapneumoniae	“	Environmental, soil	4 hours to several days
Legionella pneumophila	″	Environmental	Months in water
Mycobacterium avium	″	Environmental, water, dust, plants	Survives outdoors
Mycobacterium kansasii	″	Water, cattle, swine	Survives outdoors
Pseudomonas aeruginos	″	Environmental, sewage	Survives outdoors
Serratiam arcescens	″	Environmental	35 days or more
Staphylococcus aureus	″	Sewage	7 – 60 days
Staphylococcus epidermis	″	Sewage	—

Pathogen	Group	Natural source	Survival outside host
			—
Yersinia pestis	"	Rodents, wild animals	Limited
Coxiella burnetii	"	Cattle, sheep, goats	Years
Bacillus anthracis	"	Cattle, sheep, other animals, soil	Years
Micromonospora faeni	"	Agricultural, moldy hay	Survives outdoors
Nocardia asteroids	"	Environmental, soil, sewage	Indefinitely in soil, water
Nocardiabra siliensis	"	Environmental, soil, sewage	Survives outside host
Saccharopolyspora	"	Agriculture	Survives outdoors
Thermoactinomyces	"	Agriculture, bagasse	Survives outdoors
Thermoactinomyces vulgaris	"	Agriculture	Survives outdoor
Thermomonospora viridis	"	Agriculture	Survives outdoors

Source: Austin, 1991[14]; Mitscherlich and Marth, 1984[17]; McDade et al., 1964[42]; Wright et al., 1969[43]; Bitton,1980[44]; Hurst, 1991[45]; Jenkins,1991[46].

The process of recovery of spores is, however, not well understood. This is often recognized as a process associated with germination[47]. Some workers have justifiably stated that recovery of spores from the biocidal influence may be a self-limiting process when they are exposed to UV or ionizing radiation[30].

The death rate of any microbe under thermal exposure depends on the temperature level of exposure. Dry heat and moist heat exposure show differences in their biocidal impact[48]. The most empirical results have come from the tests done in solutions. The pH has been found to affect the rate of decay[17, 49]. The temperature may influence the susceptibility of microorganisms to other factors[50].

LIMITATIONS OF THE STUDIES

Most of the studies on microbial survival have been carried out on microbes held in liquid suspension. The results of such studies may not be precisely applicable to the airborne state of microbes. Some prototypes and replicas of aerial stay of microbes have been attempted, such as dynamic aerosol toroid[51] and micro-thread technique[52]. Some new models are required to be developed to reach some more valid generalizations.

CONCLUSION

The foregoing account on the fate of airborne microbes under the influence of diverse biocidal factors goes to establish that the population of microbes in the outdoor air may be inactivated or even killed irrevocably. Barring some chances of recovery from the biocidal impact, permanent alterations in the physicochemical or molecular components of the cellular fabric of airborne microbes may have a corresponding repercussion, even though slowly, on their pathogenicity or allergenic potential. Obviously, such biocidal influence will have remote aerobiological implications. Extensive studies are further required on the subject to obtain more accurate results.

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Review Article

"OMICS" IN CLINICAL DECISION MAKING IN ASTHMA : A PULMONOLOGY PERSPECTIVE

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"Ömics" is the integrated science of in-depth exploration of the structure-function relationship of various molecules, cells, tissues of biological systems with sophisticated technologies such as genomics, proteomics, transcriptomics, metabolomics, epigenomics, etc. The last decade has witnessed significant advancements in diagnostic and therapeutic research in obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease). Asthma genomics have reported around 1700 human genes of which certain gene (CLC, DNAse 1 L3, etc.) expression signatures in airway inflammatory cells help to predict endotypes of asthma and guide subsequent treatment of this complex disease. In proteomics, several cytokine mediators regulating cell recruitment and causing airway inflammation has been described. The SARP (severe asthma research programme) study described 18 cytokines in bronchoalveolar lavage fluid (BALF) and identified asthma clusters with variable severity and signature proteins, independent of corticosteroid use. Measurement of metabolic products (e.g., carbohydrates, amino acids, organic acids, nucleic acid, lipids, etc.) in exhaled breath, urine, serum, sputum or BALF may have novel applications in airway diseases. In this context, 'Breathomics' or profile of exhaled breathe air can recognise an asthmatic individual with their specific steroid responsiveness.

Thus, 'omics' based tests and technologies have a great potential in asthma endotyping, especially when biomarkers fail to identify a clear endotype. However, understanding longitudinal stabilities of endotypes along with validation of standardized technologies and their clinical application are essential prerequisites for the use of omics in clinical decision making in asthma.

Key words: Omics, endotyping, airway diseases, asthma.

CLINICAL PHENO-ENDOTYPING OF ASTHMA

Guideline-based (Global Initiative for Asthma, GINA) treatment of asthma assumes the disease to be homogenous and suggests the use of a step-wise approach to treatment depending on the response to an earlier step therapy[1-3]. This strategy, however, does not work well in severe asthma, which is heterogenous and requires a clear understanding of the underlying mechanism driving the disease in each patient for formulating treatment strategies. There are several ways to pheno-endotype severe asthma, and one such way is by airway inflammometry i.e. by the nature of the underlying airway inflammation or bronchitis, as this is also indicative of the mediators involved in the patient's disease[4]. Bronchitis is the result of cells infiltrating into the airways in response to a variety of stimuli, and intuitively the best method of assessing bronchitis is by the type of cellular infiltrate. Although a bronchoscopic sampling of the airways may be considered the gold standard, it may not be always feasible in the broncho-constricted severe asthma patient. Sputum quantitative assay is by far the most successful and clinically applicable and safe method for estimating bronchitis[5]. It entails selection of a small quantity of sputum from either a spontaneous or induced sample, treatment with a sputolysin (dithiothreitol) and subsequent filtering to obtain a homogenous suspension of cells. The total cell count and viability are determined in a hemocytometer, while differential counts are obtained from Wright stained cytospins[6].

Sputum quantitative assay can reliably stratify and guide treatment of airway diseases according to the cellular nature of the underlying inflammation (eosinophilic (Th2 high), neutrophilic (Th2 low), combined or paucigranulocytic (Th2 low and Th17 low)) (Figure 1). However, in certain situations, clinical inflammometry may fail to recognise an unambiguous endotype. Examples of such situations are Stage 5 (GINA) disease with good compliance and no ongoing allergy exposure having: 1) normal blood eosinophils and serum Immunoglobulin E (IgE) with raised fraction of exhaled Nitric Oxide (FeNO) and the patient cannot cough up sputum, 2) repeated exacerbations despite having normal blood tests, FeNO and a paucigranulocytic sputum. In both of these situations, the available biomarkers fail to portray the accurate endotype and suggest further therapy. Using an "omics" approach in these clinical situations is likely to provide insights into the underlying pathomechanism of disease and guide further therapy.

ENDOTYPING USING "OMICS."

Transcriptomics/Genomics

Asthma is a complex disease, and as many as 1700 genes have been reported in whole genome expression studies[7]. The challenge for researchers has thus been to identify different signatures from these genes that could predict different endotypes and guide subsequent treatment. Indeed, gene expression studies in bronchial epithelium brushings[8], induced sputum[9-13] or peripheral blood mononuclear cells (PBMC)[7] have identified distinct gene signatures for "Th2-high" and "Th2-low" endotypes and despite differences in methodology, the Th2-high endotype was found to be steroid responsive as one would expect[8]. In fact, whole genome gene expression profile studies with unsupervised hierarchical cluster analysis of induced sputum of stable asthma patients have found three distinct transcriptional asthma phenotypes (TAPs) which are similar to the previously defined sputum inflammatory phenotypes of eosinophilic (TAP1), neutrophilic (TAP2), and paucigranulocytic (TAP3)[9]. Subsequently, a sputum gene expression signature comprising of six genes (CLC, CPA3, DNASE1L3, IL1B, ALPL, and CXCR2) was described to be able to discriminate asthmatics according to their inflammatory endotype and predict ICS response[10]. Interestingly, while comparing responders vs non-responders, this signature outperformed the ability of sputum eosinophil counts to predict corticosteroid response[10]. These results may be anticipated to have great potential for reliably identifying asthma endotypes when other tools fail.

Proteomics

Proteomics in asthma usually refers to the study of mediator cytokines that regulate cell recruitment. There are numerous studies which have attempted to identify cytokines profiles from blood[14], BAL fluids[15-16], sputum[17-18] and bronchial brushings[19]. The goal is to identify patterns capable of predicting response to specific therapeutic strategies. For example, elevated serum periostin levels predict response to anti-IL-1314. The SARP study looked at 18 cytokines in BALF and described 4 clusters of asthma of varying severity based on their protein signatures, independent of corticosteroid use[20]. A protein microarray study investigated whether inflammatory endotype of induced sputum correlated with specific types of proteomic profiles in sputum supernatants. They analysed subgroups of sputum endotypes and concluded that differential protein expressions existed depending on the cellular subtype[21]. Interestingly, only two proteins were increased in the sputum eosinophil stratified analysis while 25 to 28 proteins were increased >2-fold in sputa with > or = 40% neutrophils irrespective of eosinophil counts[21]. Intuitively, an ideal situation would be to be able to measure important cytokines (e.g. 1L-5, IL-4, IL13, IL-17, CXCR2) from sputum supernatants or BAL fluids of severe asthma patients and target treatment at whichever was elevated.

Metabolomics

Metabolomics or the systematic analysis of small molecules, including carbohydrates, amino acids, organic acids, nucleotides, and lipids, have recently received great interest amongst respiratory researchers. It represents an integrated pathophysiologic profile en-

compassing genetic and environmental interactions. Metabolic profiles measured in exhaled breath, urine, plasma, and serum may have novel applications in airway diseases. "Breathomics"[22] or profiles of volatile organic compounds in exhaled breath can be measured by an electronic nose and has demonstrated the ability to discriminate asthmatics from healthy controls[23-25] and also to correlate with sputum eosinophils predicting steroid responsiveness[25].

The "omics" technologies described above have great potential in asthma endotyping especially when the existing biomarkers fail to identify a clear endotype (Figure 1). However, these technologies are not without limitations, and the results of such studies should be interpreted with caution. Most studies have cross-sectional designs and are only hypothesis generating. The longitudinal stability of the described clusters to understand how they behave over time and their response to treatment is yet to be determined. Further, generalisability of the clusters across asthma populations remains unknown as the majority of studies have defined clusters in an only small number of asthma patients and hence lack statistical robustness.

By far, the most important technical issue for using these novel technologies is to standardize the tests and increase awareness amongst healthcare providers. A 30-point checklist of criteria has been developed by McShane et al. which comprehensibly covers issues (specimens, assays, mathematical modelling, clinical trial design, and ethical, legal and regulatory aspects) related to the clinical readiness of omics-based strategies[26]. Recommendations for designing future randomized clinical trials (RCTs) have also been discussed[27].

CONCLUSION

"Omics" based tests have immense potential in management (assessing prognosis and predicting therapies) of severe asthma. Unaddressed issues include 1) understanding the longitudinal stability of endotypes, 2) randomized clinical trials to validate clinical application especially for advocating therapies, and 3) standardization of technologies to ensure uniformity of results. A consensus needs to be penned on how and when to use these tools in asthma. Meanwhile, the position of "omics" technologies in clinical decision making is unknown.

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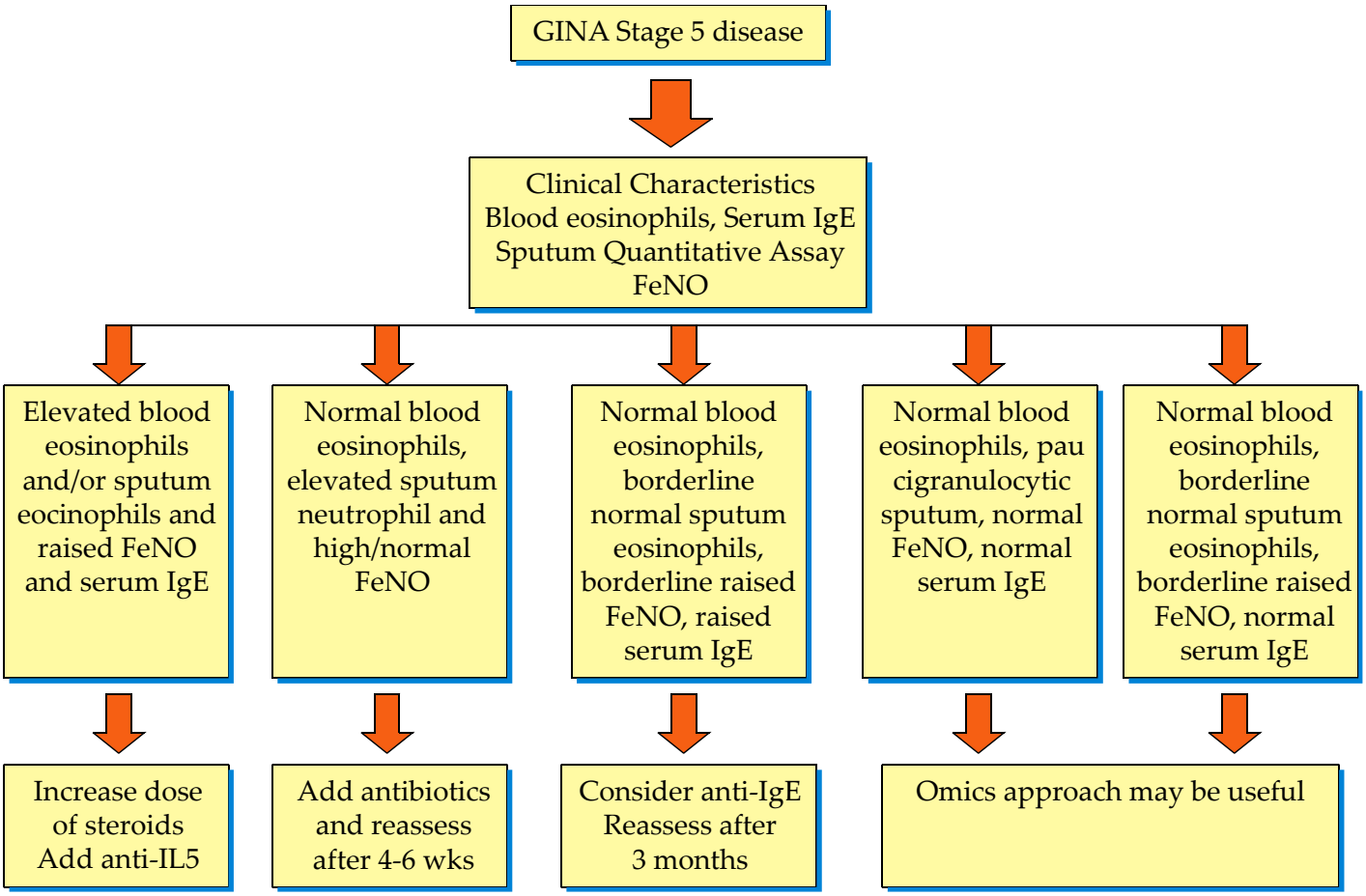

Fig.1 : An endotype based approach to severe asthma with the possible role of omics. (Eosinophilic sputum: Normal total count with >3% eosinophils; Neutrophilic sputum: Raised or normal total count with >67% neutrophils; Raised FeNO: >40ppm; IL: interleukin; IgE: immunoglobulin E)

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Review Article

EPIDEMIOLOGY AND DISEASE FORECASTING SYSTEMS OF CROPS IN INDIA

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In agro-based continental Country like India, Maharashtra is one of the leading agricultural states; concerning sugarcane, jowar, wheat, bajra, groundnut, sunflower, vegetables, grapes, mangoes, cucumbers, watermelons, beetle-vines and several other crops. Tropical environmental parameters like temperature, relative humidity, rainfall, wind direction, wind velocity and topographic geographical localities in Maharashtra harbour plenty of plant pathogens during Monsoon and other seasons. These pathogens after finding a suitable host and its appropriate growth stage attack them to cause different diseases like fungal, bacterial, viral, nematode or mite infestations. The proportion of these diseases depends upon favourability of the components of the "Disease Triangle". Accordingly, the disease may attain sporadic, endemic, severe, epiphytotic or pandemic levels causing damage and varying losses of valuable crop yields. The arid districts of Marathwada are also not an exception to this and suffer such severe crop losses.

Hence scientists gave serious thought to this problem and became successful in formulating useful strategies for disease management. The holistic approach to disease management is the present concept. It includes crop or field sanitation measures, modern cultural practices, fungicidal, bactericidal, viricidal, nematicidal, acaricidal spray programmes, disease avoidance by early or late sowing, ridged sowing or development of disease-resistant varieties by breeding and hybridization. Advance Planning of disease management is essential for proper implementation of any of these methods. Development of crop-wise disease forecasting system, through aerobiological investigations, has been found to be a boon for the planning of holistic approach to disease management.

Key words: Indian crops, diseases, epidemiology, forecasting

INTRODUCTION

Development of plant disease prediction technique is important for: economical use of chemicals for spray, reduction in environmental consequences, plant protection, crop yield and prevention of losses, quantitative disease prediction through models, simulation, the holistic approach of crop protection and increase in yield. A reliable disease forecasting system would provide a meaningful solution for some destructive diseases of major cash crops[1-3]. Such warning system would enable farmers to plan advance preventive measures for possible crop losses[4]. It may also reduce chemical pollution by its minimum use. Recurrent monsoons and typhoons assure dispersal of pathogens over large areas resulting in epiphytotics[5]. The perpetuation of pathogens is enhanced due to mono-cropping, wide cultivation, collateral hosts, weeds and viability of propagules[6].

Diurnal and seasonal weather fluctuations are minimum in the tropics favouring easy and efficient dispersal of the pathogens. These aggressive pathogens may develop diseases on suitable growth stages of susceptible hosts, under congenial environmental parameters, All the considerations pose the urgent need of developing efficient disease forecasting system[7].

PRINCIPLES

Tilak and Jogdand[8,9] enlightened the basic principles of the plant disease forecasting system, and McCartney[10-14] revealed the mechanism of dispersal of pathogens. Plant disease forecasting requires consideration of aggressive pathogen, disease incidence congenial weather parameters, nature of the host-susceptibility or resistance, suitable growth stages of the crop.

ENVIRONMENTAL FACTORS RELEVANCE IN THE PERPETUATION

Survival of pathogen, liberation, spread, deposition, germination, penetration, infection of primary and secondary inoculums and colonization are prerequisite for predicting spread and severity of epiphytotics[14-20]. The extensive and exhaustive accumulation of such a data through critical epidemiological investigations with constant prolonged efforts for many years may achieve an efficient plant disease forecasting system. Such a forecasting system aims at dealing with diseases of major cash crops used successfully on smaller geographical areas. The advance information(forecast) of the possibility of epiphytotics would be most useful for suitably organizing a preventive campaign[5,21-23].

The basis for forecasting methods includes 1) weather conditions during intercrop months affecting the survival of inocula, 2) weather conditions during the crop season, 3) the disease intensity and age of the crop, 4) the spore load of the pathogen in the air, soil and on planting martial.

Empirical Predictive System

Empirical predictive system so far developed should be tested over a wide range of environmental conditions in different geographical regions. It can be modified, if necessary, by taking into consideration all the variations of the weather affecting disease development. The reliability is considerably increased even under a wide range of environmental variations[24-26].

Fundamental Predictive Systems

Fundamental predictive systems are based on the data obtained by studying the effect of different weather factors on host and pathogen, population, singly or together/ combined under laboratory or field conditions[27-30]. The results of such studies are interpreted and applied according to prevailing conditions in the regions under consideration. Both systems require testing and evaluation under natural conditions of crop growth and require modification to suit under different environmental and geographic conditions.

Models and Simulators

Quantitative disease prediction is possible through models and simulators based on the concepts and principles[21-22]. In most of the diseases, the pathogen quickly responds to variations in weather parameters during the growing season of the crop. In such cases, disease forecasting depends on micrometeorological observations. Many models have been developed, taking into considerations, the effect of environmental factors on growth stages of the pathogen and the host.

Geophytopathological Models: Geo-phyto-pathological Model is a three-dimensional analysis involving: Disease prevalence, Severity, Space and time. Joshi et al.[31] have attempted to correlate isochrones (the imaginary line drawn to places where the disease in question has been observed at a particular time is 'isochron') with isotherm and establish a disease, Weather, time and space relationship.

Linear Models

The holistic or linear models treat the epidemic as a single biological entity and therefore one or more rate determining environmental factors. Multiple Regression Analysis (MRA) provides one of the more adaptive types of holistic models[23].

Blite-Cast

It is computerized potato late blight model in Eastern USA. Within a fraction of a second, the computer analyzes the data and send the forecast and spray recommendation to farmers. Farmers keep a "Hygrothermograph" in a shelter among the rows of their potato fields and contact "BLITE-CAST" through the phone[23]. Major diseases on main crops increase utilities of models.

Bioclimatic Models

Empirical prediction models can be developed by monitoring prevalent weather parameters and relating them to disease development. The model assumes that all other relevant conditions are congenial which may not always be true with the fluctuations in the host, time and pathogen. The deviation or error is increased. Dutch rules for *Phytophthora infestans* on

potato were evolved. Temperature-Humidity rule was modified such predictive system for potato late blight is followed all over the world[14,16].

Comments and Priorities in India

Recent advances in the system of disease forecasting analysis have been given new insight into the problems of a subsystem of epidemics, and now simulation models including the one obtained with the help of computers are possible. The system analytics models for prediction in the long term will dominate for many potentially epidemic diseases in high-value crops. Forecasting of disease epidemics is still in its infant stage in India and other SAARC countries[32-39]. In India, priorities have to be decided on the type of diseases that need to be considered for forecasting[40-44]. There is a need to organized and co-ordinate the above-mentioned information. Many destructive diseases like mildews, rusts and blights are essentially controlled by using resistance varieties. Forecasting would be highly useful to avoid indiscriminate use of the chemicals[45-49]. Variation in pathogenic spore concentration in two consecutive Kharif seasons over jowar field in relation to meteorological parameters and disease incidence has been depicted in Table 1.

Table 1: Variation in pathogenic spore concentration in two consecutive Kharif seasons over jowar field in India in relation to meteorological parameters and disease incidence

S. N.	Disease	Causal Organism	Year	Date of the first incidence of spores in the air	Period of the first appearance of disease	Highest conc./ m^3 of air (Date)	Weather conditions on the day of highest concern.			Period of maximum incidence of disease	Growth stage of the crop
							Temp (ºC)	R.H. %	Rain (mm)		
1	Ergot of jowar	*C. sorghi*	1984	25th July	3rd week of Sept.	420 (7th May)	25.2	82	0.3	4th week of Sept.	Flowering stage
			1985	13th July	1st week of Sept.	210 (23rd July)	24.85	97	31.7	2nd week of Sept.	Emergence stage
2	Rust of jowar	*P. Purpurea*	1984	11th Aug	2nd week of Oct.	742 (9th Nov)	23.3	52	-	4th week of Oct.	Dough stage
			1985	11th July	4th week of Oct.	1078 (23rd Oct)	22.8	42	-	2nd week of Oct.	Grain formation
3	Smut of jowar	*Spacelotheca. sp.*	1984	24th July	1st week of Sept.	8932 (10th Nov)	22.75	77	-	3rd week of Sept.	Emergence stage
			1985	7th July	2nd week of Sept.	312 (25th Oct)	21.95	43	-	2nd week of Sept.	Emergence stage
4	Leaf spot of jowar	*Alternaria sp.*	1984	24th July	1st week of Sept.	1876 (22nd Oct)	25.65	47	1.8	2nd week of Sept.	Tillering stage
			1985	7th July	1st week of Sept.	17038 (7th Oct)	22.55	87	-	2nd week of Sept.	Emergence stage
5	Rough leaf spot of jowar	*Ascochyta sorghina*	1984	-	3rd week of Oct.	-	-	-	-	4th week of Oct.	Flowering stage
			1985	16th July	1st week of Oct.	126 (16th July)	23.15	90	0.6	3rd week of Oct.	Emergence stage
6	Leaf spot of jowar	*Curvularia sp.*	1984	24th July	2nd week of Sept.	4186 (22nd Oct)	25.65	47	1.8	4th week of Sept.	Flowering stage
			1985	7th July	1st week of Sept.	6608 (14th Oct)	24.45	64	-	2nd week of Sept.	Emergence stage

S. N.	Disease	Causal Organism	Year	Date of the first incidence of spores in the air	Period of the first appearance of disease	Highest conc./ m^3 of air (Date)	Weather conditions on the day of highest concern.			Period of maximum incidence of disease	Growth stage of the crop
							Temp (°C)	R.H. %	Rain (mm)		
7	Seed mould of jowar	*Curvularia* sp.	1984	24th July	2nd week of Nov.	4186 (22nd Oct)	25.65	47	1.8	3rd week of Nov.	Mature grain
			1985	7th July	1st week of Oct.	6608 (14th Oct)	24.45	64	-	2nd week of Oct.	Mature grain
8	Head blight of jowar	*Fusarium sp.*	1984	7th Aug.	4th week of Sept.	56 (23rd Oct)	26.45	82	0.6	2nd week of Oct.	Grain formation
			1985	7th Aug.	3rd week of Sept.	574 (16th July)	27.35	56	-	1st week of Oct.	Grain formation
9	Seed mould of jowar	*Fusarium sp.*	1984	7th Aug.	3rd week of Oct.	56 (23rd Oct)	26.45	82	0.6	1st week of Nov.	Mature grain stage
			1985	7th Aug.	2nd week of Oct.	574 (16th July)	27.35	56	-	3rd week of Oct.	Mature grain stage
10	Leaf blight of jowar	*Helminthosporium sp.*	1984	24th July	2nd week of Sept.	938 (22nd Oct)	25.65	47	1.8	3rd week of Oct.	Grain formation
			1985	7th July	1st week of Sept.	1484 (14th Oct)	24.45	64	-	2nd week of Oct.	Grain formation

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Original Article

SEASONAL VARIATION OF *ALTERNARIA*, *ASPERGILLUS*, *CURVULARIA* AND *PENICILLIUM* SPECIES IN THE ENVIRONMENT OF NAWAPARA (RAJIM) OF RAIPUR DISTRICT, CHHATTISGARH

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Seasonal variation of airborne fungal spores was studied from July 2008 to June 2009 at Nawapara (Rajim) of Raipur District, Chhattisgarh. Gravity petriplate sampling method was used for overall survey of cultivable fungal spores. The sampling was performed twice a month at fortnightly intervals. Sterilized petriplates containing PDA media were exposed for 5-10 minutes at ground level of each sampling site of study area, which included Bus Terminus, Sadar Road and Ganj Road (market place) area. The exposed petriplates were brought in the laboratory and incubated 26±1°C for 4-5 days and the fungal colonies were counted to assess their percentage contribution and frequency. A total of 953 fungal colonies and 33 fungal species of *Alternaria, Aspergillus, Curvularia* and *Penicillium* species were identified. Maximum percentage contribution was observed for *Aspergillus niger* (24.65%), followed by *Aspergillus flavus* (19.93%), *Aspergillus vercicolar* (7.76%), *Curvularia clavata* (5.56%), *Alternaria citri* (4.40%), *Alternaria alternata* (3.56%) *Aspergillus parasiticus* and *Curvularia sengalansis* (0.73%). *Alternaria* species (0.20%) showed the least contribution of fungal bioaerosols. It was also observed that the *Aspergillus flavus, Aspergillus niger, Curvularia clavata, Alternaria alternata, Aspergillus fumigatus, Aspergillus sydowii, Penicillium chrysogenum, Aspergillus luchuiensis* were among the most frequent members, while *Alternaria crassa, Alternaria radicinia, Aspergillus nidulans, Alternaria* species were less frequent in comparison.

Keywords: Biodiversity; Aerobiology; Bioaerosol; Nawapara (Rajim)

INTRODUCTION

Fungal spores, always present in the environment, vary from season to season, month to month, day to day and place to place. Environmental conditions play an important role in distribution of fungal spores. For instance, seasonal crops and vegetation provide compost material favoring the growth and reproduction of large number of fungi irrespective of human interference[1]. Some pathogenic fungal spores when breathed in, can cause disease like pneumonia, asthma, respiratory infections and some type of skin allergies. It may also affect the crops. Fungi are heterogeneous group of organisms belonging to the group of eukaryotes. They are ubiquitous in indoor and outdoor environments. Fungi are the major part of microbial diversity. The number and type of airborne fungal spores of a locality vary according to the time of the day, weather, geographical location and presence of the local spore sources. The meteorological factors such as temperature, relative humidity and rainfall are most significant to influence the viability of microorganisms. Seasonal variation affects the distribution of fungal spores of particular area. The objective of the study is to record the seasonal periodicity of the cultivable fungal species of *Alternaria, Aspergillus, Curvularia* and *Penicillium* species in the atmosphere of study area.

MATERIALS AND METHODS

Sampling period

The present study was carried out by gravity petriplate (containing PDA medium) exposure during July 2008 to June 2009.

Sampling site

Nawapara (Rajim) is a place of historical importance, 45 kilometers southeast of Raipur on the bank of the river Mahanadi. It is located at 20°58' North latitude and 81°50' in East latitude, at the height of 297.80 meters above the sea level in the middle east of Chhattisgarh state. This area is known as the "Prayag" of Chhattisgarh because it is situated at the meeting point of the Mahanadi, Pairy and Sondur rivers. Cultural and heritage department of Chhattisgarh organize Kumbh fair every year in this place. The climate of Nawapara (Rajim) is characterized by the rainy season (July-October), winter season (November-February) and summer season (March-June).

Monitoring of cultivable fungal spores

Sterilized petriplates containing 2% potato dextrose agar (PDA) media were exposed for 5-10 minutes twice a month in fortnightly intervals in each sampling site of study area. The exposed petriplates were brought in the laboratory and incubated at 26 ± 1°C for 4-6 days. After incubation period, numbers of fungal colonies were counted. The obtained fungal colonies were subjected to grow on PDA medium and after incubation period, morphological and microscopic characteristics were examined to identify the fungal microorganism. Morphological characters included front and back colour, margin, elevation, growth rate and diameter of colonies. The lactophenol cotton blue mounted slides of fungi were observed under compound microscope attached with CCD camera. The microphotography was done by Digi Pro 2.0 software, identified with the help of available literature [2-5] and finally identified by the authentic authority.

RESULTS AND DISCUSSION

During present investigation a total of 33 cultivable fungal species and 953 colonies were recorded. *Aspergillus flavus, A. niger*, and *Curvularia clavata* were found as most frequent fungi, where *Alternaria alternata, Alternaria citri, Aspergillus fumigatus, A. luchuensis, A. versicolar, Aspergillus fumigatus, Penicillium chrysogenum, A. oryzae, A. sydowii, Penicillium notatum, P.rugulosum* and *Panicillium* sp. were moderately frequent fungal members. On the contrary *Penicillium citrinum Alternaria radicinia, Alternaria* sp., *Aspergillus chevalieri* var. *intermedius, Aspergillus japonicus*, and *Curvularia* sp. showed comparatively lower level of frequency (Table 1).

The results obtained in the present study are in agreement with several reports where *Aspergillus, Alternaria, Curvularia* and *Penicillium* were most dominant throughout the study period[6,7]. *Aspergillus* was found to be most predominant in the atmosphere of Raipur[8,9]. *Aspergillus* and *Penicillium* are most frequent fungi from Bikaner[10]. In addition, *Aspergillus niger* was found as most frequent fungi in different places[11-13] in India and abroad[14].

The result also indicates the maximum percentage contribution from *Aspergillus niger* (24.65%), *A. flavus* (19.93%), *A. versicolar* (7.76%) and *Curvularia clavata* (5.56%), while minimum percentage contribution was recorded from *Alternaria* sp. (0.20%). Meteorological factors like temperature, relative humidity and rainfall seemed to play a major role in the concentration of the bioaerosols of a particular area.

During the present investigation, maximum 32 fungal species (437 fungal colonies) were observed in the winter season possibly due to the average temperature and relative humidity (30.9°C and 84.5%), which were favorable for fungal growth. Moderate level of 23 fungal species (291 fungal colonies) were observed in rainy season indicating the episodes of the washing of the fungal propagules from atmosphere by rainwater. A minimum level of 17 fungal species (225 colonies) was observed during summer season reflecting unfavorable average temperature (40.3°C) and relative humidity (50.2%) levels.

Maximum number of fungal sp. (n = 32) were reported in the month of December while minimum number of fungal species (n = 09) were found in the month of May. *Aspergillus flavus* and *A. niger* were present throughout. Certain fungi were present in more than one month but not always like *Alternaria alternata, Aspergillus fumigatus, Curvularia clavata, Penicillium chrysogenum* and *P. notatum*. Some fungi were present only

in particular month like *Alternaria radicinia* and *Aspergillus speluneus* (November), *Aspergillus jopanicus* (December) and *Alternaria* sp. (March) (Figure 1-2).

The results of seasonal periodicity (Figure 3-4) are in agreement with several other previous reports. For example, maximum fungal species during winter and minimum level in summer season was reported from an agriculture farm in Madhyamgram, Kolkata (West Bengal)[15]. On the other hand, *Aspergillus* (19.2%), *Cladosporium* (14.5%) and *Penicillium* (11.43%) were found as predominant fungal genera in the atmosphere of Giza, Egypt[16]. *Aspergillus* and *Alternaria* were dominant contributors in the atmosphere of Athens, Greece[17]. Maximum level of fungal species (n = 35) were also found in December with minimum level in May (n =12) from the atmosphere of Panabaras region, Rajnandgaon[18]. Maximum fungal species level was recorded during winter season, moderate in rainy season and least in summer season in the air of Raipur[19] and Pondicherry region[20] too. The results clearly indicate that environmental factors play an important role in distribution of the fungal bioaerosols (Figure 5).

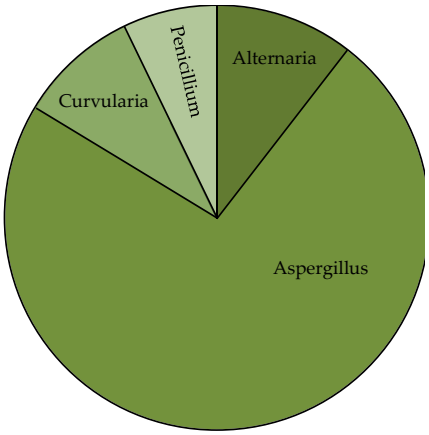

Fig. 1 : Genera wise distribution of total fungal colonies in the atmosphere of Nawapara (Rajim) of Raipur district (C.G.) in rainy season.

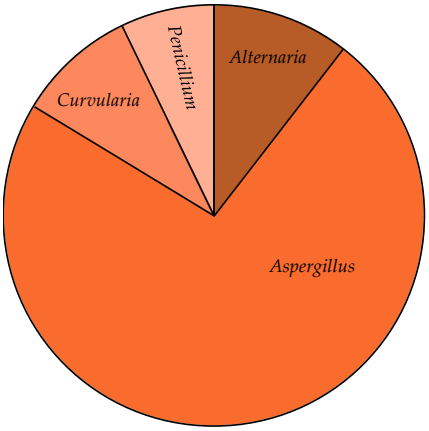

Fig. 2: Genera wise percentage contribution of Nawapara (Rajim) in winter.

Table 1 : Percentage frequency and percentage contribution of fungal bioaerosols of Nawapara (Rajim) of Raipur district (C.G.).

S.N.	Fungal species	Total No. of Fungal colonies	Percentage frequency	Percentage contribution
1	*Alternaria alternata*	34	83.33	3.56
2	*A. brassicola*	07	33.33	0.73
3	*A. cherianthi*	07	25.00	0.73
4	*A. citri*	42	58.33	4.40
5	*A. crassa*	03	16.66	0.31
6	*A. radicinia*	07	16.66	0.73
7	*Alternaria sp.*	02	8.33	0.20
8	*Aspergillus carneus*	12	50.00	1.25
9	*A. awamori*	09	25.00	0.94
10	*A. chevalieri var. intermedius*	12	33.33	1.25
11	*A. flavus*	190	100.00	19.93
12	*A. fumigatus*	24	66.66	2.51
13	*A. japonicas*	13	33.33	1.36
14	*A. luchuensis*	22	66.66	2.30
15	*A. nidulans*	06	16.66	0.62
16	*A. niger*	235	100.00	24.65
17	*A. niveus*	09	25.00	0.94

S.N.	Fungal species	Total No. of Fungal colonies	Percentage frequency	Percentage contribution
18	*A. ochraceus*	14	41.66	1.46
19	*A. oryzae*	11	41.66	1.15
20	*A. parasiticus*	07	41.66	0.73
21	*A. speluneus*	06	25.00	0.62
22	*A. sydowii*	22	66.66	2.30
23	*A. tamarii*	13	41.66	1.36
24	*A. terrieus*	17	50.00	1.78
25	*A. vercicolar*	74	75.00	7.76
26	*Curvularia clavata*	53	91.00	5.56
27	*C. lunata*	28	75.00	2.93
28	*C. senegalansis*	07	25.00	0.73
29	*Penicillium chrysogenum*	20	66.66	2.09
30	*P.citrinum*	14	33.33	1.46
31	*P. notatum*	12	41.66	1.25
32	*P. rugulosum*	13	41.66	1.36
33	*Penicillium* sp.	08	41.66	0.83

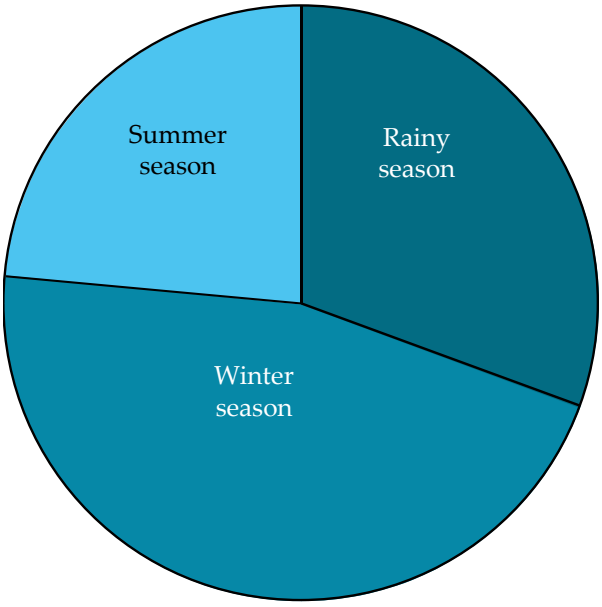

Fig. 3 : Season wise fungal colonies of aeromycoflora of Nawapara (Rajim)

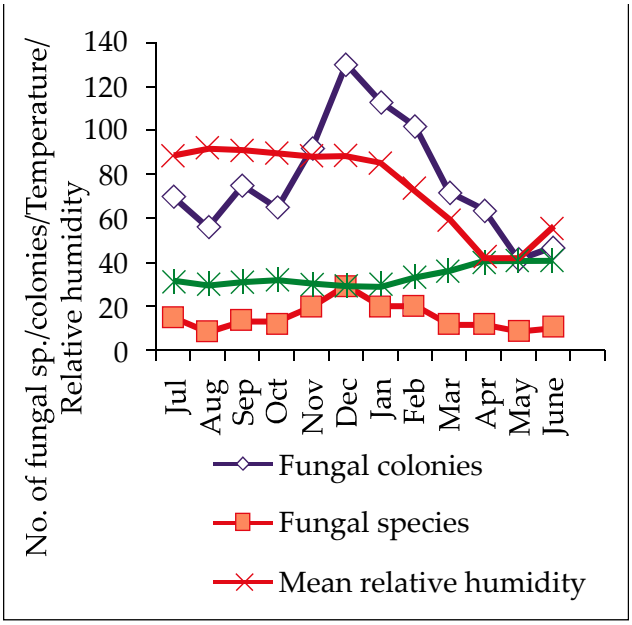

Fig. 5 : Monthly variation of fungal species and colonies in relation to Temperature and Relative humidity of aeromycoflora of Nawapara (Rajim)

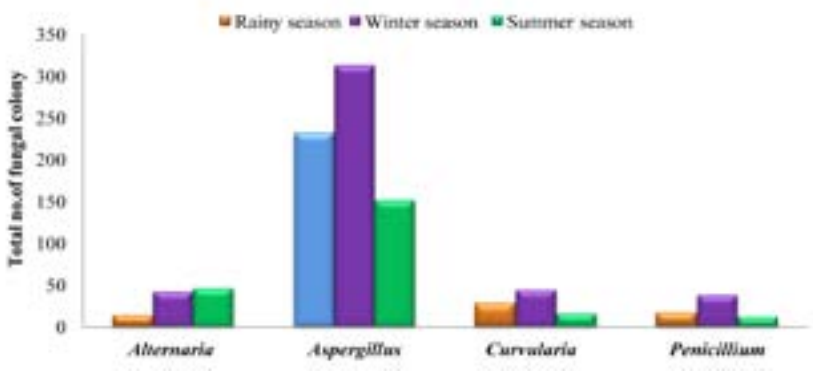

Fig. 4 : Season wise distribution of fungal species of Nawapara (Rajim)

CONCLUSION

Fungal diversity of *Aspergillus, Alternaria, Curvularia* and *Penicillium* species in the environment of Nawapara (Rajim) is of great importance in the clinical aspect, plant pathology, biodeterioration, biotechnology and environmental studies. Some groups of fungi have been studied extensively because of their economical and pathological importance.

Atmospheric monitoring of fungal members provides information about identification, concentration and seasonal variation of the bioaerosols of the study area. The results will be helpful for diagnosis and therapeutic management of fungal diseases both in plants and human beings.

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Original Article

A COMPARATIVE STUDY ON DIFFERENT AIRBORNE ASTERACEAE POLLEN GRAINS AND THEIR CROSS-REACTIVITY

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Allergy caused by air-borne pollen grains is increasing worldwide with India being not an exception. Among several air-borne pollen grains, members of the Asteraceae family are identified as most obnoxious allergy causing species globally. In the present study air-borne nature of different Asteraceae pollen grains were investigated by air-sampling using Burkard Personal sampler throughout the year and at different heights from ground level. The allergenic potential of air-borne Asteraceae pollen grains was studied by skin prick test. *Helianthus annuus* and *Parthenium hysterophorus* were found to be highly allergy causing among atopic individuals. Specific IgE ELISA and western blot were performed against *Helianthus annuus* and *Parthenium hysterophorus* total protein which identified a 32 kDa allergen present in both the species. However, the inhibition ELISA study showed only 30-35% of cross-reactivity among them.

Key words : Airborne Asteraceae pollen, allergenicity, cross-reactivity.

INTRODUCTION

Allergy is the abnormal reaction of the immune system caused by otherwise harmless substances. Pollen grains are the most common source of allergens conferring nearly 30% of allergic burden worldwide[1]. In 1819, Bostock[2] first suspected pollen as the cause of hay fever. Since then several studies have been carried out in different parts of the world which identifies clinically important pollen allergens for diagnosis and immunotherapy of allergy sufferers. According to International Union of Immunological Societies (IUIS)-allergen database, the most allergy-causing plants belong to Poaceae (grass pollen family, 43 species) Cupressaceae (Cypress family, 16 species), Betulaceae (birch family, 12 species), Arecaceae (palms, eight species), Asteraceae (ragweed family, eight species), and Fabaceae (legumes, seven species)[3]. Type I allergy associated with pollen grains of Asteraceae is common in USA and Europe, where ragweed and mugwort cause 14% and 50% of patients are suffering from pollinosis[4]. Extensive work has been done on *Ambrosia artemisiifolia* and *Artemisia vulgaris* from which eleven and six allergens were identified respectively. Although these plants are uncommon in the Indian subcontinent, several plants belonging to Asteraceae family grow all over India throughout the year, whose allergenic significance was poorly studied.

The Asteraceae family comprises of several ornamental plants, oil-yielding plants as well as weeds. Sunflower (*Helianthus annuus*) is an important oil yielding plant which is cultivated in large scale in India during summer. On the other hand, *Helianthus debilis*, garden sunflower, is grown widely throughout the year as an ornamental plant. In IUIS database 4 major allergens were reported from *Helianthus annuus*[5-7] by previous workers. *Parthenium hysterophorus* is another allergy causing weed of Asteraceae family, from which 1 allergen was reported in IUIS database[8]. However, detailed aerobiological work on this important allergy causing family is lacking. As pollen grains of the Asteraceae family members share similar ornamentation pattern, it is very difficult to differentiate between them. Moreover, there is a controversy regarding the presence of sunflower pollen grains in the air due to their large size.

The present study is aimed to characterize the aero-pollen load contributed by Asteraceae family members and contributing pollen grains at different heights. Pollen grains of diverse Asteraceae pollen grains are studied thoroughly which further facilitate to differentiate between them. Along with this aerobiological study, the allergenic significance of common Asteraceae members is studied by Skin prick test (SPT) on atopic individuals. Some of the allergy-causing members are studied in details to identify probable cross-reactivity among them.

Fig. 1 : Map of the study area

MATERIALS AND METHODS

Study area

The present study was conducted at Rajarhat area (22.57°N 88.48°E) of Kolkata (Fig. 1) megacity.

Vegetation study and preparation of reference slides

The flora around the site is typical of that of moist tropical vegetation and the vegetation associated with the Indo-Gangetic plains. An area of 9 km^2 of the sampling station was botanically explored. The vegetation was dominated by different cultivated and wild-type species of the Asteraceae family. Polliniferous materials of these taxa were collected. Acetolysis were performed by adding a mixture of acetic anhydride and concentrated sulphuric acid (9:1). The acetolysed pollen grains were mounted on a slide and checked under a light microscope. Preparation of these reference slides facilitated identification of airborne dispersed pollen types of the Asteraceae family as pollen grains of different members of this family showed the almost similar type of ornamentation and can only be distinguished by their size.

Aerobiological sampling

A continuous air sampling was conducted in Rajarhat sampling station from January to December of 2014 using a Burkard personal volumetric air sampler. The sampler was placed within 7 km of a cultivation land where different sunflower cultivars were grown at different seasons in the year. The sampling was done at 4 different heights (0.5m, 1.0m, 1.5m and 2m) from the ground level and exposed for 20 minutes. The exposed tapes were then mounted and microscopically examined according to the guidelines of The British Aerobiology Federation[9] to identify and record seasonal periodicities of the airborne Asteraceae pollen grains and percentage of Asteraceae pollen grains in the air in respect to total aero-pollen load.This study was conducted at different heights from the ground level to know the abundance of different Asteraceae pollen grains at various heights.

Collection of dominant air-borne pollen grains

Pollen grains were collected from different Asteraceae members during their flowering season (different months from January to December 2014) which were found to contribute to the aero-flora of the study area. 200-micron mesh was used to filter out unwanted debris. The batch used throughout this work contained less than 1% of non pollen contaminants.

Preparation of antigenic extract for skin prick test (SPT)

Pollen grains were defatted in diethyl ether,and total protein was extracted from 1 g of pollen in 5 ml of 0.1M phosphate buffer (PB), pH 7.2. Centrifugation was done at 12,000×g for 20 min to obtain the clear supernatant, which was passed through a 0.22 μm Millipore filter (Millipore Corp.) and used as antigen extract.

Skin prick test and patient selection

Skin prick test with a panel of 7 different species of air-borne Asteraceae pollen grains were performed on the patients visiting the Allergology unit of Institute of Child Health, Kolkata according to the method described elsewhere[10]. During SPT skin rashes were induced by placing allergen extract on the surface of the skin pricked with a sterile lancet and the reaction was measured after 20 minutes. Allergenic potential of the air-borne pollen types was recorded. The patients were selected based on their reaction in SPT and questionnaire study regarding the onset of allergy, family history, symptoms etc. and 5 ml of peripheral blood was collected for further immuno-biochemical work from the patients who had given their consent to use their blood for experimental purpose.

Specific IgE ELISA

The allergy causing species of the Asteraceae family found in the study area were selected, based on patient's reactivity in SPT, for detailed immuno-biochemical study. Indirect ELISA was performed to estimate the level of specific IgE in patient's sera against crude antigenic extracts of selected pollen grains following the standardized method[11]. Briefly, microtiter plates (Nunc, Thermo) were coated with crude antigenic extract (100 ng/µl) in carbonate-bicarbonate buffer (pH 9.2) and incubated overnight at 4°C. After repeated washing and blocking with 1% bovine serum albumin (BSA) (Sigma), wells were incubated with individual patients' sera (in 1:5 dilution) at 37°C for 16 h. Anti-human IgE tagged with alkaline phosphatase (1:1000 dilution) was used as secondary antibody. Colour was developed with para-Nitro Phenyl Phosphate (pNPP). The reaction was stopped by adding 3N NaOH and absorbance was measured at 405 nm. An individual serum is having P/N value [ratio between mean OD405 of patient sera (P) and the healthy control (N)] more than 2.5 were considered as having high specific IgE titre and selected for further studies.

Total protein profiling by SDS-PAGE and detection of IgE reactive proteins by western blot

Total pollen proteins of selected species, extracted in 0.1 M PB was run on 12% SDS-PAGE[11] using Mini-vertical gel electrophoresis apparatus (Amersham). The protein profile was visualized by CBB-R250 staining.

For immunoblot, the resolved total protein was transferred onto polyvinylidene difluoride (PVDF) membrane (GE Life Sciences) by semi-dry transfer method as described elsewhere[7]. Membrane strips were blocked with 3% BSA in Tris buffer saline or TBS (50 mM Tris-Cl, 150 mM NaCl, pH 7.5) containing 0.05% Tween-20 (TBS-T) at 4°C for 3 h. Following washing, the strips were incubated overnight with 1:10 diluted patients' sera at 4°C. Healthy serum pool was taken as negative control. Unbound IgE was removed by washing with TBS-T and strips were incubated with 1:1000 diluted secondary antibody, i.e., monoclonal anti-human IgE alkaline phosphatase conjugate (Sigma) at 4°C for 3h. Blots were developed with nitro-blue tetrazolium-5-bromo-4-chloro-3'-indolyphosphate (NBT-BCIP) (Sigma). Finally, the reaction was stopped by adding 0.5 mM ethylene diamine tetra acetic acid (EDTA).

Study of cross-reactivity by inhibition ELISA

Cross-reactivity between two members of the Asteraceae family (*Helianthus annuus* and *Parthenium hysterophorus*) were studied by inhibition ELISA as they shared some common bands in the immunoblot. For inhibition ELISA, microtitre plate was coated with 0.1 µg of *Helianthus* protein. *Parthenium* protein was used as fluid phase inhibitor. Pooled Sera (1:5 v/v dilution) was incubated overnight with a concentration range of 10 ng, 50 ng, 100 ng, 1000 ng, 10000 ng of *Parthenium, Helianthus* (auto-inhibitor, positive control), and BSA (non-inhibitor, negative control). Plate-bound *Helianthus* protein was then separately probed with these pre-incubated sera. The percentage of IgE ELISA-inhibition was determined by the following formula –

$$\left(1 - \frac{\textit{O.D. of sample with inhibitor}}{\textit{O.D. of sample without inhibitor}} \times 100\right)$$

Identification of probable cross-reactive protein by MALDI TOF

The proteins of same molecular weight from both the species which showed IgE reactivity was supposed to be probable cross-reactive proteins. These proteins

were trypsin digested, extracted from the gel by a series of chemical treatments[12] and subjected to mass spectrometry. Briefly, the gel pieces were destained with ethanol in 50 mM ammonium bicarbonate (pH 8.0) (1:1 v/v) and Acetonitrile (ACN). Reduction and alkylation were done with 10mM DTT and 55mM iodoacetamide respectively. Digestion was carried out in 12.5 ng/µl modified sequencing grade Trypsin Gold (Promega) at 37°C for 16 h. Tryptic fragments were eluted from gel pieces by vigorous vortexing in extraction buffer containing 3% TFA and 30% ACN. The final volume of the sample was reduced up to 10 times in Speed Vac (Thermo Fischer). Approximately, 1.5 µl of peptide digests were mixed with 5 volumes of 0.5 mg/ml α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (Bruker Daltonics), spotted on MTP 384 ground steel target plate (Bruker Daltonics) and air dried. Mass spectra of trypsin-digested proteins were obtained in Autoflex II MALDI TOF/TOF (Bruker Daltonics). Mass spectra were recorded in linear mode equipped with a pulsed N2 laser (λ= 337nm, 50 Hz) at 54% power in positive ion mode. The MS spectra were then analysed by MS Biotools™ 3.2(Bruker Daltonics) using in-house MASCOT search engine version 2.2. Spectra were searched against NCBInr database. *Parthenium* does not have any sequence information, rendering difficulty in the identification of the protein in MS. In this case, further confirmation was done by retrieving spectral information from the database[8].

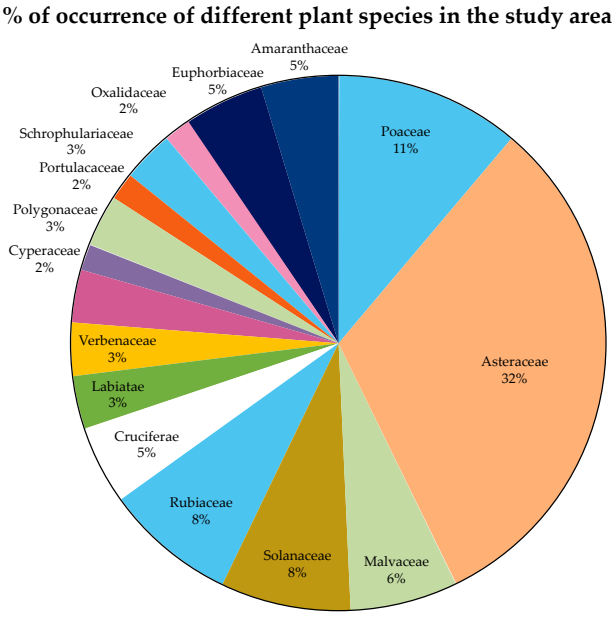


Fig. 2 : Study on local vegetation and estimation of percentage of occurrence of different plant species in the study area

RESULTS

Study on local vegetation and seasonal periodicities of different Asteraceae pollen grains

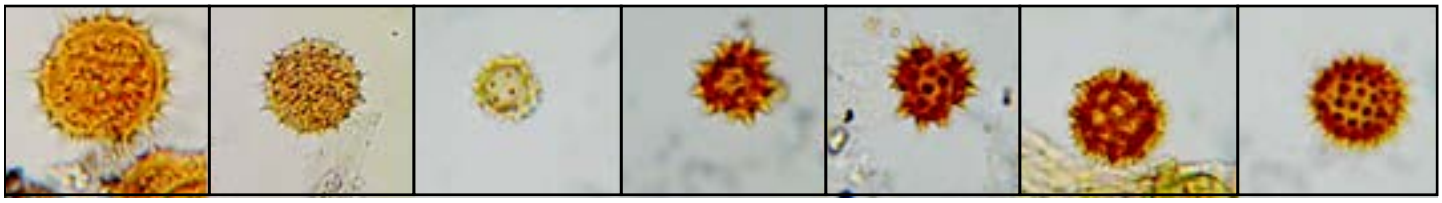
Local herbaceous vegetation was dominated by the members of the Asteraceae family, where 20 different species of Asteraceae (cultivated and wild type) were found throughout the year. Besides these, grasses, members of Solanaceae, Rubiaceae, Malvaceae, etc., were also found. The numbers of plant species under different families present in the study area were depicted in Fig. 2.The members of the Asteraceae family found in the locality were listed in Table 1.

Polliniferous materials from those species were collected to prepare reference slides for identification of air-borne dispersed pollen types. Pollen grains were echinate and tricolporate. They can be differentiated only by their size and in some cases spines were slightly different in shape and size (length, base – narrow as in *Vernonia* or broad as in *Parthenium*). By comparing reference slides it was found that among Asteraceae members *Helianthus annuus* (sunflower), *Parthenium* and *Vernonia* were different in size, shape and morphology from other Asteraceae pollen grains and can be easily distinguished. Other pollen grains showed very similar ornamentation pattern and differ in size only by 2-5 µm. So they were very hard to discriminate when trapped in Burkard sampler. Fig. 3 represents pollen grains of some of the Asteraceae members after acetolysis.

The seasonal periodicities of Asteraceae members were recorded by Burkard Personal sampler (Fig. 4). The highest number of Asteraceae pollen grains was present in the air during the month of April which represented 60% of total aero-pollen load during that season and lowest in the month of July. During the month of April the distinguishable air-borne pollen types among Asteraceae members were *Helianthus* sp., *Parthenium*, and *Vernonia*. *Helianthus annuus* pollen grains were mainly found within 5 km radius of its cultivation land and cannot fly long distance due to larger pollen size.

Table 1 : Different plant species under Asteraceae family found in the locality

Sl. No.	Name of the plant	Flowering time
1	*Acanthospermum hispidum*	August - December
2	*Acmella paniculata*	September - January
3	*Ageratum conyzoides*	October – June
4	*Bidens bipinnata*	September
5	*Blumea lacera*	October – June
6	*Calendula officinalis*	January-April
7	*Cosmos bipinnatus*	December – January
8	*Dahlia pinnata*	December-February
9	*Echinops echinatus*	November – January
10	*Eclipta prostrata*	Throughout the year in moist places
11	*Helianthus annuus*	March – June
12	*Helianthus debilis*	Throughout the year
13	*Parthenium hysterophorus*	October – May
14	*Sonchus wightianus*	January – April
15	*Sphaeranthus indicus*	November – January
16	*Tagetes erecta*	September – November
17	*Tridax procumbens*	Throughout the year
18	*Vernonia cinerea*	Throughout the year
19	*Xanthium strumarium*	October – May
20	*Zinnia elegans*	September – October



Helianthus annuns 44.2 µm x 44.2 µm | *Helianthus debilis* 33.8 µm x 36.4 µm | *Parthenium hysterophorus* 20.8 µm x 18.2 µm | *Blumea lacera* 26 µm x 26 µm | *Spilanthes acmella* 28.6 µm x 28.6 µm | *Vernonia cinerea* 29.9 µm x 26 µm | *Tridax procumbens* 33.8 µm x 29.9 µm

Fig. 3 : Photomicrographs of some pollen grains of Asteraceae after acetolysis

Prevalence of different Asteraceae pollen grains at different heights

Sampling was done at different heights to know the prevalence of air-borne pollen grains at human height, as they have the greater chances for allergy causing. The distribution pattern of Asteraceae pollen grains at 0.5 m 1.0 m, 1.5 m and 2.0 m were depicted in Fig. 5. The highest numbers of pollen grains were present at the ground level. Sunflower pollen grains were mostly present at 1.0 m and 1.5 m height, whereas *Parthenium* pollen grains were present at all the tested heights. *Vernonia* pollen grains were mainly present at 0.5 m, 1.0 m and 1.5 m height.

Patient sensitization profile of different airborne Asteraceae pollen grains

Skin Prick Test (SPT) was carried out on 258 patients visiting different Government hospitals near the study area. The most allergy-causing pollen types were *Helianthus annuus* which accounts for 20.62% of positivity and *Parthenium hysterophorus* which accounts for 13.89% of positivity among atopic individuals (Fig.6). *Helianthus debilis* causes an allergenic reaction among 4.23% of patients which may be due to cross-reactivity. The further immuno-biochemical studies were carried out with *Helianthus annuus* and *Parthenium hysterophorus*. It was evident from the questionnaire study that the patients positive to sunflower were living near sunflower cultivation land or attached in sunflower processing industry. Among the atopic individuals 10 patients had given their written consent to use their blood for research purpose and 5 ml peripheral blood was collected from them. The control group was represented by 3 healthy individuals.

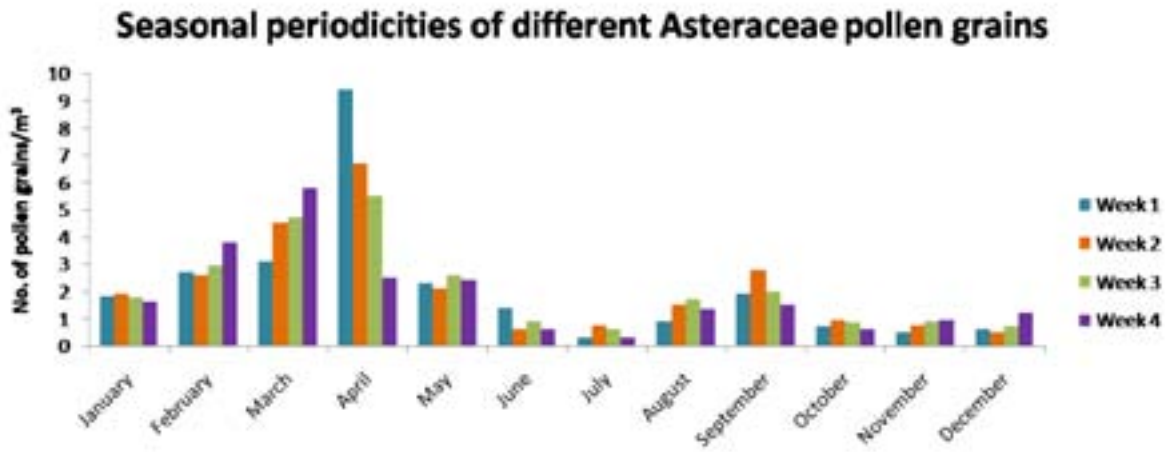


Fig. 4 : Seasonal periodicities of airborne Asteraceae pollen grains during study period

Determination of allergenic potential of most allergy-causing species of Asteraceae by specific IgE ELISA and immunoblot

The demographic features of the ten selected patients were briefly described in the Table 2. Most of the selected patients were sensitized to the pollen grain of *Helianthus annuus* (sunflower) and some were found to be sensitized to both *Helianthus annuus* and *Parthenium hysterophorus* pollen. Immuno-blotting experiment was performed using these patient sera. In case of *Helianthus annuus* pollen, IgE reactive bands were found for approximately 32 kDa, 34 kDa and 42 kDa molecular weight components (Fig. 7a) present in the pollen extracts. In case of *Parthenium* pollen extract, 32 kDa IgE reactive band was found in seven patients (Fig.7b). An IgE reactive band of 32 kDa in sunflower pollen extract was also found in case of seven patients. The overall result of immunoblot was found to be almost in accordance with the specific IgE ELISA using patient serum samples.

Determination of cross-reactivity between *Helianthus annuus* and *Parthenium hysterophorus* pollen

Inhibition ELISA was performed to detect the cross-reactivity between *Helianthus annuus* and *Parthenium hysterophorus* pollen. Around 30-35% cross-reactivity was found between them (Fig. 8). As only one IgE reactive band is present in the tested patient sera against *Parthenium*, so it can be said that the cross-reactivity is due to this protein.

Prediction of cross-reactive proteins between *Helianthus annuus* and *Parthenium hysterophorus*

The 32 kDa protein of sunflower was subjected to mass spectrometry. It showed homology with cysteine protease of *Helianthus annuus*. The MS spectra are represented in Fig. 9. The protein band is present at the same position in *Parthenium* which is also subjected to MALDI-TOF. However, due to unavailability of sequence information, it was difficult to identify in MS. So it was compared with the database,and from the previous report, it was found that a 32 kDa cysteine protease was present in *Parthenium*. So it was assumed that it might be the same protein.

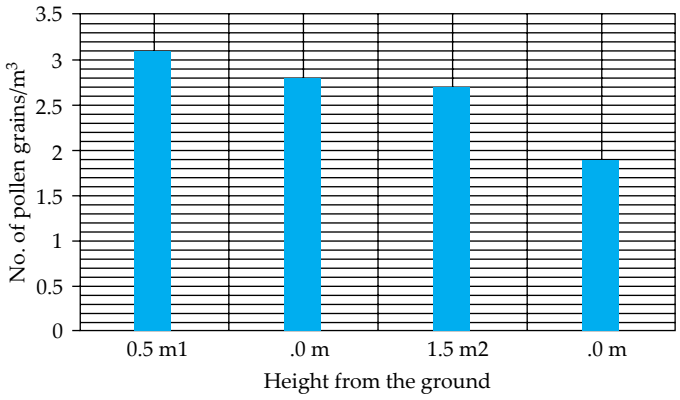


Fig. 5 : Prevalence of pollen grains in air at different heights from the ground level

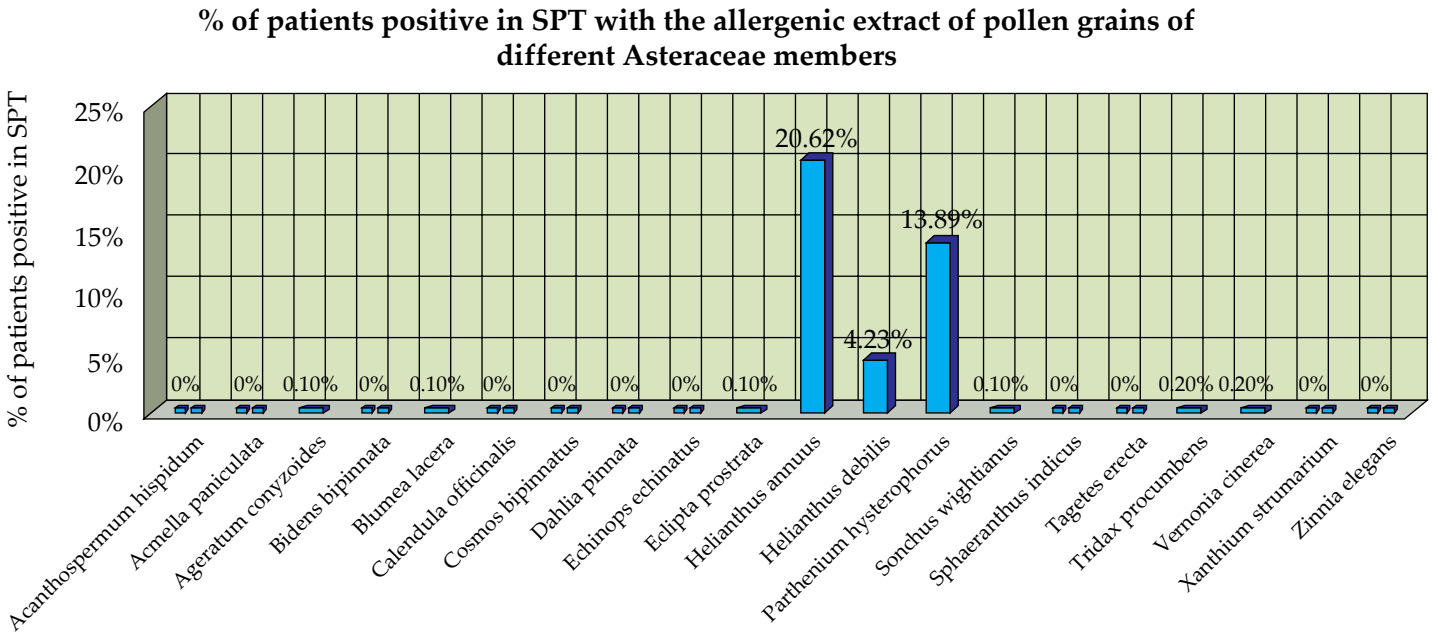


Fig. 6 : Patient sensitization profile of different Asteraceae pollen grains

Table 2 : Demographic features of the patients enrolled in the study

Patient No.	Age	Sex	Symptoms against *Helianthus annuus*	SPT against *Helianthus annuus*	SPT against *Parthenium hysterophorus*	Specific IgE against *Helianthus annuus* (P/N Value)	Specific IgE against *Parthenium hysterophorus* (P/N Value)
1	34	F	AR+ BA	+3	+1	3.62	2.74
2	49	M	U	+2	+1	3.56	3.02
3	48	F	AR+ ANG	+1	+3	2.32	4.16
4	35	M	SOB	+3	+1	3.69	2.19
5	25	F	CC	+3	+1	3.86	2.30
6	24	F	CC	+3	+1	4.26	2.85
7	20	F	AR+ BA	+3	+1	4.09	3.85
			SOB				
8	16	M	SR	+3	+1	3.77	1.75
9	30	F	CC	+1	+3	2.43	4.02
10	50	F	AR+ BA	+1	+2	2.39	3.22
C1	30	M	-	-	-	-	-
C2	42	F	-	-	-	-	-
C3	21	F	-	-	-	-	-

C1-C3: control, AR: Allergic rhinitis, BA: Bronchial asthma, U: Urticaria, SOB: Shortness of breath, CC: Cough and cold, ANG = Angioedema

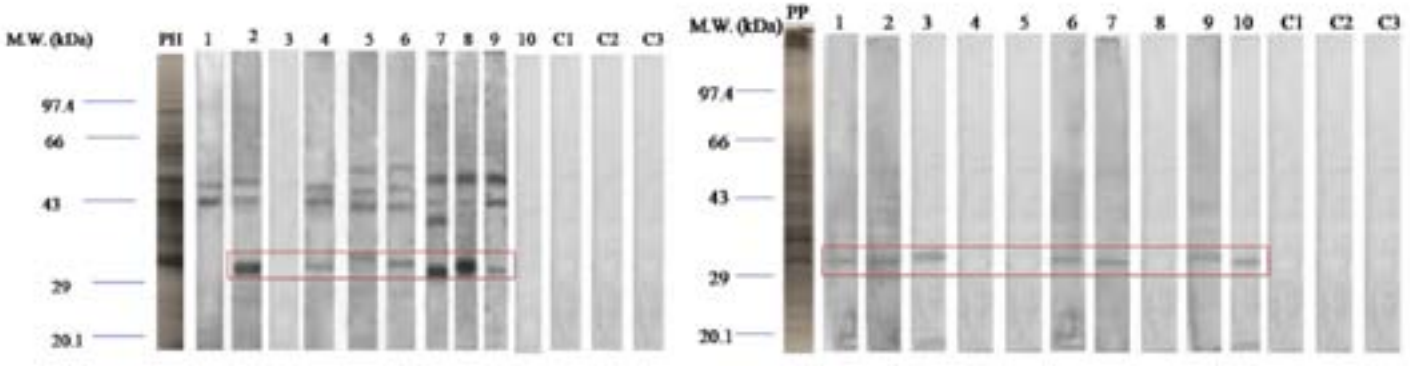


Fig. 7 : The total protein profiling and IgE reactivity pattern of *Helianthus annuus* (A) and Parthenium *hysterophorus* (B); PH: Total protein of *Helianthus* sp., PP: Total protein of *Parthenium* sp., 1-10: Patient number, C1-C3: Control. The 32 kDa common band is marked with a red box.

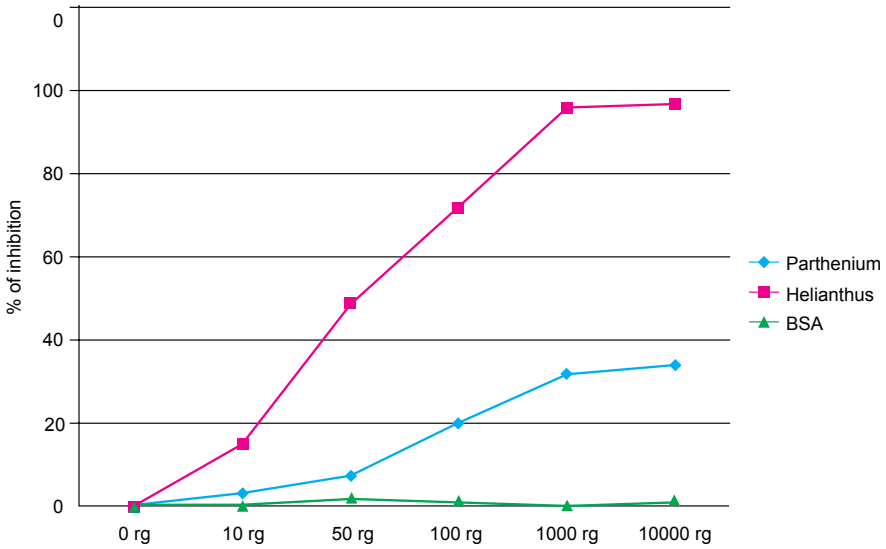


Fig. 8 : Cross-reactivity between allergenic protein extracts of Helianthus annuus (in plate bound solid phase) and Parthenium hysterophorus pollen (in fluid phase)

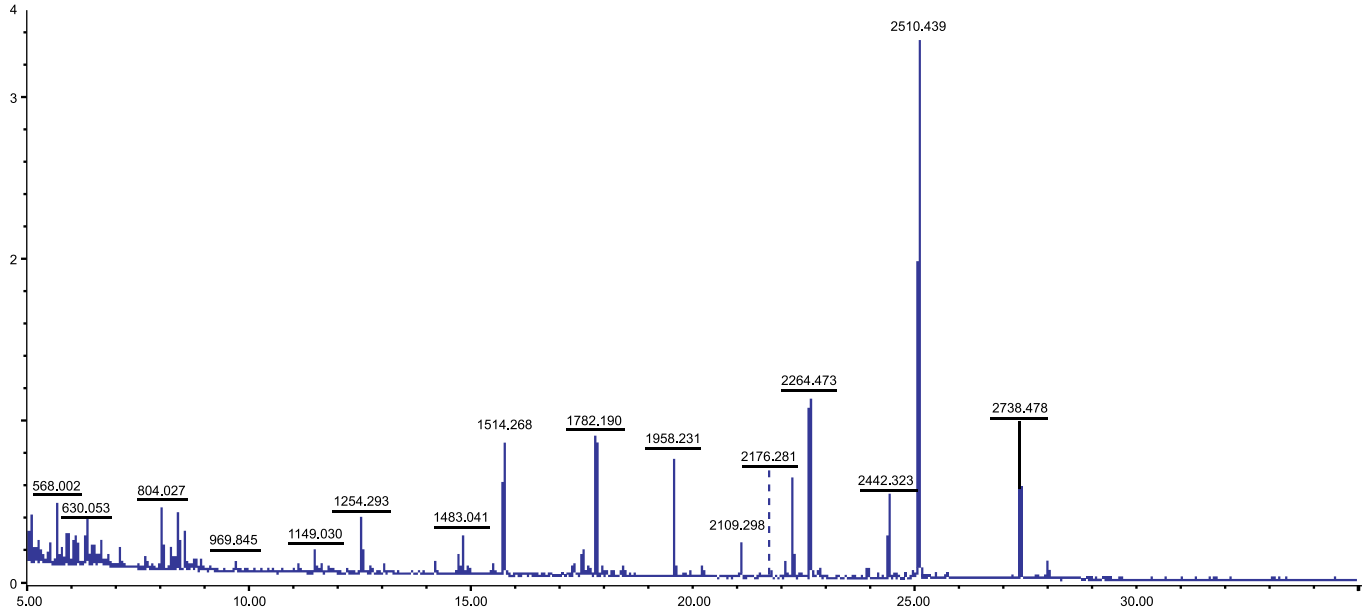


Fig. 9 : The MS spectra of 32 kDa Helianthus pollen protein matched with cysteine protease

DISCUSSION

Allergic diseases increase at an alarming rate world-wide with India being not an exception[13,14]. In the present study, the occurrence of different pollen grains in the ambient out-door environment of Kolkata and their impact on atopic population was monitored. Pol-

len grains of the Asteraceae family were found to contribute upto 60% of the aero-pollen load. Despite of similar ornamentation pattern, some Asteraceae pollen grains were found to be distinguishable from the others. Among different Asteraceae members, the pollen grains of *Helianthus* sp., *Parthenium* and *Vernonia* were present in the air of which *Helianthus* sp. and *Parthenium* were allergy causing. The present study clearly depicted the presence of sunflower pollen grains in the ambient outdoor environment in spite of its larger size. It is mainly found near the vicinity of sunflower plantation and cannot be dispersed very long distance like *Parthenium*, but cause severe effects on atopic individuals. It mainly affects the local people living near sunflower cultivation land or attached with sunflower processing. However, it is generally present at the human height (approximately 1.5 m) and thus has the higher advantage to cause allergy. On the other hand, *Parthenium* is an invasive species that grows ubiquitously in the wild and many cases replaces the natural vegetation. Due to its smaller pollen size, it can fly long distance. However, the occurrence of sensitization to *Helianthus annuus* is higher than *Parthenium hysterophorus*. It is found that most of the patients sensitized to *Helianthus annuus* did not show any allergenic reaction towards *Parthenium hysterophorus*, suggesting the fact that there may be no or very little cross-reactivity between these two plants although they belong to the same family. The cross-reactivity between them was studied by inhibition ELISA and found that there is only 30-35% cross-reactivity between them. The western blot also suggests the presence of only single common IgE reactive band between these two plants. So it may be concluded that the cross-reactivity is due to this common IgE reactive band. This band was identified as cysteine protease in sunflower by mass spectrometry. Due to unavailability of sequence information, it cannot be identified in *Parthenium*. By comparing the molecular weight and previous report, it can be assumed as the same protein in *Parthenium hysterophorus* also, which needs further detailed study.

CONCLUSION

In conclusion, the present study gives a comprehensive idea about different Asteraceae pollen grains found in Eastern India, their size, ornamentation pattern to differentiate between them as well as their airborne nature and allergy-causing capacity. *Helianthus annuus* and *Parthenium hysterophorus* were found to be the most allergy-causing species of the Asteraceae family, but very little cross-reactivity was found between them.

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Authors wish to dedicate the paper in memory of Late. Prof. Sunirmal Chanda, who was the source of inspiration of all the research works of the authors.

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Original Article

POLLEN ANALYSIS OF SPIDER WEBS FROM ALLAHABAD DISTRICT, UTTAR PRADESH

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In recent years spider webs have emerged as potent natural pollen traps, effective to study pollen rain-vegetation relationship in a particular geographical region and also to evaluate the airborne pollen grains of a region. Pollen analysis of 11 spider web samples collected from different localities of Allahabad district recorded presence of 40 pollen morphotypes belonging to 22 families. 22 pollen morphotypes originated from arboreal taxa and 18 from non-arboreal taxa. Among arboreal taxa *Holoptelea integrifolia* is the most abundant pollen type followed by *Eucalyptus citriodora, Phyllanthus emblica, Terminalia arjuna, Thuja occidentalis, Ricinus communis, Syzygium cumini, Callistemon citrinus, Aegle marmelos, Azadirachta indica* etc. Among non-arboreal taxa, pollen grains of Poaceae are most abundant followed by those of Amaranthaceae/Chenopodiaceae, *Brassica campestris, Ageratum conyzoides, Parthenium hysterophorus* and other Asteraceae. The pollen spectra of web samples collected from different localities as well as from same locality show variation in the pollen composition qualitatively as well as quantitatively. The results reveal that the pollen assemblage retrieved from the analysis of spider webs largely reflects the ground vegetation of the study area and also exhibits a reflection of the airborne pollen flora of Allahabad which in turn is also correlated with the ground vegetation.

Keywords : Pollen analysis, pollen assemblage, spider webs, airborne pollen, arboreal taxa, non-arboreal taxa.

INTRODUCTION

Various natural pollen traps viz. surface soil/sediment, moss cushions, leaves and barks have been used to understand the relationship between the local vegetation and pollen rain[1-6]. In recent years spider webs have emerged as potent natural pollen traps and their qualitative and quantitative pollen analysis has been proved to be a strong tool to assess the actual contribution of the local plants in the pollen rain and the overall representation of local and regional vegetation[7,8]. Spider webs also provide a useful indicator of environmental pollution and chemistry[9]. Recently, using spider webs aerobiological studies were conducted in Hyderabad city[10] and in Warangal District.[11] Investigations on pollen analysis of spider webs are available from Lucknow,[8] Hyderabad,[12] Khedla village, Betul[13] and Korba District[14] in India and from Yunnan in China[4].

The present investigation on pollen analysis of spider web samples collected from various localities of Allahabad district is undertaken to understand the relationship between local vegetation and pollen rain as recorded from spider webs. The pollen assemblage retrieved from the spider webs has been correlated with the airborne pollen grains of Allahabad[15-17].

MATERIALS AND METHODS

Allahabad district (25.4358° N 81.8463° E longitude) covering an area of 5482 sq. kms, is located at 103 m above the sea level. Allahabad district has humid subtropical climate. It has three seasons, a long hot summer, a short humid rainy season and a cool dry winter. It forms a part of the floristic sub - division of India, known as the Gangetic plain, with a grassland vegetation having plantations of trees mostly along roadsides, gardens, wastelands etc.

The present study is based on the pollen analysis of 11 spider web samples collected from various localities of Allahabad (Fig.1).

Out of 11 samples, Spider Web 1-6 were collected during November- December 2015 while Spider Web 7-11 were collected during May 2016.

Majority of samples were collected from the trees growing along the road sides in various localities (S.W.1–7 and S.W. 9). While S.W. 8, 10 and 11 were collected from two gardens as mentioned below:

- S.W. 1–S.W.4 were collected from Parade ground during November 2015.

Fig. 1 . Map showing sampling sites of different localities of Allahabad District.

- S.W. 5 and 6 were collected from Handia Road (December 2015).
- S.W. 7 was collected from Handia Road (May 2016).
- S.W. 8 was collected from Company garden (May 2016).
- S.W. 9 was collected from Jhunsi Road (May 2016).
- S.W. 10 and 11 were collected from wall corners in Khusrobagh garden (May 2016).

Collected web samples were placed in separate polythene bags. Field surveys around the sites of spider web collection were conducted from time to time to collect the floral material for the preparation of pollen reference slides.

Two gram of each spider web sample was transferred to a container containing concentrated HCl which dissolves the meshes instantaneously and then passed through the sieve to remove the superfluous matter. The samples were washed with distilled water several times to remove acid content by centrifugation and decantation. After centrifuging the residue was mixed with 10 ml concentrated HF in polythene test tube and kept for 2 days to remove silica. The filtrate was washed with distilled water 4 to 5 times to remove silica particle. The residue was acetolysed using the method suggested by Arora and Modi[18]. From each sample four slides were prepared in saffranin glycerine jelly and thoroughly scanned under microscope. Identification of pollen grains recovered from the spider webs were made largely with the help of reference slides and relevant literature[19].

The pollen grains were identified upto specific/generic level, except few cases where the identification could be done upto family level only (e.g. Poaceae, Chenopodiaceae, Amaranthaceae and some pollen grains of Asteraceae and Brassicaceae). Further, Amaranthaceae and Chenopodiaceae are included in a category Amaranthaceae/Chenopodiaceae because of their pollen morphological similarities.

Representation of pollen assemblage of each spider web has been shown by pollen spectrum depicting the contribution of various plant taxa (Fig. 2 and 3).

Photographs of pollen grains were taken using a Leica DM 2500 microscope with Leica DFC295 camera attachment.

RESULTS

The pollen assemblage of S.W. 1, 2, 3 and 4 demonstrates dominance of non arboreal taxa over arboreal taxa (Fig. 4)

In S.W. 1, 51.83% of pollen grains are from non-arboreal taxa, with abundance of Poaceae (25.30%) followed by *Ageratum conyzoides* (9.63%), Amaranthaceae-Chenopodiaceae (7.83%), other Asteraceae (6.02%) and *Brassica campestris* (3.01%). In arboreal pollen assemblage (39.15%) , *Cassia siamea* (16.80%) is major contributor followed by *Holoptelea integrifolia* (10.24%), *Callistemon citrinus* (6.02%), *Thuja occidentalis* (2.40%), *Azadirachta indica* (2.40%) and *Moringa* sp. (1.20%).

In S.W. 2, 51.96% of pollen grains are from non-arboreal taxa. Amongst which pollen grains of *Brassica campestris* (16.15%) ranked first followed by *Ageratum conyzoides* (13.10%), Poaceae (7.86%), Amaran-

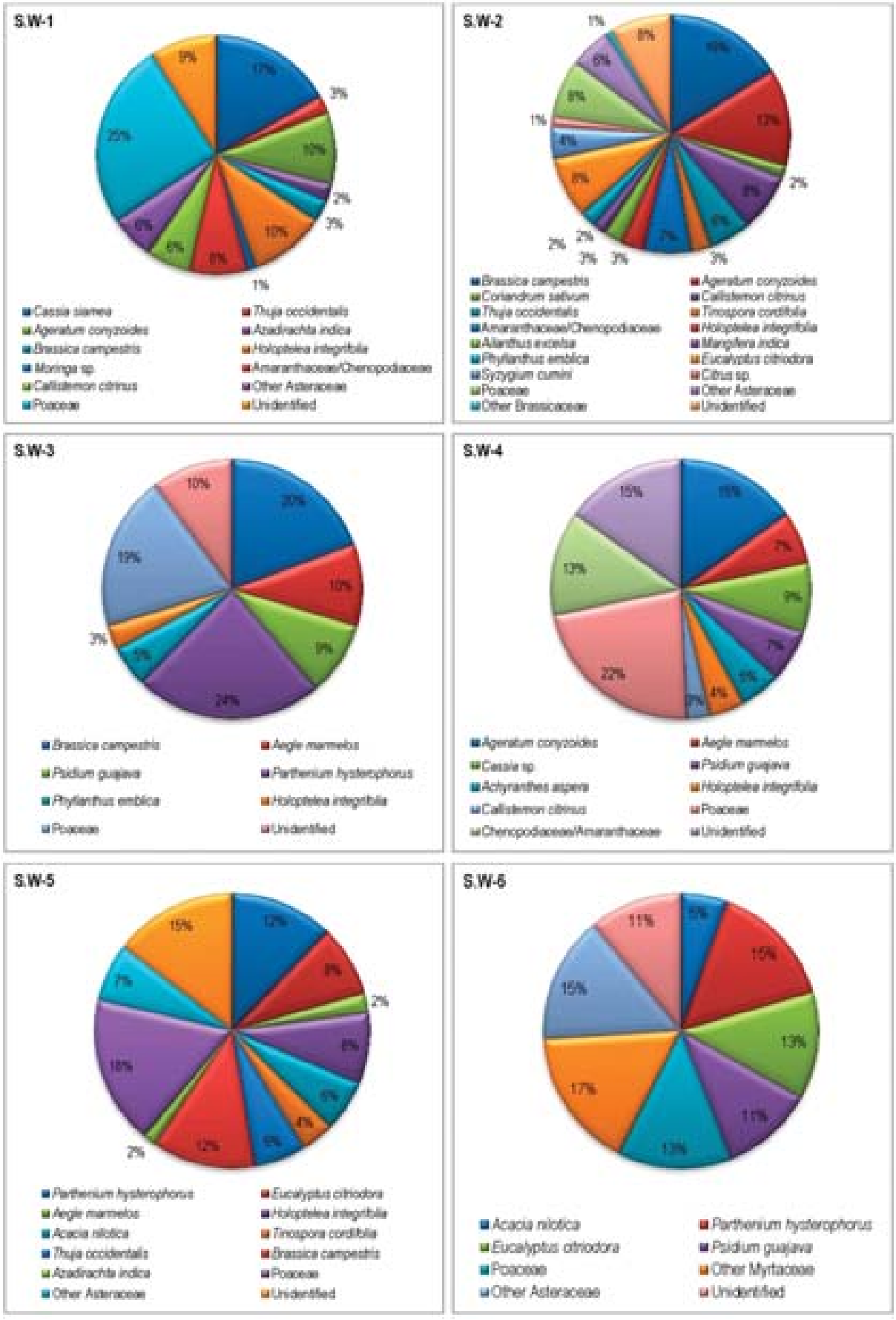

Fig.2 : Pollen spectra showing the components of pollen assemblage of spider webs(SW 1-6)

thaceae/Chenopodiaceae (6.55%) and other Brassicaceae (0.87%). Among pollen grains of arboreal taxa (39.73%), *Eucalyptus citriodora* (8.29%), *Callistemon citrinus* (7.86%) and *Thuja occidentalis* (5.67%) are better represented in comparison to other taxa like *Syzygium cumini* (4.36%), *Holoptelea integrifolia* (3.05%), *Ailanthus excelsa* (2.62%), *Tinospora cordifolia* (2.62%), *Phyllanthus emblica* (2.18%) and *Citrus* sp. (1.31%).

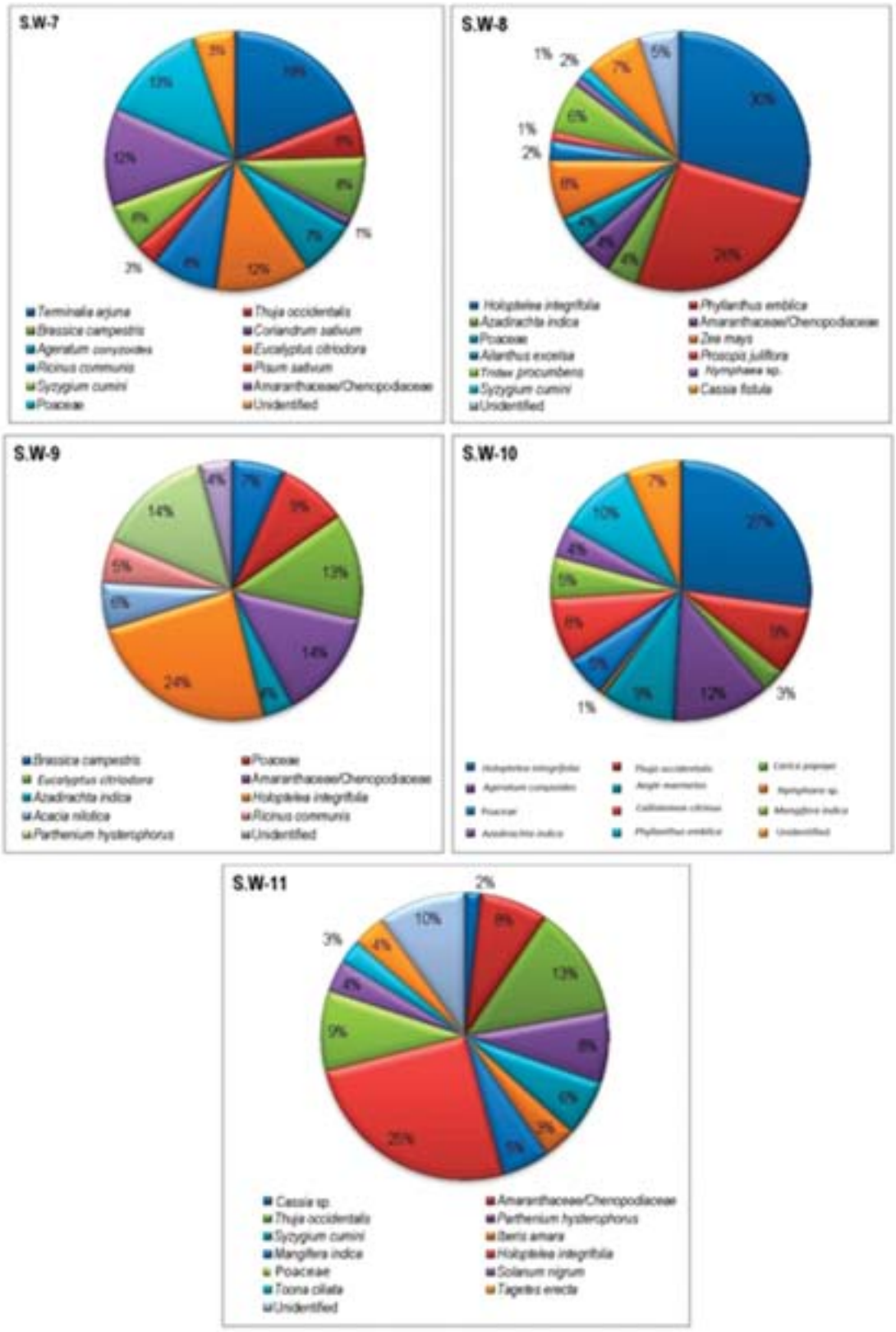

Fig.3 : Pollen spectra showing the components of pollen assemblage of spider webs(SW 7-11).

In S.W. 3, 62.24% of pollen grains were found to be originated from non arboreal taxa and are represented by *Parthenium hysterophorus* (23.46%), Poaceae (19.38%) and *Brassica campestris* (19.38%).

Arboreal taxa (27.55%) are represented by *Aegle marmelos* (10.29%), *Psidium guajava* (9.18%), *Phyllanthus emblica* (5.10%) and *Holoptelea integrifolia* (3.06%).

In S.W. 4, 55.79% of pollen grains are retrieved from the non arboreal taxa and are represented by Poaceae (24.46%), *Ageratum conyzoides* (15.21%) and Amaran/ Chenopod (13.04%). Pollen grains of arboreal taxa (28.98%) are represented by *Cassia* sp. (8.69%), *Aegle marmelos* (6.52%), *Psidium guajava* (6.52%) and *Callistemon citrinus* (2.89%).

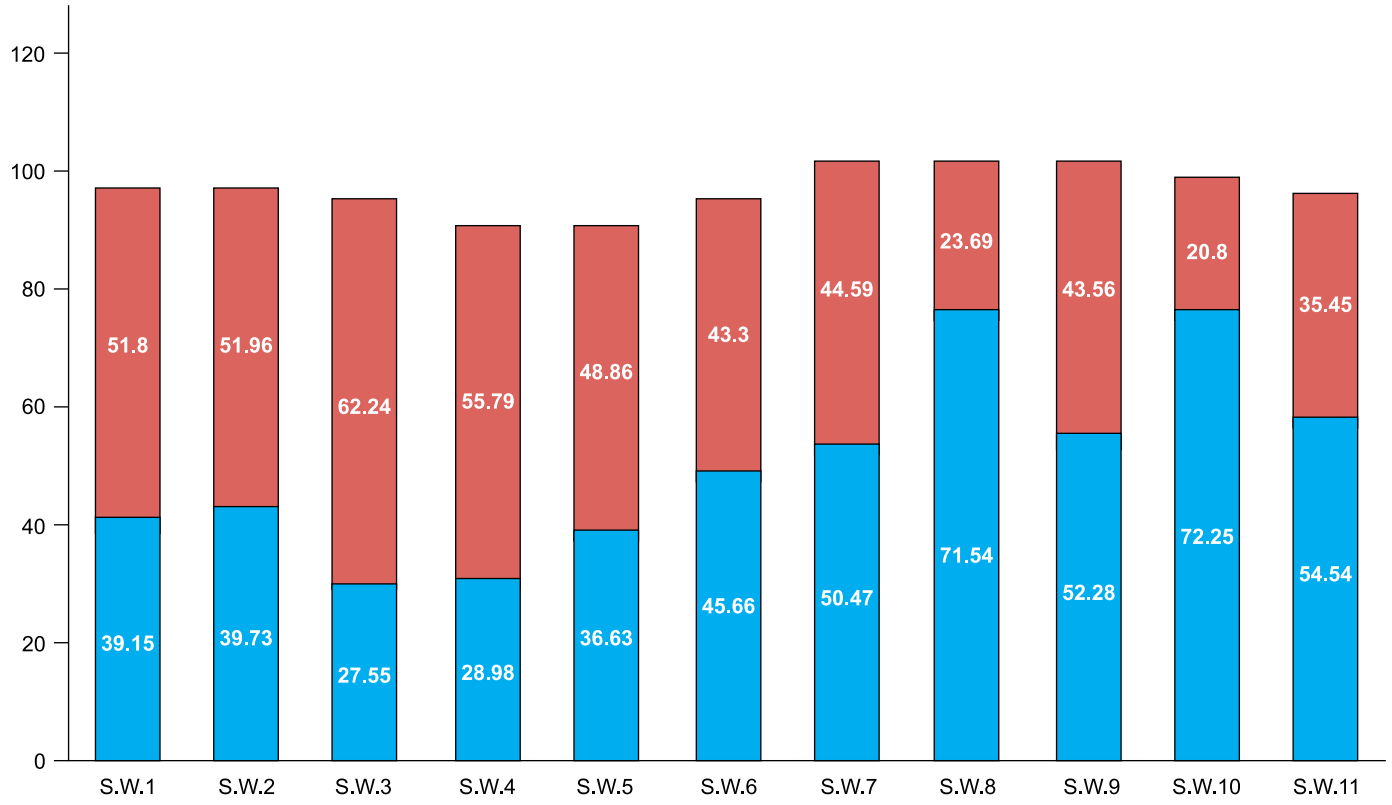

Fig. 4 : Percentage of arboreal and non arboreal taxa in the spider web samples.

The vegetation of Parade ground (sites of S.W. 1-4) comprises of *Holoptelea integrifolia, Cassia siamea, Azadirachta indica, Aegle marmelos, Eucalyptus citriodora, Ficus virens, Ficus racemosa, Ficus benghalensis, Syzygium cumini, Ailanthus excelsa, Mangifera indica*[9], *Pongamia pinnata, Madhuca indica, Phyllanthus emblica, Citrus sp., Tamarindus indica, Callistemon citrinus, Neolamarckia cadamba,* and *Tinospora cordifolia*. Herbaceous flora consists of grasses along with *Ageratum conyzoides, Amaranthus spinosus, Achyranthes aspera, Chenopodium album, Parthenium hysterophorus* and *Coriandrum sativum*. Small plots near the site of S.W. 2 & 3 are covered with *Brassica campestris* crops.

Pollen spectrum of S.W. 5 demonstrates dominance of non arboreal taxa (48.86%). Among these Poaceae (17.55%) is best represented followed by *Parthenium hysterophorus* (12.21%), *Brassica campestris* (12.21%) and other Asteraceae (6.87%) while arboreal taxa (36.63%) are represented by *Holoptelea integrifolia* (8.39%), *Eucalyptus citriodora* (8.39%), *Acacia nilotica* (6.10%), *Thuja occidentalis* (6.10%), *Tinospora cordifolia* (3.81%) and *Aegle marmelos* (2.29%).

In the pollen assemblage of S.W. 6, arboreals (45.66%) are better represented, recording dominance of pollen grains belonging to family Myrtaceae (*Eucalyptus citriodora*, 12.59%; *Psidium guajava*, 11.02% and other Myrtaceae, 16.53%) apart from *Acacia nilotica* (5.51%). Non arboreal taxa (43.30%) are represented by *Parthenium hysterophorus* (14.96%) & other Asteraceae (14.98%) and Poaceae (13.38%).

The pollen rain composition of S.W. 7 also portrays dominance of arboreal taxa (50.47%), among which *Terminalia arjuna* (18.86%) ranked first followed by *Eucalyptus citriodora* (11.79%), *Ricinus communis* (8.25%), *Syzygium cumini* (5.89%) and *Thuja occidentalis* (5.66%). Non arboreal palynoassemblage (44.59%) is comprised of Poaceae (13.20%), Amaranthaceae/Chenopodiaceae (12.26%), *Brassica campestris* (7.78%), *Ageratum conyzoides* (7.07%), *Pisum sativum* (2.83%) and *Coriandrum sativum* (1.17%).

S.W. 5, 6 and 7 are procured from the same locality (Handia Road). S.W. 5 and 6 are from the two trees growing about 20 meters apart while S.W. 7 is collected from a tree growing about half a km away from S.W. 5 and 6. The majority of vegetation is common at the three sites, consisting of *Eucalyptus citriodora, Dalbergia sissoo, Parkinsonia aculeata, Moringa oleifera, Tamarindus indica, Ailanthus excelsa, Holoptelea integrifolia, Azadirachta indica, Mangifera indica, Tectona gran-*

dis and *Artocarpus heterophyllus* etc. Ground vegetation consists of *Parthenium hysterophorus, Peristrophe bicalyculata, Brassica campestris, Ageratum conyzoides*, Amaranthaceae/Chenopodiaceae along with grasses. Taxa like *Acacia nilotica, Psidium guajava, Azadirachta indica, Brassica campestris* and *Tinospora cordifolia* are present only at sampling site of S.W. 5 and 6, while *Terminalia arjuna, Ricinus communis, Coriandrum sativum* and *Pisum sativum* are present at 7th sampling site only.

In S.W. 8, 71.54% of pollen originated from arboreals, among which *Holoptelea integrifolia* (29.64%) ranked first followed by *Phyllanthus emblica* (25.69%), *Cassia*

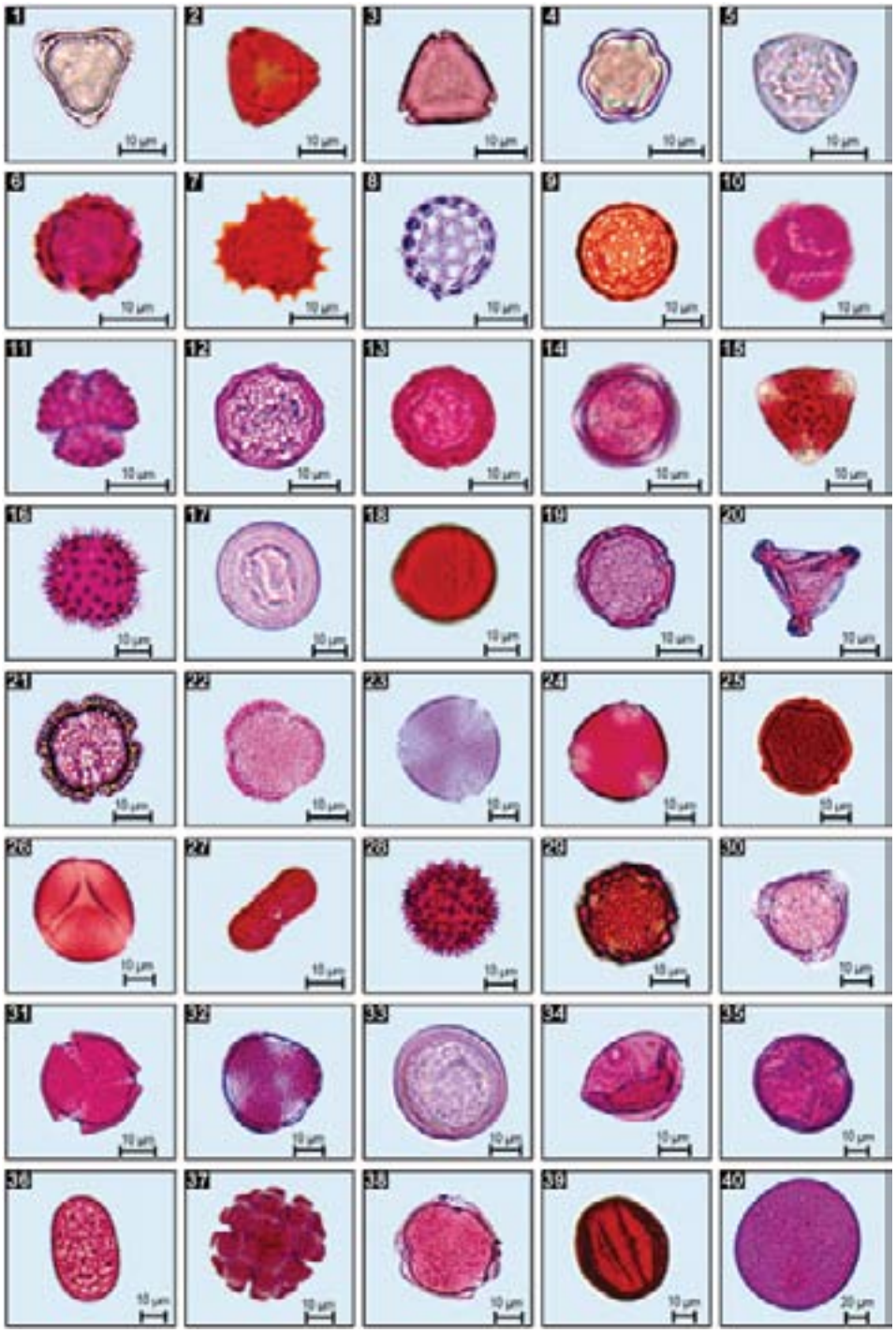

Fig. 5: Pollen types recovered from spider web samples. 1. *Syzygium cumini.* 2. *Callistemon citrinus*. 3. *Psidium guajava*. 4. *Terminalia arjuna*. 5. *Eucalyptus citriodora*. 6. *Parthenium hysterophorus*. 7. *Ageratum conyzoides*. 8. *Achyranthes aspera*. 9. Amaranthaceae/Chenopodiaceae. 10. *Tinospora cordifolia*. 11. other Asteraceae. 12. *Holoptelea integrifolia.* 13. *Phyllanthus emblica.* 14. other Brassicaceae. 15. *Mangifera indica*. 16. *Tridax procumbens*. 17. *Thuja occidentalis*. 18. *Carica papaya.* 19. *Toona ciliata*. 20. other Myrtaceae. 21. *Citrus* sp. 22. *Iberis amara*. 23. *Cassia fistula*. 24. *Cassia* sp. 25. *Ailanthus excelsa.* 26. *Prosopis juliflora*. 27. *Coriandrum sativum*. 28. *Tagetes erecta*. 29. *Aegle marmelos*. 30. *Solanum nigrum*. 31. *Ricinus communis*. 32. *Brassica campestris*. 33. *Nymphaea* sp. 34. Poaceae. 35. *Cassia siamea*. 36. *Pisum sativum*. 37. *Acacia nilotica*. 38. *Moringa* sp. 39. *Azadirachta indica*. 40. *Zea mays*.

fistula (7.11%), *Azadirachta indica* (3.95%), *Ailanthus excelsa* (2.37%), *Syzygium cumini* (1.58%) and *Prosopis juliflora* (1.18%). Non arboreals pollen grains (23.32%) are represented by those of *Zea mays* (7.50%), *Tridax procumbens* (6.32%), Amaranthaceae/Chenopodiaceae (4.34%), Poaceae (3.95%) and *Nymphaea* sp. (1.18%).

S.W. 8 is procured from Company garden which is large garden of Allahabad with diverse vegetation chiefly belonging to arboreal taxa-*Ailanthus excelsa, Ziziphus jujuba, Syzygium cumini, Cassia fistula, Eucalyptus citriodora, Dalbergia sissoo, Phyllanthus emblica, Azadirachta indica, Ficus religiosa, Bombax ceiba, Delonix regia, Peltophorum pterocarpum, Holoptelea integrifolia, Mangifera indica, Artocarpus heterophyllus, Prosopis juliflora* and *Nerium oleander* etc. Ground vegetation comprised of grasses chiefly along with *Zea mays, Parthenium hysterophorus, Nymphaea* sp., *Ageratum conyzoides, Tridax procumbens* and Amaranthaceae/Chenopodiaceae etc.

The pollen assemblage recovered from the S.W. 9 also illustrates the dominance of arboreal taxa (52.28%) over non arboreal taxa (43.56%). Among the arboreal taxa, *Holoptelea integrifolia* ranked first (24.06%) followed by *Eucalyptus citriodora* (13.27%), *Acacia nilotica* (5.80%), *Ricinus communis* (5.39%) and *Azadirachta indica* (3.73%). Non arboreal taxa are represented by *Parthenium hysterophorus* (14.52%), Amaranthaceae/Chenopodiaceae (13.69%), Poaceae (8.71%) and *Brassica campestris* (6.63%).

S.W. 9 is collected from the Jhunsi. *Citrus* sp., *Ziziphus jujuba, Syzygium cumini, Cassia fistula, Eucalyptus citriodora, Dalbergia sissoo, Phyllanthus emblica, Azadirachta indica, Ficus religiosa, Acacia nilotica, Delonix regia, Peltophorum pterocarpum, Tectona grandis, Holoptelea integrifolia, Azadirachta indica, Mangifera indica* and *Ricinus communis* etc. are common taxa. Non arboreal comprises of grasses, *Parthenium hysterophorus, Ageratum conyzoides, Brassica campestris* and Amaranthaceae/Chenopodiaceae etc.

Palynoassemblage of S.W. 10 and 11 demonstrates dominance of arboreal taxa.

In S.W. 10, 72.25% of pollen grains are from arboreal taxa belonging to *Holoptelea integrifolia* (27.16%) followed by *Phyllanthus emblica* (9.82%), *Aegle marmelos* (9.24%), *Thuja occidentalis* (8.67%), *Callistemon citrinus* (8.09%), *Mangifera indica* (5.20%) and *Azadirachta indica* (4.04%). Non arboreal palynoassemblage (20.80%) is represented by *Ageratum conyzoides* (12.13%), Poaceae (5.20%), *Carica papaya* (2.89%) and *Nymphaea* sp. (0.57%).

In S.W.11, 54.54% of pollen grains originated from arboreal taxa, represented by *Holoptelea integrifolia* (25.45%), *Thuja occidentalis* (12.72%), *Syzygium cumini* (6.36%), *Mangifera indica* (5.45%), *Toona ciliata* (2.72%) and *Cassia* sp. (1.81%). Non arboreal taxa (35.45%) are represented by Poaceae (9.09%), *Parthenium hysterophorus* (8.18%), Amaranthaceae/Chenopodiaceae (7.27%), *Solanum nigrum* (3.63%), *Iberis amara* (3.63%) and *Tagetes erecta* (3.63%).

S.W.10 and 11 are procured from Khusrobagh an old Mughal monument surrounded by walled large garden comprising of diverse vegetation viz. *Citrus* sp., *Syzygium cumini, Cassia fistula, Eucalyptus citriodora, Thuja occidentalis, Dalbergia sissoo, Phyllanthus emblica, Azadirachta indica, Ficus religiosa, Cordia dichotoma, Ficus benghalensis, Ailanthus excelsa, Citrus sp., Delonix regia, Peltophorum pterocarpum, Polyalthia longifolia, Holoptelea integrifolia, Carica papaya, Saraca asoca, Aegle marmelos, Toona ciliata, Azadirachta indica, Mangifera indica, Artocarpus heterophyllus, Artocarpus lakoocha, Nerium oleander* and *Callistemon citrinus* etc. Ground vegetation mainly consists of grasses along with *Parthenium hysterophorus, Nymphaea* sp., *Tagetes erecta, Iberis amara, Ageratum conyzoides, Tridax procumbens*, and Amaranthaceae/Chenopodiaceae etc.

DISCUSSION

The present study provides first report on pollen analysis of spider webs collected from various localities of Allahabad.

Altogether 40 pollen morphotypes belonging to 22 families are recovered from the pollen analysis of web samples (Fig. 5). Of these 22 pollen morphotypes are from arboreal taxa and 18 pollen morphotypes belong to non arboreal taxa. Web samples recorded variation in the diversity of pollen types as well as in pollen con-

tent. Diversity of pollen types in web samples varies from 7 to 17 while pollen sum varies from 98 to 424 grains per sample. Age of the spider web seems to be the major factor for these variations.

The study also demonstrates that the vegetation around the site of collection is largely represented in the pollen spectra of web meshes. Among arboreal taxa *Holoptelea integrifolia* (12%) is the most abundant pollen type recovered from the web meshes followed by *Eucalyptus citriodora* (6.12%), *Phyllanthus emblica* (4.40%), *Thuja occidentalis* (3.73%), *Syzygium cumini* (2.20%), *Callistemon citrinus* (2.20%), *Aegle marmelos* (1.67%) and *Azadirachta indica* (1.53%). Dominance of arboreal taxa in the pollen assemblage in the web samples corresponds to their abundance in local vegetation. Pollen grains of some tree taxa like those of *Terminalia arjuna* (3.82%), *Ricinus communis* (2.29%), *Psidium guajava* (1.53%), *Acacia nilotica* (1.38%) and *Cassia siamea* (1.33%) recorded intermittent presence. All these tree taxa are found in limited numbers only around some of the sites.

Absence of pollen grains of various species of *Ficus* like *Ficus religiosa, Ficus racemosa, Ficus virens and Ficus benghalensis* have been recorded in the pollen spectra, despite being fairly present around the sampling sites. *Ficus* spp. have hypanthodium inflorescence thus there is no discharge of pollen in air resulting their altogether absence in the pollen assemblage.

Among non-arboreal taxa, pollen grains of Poaceae (12.24%) are most abundant recording their presence in all the samples followed by those of Amaranth/Chenopod (7.17%), *Brassica campestris* (6.02%), *Ageratum conyzoides* (5.64%), *Parthenium hysterophorus* (4.88%) and other Asteraceae (2.44%). Poaceae, Amaranthaceae and Chenopodiaceae are stenopalynous families and their representation in the spider webs is the collective contribution of pollen grains belonging to different plants of the families. Availability of crop fields of *Brassica campestris* around the collection sites may be accounted for its good representation in the pollen assemblage while *Ageratum conyzoides* and *Parthenium hysterophorus* are the common weeds growing around the sampling sites.

Holoptelea integrifolia and plants belonging to Poaceae are anemophilous and their pollen grains are recovered in dominant numbers from the web samples. Dominance of *Holoptelea integrifolia* in the pollen assemblage may be ascribed to its high pollen producing capacity while that of Poaceae is due to availability of some or the other grasses in flowering condition round the year. About 50 grasses are known to grow in and around Allahabad[20, 21].

Abundance of the pollen grains of Poaceae, *Holoptelea integrifolia*, Amaran/Chenopod, *Ageratum conyzoides, Phyllanthus emblica, Parthenium hysterophorus, Aegle marmelos* and *Syzygium cumini* in spider web have also been reported by other workers in various parts of the country[8, 10, 11, 13, 14].

It may be mentioned here that pollen of Poaceae and *Holoptelea integrifolia* are also major component of airborne pollen spectrum of Allahabad. Among other types recorded from the web samples Amaranthaceae/ Chenopodiaceae, *Brassica campestris, Parthenium hysterophorus, Thuja occidentalis, Ricinus communis, Syzygium cumini, Aegle marmelos,* and *Azadirachta indica* are also common components of aeropalynoflora of Allahabad[15,17]. The pollen assemblage recovered from the spider webs largely reflects the ground vegetation of the study area and also exhibits the reflection of the aeropalynoflora of Allahabad which in turn is also correlated with the ground vegetation.

The pollen assemblage of spider web samples collected from different localities as well as from the same locality show variation in the pollen composition qualitatively as well as in pollen counts. These variations are found correlated with the type of vegetation and their density around the sampling sites as well as with the age of the spider webs. Four samples (S.W. 1-4) are procured from the same locality but the high representation of pollen grains of *Cassia siamea* is recorded in only one sample which can be attributed to the presence of source plants in good number around the site of S.W. 1 only. Further, among the four samples of the same locality, S.W. 2 represents highest pollen assem-

blage both qualitatively as well as quantitatively. It is noteworthy that the S.W. 2 is the oldest one, therefore age of the spider webs may also be considered as a factor affecting the pollen assemblage.

CONCLUSION

Airborne pollen grains are known to be one of the causative agents concerned with inhalant allergy in human being. Among the pollen grains recovered in dominant number from the pollen analysis of spider webs *Holoptelea integrifolia, Eucalyptus citriodora, Azadirachta indica, Ricinus communis*, Amaranthaceae/ Chenopodiaceae, *Brassica campestris, Cassia siamea, Ageratum conyzoides*, Poaceae, *Parthenium hysterophorus* are reported as allergenic pollen[10, 11, 14, 17]. It is established from the present study that spider webs can be used as an alternative method to trap the airborne pollen grain of an area.

In this way, the spider webs may be regarded as natural traps to assess the contribution of local plants in the pollen assemblage and also to evaluate the aeropalynoflora of a region comparable with the data generated through various pollen traps. Pollen grains trapped in woven web meshes can be easily collected from different localities of a region providing an effective way to study pollen rain-vegetation relationship in a particular geographical region.

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3rd Prof. Sunirmal Chanda Memorial Lecture, 20th IAS Conference, Amravati

AEROPALYNOLOGY IN NORTH EAST INDIA

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Preamble:

Prof. Sunirmal Chanda was born on January, 1932 in Mytkiana of Myanmar where his father was a Government employee. Prof. Chanda had his early education at his ancestral home - Dhaka (now in Bangladesh). As his family migrated from Dhaka to Kolkata, he did his B.Sc. (Hons) and M.Sc. in Botany from Calcutta University and joined the University of Gottingen, Germany in 1960 under the guidance of Prof. F. Firbas in a DAAD Fellowship and obtained his Dr. re.nat. Degree in the field of Quaternary Palynology. Later Prof. Chanda joined Palynological laboratory, Swedish Museum of Natural History of Stockholm under the guidance of Prof. Gunner Erdtman with a fellowship of US Atomic Energy Commission in 1961. He also works with Prof. Knut Fageri of the University of Bergen, Norway for more than one and a half years in the field of Quaternary Palynology. After his successful exposure to the realm of Palynology, Prof. Chanda started his career as a Lecturer in the Department of Botany, Bose Institute, Kolkata and became Professor and Chairman of the same Department in 1980. Prof. Chanda took initiative in establishing Aerobiological Society in India. In 1980, Aerobiologists from different parts of India assembled to attend the workshop on "Modern Trends in Aerobiology with particular reference to Plant Pathology and Medicine" held at Bose Institute, Kolkata under the leadership of Prof. Chanda where the Indian Aerobiological Society (IAS) was formed and started functioning from 31st January, 1980. The scientific contributions of Prof. Chanda were recognized by several learned and professional societies. He was elected President of International Association for Aerobiology (1986-90); Founder President, Indian Aerobiological Society (1980-83); Chairman, International Commission for Aerobiology, I.U.B.S. (1986-90), President of Indian College of Allergy and Applied Immunology (1995-96); President, National Botanical Society, Kolkata (1993-96), etc. Prof. Sunirmal Chanda, a renowned Palynologist and Aerobiologist of International repute, breathed his last on 18 January, 2015 at the age of 83 years. We met for the first time in 1975 during a national workshop on Aerobiology and Antigen preparation at the Department of Respiratory, Allergy and Applied Immunology, V.P. Chest Institute, Delhi University, Delhi. Since then, we met each other during the biannual conferences of the Indian Aerobiological Society. He was a kind hearted person. Since the Indian Aerobiological society has bestowed on me to deliver the third memorial lecture on 2018 in 20th IAS Conference held at Amravati, please accept my humble salute to you, sir.

Key words : Prof. Sunirmal Chanda Memorial Lecture, Aeropalynology, North East India

INTRODUCTION

The Pollen grains are male reproductive units of the flowering plants. After maturity they get liberated and dispersed by wind, insects and other aerial vectors to reach the ultimate destination to execute fertilization.[1] Based on differences recorded in several years of observation in airborne pollen, pollen calendars are drawn as an aid to human allergy diagnosis and management.[2] The article reviews on various aeropalynological investigation carried out at different states of North East India during the last five decades.

Aeropalynology in North-East India can be categorized under two heads, viz.,

(i) Pollen Flora of Extramural Air

(ii) Pollen Flora of Intramural Air

The first paper published from the NE India was Aerospora and Allergic Human Diseases – A Study of Certain Fungal Spores and Pollen Grains of Gauhati.[3] This was followed by - A study of atmospheric pollen grains of Gauhati.[4] Subsequently remarkable research publications appeared from different research centers of the North East India.

RESULTS AND DISCUSSION

Pollen Flora of Extramural Air

Flowering calendar of the plants growing in Shillong and its suburbs (Meghalaya) revealed 500 angiospermic species distributed under 93 families (both wild and cultivated). Out of these, 70 species belonging to 62 families were selected. They were categorized into three groups, viz., i)Trees ii)Shrubs and iii) Herbs (both wild and cultivated). From allergenic point of view, pollen grains of following plants were significant:-Trees (*Melia, Mangifera, Acacia, Callistemon, Eucalyptus, Morus, Salix*, and *Phoenix*); Shrubs and Undershrubs (*Caesalpinia, Rosa, Datura, Lantana, and Ricinus*); Herbs (both wild and cultivated) (*Papaver, Ambrosia, Brassica, Gynandropsis, Lathyrus, Cucurbita, Coriandrum, Ageratum, Artemisia, Tagetes, Ipomoea, Plantago, Amaranthus, Chenopodium, Cannabis, Cyperus, Cynodon, Digitaria, Eleusine, Oryza, Panicum, Poa, Setaria and Zea*)[5].

Shillong (Meghalaya) atmosphere was never pollen free but roughly two pollen grain seasons were noted. Herbs and shrubs predominate from June to October and tree from February to April. Great fluctuations in the quantitative incidence of pollen grains were observed[6]. Sixty pollen grain types were identified from the air of Shillong (Meghalaya). Out of which, herbs constitute 20.55%, whereas shrubs and tree constitute 5.95% and 73.5% respectively. Dominant pollen grain types were *Cannabis, Coriandrum, Datura, Eucalyptus, Gramineae, Morus, Oryza, Plantago, Salix, Zea*, etc[7].

Flowering calendar of plants growing in and around Imphal (Manipur) revealed 4 (four) groups viz. tree, Shrubs and Undershrubs, climber, and Herbs (wild and cultivated). Out of total 257 species distributed in 96 families of wild and cultivated angiospermic plants, trees contributed 57 species under 29 families, shrubs and undershrubs contributed 25 families, consisting of 68 species, herbs contributed the highest number of flowering plants with 155 species under 36 families. Whereas only 17 species of climbers and 6 families were recorded. *Hibiscus rosa- sinensis, Acacia nilotia, Callistemon, Canna, Rosa* sp., *Solanum melongena, Lantana camera, Duranta* and *Bougainvillea*, were found flowering almost all round the year[8]. A survey of airborne pollen grains of Imphal (Manipur) revealed Poaceae as the first rank with a mean contribution of 38.44% of the total pollen counts followed by *Eucalyptus* (8.87%), *Alnus* (6.43%), *Pinus* (5.87%), *Amaranthus* (2.07%), *Chenopodium* (1.68%), etc. Monthly variations were more frequent quantitatively than annual variations[9]. Study on airborne allergenic pollen grains of Imphal (Manipur) revealed 28 pollen grain types. Poaceae ranked first followed by *Eucalyptus, Oryza, Alnus, Cyperus*, Amaranth–Chenopod types, *Callistemon*, etc. Three major pollen incidence seasons (July-September, November-December and January-March) were observed[10].

A comprehensive pollination calendar of flowering plants of Kakching area (Manipur) in respect of their prevalence, mode of pollination and period of flowering were prepared. They were categorized into four groups, viz., (i) Trees (50 species), (ii) Shrubs and Undershrubs (50 species), (iii) Climbers (16 species), and (iii) Herbs (69 species). From the allergenic point of view, the following plants were common in Kakching area viz., Trees (*Melia azadirachta, Mangifera indica, Callistemon linearis, Eucalyptus globolus, Morus alba, Albizia lebbeck* and *Cassia fistula*); Shrubs and Undershrubs (*Cassia leavigata, C. sophera, Rosa indica, Datura metel, Lantana camara,* etc.); and Herbs (*Papaver, Argemone, Ageratum conyzoides, Amaranthus spinosa, Chenopodium album, Cynodon dactylon, Poa anunus, Zea mays*, etc.)[11]. Day to day, diurnal and seasonal variations on the incidence of airborne pollen grains of Kakching (Manipur) revealed 80 (eighty) pollen types. Herbs, Shrubs

and tree pollen grains contributed 5.87%, 17.4%, and 2.9% of the total pollen types identified during 1986-87, whereas herbs, shrubs and trees contributed 52.91, 17.5% and 27.7% of the total pollen types categorized during 1987-88[12].

A total of 52 (fifty two) known allergenic plants were recorded from Thoubal district (Manipur). Out of these, trees contributed 15 species; Shrubs and Undershrubs–13 species; Herbs – 20 species, and Climbers – 4 species under 33 families. Among the known allergenic plants, cultivated types include *Mangifera, Melia, Callistemon, Eucalyptus, Amaranthus, Coriandrum*, etc. Whereas the wild types include *Datura, Cyperus, Ricinus, Parthenium, Plantago,* etc [13]. Outdoor pollen airspora of Thoubal district, Manipur revealed 14 (fourteen) pollen morphotypes during 1996-1998. They are correlated with meteorological parameters. A total of 7447.3/m^3 pollen grains were trapped, out of which 7002.6m^3 were identified. The common types were *Acacia, Artemisia, Eucalyptus, Poaceae, Ricinus, Solanum*, etc. Statistical analysis revealed that pollen airspora was significantly higher in the 1st year (Y1) (1st October, 1996 to 30th September, 1997) than that of the 2nd Year (Y2) (1st October, 1997 to 30th September, 1998)[14].

Aeropalynology of two semi-urban sites of Guwahati city (Assam) revealed maximum incidence of pollen grains in the Month of April (Khanapara poultry firm) whereas maximum incidence of pollen grains in the month of May and September (Kahikuchi horticultural farm). The dominant pollen types recorded were *Drypetes roxburghii* and Poaceae [15]. Incidence of airborne pollen grains in Central Guwahati (Assam) revealed Poaceae type as dominant pollen type followed by Amaranth- Chenopod type and Asteraceae. It was observed that anemophilous pollen ranked the first in order of dominance. Herbaceous pollen showed maximum incidence. Some allergenically significant pollen recorded were Amaranth- Chenopod type, Asteraceae, Poaceae, *Azadirachta indica, Mangifera indica, Drypetes roxburghii, Eucalyptus*, and *Terminalia cuneata*. Highest pollen catch was observed during the month of April. The total pollen as well as individual pollen types displayed distinct seasonal periodicity in their incidence. The results were statistically found to be significant[16].

Atmospheric pollen concentration in humid tropical climate of Silchar (South Assam) revealed Poaceae pollen grains as dominant types followed by Cassia, Asteraceae, Mimosaceae, etc. The maximum pollen concentration was recorded during winter season – February (717.2m^3 air) followed by December (660/m^3 air), September (493.3/m^3 air) respectively. The dry period (September to February), recorded maximum pollen concentration per m^3 of air. Known allergenic pollen types like *Acacia, Cocos, Cassia, Ricinus*, etc. were common in the atmosphere during the survey period. Winter and spring seasons were found to favour higher, atmospheric pollen concentration in Silchar (South Assam)[17].

Floristic composition and aerobiology of Udaipur town (Tripura) revealed 467 angiosperm species under 238 genera and 102 families. Asteraceae was found to be predominant followed by Poaceae, Cyperaceae, Fabaceae, Euphorbiaceae, Lamiaceae, Acanthaceae, and Scrophulariaceae. Most of the plants showed flowering during spring followed by rainy season and winter. Aerobiolgical survey revealed 27 pollen grains types. The dominant types were grasses followed by *Acacia, Cocos, Areca* and Cyperaceae[18]. Aerobiological survey in Agartala (Tripura) revealed two allergenic pollen types (*Trema orientalis, Peltophorum inerme*) with peak concentration of *Trema*. Its pollen grains occurred during February – March, whereas *Peltophorum* pollen grains occurred during April-May. The environmental factors such as temperature, rainfall and relative humidity played a vital role in pollen release and dispersal [19]. Flowering calendar of trees in Agartala (Tripura) revealed 121 species belonging to 87 genera. Among the 121 species, 93 species show entomophilous pollination while the remaining species are anemophilous and some species are allergenically significant [20]. Airborne pollen grains at Suryamaninagar (Agartala) revealed 43 pollen grain types. Most dominant taxa were Poaceae (67/m^3), *Syzygium cumuni* (58/m^3), *Ailanthus integrifolia* (50/m^3), *Trema orientalis* (47/m^3) , *Toona ei-*

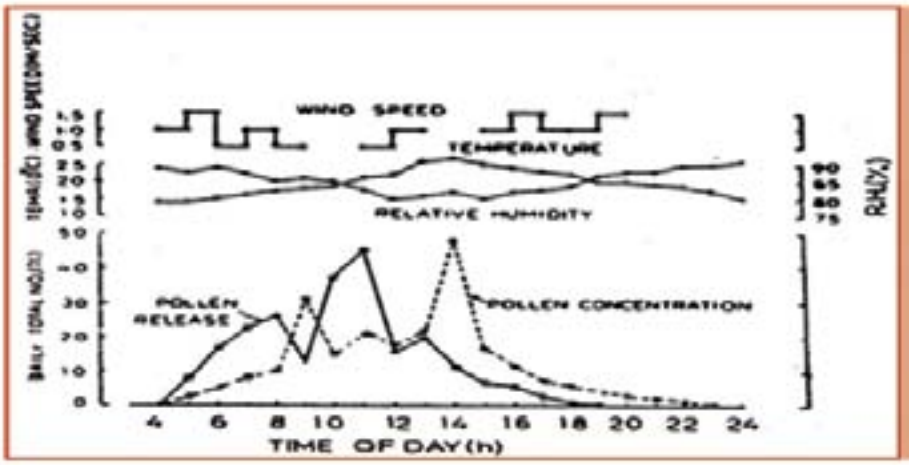


Fig 1 . Pollen release (%) and pollen concentration (%) of Ricinus communis in relation to meteorological parameters

hita (92/m^3) and *Schima wallichii* (92/m^3). The low incidence of Chenopod - Amaranth pollen grain types may be due to less number of plants in the surrounding area [21].

Pollen release and Pollen Concentration

The relation between pollen release and pollen concentration in the atmosphere of Kakching (Manipur) was determined by simultaneous measurement of pollen release and pollen trapping by employing Rotorod air sampler. The quantity of pollen in the ambient air was the outcome of the pollen grains discharged from the mature anthers. The pollen grains were discharged through splitting of anthers or through special pores of the anthers. Flowering phenology of the particular taxon helps in the quantification of daily pollen release. The time of pollen release was affected by the meteorological parameters (Fig: 1)[22].

Pollen production

Pollen production data per flower of 10 (ten) human allergenic plants of Shillong (Meghalaya) revealed *Datura metel* produce maximum (257,250 per anther and 12, 86,250 per flower) while *Lantana camara* (300 per anther and 3000 per flower) produce the lowest[23]. Further, pollen production data per flower of 5 (five) *Cassia* species of Kakching

Table 1 : Pollen Productivity of some common species of Poaceae growing in the Gauhati University Campus (Assam)

Name of species	No. of stamen (anther) per floret	No. of pollen per anther	No. of pollen per floret	Pollen size (μ)
Aronopus compressus (Sw.) Beauv.	3	162	486	22.5-27.5
Chrysopogon aciculatus (Retz.) Trin	3	512	1536	22.5-35
Cynodon dactylon (L.) Pars.	3	864	2592	22.5-32.5
Dactyloctenium aegyptium (L.) Willed	3	108	324	31.2-36.3
Eleusine indica (L.) Gaertn.	3	212	636	17.5-25
Eragrostis unioliodes (Retz.) Nees ex Steud	2	184	368	25-32.5
Imperata cylindrica (L.) Beauv.	2	1,231	2462	60-85
Oplismenus burmanii (Retz Beauv.)	3	227	681	25-27.5
Oryza meyriana (Zollinger & Moretti ex Steud) Baill.	6	3,836	23,016	39-40
Paspalidium flavidum (Retz.) A. Camus	3	1,020	3060	27.5-37.5
Paspalum conjugatum Berg.	3	40	120	25-40
Pogonatherum crinitum (Thunb.) Kunth	1	422	422	22.5-28.8
Setaria glauca (L.) Beauv.	3	284	852	35-46

Table 2 : Frequency of Pollen fall picture for *Cannabis sativa* L.

Pollen units	Observations					
	I	II	III	IV	V	Average
1.	35	23	37	30	28	30
2.	6	1	2	1	2	2
3.	1	1	0	1	0	1
4.	0	0	0	0	1	1
5.	1	0	0	0	0	1
6.	0	0	0	0	0	0
7.	0	0	0	0	0	0
8.	0	0	0	0	0	0
9.	0	0	0	0	0	0
10.	0	0	0	0	0	0

area (Manipur) revealed maximum production by *C. laevigata* (22,700 per anther and 45,280 per flower) while *C. tora* produce the lowest (324 per anther and 15,000 per flower) [24]. Pollen production per anther and per floret of certain common species of Poaceae growing in the Gauhati University Campus (Assam) revealed pollen production per anther and per floret range 40 to 1,231 per anther and 120 to 23,016 per floret respectively (Table 1).The maximum incidence of grass pollen in the air was during September to November [25].

Pollen fall picture

In pollen fall picture[26] of nine angiospermic plants of Kakching area (Manipur) revealed 1-2 unit for 5 taxa (*Artemesia vulgaris, Cannabis sativa, Cynodon dactylon, Cyperus rotundus,* and *Ageratum conyzoides*), 1- ≥2 for one taxa (*Plantago major*) and 1 or 2 or ≥10 for 2 taxa (*Carica papaya, Ricinus communis*) (Table 2).

Horizontal and Vertical profile of airborne pollen grain

Monthly average number of *Parthenium hysterophorus* pollen at four sampling sites (Tiddim road, Heiran

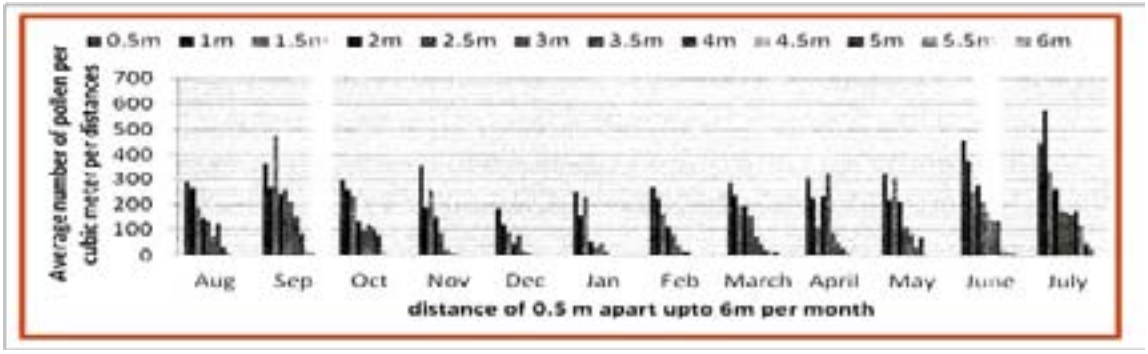


Fig 2 : Month wise average number of *Parthenium* pollen grains of four different sites in Imphal city (m^3 /distances of *0.5m apart) in horizontal profile studies (Aug., 2009 – July, 2012)*

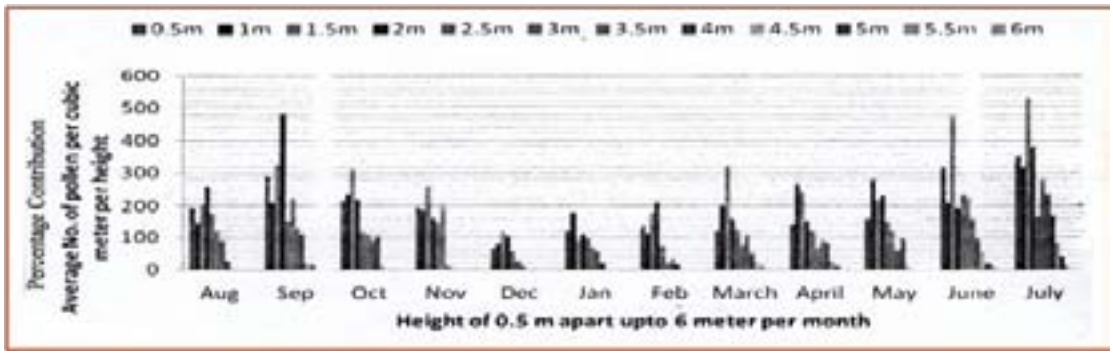


Fig 3 : Month wise average number of *Parthenium* pollen grains at four different sites in Imphal city (m^3 /height of 0.5 m apart) in vertical profile studies (Aug., 2009– July, 2012)

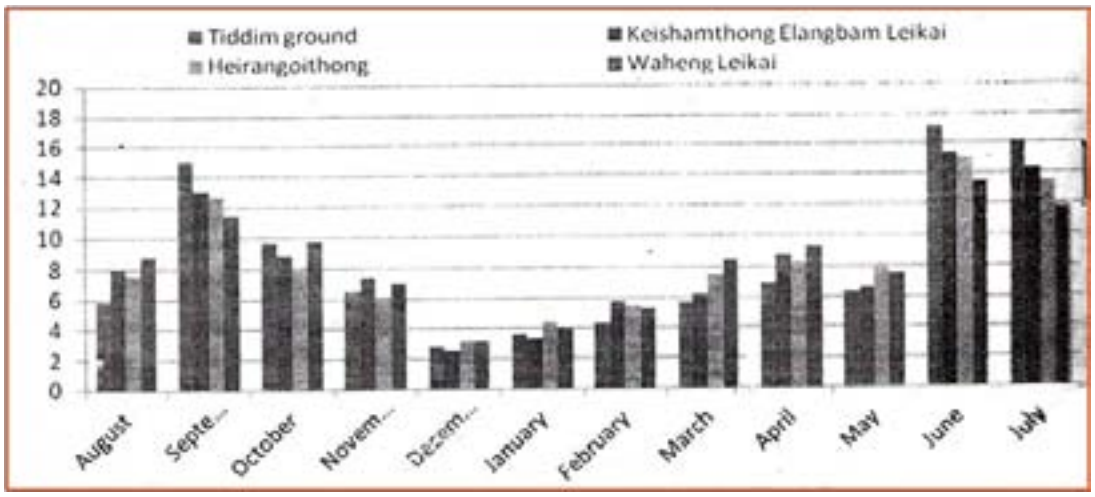


Fig 4 : Month wise percentage contribution of *Parthenium* pollen out of annual total *Parthenium* pollen count in horizontal profile studies (Aug., 2009 – July, 2012)

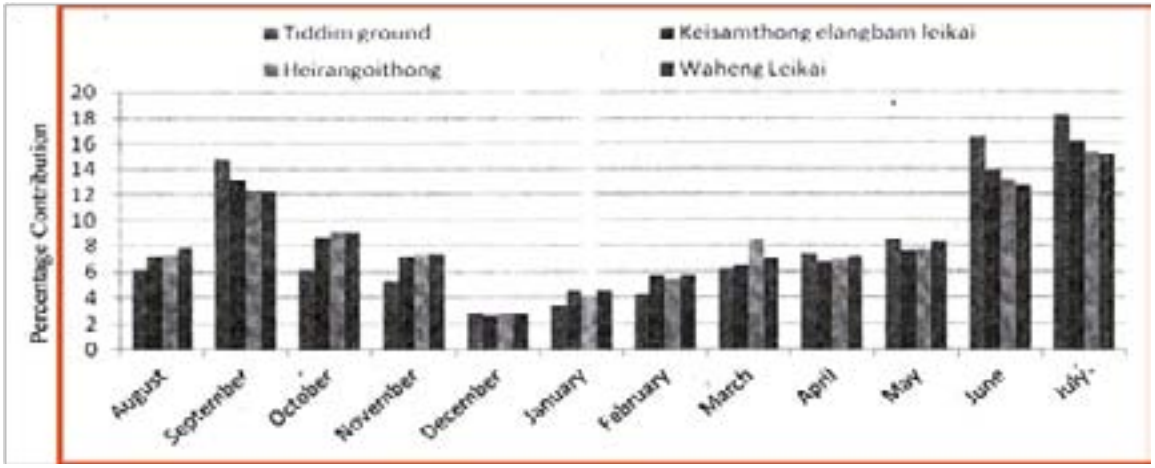


Fig 5 : Month wise percentage contribution of *Parthenium* pollen out of annual total *Parthenium* pollen count in vertical profile studies (Aug., 2009 – July, 2012)

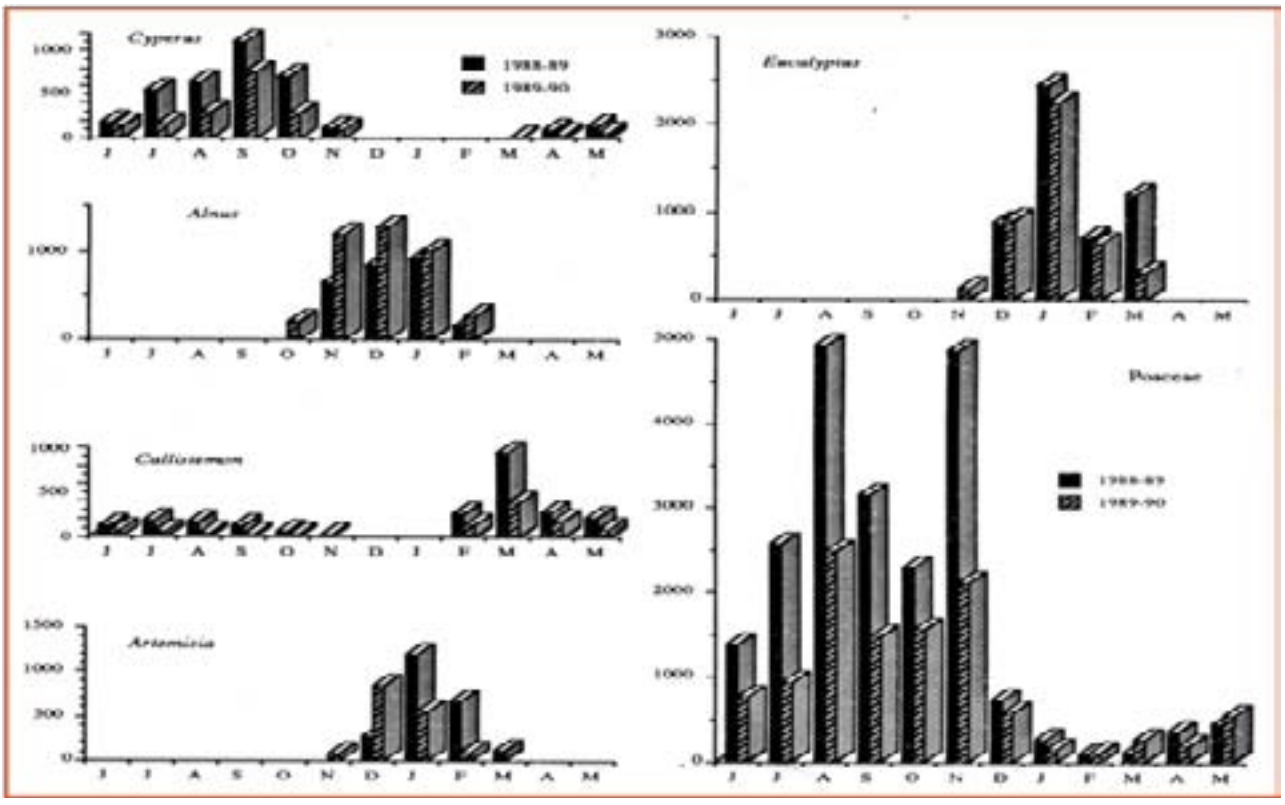


Fig. 6a : Monthly collection of allergenic pollen grains for 2 consecutive years (June, 1988 to May, 1990)

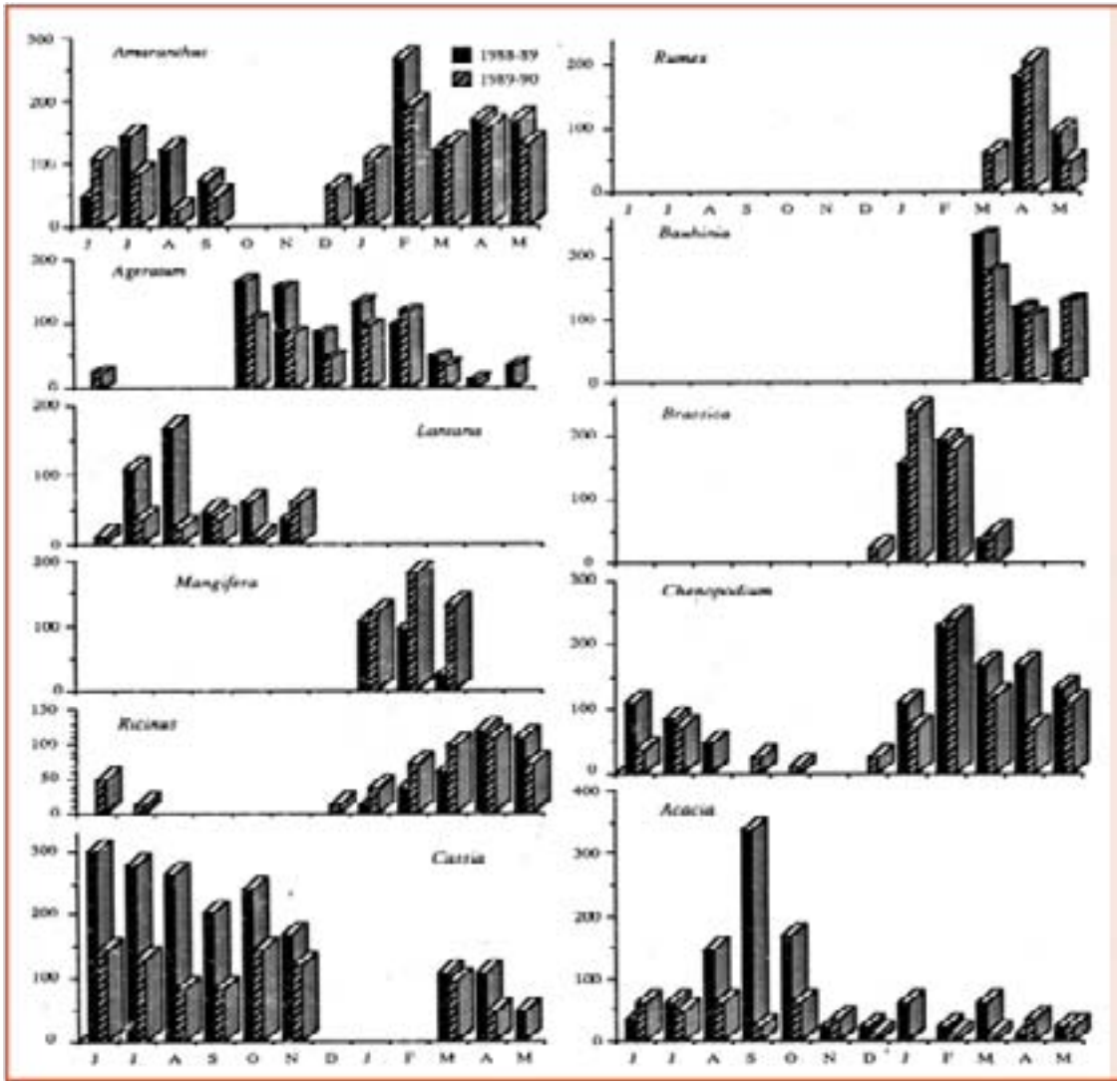


Fig. 6b : Monthly collection of allergenic pollen grains for 2 consecutive years (June, 1988 to May, 1990)

goithong, Keishamthong Elangbam Leikai and Waheng Leikai) were found high at a distance of 1m from the plants in horizontal (580 pollen/m^3) (Fig:2) and 1.5 m in vertical (530 pollen/m^3) in July (Fig:3). Pollen grains of *Parthenium* were present round the year. The highest monthly pollen concentration was found in June and September in horizontal profile (Fig: 4), whereas in the vertical profile the highest pollen concentration was found in July followed by June and September respectively (Fig: 5) in the four sampling sites. The correlation between the frequency of the average *Pathenium* Pollen grain number of horizontal and vertical for 4 different sampling sites using t-test was found to be 3.3 at 0.05% significance. It was observed that temperature (25-28 ^oC); wind speed (4-5 km/h); RH (60-70 %); bright sun and rain free days were favorable for high *Parthenium hysterophorus* pollen grain catch [27].

Aeropalynology and Allergic human diseases

The roles of aeroallergens such as Pollen grains, etc. in causing allergic human diseases are well known. Some of the important and outstanding research contributions in this field from the NE India are described below: Monthly, seasonal and year wise variations in the airborne allergic pollen grains of Imphal were studied. Monthly variations were more frequent than year wise variations. The monthly frequency of allergenic significant pollen are listed graphically (Fig. 6a-c) [28].

Serological and Amino acid analysis studies

Pollen grains of Grass, *Amaranthus*, Goldmohor, Paddy, *Ricinus*, and Garden poppy induce antibody production in the blood of rabbits as characterized by serological reactions, thus showing their antigenic and protein nature[29]. The allergenic factor in the above noted pollen grains were chemically analyzed and found the presence of amino acids such as Cystine, Lysine, Histidine, Aspergine, Tyrosine, Ar-

Table 3 : Amino acids from surface washing of Pollen grains

Spot number	Amino acids mixture Rf value control	Rf value of pollen washing Amino acid						Amino acid
		Grass	Paddy	Amaranthus	Goldmohor	Ricinus	Garden poppy	
1	0.16	0.16	0.16	-	-	-	-	Cystine
2	0.19	-	-	-	-	-	-	Lysine
3	0.21	0.21	-	0.22	-	-	-	Histidine
4	0.25	-	-	-	-	-	-	Aspergine
5	0.34	-	-	-	-	-	-	Glycine
6	0.39	-	-	-	-	-	-	Proline
7	0.42	-	-	-	-	-	-	Alanine
8	0.52	-	-	-	-	-	-	Tyrosine
9	0.63	-	-	-	-	-	-	Methionine
10	0.67	-	-	-	-	-	-	Tyrptophan

** From Controls the identity of the unknown amino acids have been fixed

Table 4 : Results of agglutination between homologous antigens and antibody

Antigen used	Antibody litre							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Grass	-	-	-	-	-	-	-	-
Paddy	-	-	-	-	-	-	-	-
Ricinus	-	-	-	-	-	-	-	-
Amaranthus	-	-	-	-	-	-	-	-
Goldmohor	-	-	-	-	-	-	-	-
Garden poppy	-	-	-	-	-	-	-	-

(++) = 50% agglutination, (+) = 25% agglutination, (-) = not agglutinate

ginine, Glycine, Alanine, Proline, and Tryptophan in varying proportions[30]. The pollen grains of above mentioned types were also found in the atmosphere of rooms occupied by Asthmatic patients. The surface washings of the pollen grains of Grass, Paddy, Ricinus, Amaranthus, Goldmohor and Garden poppy were chromatographically studied for the presence of amino acids[31] (Table 3).

Further, serological studies were also made to determine the nature of antigenic reaction by injection of surface washings of pollen grains in chickens. In that, the experimental chickens were seen retardation of growth (Table 4)[31].

Clinical investigation and allergenic skin test

An allergenic diagnostic camp was organized for clinical investigation and diagnosis of suspected allergic patients of greater Silchar, Assam. A total of 140 allergic patients had attended the diagnostic camp (Plate IA-C. A total of 15 (fifteen) patients were selected for the allergy skin test. Intra-dermal skin test were done on 2 (two) patients and skin pick test was done on the rest of 13 selected allergic patients. Out of the 15 patients tested, 50% were having skin allergy. Most of the patients have shown skin positive reaction to pollen grains (Plate 2A and 2B).

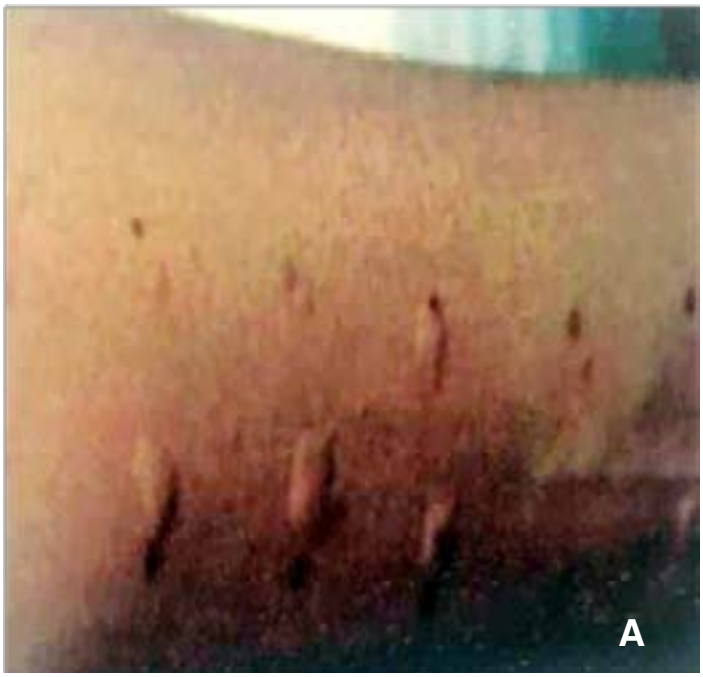
Among the antigen tested, *Acacia auriculiformis* had shown positive result in maximum number of the patients tested. Higher grade of allergencity was recorded in the extract of *Cleome gynaendra, Cocos nucifera, Trewia nudiflora,* etc. all showing +3 and +4 grade of result [32].

Plate 1A

Plate1B

Plate1C

Plate 1A,1B and 1C : Patients attending the diagnostic camp



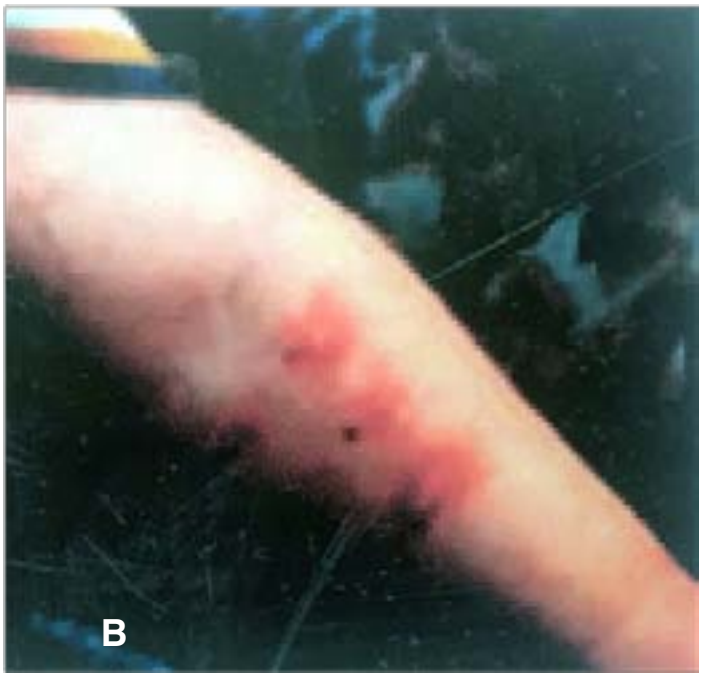


Plate 2 : Development of wheal (A) and erythrema (B)

Pollen flora of Intra-mural air

Pollen flora of two intramural sites in Shillong (Meghalaya) viz., a)hospital ward (b) Cinema hall were investigated. In all, 35 and 37 pollen grain types were identified from the air of hospital and cinema hall respectively. Pollen grains of Amaranth-Chenopod types identified. Pollen grains of Gramineae or Poaceae, Cyperaceae, *Pinus khasya, Salix babylonica,* etc. were found in both indoor sites[33].Further, analysis of aerospora inside the rooms occupied by patients (Male medical ward, Civil hospital, Shillong) revealed 34.24% *Pinus* pollen while 33.19% Gramineae or Poaceae pollen grains of the yearly total counts [34]).

Aeropalynology and Bioinformatics

The aerobiological database developed contain morphological feature, key characters for identification, their seasonal occurrence and structures of airborne micro data like spores fungi, blue green algae and pollen grains (outdoor and indoor air). The database is implemented using 3-tier architecture utilizing MySQL for storing data, PHP as open source middle layer between database and web server, HTML and CSS for designing web interface. Apache is used as Web Server which is installed in Windows XP. ADMAN database is divided into three sections viz (a) Airborne Blue green algae Database for Manipur (b) Airborne fungal

Database for Manipur and (c) Airborne pollen grains Database for Manipur. Approximately 186 airborne biota have been uploaded in this databe[35]. Using the same technique noted above, 97 air borne pollen types have been uploaded into database from works done in the Manipur University. The database will act as a resource for the information about species found in Manipur and toxin containing in 6 (six) pollen family types[36]. These databases will provide details relating to pollen morphology, quantitative morphometry, plant habitat, growth characteristics and geographical distributions and each species will be illustrated through several images [37].

CONCLUSION

Aeropalynological studies in North-East India have been reviewed under two heads, viz., (i) Pollen flora of extramural air and (ii) Pollen flora of intramural air. Under pollen flora of extramural air, compiled the flowering calendar of regional flora and correlated with the incidence of airborne pollen grains. And also studied pollen production, pollen fall picture, pollen release and concentration including horizontal and vertical profile of airborne pollen grains. The role of airborne pollen grains in causing allergenic human diseases were studied in the context of (a) monthly, seasonal and year wise variations, (b) surface washing for the presence of amino acids, (c) serological nature of antigenic reaction and (d) clinical investigation followed by skin test. Under pollen flora of intramural air, the pollen flora of hospital and cinema hall has been identified. Digitalized aeropalynological data for regional pollen identification.

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