FINAL REPORT OF UGC MAJOR RESEARCH PROJECT

"EPITOPE MAPPING OF THE ALLERGENIC PROTEINS OF THE POLLEN OF A FEW ALLERGENIC PLANTS GROWING IN KOLKATA: IMPLICATIONS FOR IMMUNOTHERAPY AND STUDIES ON THE AIR POLLUTION IMPACT ON THEIR ETIOLOGY"

Ref. No. F.42-559/2013 (SR), dt. 22.03.13

Dr. Sanjukta Mondal (Parui)

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Annexure – IX
UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Final Report of the work done on the Major Research Project

1	**Title of research project**	"Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: implications for immunotherapy and studies on the air pollution impact on their etiology"
2	**(a) Name of the Principal Investigator**	Dr. Sanjukta Mondal (Parui)
	(b) Address	Tower 4, Flat no. 3H Diamond City West 18 Ho Chi Minh Sarani Kolkata – 700 061, W.B.
3	**Name and Address of the Institution**	Department of Zoology Lady Brabourne College P1/2, Suhrawardy Avenue Kolkata – 700 017, W.B.
4	**UGC approved Reference No. and Date**	F.42-559/2013 (SR), dt. 22.03.13
5	**Date of implementation**	15.7.2013
6	**Tenure of the Project**	2013 – 2017 (March)
7	**Total grant allocated**	Amount approved: Rs. 13,05,800/- (according to sanction letter dated 22.3.2013) Amount allocated: Rs. 12,50,574/- (according to sanction letter dated 15.10.2015)
8	**Total grant received**	Rs. 11,67,297/-
9	**Final expenditure**	Rs. 13,05,800/-

10. TITLE OF THE PROJECT

Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: implications for immunotherapy and studies on the air pollution impact on their etiology

11. OBJECTIVES OF THE PROJECT

The present study was undertaken to identify the allergenic protein fractions of the pollen of three related species of *Datura* (*Datura metel, Datura inoxia* and *Datura stramonium*) and Epitope mapping of the allergenic proteins of the pollen of *Datura* sp. in order to identify and characterize the binding sites of antibodies which can aid in the development of new therapeutic vaccines for successful immunotherapy. Thus the overall study involved the following:

- Ultrastructure study of the pollen by Light microscopy, SEM and TEM for proper identification of the airborne pollen to which a patient is exposed to in his or her immediate vicinity and to study any specific features of pollen morphology as well as the ultrastructure that might have the potential to influence its allergenicity.
- Extract, quantify and study the soluble protein profile of the pollen of the three related species of *Datura* using standard methods and gel electrophoresis and partial characterization of the proteins (determining the molecular weights).
- Study the variation in protein profile with maturity of pollen (before and after anthesis).
- Isolation of the individual protein fractions by gel filtration.
- Identification of the allergenic protein fractions by performing Ouchterlony Immunodiffusion and ELISA with blood plasma of *Datura* sensitive patients.
- Development of antibodies in Male LOBUND –Wistar rats and study cross reactivity among the 3 species of *Datura* by Ouchterlony Immunodiffusion and ELISA to identify the common proteins fractions.
- Crosslinking coupled Mass Spectrometry for epitope mapping. Detection and identification of antigen-antibody binding location with high mass MALDI detection (high resolution mass spectrometry or MS/MS techniques) and amino acid sequencing of the epitopes.

12. WHETHER OBJECTIVES WERE ACHIEVED

Yes, the progress has been according to original plan of work and towards achieving the objective. However more number of species could be investigated. But due to lack of time and the extensive investigations to be done with each species, the study was restricted to 3 species.

13. ACHIEVEMENTS FROM THE PROJECT

- The investigation carried out under the project helped to identify the allergenic proteins of the pollen of three species of *Datura* which has been found to have a role in allergenic rhinitis causing allergy in sensitive patients. Till date this pollen has been largely ignored as a major airborne allergen keeping in mind its entomophilous nature. Yet the present investigation on its pollen ultrastructure shows several characters exhibited by anemophilous or wind borne pollen and this pollen has been reported by several workers in their routine aeropalynological surveys in the airspora of Kolkata and its adjoining areas.
- Isolation and identification of the actual allergenic proteins by gel electrophoresis, Ouchterlony Immunodiffusion and ELISA and Epitope mapping by Crosslinking coupled Mass Spectrometry with high mass MALDI detection helped to identify the binding location and sequencing of the amino acids. This will help in the preparation of appropriate and suitable vaccines for Allergen Specific Immunotherapy and reduce the risk of the contaminating constituents unrelated to the few allergens to which a patient is actually allergenic which can lead to the induction of IgE antibodies with new specificities and to untoward effects including anaphylaxis.

14. SUMMARY OF THE FINDINGS

Based on the present work, it can be concluded that the three species of *Datura* studied were palynologically distinct. The results demonstrated variations in the exine ornamentation, a character usually utilized in taxonomic delimitations. However there are examples where minimal morphological variation precludes distinguishing pollen of individual taxa from one another, resulting in all types being lumped together into one class. In case of *Datura* even if differences between the three closely related species can be identified, there remains a degree of overlap that prevents firm classification of every grain. Hence additional studies are necessary (principally employing TEM) to provide more detailed observations of some structures particularly the detailed structure through the pollen wall.

A comparative account of the SDS-PAGE protein profile of the pollen of the three species shows that *Datura metel* exhibited the maximum number of bands (20) and the remaining two species has 16 bands each. The molecular weights of the proteins ranged between 205 kDa and 15 kDa. Immunodiffusion and ELISA with the individual protein fractions obtained by gel filtration and the pooled blood serum of sensitive patients (patients sensitive to the pollen of *Datura*) helped to identify the allergenic fractions of the three species of *Datura.* The precipitation arcs were obtained with 11 protein fractions having molecular weights of 205 kDa, 183.5 kDa, 172.7 kDa,, 108.2 kDa, 66 kDa, 59.4 kDa, 29 kDa, 21 kDa, 17.6 kDa, 16.4 kDa

and another protein having molecular weight >205 kDa. Of these the 205 kDa, 66 kDa and 59.4 kDa protein fractions were the major allergenic fractions found in all the 3 species.

The allergenic proteins were analyzed by MALDI and the protein sequencing of twelve allergenic fractions done. The thirteenth allergenic fraction having a molecular weight greater than 205 kDa could not be sequenced.

15. CONTRIBUTION TO THE SOCIETY

Significance of the study:

Pollen of *Datura* sp.

Datura is an herbaceous, leafy annual herb growing in the wild in all the warmer parts of the world, such as India and is cultivated worldwide for its chemical and ornamental properties. It is a common herb growing along road sides, in open fields, wastelands and even grown in gardens for the erect or spreading, trumpet-shaped, 6-8 inches, pleasantly-scented flowers varying in color from white to yellow, pink, and pale purple. This plant can propagate easily as the fruits which are a spiny capsule split open releasing the numerous seeds. The plants tend to reseed themselves and may become invasive. Hence this plant has been found to grow as a weed in certain waste lands. According to Hindu rituals the flowers of this plant are used in prayers to Lord Shiva. Hence the flowers of this plant are used extensively by Hindu women to offer prayers to Lord Shiva and is, a common flower sold in most road side flower stalls.

The pollen of *Datura* sp. has been found to have a role in allergenic rhinitis causing allergy in sensitive patients. In spite of this, this plant has till date not been considered to be a serious allergenic hazard (Parui and Mandal, 1998). One of the major reasons behind this is the entomophilous nature of this taxa. In case of pollen allergy, greater attention has been given to anemophilous pollen by aerobiologists, with the entomophilous pollen being neglected in their routine aeropalynological surveys because of their rare occurrence. Contrary to this belief, surveys have reported the presence of entomophilous pollen from the air-spora (Agashe, 1989; Agashe et al., 1983, Atluri et al., 1992; Singh and Babu, 1982; Singh and Devi, 1992; etc.). Moreover pollen grains tend to be distributed in dense concentrations around their sources and therefore tend to be of local occurrence (Gregory, 1961). This is seen more in case of entomophilous pollen, which remain in high concentrations in air near the source plants (Durham, 1947). The pollen of *Datura* has been reported in the air by several workers (Santra et al., 1991; Jain et al., 1992) and the allergenic potency has been proved (Santra et al., 1991; Jain et al., 1992; Parui and Mandal, 1998). However one has to keep in mind that although this plant can potentially cause allergies in some individuals, not everyone is affected by them. An important factor for sensitive patients is that how common and widespread these plants are around the place where they live. With the different species of *Datura* being dominant in the different localities particularly in West Bengal, it seems impossible for the sensitive patients to avoid this allergen. An earlier study on

the skin sensitivity tests with the extracts of the pollen of *Datura metel* revealed that women were almost equally affected as males and most of the affected patients were between the age group of 31-40 years, although patients were reported between the age group of 9-56 years (Parui and Mandal, 1998). Allergenic incidences were common among housewives, most of whom used the flowers of this plant on a daily basis for worship or those who cultivated this plant in their gardens either for ornamental purpose or flowers for ritual purposes. Gardeners don't let a little physical discomfort stop them from gardening. One can avoid these allergies and discomforts, if one chooses the right plants. The severity of symptoms tends to vary throughout life; many people experience periods when they have no symptoms at all. Unfortunately the different species of *Datura* are found to flower round the year and the people sensitive to the pollen of this plant have very little chance to avoid this allergen.

Pollen morphology

One of the important aspects of aerobiological studies with the subsequent diagnosis of allergy, is determining the source of these airborne biopollutants. However, the vastness of the Indian flora together with the incidence of abundant airborne pollen grains have posed to be a serious problem to aerobiologists, for whom identification of the source materials in context of their release process in time and space, has become a tedious task. The type of pollen prevalent in the atmosphere of a particular region, generally belong to the plants growing in that region and have been found to synchronize with the flowering periods i.e. the pollen generally belong to the plants flowering during that period. Further, allergy is not always an “all or none” phenomenon, as allergy to a number of different allergens in varying degrees is a rule rather than an exception in case of chronic allergic disorders, where total allergen load, rather than single allergen is responsible for producing the symptoms. In such cases, an attempt to pinpoint an allergen as the cause of a patient’s symptoms becomes an exercise in futility (Bapat and Bapat, 1994).

Pollen morphology plays an important role in the identification of the plant taxa to which a airborne pollen belongs to i.e. the mother plants. This is very important as lack of sufficient knowledge in the palynology of the present day plants can lead to erroneous identification of airborne pollen, subsequently leading to wrong interpretation or diagnosis of a patient’s symptoms. The morphological characteristics of pollen grains are manifested in the exine. Important pollen atlases have been published based on morphological characters of the native flora. The basic structure of pollen grains is well known as many publications give a detailed description of it. However detailed descriptions and highly magnified images of the specimens analyzed in such accounts are often lacking leading to error in identification of the pollen allergen.

Secondly many investigators have used different palynological terms to describe their results, which create a lot of confusion. The exoaperture and endoaperture are the

highly determined constituent parts of pollen grains, containing colpi, costae and fastigia (Kosonko and Sventorzhetskaya, 1999; Larsen and Barrett, 2000). Generally pollen grains have a double wall - intine or endospore and exine or exospore. The exine often bears spines or is variously sculptured giving the characteristic difference between different species often termed as echinae or microechinae. But this exine structures are point of variations and contradictory terms because some investigators have used scabrae in place of microehinae. Colpi appears to be surrounded by halo like structure under LM because of a space like girdle or band that separates the colpi margins from the other parts of the mesocolpium (Harley and Baker, 2001; Hesse, 2002). These apertures are one of the route for release of allergens by expulsion of the cytoplasmic contents through rupture of the pollen grains upon contact with water (Bacsi et. al., 2006; El-Ghazaly et. al., 1999; Grote et. al., 2000; Taylor et. al., 2004). So for the palynological study and to better understand the allergenicity of pollen, it is necessary to investigate not only the morphological structure of pollen but also pollen class, polar equatorial measurements, P/E ratio, pollen outline, endoaperture and ectoaperture structures, to see whether some palynological variations among the examined species will exist and will be relied on for such differentiation (Moore and Web, 1978; Victor and Van Wyk, 2000).

Thirdly, the circumscription and taxonomic relationships between very similar species has been sometimes controversial. Along with other characters, the differences in pollen grain wall structure and exine ornamentation (sculpture) are found to be valuable characteristics for distinguishing between such species. The present study will contribute to our understanding of pollen systematic determinations and allergenic analysis but also in studies related to aerobiology, melissopalynology and reproductive biology.

Importance of epitope mapping in Immunotherapy

One of the most successful treatment for allergic rhinitis is immunotherapy, also called hypo-sensitization, which involves repeated subcutaneous injections of gradually increasing concentrations of the allergen(s) considered to be specifically responsible for the symptom complex. Controlled studies have established that patients are partially relieved of their symptoms by such treatments applied over a period of years. Unfortunately, allergen specific immunotherapy (ASIT) as practiced today has not only proved to be cumbersome and expensive, but is also associated with the risk of fatal side reactions. This is because commercial antigenic extracts used for immunotherapy consists of heterogeneous (crude) extracts of the pollen (complex mixture of several proteins, lectins, complex carbohydrates, lipids, nucleic acids, enzymes etc.) and may contain the relevant allergens in minute, irreproducible amounts leading up to 100 X variations in allergenic potency. Pollen antigens generally comprise 0.5 to 1.0% of the total extractable pollen proteins. To standardize such extracts, it is important to analyze their component allergens and establish a quantification system for major allergens

(American Academy of Allergy, Asthma and Immunology, 1997). The World Allergy Organization (WAO) recommends that standardized vaccines be used for immunotherapy. However, the protocols and methods for the standardization of allergen extract are different among different suppliers, which use their own in-house reference materials and their own unique allergen units. This makes it difficult to compare the therapeutic effects and safety among clinical trials involving different products. It has been proposed that the active ingredients of the treatment be quantified. To improve the safety and clinical therapeutic effects of a vaccine, the selection of allergens for vaccination is an important issue.

Further only limited areas in the antigen take part in the forming of immune complexes. These sites are known as antigenic determinants or epitopes. Therefore, the contaminating constituents unrelated to the few allergens to which a patient is actually allergenic can lead to the induction of IgE antibodies with new specificities and lead to untoward effects including anaphylaxis. The cross-reactive allergens have to be removed from vaccines in order to avoid severe systematic adverse reactions caused by crossreactivity for safer ASIT. Thus identification of the actual allergenic components of pollen, their characterization and epitope mapping of these allergenic fractions is very essential for preparation of vaccines for successful immunotherapy.

This will help improve the safety and clinical therapeutic efficacy of the vaccines in comparison to traditional immunotherapy using crude extract. Such an allergen diagnosis will enable us to choose only IgE-binding allergens that are individually sensitized for antigen-specific immunotherapy. This approach, in which only sensitized allergens are used for immunotherapy, avoids secondary additional sensitization against nonreactive proteins that can occur with the use of crude extracts or a mixture of allergens.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

The Project Fellow Barnali Bera has been registered for her Ph. D. under the joint supervision of Professor Jayanta Kumar Kundu, Department of Zoology, Vidyasagar University.

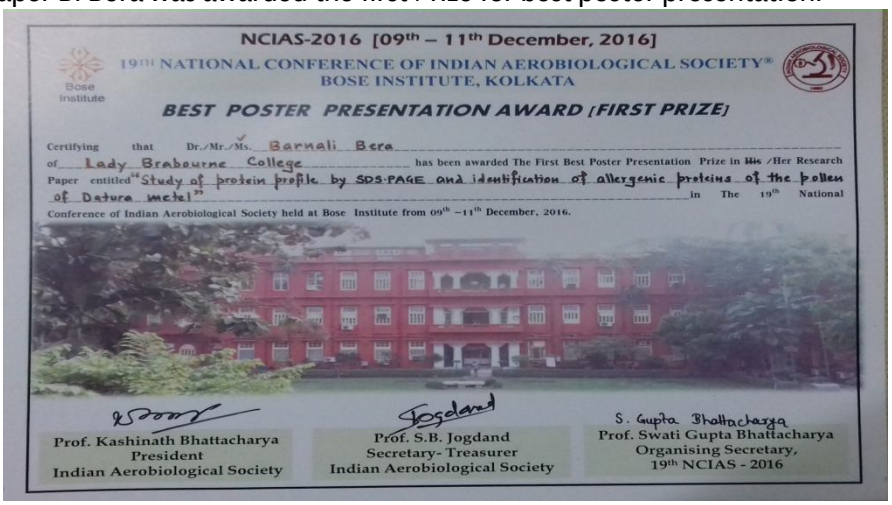
17. NO. OF PUBLICATIONS OUT OF THE PROJECT

Published Papers

1. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2015). Variation in The Protein Profile of the Allergenic Pollen of the *Datura metel* with Different Stages of Maturity. International Journal of Current Research, 7(6): 17174-17180.
2. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2016). The developmental variation of the protein profile of the pollen of *Datura inoxia* : A comparative study. International journal of Bioassays, 5.6: 4625-4629.
3. Bera, B., Mondal (Parui), S. and Kundu, J.K., Mondal, A.K. (2017). *Datura stramonium* pollen and its possible role in allergenic etiology- Recommendations for standardizations of pollen extracts for ASIT, 12(3): 62-69.

Abstracts published in proceedings of Seminars/Conferences attended

1. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2014). Variation in The Protein Profile of The Allergenic Pollen of *Datura metel* with Different Stages of Maturity. In: the proceedings of the National Conference on Environmental Issues and Food Security in India: Let's voice together towards a sustainable future organized by Foundation for Science and Environmental Research Institute, Kolkata in Association with Scientific & Environmental Research Institute, Kolkata on 10th August, 2014. Abst. No. P-20, p.144.
2. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2014). A Comparative Study of the Developmental and Seasonal Variation of the Protein Profile of the Pollen of *Datura inoxia* and *Datura metel*. In: the proceedings of the National Conference on Nanoscience and Nanotechnology (NS & NT-2014) organized by Centre for Research in Nanoscience and Nanotechnology (CRNN) University of Calcutta, Kolkata on September 18 & 19, 2014. Abst. No. 36, p.52.
3. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2015). A comparative study of the developmental and seasonal variation of the protein profile of the pollen of *Datura stramonium* and *Datura metel*. In: the proceedings of the West Bengal State Science & Technology Congress 2015 from 28 February -1 March, 2015.
4. Bera, B., Mondal (Parui), S. and Kundu, J. K. (2016). Study of Protein Profile By SDS-PAGE and Identification of The Pollen of *Datura metel.* In: the proceedings of 19th National Conference of Indian Aerobiological Society, Bose Institute, Kolkata from 9-11th December, 2016, Abst. No. 2, p. 63. For this paper B. Bera was awarded the first Prize for best poster presentation.


NCIAS-2016 [09th – 11th December, 2016]

19TH NATIONAL CONFERENCE OF INDIAN AEROBIOLOGICAL SOCIETY®
BOSE INSTITUTE, KOLKATA

Bose Institute

BEST POSTER PRESENTATION AWARD (FIRST PRIZE)

Certifying that Dr./Mr./Ms. Barnali Bera of Lady Brabourne College has been awarded The First Best Poster Presentation Prize in ~~His~~ /Her Research Paper entitled "Study of protein profile by SDS-PAGE and identification of allergenic proteins of the pollen of Datura metel" in The 19th National Conference of Indian Aerobiological Society held at Bose Institute from 09th –11th December, 2016.

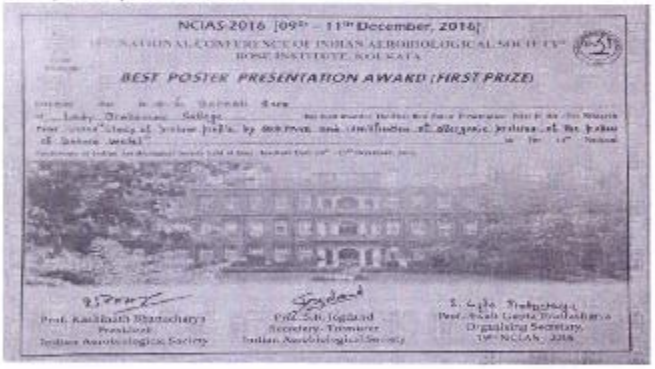
Prof. Kashinath Bhattacharya
President
Indian Aerobiological Society

Prof. S.B. Jogdand
Secretary- Treasurer
Indian Aerobiological Society

S. Gupta Bhattacharya
Prof. Swati Gupta Bhattacharya
Organising Secretary,
19th NCIAS - 2016

proceedings of the National Conference on Environmental Issues and Food Security in India: Let's voice together towards a sustainable future organized by Foundation for Science and Environmental Research Institute, Kolkata in Association with Scientific & Environmental Research Institute, Kolkata on 10th August, 2014. Abst. No. P-20, p.144.

2. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2014). A Comparative Study of the Developmental and Seasonal Variation of the Protein Profile of the Pollen of *Datura inoxia* and *Datura metel*. In: the proceedings of the National Conference on Nanoscience and Nanotechnology (NS & NT-2014) organized by Centre for Research in Nanoscience and Nanotechnology (CRNN) University of Calcutta, Kolkata on September 18 & 19, 2014. Abst. No. 36, p.52.
3. Bera, B., Mondal (Parui), S. and Mondal, A.K. (2015). A comparative study of the developmental and seasonal variation of the protein profile of the pollen of *Datura stramonium* and *Datura metel*. In: the proceedings of the West Bengal State Science & Technology Congress 2015 from 28 February -1 March, 2015.
4. Bera, B., Mondal (Parui), S. and Kundu, J. K. (2016). Study of Protein Profile By SDS-PAGE and Identification of The Pollen of *Datura metel*. In: the proceedings of 19th National Conference of Indian Aerobiological Society, Bose Institute, Kolkata from 9-11th December, 2016, Abst. No. 2, p. 63. For this paper B. Bera was awarded the first Prize for best poster presentation.


NCIAS-2016 [09th – 11th December, 2016]

BEST POSTER PRESENTATION AWARD (FIRST PRIZE)

Prof. Kalidas Bhattacharya
President
Indian Aerobiological Society

Prof. S.K. Jagtap
Secretary-Treasurer
Indian Aerobiological Society

Organizing Secretary
19th NCIAS - 2016

Sanjukta Mondal (Parui)
SIGNATURE OF THE PRINCIPAL INVESTIGATOR

DR. SANJUKTA MONDAL (PARUI)
Associate Professor in zoology, WBES
Department of Zoology
LADY BRABOURNE COLLEGE,
KOLKATA-700 017

SIGNATURE OF THE REGISTRAR/PRINCIPAL

Principal
Lady Brabourne College
Kolkata

Annexure – X

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
ASSESSMENT CERTIFICATE

It is certified that the proposal entitled "Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: implications for immunotherapy and studies on the air pollution impact on their etiology" by Dr. Sanjukta Mondal (Parui), Department of Zoology, Lady Brabourne College has been assessed by the Expert committee consisting the following members for submission to the University Grants Commission, New Delhi for financial support under the scheme of Major Research Projects.

Details of Expert Committee:

Name of Expert	Name of Department	Signature with Date
Professor Goutam Saha	Department of Zoology University of Calcutta	13.12.17 Prof. Goutam Kr. Saha Professor in Zoology University of Calcutta
Dr. Tushar Kanti Mukhopadhyay Retired Professor of Zoology	Department of Zoology Presidency College (now Presidency University) Kolkata	13/12/17 Dr. Tushar Kanti Mukherjee Associate Prof. (Retired), W.B.E.S Presidency University (Formerly Presidency College) 86/1 College Street, Kol-7

The proposal is as per the guidelines.

(REGISTRAR/ PRINCIPAL)

Principal
Lady Brabourne College
Kolkata

Annexure – XI

Final Report Assessment / Evaluation Certificate

(Two Members Expert Committee Not Belonging to the Institute of Principal Investigator)

It is certified that the final report of Major Research Project entitled "Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: implications for immunotherapy and studies on the air pollution impact on their etiology" by Dr. Sanjukta Mondal (Parui), Department of Zoology, Lady Brabourne College has been assessed by the committee consisting the following members for final submission of the report to the UGC, New Delhi under the scheme of Major Research Project.

Comments/Suggestions of the Expert Committee: Annexure 1

Name & Signatures of Experts with Date

Name of Expert	Name of Department	Signature with Date
Professor Goutam Saha	Department of Zoology University of Calcutta	Prof. Goutam Kr. Saha Professor in Zoology University of Calcutta 3.12.17
Dr. Tushar Kanti Mukhopadhyay Retired Professor of Zoology	Department of Zoology Presidency College (now Presidency University) Kolkata	3/12/2017 Dr. Tushar Kanti Mukherjee Associate Prof. (Retired), W.B.E.S. Presidency University (Formerly Presidency College) 86/1 College Street, Kol-7

It is certified that the final report has been uploaded on UGC-MRP portal on 24/07/2018

It is also certified that final report, Executive summary of the report, Research documents, monograph academic papers provided under Major Research Project have been posted on the website of the University/College.

(Registrar/Principal)

Seal

Principal
Lady Brabourne College
Kolkata

Adjudication Report on evaluation of the final report of the UGC Major Research Project entitled "Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: Implications for immunotherapy and studies on the air pollution impact on the etiology" by Dr. Sanjukta Mondal (Parui)

The report submitted by Dr. Sanjukta Mondal (Parui) has been evaluated and strongly recommended for the Final Assessment and Submission of the report of the Project based on the following points:

- The work is a comprehensive on a very important aspect of pollen allergy, characterization of the allergenic proteins and its implication in immunotherapy. The report provides a clear overview of the work done and the reasons behind selecting the topic for the research work. A very good attempt has been made at a concise presentation of the total work done with very clear aims and objectives.
- The materials and methods section includes details of the studies. Her effort is praiseworthy because she has been able to achieve beneficent results and effective conclusions.
- The illustrations and the photographs illuminate the findings and the results of the experiments.
- The results, discussion and conclusion are clear and precise. She has highlighted on the main findings and analyzed the data to come to very decisive conclusions.
- The work would have been more fruitful if more species could be taken into consideration. Given further assistance, the work could be carried forward for more scope in research in this applied science.
- I have no further observation in this report and again, strongly recommend her research.

Tushar K Mukherjee 10/11/2017

Dr. Tushar Kanti Mukherjee
Retired Associate Professor of Zoology
Department of Zoology
Presidency College (now Presidency University)
Kolkata

Dr. Tushar Kanti Mukherjee
Associate Prof. (Retired), W.B.E.S.
Presidency University
(Formerly Presidency College)
86/1 College Street, Kol-73

I have critically gone through the Final Report of the UGC Major Research Project entitled "**Epitope mapping of the allergenic proteins of the pollen of a few allergenic plants growing in Kolkata: implications for immunotherapy and studies on the air pollution impact on their etiology**" **[Ref. No. F.42-559/2013 (SR), dt. 22.03.13]** of Dr. Sanjukta Mondal (Parui), Associate Professor & Head of the Department of Zoology, Lady Brabourne College, Kolkata. The report has been evaluated and recommended for submission to UGC from the following points of view:

It's a fact that the incidence of nasobronchial allergic manifestations are increasing day by day in an unprecedented manner, particularly in urban settings of developing countries like India. The bronchi of subjects with allergic complaints are sensitive to various external substances, of which pollens from good number of plant species are important to initiate the problems. The present research is a honest effort towards characterization and epitope mapping of the allergenic proteins of pollen grain of selected plant species available in Kolkata metropolis, (taking *Datura* sp. as a model) to provide best possible diagnosis and treatment to the sufferers. Empirical studies demonstrate that only limited areas in the antigen take part in the formation of immune complexes. These sites are known as antigenic determinants or epitopes. One of the most possible treatment for allergic rhinitis is immunotherapy, also called hypo-sensitization, which involves repeated subcutaneous injections of gradually increasing concentrations of the allergen(s) considered to be specifically responsible for the symptom complex. Controlled studies have established that patients are partially relieved of their symptoms by such treatments applied over a period of years. Unfortunately, allergen specific immunotherapy (ASIT) as practiced today has not only proved to be cumbersome and expensive, but is also associated with the risk of fatal side reactions. This is because commercial antigenic extracts used for immunotherapy consists of heterogeneous (crude) extracts of the pollen (complex mixture of several proteins, lectins, complex carbohydrates, lipids, nucleic acids, enzymes etc.) and may contain the relevant allergens in minute, irreproducible amounts. Therefore, the contaminating constituents unrelated to the few allergens to which a patient is actually allergenic can lead to the induction of IgE antibodies with new specificities and lead to untoward effects including anaphylaxis. The cross-reactive allergens have to be removed from vaccines in order to avoid severe systematic adverse reactions caused by cross reactivity for safer ASIT. Thus identification of the actual allergenic components of pollen, their characterization and epitope mapping of these allergenic fractions is very essential for preparation of vaccines for successful immunotherapy. This will help to improve the safety and clinic-therapeutic efficacy of the vaccines in comparison to traditional immunotherapy using crude extract. Thus the selection of topic is very much relevant to the present day scenario. The aims and objectives are very clear and logically sound. I congratulate the researcher for the selection of such a topic of great medical importance.

- A very good attempt has been made in the total work done with very clear aims and objectives and substantial and sophisticated experimental work deployed to support the findings. The conclusion gives a clear overview of the work done and the reasons behind selecting the topic for the research work.

- The materials and methods section includes details of the experiments. The results have given a vantage point from which one can gain a rational view on the actual allergenic fractions of *Datura* pollen.
- Isolation and identification of the actual allergenic proteins by gel electrophoresis, Ouchterlony Immuno-diffusion and ELISA and Epitope mapping by Cross linking coupled Mass Spectrometry with high mass MALDI detection helped to identify the binding location and sequencing of the amino acids. This will help in the preparation of appropriate and suitable vaccines for Allergen Specific Immunotherapy and reduce the risk of the contaminating constituents unrelated to the few allergens to which a patient is actually allergenic. I believe the outcome of the study might enrich the present state of knowledge in the relevant field.

- The researcher has successfully completed the three basic objectives within stipulated time period and she has made considerable achievements in her field of research as evidenced from her two excellent publications and I believe there are enough scope and potential for more good publications.

- The part of the title "studies on the air pollution impact on their etiology" could not be completed due to lack of time as communicated by the researcher. I am sure with proper funding and time, this aspect of the investigation will be carried out by her in near future.

On the whole, in my opinion Dr. Mondal has done a commendable job and I am extremely satisfied with the quality of data collected, analyzed and inferences made. Considering all aspects, I strongly recommend the present report

16.01.16

(Prof. Goutam Kumar Saha)
Department of Zoology
University of Calcutta

Prof Goutam Kr Saha
Professor in Zoology
University of Calcutta